Royal Netherlands Navy

Advances in diagnosis and treatment of cerebral arterial gas embolism

R.P. Weenink

Advances in diagnosis and treatment of cerebral arterial gas embolism

Advances in diagnosis and treatment of cerebral arterial gas embolism
Thesis, University of Amsterdam, The Netherlands 2013

ISBN: 978-90-9027688-5

Cover design: Martin Risseeuw, Martinr.nl, Den Haag, The Netherlands
Printed by: Drukkerij Ridderprint, Ridderkerk, The Netherlands

All studies described in this thesis were performed at the Department of Surgery and the Department of Anesthesiology, Academic Medical Center, Amsterdam, The Netherlands, and at the Diving Medical Center, Royal Netherlands Navy, Den Helder, The Netherlands.

The research described in this thesis was funded by the Netherlands Ministry of Defense (grant 009-07-5041-01).

Printing of this thesis was funded by the Royal Netherlands Navy, the Department of Surgery of the Academic Medical Center, Amsterdam, and Roba Metals BV.

ADVANCES IN DIAGNOSIS AND TREATMENT OF CEREBRAL ARTERIAL GAS EMBOLISM

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom
ten overstaan van een door het college voor promoties
ingestelde commissie,
in het openbaar te verdedigen in de Aula der Universiteit
op woensdag 18 september 2013, te 11:00 uur

door

Robert Paul Weenink
geboren te Amsterdam

Promotiecommissie

Promotores:	Prof. dr. dr. M.W. Hollmann
	Prof. dr. T.M. van Gulik
Co-promotores:	Dr. R.A. van Hulst
	Dr. M.F. Stevens
Overige leden:	Prof. dr. J.T.A. Knape
	Prof. dr. B.A.J.M. de Mol
	Prof. dr. R.E. Moon
	Prof. dr. P. Radermacher
	Prof. dr. W.S. Schlack
	Prof. dr. M.J. Schultz

Faculteit der Geneeskunde

Contents

Chapter 1	General introduction and outline (submitted)	9
Chapter 2	Animal models of cerebral arterial gas embolism (J Neurosci Methods 2012; 205:233-45)	27
Chapter 3	Cerebral arterial gas embolism in swine; comparison of two sites for air injection (J Neurosci Methods 2011; 194:336-41)	71
Chapter 4	Quantitative electroencephalography in a swine model of cerebral arterial gas embolism (Clin Neurophysiol 2012; 132:411-7)	89
Chapter 5	Detection of cerebral arterial gas embolism using regional cerebral oxygen saturation, quantitative electroencephalography, and brain oxygen tension in the swine (submitted)	109
Chapter 6	Hyperbaric oxygen does not improve cerebral function when started two or four hours after cerebral arterial gas embolism in swine (Crit Care Med; 2013; 41:1719-27)	133
Chapter 7	A retrospective cohort study of lidocaine in divers with neurological decompression illness (Undersea Hyperb Med; in press)	157
Chapter 8	Acute neurological symptoms during hypobaric exposure: consider cerebral air embolism (Aviat Space Environ Med 2012; 83:1084-91)	173
	Summary and conclusions	197
	Samenvatting en conclusies	205
	Dankwoord	215
	Portfolio	221

General introduction and outline

1

An adapted version of this chapter has been submitted to “Nederlands Tijdschrift voor Anesthesiologie” (Netherlands Journal of Anesthesiology).

1 Introduction

The underwater environment presents its human visitors with many unique and interesting challenges. Many of these problems are direct or indirect effects of the increased pressure involved in hyperbaric exposure. The higher partial pressure of oxygen may cause oxygen toxicity, the elevated levels of nitrogen pose a risk of inert gas narcosis as well as decompression sickness, and trace amounts of contaminants in the breathing gas may rise to dangerously high levels when breathed under pressure. Barotrauma may occur due to pressure differences between air spaces and the surrounding environment. The best known example of barotrauma is middle ear squeeze, but damage may occur in any air filled cavity that is not (sufficiently) communicating with the outside world. When such an area of air trapping exists in the lungs, pulmonary barotrauma may occur during decompression from depth. The most intriguing and feared result of pulmonary barotrauma occurs when the escaping air flows into the pulmonary veins and is then transported via the heart to the cerebral arteries. This is termed cerebral arterial gas embolism (CAGE). Apart from pulmonary barotrauma, CAGE may result from direct introduction of air into the systemic circulation. These cases of CAGE are usually the result of some kind of invasive medical procedure, for instance cardiac surgery.

The divers in the service of the Royal Netherlands Navy are involved in a multitude of high-demanding diving operations. Furthermore, the Navy's submarine personnel is regularly trained on disabled submarine situations, which includes rapid ascent from various depths. These activities carry a risk of pulmonary barotrauma and therefore CAGE. Despite the stringent safety precautions employed in all training and operational underwater activities, occasional cases of CAGE have occurred in active duty Dutch military personnel. It is therefore not surprising that the Navy has great interest in this disorder.

This thesis is the result of a continuous Navy research program into CAGE. In this first chapter, we will provide a general overview of this dis-

ease, outlining etiology, pathophysiology, diagnosis, and treatment. We then proceed with a synopsis of the remaining chapters of this thesis, in which we describe our investigations on diagnosis and treatment of this disorder.

Etiology

There are four possible methods for air bubbles to reach the cerebral circulation (figure 1).

1. Pulmonary barotrauma resulting in air entrance in the pulmonary veins (1). The best known cause of this form of CAGE is diving. When a diver

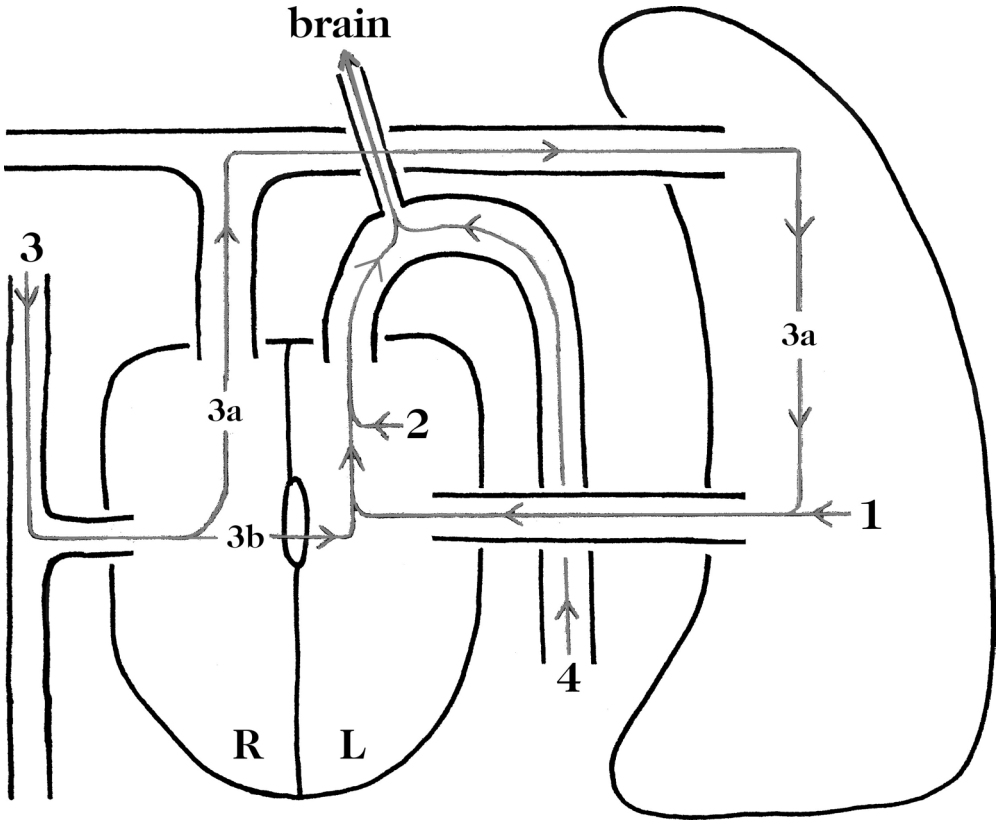


Figure 1. Etiological mechanisms of CAGE. The numbers in the figure correspond to the paragraph numbers in the text.

ascends, the surrounding pressure decreases which causes the air in the lungs to expand (Boyle's law). This expanding air has to escape through the airways. While this occurs without difficulty in persons without pulmonary abnormalities, problems may arise in cases of air trapping. Air trapping may be present in a variety of pulmonary disorders, such as asthma, emphysema, cyts, bullae, etcetera. Even without pulmonary abnormalities, when a divers fails to exhale during ascent, the whole lung volume becomes a closed compartment. Trapped air during ascent can cause rupture of alveoli, resulting in pneumothorax, pneumomediastinum, subcutaneous emphysema, and/or entrance of air into the pulmonary veins. In the latter case, this air will travel to the left heart and thence to the brain.

2. Direct introduction in the circulation somewhere in the trajectory from the pulmonary veins to the brain (1). These cases are usually iatrogenic in origin. The best known example of this category is cardiac surgery, which may lead to air introduction due to inadequate de-airing of the cardiac chambers (in cases of open chamber surgery) or due to air bubbles originating from the cardiopulmonary bypass system. Other causes of CAGE due to iatrogenic air introduction are for instance carotid and pulmonary surgery.

3. Arterialization of air introduced in systemic veins (2). Air introduced into the systemic venous vasculature will generally flow to the lungs. Since the lung is an excellent filter for air bubbles, this will normally not cause clinical symptoms, although large amounts of air may cause clinically evident pulmonary embolism. Also, these large amounts of air may overload the pulmonary arteries, which results in shunting of air from the pulmonary arteries to the pulmonary veins (3a in figure 1). From there the air may be propelled to the brain. A second method for arterialization of systemically introduced air is through a right-left shunt in the heart, most commonly a patent foramen ovale (3b in figure 1). Arterialization of venous air is termed paradoxical air embolism.

4. Retrograde movement of air in systemic arteries (3, 4). It has been demonstrated that large amounts of air can travel retrogradely through arteries and may thus reach the brain. This can either be due to buoyancy of the air bubbles or due to a volume effect. This is mostly a theoretical possibility, since only very few studies have been published that report this way of embolization of the cerebral arteries.

Distinction between CAGE and similar disorders

Other forms of air embolism exist, some bearing close resemblance to CAGE. It is important to understand the differences between these disorders and CAGE.

Non-cerebral arterial gas embolism

Air that is propelled into the systemic arterial circulation from the heart may not only lead to CAGE, but may also disperse to the rest of the systemic circulation. In theory, this could lead to ischemia in all parts of the body. The main reason that systemic arterial gas embolism usually doesn't result in clinical symptoms is the fact that in most organs some degree of collateral circulation exists. One important exception – apart from the brain – is the coronary circulation. In fact, many patients with CAGE also suffer from cardiac ischemia, indicating that some of the air has ended up in the coronary circulation (5).

Venous gas embolism

Air may be introduced into systemic veins through various mechanisms (6). An example is opening of non-collapsing cerebral venous sinuses during neurosurgery. When this is combined with a negative pressure (as compared to the heart) in these sinuses, large amounts of air may be sucked in. Air introduced into these and other systemic veins will lodge in the pulmonary arteries and be resorbed there. Large amounts of air may cause symptomatic pulmonary embolism and should be acted upon. Furthermore, as described above, an overloading of the pulmonary arteries may lead to shunting of the

air to the pulmonary veins, possibly leading to CAGE. Secondly, air flowing to the right heart from the systemic veins may pass to the left heart through a patent foramen ovale, leading to paradoxical CAGE.

Decompression sickness

While the contents of the gas bubbles in CAGE is determined by the mixture of the gas that was introduced into the circulation (most often air), the bubbles in decompression sickness (also known as caisson disease) contain only nitrogen (7). These bubbles appear when a person is decompressed, for instance when returning to surface after diving. Henry's law states that the solubility of a gas is proportional to the pressure of the gas. Therefore, during a dive the large partial pressure of inhaled nitrogen causes no problems, since all nitrogen is dissolved in the body. When the person ascends, pressure decreases, causing the nitrogen to come out of solution. When this occurs too quickly, nitrogen bubbles may form. These bubbles can cause local tissue damage but may also lead to vascular occlusion. Although neurological damage due to decompression sickness mostly occurs at the spinal level, some patients may exhibit cerebral neurological abnormalities resembling CAGE.

Pathophysiology

In CAGE, the contents of the gas that was introduced into the circulation determines the composition of the bubbles. In most cases, the gas will be air. This is the case in the diver who breathes compressed air, as well as in most iatrogenic cases. However, the bubbles may contain other gasses, for example in a diver who uses alternative breathing mixtures or when paradoxical CAGE occurs during laparoscopy (in which case the gas will be carbon dioxide). While the behavior of gas bubbles may differ depending on the contents of the bubbles, the general principles of cerebral damage induced by CAGE are always the same. Throughout this thesis, we will consitently use the term CAGE, although the specific gas used in our studies was air.

CAGE causes cerebral injury in two ways (1). Firstly, the bubbles may lodge in small cerebral arterioles and cause ischemic injury much like solid thrombi do. The difference between gaseous and solid thrombi is the fact that the occlusion caused by gaseous bubbles is always temporary. Small bubbles may lodge for only very short periods, being propelled through the capillaries into the veins in a matter of seconds. On the other hand, large amounts of gas may stay in the vessels for long periods, possibly hours (8). Eventually, all bubbles will shrink due to resorption of the gas, followed by propulsion of the bubble to the venous circulation. The second cause of cerebral injury due to CAGE is damage to the endothelium by the bubbles. It has been demonstrated that even bubbles that are too small to lodge in the arteries for a significant amount of time cause endothelial damage, due to stripping of the glycocalyx and the endothelial cells themselves (9). The resulting leakage is clinically evident as cerebral edema. The endothelial damage furthermore results in an inflammatory and protrombotic response (1, 2).

Epidemiology

At first glance, CAGE is a rare disorder. The incidence in diving is difficult to estimate, since generally the denominator (total number of dives) is not known. One study estimated the incidence of CAGE to be approximately 0.025 per 100,000 dives. This number was much higher (11 to 32 per 100,000) in training dives involving rapid ascent (10). A large retrospective series of iatrogenic CAGE estimated the complication to occur in 0.57 per 100,000 hospital admissions (5). However, it is possible that cases involving small amounts of air are regularly missed. This can be illustrated using the example of postoperative cognitive dysfunction. As much as 20-40% of patients undergoing cardiac surgery experience long lasting cognitive decline (11, 12). Although the etiology of this disorder is no doubt multifactorial, CAGE has been implicated as an important contributing factor in several studies (13, 14). Multiple reasons are conceivable why not all patients suffering peroperative CAGE are identified, such as difficulty in

adequately diagnosing cerebral air embolization, delayed recovery due to extended postoperative anesthesia, the difficulty distinguishing CAGE from other causes of peroperative cerebral damage, and insufficient clinical awareness. All in all, the exact incidence of CAGE cannot be determined, but is likely to be higher than reported.

Clinical presentation

The symptomatology of CAGE is heterogeneous and primarily depends on the brain regions and amount of air involved. Symptoms range from slight transient neurological dysfunction, such as sensory or motor deficit, to coma and brain herniation. In a recent clinical study 82% of patients diagnosed with clinical CAGE had impaired consciousness, 63% of this group was comatose (15). Symptoms develop suddenly after introduction of the air embolism and may be biphasic, when the initial insult is followed by the development of cerebral edema and increasing intracranial pressure. In surgical patients, symptoms may be masked by general anesthesia (1). Some authors have reported higher incidence of right hemispheric lesions compared to left hemispheric lesions (15). This is supposed to be due to the fact that the brachiocephalic artery, which is the first branch of the aortic arch, catches the largest amount of air coming from the heart. The distinction between CAGE and decompression sickness can usually be made by regarding the timespan between provoking exposure (e.g., diving) and start of symptoms. In CAGE, symptoms generally start during the dive or within 5 min after surfacing, while in decompression sickness it may take up to several hours for symptoms to develop.

Since air bubbles originating from the pulmonary veins or heart can distribute to all of the systemic circulation, signs of systemic embolization can accompany CAGE. One of the most important organs in this regard is the heart, and cardiac ischemia is a frequently encountered concurrent problem in patients with CAGE (5). A second set of problems occurs in patients with pulmonary barotrauma as the cause of CAGE, most often

divers. Apart from air entry into the vessels, the lung damage these patients have sustained may lead to pneumothorax, pneumomediastinum, and subcutaneous emphysema.

Diagnosis

The single key element in correctly identifying patients with CAGE is index of suspicion. CAGE should be included in the differential diagnosis in all patients with acute neurological symptoms in whom a possibility of entrance of air into the vasculature has existed (2). This includes divers who surface with neurological symptoms, but also surgical patients, especially in procedures with a high risk of CAGE, such as open chamber cardiac surgery. Concurrent abnormalities such as cardiac ischemia or pulmonary barotrauma (in case of a diver) may point in the direction of CAGE. Imaging of the brain may show air bubbles, but false negatives are common. Cerebral ischemia may be demonstrated on CT or MRI, but all techniques require a certain time before ischemia is adequately visible. In cases where there is a high likelihood of CAGE, such as a diver surfacing with neurological abnormalities, it is therefore advised to initiate treatment immediately, without inducing unnecessary delay due to imaging (7).

Treatment

In cases of severe CAGE, especially when concurrent coronary air embolization is present, hemodynamic support may be necessary. Normobaric oxygen therapy should be instituted immediately. Although the use of oxygen in other ischemic conditions is increasingly discussed (16), there exists little doubt on the use of oxygen in CAGE. The reason for this is the fact that in CAGE the primary reason for supplemental oxygen is not oxygenation of the penumbra but creation of a favorable gradient for outflow of nitrogen from the air bubbles (denitrogenation) (2). All other necessary

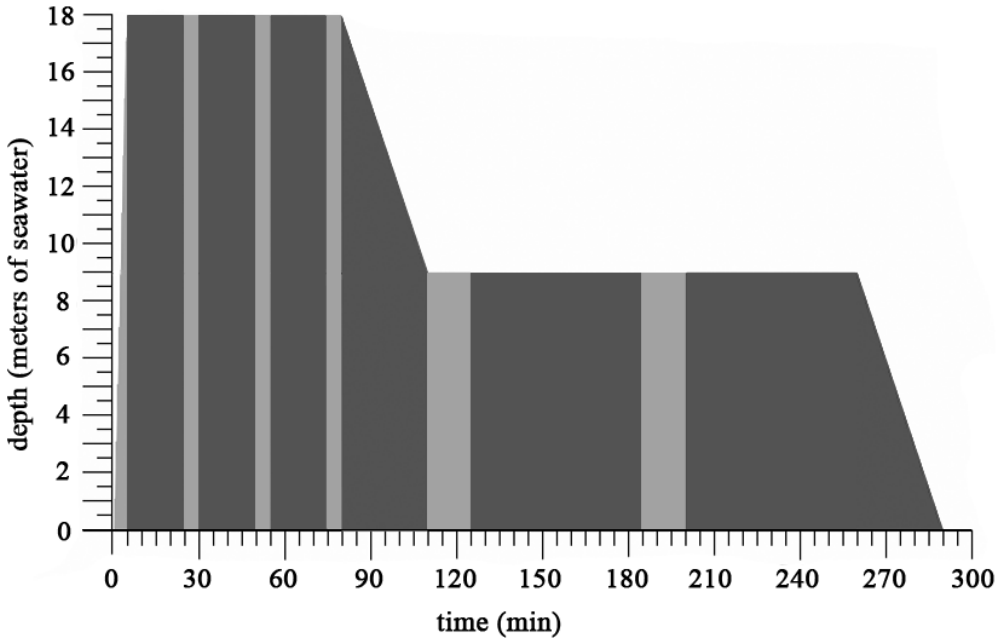


Figure 2. Profile of the US Navy Treatment Table 6. The therapy starts with rapid compression to 18 meters of seawater (2.8 atmospheres absolute, 280 kPa) on air (light grey), followed by three 20 min periods on oxygen (dark grey) each followed by 5 min air breaks. Then ascent to 9 meters of seawater (1.9 atmospheres absolute, 190 kPa) on oxygen is performed in 30 min, after which two blocks of 15 min air and 60 min oxygen follow. The ascent to the surface is performed on oxygen in 30 min. The total treatment takes 4 h 48 min.

measures to prevent secondary brain injury (prevention of hypotension, hypo- or hypercapnia, and hyperthermia) should be taken. The only specific treatment for CAGE is hyperbaric oxygen therapy (HBOT), which has important advantages over normobaric hyperoxia. Firstly, the increased atmospheric pressure compresses air bubbles that are still present in the circulation, which promotes passage of these bubbles through the capillaries to the venous circulation. Secondly, 100% oxygen combined with increased atmospheric pressure results in arterial and cerebral oxygen tensions that are significantly higher than in normobaric hyperoxia, leading to higher oxygen availability in the brain (2). In the third place, HBOT has been shown to have anti-inflammatory properties, which mediate the inflammatory response that follows the damage done to the endothelium by the air bubbles (17). Initial HBOT in CAGE is generally performed using US Navy Treatment Table 6, which is comprised of two stages of compres-

sion, the first stage at 2.8 atmospheres absolute and the second stage at 1.9 atmospheres absolute. During the treatment the patient breathes 100% oxygen, with intermittent air breaks to reduce the risk of cerebral oxygen toxicity (18) (figure 2). The treatment can be extended or repeated based on the clinical status of the patient.

Outcome

Outcome in patients suffering CAGE varies widely between studies. Four retrospective studies report good outcome (defined as no neurological sequelae or complete resolution) in 7%, 21%, 35%, and 77%, respectively (15, 19-21). Outcome in CAGE likely depends on the amount of air involved, affected brain regions, whether or not HBOT is administered, and delay between insult and initiation of HBOT. It must be noted, though, that only few studies have adequately investigated the factors influencing outcome. A study including both arterial and venous embolism found that cardiac arrest at the time of embolism and a Simplified Acute Physiology Score II of more than 33 were the only independent predictors of mortality. Focal motor deficits or Babinski sign at the time of admission to the intensive care unit, and mechanical ventilation of more than five days were independent predictors of neurological sequelae. Furthermore, in the univariate analysis a delay between CAGE and HBOT of more than 7 h was associated with more neurological sequelae (5). This relationship between delay to HBOT and poor outcome was also demonstrated in another retrospective study (22). However, yet another study could not demonstrate an effect of delay on outcome (21).

Difficulties and controversies

The previous paragraphs have focused on well-known aspects of CAGE. However, many questions regarding diagnosis and treatment of CAGE are currently unanswered. This paragraph names some of these issues,

specifically the ones that were the subjects of the research presented in this thesis.

Adequate treatment requires an adequate diagnosis. Historically, CAGE is best known in the diving community, and every diver who experiences neurological dysfunction during of shortly after a dive will be regarded to have suffered CAGE until proven otherwise. Although it not always possible to confirm or reject the diagnosis of CAGE with certainty (for instance when decompression sickness is a likely alternative), most divers with neurological injury will immediately receive normobaric oxygen, followed by HBOT. In regard to diagnosis, we have therefore focused on iatrogenic CAGE, since in these patients the diagnosis is more difficult, for example because symptoms are masked by general anesthesia. This issue is addressed in chapters 4 and 5, in which we report our studies on the use of electroencephalography and regional cerebral oximetry to detect CAGE.

A second discussion in CAGE regards the optimal timing of HBOT. Several studies show that earlier treatment is associated with better outcome (5, 22), but the maximum tolerable delay is unknown. Since in almost all cases of CAGE a significant delay will be present (an exception is professional diving where a recompression facility is available on the location), it is important to gather information on the effectiveness of delayed HBOT. This could provide guidance on the amount of urgency that should be applied when a possible case of CAGE is encountered. This issue is addressed in chapter 6.

The last controversy that we will discuss here is the use of adjuvant therapy in the treatment of CAGE. A number of adjunctive treatments have been studied in an effort to improve outcome. We have focused on the most widely studied substance, intravenous lidocaine. Several animal studies have shown a positive effect of this substance on the recovery of cerebral function after CAGE (23). This effect is supposed to result from a combination of neuronal membrane stabilization, neuronal metabolism

depression, and anti-inflammatory action (24). Four human studies on the use of intravenous lidocaine as a neuroprotective agent have been performed, yielding conflicting results (25). Currently, the use of lidocaine in CAGE is neither promoted nor discouraged by the Undersea & Hyperbaric Medical Society (26). This issue is addressed in chapter 7.

Outline of this thesis

Chapter 2 provides an introductory overview of all animal models used in CAGE research, discussing their advantages and disadvantages. It provides the researcher with an overview of the factors that should be taken into account in the design of a CAGE model. This review has served as the basis for the improvements of the CAGE model presented in the remainder of the thesis.

Chapter 3 elaborates on an important issue in the use of the pig as a model for CAGE. Since the pig possesses a finely entangled network of arterioles in the carotid system proximal to the brain, direct entrance to the Circle of Willis with a microcatheter to deliver the air is not possible. Two possible ways to overcome this problem, and adequately inject air into the pig's cerebral arteries, are presented and compared.

Chapter 4 reports the use of quantitative electroencephalography (qEEG) in quantifying the effects of CAGE on cerebral function. Several qEEG parameters are studied in the setting of acute CAGE.

Chapter 5 reports our investigation on the optimal way of peroperative detection of CAGE. We investigate two different methods to detect peroperative CAGE, namely qEEG and non-invasive regional cerebral oximetry using near-infrared spectroscopy. These methods are compared to results obtained with invasive measurement of brain oxygen tension and the cerebral microdialysis markers lactate and glycerol.

Chapter 6 presents our study on the effect of delay on the effectiveness of HBOT in CAGE. We commenced HBOT either two or four hours after induction of CAGE. Results were compared to control animals that did not receive HBOT.

Chapter 7 is a clinical study on the use of intravenous lidocaine as adjunctive treatment in neurological decompression illness (which includes CAGE and neurological decompression sickness). Divers suffering decompression illness who received HBOT and adjuvant lidocaine were retrospectively compared to a group of divers who received only HBOT.

Chapter 8 is a review study on CAGE due to hypobaric exposure. Diving related CAGE originates from pulmonary barotrauma following decompression from depth. Hypobaric activities such as flying and mountaineering also involve decompression, not from depth but from normal pressure to altitude. In this chapter all published cases that involve CAGE due to hypobaric activities are reviewed, and diagnostic and therapeutic implications are discussed.

References

1. Muth CM, Shank ES. Gas embolism. N Engl J Med 2000; 342:476-82.
2. van Hulst RA, Klein J, Lachmann B. Gas embolism: pathophysiology and treatment. Clin Physiol Funct Imaging 2003; 23:237-46.
3. Chang C, Dughi J, Shitabata P, Johnson G, Coel M, McNamara JJ. Air embolism and the radial arterial line. Crit Care Med 1988; 16:141-3.
4. Lowenstein E, Little JW, III, Lo HH. Prevention of cerebral embolization from flushing radial-artery cannulas. N Engl J Med 1971; 285:1414-5.
5. Bessereau J, Genotelle N, Chabbaut C, Huon A, Tabah A, Aboab J et al. Long-term outcome of iatrogenic gas embolism. Intensive Care Med 2010; 36:1180-7.
6. Mirski MA, Lele AV, Fitzsimmons L, Toung TJ. Diagnosis and treatment of vascular air embolism. Anesthesiology 2007; 106:164-77.
7. Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. Lancet 2011; 377:153-64.
8. Dexter F, Hindman BJ. Recommendations for hyperbaric oxygen therapy of cerebral air embolism based on a mathematical model of bubble absorption. Anesth Analg 1997; 84:1203-7.
9. Hills BA, James PB. Microbubble damage to the blood-brain barrier: relevance to decompression sickness. Undersea Biomed Res 1991; 18:111-6.
10. Lafere P, Germonpre P, Balestra C. Pulmonary barotrauma in divers during emergency free ascent training: review of 124 cases. Aviat Space Environ Med 2009; 80:371-5.
11. Newman MF, Mathew JP, Grocott HP, Mackensen GB, Monk T, Welsh-Bohmer KA et al. Central nervous system injury associated with cardiac surgery. Lancet 2006; 368:694-703.
12. Lombard FW, Mathew JP. Neurocognitive dysfunction following cardiac surgery. Semin Cardiothorac Vasc Anesth 2010; 14:102-10.
13. Carrascal Y, Guerrero AL. Neurological damage related to cardiac surgery: pathophysiology, diagnostic tools and prevention strategies. Using actual knowledge for planning the future. Neurologist 2010; 16:152-64.

14. Likosky DS, Marrin CA, Caplan LR, Baribeau YR, Morton JR, Weintraub RM et al. Determination of etiologic mechanisms of strokes secondary to coronary artery bypass graft surgery. Stroke 2003; 34:2830-4.
15. Griese H, Seifert D, Koerfer R. Cortical infarction following cardiosurgical procedures - air embolism as a probable cause. Eur Neurol 2009; 61:343-9.
16. Meyhoff CS, Staehr AK, Rasmussen LS. Rational use of oxygen in medical disease and anesthesia. Curr Opin Anaesthesiol 2012; 25:363-70.
17. Thom SR, Mendiguren I, Hardy K, Bolotin T, Fisher D, Nebolon M et al. Inhibition of human neutrophil beta2-integrin-dependent adherence by hyperbaric O2. Am J Physiol 1997; 272:C770-C777.
18. U.S. Department of the Navy, Naval Sea Systems Command: U.S. Navy Diving Manual. Volume 5: Diving Medicine & Recompression Chamber Operations. Washington, D.C.: Naval Sea Systems Command, 2008.
19. Massey EW, Moon RE, Shelton D, Camporesi EM. Hyperbaric oxygen therapy of iatrogenic air embolism. J Hyperbaric Med 1990; 5:15-21.
20. Trytko BE, Bennett MH. Arterial gas embolism: a review of cases at Prince of Wales Hospital, Sydney, 1996 to 2006. Anaesth Intensive Care 2008; 36:60-4.
21. Blanc P, Boussuges A, Henriette K, Sainty JM, Deleflie M. Iatrogenic cerebral air embolism: importance of an early hyperbaric oxygenation. Intensive Care Med 2002; 28:559-63.
22. Ziser A, Adir Y, Lavon H, Shupak A. Hyperbaric oxygen therapy for massive arterial air embolism during cardiac operations. J Thorac Cardiovasc Surg 1999; 117:818-21.
23. Evans DE, Kobrine AI, LeGrys DC, Bradley ME. Protective effect of lidocaine in acute cerebral ischemia induced by air embolism. J Neurosurg 1984; 60:257-63.
24. Mitchell SJ. Lidocaine in the treatment of decompression illness: a review of the literature. Undersea Hyperb Med 2001; 28:165-74.
25. Mitchell SJ, Merry AF. Lignocaine: neuro-protective or wishful thinking? J Extra Corpor Technol 2009; 41:37-42.
26. Undersea Hyperbaric Medical Society. UHMS best practice guidelines.

Prevention and treatment of decompression sickness and arterial gas embolism. Retrieved 1 September 2012 from http://c.ymcdn.com/sites/membership.uhms.org/resource/resmgr/dcsandage_prevandmgt_uhms-fi.pdf.

Animal models of cerebral arterial gas embolism

2

Weenink RP, Hollmann MW, van Hulst RA
J Neurosci Methods 2012; 205:233-45

2

Abstract

Cerebral arterial gas embolism is a dreaded complication of diving and invasive medical procedures. Many different animal models have been used in research on cerebral arterial gas embolism. This review provides an overview of the most important characteristics of these animal models. The properties discussed are species, cerebrovascular anatomy, method of air embolization, amount of air, bubble size, outcome parameters, anesthesia, blood glucose, body temperature, and blood pressure.

Introduction

Cerebral arterial gas embolism (CAGE) is defined as the presence of gas in the cerebral vessels. It can either occur after pulmonary barotrauma, which is the most common etiology in divers, or iatrogenically, for instance during cardiopulmonary bypass and interventional radiology (1, 2). CAGE can be regarded as multifocal cerebral ischemia with specific characteristics, for instance a transient nature and the immediate occurrence of blood-brain barrier damage (2).

In the past, reviews have addressed the different ways of induction of focal cerebral ischemia in animals (3, 4). However, these reviews did not or only briefly discuss CAGE. The clinical picture of CAGE is heterogeneous and patients can present with signs ranging from slight transient neurological dysfunction to coma with brain herniation (5). Many different animal models have been used in CAGE research, which is in line with the large clinical variance of the disease. In this review we discuss the existing animal models of CAGE, by analyzing the most important parameters to take into account in the development and evaluation of these models.

Methods

We searched MEDLINE and Embase for relevant articles (figure 1). The MEDLINE search strategy was ("embolism, air"[mesh] OR "air embolism"[tiab] OR "gas embolism"[tiab] OR (("embolism"[mesh] OR "embolism"[tiab]) AND ("air"[mesh] OR "air"[tiab] OR "gas"[tiab]))) AND ("brain"[mesh] OR "brain"[tiab] OR "cerebrum"[tiab] OR "cerebral"[tiab] OR "arteries"[mesh] OR "arterial"[tiab]), and a similar strategy was used for Embase. The search was last performed august 2011. Search results were limited to studies on animals, written in English. No constraints on publication date were imposed. Articles were initially selected based on title and abstract, after which the fulltext article was read to confirm article relevance. Relevant studies were defined as experiments in which air was

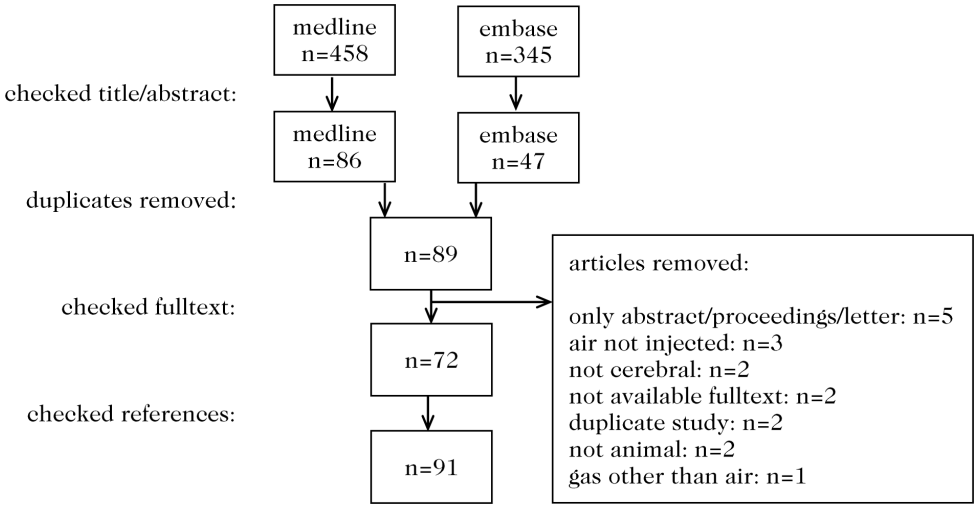


Figure 1. Search strategy.

actively administered to the circulation with the intent of entrance of air into the cerebral arteries. We excluded studies in which gases other than air were used, as well as studies that were only available as an abstract or as proceedings. References of included papers were checked to identify additional articles. In total, this search strategy resulted in 91 animals studies of CAGE (table 1). In this review, we provide an overview of these animal models by summarizing their most important characteristics:

- species
  - cerebrovascular anatomy
- location of air administration
- amount of air
- bubble size
- outcome parameters
- anesthesia
- other factors influencing outcome in cerebral ischemia
  - temperature
  - blood glucose
  - blood pressure

Species

As in all animal research, the first question to answer in the development of a CAGE model is which species to use. Naturally, no animal perfectly resembles the human situation. Generally, animals can be divided into small and large species, although this is a somewhat arbitrary division. Both groups have distinct advantages and disadvantages (97), and it is therefore not possible to prefer one species over the others. Larger brains usually bear more anatomic similarity to the human brain than smaller brains, for instance with regard to gyration. Cardiovascular and cerebral physiology more closely resemble the human situation in larger animals as compared to smaller animals. Furthermore, the closer brain size approaches human brain size, the easier techniques used in humans, such as imaging modalities and placement of cerebral probes, are applied. Advantages of smaller animals are lower cost and greater ease of breeding and handling. In addition, small animals, especially mice and rats, are much better known from a genetic and metabolic point of view. Figure 2 displays the various species used in CAGE research. The dog, cat, and rabbit are the most widely used animals. It is interesting to note a shift in the large animals used. Almost no studies using monkeys, dogs, or cats have been performed since 1995. Instead, the pig has gained much interest as a large animal model. One of the reasons for this change may be the lower emotional value of pigs as opposed to monkeys, cats, and dogs.

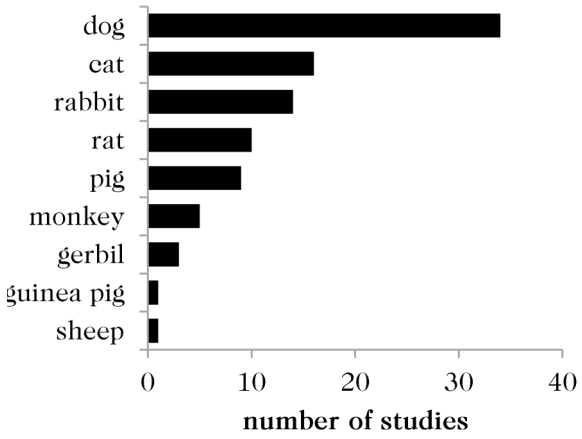


Figure 2. Species used in animal studies of CAGE. In two studies more than one species was used.

References	First use	Animal	Weight	Anesthesia
(6)	1929	dog	5.5-22.4kg	ether
(7)	1940	rabbit	unknown	ether
(8)	1957	dog	unknown	pentobarbital
(9)	1958	dog	unknown	unknown
(10)	1959	cat, rabbit	0.9-2.8kg	pentobarbital
(11)	1962	cat, monkey	unknown	ether d-tubo-cumarine pentobarbital
(12, 13)	1962	dog	15-20kg	pentobarbital
(14)	1963	cat	adult	pentobarbital
(15)	1963	dog	unknown	pentobarbital
(16)	1963	rabbit	2.2-3.6kg	pentobarbital succinylcholine
(17)	1964	cat	1.5-2.7kg	ether
(18)	1966	dog	unknown	pentobarbital
(19)	1966	rabbit	1-3kg	urethane
(20)	1967	dog	10-15kg	pentobarbital
(21)	1969	dog	10-15kg	unknown
(22)	1970	dog	adult	thiopental
(23, 24)	1971	dog	10-21kg	gallamine-triethiodide pentobarbital
(25)	1971	monkey (baboon)	3-6kg	pentobarbital
(26)	1973	dog	10-12kg	thiopental
(27-30)	1977	rat	200-300g	pentobarbital
(31-34)	1978	cat	1.9-5kg	gallamine-triethiodide pentobarbital
(35)	1978	dog	8-14kg	alpha-chloralose hexobarbital urethane
(36-38)	1978	gerbil	50-80g	pentobarbital
(39)	1978	monkey	unknown	pentobarbital
(40-48)	1979	dog	8-16kg	pentobarbital xylazine
(49)	1981	cat	2.5-4.0kg	alpha-chloralose keta
(50)	1982	rat	300-450g	halothane
(51)	1983	dog	unknown	morphine nitrous-oxide succinylcholine
(52, 53)	1984	cat	2.5-4.0kg	alpha-chloralose keta
(54-60)	1984	dog	8-16kg	pentobarbital xylazine
(61)	1985	rat	300g	gallamine-triethiodide pentobarbital
(62)	1986	rabbit	2-5kg	halothane
(63)	1986	rabbit	2-3kg	acepromazine atracurium halotane keta
(64)	1988	dog	14-31kg	halothane isoflurane thiopental
(65)	1988	monkey (macaque)	±7kg	keta thiopental
(66-69)	1989	cat	2.4-4.5kg	alpha-chloralose ketamine
(70-72)	1990	rabbit	2.1-2.4kg	gallamine-triethiodide urethane
(73)	1991	guinea pig	850±50g	pentobarbital
(74, 75)	1994	dog	10.3±2.5kg	thiopental

table continued on next page

Location	Air dosing	Outcome	Remark
pulmonary vein	weight	clinical	
'carotid artery'	fixed	clinical	
CCA	weight	CBF clinical	
CCA	titrated (death)	craniect EEG	
CCA	fixed	BBB	
ICA	fixed	BrOx CBF craniect EEG	
ICA	fixed	BBB clinical hist ICP	1 2 4
lung overpressure	n/a	craniect	
ICA	weight	hist WD	
'carotid artery'	titrated (EEG)	EEG	
'carotid artery'	weight	neuro-qual	
CCA	weight	EEG ICP	
CCA	fixed	BBB	5
ascending aorta	fixed	neuro-qual	
innominate artery / CPB	fixed	EEG	
CCA	fixed	neuro-qual	
VA	weight	AvOx CBF CSFOx ICP	1 3
CCA	fixed	CBF EEG	
multiple	weight	clinical EEG neuro-qual	6
CCA	fixed	BBB EM WD	1 2 3 5
innominate artery	fixed	AvOx BBB CBF craniect EEG EM ICP qEEG	1 2 3
ICA	fixed	BBB	
ICA	fixed	BBB BrMetab CBF EM hist neuro-qual WD	2 3
ICA	unknown	craniect EEG EM	
ICA	titrated (SSEP)	CBF EM hist SSEP	1 2 3
VA	weight	clinical	
ICA	fixed	CBF EEG	
descending aorta	weight	CBF clinical neuro-quant	
VA	fixed	ICP SSEP WD	1 2
ICA	fixed	CBF ICP SSEP WD	1 2
CCA	titrated (EEG)	BrOx CBF EEG	
femoral artery	fixed	craniect	
ICA	titrated (EEG)	EEG neuro-qual	
left ventricle / asc. aorta	fixed	Doppler (carotid)	
radial artery	fixed	gammascan	
CCA	titrated (SSEP)	SSEP	1 3 7
CCA	fixed	CBF craniect SSEP	8
ICA	fixed	BBB	
ICA	titrated (CT)	clinical CT EEG EM hist neuro-quant	2

table continued on next page

(76)	1994	monkey (macaque)	5-8kg	pentobarbital
(77-81)	1996	rabbit	2.5-3.4kg	isoflurane methohexital succinylcholine
(82)	1998	pig	25.8±3.2kg	keta lidocaine sufent
(83)	2001	sheep	±50kg	halothane thiopenthal
(84)	2002	pig	24.1±2.9kg	azaperonium diazepam keta thiopental
(85)	2003	cat	2.3-3.5kg	alpha-chloralose keta pancuronium
(86-89)	2003	pig	30-35kg	keta midazolam pancuronium
(90)	2004	pig	37.7±6.1kg	buprenorphine keta midazolam xylazine
(91-93)	2006	rat	363±17g	atracurium fentanyl isoflurane midazolam
(94)	2010	rat	255-326g	isoflurane
(95, 96)	2011	pig	35-40kg	keta midazolam pancuronium sufent

Table 1. Animal models of CAGE. When a model was used in multiple studies, this can be seen in column. When too few model properties were shared between studies, the studies were regarded as 'carotid artery' is mentioned as the location of air injection, it is not known whether this was the

Abbreviations: keta = ketamine; sufent = sufentanil; CCA = common carotid artery; ICA = internal craniect = craniectomy (with visualization of cerebral vessels); EEG = electroencephalography tension; hist = histology; ICP = intracranial pressure; WD = wet/dry ratio; neuro-qual = qualitative CSF oxygen tension; EM = electron microscopy; qEEG = quantitative electroencephalography; neurological scoring; CT = computed tomography; TCD = transcranial Doppler; BrT = brain resonance imaging; CPB = cardiopulmonary bypass.

Remarks: 1) weight varied slightly between studies; 2) not all outcome parameters mentioned with and without clipping of cerebral arteries; 5) used CCA with ECA (and in some studies other maxillary artery ligated; 8) tip of catheter at origo of ICA; 9) also used alcuronium, clonidine and arteries cauterized; 12) Ref (95) also used ECA.

Cerebrovascular anatomy and the carotid rete

An important consideration in the choice of a model for CAGE research is the cerebrovascular anatomy of the selected species. As will be shown below, the majority of animal models involve active injection of air into an artery feeding the brain. After injection of air, the specific cerebrovascular anatomy of the animal under study will determine distribution of the bubbles through the brain. In this paragraph we will mostly consider the five species that have most frequently been used in CAGE research, namely the dog, cat, rabbit, rat, and pig. In all these animals the blood supply to the brain is provided through an arterial circle comparable to the circle of Willis as present in man (98). Considerable differences exist between species in regard to which vessels supply the circle of Willis (figure 3). While in man the internal carotid

CCA with ECA ligated	fixed	TCD	
ICA	weight	BrT hist neuro-quant SSEP	1 2
CCA	fixed	BAER	9
CCA	fixed	ICP TCD	
CCA with ECA ligated	fixed	BrMetab(MD) CBF hist	
CCA	weight	intravital-microscopy	
CCA	weight	BrMetab(MD) BrOx BrT ICP qEEG	1 2
CCA	weight	MRI	
ICA	fixed	hist neuro-quant	1 3 10
CCA	fixed	hist neuro-quant	11
ascending pharyngeal art.	weight	BrMetab(MD) BrOx ICP qEEG	2 12

the first column. Differences between studies within a specific animal model can be seen in the last using different models, even when the studies were performed by the same research group. When common carotid artery or one of its branches.

carotid artery; VA = vertebral artery; ECA = external carotid artery; CBF = cerebral blood flow; (qualitative); BBB = blood-brain barrier damage (by injection of a tracer); BrOx = brain oxygen neurological examination; AVox = arteriovenous oxygen difference across the brain; CSFox = BrMetab = brain metabolism; SSEP = somatosensory evoked potentials; neuro-quant = quantitative temperature; BAER = brainstem auditory evoked responses; MD = microdialysis; MRI = magnetic

were used in all studies; 3) anesthetic agents varied between studies; 4) Ref (13) used ICA/VA branches) ligated; 6) used CCA / innominate artery / aorta / heart / CPB system; 7) with external flunitrazepam; 10) with pterygopalatine artery ligated; 11) tip of catheter at origo of ICA with other

artery is the most important artery, this vessel is only present as such in the rabbit, dog, and rat. In the dog the internal carotid artery is very small and the largest contribution to cerebral perfusion is through the internal maxillary artery, a branch of the common carotid artery (99). In the cat, the internal carotid artery is only present as a thin fibrous cord and the main blood supply is again through the internal maxillary artery (99). In the pig, the majority of blood supply to the circle of Willis comes from the ascending pharyngeal artery, a branch of the common carotid artery (98). The contribution of the vertebral arteries to total cerebral perfusion varies between the species, but is less than that of the carotid system in all species (98).

Some larger animals are known to possess bilateral networks of intertwined arterioles in the arterial system proximal to the circle of Wil-

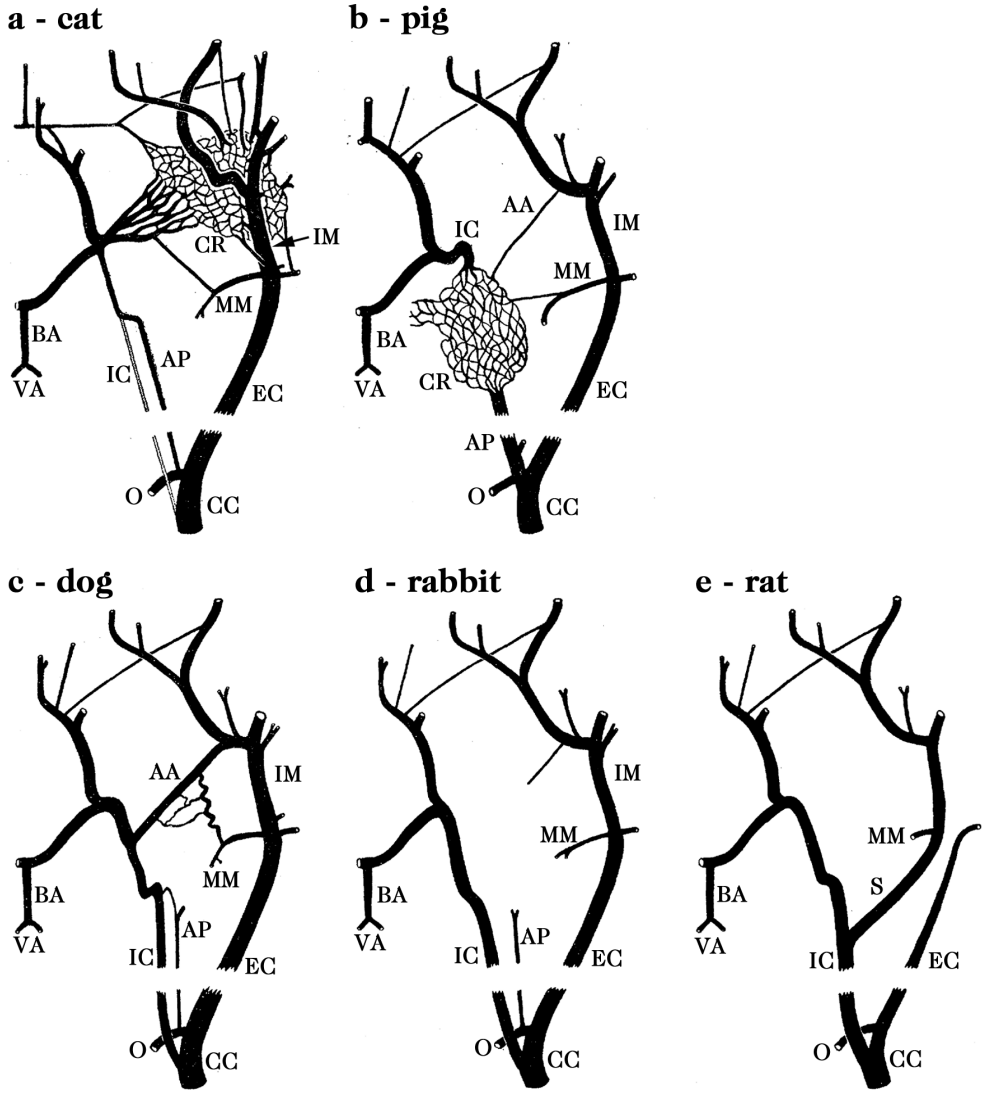


Figure 3. Schematic comparative cerebrovascular anatomy of the five most frequently used species in CAGE animal studies. Adapted (with permission) from ref (98). AA = anastomotic artery; AP = ascending pharyngeal artery; BA = basilar artery; CC = common carotid artery; CR = carotid rete; EC = external carotid artery; IC = internal carotid artery; IM = internal maxillary artery; MM = medial meningeal artery; O = occipital artery; S = stapedial artery; VA = vertebral artery.

lis, termed the carotid rete. Species with such a rete include the cat, pig, ox, goat, and sheep (98). A carotid rete does not exist in rabbits or rats, and only a few vessels that may represent a remnant rete are

present in the dog. Important to consider, the carotid rete precludes direct access with a catheter to the circle of Willis from the common carotid artery, and thus air has to be injected into arteries proximal to the rete. Due to the small size of the arterioles of which the carotid rete is comprised (200-300 µm in cats (98), reported as 74 µm (100) and 154 µm (101) in pigs), injection of air proximal to this structure may result in retention of the air in the plexus, which prevents access of air into the cerebral arteries. Hence, this would not result in cerebral ischemia, since the circle of Willis would still be supplied through the contralateral carotid system and the vertebral arteries.

Only few studies on CAGE in animals with a carotid rete have directly addressed this issue. We have recently shown in the pig that air injected into the artery proximal to the carotid rete does enter the cerebral vessels and results in injury measured as increased brain lactate and intracranial pressure (ICP) (95). Other researchers have chosen to inject air proximal to the carotid rete after ligation of branches that do not contribute to cerebral circulation, in order to force air through the plexus. In these studies, performed in cats, entrance of air into the cerebral vessels was evident by a decrease of somatosensory evoked potentials (SSEP) (66). In some models air was injected into the common carotid artery, or a more proximal artery, without measures to prevent flow of air to the external carotid territory (10, 17, 31, 82, 85, 86, 90). Other than cats and pigs, species that possess a carotid rete have not been studied in CAGE, except in one study in which air was injected into the common carotid artery in sheep (83) and entrance of air into the brain vessels was demonstrated by ultrasonic Doppler. In general it can be stated that if no measures are taken to prevent flow of air into the extracerebral arteries, it can not be ascertained that all air passes through the rete into the brain. The amount of undesired air flow will depend on various parameters, such as diameter of the arterioles and local perfusion characteristics, and may result in heterogeneous effects of the embolism. We have recently observed that air can be selectively directed through the carotid rete of the pig by

inflating a balloon in the ascending pharyngeal artery and injecting the air between the balloon and the carotid rete. After embolization this balloon is deflated in order to restore the normal cerebral perfusion situation (unpublished results).

Another way of circumventing the problems caused by the carotid rete is by introduction of air through the vertebral artery. This is not possible in the pig, since the vertebral arteries of the pig contain a rete of their own (102). In dogs, however, it has been shown that the middle cerebral artery can be reached with a catheter through the vertebral artery (103). Indeed, in one CAGE model air has been successfully injected into the vertebral artery in dogs (23). The vertebral artery in cats does not have a carotid rete, and although the size of this artery is very small, CAGE has been generated by injection of air into the cat vertebral artery (49, 52). The problems that may potentially arise after embolization of the vertebral artery, namely respiratory and cardiovascular compromise due to brain stem infarction, will be discussed in the next paragraph.

Avoiding injection of air proximal to the rete is also possible by directly accessing one of the cranial arteries through opening of the skull and subsequent microsurgical approach to the cranial base. Although such techniques have been described for many species and have been used in other animal models, this approach has not been used in any of the CAGE models. Reasons may be the surgical complexity of these procedures or the fact that the operation itself may have an effect on cerebral metabolism, which complicates analysis of injury due to CAGE.

In summary, while the intracranial cerebrovascular anatomy is grossly similar between most species used in CAGE research, there are important differences in the more proximal vasculature. The presence of a carotid rete in the species under study is of great significance in the development of an adequate animal model. There are several ways to inflict CAGE in animals with this structure.

Location of air administration

One of the most important factors of influence on the effects of CAGE on the brain and the stability and reproducibility of the animal model is the method of administration of the air. Throughout the published animal studies, the location of air administration into the circulation can be classified as 1) the arterial system proximal to the brain 2) the pulmonary venous circulation 3) an artery not responsible for brain perfusion 4) a cardiopulmonary bypass (CPB) system.

The vast majority of researchers has employed the first method. It makes sense to inject the air as close to the cerebral vasculature as possible, in order to generate the most standardized effects on the brain. In most models the tip of the catheter is therefore placed in the main artery supplying the cerebrum, usually the internal carotid artery, either with or without ligation of vessels not supplying the brain (11, 12, 15, 35, 36, 39, 40, 50, 54, 63, 73, 74, 77, 91, 95). In some studies the vertebral artery was chosen instead of the carotid (23, 49, 52). Others have administered air in a more proximal artery such as the common carotid artery with ligation of vessels not supplying the brain (19, 27, 66, 76, 84, 94). One would suspect that air injection into a proximal artery without ligation of external carotid vessels is likely to result in larger variance of results, because of distribution of air between external and internal carotid territories. This might be especially true in species that are known to have a smaller internal carotid artery (or equivalent main supplier of the brain) as compared to the external carotid artery, such as the pig (102). Nevertheless, in many models adequate results have been obtained after embolization of the common carotid artery (7-10, 16-18, 22, 25, 61, 70, 82, 83, 85, 86, 90) or even the innominate artery (21, 26, 31), ascending aorta (20, 26, 64), or heart (26, 64).

Although it makes sense to ligate certain branches not participating in cerebral perfusion when air is injected into a more proximal artery, this practice has disadvantages, as mentioned by Furlow (50). Disturbance of

normal cerebral perfusion characteristics may result in undesired flow changes. For instance, if the external carotid artery is ligated and the animal has anastomoses between external and internal carotid territories (as are present in many animals), then shunting of blood (and air) from cerebral to extracerebral tissues may occur. It is desirable to minimize alterations to cerebral perfusion characteristics in CAGE models.

With regard to the choice between carotid or vertebral artery, the study of De la Torre et al. (13) is of interest. Blood foam (blood thoroughly shaken with air) was injected into the internal carotid or vertebral artery of anesthetized spontaneously breathing dogs, with or without clipping of both posterior communicating arteries. Without clipping, immediate effects were summarized as apnea and hypertension with bradycardia in most animals, with no obvious differences between injection sites. However, in dogs in which the posterior communicating arteries were effectively clipped, no respiratory or cardiovascular changes were seen after injection into the internal carotid artery. These animals showed severe reactions after injection into the vertebral artery. These findings, which are explained by the presence of respiratory and autonomic nuclei in the brainstem, demonstrate the different reactions that can occur after embolization through the internal carotid versus the vertebral artery.

The second mentioned method for induction of CAGE, entrance of air through the pulmonary venous circulation, is of interest since in some instances – mostly in divers – CAGE is caused by pulmonary barotrauma. Only two studies were performed using this method, probably because it generates heterogeneous results due to dispersion of air through essentially the whole body. In their classic study Van Allen et al. (6) introduced air into the pulmonary vein of dogs and demonstrated that the air can be directed somewhat selectively to the cerebrum by positioning the subject in an upright posture. Another mode of administration of air in the pulmonary veins was used by Atkinson (14), who induced pulmonary barotrauma by adding one vital capacity to the lungs of cats, after which air bubbles were seen in the cerebral vessels.

Some authors have demonstrated that CAGE can be induced by introducing air into an artery not responsible for brain perfusion such as the descending aorta (51), femoral artery (62) or radial artery (65). In these studies, the purpose of generating CAGE was to demonstrate movement of air bubbles against the direction of arterial flow. The fact that these air bubbles can potentially reach the brain is either caused by their buoyancy or reflects a volume effect (i.e. the volume and speed of air injection result in a temporary reversal of arterial flow) (104).

Historically, air embolism has been a risk in CPB. This risk has been largely abolished since bubble oxygenators have been replaced by membrane oxygenators, although gaseous micro-emboli can still be generated in these systems (105). Occasionally, cases of massive CAGE due to malfunctioning of CPB machinery or human error in handling the system continue to be reported (106). A review on animal CPB models has been published recently (107). We do not report on studies in which air was passively generated by the oxygenator in a CPB system, since we only included studies in which air was actively administered to the circulation. There is essentially no difference in results between injection into a proximal artery and introduction of air in the arterial part of the CPB system, with the same remark regarding dispersion of air to unwanted vessels as mentioned above (26).

In order to simulate human CAGE as closely as possible, it would be interesting to know about patterns of cerebral infarction in clinical or diving-related CAGE. Griese et al. (108), in a retrospective analysis of probable CAGE cases after CPB, reported a higher incidence of right hemispheric lesions, mostly located in the cortex of the border zone between middle and anterior cerebral artery territories. They hypothesized this preponderance of right sided infarction to result from the fact that the brachiocephalic artery is the first artery branching from the aortic arch, thus catching the largest amount of air. No further studies on human CAGE infarction patterns are available, which makes resemblance of the human situation in animal models difficult. It may be advantageous to create uni-

lateral injury, so that the contralateral hemisphere can be used as internal control. Some authors have demonstrated unilateral injury in their model (94), but in general strict unilateral distribution is difficult to obtain. The first reason for this difficulty is the fact that changes in cerebral perfusion characteristics occur due to air introduction, which may shunt part of the air to the contralateral hemisphere (50). Secondly, in animals with a carotid rete there are usually many anastomoses between the left and right rete, which results in bilateral dispersion of air after unilateral injection (96). Therefore, one can not assume that unilaterally injected air remains in the ipsilateral hemisphere.

In conclusion, most CAGE models involve air injection through an intravascular catheter placed close to the cerebral circulation. This is obviously different from the etiology of CAGE in almost all clinical and diving-related cases, but is the best way of ensuring the most standardized effect on the cerebrum. Little is known about infarction patterns in human CAGE, which complicates accurate mimicking of the human situation in animal models.

Amount of air

With a given species and location of air administration, one of the important factors determining the level of injury inflicted in the animal is the amount of air injected. The main limitation in determining the optimal volume of air is that the amount of air involved in clinical cases of CAGE is usually not known. Furthermore, in many instances the duration of the air flow into the vessels is unknown. This especially holds true for cases in which air enters the circulation due to lung damage, for instance in pulmonary barotrauma in divers. The amount of air involved and the duration of the exposure in human cases of CAGE are probably quite variable, given the great scattering of symptoms and signs encountered in CAGE (5). The lower end of the CAGE spectrum is characterized by the subtle neuropsychological dysfunction that may

be encountered after CPB. This type of CAGE has been coined cerebral air microembolization (94). There is no clear transition between cerebral air microembolization and 'normal' CAGE in regard to the amount of air involved. The higher end of CAGE symptomatology involves widespread cerebral infarction visible on computed tomography (CT) or magnetic resonance imaging (MRI) scans, severe cytotoxic and vasogenic edema with ensuing rise in ICP, followed by brain herniation and death. Clinical and diving-related patients can present anywhere between these extremes.

Given this heterogeneous clinical presentation, it is not surprising that the amount of air introduced varies greatly between animal models of CAGE. The amount depends on the desired level of damage, which in turn depends on the research question and the outcome parameters under study. Models that rely on very sensitive measures of cerebral function, such as SSEP or electroencephalography (EEG), require less air than models that use for instance ICP to quantify brain injury. The largest amount of air used in a CAGE model was by Martens et al. (90), being an average of 37 ml into the common carotid artery of pigs. On the opposite side of the spectrum, Gerriets et al. (94) have recently shown that amounts as little as 0.1 µl injected into the common carotid artery of rats (with extracerebral arteries ligated) can cause quantifiable neurological deficits. In general, studies in which only effects of CAGE on the blood-brain barrier were investigated used small amounts of air while research into the effects of CAGE on cerebral metabolism (brain oxygenation, brain glucose metabolism) and cerebral hemodynamics (cerebral blood flow (CBF), ICP) required larger amounts of air. It is, however, difficult to directly compare amounts of air used between publications. The reason for this is the fact that most authors either choose a standard amount of air, or correct the volume for the animal's weight. One may question the validity of these approaches to air dosing. In most species brain weight does not increase linearly with body weight as the animal grows. Brain weight tends to reach a plateau in adolescence while body weight can increase greatly thereafter (109). Furthermore, smaller

animals tend to have proportionally larger brain mass relative to body mass than larger animals (110), so comparing volume based on body weight between species can be misleading. It would be more appropriate to base the amount of air on the animals brain weight or perhaps on its cerebral blood flow, but these approaches have not been undertaken in any of the CAGE animal models.

Despite the disadvantages of fixed amounts of air and relating air volume to body weight, some authors have published adequate volume-response relationship after embolization using these approaches in the rabbit (77, 78) and the rat (92, 94). In contrast with these positive results, it has been shown that even within a single species and a narrow weight range the cerebral effects of a standardized air bolus can be quite variable (96). This was also demonstrated by Simms et al. (24), who injected various amounts of air (ranging form 0.05 ml/kg to 1.00 ml/kg) into the vertebral artery in dogs weighing 13 to 24 kg. There was no statistically significant difference between any of the air volumes with regard to cerebral venous blood oxygen tension and cerebrospinal fluid oxygen tension.

One way to circumvent these problems is to titrate the amount of air injected to a certain parameter. Repeated air boluses are administered until the desired amount of damage has been inflicted. This results in varying amounts of injected air, but possibly more standardized effects on the brain. The parameters used to titrate the air volume should have a high temporal resolution, therefore this approach has been followed using SSEP and EEG. These methods are discussed in greater detail below.

To summarize this paragraph, the amount of air used in CAGE models varies greatly and depends primarily on the desired amount of cerebral injury and the parameters used to monitor the brain. Fixed or weight-related amounts of air have been used with success, but have in some models resulted in large variances between animals. Titration of air volume to obtain a certain amount of cerebral injury may increase reproducibility of results.

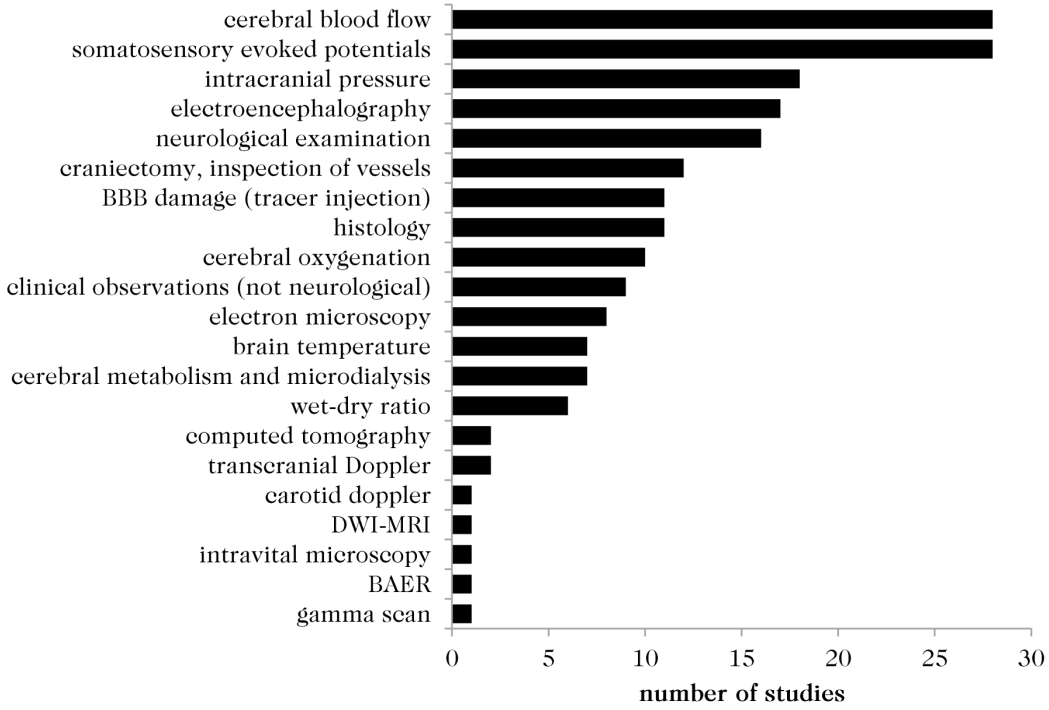


Figure 4. Methods used for monitoring of cerebral function in CAGE animal studies. In most studies more than one method was used. Methods that were only used in one study were omitted from this figure for the sake of conciseness. BBB = blood brain barrier; DWI-MRI = diffusion-weighted magnetic resonance imaging; BAER = brainstem auditory evoked response.

Bubble size

Not only the amount of air injected, but also the size of the bubbles may have influence on the effects on the brain. Furlow (50) has described the properties that have to be controlled in order to generate uniform bubble size, namely the size of the orifice from which the bubble emerges, the pressure of injection, the blood flow velocity behind the orifice, and the surface tension of the blood. Although most authors inject a known volume of air without paying attention to the size of the bubbles generated, some researchers have used uniform bubble sizes in their animal model (94). However, the size and homogeneity of the size of bubbles generated in clinical cases of CAGE is unknown. It is therefore debatable whether

or not generating bubbles with a known and uniform size is useful in experimental research.

In this regard, several aspects of intravascular bubble behavior are of importance (111). Intravascular bubbles that are larger than the diameter of the vessel they occupy are present not as spheres but as cylinders of air with rounded caps. Bubbles may not stay intact when they encounter a branch in the vessel, but can split up with both parts following their own way. Consequential air bubbles in the same vessel will either merge or line up one after the other, forming a 'string of pearls' (named for its appearance as viewed through a craniectomy). The position where the embolus ultimately lodges is determined by the tradeoff between the surface tension at the leading edge of the bubble (promoting trapping) and the sum of the surface tension and the arterial pressure at the trailing edge (both promoting propagation). Small air bubbles (diameter <15 µm) may not lodge at all and pass to the venous circulation immediately (112). However, this does not imply that these small bubbles do not cause cerebral injury, because even bubbles as small as 10-20 µm have been shown to cause blood-brain barrier disruption (73).

Air bubbles are often reported to lodge in arterioles of 30-60 µm diameter, although the articles referred to in support of this statement do not actually provide proof of this allegation. Gorman and Browning have shown that bubbles smaller than 200 µm diameter generally lodge in arterioles of 50-200 µm diameter (62). It has been demonstrated that bubbles preferentially lodge in areas with low cerebral perfusion pressure, namely the watershed areas between arterial territories and the grey-white matter junction (46). Although it is reasonable to assume that bubbles of different size will lodge in vessels of different size, this hypothesis has not been tested. Given the abovementioned characteristics of intravascular air behavior a clear size-response relationship seems unlikely. Until studies investigating the effect of bubble size on cerebral damage have been performed, it may be more practical to titrate air introduction to a certain level of injury, as mentioned in an earlier paragraph.

Outcome parameters

Many different outcome parameters have been studied in CAGE animal studies. For general monitoring of the anesthetized animal, most researchers report on the use of one or more parameters, generally heart rate, blood pressure, body temperature, and/or electrocardiogram. In this paragraph, we will focus on the methods that have been used for assessment of cerebral condition. Figure 4 displays the most commonly used parameters, which will be discussed here. Rogatsky et al. (113) have reviewed the methods used for physiologic and biochemical monitoring during hyperbaric oxygen therapy (HBOT) in human studies, including some methods for assessing cerebral functioning. This study is of interest, since HBOT is the generally accepted treatment for CAGE and is therefore applied in many of the animal studies under review here.

Together with SSEP, the most commonly studied parameter in CAGE models is CBF. Several different techniques have been used, of which [14C]-iodoantipyrine is the most common (114). This technique relies on the diffusion of a chemically inert tracer into the brain tissue. The amount of tracer can be visualized by an autoradiographic procedure and CBF can be quantified from the optical density. This method requires removal and sectioning of the brain and can thus only be performed postmortem. Methods used in CAGE research that provide continuous or repetitive CBF values include xenon (25) or hydrogen (50) clearance, and thermocouple (31) methods. Modern techniques for measuring CBF such as xenon CT, perfusion CT, and functional MRI have not yet been employed in CAGE animal studies.

Another frequently used method for assessing neuronal function in CAGE models is SSEP. The most important advantage of this method is that it provides information on the functional status of the brain, albeit in a specific neuronal pathway. The most frequently used model measures SSEP after median nerve stimulation in the dog (40, 54). Other researchers stimulated the sciatic nerve in the cat (52, 66) or the median

nerve in the rabbit (70, 77). The high temporal resolution of SSEP measurements enables the use of this technique to determine appropriate air volume, i.e. titrating the amount of air to obtain a certain degree of response depression. The most commonly used protocol involves injection of 50 µl of air into the internal carotid artery of the dog, followed by 20-50 µl boluses in order to suppress the amplitude between the first positive peak and the first negative peak to 10-20% of baseline value. This suppression by means of repeated air boluses is maintained during one hour (40, 54). In the cat model the amplitude between the second positive peak and the first negative peak is suppressed to less than 10% of baseline value during 15 minutes by repeated infusion of 80 µl air boluses into the common carotid artery with ligation of the external maxillary artery (52, 66). In the rabbit model SSEP was not used for titration of air volume, instead fixed amounts of air were injected (70, 77). After completion of the embolization period, recovery of the SSEP amplitude can be followed and used as a measure of cerebral function. One has to bear in mind that latency and amplitude of SSEP waves can be altered by certain anesthetics, which will be discussed later.

EEG, or sometimes electrocorticography, is another frequently employed method to study cerebral function in CAGE models. It has the advantage of rendering a more general image of neuronal function than SSEP. Furthermore, EEG can be measured non-invasively and therefore is one of the few techniques that can be readily applied in the clinical situation. EEG has a very high temporal resolution. Traditionally, however, EEG has only been analyzed in a qualitative fashion and therefore results have been difficult to compare between studies (9, 11, 16, 18, 25, 26, 39, 50, 61, 63, 74). Development and application of quantitative methods for EEG analysis (qEEG) may expand the use of this technique in CAGE models. Little information on qEEG in CAGE is available, since quantitative analysis was only conducted in 4 of 17 studies in which EEG was performed (31, 32, 87, 96). We have recently demonstrated the use of the temporal brain symmetry index in monitoring the acute effects of CAGE on the pig brain (96). Like SSEP, EEG signals are altered by the

use of anesthetic agents. Recent research has evolved in the application of bispectral index for monitoring depth of anesthesia. However, this method is not developed for monitoring cerebral function in disease states and should in its present form not be used for this purpose (115).

Histological procedures performed in CAGE models can be divided into two groups. In the first group histology is used for infarct delineation. This is done either with heamatoxylin and eosin staining or with triphenyl tetrazolium chloride staining. Tripenyl tetrazolium chloride is a white redox indicator that is reduced to a red substance in living cells. Infarcted areas remain pale and can be quantified after manual or automated delineation (81). The second goal of histology in CAGE research is determination of blood-brain barrier damage. Methods used include injection of Evans blue-labeled albumin (27), Trypan blue (12), or horseradish peroxidase (28), and autoradiography of labeled albumin (29). Others have performed electron microscopy to investigate blood-brain barrier damage at the microscopic level (39). The obvious disadvantage of histological methods is that they can only be performed postmortem.

Some methods used in CAGE research require placement of a probe in the cerebrum. These methods all provide the advantage of rendering (near) real time results. Of these, ICP is the most frequently obtained measurement (12, 18, 23, 31, 52, 54, 83, 86, 95). ICP is a commonly used parameter in traumatic brain injury with a well defined upper limit of normal and a high clinical relevance. Although ICP is generally evenly distributed in the normal situation, pressure gradients between brain regions may exist in certain pathological states such as unilateral space-occupying lesions (116). This should be kept in mind when performing unilateral ICP measurements in CAGE experiments. Intraventricular probe placement (as opposed to intraparenchymal measurement) renders the most reliable results (116). A drawback is the fact that ICP increase is a late event in the cascade of neuronal injury. Other techniques such as SSEP, EEG, microdialysis, and cerebral oxymetry provide earlier insight into neuronal function.

Determination of cerebral oxygenation by means of cerebral oxygen tension monitoring is increasingly used clinically for oxygenation targeted therapy in traumatic brain injury (117). Since in most CAGE models the location where the bubbles lodge and the resulting ischemic area can not be entirely controlled, it is theoretically possible that the probe is placed in unaffected tissue. Nevertheless, this technique has been successfully used in several CAGE animal models (86, 95). Other researchers measured oxygen tension of cerebrospinal fluid (24) or assessed total cerebral oxygen consumption by calculation of the arteriovenous oxygen difference across the brain (24, 31). More modern techniques for assessing cerebral oxygenation such as near-infrared spectroscopy have not yet been used in CAGE studies.

Microdialysis is a technique first described by Ungerstedt (118), requiring placement of a double lumen probe with a semi-permeable outer membrane in the cerebral tissue. This probe is continuously flushed with a liquid resembling cerebrospinal fluid. Substances in the extracellular tissue diffuse passively over the membrane into the perfusate and can be measured after collection, bedside if necessary. Most frequently this technique is used to monitor glucose metabolism by measuring glucose, lactate, and sometimes pyruvate in the dialysate (84, 86, 95). Some researchers have additionally measured glycerol, a marker for cell wall damage (84, 89). The amount of a substance retrieved from the extracellular matrix into the perfusate is termed the recovery rate and depends on several factors, of which probe length and perfusion speed are the most important. Values as determined in the microdialysate must be corrected with this recovery rate, which has to be determined in vitro or in vivo (119). A disadvantage of the technique is the fact that the probe itself injures cerebral tissue, although it has been demonstrated that this damage persists only for little more than an hour after implantation (120), and blood-brain barrier disruption can be largely prevented by slow placement of the probe (121). When used as a global parameter of cerebral functioning it has the same disadvantage as cerebral oxygen tension monitoring, namely that the probe may be placed in unaffected

tissue and that measurements may thus not be representative of the entire brain. However, we have recently shown in a swine model of CAGE that microdialysis values correlate well with other measures of cerebral function (95).

Standardized neurological examination can be regarded as the gold standard for the assessment of cerebral functioning after neuronal injury. Qualitative neurological examination has often been part of outcome assessment in CAGE models (17, 20, 22, 26, 38, 63), and some researchers have performed neurological assessment using quantitative scoring systems (51, 75, 77, 91, 94). Studies investigating correlation of measured parameters with neurological outcome are few, which can pose difficulties in determining the clinical relevance of results obtained. An exception is SSEP, which in New Zeeland White rabbits has been shown to correlate well with neurological outcome after administration of 50, 100, or 150 µl/kg air (78). Annane et al. (75) aimed to correlate CT findings after CAGE with clinical symptoms. CT results did not correspond well with clinical symptoms. CT scanning of the brain can therefore be regarded as an inferior method of quantification of injury due to CAGE.

In conclusion, the choice of which methods to use for assessment of the effects of CAGE on the animal brain has a close relationship to the desired amount of injury. Researchers interested in the upper limit of CAGE symptomatology could for instance obtain CBF and ICP measurements and perform histological procedures to assess macroscopic infarction. More subtle changes in cerebral function could be quantified using SSEP and qEEG, while even smaller amounts of injury might need for instance electron microscopy to investigate blood-brain barrier damage. Overall, frequently used methods are CBF measurement, electrophysiological recordings, and histological procedures. The fact that many parameters have not been correlated to clinical outcome in the setting of CAGE implicates that all extrapolations to the human situation must be made with caution.

Anesthesia

In all non-awake animal experiments, the choice of an appropriate anesthetic regimen is of crucial importance. This is even more so in experiments in which the brain is the subject of study, since anesthetics influence cerebral function by definition. The researcher should recognize the possible influences of the anesthetics on the measurements obtained. It is beyond the scope of this review to discuss in detail the effects of each anesthetic agent in the several species used in CAGE studies. In general, anesthetic agents lower cerebral metabolic rate and thus lower CBF, due to coupling of metabolism and blood flow in the brain. Notable exceptions are ketamine and nitrous oxide, which are known to increase cerebral metabolism and CBF in parts of the brain. Effects are usually concentration-dependent, and some volatile anesthetics are known to increase CBF in low concentrations while decreasing CBF at higher concentrations (122). Nitrous oxide is an exceptionally bad choice in CAGE, not only because it raises ICP but also because this gas diffuses rapidly into air bubbles resulting in increased size of the bubbles (123). Muscle relaxants have little effect on cerebral metabolic rate.

The effects of anesthetics on cerebral metabolic rate are most directly notable in electrophysiological recordings (124). Most anesthetics cause concentration-dependent changes in the EEG, starting with an excitatory phase and followed by decrease in frequency and amplitude. Effects of anesthetics, for instance volatile agents, on SSEP are summarized as decreased amplitude and increased latency. Again, ketamine is an exception, producing high amplitude theta and beta activity in the EEG and increased amplitude of SSEP waves. Opioid analgesics have only little effect on EEG, although they can produce a decrease in frequency. Barbiturates are known for their ability to produce an isoelectric EEG, while SSEP responses can still be evoked when the EEG is already isoelectric. The main effect of muscle relaxants on EEG and SSEP is decrease of artifacts due to muscle activity.

Another matter that requires attention in this regard is the neuroprotective effect of anesthetic agents. All anesthetics provide at least some degree of neuroprotection which is inherent to their property of lowering cerebral metabolism (122). In a recent review Schifilliti et al. (125) conclude that many anesthetic drugs, such as barbiturates, propofol, xenon, and most volatile inhalational agents have neuroprotective effects, although there is little evidence at this moment to choose any substance over the others in cases where neuroprotection is desirable. Of interest, some substances, of which isoflurane and ketamine are the most notable, have shown neurodegenerative effects next to their neuroprotective properties in animal studies (125, 126).

Since HBOT is the generally accepted treatment modality for CAGE, many studies have involved subjecting animals to HBOT after induction of air embolism. The use of hyperbaric oxygenation in an anesthetized and ventilated animal presents the researcher with interesting challenges. Many of these concerns are practical or technical by nature and have been summarized by several authors (123, 127-129). One of the important anesthesiological issues is that volatile anesthetics are generally not allowed inside hyperbaric chambers because of the closed environment and the consequential risk of exposure of personnel to the anesthetic, as well as the increased risk of fire due to high oxygen tensions. Total intravenous anesthesia is recommended in HBOT, use of intramuscular medication is discouraged because availability of the substance is uncertain due to vasoconstriction caused by hyperbaric oxygenation (128). There is no clinically relevant effect on the efficacy of anesthetic and analgesic agents in the pressure range used for treatment of CAGE (up to 2.8 atmospheres absolute (152 kPa)) (130). Apart from the incompatibility of nitrous oxide with CAGE as mentioned above, administration of this gaseous anesthetic induces a great risk of decompression sickness due to its high solubility under pressure. Nitrous oxide can therefore not be used in HBOT (127).

To summarize this paragraph, the choice for an anesthesiological regimen in an animal model of CAGE should take into account the effects of the

drugs on cerebral metabolism and the parameters monitored. Some extra considerations apply when HBOT is part of the experimental protocol. By definition, no anesthetic protocol is without effects on the brain.

Other factors influencing outcome in cerebral ischemia

In the previous paragraphs we discussed considerations that are of specific interest in CAGE research, except of species and anesthesia, which are factors that need to be taken into account in all animal models. There are a few more variables that are known to influence outcome in cerebral ischemia. Of these factors the most extensively studied are blood glucose and body temperature, although it must be noted that neither of these parameters have been studied in the context of CAGE. Hyperglycemia (131) and hyperthermia (132) are independent predictors of worse outcome in ischemic stroke in humans while hypothermia protects against neuronal injury in cerebral ischemia (132). Since CAGE is a type of multifocal cerebral ischemia, it may well be that these factors influence outcome in CAGE and it is important for the researcher to realize this. A special remark must be made on the effect of blood pressure on outcome in CAGE. Hypotension as well as hypertension can result in detrimental effects on the brain. In the case of severe hypotension cerebral perfusion will diminish, which may lead to aggravation of ischemia. In a recent animal study addressing the effect of different levels of mean arterial pressure (MAP) on neurological outcome after CAGE during CPB in rats, hypotension (MAP=50 mmHg) was associated with worse neurological scores than normal (MAP=60-70 mmHg) or increased (MAP=80 mmHg) blood pressure (93). On the other hand hypertension, although promoting bubble redistribution to the venous circulation (133), increases damage to the already injured blood-brain barrier which results in increased cerebral edema (58). Hypertension commonly occurs after air injection, as discussed above. The researcher should recognize the possible influence of both low and high blood pressure on experimental results.

Conclusions

In this review article, we have summarized the most important characteristics of animal models that have been used in CAGE research. It is interesting to notice the large variation in the published animal models with respect to species, method of air introduction, amount of air, measurement of outcome, and anesthesiological protocol. This variation exists despite the relatively small amount of literature available on this subject, as compared to studies on other causes of cerebral ischemia. The main reason for the large differences between animal models is the fact that CAGE is a heterogeneous clinical entity. From this observation it can be inferred that there is not one ideal animal model for CAGE. The research question under study determines the desired amount of injury which in turn influences the choice of parameters to monitor. Creating the correct amount of damage can be done by injection of fixed or weight-related air volumes, but titrating the amount of air to achieve a certain amount of SSEP or EEG depression has resulted in the most standardized amounts of cerebral injury. The relevance of controlling air bubble size remains to be elucidated. Measures must be taken to deliver the air as selectively to the brain as possible, taking into account the cerebrovascular characteristics of the species under study. The carotid rete can prove troublesome in this regard, but when appropriate precautions are taken, CAGE can be effectively induced in species that possess this anatomical structure. Anesthesia can produce unwanted effects on cerebral metabolism and the anesthetic regimen must therefore be chosen carefully.

We do not pretend to have produced an exhaustive review on all methodological subjects of interest in CAGE research. We have merely extracted important factors from existing animal studies on CAGE, while many interesting topics may be derived from research on human CAGE or in the field of decompression sickness and other causes of ischemic stroke. Researchers should therefore keep informed on these subjects as well.

Adequate animal models will always be of crucial importance in CAGE re-

search, mostly because the relatively low incidence of the disease hampers collection of large patient series (133). Moreover, in obvious cases of CAGE the patient is generally expeditiously treated with HBOT despite the lack of level I evidence for its efficacy. Withholding HBOT from patients would be unethical, which further limits the conduction of randomized clinical trials (133). It is not possible to rate one animal model as superior to the others, based on the data in this review. We have merely aimed to provide the researcher with an overview upon which to make his own decisions regarding species, method of embolization, parameters to monitor, and other important factors. We hope that the continuing development and improvement of animal models will result in a better understanding of pathophysiologic processes in CAGE and lead to advances in diagnostic and therapeutic possibilities for this disease.

References

1. Muth CM, Shank ES. Gas embolism. N Engl J Med 2000; 342:476-82.
2. van Hulst RA, Klein J, Lachmann B. Gas embolism: pathophysiology and treatment. Clin Physiol Funct Imaging 2003; 23:237-46.
3. Hossmann KA. Experimental models for the investigation of brain ischemia. Cardiovasc Res 1998; 39:106-20.
4. Howells DW, Porritt MJ, Rewell SS, O'Collins V, Sena ES, van der Worp HB, et al. Different strokes for different folks: the rich diversity of animal models of focal cerebral ischemia. J Cereb Blood Flow Metab 2010; 30:1412-31.
5. Bessereau J, Genotelle N, Chabbaut C, Huon A, Tabah A, Aboab J, et al. Long-term outcome of iatrogenic gas embolism. Intensive Care Med 2010; 36:1180-7.
6. van Allen CM, Hrdina LS, Clark J. Air embolism from the pulmonary vein. A clinical and experimental study. Arch Surg 1929; 19:567-99.
7. Fine J, Fischmann J. An experimental study of the treatment of air embolism. N Engl J Med 1940; 223:1054-7.
8. Fries CC, Levowitz B, Adler S, Cook AW, Karlson KE, Dennis C. Experimental cerebral gas embolism. Ann Surg 1957; 145:461-70.
9. Redo SF. The effects of intraarterial air injection on the electroencephalogram, electrocardiogram, arterial and venous blood pressure. Surg Forum 1958; 9:166-71.
10. Lee JC, Olszewski J. Effect of air embolism on permeability of cerebral blood vessels. Neurology 1959; 9:619-25.
11. Meyer JS, Gotoh F, Tazaki Y. Circulation and metabolism following experimental cerebral embolism. J Neuropathol Exp Neurol 1962; 21:4-24.
12. De la Torre E, Meredith J, Netsky MG. Cerebral air embolism in the dog. Arch Neurol 1962; 6:307-16.
13. De la Torre E, Mitchell OC, Netsky MG. The seat of respiratory and cardiovascular responses to cerebral air emboli. Neurology 1962; 12:140-7.
14. Atkinson JR. Experimental air embolism. Northwest Med 1963;

62:699-703.
15. Danis RK, Willman VL. Cerebral air embolism effects on brain water and serotonin content. Surg Forum 1963; 14:63-4.
16. Meijne NG, Schoemaker G, Bulterijs AB. The treatment of cerebral gas embolism in a high pressure chamber. An experimental study. J Cardiovasc Surg (Torino) 1963; 4:757-63.
17. Pate JW, Birdsong S. Carotid air embolism. An experimental study in cats. Arch Surg 1964; 89:685.
18. Worman LW, Seidel B. Treatment of cerebral air embolism in the dog. Am J Surg 1966; 111:820-4.
19. Broman T, Branemark PI, Johansson B, Steinwall O. Intravital and postmortem studies on air embolism damage of the blood-brain barrier tested with trypan blue. Acta Neurol Scand 1966; 42:146-52.
20. Wyant JD, Dobell AR. Epileptogenic threshold of parenteral penicillin in dogs. J Thorac Cardiovasc Surg 1967; 54:579-81.
21. Hollon CF, Maginn RR. Detection of carotid air emboli. Surg Forum 1969; 20:182-3.
22. Currie TT, Hayward NJ, Campbell PC, Westlake G, Williams J. Epilepsy in dogs caused by large doses of penicillin and concurrent brain damage. Br J Exp Pathol 1970; 51:492-7.
23. Simms NM, Kush GS, Long DM, Loken MK, French LA. Increase in regional cerebral blood flow following experimental arterial air embolism. J Neurosurg 1971; 34:665-71.
24. Simms NM, Long DM, Matthews JH, Chou SN. Hyperoxia of cerebral venous blood and cisternal cerebrospinal fluid following arterial air embolism. J Neurosurg 1972; 37:30-5.
25. Meldrum BS, Papy JJ, Vigouroux RA. Intracarotid air embolism in the baboon: effects on cerebral blood flow and the electroencephalogram. Brain Res 1971; 25:301-15.
26. Gomes OM, Pereira SN, Castagna RC, Bittencourt D, Amaral RV, Zerbini EJ. The importance of the different sites of air injection in the tolerance of arterial air embolism. J Thorac Cardiovasc Surg 1973; 65:563-8.
27. Rosengren LE, Persson LI, Johansson B. Enhanced blood-brain

barrier leakage to evans blue-labelled albumin after air embolism in ethanol-intoxicated rats. Acta Neuropathol 1977; 38:149-52.
28. Persson LI, Johansson BB, Hansson HA. Ultrastructural studies on blood-brain barrier dysfunction after cerebral air embolism in the rat. Acta Neuropathol 1978; 44:53-6.
29. Johansson BB. Cerebral air embolism and the blood-brain barrier in the rat. Acta Neurol Scand 1980; 62:201-9.
30. Johansson BB. Indomethacin and cerebrovascular permeability to albumin in acute hypertension and cerebral embolism in the rat. Exp Brain Res 1981; 42:331-6.
31. Hossmann KA, Fritz H. Coupling of function, metabolism, and blood flow after air embolism of the cat brain. Adv Neurol 1978; 20:255-62.
32. Fritz H, Hossmann KA. Arterial air embolism in the cat brain. Stroke 1979; 10:581-9.
33. Haller C, Kuschinsky W, Reimnitz P. Effect of gamma-hydroxybutyrate on the reactivity of pial arteries before and after ischemia. J Cereb Blood Flow Metab 1986; 6:658-66.
34. Haller C, Sercombe R, Verrecchia C, Fritsch H, Seylaz J, Kuschinsky W. Effect of the muscarinic agonist carbachol on pial arteries in vivo after endothelial damage by air embolism. J Cereb Blood Flow Metab 1987; 7:605-11.
35. Herbaczynska-Cedro K, Truskolaski P, Ruszczewski P. Inhibitory effect of hydrocortisone on the release of prostaglandins from dog's brain in hypoxia and cerebral embolism. Acta Physiol Pol 1978; 29:501-7.
36. Nishimoto K, Wolman M, Spatz M, Klatzo I. Pathophysiologic correlations in the blood-brain barrier damage due to air embolism. Adv Neurol 1978; 20:237-44.
37. Lossinsky AS, Garcia JH, Iwanowski L, Lightfoote WE, Jr. New ultrastructural evidence for a protein transport system in endothelial cells of gerbil brains. Acta Neuropathol 1979; 47:105-10.
38. Garcia JH, Klatzo I, Archer T, Lossinsky AS. Arterial air embolism: structural effects on the gerbil brain. Stroke 1981; 12:414-21.
39. Hekmatpanah J. Cerebral microvascular alterations in arterial air

embolism. Adv Neurol 1978; 20:245-53.

40. Hallenbeck JM, Furlow TW, Jr., Ruel TA, Greenbaum LJ, Jr. Extracorporeal glass-wool filtration of whole blood enhances post-ischemic recovery of the cortical sensory evoked response. Stroke 1979; 10:158-64.
41. Hallenbeck JM, Leitch DR, Dutka AJ, Greenbaum LJ, Jr., McKee AE. Prostaglandin I2, indomethacin, and heparin promote postischemic neuronal recovery in dogs. Ann Neurol 1982; 12:145-56.
42. Hallenbeck JM, Obrenovitch TP, Kumaroo KK, Thompson C, Leitch DR. Several new aspects of bubble-induced central nervous system injury. Philos Trans R Soc Lond B Biol Sci 1984; 304:177-84.
43. Obrenovitch TP, Kumaroo KK, Hallenbeck JM. Autoradiographic detections of 111indium-labeled platelets in brain tissue sections. Stroke 1984; 15:1049-56.
44. Hallenbeck JM, Dutka AJ, Tanishima T, Kochanek PM, Kumaroo KK, Thompson CB, et al. Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early postischemic period. Stroke 1986; 17:246-53.
45. Kochanek PM, Dutka AJ, Kumaroo KK, Hallenbeck JM. Platelet activating factor receptor blockade enhances recovery after multifocal brain ischemia. Life Sci 1987; 41:2639-44.
46. Dutka AJ, Kochanek PM, Hallenbeck JM, Storey JR. Air embolism may cause unrecognized ischemia of the gray-white junction. Undersea Biomed Res 1988; 15:99-106.
47. Kochanek PM, Dutka AJ, Kumaroo KK, Hallenbeck JM. Effects of prostacyclin, indomethacin, and heparin on cerebral blood flow and platelet adhesion after multifocal ischemia of canine brain. Stroke 1988; 19:693-9.
48. Dutka AJ, Kochanek PM, Hallenbeck JM. Influence of granulocytopenia on canine cerebral ischemia induced by air embolism. Stroke 1989; 20:390-5.
49. Evans DE, Kobrine AI, Weathersby PK, Bradley ME. Cardiovascular effects of cerebral air embolism. Stroke 1981; 12:338-44A.
50. Furlow TW, Jr. Experimental air embolism of the brain: an analysis

of the technique in the rat. Stroke 1982; 13:847-52.
51. Huddleston CB, Wareing TH, Yu S, Phythyon JM, Hammon JW, Stoney WS. Experimental treatment of cerebral air embolism. Surg Forum 1983; 34:344-6.
52. Evans DE, Kobrine AI, LeGrys DC, Bradley ME. Protective effect of lidocaine in acute cerebral ischemia induced by air embolism. J Neurosurg 1984; 60:257-63.
53. Evans DE, Kobrine AI. Reduction of experimental intracranial hypertension by lidocaine. Neurosurgery 1987; 20:542-7.
54. Leitch DR, Greenbaum LJ, Jr., Hallenbeck JM. Cerebral arterial air embolism: I. Is there benefit in beginning HBO treatment at 6 bar? Undersea Biomed Res 1984; 11:221-35.
55. Leitch DR, Greenbaum LJ, Jr., Hallenbeck JM. Cerebral arterial air embolism: II. Effect of pressure and time on cortical evoked potential recovery. Undersea Biomed Res 1984; 11:237-48.
56. Leitch DR, Greenbaum LJ, Jr., Hallenbeck JM. Cerebral arterial air embolism: III. Cerebral blood flow after decompression from various pressure treatments. Undersea Biomed Res 1984; 11:249-63.
57. Leitch DR, Greenbaum LJ, Jr., Hallenbeck JM. Cerebral arterial air embolism: IV. Failure to recover with treatment, and secondary deterioration. Undersea Biomed Res 1984; 11:265-74.
58. Dutka AJ, Hallenbeck JM, Kochanek PM. A brief episode of severe arterial hypertension induces delayed deterioration of brain function and worsens blood flow after transient multifocal cerebral ischemia. Stroke 1987; 18:386-95.
59. Dutka AJ, Mink RB, Pearson RR, Hallenbeck JM. Effects of treatment with dexamethasone on recovery from experimental cerebral arterial gas embolism. Undersea Biomed Res 1992; 19:131-41.
60. Dutka AJ, Mink R, McDermott JJ, Clark JB, Hallenbeck JM. Effect of lidocaine on somatosensory evoked response and cerebral blood flow after canine cerebral air embolism. Stroke 1992; 23:1515-20.
61. Menasche P, Pinard E, Desroches AM, Seylaz J, Laget P, Geyer RP, et al. Fluorocarbons: a potential treatment of cerebral air embolism in open-heart surgery. Ann Thorac Surg 1985; 40:494-7.

62. Gorman DF, Browning DM. Cerebral vasoreactivity and arterial gas embolism. Undersea Biomed Res 1986; 13:317-35.
63. Spiess BD, Braverman B, Woronowicz AW, Ivankovich AD. Protection from cerebral air emboli with perfluorocarbons in rabbits. Stroke 1986; 17:1146-9.
64. Butler BD, Laine GA, Leiman BC, Warters D, Kurusz M, Sutton T, et al. Effect of the Trendelenburg position on the distribution of arterial air emboli in dogs. Ann Thorac Surg 1988; 45:198-202.
65. Chang C, Dughi J, Shitabata P, Johnson G, Coel M, McNamara JJ. Air embolism and the radial arterial line. Crit Care Med 1988; 16:141-3.
66. Evans DE, Catron PW, McDermott JJ, Thomas LB, Kobrine AI, Flynn ET. Effect of lidocaine after experimental cerebral ischemia induced by air embolism. J Neurosurg 1989; 70:97-102.
67. McDermott JJ, Dutka AJ, Evans DE, Flynn ET. Treatment of experimental cerebral air embolism with lidocaine and hyperbaric oxygen. Undersea Biomed Res 1990; 17:525-34.
68. McDermott JJ, Dutka AJ, Koller WA, Pearson RR, Flynn ET. Comparison of two recompression profiles in treating experimental cerebral air embolism. Undersea Biomed Res 1992; 19:171-85.
69. McDermott JJ, Dutka AJ, Koller WA, Flynn ET. Effects of an increased PO2 during recompression therapy for the treatment of experimental cerebral arterial gas embolism. Undersea Biomed Res 1992; 19:403-13.
70. Helps SC, Parsons DW, Reilly PL, Gorman DF. The effect of gas emboli on rabbit cerebral blood flow. Stroke 1990; 21:94-9.
71. Helps SC, Meyer-Witting M, Reilly PL, Gorman DF. Increasing doses of intracarotid air and cerebral blood flow in rabbits. Stroke 1990; 21:1340-5.
72. Helps SC, Gorman DF. Air embolism of the brain in rabbits pretreated with mechlorethamine. Stroke 1991; 22:351-4.
73. Hills BA, James PB. Microbubble damage to the blood-brain barrier: relevance to decompression sickness. Undersea Biomed Res 1991; 18:111-6.
74. Annane D, Troche G, Delisle F, Devauchelle P, Paraire F, Raphael

JC, et al. Effects of mechanical ventilation with normobaric oxygen therapy on the rate of air removal from cerebral arteries. Crit Care Med 1994; 22:851-7.

75. Annane D, Troche G, Delisle F, Devauchelle P, Hassine D, Paraire F, et al. Kinetics of elimination and acute consequences of cerebral air embolism. J Neuroimaging 1995; 5:183-9.
76. Bunegin L, Wahl D, Albin MS. Detection and volume estimation of embolic air in the middle cerebral artery using transcranial Doppler sonography. Stroke 1994; 25:593-600.
77. Ryu KH, Hindman BJ, Reasoner DK, Dexter F. Heparin reduces neurological impairment after cerebral arterial air embolism in the rabbit. Stroke 1996; 27:303-9.
78. Reasoner DK, Dexter F, Hindman BJ, Subieta A, Todd MM. Somatosensory evoked potentials correlate with neurological outcome in rabbits undergoing cerebral air embolism. Stroke 1996; 27:1859-64.
79. Reasoner DK, Hindman BJ, Dexter F, Subieta A, Cutkomp J, Smith T. Doxycycline reduces early neurologic impairment after cerebral arterial air embolism in the rabbit. Anesthesiology 1997; 87:569-76.
80. Hindman BJ, Dexter F, Enomoto S, Subieta A, Smith T, Cutkomp J. Recovery of evoked potential amplitude after cerebral arterial air embolism in the rabbit: a comparison of the effect of cardiopulmonary bypass with normal circulation. Anesthesiology 1998; 88:696-707.
81. Hindman BJ, Dexter F, Subieta A, Smith T, Cutkomp J. Brain injury after cerebral arterial air embolism in the rabbit as determined by triphenyltetrazolium staining. Anesthesiology 1999; 90:1462-73.
82. Juncker C, Petroianu G, Bergler W, Maurer J, Rufer R, Hormann K. The influence of an arterial and venous air embolism on the hearing level in laboratory mini pigs. Acta Otolaryngol 1998; 118:803-7.
83. Williams DJ, Doolette DJ, Upton RN. Increased cerebral blood flow and cardiac output following cerebral arterial air embolism in sheep. Clin Exp Pharmacol Physiol 2001; 28:868-72.
84. Medby C, Ro H, Koteng S, Juul R, Krossnes BK, Brubakk AO. Microdialysis in cisterna magna during cerebral air embolism in

swine. Undersea Hyperb Med 2002; 29:226-34.

85. Yamaguchi S, Yamakawa T, Niimi H. Cerebral microvascular responses to air embolism-reperfusion in the cat using fluorescence videomicroscopy. Clin Hemorheol Microcirc 2003; 28:59-70.
86. van Hulst RA, Lameris TW, Hasan D, Klein J, Lachmann B. Effects of cerebral air embolism on brain metabolism in pigs. Acta Neurol Scand 2003; 108:118-24.
87. Drenthen J, van Hulst RA, Blok JH, van Heel MD, Haitsma JJ, Lachmann B, et al. Quantitative EEG monitoring during cerebral air embolism and hyperbaric oxygen treatment in a pig model. J Clin Neurophysiol 2003; 20:264-72.
88. van Hulst RA, Haitsma JJ, Lameris TW, Klein J, Lachmann B. Hyperventilation impairs brain function in acute cerebral air embolism in pigs. Intensive Care Med 2004; 30:944-50.
89. van Hulst RA, Drenthen J, Haitsma JJ, Lameris TW, Visser GH, Klein J, et al. Effects of hyperbaric treatment in cerebral air embolism on intracranial pressure, brain oxygenation, and brain glucose metabolism in the pig. Crit Care Med 2005; 33:841-6.
90. Martens S, Theisen A, Balzer JO, Dietrich M, Graubitz K, Scherer M, et al. Improved cerebral protection through replacement of residual intracavital air by carbon dioxide: a porcine model using diffusion-weighted magnetic resonance imaging. J Thorac Cardiovasc Surg 2004; 127:51-6.
91. Jungwirth B, Gordan ML, Blobner M, Schmehl W, Kochs EF, Mackensen GB. Xenon impairs neurocognitive and histologic outcome after cardiopulmonary bypass combined with cerebral air embolism in rats. Anesthesiology 2006; 104:770-6.
92. Jungwirth B, Kellermann K, Blobner M, Schmehl W, Kochs EF, Mackensen GB. Cerebral air emboli differentially alter outcome after cardiopulmonary bypass in rats compared with normal circulation. Anesthesiology 2007; 107:768-75.
93. Qing M, Shim JK, Grocott HP, Sheng H, Mathew JP, Mackensen GB. The effect of blood pressure on cerebral outcome in a rat model of cerebral air embolism during cardiopulmonary bypass. J Thorac

Cardiovasc Surg 2011; 142:424-9.
94. Gerriets T, Walberer M, Nedelmann M, Doenges S, Ritschel N, Bachmann G, et al. A rat model for cerebral air microembolisation. J Neurosci Methods 2010; 190:10-3.
95. Weenink RP, Hollmann MW, Stevens MF, van Lienden KP, Ghazi-Hosseini E, van Gulik TM, et al. Cerebral arterial gas embolism in swine. Comparison of two sites for air injection. J Neurosci Methods 2011; 194:336-41.
96. Weenink RP, Vrijdag XC, van Putten MJ, Hollmann MW, Stevens MF, van Gulik TM, et al. Quantitative electroencephalography in a swine model of cerebral arterial gas embolism. Clin Neurophysiol 2012; 123:411-7.
97. Duhaime AC. Large animal models of traumatic injury to the immature brain. Dev Neurosci 2006; 28:380-7.
98. Daniel PM, Dawes JD, Prichard ML. Studies of the carotid rete and its associated arteries. Philos Trans R Soc Lond B Biol Sci 1953; 237:173-208.
99. Gillilan LA. Extra- and intra-cranial blood supply to brains of dog and cat. Am J Anat 1976; 146:237-53.
100. McGrath P. Observations on the intracranial carotid rete and the hypophysis in the mature female pig and sheep. J Anat 1977; 124:689-99.
101. Lee DH, Wriedt CH, Kaufmann JC, Pelz DM, Fox AJ, Vinuela F. Evaluation of three embolic agents in pig rete. AJNR Am J Neuroradiol 1989; 10:773-6.
102. Reinert M, Brekenfeld C, Taussky P, Andres R, Barth A, Seiler RW. Cerebral revascularization model in a swine. Acta Neurochir Suppl 2005; 94:153-7.
103. Rink C, Christoforidis G, Abduljalil A, Kontzialis M, Bergdall V, Roy S, et al. Minimally invasive neuroradiologic model of preclinical transient middle cerebral artery occlusion in canines. Proc Natl Acad Sci U S A 2008; 105:14100-5.
104. Lowenstein E, Little JW, III, Lo HH. Prevention of cerebral embolization from flushing radial-artery cannulas. N Engl J Med 1971; 285:1414-5.

105. Kurusz M, Butler BD. Bubbles and bypass: an update. Perfusion 2004; 19 Suppl 1:S49-S55.
106. Guy TS, Kelly MP, Cason B, Tseng E. Retrograde cerebral perfusion and delayed hyperbaric oxygen for massive air embolism during cardiac surgery. Interact Cardiovasc Thorac Surg 2009; 8:382-3.
107. Jungwirth B, de Lange F. Animal models of cardiopulmonary bypass: development, applications, and impact. Semin Cardiothorac Vasc Anesth 2010; 14:136-40.
108. Griese H, Seifert D, Koerfer R. Cortical infarction following cardiosurgical procedures - air embolism as a probable cause. Eur Neurol 2009; 61:343-9.
109. Blinkov SM, Glezer II. Gewicht, Volumen und Abmessungen des Gehirns. In: Blinkov SM, Glezer II, eds. Das Zentralnervensystem in Zahlen und Tabellen. Jena, Germany: VEB Gustav Fischer Verlag, 1968:125-36.
110. Williams MF. Primate encephalization and intelligence. Med Hypotheses 2002; 58:284-90.
111. Mitchell S, Gorman DF. The pathophysiology of cerebral arterial gas embolism. J Extra Corpor Technol 2002; 34:18-23.
112. Feinstein SB, Shah PM, Bing RJ, Meerbaum S, Corday E, Chang BL, et al. Microbubble dynamics visualized in the intact capillary circulation. J Am Coll Cardiol 1984; 4:595-600.
113. Rogatsky GG, Shifrin EG, Mayevsky A. Physiologic and biochemical monitoring during hyperbaric oxygenation: a review. Undersea Hyperb Med 1999; 26:111-22.
114. Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L. Measurement of local cerebral blood flow with iodo [14C] antipyrine. Am J Physiol 1978; 234:H59-H66.
115. Souter MJ, Lam AM. Neurocritical care. In: Miller RD, Eriksson LI, Fleisher LA, Wiener-Kronish JP, Young WL, eds. Miller's anesthesia. Philadelphia, PA: Churchill Livingstone, 2009:2899-922.
116. Smith M. Monitoring intracranial pressure in traumatic brain injury. Anesth Analg 2008; 106:240-8.
117. Narotam PK, Morrison JF, Nathoo N. Brain tissue oxygen monitoring

in traumatic brain injury and major trauma: outcome analysis of a brain tissue oxygen-directed therapy. J Neurosurg 2009; 111:672-82.
118. Ungerstedt U. Microdialysis - a new bioanalytical sampling technique. Curr Sep 1986; 7:43-6.
119. Peerdeman SM, Girbes AR, Vandertop WP. Cerebral microdialysis as a new tool for neurometabolic monitoring. Intensive Care Med 2000; 26:662-9.
120. Ungerstedt U, Rostami E. Microdialysis in neurointensive care. Curr Pharm Des 2004; 10:2145-52.
121. Allen DD, Crooks PA, Yokel RA. 4-Trimethylammonium antipyrine: a quaternary ammonium nonradionuclide marker for blood-brain barrier integrity during in vivo microdialysis. J Pharmacol Toxicol Methods 1992; 28:129-35.
122. Patel PM, Drummond JC. Cerebral physiology and the effects of anesthetic drugs. In: Miller RD, Eriksson LI, Fleisher LA, Wiener-Kronish JP, Young WL, eds. Miller's anesthesia. Philadelphia, PA: Churchill Livingstone, 2009:305-40.
123. Muth CM, Radermacher P, Shank ES. When HBO meets the ICU: intensive care patients in the hyperbaric environment. In: Bakker DJ, Cramer FS, eds. Hyperbaric surgery: perioperative care. Flagstaff, AZ: Best Publishing Group, 2001:111-57.
124. Sloan TB. Anesthetic effects on electrophysiologic recordings. J Clin Neurophysiol 1998; 15:217-26.
125. Schifilliti D, Grasso G, Conti A, Fodale V. Anaesthetic-related neuroprotection: intravenous or inhalational agents? CNS Drugs 2010; 24:893-907.
126. Hudetz JA, Pagel PS. Neuroprotection by ketamine: a review of the experimental and clinical evidence. J Cardiothorac Vasc Anesth 2010; 24:131-42.
127. Severinghaus JW. Hyperbaric oxygenation: anesthesia and drug effects. Anesthesiology 1965; 26:812-24.
128. Camporesi EM. Anesthesia in the hyperbaric environment. In: Jain KK, ed. Textbook of hyperbaric medicine. Cambridge, MA: Hogrefe & Huber Publishing, 2004:415-20.

129. Weaver LK. Hyperbaric oxygen in the critically ill. Crit Care Med 2011; 39:1784-91.
130. Moon RE, Camporesi EM. Clinical care in extreme environments: at high and low pressure and in space. In: Miller RD, Eriksson LI, Fleisher LA, Wiener-Kronish JP, Young WL, eds. Miller's anesthesia. Philadelphia, PA: Churchill Livingstone, 2009:2485-517.
131. Quinn TJ, Lees KR. Hyperglycaemia in acute stroke – to treat or not to treat. Cerebrovasc Dis 2009; 27 Suppl 1:148-55.
132. Reith J, Jorgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppesen LL, et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. Lancet 1996; 347:422-5.
133. Moon RE. Bubbles in the brain: what to do for arterial gas embolism? Crit Care Med 2005; 33:909-10.

Cerebral arterial gas embolism in swine; comparison of two sites for air injection

3

Weenink RP, Hollmann MW, Stevens MF, van Lienden KP,
Ghazi-Hosseini E, van Gulik TM, van Hulst RA
J Neurosci Methods 2011; 194:336-41

3

Abstract

Cerebral arterial gas embolism is a risk in diving and occurs as a complication in surgery and interventional radiology. Swine models for cerebral arterial gas embolism have been used in the past. However, injection of air into the main artery feeding the pig brain – the ascending pharyngeal artery – might be complicated by the presence of the carotid rete, an arteriolar network at the base of the brain. On the other hand, anastomoses between external and internal carotid territories are present in the pig. In order to determine the most appropriate vessel for air injection, we performed experiments in which air was injected into either the ascending pharyngeal artery or the external carotid artery. We injected 0.25 ml/kg of room air selectively into the ascending pharyngeal artery or the external carotid artery of 35-40 kg Landrace pigs (n=8). We assessed the effect on cerebral metabolism by measuring intracranial pressure, brain oxygen tension, and brain glucose and lactate concentrations using cerebral microdialysis. Intracranial pressure and brain oxygen tension changed significantly in both groups, but did not differ between groups. Brain lactate increased significantly more in pigs in which air was injected into the ascending pharyngeal artery. Intracranial pressure, brain oxygen tension, and brain lactate correlated after injection of air into the ascending pharyngeal artery, but not after injection into the external carotid artery. Our model is suitable for investigation of cerebral arterial gas embolism. The ascending pharyngeal artery is the most appropriate vessel for air injection.

Introduction

Cerebral arterial gas embolism (CAGE) can occur in divers when air trapped in the lungs expands during the ascent and causes lung overdistension damage (1, 2). It can also occur as a complication in surgery or interventional radiology. CAGE is a serious disorder often resulting in permanent neurological dysfunction. Current treatment mainly relies on hyperbaric oxygen therapy which ameliorates the injury by decreasing bubble size and increasing oxygenation. Hyperbaric oxygen therapy also attenuates the inflammatory process that follows the passage of bubbles through the vascular bed (3). Most of the clinical evidence on the effectiveness of HBOT in CAGE comes from case reports and retrospective studies including small groups of patients. These reports have been convincing to such an extent that performing prospective trials in humans seems unethical. In order to facilitate further advances in CAGE research, an adequate animal model is needed. Although several other animals such as cats (4), rabbits (5), and dogs (6) have been used in the past, pigs were frequently used in different models of air embolism (7-11). Unfortunately, the application and volume of air and its effects varied over a wide range.

We have developed a porcine model of CAGE, using clinically relevant measurements such as intracranial pressure (ICP), brain oxygen tension ($PbtO_2$), and microdialysis to assess the effects of air embolism on cerebral metabolism (12). This model has proven its usefulness in CAGE research (13). We primarily chose the pig because of the similarities between human and swine anatomy and cardiovascular physiology (14). Moreover, the brain of smaller laboratory animals cannot accommodate the probes we require for our measurements.

Since the pig has extensive anastomoses between external and internal carotid territories (15), injection into the external carotid artery (ECA) results in entrance of air into the pig brain. However, the primary vessels supplying the pig brain are the bilateral ascending pharyngeal arteries

(APA). We were interested if the APA would be a more appropriate vessel for air embolization. Our main concern in this regard was the carotid rete, a network of finely entangled arterioles that forms out of each APA (15). The internal carotid arteries are formed out of these retia. The carotid rete cannot be passed with an angiography catheter, so the air has to be delivered proximal to this structure. The small size of the vessels in the rete might retain all injected air, thus preventing it from entering the cerebral circulation.

In order to assess which of the vessels would be the most appropriate for air embolization experiments, we performed experiments in which we injected equal amounts of air into either the APA or the ECA of the pig. We assessed the effect of air embolism on cerebral metabolism by measuring ICP, $PbtO_2$, and brain glucose and lactate. A larger effect on neuronal metabolism after injection of air in one of the two target vessels would indicate that more air reaches the brain following injection in this particular artery. Furthermore, since the measurements acquired with the brain oxygen and microdialysis probes only represent cerebral metabolism in the immediate vicinity of the probe, we were interested in the degree of correlation between ICP, $PbtO_2$, and brain lactate. A higher degree of correlation after injection in one of the vessels would suggest this vessel as being the most appropriate for our experiments.

Material and Methods

Animal handling

Approval of this study was obtained from the Animal Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands. Animal care was in accordance with European Union guidelines. Subjects were 16 female 35-40 kg crossbred Landrace pigs. Anaesthesia was induced with intramuscular ketamine 15 mg/kg (Eurovet Animal Health, Bladel, The Netherlands), midazolam 2 mg/kg (Actavis, Hafnarfjordur, Iceland), and atropine sulfate 0.01 mg/kg (Pharmachemie, Haarlem, The

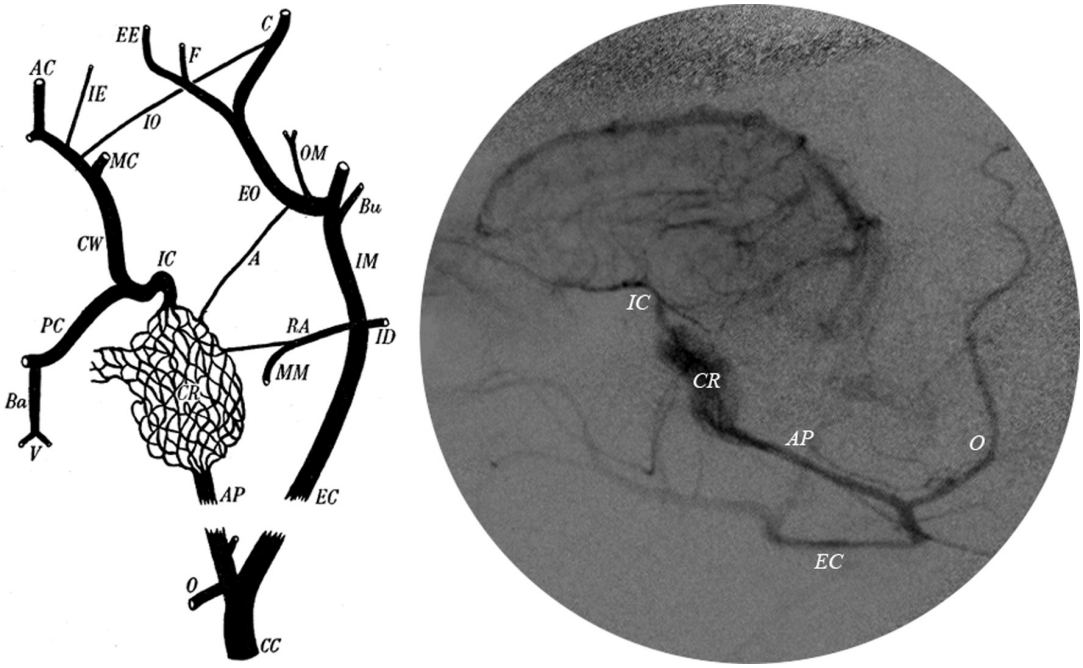


Figure 1. Left: Schematic drawing of the cerebral vascularization in the pig. Copied (with permission) from (15). Right: Lateral selective angiogram of the ascending pharyngeal artery. CC = common carotid artery; AP = ascending pharyngeal artery; EC = external carotid artery; CR = carotid rete; IC = internal carotid artery; CW= circle of Willis; O = occipital artery.

Netherlands) after which the animals were intubated and anaesthesia continued with intravenous ketamine 10-15 mg/kg/h, sufentanil 5-6 µg/kg/h (Hameln Pharmaceuticals, Hameln, Germany), midazolam 1.5 mg/kg/h, and pancuronium bromide 0.1 mg/kg/h (Organon, Oss, The Netherlands). After intubation, animals were connected to a ventilator (Servo Ventilator 900C, Siemens-Elema, Sweden) and ventilated in a volume-controlled mode. Ventilation parameters were: frequency 18/min, inspiratory oxygen fraction 0.4, inspiration time 25%, pause time 10%, positive end-expiratory pressure 4 mmHg. Blood gases were taken hourly and $PaCO_2$ was maintained at 35-40 mmHg by adjusting minute volume (usually 7.5±0.5 l). Arterial blood pressure (measured by means of a catheter placed in one of the brachial arteries), oxygen saturation, capnography, pulse rate, and rectal temperature were continuously monitored. A bladder catheter was placed in all animals. Body tempera-

ture was maintained at 37-38 ^{0}C by means of an aluminum emergency blanket. At the end of the experiment, animals were sacrificed with potassium chloride.

Arterial catheter

Access to the right femoral artery was obtained using the Seldinger technique. A 4F angiography catheter (Radiofocus Glidecath, Terumo, Tokyo, Japan) was advanced over a guide wire (Radiofocus, Terumo, Tokyo, Japan) under fluoroscopic guidance using Ultravist 300 (Bayer, Mijdrecht, The Netherlands). The tip of the catheter was placed either in one of the ascending pharyngeal arteries (APA group) or in one of the external carotid arteries (ECA group) (figure 1).

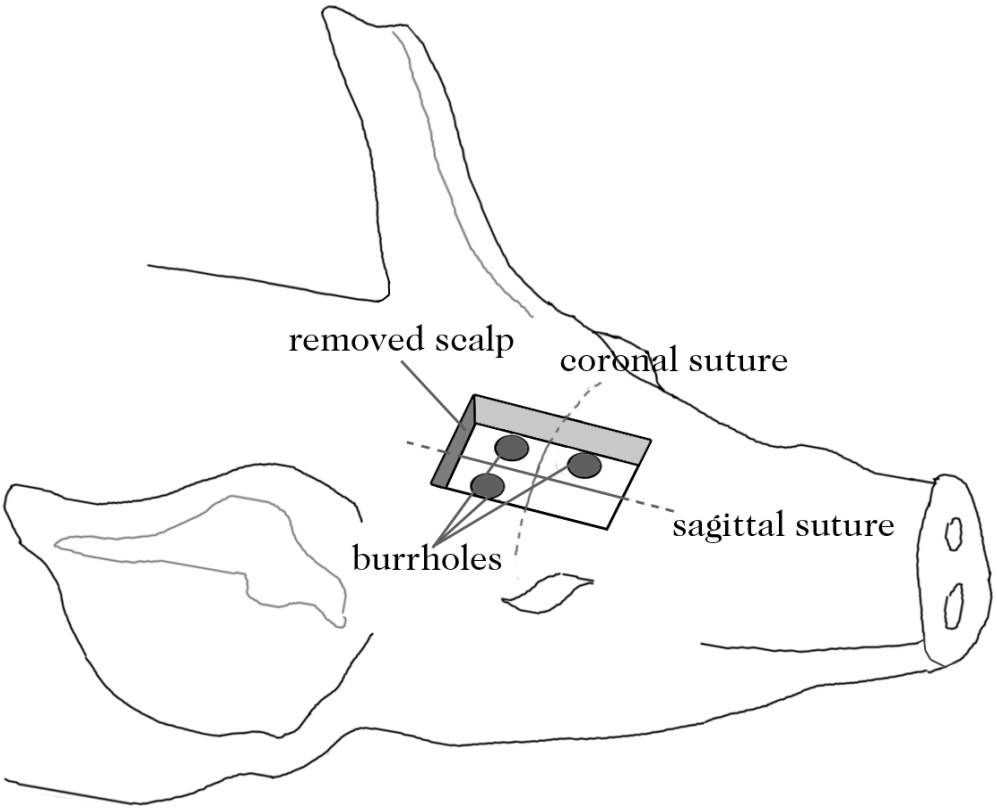


Figure 2. Schematic view of the head of the pig after preparation of the burr holes.

Intracerebral probes

The techniques used have described before (12). In short, after removal of a 2 by 3 cm sized scalp flap centered over the sagittal and coronal sutures, the skull was exposed. Three burr holes were created as depicted in figure 2. After calibration of the probes two Licox (Integra, Plainsboro, NJ, USA) brain oxygen catheter-micro-probes (one in each dorsal burr hole), two CMA 20 Elite (Carnegie Medicine AB, Solna, Sweden) microdialysis probes (one in each dorsal burr hole), one Licox brain temperature catheter-micro-probe (frontal burr hole), and one Codman (Raynham, MA, USA) ICP probe (frontal burr hole) were advanced 15-20 mm through the dura. Left and right $PbtO_2$ were corrected for brain temperature as measured by the temperature probe. The oxygen probes were advanced a few millimeters more into the brain if the recorded value did not increase at least 15 mmHg after increasing the inspired oxygen fraction to 1.0 for 15 min. The microdialysis probes used had a length of 10 mm, a 0.5 mm diameter, and a cut-off value of 20 kDa. The probes were perfused by a CMA 100 microdialysis pump with artificial cerebrospinal fluid (CMA, Carnegie Medicine AB, Solna, Sweden). The vials containing the dialysate were changed every 15 min and analyzed immediately for the amount of glucose and lactate using a CMA 600 analyzer. Values presented here have been corrected for the recovery rate (16) as determined in a preliminary in vitro experiment. Recovery rate was 76 percent for glucose and 89 percent for lactate.

Embolization

A 1 h stabilization period was provided after completion of all surgical procedures, after which 0.25 ml/kg of room air was injected in 60 s through the catheter placed in either the APA or the ECA. ICP and $PbtO_2$ were recorded 30, 60, 90, and 120 min after embolization. Glucose and lactate concentration were measured in the microdialysis vials every 15 min. The animals were sacrificed 120 min after air injection.

Statistical analysis

Data was analyzed using SPSS for Windows software. Values are given

	baseline		t=120 min	
	ascending pharyngeal artery	external carotid artery	ascending pharyngeal artery	external carotid artery
heart rate (min^{-1})	84 ± 21	93 ± 31	91 ± 22	114 ± 34
MAP (mmHg)	88 ± 17	96 ± 11	77 ± 13	91 ± 18
body temperature (^{0}C)	37,1 ± 0,3[b]	37,5 ± 0,3[b]	37 ± 0,4	38 ± 0,4
blood pH	7,46 ± 0,05	7,47 ± 0,04	7,47 ± 0,03	7,44 ± 0,03
PaO_2 (mmHg)	234 ± 52	234 ± 23	199 ± 45	210 ± 12a
$PaCO_2$ (mmHg)	39 ± 3	40 ± 2	39 ± 2	40 ± 1
ICP (mmHg)	7 ± 2	8 ± 3	25 ± 17[a]	32 ± 20[a]
CPP (mmHg)	81 ± 16	87 ± 13	52 ± 28	67 ± 23[a]
$PbrO_2$ (mmHg)	22 ± 6	26 ± 5	11 ± 5a	15 ± 10[a]
brain glucose (mmol/l)	1,3 ± 0,7	0,6 ± 0,2	0,6 ± 0,3	0,3 ± 0,3
brain lactate (mmol/l)	0,7 ± 0,2	0,5 ± 0,2	2,7 ± 2,0[ab]	1,0 ± 0,5[b]

Table 1. Data of variables in both groups (both n=8) at baseline and two hours after embolization. Values are mean ± SD. MAP = mean arterial pressure; ICP = intracranial pressure; CPP = cerebral perfusion pressure; [a] = significant difference between baseline and t=120; [b] = significant difference between groups.

as average ± SD unless stated otherwise. Differences between baseline and 120 min after embolization were calculated using two sided paired t-tests. Differences between groups at a single time point were calculated using two sided non-paired t-tests. Differences between groups for repeated measurements were calculated using the area under the curve. Correlation between parameters was quantified using Spearman's rho. Statistical significance was accepted at $p<0.05$.

Results

All animals survived the experimental protocol. Values obtained at baseline and at the end of the experiment are presented in table 1. There were no differences between the groups at t=0, except for a small but significant difference between body temperature (37.1 ^{0}C in the APA group versus 37.5 ^{0}C in the ECA group) as well as a small but significant

decrease in arterial oxygen tension during the experiment in the ECA group (234±23 mmHg at t=0, 210±12 mmHg at t=120).

ICP increased significantly in both groups but there was no difference in ICP between groups. Since $PbtO_2$, brain glucose, and brain lactate did not differ significantly between both hemispheres, the values of both sides of the brain were averaged for each time point. Brain oxygen at t=120 was significantly lower than baseline in both groups, but the difference between the APA and ECA group was not significant. Brain glucose values did not change significantly during the experiments in both groups. There was a significant difference in lactate concentration between groups, the APA group showing a larger increase in lactate (figure 3, p=0.03). We determined correlation of our outcome measures at the end of the experiment (figure 4). There was a significant correlation between ICP, $PbtO_2$, and brain lactate in the APA group, while no such correlation was found in the ECA group.

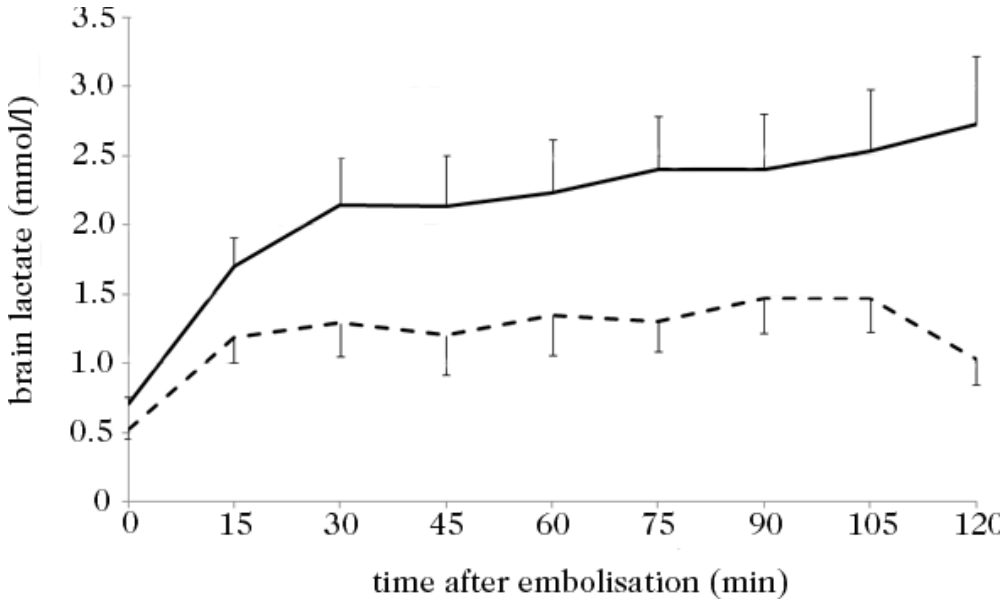


Figure 3. Brain lactate concentration ± SEM during two hours after air embolism. Solid line = ascending pharyngeal artery; interrupted line = external carotid artery. Difference is significant (p=0.03).

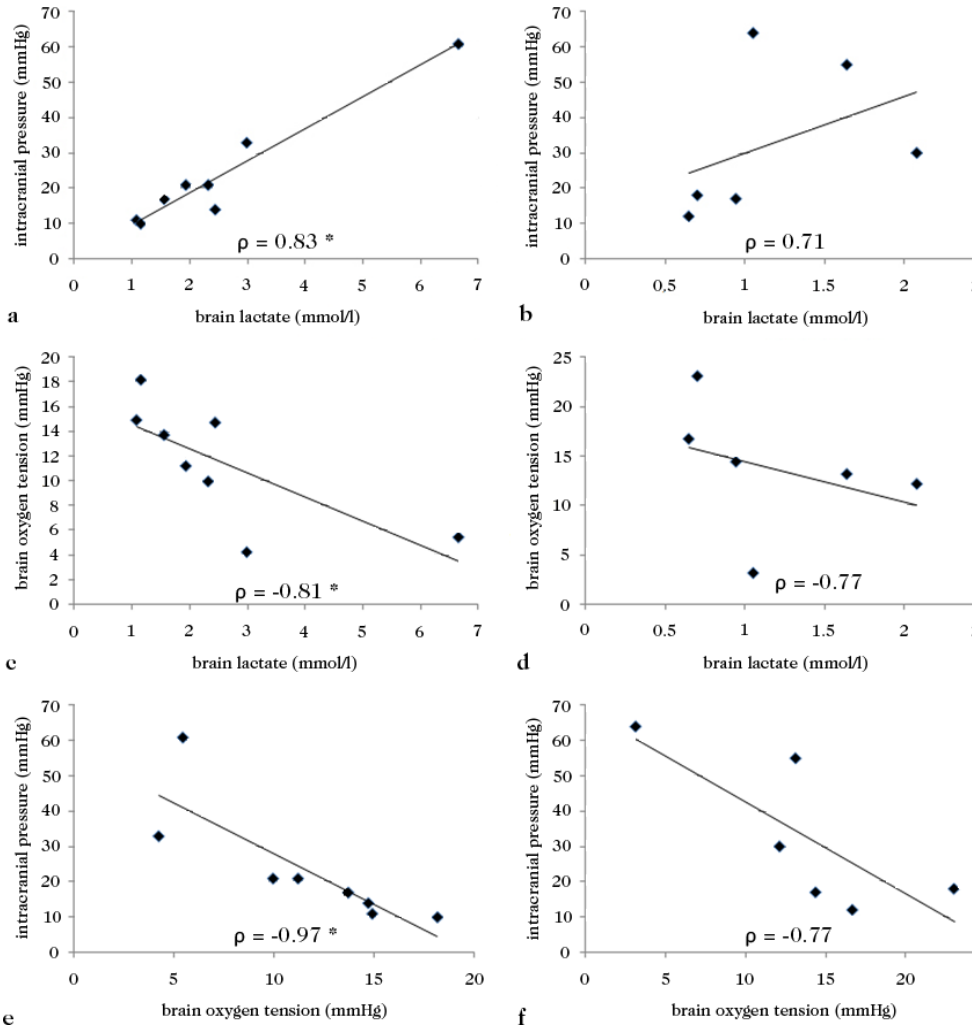


Figure 4. Correlation of measured variables at t=120 min. Spearman's rho values shown in each graph. Left column = ascending pharyngeal artery; right column = external carotid artery; * = $p<0.05$.

Discussion

In this study we investigated whether injection of air into the APA or the ECA would be most appropriate for CAGE experiments. The most appropriate vessel was predefined as being the artery that resulted in the largest effects on neuronal metabolism after air injection. Although

ICP and $PbtO_2$ did not differ between groups, we did observe a larger increase in brain lactate level after embolization of the APA. Furthermore, we observed a significant correlation between ICP, $PbtO_2$, and brain lactate after injection into the APA, which was not the case after embolization of the ECA. Together, these results suggest that use of the APA is more appropriate in our model.

In order to properly investigate the effect of CAGE on the brain, it is essential to inject air into the cerebral vasculature. Although ligation of multiple cerebral vessels would also produce global ischemia (17, 18), the effect of intravascular air on cerebral blood flow and the blood brain barrier as well as the inflammatory response that follows passage of air would not be adequately modeled in this way (19-22). In order to determine which vessel is the most appropriate for injection of air into the pig cerebral vasculature, we measured cerebral function after air embolization into two different arteries. Our main concern was the presence of the carotid rete at the cranial base. The size of the vessels in this carotid rete has been reported to range from 74 µm (23) to 154 µm (24). We hypothesized that air injected proximal of the carotid rete might lodge in these vessels, preventing entrance of air into the circle of Willis. Our results show that this is not the case and that a substantial part of the air passes through the carotid rete into the cerebral vasculature.

Brain glucose and lactate concentrations were measured using cerebral microdialysis. A drawback of this technique is that it only samples the extracellular fluid in an area quite close to the probe (25). The same holds for our measurement of $PbtO_2$. Focal changes might therefore be under- or overestimated due to sampling error. However, there was no correlation between the variables measured after embolization of the ECA, whereas a significant correlation was observed after air injection into the APA. This finding might suggest that air injected into the latter artery disperses more globally through the brain, while embolization of the ECA results in more local distribution of the air.

Brain lactate increased significantly more when air was injected in the APA. This indicates that more air reaches the cerebral vessels when embolization takes place through this artery. We believe this difference can be explained by the fact that when the air is injected in the ECA, it can only reach the circle of Willis through small anastomoses between the ECA and the carotid rete. It would therefore also be expected that the variance of the values obtained would be lower after injection into the APA than after injection into the ECA. We were not able to confirm that in our study. This might be explained by the fact that the cerebral vascular anatomy of the swine does not allow determination of the exact distribution of air within the brain. Variation in cerebral perfusion dynamics between individual pigs might result in different dispersion of air through the vasculature, giving rise to diverse reactions. If, for instance, a large amount of air accumulates in the posterior circulation, a larger cardiovascular response can be expected (26).

The larger increase in brain lactate in the APA group was not associated with a larger increase in ICP or a larger decrease in $PbtO_2$. A recent study in patients with severe traumatic brain injury has shown that lactate increase precedes ICP increase in these patients by more than 2 h (27). This suggests that if the duration of our experiments would have been longer, it might well be possible that the larger increase in brain lactate in the APA groups would be reflected by a larger increase in ICP.

The amount of glucose in brain microdialysate is indicative of the energy available for cerebral metabolism (28). We have previously shown a decrease in brain glucose after CAGE in the pig (12). These results are in line with the findings in the present study, although the decrease in brain glucose we found was not significant and did not differ between groups. Brain lactate increased more in animals embolized via the APA than in those in which the air was injected in the ECA. In the latter group the lactate level seemed to be decreasing by the end of the experiment, while in the former group it increased to levels comparable to those found in patients admitted with severe traumatic brain injury

(29). It must be noted that an increase in lactate concentration is not purely indicative of anaerobic metabolism. A state of hypermetabolism as occurs during cellular stress is also accompanied by a rise in lactate (30). In order to differentiate between hypermetabolism and ischemia, one might argue to measure the lactate/pyruvate ratio as an indicator of true ischemia (16). However, it has been demonstrated that brain lactate is a better predictor of ICP increase than lactate/pyruvate ratio in patients with severe traumatic brain injury (27).

There was a baseline difference between groups in regard to body temperature. Furthermore, we observed a significant decrease in arterial oxygen tension during the experiment in the ECA group but not in the APA group. However, since all values remained well within normal limits for the duration of the experiments, we believe it to be unlikely that these differences have influenced our results.

We did not investigate the effect of air injection into the vertebral artery, because the vertebral arteries in pigs are very small and have only little contribution to cerebral perfusion (31). Furthermore, anastomoses between vertebrobasilar and carotid vasculature and thus the carotid rete exist. Finally, injection into the vertebrobasilar system will probably result in brain stem infarction, with subsequent unpredictable and severe hemodynamic changes (26). Although we did observe an increase in blood pressure and heart rate in most of our animals of both groups, these changes were usually moderate and always transient, with values returning to normal within 10 min.

In conclusion, changes in ICP and $PbtO_2$ were comparable between the groups, but brain lactate increased significantly more when the air was injected in the APA. Only embolization of the APA resulted in significant correlation between ICP, $PbtO_2$ and brain lactate. We believe the swine to be a suitable animal for cerebral arterial gas embolism experiments and suggest the ascending pharyngeal artery as the preferred vessel for air injection.

References

1. Muth CM, Shank ES. Gas embolism. N Eng J Med 2000; 342:476-82.
2. van Hulst RA, Klein J, Lachmann B. Gas embolism: pathophysiology and treatment. Clin Physiol Funct Imaging 2003; 23:237-46.
3. Thom SR, Mendiguren I, Hardy K, Bolotin T, Fisher D, Nebolon M et al. Inhibition of human neutrophil beta2-integrin-dependent adherence by hyperbaric O2. Am J Physiol 1997; 272:C770-C777.
4. Evans DE, Kobrine AI, Weathersby PK, Bradley ME. Cardiovascular effects of cerebral air embolism. Stroke 1981; 12:338-44.
5. Helps SC, Meyer-Witting M, Feilly PL, Gorman DF. Increasing doses of intracarotid air and cerebral blood flow in rabbits. Stroke 1990; 21:1340-5.
6. Dutka AJ, Mink R, McDermott J, Clark JB, Hallenbeck JM. Effect of lidocaine on somatosensory evoked response and cerebral blood flow after canine cerebral air embolism. Stroke 1992; 23:1515-21.
7. Herren JI, Kunzelman KS, Vocelka CR, Cochran RP, Spiess BD. Horseradish peroxidase as a histological indicator of mechanisms of porcine retinal vascular damage and protection with perfluorocarbons after massive air embolism. Stroke 1997; 28:2025-30.
8. Herren JI, Kunzelman KS, Vocelka CR, Cochran RP, Spiess BD. Angiographic and histological evaluation of porcine retinal vascular damage and protection with perfluorocarbons after massive air embolism. Stroke 1998; 29:2396-403.
9. Juncker C, Petroianu G, Bergler W, Maurer J, Rufer R, Hormann K. The influence of an arterial and venous air embolism on the hearing level in laboratory mini pigs. Acta Otolaryngol 1998; 118:803-7.
10. Medby C, Ro H, Koteng S, Juul R, Krossnes BK, Brubakk AO. Microdialysis in cisterna magna during cerebral air embolism in swine. Undersea Hyperb Med 2002; 29:226-34.
11. Martens S, Theisen A, Balzer JO, Dietrich M, Graubitz K, Scherer M et al. Improved cerebral protection through replacement of residual intracavital air by carbon dioxide: a porcine model using diffusion-weighted magnetic resonance imaging. J Thorac Cardiovasc Surg

2004; 127:51-6.
12. van Hulst RA, Lameris TW, Hasan D, Klein J, Lachmann B. Effects of cerebral air embolism on brain metabolism in pigs. Acta Neurol Scand 2003; 108:118-24.
13. van Hulst RA, Drenthen J, Haitsma JJ, Lameris TW, Visser GH, Klein J et al. Effects of hyperbaric treatment in cerebral air embolism on intracranial pressure, brain oxygenation, and brain glucose metabolism in the pig. Crit Care Med 2005; 33:841-6.
14. Swindle MM. Cardiothoracic and vascular sugery/chronic intravascular catheterization. In Swindle MM, ed. Swine in the laboratory. Boca Raton, FL: CRC Press, 2007:195-259.
15. Daniel PM, Dawes JD, Prichard ML. Studies of the carotid rete and its associated arteries. Philos Trans R Soc Lond B Biol Sci 1953; 237:173-208.
16. Ungerstedt U, Rostami E. Microdialysis in neurointensive care. Curr Pharm Des 2004; 10:2145-52.
17. Haaland K, Orderud WJ, Thoresen M. The piglet as a model for cerebral circulation: an angiographic study. Biol Neonate 1995; 68:75-80.
18. Burbridge B, Matte G, Remedios A. Complex intracranial arterial anatomy in swine is unsuitable for cerebral infarction projects. Can Assoc Radiol J 2004; 55;326-9.
19. Fries CC, Levowitz B, Adler S, Cook AW, Karlson KE, Dennis C. Experimental cerebral gas embolism. Ann Surg 1957; 145:461-70.
20. Meyer JS, Gotoh F, Tazaki Y. Circulation and metabolism following experimental cerebral embolism. J Neuropathol Exp Neurol 1962; 21:4-24.
21. Johansson BB. Cerebral air embolism and the blood-brain barrier in the rat. Acta Neurol Scand 1980; 62:201-9.
22. Hallenbeck JM, Obrenovitch TP, Kumaroo KK, Thompson C, Leitch DR. Several new aspects of bubble-induced central nervous system injury. Philos Trans R Soc Lond B Biol Sci 1984; 304:177-84.
23. McGrath P. Observations on the intracranial carotid rete and the hypophysis in the mature female pig and sheep. J Anat 1977; 124:689-99.

24. Lee DH, Wriedt CH, Kaufmann JC, Pelz DM, Fox AJ, Vinuela F. Evaluation of three embolic agents in pig rete. AJNR Am J Neuroradiol 1989; 10:773-6.
25. Peerdeman SM, Girbes AR, Vandertop WP. Cerebral microdialysis as a new tool for neurometabolic monitoring. Intensive Care Med 2000; 26:662-9.
26. De la Torre E, Mitchell OC, Netsky MG. The seat of respiratory and cardiovascular responses to cerebral air emboli. Neurology 1962; 12:140-7.
27. Adamides AA, Rosenfeldt FL, Winter CD, Pratt NM, Tippett NJ, Lewis PM et al. Brain tissue lactate elevations predict episodes of intracranial hypertension in patients with traumatic brain injury. J Am Coll Surg 2009; 209:531-9.
28. Siesjo BK. Pathophysiology and treatment of focal cerebral ischemia. Part I: Pathophysiology. J Neurosurg 1992; 77:169-84.
29. Zauner A, Doppenberg EM, Woodward JJ, Choi SC, Young HF, Bullock R. Continuous monitoring of cerebral substrate delivery and clearance: initial experience in 24 patients with severe acute brain injuries. Neurosurgery 1997; 41:1082-91.
30. Mizock BA. Alterations in carbohydrate metabolism during stress: a review of the literature. Am J Med 1995; 98:75-84.
31. Reinert M, Brekenfeld C, Taussky P, Andres R, Barth A, Seiler RW. Cerebral revascularization model in a swine. Acta Neurochir Suppl 2005; 94:153-7.

Quantitative electroencephalography in a swine model of cerebral arterial gas embolism

4

Weenink RP, Vrijdag XC, van Putten MJ, Hollmann MW,
Stevens MF, van Gulik TM, van Hulst RA
Clin Neurophysiol 2012; 132:411-7

Abstract

Introduction: Cerebral arterial gas embolism (CAGE) is a serious hazard in cardiovascular surgery and other invasive procedures. We used a swine model of CAGE to determine if quantitative electroencephalography (qEEG) is a useful tool in diagnosis and prognostication of CAGE. **Methods**: 0.05 ml/kg of air was injected into the ascending pharyngeal artery in 16 pigs. Intracranial pressure, lactate in brain microdialysate, and brain oxygen tension were measured during 4 h after embolization. The qEEG parameters mean amplitude (MAMP), alpha-delta ratio (ADR), spectral edge frequency (SEF_{90}), spatial brain symmetry index (sBSI), and temporal brain symmetry index (tBSI) were calculated. **Results**: MAMP and tBSI, but not ADR, SEF_{90}, and sBSI correlate with intracranial pressure, brain lactate, and brain oxygen tension after 4 h. Early levels of MAMP and tBSI can predict intracranial pressure, brain lactate and brain oxygen tension after 4 h. **Conclusions**: MAMP and tBSI can diagnose and predict outcome in a swine model of CAGE. This study provides evidence for the utility of qEEG for diagnosis and prognosis in CAGE. Further studies are necessary to investigate the use of this method in patients.

Introduction

Cerebral arterial gas embolism (CAGE) is a risk in cardiovascular surgery and other invasive procedures (1, 2). CAGE may be regarded as a type of transient multifocal ischemia resulting in grossly inhomogeneous cerebral blood flow, profound changes in cerebral metabolism and electrophysiological status, and immediate disruption of the blood-brain barrier (3). CAGE is a serious disorder often leading to permanent neurological deficits. In a recent study half of CAGE patients had a Glasgow Coma Score <8 on admission (4). In these patients only limited clinical examination is possible. Early monitoring of CAGE patients is therefore a prerequisite. Application of invasive measurements such as intracranial pressure (ICP) is not indicated or technically feasible in all patients. It would therefore be interesting to use a non-invasive method to assess neuronal function in comatose CAGE patients.

Electroencephalography (EEG) is a commonly used method to study cerebral function. Analysis of EEG data is usually performed qualitatively, which requires much training and limits comparison between patients. Quantitative analysis of EEG data (qEEG) reduces the amount of data and produces results that are easy to interpret without much knowledge on the background of EEG. Since qEEG is a very sensitive tool for detecting acute cerebral ischemia (5), it might be of special interest in CAGE.

As the collection of large series of patients with arterial gas embolism is limited by the prevalence of only 0.57 per 100,000 hospital admissions (4), an adequate animal model is needed in CAGE research. Our group has established a swine model of CAGE, using relevant measurements such as ICP, brain oxygen tension ($PbtO_2$), and cerebral microdialysis to assess neuronal function (6, 7). The current paper reports on the use of qEEG in this model. We determined correlations between ICP, $PbtO_2$, brain lactate, and several qEEG features to determine which parameters are of most interest in assessing the neuronal effects of CAGE. Furthermore, we were interested if qEEG values obtained early after induction of CAGE can predict outcome.

Methods

Experimental setup

This study was approved by the Animal Ethics Committee of the Academic Medical Center Amsterdam, The Netherlands. Care and handling of the animals was in accordance with the latest European Community guidelines. Our group recently published a detailed description of the surgical and analytical procedures of the model (7). Briefly, 16 Landrace pigs (35-40 kg) were anesthetized with intramuscular ketamine 15 mg/kg (Eurovet Animal Health, Bladel, The Netherlands), midazolam 2 mg/kg (Actavis, Hafnarfjordur, Iceland), and atropine sulfate 0.01 mg/kg (Pharmachemie, Haarlem, The Netherlands). Animals were intubated and ventilated to maintain normocapnia. Anesthesia was continued with intravenous ketamine 10-15 mg/kg/h, sufentanil 5-10 µg/kg/h (Hameln Pharmaceuticals, Hameln, Germany), midazolam 1.5 mg/kg/h, and pancuronium bromide 0.15 mg/kg/h (Organon, Oss, The Netherlands). Blood pressure was measured invasively through a catheter placed in the brachial artery, rectally measured body temperature was regulated by means of an aluminum blanket, and a bladder catheter was placed in all animals. A 4F angiography catheter (Radiofocus Glidecath, Terumo, Tokyo, Japan) was placed under fluoroscopic guidance in one of the ascending pharyngeal arteries, which are the most important arteries supplying the pig brain. Three burr holes were made in the skull, and a calibrated ICP sensor (Codman, Raynham, MA, USA), a temperature sensor (Integra, Plainsboro, NJ, USA), a left and right $PbtO_2$ sensor (Integra), and a left and right microdialysis probe (Carnegie Medicine AB, Solna, Sweden) were inserted into the brain. Microdialysis probes were continuously flushed with artificial cerebrospinal fluid (Carnegie Medicine AB). Subdermal wire electrodes (Ives EEG Solutions Inc., Manotick, Canada) were placed in the skin in the positions shown in figure 1.

Experimental protocol

A 1 h stabilization period was provided after all surgical procedures, after

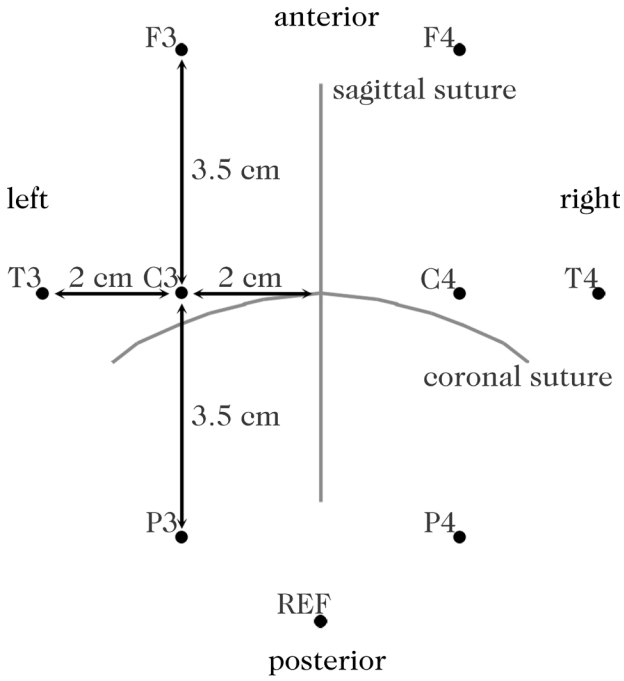


Figure 1. Position of the EEG electrodes is related to the crossing of coronal and sagittal sutures (bregma point). Nomenclature of electrodes corresponds with the international 10-20 system, distances are adapted to fit the pig skull.

which 0.05 ml/kg of room air was injected through the catheter placed in the ascending pharyngeal artery over a period of 60 s. Heart rate, blood pressure, body temperature, ICP, brain temperature, and left and right hemispheric $PbtO_2$ were recorded at t=15 min, t=30 min, and every 30 min thereafter. Blood gas analysis was performed every 60 min. EEG was recorded continuously. Vials containing the effluent of the microdialysis probes were changed at t=15 min, t=30 min, and every 30 min thereafter. The microdialysate was analysed for glucose and lactate with a CMA 600 analyzer (Carnegie Medicine AB), and values were corrected for the recovery rate as determined in an earlier in vitro experiment (76% for glucose and 89% for lactate). Position of the arterial catheter was reassessed using angiography if no change in any of the measurements was observed after air embolization. The animal was excluded from analysis if the catheter had dislocated from the ascending pharyngeal artery. Animals were terminated using potassium chloride at t=240 min, or earlier if they developed an iso-electric EEG in combination with a cerebral perfusion pressure (CPP, equals mean arterial pressure minus ICP) <30 mmHg for 30 min.

Quantitative EEG analysis

A personal computer system was used for the recording of the EEG signal from a Schwarzer PTMS1-EEG headbox (OSG, Rumst, Belgium), with a sample frequency of 500 Hz, an analog bandpass filter of 0.53 to 70 Hz (-3 dB), and a sensitivity of 70 µV/cm. All signals were recorded with the average of all electrodes as the common reference. Offline quantitative EEG analysis was performed using Matlab 2010a (The Mathworks Inc., Boston, MA, USA). EEG signals were bandpass filtered using a zero-phase 6th order Butterworth filter with cut-off frequencies 0.5 and 30 Hz. A Hamming window was applied before fast Fourier transform of each EEG epoch (10 s of EEG, using Welch's method). The following qEEG parameters were calculated: mean amplitude (MAMP), alpha-delta ratio (ADR), spectral edge frequency (SEF_{90}), spatial brain symmetry index (sBSI), and temporal brain symmetry index (tBSI). MAMP was calculated for each epoch by averaging the mean of the absolute value of the EEG from the eight channels. The estimate of the power spectral density was calculated using Welch's method with an epoch length of 2 s and 25% overlap resulting in a spectral resolution of 0.5 Hz. From the power spectral density the ADR was calculated (8), which was defined as the power in the alpha band (8-13 Hz) divided by the power in the delta band (0.5-4 Hz). SEF90 was calculated as the frequency below which 90% of the power between 0.5 and 20 Hz was contained (9). sBSI and tBSI were calculated as proposed by Van Putten (10). The sBSI calculates the difference between the spectral characteristics of the left and right hemispheres and is defined as:

$$\mathrm{sBSI}(t) = \frac{1}{K}\sum_{n=1}^{K}\left|\frac{R_n(t) - L_n(t)}{R_n(t) + L_n(t)}\right|$$

with

$$R_n(t) = \frac{1}{M}\sum_{\mathrm{ch}=1}^{M} a^2_{n,\mathrm{ch}}(t)$$

for the right hemisphere and a similar expression $L_n(t)$ for the left hemisphere. M stands for the number of channel pairs, while $a_{n,ch}(t)$ is the

Fourier coefficient with index n of channel ch, evaluated at time t, corresponding to a particular epoch with a duration of 10 s. sBSI was calculated in the frequency range 0.5–25 Hz with a spectral bandwidth of 0.5 Hz. The tBSI defines the BSI as the normalized difference between the actual spectral characteristics and a baseline EEG epoch, in our experiments a segment prior to embolization. The tBSI is defined as:

$$\mathrm{tBSI}(t) = \sqrt{|(\Delta R(t) - \gamma) \cdot (\Delta L(t) - \gamma)|}$$

with

$$\Delta R(t) = \frac{1}{K} \sum_{n=1}^{K} \left| \frac{R_n(t) - R_n(t_0)}{R_n(t) + R_n(t_0)} \right|$$

and with a similar expression for ΔL(t). t0 is the baseline time point, in our experiments an EEG segment prior to embolization. The expressions for both hemispheres quantify the relative hemispheric changes in the mean spectral characteristics of that hemisphere. Only if both of these expressions significantly differ from zero, the tBSI will change, indicating a diffuse change in the spectral characteristics. The offset correction factor γ was introduced to account for the systematic error in the estimation of the spectral difference due to the finite epoch size that is used to estimate the spectrum. In our experiments γ=0.2. For each 10 s and a frequency range from 0.5 to 25 Hz the power spectral density is used for calculation. For all features a moving average smoothing filter was applied over the current and the five previous epochs of 10 s.

Statistical analysis

SPSS 17.0 (SPSS Inc., Chicaco, IL, USA) and R (R Development Core Team, Vienna, Austria) were used for data analysis. If the endpoint of iso-electricity and CPP<30 mmHg was reached before t=240 min, the last recorded values were used as the t=240 min values. The Shapiro-Wilk test was used to test for normality, and parametric or non-parametric tests were used appropriately. $PbtO_2$ values are reported as relative to baseline values because of large variation at t=0. This large

	ICP (mmHg)	$rPBrO_2$ (%)	brain lactate (mmol/l)
MAMP (μV)	-0.817 (p=0.001)	0.725 (p=0.005)	-0.797 (p=0.002)
ADR	0.463 (NS)	-0.720 (p=0.006)	0.503 (NS)
SEF_{90} (Hz)	0.477 (NS)	-0.724 (p=0.004)	0.476 (NS)
sBSI	0.665 (p=0.013)	-0.495 (NS)	0.448 (NS)
tBSI	0.775 (p=0.002)	-0.703 (p=0.007)	0.825 (p=0.001)

Table 1. Correlation of outcome parameters with qEEG features at t=240 min. Values are displayed as spearman's rho (p-value). NS = non significant.

variation is caused by the fact that $PbtO_2$ varies greatly between brain regions and the probe only samples $PbtO_2$ in its immediate vicinity. Left and right brain relative $pBtO_2$ and microdialysis values were not significantly different and were therefore pooled. ICP, $pBtO_2$, and brain lactate were the outcome parameters of this study. Spearman's rho was used to determine correlation between outcome parameters and qEEG parameters. Accuracy of tBSI and MAMP values for diagnosing outcome in respect to ICP was tested using ROC analysis with ICP as a continuous gold standard based on the methods proposed by Obuchowski (11) using the nonbinROC package in R. Animals were further categorized as having either good outcome (low-ICP group, ICP≤20 mmHg at t=240 min) or bad outcome (high-ICP group, ICP>20 mmHg at t=240 min). 20 mmHg was chosen as the cut-off point since in the clinical situation this value is usually regarded as the upper level of normal, above which treatment to lower ICP should be initiated (12). Significance of differences within groups at different time points was calculated using paired tests (paired t-test or Wilcoxon signed-rank test as appropriate), significance of differences between groups was calculated using unpaired tests (unpaired t-test or Mann–Whitney U test as appropriate). Significance of change in qEEG features during the experiments was calculated using a linear mixed model with outcome (high-ICP or low-ICP group), time and outcome by time interaction as fixed effects, using an AR(1) covariance structure to account for repeated measures within the same animal. All tests were two-sided and statistical significance was accepted at $p<0.05$. Reported confidence intervals are 95% confidence intervals.

Results

Of the 16 animals in which air was injected, three were excluded from the analysis because the arterial catheter had dislocated from the ascending pharyngeal artery to the external carotid artery (in two cases), or the catheter had caused thrombo-embolism of the cerebral vessels (one case). Thus, the data presented here is based on the results of 13 animals. Four animals reached the pre-defined endpoint of iso-electric EEG and CPP<30 mmHg before t=240 min. Despite the fact that the same dose of air (in ml/kg body weight) was used in all experiments, we observed a wide variation of results, with ICP at t=240 min ranging from 5 to 67 mmHg.

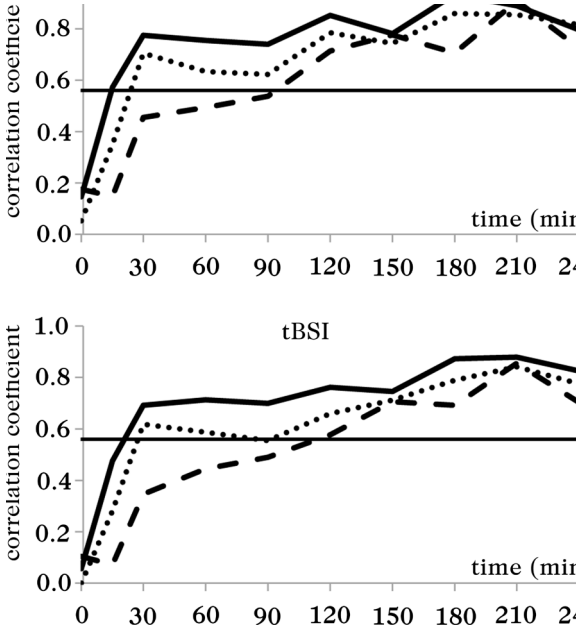


Figure 2. Absolute spearman's rho values (y-axis) of qEEG parameter values during the experiment (x-axis) with outcome parameters at t=240 min. Horizontal line indicates correlation coefficient (0.56) above which the correlation becomes significant ($p<0.05$) based on n=13. Dotted line = ICP; dashed line = $rPBrO_2$; solid line = brain lactate.

As illustrated in table 1, only MAMP and tBSI showed strong correlations ($|\rho|\approx 0.8$) with the outcome parameters at t=240 min. Figure 2 shows that early levels of MAMP and tBSI correlate with outcome parameters at t=240 min. Using non-binary gold standard ROC analysis, the accuracy of the qEEG parameters to diagnose ICP at t=240 min was determined to be 83% (CI 70–95%) for MAMP, 64% (CI 45–85%) for ADR, 62% (CI 42–91%) for SEF_{90}, 75% (CI 60–90%) for sBSI, and 80% (CI 66–94%) for tBSI. After categorization of animals into either high-ICP group (n=5) or low-ICP

group (n=8), there were no significant differences between these groups in respect to ADR, SEF_{90}, and sBSI. MAMP and tBSI changed significantly during the experiments in both groups but the difference was much more pronounced in the high-ICP group (table 2 and figure 3). Figure 4 shows that MAMP and tBSI differed significantly between groups from t=15 min (tBSI) or t=30 min (MAMP) onward.

Discussion

We used a swine model of CAGE to determine the value of several qEEG parameters for diagnosis and prognostication in CAGE. A standardized air embolus led to a large spectrum of brain damage, varying from only slight EEG disturbances to iso-electricity with brain herniation. Thus, our model mirrors the clinical situation where patients with CAGE can present with signs ranging from subtle transient neurological dysfunction to coma (4). The non-invasively obtained qEEG variables MAMP and tBSI could diagnose and predict severe brain damage in our model and may thus be of clinical value.

	high-ICP group		low-ICP group	
	t=0 min	t=240 min	t=0 min	t=240 min
heart rate (min^{-1})	79 (8)[a]	122 (26)[ab]	76 (10)	81 (11)[b]
MAP (mmHg)	83 (12)	82 (21)	85 (8)	78 (11)
temp (^{0}C)	37.9 (0.7)	37.7 (0.6)	37.6 (0.8)[a]	38.0 (0.3)[a]
PaO_2 (mmHg)	227 (27)[a]	211 (35)[a]	225 (25)	228 (14)
$PaCO_2$ (mmHg)	36.6 (1.4)[a]	37.4 (1.0)[a]	38.0 (1.5)	37.9 (1.3)
pH	7.51 (0.01)[a]	7.48 (0.02)[a]	7.49 (0.03)	7.46 (0.02)
brain temp (^{0}C)	38.2 (0.4)	37.9 (0.5)	37.9 (0.9)	38.3 (0.7)
brain glucose (mmol/l)	1.4 (0.7)[a]	0.5 (0.3)[a]	1.5 (0.4)[a]	0.5 (0.3)[a]

Table 2. General parameters in high-ICP group and low-ICP group animals at t=0 min and t=240 min, displayed as value (SD). [a] = significant difference between t=0 min and t=240 min; [b] = significant difference between high-ICP group and low-ICP group; MAP = mean arterial pressure.

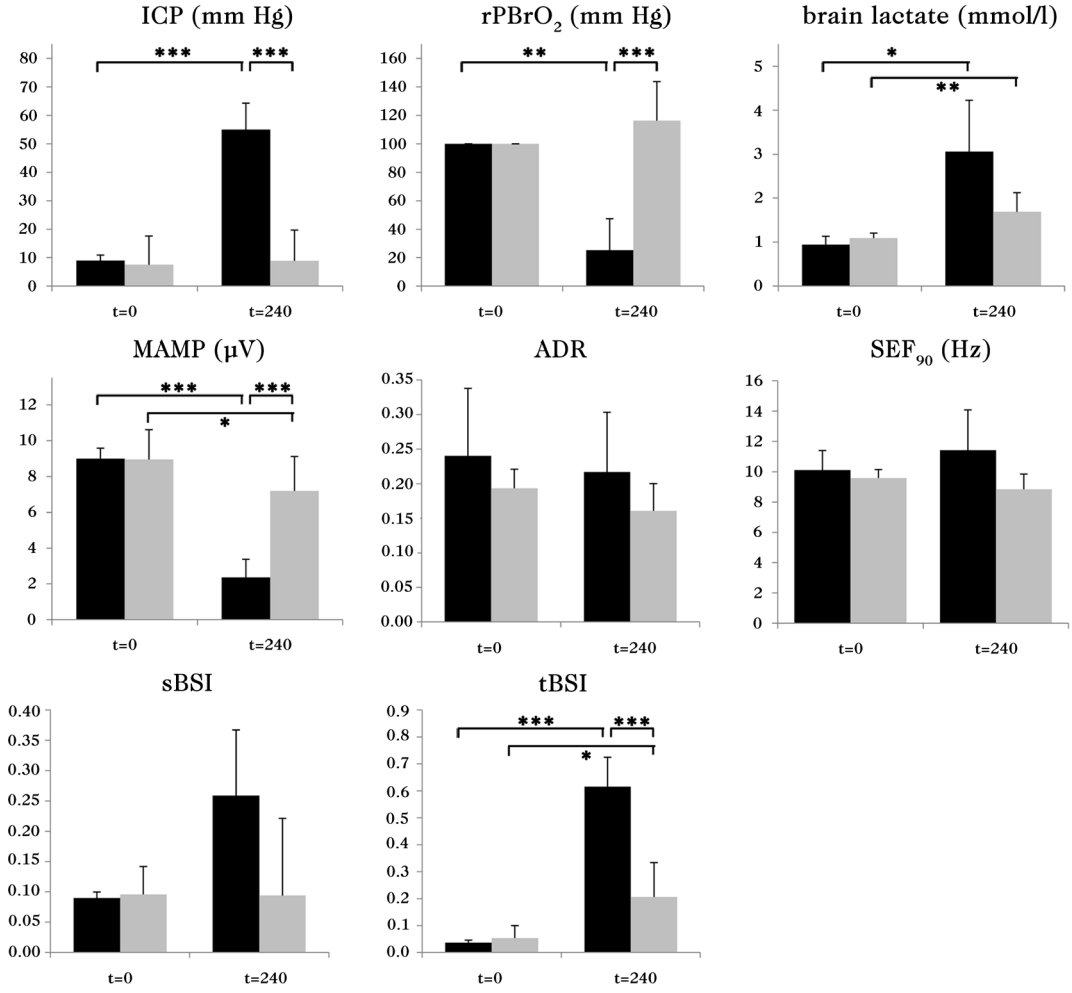


Figure 3. Bar charts displaying values of outcome parameters and qEEG features as measured in high-ICP group (black bars) versus low-ICP group (grey bars) at t=0 min and t=240 min. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

In the high-ICP group we observed a significant increase in heart rate and changes in blood gas values (decrease in PaO_2 and increase in $PaCO_2$ with decrease in pH) during the experiments. Since such changes did not occur in the low-ICP group we expect these changes to result from the extremely elevated ICP and ensuing hemodynamic and pulmonary alterations (13). For unknown reasons body temperature increased slightly but significantly in the low-ICP group. However, this might have only diminished the observed differences between the groups, since hypothermia is a robust protectant

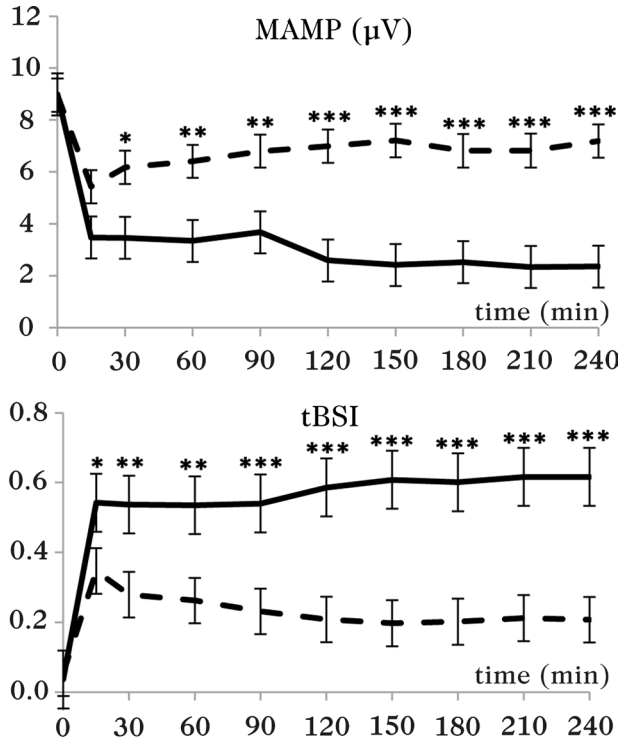


Figure 4. Estimated marginal means ± SE of MAMP and tBSI as modeled in the linear mixed model. Solid line is high-ICP group, dashed line is low-ICP group. Asterisks show significance of difference between groups. * = p<0.05; ** = p<0.01; *** = p<0.001.

against brain ischemia (14).

MAMP and tBSI showed strong correlation with our outcome parameters. Furthermore, early changes of MAMP and tBSI could predict outcome as judged by ICP, $pBtO_2$, and brain lactate. Although the relation of MAMP to neuronal damage is intuitively clear, the nature of tBSI as well as the related sBSI require more explanation. sBSI and tBSI were developed by van Putten et al. (10, 15) which group has shown the utility of sBSI and tBSI in determining shunt requirement in carotid endarterectomy (16). Furthermore, they have demonstrated the value of sBSI and a related parameter for monitoring stroke patients (17, 18). sBSI is sensitive to changes in spatial (left-right) EEG changes, while tBSI reflects temporal changes in the EEG spectrum that are not due to changes in spatial symmetry. The main advantage of both parameters is that they are normalized ranging from [0–1]. Results can therefore be easily interpreted and compared between patients, which provides an advantage over the use of non-normalized parameters such as MAMP.

In our experiments sBSI did not significantly correlate with the outcome parameters. This is somewhat surprising, since the air is injected uni-

laterally. However, it is worth mentioning that, as opposed to humans, the pig has a bilateral network of fine arterioles, called the carotid rete, at the base of the brain (19). The air is injected proximal to this carotid rete. The large amount of anastomoses between both retia probably results in bilateral dispersion of the air into the circle of Willis, accounting for the diffuse EEG changes, and explaining the lack of sBSI sensitivity as well as the absence of differences between left and right pBtO2 and brain lactate.

Previous studies have pointed out ADR and SEF_{90} as being useful in cerebral ischemia (20, 21). Mostly, general slowing of the EEG is described which is reflected by a decrease in high frequency (alpha) and an increase in low frequency (delta) activity, resulting in a decrease of ADR and SEF_{90}. In the present study no significant correlations were observed between ADR or SEF_{90} and ICP or brain lactate. Both qEEG features were negatively correlated with $pBtO_2$, which is the opposite of the expected outcome. However, when animals were categorized as having either good or bad outcome according to ICP, there was no difference between these groups in regard to ADR, SEF_{90}, and sBSI, while MAMP and tBSI did show a significant difference. Apparently, only the latter two qEEG features have true relationships with our outcome parameters.

Our experiments were performed under general anesthesia with sufentanil, midazolam and ketamine. The former two substances are known to induce dose-dependent changes in EEG pattern, mostly consisting of decrease in high-frequency activity and increase in low-frequency activity (22). Ketamine can induce an active EEG signal with increase in theta and beta activity (23). Overall, the combination of substances used in this study might well result in slowing of the EEG, which may provide an explanation for the lack of sensitivity of ADR and SEF_{90} to detect cerebral ischemia. However, we did not perform experiments in awake animals to test this hypothesis. Despite the probable changes in EEG due to general anesthesia, detection of CAGE was possible in our model as shown by the changes of MAMP and tBSI after embolization.

This study has some limitations. First of all, we did not correlate our qEEG findings with clinical outcome. We aimed to address this problem by correlating the qEEG results with other clinically relevant outcome measures such as ICP. qEEG findings have been reported to correlate with clinical outcome in human studies of stroke (18). Secondly, the duration of our experiments was only 4 h. It is well known that neuronal damage following stroke develops during several days (24), thus a longer timeframe to test the application of qEEG in CAGE would be interesting. A third limitation pertains to the tBSI, requiring a baseline EEG segment to which later changes in the EEG are compared. Although under many circumstances, such as surgical procedures known to carry a risk of CAGE induction, the definition of a normal baseline EEG epoch is possible, this is not the case in all situations. We believe the results of the present study merit further investigation of the tBSI in a form that does not require supervised definition of a baseline EEG segment. Lastly, a limitation of the employed model is that $PbtO_2$ and microdialysis values as acquired by the cerebral probes only represent the cerebral tissue in the immediate vicinity of the probe. Changes in these values are therefore highly dependent on the dispersion of air in the brain, which might differ between animals. However, we have demonstrated in an earlier study that microdialysis $PbtO_2$ values show a good correlation with ICP when air is injected in the ascending pharyngeal artery, which is the vessel used in this study (7).

CAGE due to pulmonary barotrauma is regarded as one of the most serious risks of diving (2). Although diagnosis of CAGE in the diving situation is usually very well possible based on the clinical situation, the monitoring of non-responsive patients faces the same problems as in patients with iatrogenically induced CAGE. Monitoring is further limited by the fact that hyperbaric oxygen therapy is generally commenced rapidly in these patients, which precludes application of many diagnostic (especially invasive) procedures. Therefore, qEEG might be useful in this patient category. Two abovementioned properties of our study pose difficulties in extrapolating our results to the diving popu-

lation. Firstly, the possible changes caused by the anesthetics we used may limit translation of our results to the awake situation. However, many patients with CAGE due to diving will be under general anesthesia upon arrival at a hyperbaric facility. Secondly, in out-of-hospital CAGE a normal baseline EEG segment as required for the tBSI will not be available, and thus the tBSI cannot be obtained. This difficulty may be overcome by the development of a form of the tBSI that does not require a baseline epoch.

Multiple animal studies on CAGE incorporating EEG have been published. Redo et al. found decrease in fast activity and increase in slow activity after injection of 40 ml of air into the common carotid artery in dogs, followed by decrease in amplitude when increasing amounts of air were injected (25). After injection of 0.5 ml of air into the internal carotid artery of the monkey, severe EEG changes were present which recovered after 10-20 min (26). In another study in monkeys 1-2 ml of air into the internal carotid artery was needed to obtain diffuse slowing, and marked slowing was only observed after injection of 2-4 ml (27). Fritz and Hossmann required only 0.6 ml of blood foam in the innominate artery of the cat to produce a flat electrocorticogram, which recovered in 30–60 min (3). Of note, they observed a larger recovery of fast frequency bands as compared to delta activity, resulting in a faster electrocorticogram during recovery than before embolization. Differences in tolerance to CAGE between these studies might be explained by differences in species, weight, vessel used, and volume of air injection. Drenthen et al. induced CAGE in a model much like the model used in the present study and demonstrated two independent frequency bands in which power was significantly reduced following air embolism (28). The first band, 0.5–7.3 Hz, contains delta plus theta activity while the other band, 26.4– 30.3 Hz, corresponds with fast beta activity. Interestingly, the study does not report on the alpha band or ADR, which have been shown to be of significance in other studies on EEG in cerebral ischemia (20). Nevertheless, Drenthen et al. describe EEG as a valuable tool in monitoring the treatment of CAGE by hyperbaric oxygen therapy.

No study demonstrating the acute effects of CAGE on the EEG in humans has been published so far. Ingvar et al. performed EEG measurements in submarine escape trainees, who are at risk of developing pulmonary barotrauma with ensuing CAGE because of the rapid depressurization to which they are subjected (29). They found EEG disturbances, mostly consisting of focal slowing, in seven of nine subjects with subjective and/or objective signs of neurological injury. However, EEG's were performed after treatment with hyperbaric oxygen therapy and therefore do not provide information on the acute effects of CAGE on the EEG.

We believe application of EEG in the clinical situation of a patient at risk for CAGE, for instance during cardiac surgery, may provide advantages over other methods such as transcranial Doppler ultrasonography (TCD) for detection of air bubbles. TCD does only provide information at the acute moment of bubble passage. Although signals from microemboli can be quantified, these results have not proven to correlate well with functional outcome in CPB patients (30). Since EEG provides information on the functional status of the brain, this method may have more relevance to outcome. We are well aware that the present study does not shed light on the correlation between qEEG parameters and outcome at the lower end of the damage spectrum of CAGE, but the sensitivity of the tBSI in other clinical situations (16, 18) suggests that this feature may prove interesting in this area.

The present study demonstrates the diagnostic and prognostic value of qEEG in experimental CAGE. The tBSI seems to be the most interesting parameter and may especially be of use in situations where definition of a normal baseline EEG segment is possible. Further research into a form of tBSI that does not depend on the supervised definition of a baseline EEG segment is necessary to potentiate the use of this parameter in situations where CAGE has been sustained before start of EEG measurement. Human studies are necessary to prove the value of this method in patients.

References

1. Muth CM, Shank ES. Gas embolism. N Engl J Med 2000; 342:476-82.
2. van Hulst RA, Klein J, Lachmann B. Gas embolism: pathophysiology and treatment. Clin Physiol Funct Imaging 2003; 23:237-46.
3. Fritz H, Hossmann KA. Arterial air embolism in the cat brain. Stroke 1979; 10:581-9.
4. Bessereau J, Genotelle N, Chabbaut C, Huon A, Tabah A, Aboab J, et al. Long-term outcome of iatrogenic gas embolism. Intensive Care Med 2010; 36:1180-7.
5. Jordan KG. Emergency EEG and continuous EEG monitoring in acute ischemic stroke. J Clin Neurophysiol 2004; 21:341-52.
6. van Hulst RA, Drenthen J, Haitsma JJ, Lameris TW, Visser GH, Klein J, et al. Effects of hyperbaric treatment in cerebral air embolism on intracranial pressure, brain oxygenation, and brain glucose metabolism in the pig. Crit Care Med 2005; 33:841-6.
7. Weenink RP, Hollmann MW, Stevens MF, van Lienden KP, Ghazi-Hosseini E, van Gulik TM, et al. Cerebral arterial gas embolism in swine. Comparison of two sites for air injection. J Neurosci Methods 2011; 194:336-41.
8. Claassen J, Hirsch LJ, Kreiter KT, Du EY, Connolly ES, Emerson RG, et al. Quantitative continuous EEG for detecting delayed cerebral ischemia in patients with poor-grade subarachnoid hemorrhage. Clin Neurophysiol 2004; 115:2699-710.
9. Lee SJ, Hatran DP, Tomimatsu T, Pereyra PJ, McAuley G, Longo LD. Fetal cerebral blood flow, electrocorticographic activity, and oxygenation: responses to acute hypoxia. J Physiol 2009;2033-47.
10. van Putten MJ. The revised brain symmetry index. Clin Neurophysiol 2007; 118:2362-7.
11. Obuchowski NA. Estimating and comparing diagnostic tests' accuracy when the gold standard is not binary. Acad Radiol 2005; 12:1198-204.
12. Ghajar J. Traumatic brain injury. Lancet 2000; 356:923-9.
13. Bugge JF. Brain death and its implications for management of the potential organ donor. Acta Anaesthesiol Scand 2009; 53:1239-50.

14. Yenari MA, Hemmen TM. Therapeutic hypothermia for brain ischemia: where have we come and where do we go? Stroke 2010; 41:S72-S74.
15. van Putten MJ, Peters JM, Mulder SM, de Haas JA, Bruijninckx CM, Tavy DL. A brain symmetry index (BSI) for online EEG monitoring in carotid endarterectomy. Clin Neurophysiol 2004; 115:1189-94.
16. van Putten MJ. Extended BSI for continuous EEG monitoring in carotid endarterectomy. Clin Neurophysiol 2006; 117:2661-6.
17. van Putten MJ, Tavy DL. Continuous quantitative EEG monitoring in hemispheric stroke patients using the brain symmetry index. Stroke 2004; 35:2489-92.
18. Sheorajpanday RV, Nagels G, Weeren AJ, van Putten MJ, De Deyn PP. Quantitative EEG in ischemic stroke: correlation with functional status after 6 months. Clin Neurophysiol 2011; 122:874-83.
19. Daniel PM, Dawes JD, Prichard ML. Studies of the carotid rete and its associated arteries. Philos Trans R Soc Lond B Biol Sci 1953; 237:173-208.
20. Finnigan SP, Walsh M, Rose SE, Chalk JB. Quantitative EEG indices of sub-acute ischaemic stroke correlate with clinical outcomes. Clin Neurophysiol 2007; 118:2525-32.
21. Florence G, Guerit JM, Gueguen B. Electroencephalography (EEG) and somatosensory evoked potentials (SEP) to prevent cerebral ischaemia in the operating room. Neurophysiol Clin 2004; 34:17-32.
22. Brown EN, Lydic R, Schiff ND. General anesthesia, sleep, and coma. N Engl J Med 2010; 363:2638-50.
23. Sloan TB. Anesthetic effects on electrophysiologic recordings. J Clin Neurophysiol 1998; 15:217-26.
24. Lipton P. Ischemic cell death in brain neurons. Physiol Rev 1999; 79:1431-568.
25. Redo SF. The effects of intraarterial air injection on the electroencephalogram, electrocardiogram, arterial and venous blood pressure. Surg Forum 1958; 9:166-71.
26. Meyer JS, Gotoh F, Tazaki Y. Circulation and metabolism following experimental cerebral embolism. J Neuropathol Exp Neurol 1962; 21:4-24.

27. Hekmatpanah J. Cerebral microvascular alterations in arterial air embolism. Adv Neurol 1978; 20:245-53.
28. Drenthen J, van Hulst RA, Blok JH, van Heel MD, Haitsma JJ, Lachmann B, et al. Quantitative EEG monitoring during cerebral air embolism and hyperbaric oxygen treatment in a pig model. J Clin Neurophysiol 2003; 20:264-72.
29. Ingvar DH, Adolfson J, Lindemark C. Cerebral air embolism during training of submarine personnel in free escape: an electroencephalographic study. Aerosp Med 1973; 44:628-35.
30. Kruis RW, Vlasveld FA, Van DD. The (un)importance of cerebral microemboli. Semin Cardiothorac Vasc Anesth 2010; 14:111-8.

Detection of cerebral arterial gas embolism using regional cerebral oxygen saturation, quantitative electroencephalography, and brain oxygen tension in the swine

5

Weenink RP, Hollman MW, Stevens MF,
Kager J, van Gulik TM, van Hulst RA
submitted for publication

Abstract

Introduction: Cerebral air emboli are a contributing factor in the development of neurological injury during cardiac surgery. Although peroperative cerebral monitoring is increasingly used to detect insults to the brain during high-risk procedures, the ability of these methods to detect cerebral air emboli is not known. This study investigates the utility of processed electroencephalography (EEG) and near-infrared spectroscopy derived regional cerebral oxygen saturation (rSO_2) in the detection of various volumes of intracerebrovascular air. Results were compared to invasively measured brain oxygen tension ($PbtO_2$) and brain lactate and glycerol. **Methods**: In 12 pigs a catheter was placed in one of the main arteries feeding the brain. Probes were placed to perform microdialysis and to measure rSO_2 and $PbtO_2$. An eight channel EEG was recorded and the quantitative parameter temporal brain symmetry index (tBSI) was calculated. Doses of 0.2, 0.4, 0.8, and 1.6 ml of air were injected into the cerebral arterial vasculature. **Results**: rSO_2 and tBSI were both able to detect the effects of air emboli on the brain almost instantaneously, but were less sensitive than $PbtO_2$. There was reasonable correlation between rSO_2, tBSI, $PbtO_2$, brain lactate, and brain glycerol. **Conclusions**: Our results show that regionally measured rSO_2 and processed EEG can detect the local effects of air emboli on cerebral oxygenation, but with reduced sensitivity as compared to intraparenchymal $PbtO_2$. Prospective human studies using multimodal monitoring incorporating EEG and rSO_2 should be performed.

Introduction

Cerebral injury is one of the most devastating complications of cardiac surgery. Approximately 3% of patients undergoing cardiac surgery suffers stroke, while 20-40% develops long-lasting postoperative neurocognitive deficits (1, 2). Cerebral damage due to cardiac surgery is a multifactorial process, in which embolization of solid and gaseous material is a relevant contributing factor (3). Air emboli can arise during cardiac surgery from various sources, for instance due to inadequate de-airing of the cardiac chambers or during cardiopulmonary bypass. Furthermore, cerebral air emboli can occur in other types of surgery, usually after introduction of air in systemic veins and subsequent arterialization through a patent foramen ovale (paradoxical embolism).

In an effort to decrease the number of surgical cerebral complications, there is an increasing tendency to monitor brain function during high-risk procedures, especially in cardiac surgery (4). Two commonly used non-invasive methods for brain monitoring are electroencephalography (EEG) and regional cerebral oximetry (rSO_2) using near-infrared spectroscopy (NIRS). However, the sensitivity of these methods to detect air embolism has never been investigated, and therefore, these techniques are currently not used to detect embolic insults to the brain (5).

In the present study, we use an established porcine model of cerebral arterial gas embolism (6, 7) to test the hypothesis that rSO_2 and EEG can detect air embolization. As secondary aim we compared these non-invasive cerebral monitoring methods with invasive intracerebral measurements. We injected increasing amounts of air into the cerebral arterial vasculature and continuously measured rSO_2, EEG, intraparenchymal brain oxygen tension ($PbtO_2$), as well as lactate and glycerol concentrations derived from intracerebral microdialysis catheters.

Methods

General handling and surgical preparation

After approval of the animal ethics committee of the Academic Medical Center, Amsterdam, The Netherlands and in accordance with European Community guidelines, 12 female Landrace pigs weighing on average 49 kg (SD 2.1 kg) were used for this study. Animals were premedicated with intramuscular ketamine 15 mg/kg (Eurovet Animal Health, Bladel, The Netherlands), midazolam 2 mg/kg (Actavis, Hafnarfjordur, Iceland) and atropine sulfate 0.01 mg/kg (Pharmachemie, Haarlem, The Netherlands), followed by tracheal intubation and volume controlled ventilation with an inspiratory oxygen fraction of 0.4, positive end-expiratory pressure of 4 mm Hg, frequency of 18/min, and tidal volume adjusted to maintain end-tidal carbon dioxide of 38-42 mm Hg. Anaesthesia was continued with ketamine 10-15 mg/kg/h, sufentanil 5-10 µg/kg/h (Hameln Pharmaceuticals, Hameln, Germany), midazolam 1.5 mg/kg/h, and pancuronium bromide 0.15 mg/kg/h (Organon, Oss, The Netherlands). A single bolus of 2 g ceftriaxone (Fresenius Kabi, Zeist, The Netherlands) was given as antibiotic prophylaxis. Glucose was continuously administered through a catheter in the right cephalic vein at a rate of 170-300 mg/min, targeted to maintain normoglycaemia (4-8 mmol/l) as measured in arterial blood every 30 min. Further preparation was as described earlier (7) and included invasive blood pressure measurement, a urinary catheter, temperature management to maintain normothermia, and placement of an Ascent Occlusion Balloon catheter (Johnson & Johnson, New Brunswick, NJ) in the right ascending pharyngeal artery. The ascending pharyngeal arteries are the primary feeding arteries of the porcine brain, feeding the bilateral internal carotid arteries through the rete mirabile (8). The balloon catheter allows for selective introduction of air into the ascending pharyngeal artery, while preventing backflow of air into the external carotid vasculature.

Cerebral probes and electrodes

In the prone position, a midsagittal incision was made over the cranium,

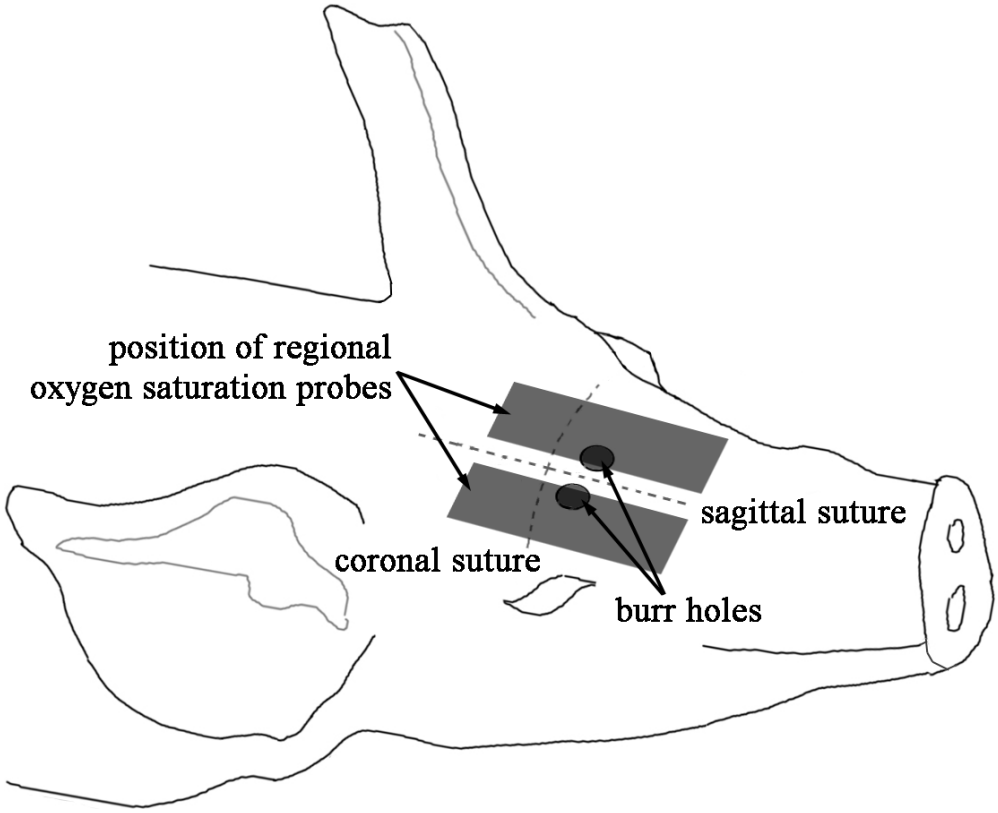


Figure 1. Schematic drawing showing the setup of the cerebral probes. Each burr hole contains one brain oxygen tension probe and one microdialysis probe. In addition, the left burr hole contains an intracranial pressure probe and the right burr hole contains a brain temperature probe. The regional oxygen saturation probes were subcutaneously tunneled to the positions indicated by the grey rectangles.

after which two burr holes were created in the skull (figure 1). Both burr holes were positioned 1 cm in front of the crossing of the coronal and sagittal sutures, with one burr hole 1 cm to the left and the other 1 cm to the right of the sagittal suture. After piercing of the dura, in each burr hole a Licox $PbtO_2$ probe (Integra, Plainsboro, NJ) and a microdialysis catheter (Carnegie Medicine AB, Solna, Sweden) were advanced approximately 1 cm into the brain parenchyma. An intracranial pressure (ICP) sensor (Codman, Raynham, MA) was advanced 2 cm into the brain through the left burr hole and a brain temperature probe (Integra) was advanced 2 cm into the brain through the right burr hole.

The temperature probe was needed to correct $PbtO_2$ for the actual brain temperature. The microdialysis probes were continuously flushed with artificial cerebrospinal fluid (Carnegie Medicine AB) at a rate of 1 µl/min. After careful hemostasis the probes were fixed in the burr holes using bone wax. Investigations by other researchers (9) as well as preliminary experiments performed in our own laboratory showed that in pigs of the size used in our experiments, rSO_2 obtained with transcutaneous measurement (as done in humans) is largely influenced by skin instead of cerebral oxygenation. Although this phenomenon has also been described in humans (10), the effect is larger in pigs, probably due to the thicker skin and skull. We avoided this problem by placing the sensors subcutaneously. At the posterior end of the midsagittal skin incision a transversal incision was created, after which two Adult SomaSensor rSO_2 probes (Covidien plc, Dublin, Ireland) were subcutaneously. Before tunneling, part of the adhesive portion of the sensors was cut away to fit the probes into the tunnel, taking care not to damage the electrical structure of the sensors. The orientation of the rSO_2 sensors was dorsal-ventral and the center of the sensors was positioned over the burr holes (figure 1). After careful hemostasis, the skin incisions were closed to prevent ambient light contamination. Nine needle electrodes (Ives EEG Solutions, Newburyport, MA) were placed in the skin as described earlier (7), to measure EEG.

Data acquisition

General parameters (heart rate, blood pressure, end-tidal carbon dioxide, body temperature, ICP, blood glucose) were recorded at 30 min intervals. Blood gas analysis for pH, arterial oxygen tension, and arterial carbon dioxide tension was performed hourly. rSO_2 was continuously displayed on and stored in an INVOS 5100C monitor (Covidien) for offline analysis. $PbtO_2$ was extracted from the Licox monitors (Integra) through a NI USB-6009 data acquisition device (National Instruments Corporation, Austin, TX) and stored for offline analysis. The eight-channel EEG signal was continuously stored for offline analysis. Temporal brain symmetry index (tBSI) was calculated over each 10 s of EEG data

as described earlier (7). In brief, the tBSI calculates spectral changes in EEG by comparing the current EEG with a defined normal baseline. It is a normalized parameter within the range [0-1]. A higher tBSI value represents a larger deviation from the baseline EEG (11). For final data presentation one value of rSO_2, $PbtO_2$, and tBSI was calculated for every 30 s of data. The vials containing the effluent of the microdialysis probes were changed every 15 min. Microdialysate was analyzed for glucose, lactate, and glycerol using a CMA 600 analyzer (Carnegie Medicine AB). Values were corrected for their in vitro recovery rates as determined in previous experiments (8). Before start of the embolizations, a 10 min increase in inspiratory oxygen fraction to 1.0 was performed to check correct placement of the sensors. If bilateral $PbtO_2$ did not increase at least 10 mm Hg or bilateral absolute rSO_2 did not increase at least 5%, the sensors were carefully repositioned until the required conditions were met.

Experimental protocol

Preliminary experiments (not published) showed that injection of 0.5 ml air generally results in detectable changes in EEG and $PbtO_2$. We were interested in the smallest amount of air that would still generate detectable injury, and therefore we chose 0.2 ml as the lowest dose. In order to investigate the effect of larger doses as well as the effect of cumulative doses of air, we designed the following experimental setup using three groups. Group A received only the two largest doses (0.8 ml and 1.6 ml), group B started with a lower dose (0.4 ml, then 0.8 ml, then 1.6 ml), and group C started with the lowest dose (0.2 ml, then 0.4 ml, then 0.8 ml, then 1.6 ml). 1.6 ml was chosen as the positive control in all groups, since we hypothesized that this amount of air would induce severe injury in all animals. Before start of the series of experiments, animals were randomly assigned to one of the three groups.

After the preparations described above, at least 1 h of stabilization was allowed before start of embolization. The balloon of the catheter in the ascending pharyngeal artery was inflated and correct position of the tip

of the catheter was confirmed using contrast angiography. Air boluses were introduced by first injecting them into the catheter using a syringe, followed by flushing of the catheter with saline. The saline used to push the emboli into the vasculature was injected manually at a rate of approximately 0.1 ml/s. After each embolization, the next embolization was delayed until rSO_2, tBSI, and $PbtO_2$ had returned to their baseline values, or, if this did not occur, until these values had stabilized for at least 10 min. At the end of the experiment, the animals were sacrificed using an overdose of pentobarbital.

	start			end		
	group A	group B	group C	group A	group B	group C
mass (kg)	50.0 (2.4)	49.3 (2.6)	49.5 (1.7)			
$etCO_2$ (mmHg)	40 (1.0)	41 (0.5)	40 (1.0)	40 (0.6)	40 (0.8)	40 (0.0)
heart rate (min^{-1})	82 (15)	85 (13)	74 (13)	80 (17)	84 (14)	73 (14)
MAP (mmHg)	104 (11)	115 (7)	116 (12)	102 (10)	109 (8)*	111 (14)*
body temperature (^{0}C)	37.6 (0.5)	37.6 (0.5)	37.2 (0.3)	37.4 (0.3)	37.4 (0.3)	37.3 (0.2)
pH	7.47 (0.02)	7.43 (0.04)	7.42 (0.04)	7.46 (0.03)	7.43 (0.05)	7.42 (0.05)
PaO_2 (mmHg)	229 (19)	226 (17)	218 (19)	228 (23)	231 (16)	220 (10)
$PaCO_2$ (mmHg)	41 (2.5)	43 (2.5)	44 (3.7)	41 (2.7)	42 (2.2)	42 (2.5)
blood glucose (mmol/l)	5.1 (0.5)	4.8 (0.6)	4.7 (1.4)	5.0 (0.8)	4.9 (0.2)	5.7 (0.9)
ICP (mmHg)	9.3 (4.0)	9.3 (2.1)	7.3 (1.7)	18 (2.2)*	17 (4.1)*	14 (7.5)
av br glucose (mmol/l)	1.8 (0.8)	2.2 (0.3)	1.5 (0.2)	1.1 (0.6)	0.9 (0.6)	1.5 (0.5)
av br lactate (mmol/l)	1.1 (0.7)	0.9 (0.2)	0.9 (0.3)	3.4 (1.4)*	3.9 (1.1)*	3.6 (1.8)*
av br glycerol (µmol/l)	20.6 (4.6)	33 (21)	22.4 (6.9)	106 (72)	124 (50)	94.1 (66)

Table 1. General parameters at start and end of the experiment in the three groups, displayed as average (SD). Group A received 0.8 and 1.6 ml of air, group B received 0.4, 0.8, and 1.6 ml of air, group C received 0.2, 0.4, 0.8, and 1.6 ml of air. Values for brain glucose, brain lactate and brain glycerol are the averages of values obtained in left and right hemisphere. There were no significant differences between the three groups at start or end of the experiment. Asterisks denote significant change between start and end of the experiment for the given parameter in the given group. $etCO_2$ = end-tidal CO_2; MAP = mean arterial pressure; ICP = intracranial pressure; av = average; br = brain.

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL). The outcome parameters of the study were rSO_2, tBSI, $PbtO_2$, brain lactate, and brain glycerol. Differences between groups A, B, and C at the start and end of the experiments were analyzed using one way ANOVA. In exception, the Kruskal-Wallis test was used when ANOVA could not be performed because the requirement for homogeneity of variance was not met. Differences between start and end of the experiment in each group were tested using t-tests for paired samples.

Absolute values of $PbtO_2$ and rSO_2 at the beginning of each embolization varied between animals, but did not change significantly between embolizations within each animal (tested using one way ANOVA). Therefore, in order to define a standardized baseline for each embolization, the values of $PbtO_2$ and rSO_2 at the beginning of each embolization were defined as 100% for that embolization.

In consultations with a statistician, the influence of side (left or right hemisphere), bolus volume (0.2, 0.4, 0.8, or 1.6 ml), and group (A, B, or C) on rSO_2, tBSI, $PbtO_2$, brain lactate, and brain glycerol was tested using linear mixed models. Since the behavior of the dependent variables over time was not linear and could not be linearized by transformation, time would have to be treated as a factor in the models, which would have resulted in a large number of parameters because of the short sampling interval of our measurements. Therefore, it was decided to reduce the number of parameters of the models by summarizing the reactions to the embolization as one value per embolization per side. For brain lactate and brain glycerol this value was the maximum value reached after the embolization. For rSO_2, tBSI, and $PbtO_2$ this value was the area under the curve (AUC) for the first 30 min after each embolization. rSO_2 AUC was calculated for the duration of desaturation under baseline value, thereby omitting any rebound increase above baseline. This is the procedure for data analysis recommended by the manufacturer of the INVOS device. For comparability, $PbtO_2$ AUC was calculated using the

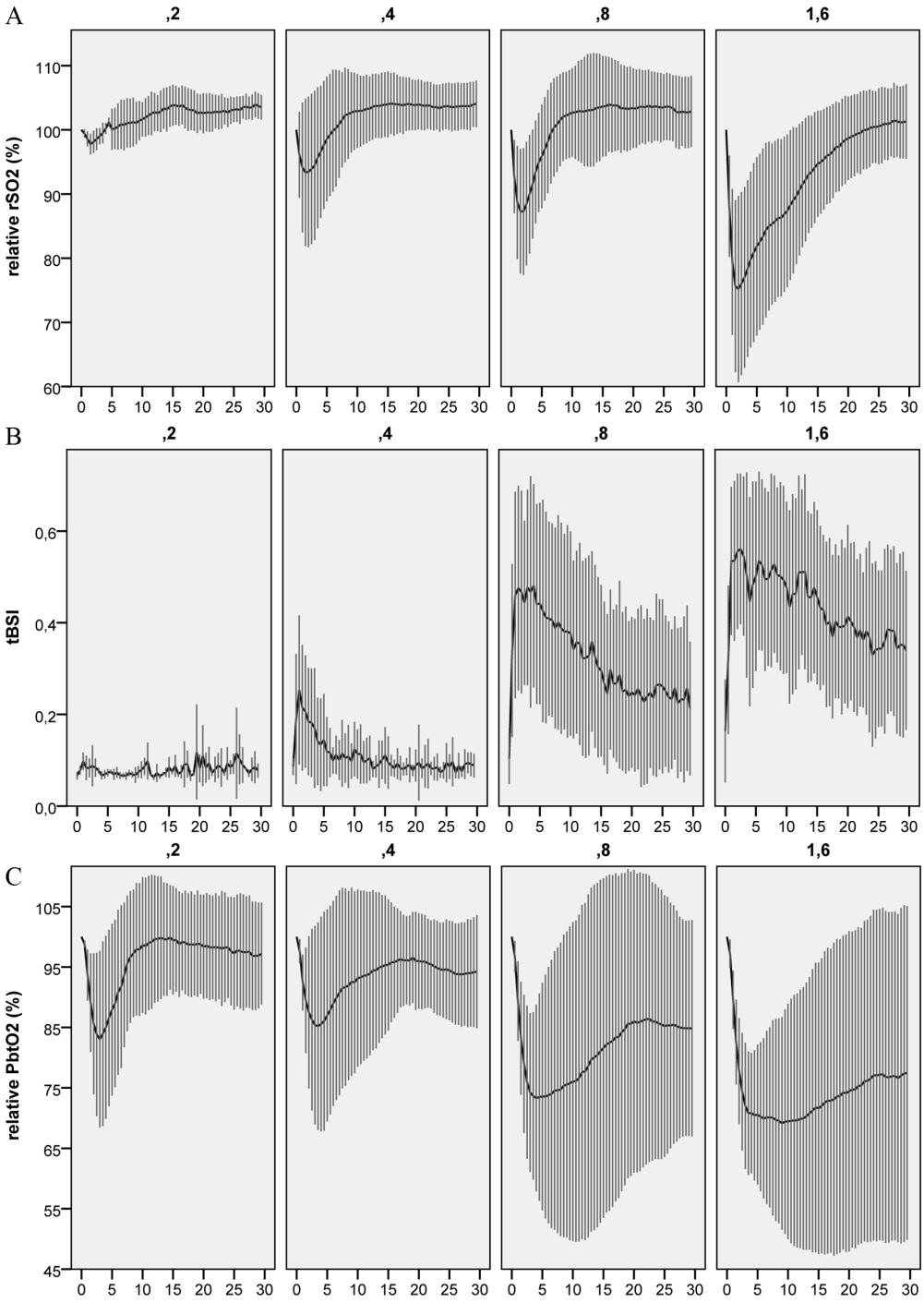
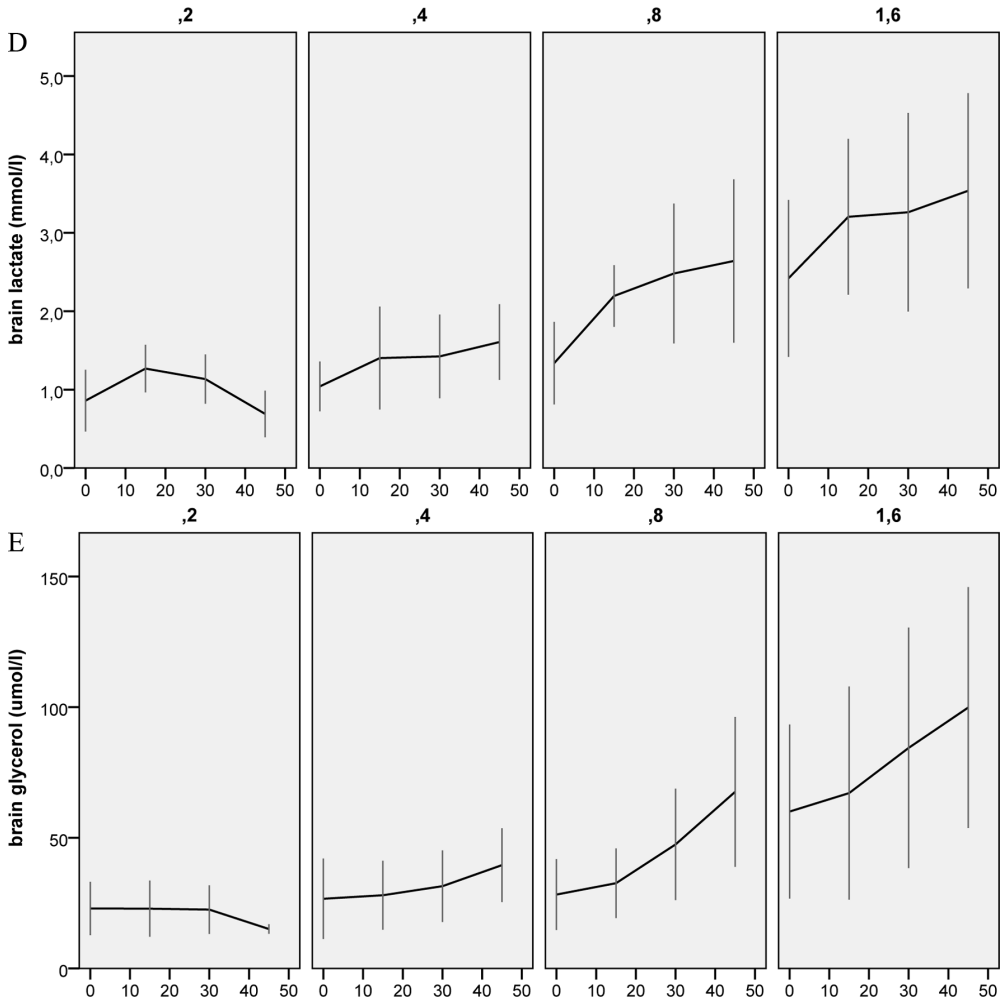
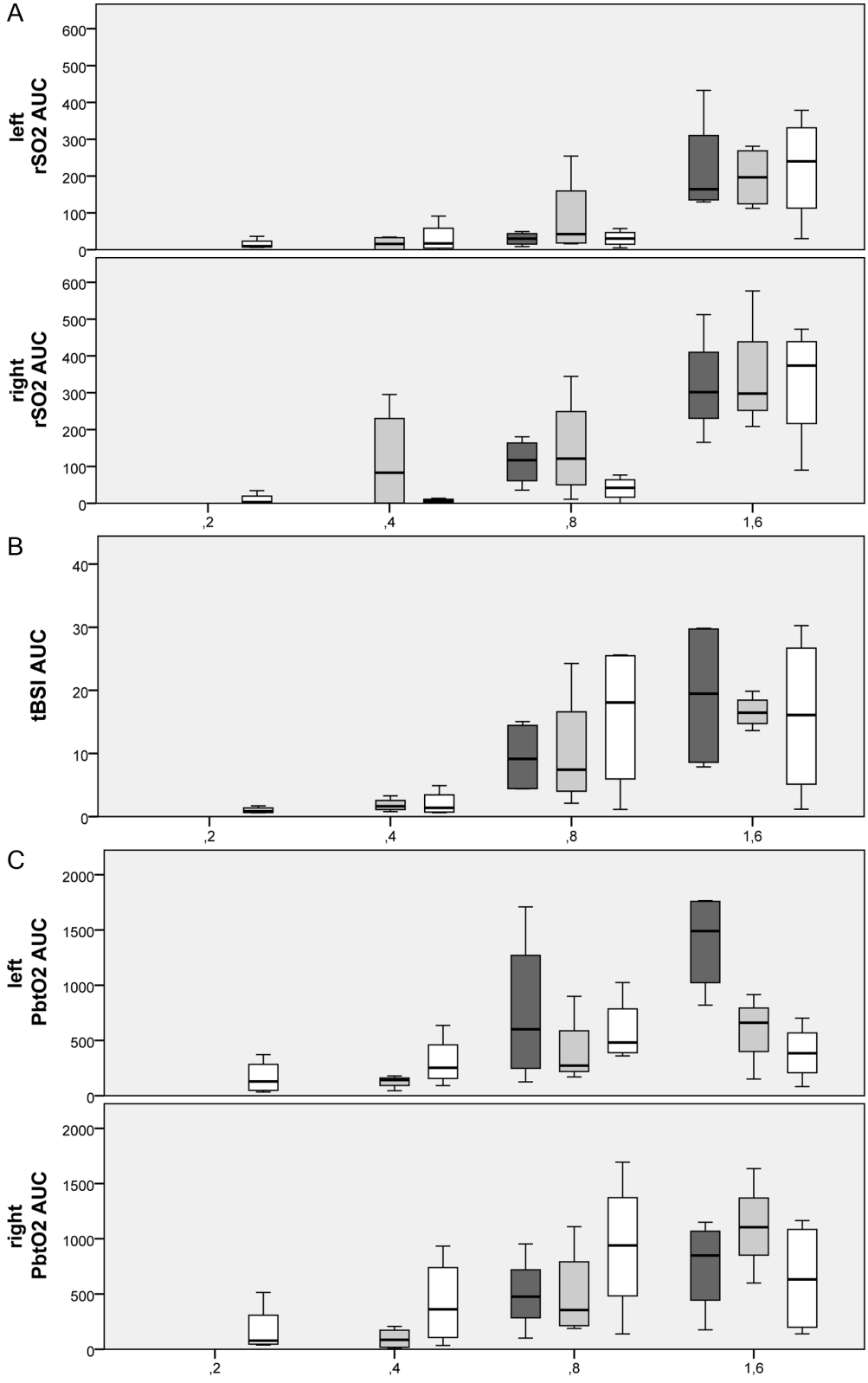


Figure 2 (continued on opposite page). Time course of rSO_2, tBSI, $PbtO_2$, brain lactate, and brain glycerol. Side (left or right hemisphere) and group (A, B, or C) were not taken into account in these graphs. X-axes show time after embolization in min. Panels show the different bolus volumes. All error bars indicate 1 standard deviation. (A) rSO_2. (B) tBSI. (C) $PbtO_2$. (D) brain lactate. (E) brain glycerol.



same method. Factors used in the linear mixed models were group, side (not for tBSI), and bolus volume. Group, side, bolus volume, and group by bolus volume were included as fixed effects. A random intercept for each animal was included. Since the linear mixed model for $PbtO_2$ did not meet the normality requirements, $PbtO_2$ AUC values had to be log-transformed. All other linear mixed models met the normality requirements without transformations.

Correlations between outcome parameters are expressed as Spearman's rho correlation coefficients. All tests were two-sided and statistical significance was accepted at $p < 0.05$.


A
left rSO2 AUC
600
500
400
300
200
100
0
right rSO2 AUC
600
500
400
300
200
100
0
,2
,4
,8
1,6
B
tBSI AUC
40
30
20
10
0
,2
,4
,8
1,6
C
left PbtO2 AUC
2000
1500
1000
500
0
right PbtO2 AUC
2000
1500
1000
500
0
,2
,4
,8
1,6

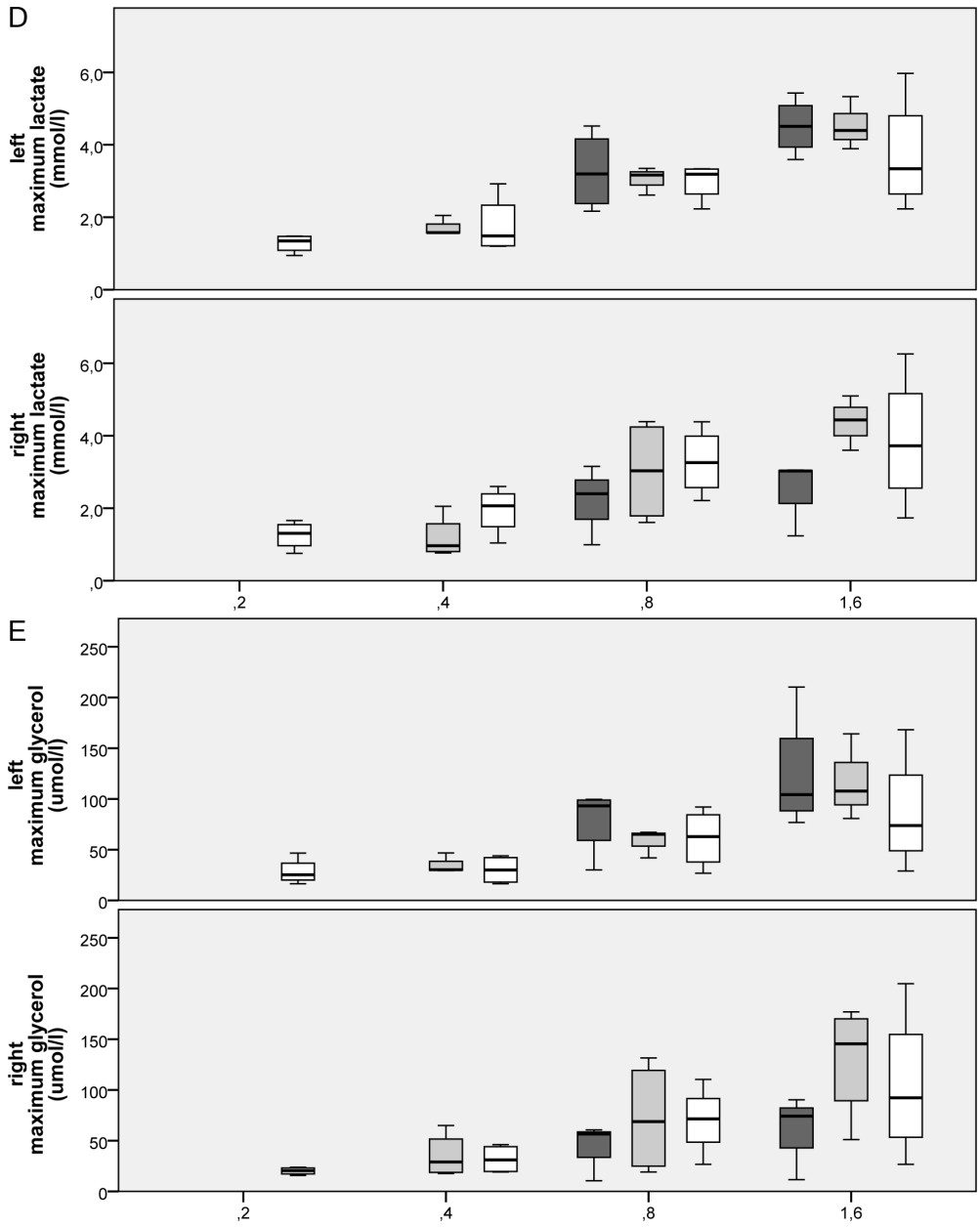


Figure 3 (continued from opposite page). Boxplots of rSO_2, tBSI, $PbtO_2$, brain lactate, and brain glycerol, quantified using area-under-the-curve for rSO_2, tBSI, and $PbtO_2$, and using maxium value after each embolization for brain lactate and brain glycerol. Graphs are split out by bolus volume (x-axis), group (dark grey = group A, light grey = group B, white = group C), and side (not for tBSI). (A) rSO_2. (B) tBSI. (C). $PbtO_2$. (D) brain lactate. (E) brain glycerol.

Results

General and microdialysis values of the three groups are shown in table 1. There were no statistically significant differences between groups A, B, and C at start and end of the experiments. There were small but significant decreases in mean arterial pressure during the experiments in groups B and C. There were significant increases in ICP in groups A and B, and in brain lactate in groups A, B, and C.

Graphs displaying the time course of rSO_2, tBSI, $PbtO_2$, brain lactate, and brain glycerol are shown in figure 2. The values displayed in these graphs are averaged over group and side. The general reaction of rSO_2 and $PbtO_2$ to the embolizations consisted of a sharp decrease followed by a slower return to baseline, sometimes including a rebound increase above 100% before the return to baseline. The reaction of tBSI was generally a sharp increase followed by slower return to baseline. In all boluses that resulted in changes of rSO_2, tBSI, and $pBtO_2$, these changes started almost immediately after the embolization.

A clinically detectable decrease (defined as <90% of baseline) of $PbtO_2$ was seen in conjunction with all boluses (of either 0.2 ml, 0.4 ml, 0.8 ml, or 1.6 ml) and clinically detectable decreases (defined <90% of baseline) of rSO_2 were seen in conjunction with 0% of 0.2 ml boluses, 38% of 0.4 ml boluses, 83% of 0.8 ml boluses, and 100% of 1.6 ml boluses. Clinically detectable increases of tBSI (defined as >0.1) were seen after 25% of 0.2 ml boluses, 63% of 0.4 ml boluses, 92% of 0.8 ml boluses, and 92% of 1.6 ml boluses.

Figure 3 displays the responses of all outcome parameters to the embolizations (quantified as described in the methods section), split out according to bolus volume, group, and side (left or right hemisphere). These values were used as the dependent variables of the linear mixed models, to determine the significance of the effect of bolus volume, group, and side. For all outcome parameters there was a significant

	rSO_2 AUC	$PbtO_2$ AUC	tBSI AUC	maximum brain lactate	maximum brain glycerol
rSO_2 AUC	1	0.417	0.616	0.597	0.540
$PbtO_2$ AUC	0.417	1	0.695	0.627	0.585
tBSI AUC	0.616	0.695	1	0.767	0.755
maximum brain lactate	0.597	0.627	0.767	1	0.898
maximum brain glycerol	0.540	0.585	0.755	0.898	1

Table 2. Spearman's rho correlation coefficients of correlations between outcome parameters. For all correlations $p < 0.001$.

effect of bolus volume (for all outcome parameters $p<0.001$). Only for $PbtO_2$ a significant group by bolus size effect was found ($p=0.024$), which indicates that only for $PbtO_2$ the reaction to a specific bolus volume was influenced by the preceding boluses. rSO_2 was significantly lower in the left hemisphere ($p=0.005$), there were no significant differences between left and right hemisphere with regard to $PbtO_2$, tBSI, brain lactate, and brain glycerol.

Correlations between rSO_2, tBSI, and $PbtO_2$ as calculated across all time points until 30 min after each embolization was weak to intermediate (correlation coefficient of rSO_2 vs $PbtO_2$ 0.137, rSO_2 vs tBSI 0.232, $PbtO_2$ vs tBSI 0.506). In order to compare our different outcome parameters with each other, all parameters were condensed to one value per embolization per side as described in the methods section. The resulting correlations were intermediate to good, as displayed in table 2.

Discussion

This study is the first to test the utility of NIRS and EEG in the setting of cerebral arterial gas embolism. We have shown that rSO_2 and tBSI can detect air emboli as early as more invasive measurements, but with a reduced sensitivity as compared to $PbtO_2$, especially when using low air

volumes. We observed reasonable correlations between rSO_2, tBSI, $PbtO_2$, brain lactate, and brain glycerol.

The incidence of cerebral injury during surgery, especially cardiac surgery, has been a concern to clinicians and researchers for decades. The etiology of perioperative stroke, encephalopathy, and cognitive decline has been the subject of extensive reviews (1, 2, 12). Current opinion suggests is that these cerebral insults arise from a multifactorial process, involving hypoperfusion, alterations in neuronal metabolism, inflammation, and embolization (3, 13). One study stated that 62% of strokes related to coronary artery bypass surgery are due to solid or gaseous emboli (14). Air emboli can arise during cardiac surgery in various ways, for instance through inadequate de-airing of cardiac chambers (15) or due to cardiopulmonary bypass, although the latter is subject to ongoing debate (16-19).

Several strategies for cerebral monitoring during high-risk procedures are clinically employed. In this study two of the three most commonly used methods were tested, namely measurement of rSO_2 using NIRS, and EEG (20). The other frequently used method is transcranial Doppler (TCD). Although this technique is known to be very sensitive to even small amounts of emboli, TCD embolic counts correlate poorly with postoperative neurocognitive function (21). A possible explanation might be that this highly sensitive method detects even the smallest emboli, which are unlikely to cause neurological injury. A second disadvantage of TCD is that it does not provide functional information on the brain. In our model, the use of TCD was not possible because the large thickness of the skulls of our pigs precluded obtaining a reliable temporal window.

Cerebral oximetry using NIRS relies on the different absorption of near-infrared light by oxygenated versus deoxygenated hemoglobin (22). Since tissue is relatively transparent to near-infrared light, applying the sensor to the human skin allows transcranial measurement of cerebral mixed arterial and venous oxygen saturation. Maintaining adequate rSO_2 has been shown to decrease morbidity after coronary artery bypass surger y(23).

Interestingly, however, a published algorithm developed to determine the correct course of action when cerebral desaturation occurs, does not include the possibility of cerebral embolism as a cause of these desaturations (5). The fact that rSO_2 values represent only a small area of brain tissue may have generated the idea that measuring rSO_2 is not capable of detecting localized events as occur in cerebral embolism. In our study we used a commercially available apparatus and used the sensors, monitor, and data analysis methods as advised by the manufacturer. The only difference was the subcutaneous placement of the sensors, which was done to reduce the influence of the porcine extracranial tissues to the signal. We took great care in positioning the tip of the $PbtO_2$ and microdialysis probes in the region covered by the rSO_2 sensors.

EEG has been used to monitor the brain since the first years of open heart surgery (24). Theoretically, measuring cerebral activity can detect changes in cerebral function due to embolic processes, but in practice, a reliable indicator of cerebral injury has been difficult to find (20). Problems include the influence of anesthesia and hypothermia on the EEG, the fact that many electrodes are needed to cover the whole brain, and the large amount of data generated in classic EEG measurement. In the current study we used an eight channel EEG to obtain a global overview of cerebral function and used tBSI to quantify the signal. The main advantage of this parameter is that it requires definition of a normal baseline (11). This baseline can be determined after induction of anesthesia and hypothermia, which reduces the influence of these factors on the results obtained. A second advantage of tBSI is that it is a normalized parameter, which allows for easy comparison between patients. Thirdly, the utility of tBSI in cerebral arterial gas embolism has been demonstrated in a previous study (7). It must be noted, however, that tBSI has thus far not been used in clinical studies.

Our study has produced a number of interesting results. It seems that of the techniques used in this study, $PbtO_2$ is the most sensitive method to detect air embolism. This is somewhat surprising, since the localized na-

ture of the $PbtO_2$ measurements had led us to hypothesize that ischemic events occurring elsewhere in the brain would be easily missed. We believe it is unlikely that the smallest amount of air injected in our animals was large enough to disperse through the whole brain, causing widespread ischemia. More probably the position of the $PbtO_2$ probes – localized in the central gyri, in the middle cerebral artery territory – was in an area that is vulnerable to the ischemia as induced in our model. Given the sensitivity of the $PbtO_2$ probes, it is interesting to note the lower sensitivity of our rSO_2 and EEG measurements. As for EEG, the global nature of this measurement may cause small ischemic events to remain unrecognized in the middle of the normal electrical activity of the rest of the brain. In regard to rSO_2, we hypothesized that these more regional measurements, which covered areas that included the areas in which the $PbtO_2$ probes were located, would be able to pick up the same ischemic effects that were recorded by the $PbtO_2$ probes. Our results may in part be explained by the influence of extracerebral tissues on the rSO_2 measurements, despite our efforts to reduce these effects by subcutaneous sensor placement (25). Nevertheless, these disadvantages are offset by the most important benefit of this technique, which is its non-invasive nature.

Correlations between rSO_2, tBSI, and $PbtO_2$ during the entire 30 min after each embolization were weak and less than reported in previous studies (26). Clustering of the dependent variables to one value per embolization per side increased correlation up to a level comparable with previous research (26), indicating that $PbtO_2$ and rSO_2 followed the same trend and thus contain essentially the same information, as demonstrated by others before (27).

In earlier studies using this model, we observed no significant differences between left and right hemisphere (7). This is explained by the fact that the air is injected proximal to the rete mirabile, a network of arterioles anastomosing across the midline at the base of the brain (8). While $PbtO_2$, brain lactate, and brain glycerol indeed showed no significant interhemispheric differences in the current study, rSO_2 was significantly lower in

the left hemisphere. This is an interesting and unexplained result, since the catheter was placed in the right ascending pharyngeal artery, and we had therefore hypothesized that if any difference between embolization of left and right hemisphere would occur, the right hemisphere would receive a larger amount of air.

The relationships between air dose and effects found in our study cannot be translated to the human situation one-to-one. Firstly, the subcutaneous placement of our sensors circumvented part of the contamination by extracranial tissues that occurred when sensors were placed on the skin. It remains to be seen if the NIRS sensors are as sensitive in humans, where extracranial contamination is also a matter of concern (10). Secondly, the pig brain is much smaller than the human brain, so a given amount of air will disperse to a relatively larger portion of the brain in pigs and may therefore be more easily detected by local measurements. One might argue that the chance that a small amount of air will end up in exactly the two regions covered by the rSO_2 sensors is small and that any use of NIRS in detecting air emboli is therefore futile. However, NIRS sensors are generally placed on the forehead, overlying the watershed areas between anterior and middle cerebral territories. Exactly these regions of the brain are known to be specifically vulnerable to ischemia in the setting of cerebral air embolism (28). rSO_2 measurement may therefore detect a relatively large proportion of air emboli, and for this reason we believe the use of rSO_2 for detecting cerebral air emboli should be considered. Specific therapies for peroperative air embolism are available, such as more extensive de-airing, retrograde cerebral perfusion, and ultimately, hyperbaric oxygen therapy (29). Since detection is the first step towards outcome modification we believe it to be crucially important to increase clinical awareness of air embolization by optimizing detection methods for gaseous emboli (20).

In conclusion, our study shows that transcranially measured rSO_2 and EEG quantified using tBSI can detect small volumes of cerebral air emboli almost instantaneously, albeit with less sensitivity than $PbtO_2$ measurement.

Future clinical research should focus on prospective trials employing multimodal monitoring with TCD, NIRS, and EEG (30), in order to extend our knowledge of the influence of air emboli on cerebral outcome in surgery.

References

1. Newman MF, Mathew JP, Grocott HP, Mackensen GB, Monk T, Welsh-Bohmer KA et al. Central nervous system injury associated with cardiac surgery. Lancet 2006; 368:694-703.
2. Lombard FW, Mathew JP. Neurocognitive dysfunction following cardiac surgery. Semin Cardiothorac Vasc Anesth 2010; 14:102-10.
3. Carrascal Y, Guerrero AL. Neurological damage related to cardiac surgery: pathophysiology, diagnostic tools and prevention strategies. Using actual knowledge for planning the future. Neurologist 2010; 16:152-64.
4. Vohra HA, Modi A, Ohri SK. Does use of intra-operative cerebral regional oxygen saturation monitoring during cardiac surgery lead to improved clinical outcomes? Interact Cardiovasc Thorac Surg 2009; 9:318-22.
5. Denault A, Deschamps A, Murkin JM. A proposed algorithm for the intraoperative use of cerebral near-infrared spectroscopy. Semin Cardiothorac Vasc Anesth 2007; 11:274-81.
6. van Hulst RA, Drenthen J, Haitsma JJ, Lameris TW, Visser GH, Klein J et al. Effects of hyperbaric treatment in cerebral air embolism on intracranial pressure, brain oxygenation, and brain glucose metabolism in the pig. Crit Care Med 2005; 33:841-6.
7. Weenink RP, Vrijdag XC, van Putten MJ, Hollmann MW, Stevens MF, van Gulik TM et al. Quantitative electroencephalography in a swine model of cerebral arterial gas embolism. Clin Neurophysiol 2012; 123:411-7.
8. Weenink RP, Hollmann MW, Stevens MF, van Lienden KP, Ghazi-Hosseini E, van Gulik TM et al. Cerebral arterial gas embolism in swine. Comparison of two sites for air injection. J Neurosci Methods 2011; 194:336-41.
9. Bein B, Cavus E, Stadlbauer KH, Tonner PH, Steinfath M, Scholz J et al. Monitoring of cerebral oxygenation with near infrared spectroscopy and tissue oxygen partial pressure during cardiopulmonary resuscitation in pigs. Eur J Anaesthesiol 2006; 23:501-9.

10. Davie SN, Grocott HP. Impact of extracranial contamination on regional cerebral oxygen saturation: a comparison of three cerebral oximetry technologies. Anesthesiology 2012; 116:834-40.
11. van Putten MJ. The revised brain symmetry index. Clin Neurophysiol 2007; 118:2362-7.
12. van Dijk D, Keizer AM, Diephuis JC, Durand C, Vos LJ, Hijman R. Neurocognitive dysfunction after coronary artery bypass surgery: a systematic review. J Thorac Cardiovasc Surg 2000; 120:632-9.
13. Hogue CW, Jr., Palin CA, Arrowsmith JE. Cardiopulmonary bypass management and neurologic outcomes: an evidence-based appraisal of current practices. Anesth Analg 2006; 103:21-37.
14. Likosky DS, Marrin CA, Caplan LR, Baribeau YR, Morton JR, Weintraub RM et al. Determination of etiologic mechanisms of strokes secondary to coronary artery bypass graft surgery. Stroke 2003; 34:2830-4.
15. Wolman RL, Nussmeier NA, Aggarwal A, Kanchuger MS, Roach GW, Newman MF et al. Cerebral injury after cardiac surgery: identification of a group at extraordinary risk. Multicenter Study of Perioperative Ischemia Research Group (McSPI) and the Ischemia Research Education Foundation (IREF) Investigators. Stroke 1999; 30:514-22.
16. Karthik S, Musleh G, Grayson AD, Keenan DJ, Pullan DM, Dihmis WC et al. Coronary surgery in patients with peripheral vascular disease: effect of avoiding cardiopulmonary bypass. Ann Thorac Surg 2004; 77:1245-9.
17. Patel NC, Deodhar AP, Grayson AD, Pullan DM, Keenan DJ, Hasan R et al. Neurological outcomes in coronary surgery: independent effect of avoiding cardiopulmonary bypass. Ann Thorac Surg 2002; 74:400-5.
18. Zamvar V, Williams D, Hall J, Payne N, Cann C, Young K et al. Assessment of neurocognitive impairment after off-pump and on-pump techniques for coronary artery bypass graft surgery: prospective randomised controlled trial. BMJ 2002; 325:1268.
19. Alston RP. Brain damage and cardiopulmonary bypass: is there really any association? Perfusion 2011; 26 Suppl 1:20-6.
20. Arrowsmith JE, Ganugapenta MS. Intraoperative brain monitoring in cardiac surgery. In: Bonser RS, Pagano D, Haverich A, eds. Brain

protection in cardiac surgery. London: Springer-Verlag, 2011:83-111.
21. Rodriguez RA, Rubens FD, Wozny D, Nathan HJ. Cerebral emboli detected by transcranial Doppler during cardiopulmonary bypass are not correlated with postoperative cognitive deficits. Stroke 2010; 41:2229-35.
22. Murkin JM, Arango M. Near-infrared spectroscopy as an index of brain and tissue oxygenation. Br J Anaesth 2009; 103 Suppl 1:i3-13.
23. Murkin JM, Adams SJ, Novick RJ, Quantz M, Bainbridge D, Iglesias I et al. Monitoring brain oxygen saturation during coronary bypass surgery: a randomized, prospective study. Anesth Analg 2007; 104:51-8.
24. Theye RA, Patrick RT, Kirklin JW. The electro-encephalogram in patients undergoing open intracardiac operations with the aid of extracorporeal circulation. J Thorac Surg 1957; 34:709-17.
25. Rohlwink UK, Figaji AA. Methods of monitoring brain oxygenation. Childs Nerv Syst 2010; 26:453-64.
26. Tyree K, Tyree M, DiGeronimo R. Correlation of brain tissue oxygen tension with cerebral near-infrared spectroscopy and mixed venous oxygen saturation during extracorporeal membrane oxygenation. Perfusion 2009; 24:325-31.
27. Brawanski A, Faltermeier R, Rothoerl RD, Woertgen C. Comparison of near-infrared spectroscopy and tissue p(O2) time series in patients after severe head injury and aneurysmal subarachnoid hemorrhage. J Cereb Blood Flow Metab 2002; 22:605-11.
28. Griese H, Seifert D, Koerfer R. Cortical infarction following cardiosurgical procedures - air embolism as a probable cause. Eur Neurol 2009; 61:343-9.
29. Gibson AJ, Davis FM. Hyperbaric oxygen therapy in the treatment of post cardiac surgical strokes--a case series and review of the literature. Anaesth Intensive Care 2010; 38:175-84.
30. Austin EH, III, Edmonds HL, Jr., Auden SM, Seremet V, Niznik G, Sehic A et al. Benefit of neurophysiologic monitoring for pediatric cardiac surgery. J Thorac Cardiovasc Surg 1997; 114:707-15, 717.

Hyperbaric oxygen does not improve cerebral function when started two or four hours after cerebral arterial gas embolism in swine

6

Weenink RP, Hollmann MW, Vrijdag XC, van Lienden KP,
de Boo DW, Stevens MF, van Gulik TM, van Hulst RA
Crit Care Med 2013; 41:1719-27

Abstract

Introduction: Hyperbaric oxygen therapy (HBOT) is the accepted treatment for cerebral arterial gas embolism (CAGE). Although earlier start of HBOT is associated with better outcome, it is unknown how much delay can be tolerated before start of HBOT. This study investigates the effect of HBOT on cerebral function in swine when initiated 2 or 4 h after CAGE. **Methods**: Under general anesthesia, probes to measure intracranial pressure (ICP), brain oxygen tension ($PbtO_2$), and brain microdialysis, and electrodes for electroencephalography were placed. The electroencephalogram (quantified using temporal brain symmetry index (tBSI)) was suppressed during 1 h by repeated injection of air boluses through a catheter placed in the right ascending pharyngeal artery. HBOT was administered using US Navy Treatment Table 6 after 2 or 4 h delay. Control animals were maintained on an inspiratory oxygen fraction of 0.4. **Results**: ICP increased to a mean maximum of 19 mmHg (SD 4.5 mmHg) due to the embolization procedure. HBOT significantly increased $PbtO_2$ in both groups treated with HBOT (mean maximum $PbtO_2$ 390 mmHg, SD 177 mmHg). There were no significant differences between groups in regard to tBSI (control vs 2 h delay p=0.078, control vs 4 h delay p=0.150), ICP and microdialysis values. **Conclusions**: We did not observe an effect of HBOT on cerebral function after a delay of 2 or 4. The injury caused in our model could be too severe for a single session of HBOT to be effective. Our study should not change current HBOT strategies for CAGE but further research is necessary to elucidate our results. Whether less severe injury benefits from HBOT should be investigated in models employing smaller amounts of air and clinical outcome measures.

Introduction

Cerebral arterial gas embolism (CAGE) is a feared complication of invasive medical procedures, for instance cardiac surgery (1). It can also occur in diving when air trapped in the lungs expands during ascent and causes pulmonary barotrauma with subsequent flow of air into the pulmonary venous system and thence to the brain. The generally accepted treatment for CAGE is hyperbaric oxygen therapy (HBOT) which ameliorates injury by decreasing bubble size and providing high partial oxygen pressure to critically perfused cerebral tissue (2). Although HBOT is a relatively safe procedure, the treatment has moderate risks. Problems may occur during transportation of patients to a hyperbaric facility as well as due to the suboptimal clinical care that can be delivered to the patient during HBOT. Furthermore HBOT itself can cause barotrauma, and the high partial oxygen pressures involved carry a risk of cerebral and pulmonary oxygen toxicity (3). Research into the use of HBOT in CAGE is therefore necessary to identify the most optimal treatment strategy in this disease.

One of the unanswered questions in this field is the optimal timing of HBOT. Although earlier start of treatment is associated with better outcome (4), no data are available on the maximum delay that can be tolerated before HBOT must be started. Because of the low incidence of CAGE and its heterogeneous presentation (4), the use of adequate animal models is of great importance in CAGE research (5). We previously published a swine model that employs clinically relevant methods such as intracranial pressure (ICP) and quantitative electroencephalography (qEEG) for assessment of the effects of CAGE on cerebral hemodynamics, metabolism and function (6, 7). In the present article, we report on the effect of HBOT when started 2 or 4 h after induction of CAGE in an adapted version of this model.

Materials and Methods

General handling

After approval of the Animal Ethics Committee of the Academic Medical Center, Amsterdam, The Netherlands and in accordance with European Community guidelines, 22 female Landrace pigs weighing 35-44 kg were used for this study. Animals were premedicated with intramuscular ketamine 15 mg/kg (Eurovet Animal Health, Bladel, The Netherlands), midazolam 2 mg/kg (Actavis, Hafnarfjordur, Iceland) and atropine sulfate 0.01 mg/kg (Pharmachemie, Haarlem, The Netherlands). After intubation, the animals were ventilated (Servo 900C, Siemens, München, Germany) in volume controlled mode with an inspiratory oxygen fraction (FiO_2) of 0.4, frequency 18/min and positive end-expiratory pressure of 4 mmHg. Arterial carbon dioxide tension was maintained between 35 and 40 mmHg by changing minute volume. Anesthesia was continued with intravenous ketamine 10-15 mg/kg/h, sufentanil 5-10 µg/kg/h (Hameln Pharmaceuticals, Hameln, Germany), midazolam 1.5 mg/kg/h and pancuronium bromide 0.15 mg/kg/h (Organon, Oss, The Netherlands). An aluminum emergency blanket was used to maintain normothermia (37-38 0C in the swine). Blood pressure was measured invasively through a catheter placed in the brachial artery. A urinary catheter was placed in all animals.

Cerebral catheter, probes and electrodes

Access to the right femoral artery was obtained using the Seldinger technique. Under fluoroscopic guidance a 5F guiding catheter (Guider Softip XF, Boston Scientific, Natick, MA) was advanced to the right common carotid artery. Through this catheter an Ascent Occlusion Balloon Catheter (Johnson & Johnson, New Brunswick, NJ) was positioned in the right ascending pharyngeal artery (the ascending pharyngeal arteries are the most important arteries supplying the pig brain). This balloon catheter allows for air injection distal to the balloon when it is inflated. One calibrated ICP sensor (Codman, Raynham, MA), one Licox temperature probe (Integra, Plainsboro, NJ), two Licox brain oxygen tension ($PbtO_2$) probes (Integra) and two microdialysis probes (Carnegie Medicine AB, Solna, Sweden) were

positioned in the cerebral tissue as described earlier (8). The Licox temperature probe was necessary to continuously correct $PbtO_2$ for the actual brain temperature. The microdialysis probes were continuously flushed with artificial cerebrospinal fluid (Carnegie Medicine AB) at 1 µL/min. 9 subdermal wire electrodes (Ives EEG Solutions, Newburyport, MA) were placed according to a method adapted from the international 10-20 system as described earlier (7). The EEG signal was recorded and analyzed as described earlier (7). Temporal brain symmetry index (tBSI) was calculated over each 10 s of EEG data and was continuously displayed in the operating room. The tBSI calculates spectral changes in the EEG by comparing the current EEG with a defined normal baseline. It is a normalized parameter within the range [0-1]. A higher tBSI value represents a larger deviation from the baseline EEG (9).

Embolization and data acquisition

After a stabilization period of at least 1 h and confirmation of correct positioning of the tip of the balloon catheter by angiography, the baseline as required for tBSI calculations was defined. This was followed by inflation of the balloon in the ascending pharyngeal artery. Balloon inflation did not cause change of the recorded parameters, including EEG signals, in any of the animals. Air embolism was inflicted according to the following protocol. Initially 0.5 ml room air was injected, followed by repeated injection of 0.2, 0.3 or 0.5 ml to reach and maintain a tBSI of at least 0.5. The catheter was flushed with saline between injections. The elevated tBSI was maintained by repeated air injections for 1 h, after which air embolism was stopped and the balloon deflated to restore normal cerebral perfusion.

Animals were randomly assigned to one of three groups. In groups 2HOURS and 4HOURS, HBOT was commenced 2 or 4 h after start of air injection, respectively. 45 min before start of HBOT these animals were transported to the hyperbaric facilities, while connected to a portable ventilator (Pneupac 2R, Smith Medical, St Paul, MN), using FiO_2=0.4. End tidal carbon dioxide level was maintained stable by adjusting minute volume. No other manipulations needed to be performed since the transport-cart was the

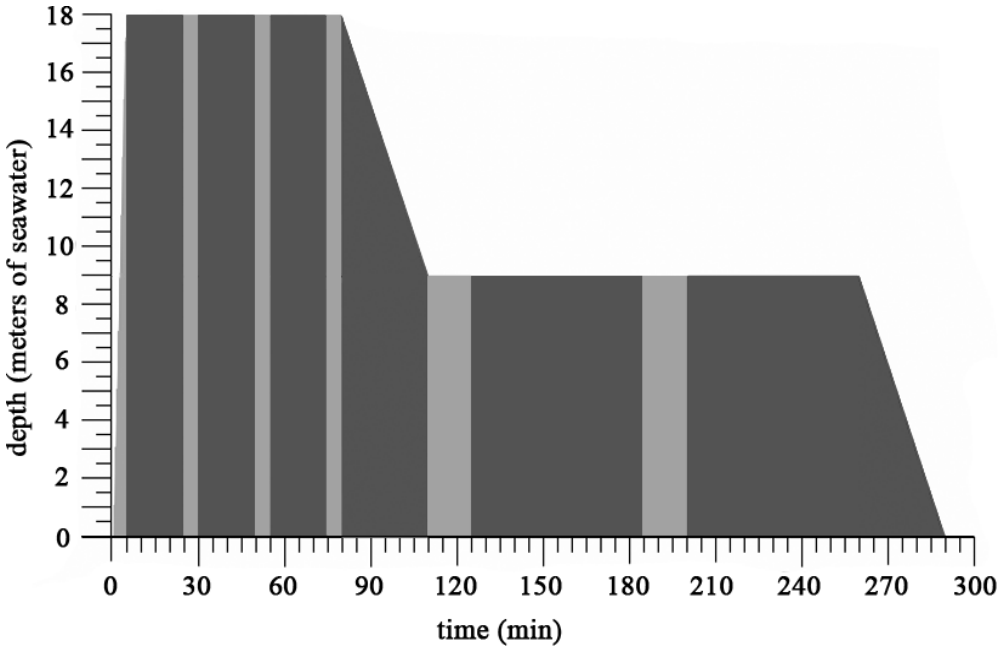


Figure 1. Profile of the US Navy Treatment Table 6. The therapy starts with rapid compression to 18 meters of seawater (2.8 atmospheres absolute, 280 kPa) on air (light grey), followed by three 20 min periods on oxygen (dark grey) each followed by 5 min air breaks. Then ascent to 9 meters of seawater (1.9 atmospheres absolute, 190 kPa) on oxygen is performed in 30 min, after which two blocks of 15 min air and 60 min oxygen follow. The ascent to the surface is performed on oxygen in 30 min. The total treatment takes 4 h 48 min.

same as the operating table used during the preparations and all monitoring equipment and intravenous pumps were transported with the pig. Transportation took approximately 10 min, after which the pig arrived in the hyperbaric chamber and was connected to the same type of ventilator and using the same settings as in the preparation phase.

HBOT was administered using a single session of US Navy Treatment Table 6, which is the most commonly used treatment table for CAGE (figure 1). At the end of the treatment FiO_2 was switched back to 0.4, and the experiment was terminated 12 min later. Total duration of the experiment after start of air embolism was 7 h (2 h delay plus 4 h 48 min treatment plus 12 min after return to normal atmospheric pressure) in the 2HOURS group and 9 h (4 h delay plus 4 h 48 min treatment plus 12 min after return to normal atmospheric pressure) in the 4HOURS group. Animals in the

CONTROL group did not receive HBOT and were maintained on FiO_2=0.4 for 9 h. Investigators could not be blinded for group allocation because of the necessary transportation of the 2HOURS and 4HOURS animals to the hyperbaric facility and the obvious difference between the 2HOURS and 4HOURS group with regard to the elapsed time from start of air embolism to start of HBOT.

Heart rate, blood pressure, body temperature, and ICP were recorded at t=15 min and t=30 min after air embolism and every 30 min thereafter. Blood gas analysis was performed hourly. The EEG was recorded continuously and analyzed offline. Average tBSI and mean amplitude were calculated for the 10 min period around t=15 min, t=30 min, and every 30 min thereafter, as described earlier (7). Vials containing the effluent of the microdialysis probes were changed every 15 min for 2 h after air embolism and every 30 min thereafter. Vials were analyzed for glucose, lactate, glycerol and pyruvate concentrations, which were corrected for recovery rate as determined in a preliminary in vitro experiment (results not displayed). Left and right $PbtO_2$ were recorded every 30 min in all groups, except during HBOT when the values were recorded halfway during each oxygen and each air period. The last $PbtO_2$ values in the HBOT groups were recorded 10 min after return to FiO_2=0.4 at normal atmospheric pressure. At the end of the experiments, animals were sacrificed using potassium chloride.

Statistics

Preliminary control experiments (not published) resulted in an average tBSI of 0.59 with a standard deviation of 0.1 at the end of the experiments. These numbers and an expected intervention effect of 25% in both HBOT groups resulted in a variance of means of 0.07. Sample size calculation using one way analysis of variance showed that this variance of means could be detected with 80% power at the 0.05 level by using 6 animals per group and 3 groups.

In previous experiments we observed that in some animals the embolization process was complicated by transient massive hypertension, some-

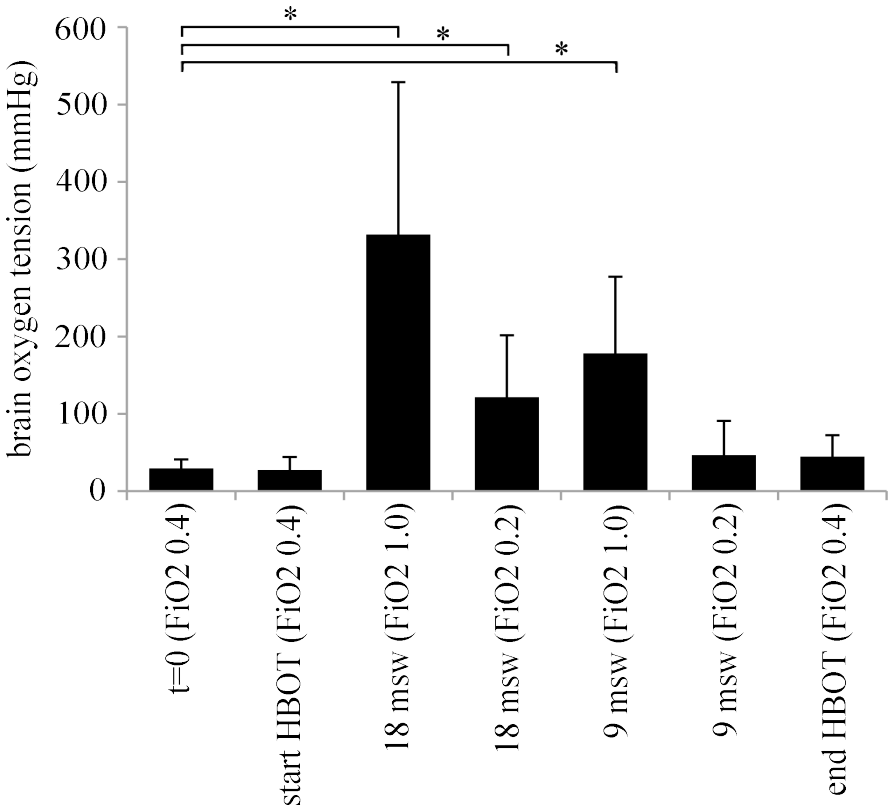


Figure 2. Brain oxygen tension in the various phases of the experiment in the animals treated with HBOT (2HOURS and 4HOURS groups). The data from these two groups are merged in this figure. All values are averages of left and right brain oxygen tensions, error bars represent 1 standard deviation. Values of the conditions during hyperbaric oxygenation are averages of all blocks during which this condition was present (e.g., as can be seen in figure 1, there are three oxygen blocks at 18 meters of seawater, the displayed value is the average of these three blocks). Asterisks mark significant changes from baseline ($p<0.008$). msw = meters of seawater.

times with tachycardia. During the following hours, this usually led to excessive ICP increase with concurrent decrease in cerebral perfusion pressure and development of an isoelectric EEG. Unfortunately, we have not been able to optimize our model in such a way that these adverse events were completely avoided, and therefore we decided to exclude animals in which ICP≥40 mmHg developed. In these cases the experiment was terminated and the animal was not used for the analysis. Excluded experiments were repeated to maintain group size of n=6.

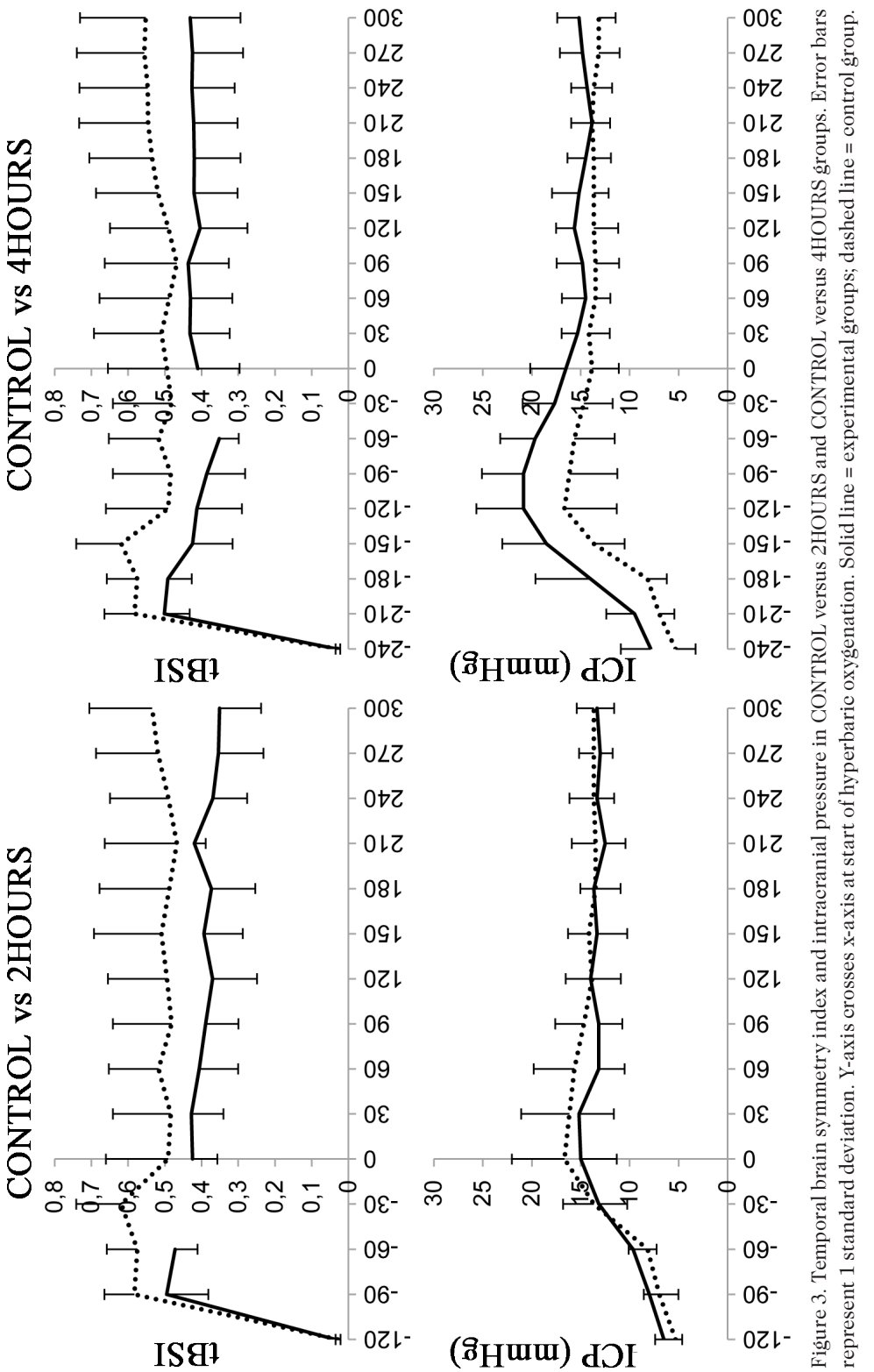


Figure 3. Temporal brain symmetry index and intracranial pressure in CONTROL versus 2HOURS and CONTROL versus 4HOURS groups. Error bars represent 1 standard deviation. Y-axis crosses x-axis at start of hyperbaric oxygenation. Solid line = experimental groups; dashed line = control group.

	t=0		
	CO	2H	4H
body mass (kg)	38±2.2	39±2.5	42±2.8
injected air volume (ml)	4.8±1.9	3.8±1.9	5.6±1.3
heart rate (min^{-1})	80±4.7[a]	76±6.3	71±6.5
MAP (mmHg)	88±6.4	89±11	97±4.4
body temperature (^0C)	37.2±0.6	37.4±0.5	37.4±0.5
PaO_2 (mmHg)	230±17	219±15	224±21
$PaCO_2$ (mmHg)	38±2.0	39±1.6	39±1.6
pH	7.5±0.02	7.5±0.02	7.5±0.02
ICP (mmHg)	5±2.1	7±1.9	8±3.1
$PbtO_2$ L (mmHg)	26±6.3	28±10	28±13
$PbtO_2$ R (mmHg)	29±10	32±12	34±11
Br glucose (mmol/l)	1.6±0.4[a]	0.9±0.4	1.2±0.4
Br lactate R (mmol/l)	0.9±0.3	0.7±0.1	1.1±0.4
Br lactate L (mmol/l)	0.9±0.3	1.1±0.6	1.0±0.1
Br glycerol R (µmol/l)	22.2±12	20.1±14	16.3±5.9
Br glycerol L (µmol/l)	17.2±5.3	62.4±56	13.9±4.9
Br L/P ratio R	5.9±1.4[a]	11±3.2	12±4.2
Br L/P ratio L	9.6±4.4	13±4.9	9.2±3.4
MAMP (µV)	8.9±1.1	8.8±2.1	8.6±1.2
tBSI	0.03±0.0	0.04±0.0	0.03±0.0

table continued on opposite page
-->

Table 1. General and cerebral parameters at the start of the experiment and start and end of group; MAP = mean arterial pressure; L = left; R = right; Br = brain; L/P = lactate/pyruvate; MAMP t=0; [b] = significant difference between CONTROL and 4HOURS groups at t=END; [c] = significant

tBSI was defined as the primary outcome measure of the study. ICP, brain lactate, brain glycerol and brain lactate/pyruvate ratio were secondary outcomes. For comparison between CONTROL and 2HOURS group, the first 7 h of data from the CONTROL group were used. For comparison between CONTROL and 4HOURS group, the full 9 h of data from the CONTROL group were used. Significance of differences between groups at the same time point was calculated using non-parametric tests for independent samples (Mann-Whitney when comparing two groups, Kruskal-Wallis when comparing three groups); differences between time points within a single group were analyzed using Wilcoxon signed-rank test. Significance

t=START				t=END			
CO	2H	CO	4H	CO	2H	CO	4H
80±5.4	78±13	92±18	82±26	91±12[c]	92±18	93±11	93±8.5
88±11	86±12	83±10	94±7.4	79±6.9[c]	77±8.1c	78±5.5	79±1.7[c]
37.7±0.5	37.5±0.4	37.7±0.4	37.2±0.4	37.7±0.3	37.7±0.4	37.5±0.3	37.5±0.5
219±16	212±10	217±17	218±16	216±12	200±17	219±14	224±39
39±1.3	37±4.9	39±1.2	41±2.6	38±2.0	39±3.4[c]	37±1.5[bc]	40±2.5
7.5±0.02	7.5±0.05	7.4±0.02	7.4±0.04	7.4±0.01[c]	7.4±0.02	7.4±0.01[b]	7.4±0.02
17±5.4	15±3.7	14±2.7	17±3.7	14±1.8	13±1.8	13±1.7	15±2.2
28±17	28±14	27±21	21±17	28±14	37±18	40±19[c]	78±57
24±11	30±22	23±10	32±6.7	36±17	33±18	38±11[c]	58±31
0.9±0.7	0.7±0.5	0.5±0.4	0.4±0.4	0.2±0.2	0.3±0.4	0.3±0.2	0.4±0.6
4.0±1.2	4.2±2.7	3.1±1.3	4.3±2.1	3.2±1.0	2.6±1.0	3.2±1.0	3.2±1.2
2.9±0.9	3.5±1.6	2.3±0.7	2.5±0.9	2.1±0.5	2.2±0.7	2.3±0.5	2.3±0.8
189±67	255±87	146±73	211±90	73.2±40[c]	68.0±35	62.3±34[c]	114±49[c]
108±36	105±59	79.0±36	73.6±53	45.0±28[c]	53.2±37	38.4±25[c]	37.9±38[c]
20±9.7	27±17	16±7.6	18±1.9	18±7.3	25±6.8	20±7.5[c]	21±7.4
17±5.4	26±8.9	16±6.4	12±3.2	13±6.0	25±9.2	15±4.8	17±4.4[c]
3.5±1.7	4.0±0.9	3.6±1.9	4.3±0.9	3.6±1.9	4.3±0.5	3.4±2.0	4.1±1.4
0.49±0.2	0.42±0.1	0.49±0.2	0.41±0.1	0.53±0.2	0.35±0.1	0.55±0.2	0.43±0.1

hyperbaric oxygenation. Values are mean ± SD. CO = control group; 2H = 2HOURS group; 4H = 4HOURS = mean amplitude; [a] = significant difference between CONTROL, 2HOURS, and 4HOURS groups at difference between t=START and t=END in this group.

of changes in tBSI were further analyzed using a linear mixed model with group (CONTROL vs 2HOURS or CONTROL vs 4HOURS), time (treated as covariate) and group by time interactions as fixed effects, using a first order autoregressive covariance structure to account for repeated measurements within the same animal. Since we were interested in the effects of HBOT, only the time points from start to end of HBOT were used for the linear mixed models. All tests were two-sided, and statistical significance was accepted at $p<0.05$. When analyzing differences between $PbtO_2$ significance was accepted at $p<0.008$ (Bonferroni correction for six comparisons).

Results

Of the 22 animals, 4 animals reached an ICP≥40 mmHg (3 in the 2HOURS group and 1 in the 4HOURS group) and were thus excluded from further analysis as determined before start of the study. In all these animals, the progressive ICP increase was already evident before start of HBOT. Thus, a total of 18 animals (n=6 per group) were analyzed. Body weight and amount of air injected were not significantly different between the three groups. Table 1 shows the values for general and brain specific values in the three groups at the start of the experiment, start of HBOT, and end of HBOT. Although there was a statistically significant difference in regard to heart rate, brain glucose, and right brain lactate/pyruvate ratio between the three groups at t=0, at the start of HBOT no significant differences between the groups were present. There were no differences between CONTROL and 2HOURS group at the end of HBOT. There were small but significant differences between $PaCO_2$ and pH between CONTROL and 4HOURS group at the end of HBOT.

HBOT resulted in large and significant increase of $PbtO_2$ during the treatment session (figure 2), while in the control group no clinically relevant changes in $PbtO_2$ occurred. However, $PbtO_2$ values in the 2HOURS and 4HOURS group were equal to values in the CONTROL group after return to normal atmospheric pressure and FiO_2=0.4 (table 1). While tBSI in the CONTROL group tended to increase after approximately 2 h after embolization, tBSI in the 2HOURS and 4HOURS group showed a decreasing trend, most notably in the 2HOURS group (figure 3). However, the differences of tBSI at the end of HBOT between groups failed to reach statistical significance (CONTROL vs 2HOURS p=0.078, CONTROL vs 4HOURS p=0.150). Additionally, linear mixed models analysis resulted in non-significant differences between the groups (group by time interaction p=0.197 for 2HOURS vs CONTROL and p=0.597 for 4HOURS vs CONTROL). ICP in all animals showed increase in the first hours after embolization (mean ICP increase 12 mmHg, not significantly different between groups), followed by slight decrease (figure 3). The microdialysis

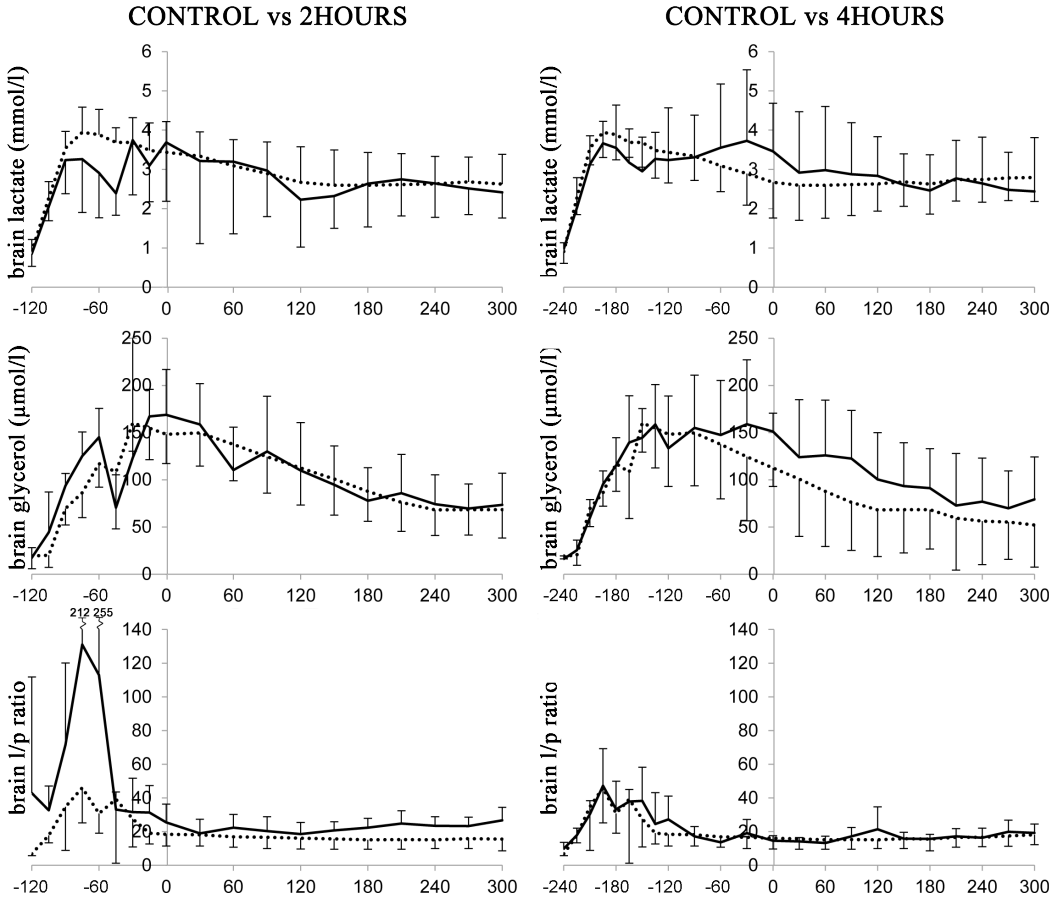


Figure 4. Microdialysis values in CONTROL versus 2HOURS and CONTROL versus 4HOURS groups. Error bars represent 1 standard deviation. Y-axis crosses x-axis at start of hyperbaric oxygenation. Solid line = experimental groups; dashed line = control group; l/p = lactate/pyruvate.

markers lactate, glycerol, and lactate/pyruvate ratio are displayed in figure 4 (averages and standard deviations are given in table 1, ICP and microdialysis data were not analyzed using linear mixed models).

Discussion

We used an EEG based strategy to inflict CAGE in swine, in order to determine the effect of HBOT after 2 and 4 h delay. Despite the fact that the treated animals were subjected to significantly higher $PbtO_2$ than

the control animals, there were no significant differences in regard to tBSI, ICP, brain lactate, brain glycerol, and brain lactate/pyruvate ratio between groups.

The amount of delay that can be tolerated before commencement of HBOT in CAGE is unknown. In humans only retrospective studies have been performed. Ziser et al. (10) reported on 17 patients with CAGE and found a significant relationship between time to HBOT and outcome. The recent study of Bessereau et al. (4) showed that patients with neurological sequelae in their mixed group of arterial and venous cerebral air embolism were more likely to have received HBOT more than 7 h after injury. In some case reports, recovery after a delay of several days have been reported (11, 12). While many different animal models have been used in CAGE research (13), in all of the studies that included HBOT the therapy was started within 0 to 60 min after induction of CAGE (6, 14-18). Thus, while in the clinical situation a delay of several hours is common, no data are available on the result of these delays on the effectiveness of HBOT. Animal studies on HBOT in non-CAGE transient ischemic stroke suggest that HBOT may be effective up to 6 h after onset of ischemia, but these results have as of yet not been confirmed in human studies (19).

The current study is based on the article by Van Hulst et al. (6) in which a similar animal model was used to demonstrate the effectiveness of HBOT in reducing ICP after a delay of 3 and 60 min after CAGE. However, in this study the embolization resulted in large increases in ICP, which in time would probably not have been compatible with life. Since our goal was to extend the duration of delay after CAGE, we were required to use smaller amounts of damage to the brain than had been used in this previous study. Based on previous research (7, 17, 20, 21) we chose qEEG (specifically tBSI) as the primary outcome measure because of its global character, high temporal resolution, easy applicability in the clinical situation, and demonstrated usefulness in our animal model. By using qEEG we tried to inflict a constant amount of damage in which HBOT would still be expected to be effective. We specifically chose not to inject a fixed

(or weight based) amount of air, since our previous investigations demonstrated that in our model this leads to a wide variation of the effects of the air embolism on the brain (7, 8). We believe this to be caused by the random distribution of the air bubbles through the cerebral vasculature. Our strategy using titrated embolization was based on work of other researchers who demonstrated reproducible injury by titrating embolization based on EEG or somatosensory evoked potentials (14, 16, 22-34).

Further advantages of the current model include the fact that the pig is a large animal and is known for its conformity with human anatomy and physiology (35). The pig brain (albeit much smaller than the human brain) allows the application of human techniques for assessment of cerebral condition, in our model ICP, $PbtO_2$, microdialysis, and qEEG. This enables comparison of our results with human studies. Furthermore, the specific cerebrovascular anatomy of the pig – with a freely anastomosing network of arterioles at the base of the brain, the rete mirabile – allows for unilateral occlusion of the carotid circulation without alteration of the EEG signal. This allowed us to inflate a balloon in one of the ascending pharyngeal arteries (the equivalents of the human internal carotid arteries) without disturbing cerebral function. By inflating the balloon, we prevented retrograde flow of air into the external carotid circulation, thereby allowing for more selective administration of the air.

Despite the abovementioned efforts and the fact that HBOT resulted in large and significant increase in $PbtO_2$, we have not been able to demonstrate an effect of HBOT on tBSI and microdialysis values in this study. There are three possible explanations for these results.

Firstly, the failure to demonstrate a significant difference between the groups may be due to type II error. The data on tBSI (figure 3) suggest that there may have been some effect of HBOT on the EEG recordings in our study, although tBSI is already somewhat lower in the intervention groups at the start of HBOT. Therefore, our study could have been underpowered, resulting in failure to detect the difference in tBSI be-

tween groups. The minimum effect size that could be detected with 80% power with the current study setup was 0.66, while the actual effect size observed was only 0.34, mostly due to larger variance of the data. This would suggest that despite our efforts the amount of damage inflicted was not consistent enough to result in predictable changes to the cerebral parameters. Further improvements to our model should therefore focus on more selective methods of embolization.

The second explanation is that there is actually no difference between the groups. A negative effect of delay on the effectiveness of HBOT in CAGE is understandable. Early after induction of CAGE air bubbles are present in the cerebral vessels. The increased atmospheric pressure used in HBOT compresses these bubbles and promotes passage to the venous circulation. Although large bubbles can remain in the arterial vasculature for hours (36), most bubbles introduced in our model will have disappeared during the 2 or 4 h delay. Thrombi may have formed due to prolonged stasis of blood (37). Under these circumstances HBOT may still have an effect, but only because of the hyperoxygenation, immunomodulation and ICP reduction it causes (1). Nevertheless, several reports have suggested beneficial effects of HBOT in CAGE, even after a delay of hours to days (11, 12). This would suggest that the amount of damage inflicted in our model was too severe for HBOT to be effective. Despite the fact that our model did not include progressive ICP increases (these animals were excluded), our embolization resulted in prolonged severe EEG disturbances and an average maximum brain lactate of 4.6 mmol/l. The clinical equivalent of these measurements in our animals are unknown since we did not awake the animals from anesthesia. It is difficult to compare our microdialysis results with human studies, since microdialysis probe characteristics and settings (and therefore recovery rates) vary widely throughout the studies. In general it can be stated that our brain lactate value of 4.6 mmol/l is in line with or even higher than values found in patients with severe traumatic brain injury (38-41). This may indicate that the injury inflicted in our model is too severe for HBOT to be beneficial. On the other hand, previous studies used an embolization protocol in which the

somatosensory evoked potential in cats was decreased to 10% of baseline (16, 22, 23). While no EEG measurements were performed in these animals, it may be expected that the electrochemical disturbances in these animals were even more profound than in our model. Nevertheless, these studies did show a beneficial effect of HBOT in CAGE, possibly because the delay between CAGE and HBOT was only 15 min. In contrast to the clinical situation, where HBOT is usually extended or repeated based on clinical or ancillary examinations, we only provided one session of HBOT. Furthermore, some patients demonstrate improvement during follow up after treatment, while we sacrificed the animals soon after termination of HBOT. It is conceivable that additional treatments with HBOT or extended follow-up would have resulted in beneficial effects, but we believe this to be impracticable from a biotechnical point of view.

A third explanation for our results may be that our outcome parameter tBSI is an inadequate representative of cerebral function and therefore an inappropriate surrogate for human outcome. The effects of CAGE on the cerebrum are multifaceted and not only include infarction leading to electrochemical dysfunction, cerebral edema, and cell death, but also an inflammatory response. We hypothesized that by quantifying brain metabolism, electrical function, and edema we would obtain a general assessment of cerebral status. Unfortunately, we did not perform histological examinations or cerebral imaging nor did we quantify the inflammatory response. Furthermore, several important aspects of outcome, such as cognitive effects of CAGE, were impossible to test in our animal study.

We have not included a control group in which HBOT was started immediately after induction of CAGE. The main reason for not including such a group is the fact that it was practically impossible to perform the experiments in this fashion, since the hyperbaric facilities were only available for the actual treatment sessions and not for the necessary preparations and embolization procedure. Moreover, the effectiveness of direct commencement of HBOT has previously been demonstrated in a model almost identical to ours, in which an even larger amount of cere-

bral injury was inflicted (6).

Only the 2HOURS and 4HOURS animals were transported to the hyperbaric facilities. We undertook great care in preventing any influence of transportation on the animals by minimizing transport time, ventilating the animal with the same FiO_2, maintaining end tidal carbon dioxide tension equal to before transportation, and moving all monitoring and other equipment together with the animal. We did not observe any influences of transportation on all measured parameters, although our methodology precludes definitive exclusion of bias due to transportation.

Recommendations on the treatment of CAGE advise prompt administration of 100% oxygen when the diagnosis is suspected (2). In the current study we maintained the animals on FiO_2=0.4 until start of HBOT. This was done because in many clinical scenarios the suspected diagnosis of CAGE is delayed for a certain period of time after the insult has occurred (4). This especially occurs in clinical cases of CAGE, where the immediate effects of the embolism may be obscured by general anesthesia. In these cases 100% oxygen will not immediately be given. Secondly, we hypothesized that the beneficial effects of 100% oxygen given during 2 or 4 h after the insult might negate the additional positive effects of subsequent HBOT.

Despite efforts to refine the model by delivering small amounts of air as directly into the carotid cerebral circulation as possible (8) and choosing a highly sensitive primary outcome measure (7), 4 of the 22 animals (18%) experienced progressive ICP increase in the hours following embolization. In three of these animals, the embolization process had been complicated by a period of massive hypertension with tachycardia. In concordance with previous studies (42) we believe these autonomic disorders to be caused by brainstem ischemia, a known issue in CAGE models (43). Although hypertension promotes passage of bubbles through the capillaries in the acute phase of embolization (2), it is known to be detrimental in the following hours since hypertension leads to increased damage to

the blood-brain barrier, which results in more cerebral edema and ICP increase (44). We chose to exclude all animals in which ICP≥40 mmHg developed in order to keep the amount of damage inflicted as consistent as possible.

The most important question is what the current study contributes to the discussion on the effectiveness of HBOT in CAGE. The use of HBOT in this disease is rational from a theoretical point of view, and its effect has been documented in animal studies and retrospective clinical series. Thus, for ethical reasons a placebo controlled trial has never been performed and probably never will be. This makes the development and use of animal models vitally important (5). We are the first to study the effect of delay in HBOT using an animal model, moreover a large animal model that has proven its use in CAGE research. This makes our results interesting and, despite the fact that we recognize that the present study should not change the current treatment strategies for CAGE, asks for more research using even more refined animal models. The use of clinical outcome parameters seems to be of vital importance as we conclude that this is the only way to reliably determine the clinical equivalent of the damage inflicted. Although difficult from a biotechnical point of view, repeat sessions of HBOT or extended observation after treatment may reveal beneficial effects not detectable in this study.

Conclusions

In our swine model of CAGE, we were not able to demonstrate improvement in qEEG, ICP and microdialysis values when HBOT was started after a delay of 2 or 4 h. This may be caused by type II error or by the fact that there is actually no effect of HBOT in this situation. If the latter is the case, then the injury inflicted in our model may have been too severe for HBOT to be effective. Further research using clinical outcome measures should be performed in order to answer the question regarding the maximum tolerable delay until start of HBOT in CAGE.

References

1. Muth CM, Shank ES. Gas embolism. N Engl J Med 2000; 342:476-82.
2. van Hulst RA, Klein J, Lachmann B. Gas embolism: pathophysiology and treatment. Clin Physiol Funct Imaging 2003; 23:237-46.
3. Weaver LK. Hyperbaric oxygen in the critically ill. Crit Care Med 2011; 39:1784-91.
4. Bessereau J, Genotelle N, Chabbaut C, Huon A, Tabah A, Aboab J et al. Long-term outcome of iatrogenic gas embolism. Intensive Care Med 2010; 36:1180-7.
5. Moon RE. Bubbles in the brain: what to do for arterial gas embolism? Crit Care Med 2005; 33:909-10.
6. van Hulst RA, Drenthen J, Haitsma JJ, Lameris TW, Visser GH, Klein J et al. Effects of hyperbaric treatment in cerebral air embolism on intracranial pressure, brain oxygenation, and brain glucose metabolism in the pig. Crit Care Med 2005; 33:841-6.
7. Weenink RP, Vrijdag XC, van Putten MJ, Hollmann MW, Stevens MF, van Gulik TM et al. Quantitative electroencephalography in a swine model of cerebral arterial gas embolism. Clin Neurophysiol 2012; 123:411-7.
8. Weenink RP, Hollmann MW, Stevens MF, van Lienden KP, Ghazi-Hosseini E, van Gulik TM et al. Cerebral arterial gas embolism in swine. Comparison of two sites for air injection. J Neurosci Methods 2011; 194:336-41.
9. van Putten MJ. The revised brain symmetry index. Clin Neurophysiol 2007; 118:2362-7.
10. Ziser A, Adir Y, Lavon H, Shupak A. Hyperbaric oxygen therapy for massive arterial air embolism during cardiac operations. J Thorac Cardiovasc Surg 1999; 117:818-21.
11. Muller MC, Lagarde SM, Germans MR, Juffermans NP. Cerebral air embolism after arthrography of the ankle. Med Sci Monit 2010; 16:CS92-CS94.
12. Wherrett CG, Mehran RJ, Beaulieu MA. Cerebral arterial gas embolism following diagnostic bronchoscopy: delayed treatment with hyperbaric

oxygen. Can J Anaesth 2002; 49:96-9.
13. Weenink RP, Hollmann MW, van Hulst RA. Animal models of cerebral arterial gas embolism. J Neurosci Methods 2012; 205:233-45.
14. Meijne NG, Schoemaker G, Bulterijs AB. The treatment of cerebral gas embolism in a high pressure chamber. An experimental study. J Cardiovasc Surg (Torino) 1963; 4:757-63.
15. Worman LW, Seidel B. Treatment of cerebral air embolism in the dog. Am J Surg 1966; 111:820-4.
16. McDermott JJ, Dutka AJ, Evans DE, Flynn ET. Treatment of experimental cerebral air embolism with lidocaine and hyperbaric oxygen. Undersea Biomed Res 1990; 17:525-34.
17. Drenthen J, van Hulst RA, Blok JH, van Heel MD, Haitsma JJ, Lachmann B et al. Quantitative EEG monitoring during cerebral air embolism and hyperbaric oxygen treatment in a pig model. J Clin Neurophysiol 2003; 20:264-72.
18. Leitch DR, Greenbaum LJ, Jr., Hallenbeck JM. Cerebral arterial air embolism: I. Is there benefit in beginning HBO treatment at 6 bar? Undersea Biomed Res 1984; 11:221-35.
19. Matchett GA, Martin RD, Zhang JH. Hyperbaric oxygen therapy and cerebral ischemia: neuroprotective mechanisms. Neurol Res 2009; 31:114-21.
20. Hossmann KA, Fritz H. Coupling of function, metabolism, and blood flow after air embolism of the cat brain. Adv Neurol 1978; 20:255-62.
21. Fritz H, Hossmann KA. Arterial air embolism in the cat brain. Stroke 1979; 10:581-9.
22. McDermott JJ, Dutka AJ, Koller WA, Pearson RR, Flynn ET. Comparison of two recompression profiles in treating experimental cerebral air embolism. Undersea Biomed Res 1992; 19:171-85.
23. McDermott JJ, Dutka AJ, Koller WA, Flynn ET. Effects of an increased PO2 during recompression therapy for the treatment of experimental cerebral arterial gas embolism. Undersea Biomed Res 1992; 19:403-13.
24. Dutka AJ, Kochanek PM, Hallenbeck JM, Storey JR. Air embolism may cause unrecognized ischemia of the gray-white junction. Undersea Biomed Res 1988; 15:99-106.

25. Dutka AJ, Kochanek PM, Hallenbeck JM. Influence of granulocytopenia on canine cerebral ischemia induced by air embolism. Stroke 1989; 20:390-5.
26. Hallenbeck JM, Furlow TW, Jr., Ruel TA, Greenbaum LJ, Jr. Extracorporeal glass-wool filtration of whole blood enhances post-ischemic recovery of the cortical sensory evoked response. Stroke 1979; 10:158-64.
27. Hallenbeck JM, Leitch DR, Dutka AJ, Greenbaum LJ, Jr., McKee AE. Prostaglandin I2, indomethacin, and heparin promote postischemic neuronal recovery in dogs. Ann Neurol 1982; 12:145-56.
28. Hallenbeck JM, Obrenovitch TP, Kumaroo KK, Thompson C, Leitch DR. Several new aspects of bubble-induced central nervous system injury. Philos Trans R Soc Lond B Biol Sci 1984; 304:177-84.
29. Hallenbeck JM, Dutka AJ, Tanishima T, Kochanek PM, Kumaroo KK, Thompson CB et al. Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early postischemic period. Stroke 1986; 17:246-53.
30. Kochanek PM, Dutka AJ, Kumaroo KK, Hallenbeck JM. Platelet activating factor receptor blockade enhances recovery after multifocal brain ischemia. Life Sci 1987; 41:2639-44.
31. Kochanek PM, Dutka AJ, Kumaroo KK, Hallenbeck JM. Effects of prostacyclin, indomethacin, and heparin on cerebral blood flow and platelet adhesion after multifocal ischemia of canine brain. Stroke 1988; 19:693-9.
32. Menasche P, Pinard E, Desroches AM, Seylaz J, Laget P, Geyer RP et al. Fluorocarbons: a potential treatment of cerebral air embolism in open-heart surgery. Ann Thorac Surg 1985; 40:494-7.
33. Spiess BD, Braverman B, Woronowicz AW, Ivankovich AD. Protection from cerebral air emboli with perfluorocarbons in rabbits. Stroke 1986; 17:1146-9.
34. Evans DE, Catron PW, McDermott JJ, Thomas LB, Kobrine AI, Flynn ET. Effect of lidocaine after experimental cerebral ischemia induced by air embolism. J Neurosurg 1989; 70:97-102.
35. Swindle MM. Cardiothoracic and vascular sugery/chronic intravascular

catheterization. In: Swindle MM, ed. Swine in the laboratory. Boca Raton: CRC Press, 2007:195-259.
36. Dexter F, Hindman BJ. Recommendations for hyperbaric oxygen therapy of cerebral air embolism based on a mathematical model of bubble absorption. Anesth Analg 1997; 84:1203-7.
37. Obrenovitch TP, Kumaroo KK, Hallenbeck JM. Autoradiographic detections of 111indium-labeled platelets in brain tissue sections. Stroke 1984; 15:1049-56.
38. Menzel M, Doppenberg EM, Zauner A, Soukup J, Reinert MM, Bullock R. Increased inspired oxygen concentration as a factor in improved brain tissue oxygenation and tissue lactate levels after severe human head injury. J Neurosurg 1999; 91:1-10.
39. Zauner A, Doppenberg EM, Woodward JJ, Choi SC, Young HF, Bullock R. Continuous monitoring of cerebral substrate delivery and clearance: initial experience in 24 patients with severe acute brain injuries. Neurosurgery 1997; 41:1082-91.
40. Robertson CS, Gopinath SP, Uzura M, Valadka AB, Goodman JC. Metabolic changes in the brain during transient ischemia measured with microdialysis. Neurol Res 1998; 20 Suppl 1:S91-S94.
41. Stahl N, Ungerstedt U, Nordstrom CH. Brain energy metabolism during controlled reduction of cerebral perfusion pressure in severe head injuries. Intensive Care Med 2001; 27:1215-23.
42. De la Torre E, Mitchell OC, Netsky MG. The seat of respiratory and cardiovascular responses to cerebral air emboli. Neurology 1962; 12:140-7.
43. Furlow TW, Jr. Experimental air embolism of the brain: an analysis of the technique in the rat. Stroke 1982; 13:847-52.
44. Dutka AJ, Hallenbeck JM, Kochanek PM. A brief episode of severe arterial hypertension induces delayed deterioration of brain function and worsens blood flow after transient multifocal cerebral ischemia. Stroke 1987; 18:386-95.

A retrospective cohort study of lidocaine in divers with neurological decompression illness

7

Weenink RP, Hollmann MW, Zomervrucht A, van Ooij PJ, van Hulst RA
Undersea Hyperb Med; in press

Abstract

Lidocaine is the most extensively studied substance for adjuvant therapy in neurological decompression illness (DCI), but results have been conflicting. In this retrospective cohort study, we compared 14 patients who received adjuvant intravenous lidocaine for neurological decompression sickness and cerebral arterial gas embolism between 2001 and 2011, against 21 patients who were treated between 1996 and 2001 and did not receive lidocaine. All patients were treated with hyperbaric oxygen therapy (HBOT) according to accepted guidelines. Groups were comparable for all investigated confounding factors, except that significantly more lidocaine treated patients had made an unsafe dive (62% vs 14%, p=0.007). Groups had comparable injury severity as measured by Dick and Massey score (lidocaine 2.7±1.7, control 2.0±1.6), an adapted version of the Dick and Massey score, and Blatteau score. Number of HBOT sessions given was comparable in both groups (lidocaine 2.7±2.3, control 2.0±1.0). There was neither a positive nor a negative effect of lidocaine on outcome (relative risk for objective neurological signs at follow up in the lidocaine group was 1.8, 95% CI 0.2-16). This is the first retrospective cohort study of lidocaine in neurological DCI. Since our study is underpowered to draw definitive conclusions, a prospective multicenter study remains the only way to reliably determine the effect of lidocaine in neurological DCI.

Introduction

Neurological decompression illness (DCI) is one of the most serious complications of diving, at times resulting in mortality and permanent morbidity (1). Neurological DCI encompasses two disease entities, neurological decompression sickness (DCS) and cerebral arterial gas embolism (CAGE). Although pathophysiology and clinical presentation of these two diseases are different, treatment for both conditions is the same and consists of prompt administration of 100% oxygen and intravenous fluids followed by expeditious administration of hyperbaric oxygen therapy (HBOT) (2).

The search for adjuvant therapies to improve outcome in neurological DCI has led to the investigation of intravenous lidocaine as a neuroprotective agent. This sodium channel blocking and anti-inflammatory agent has shown promising results in several animal studies (3, 4), but subsequent animal and human investigations have yielded conflicting results (5-9). Based on the positive effects of lidocaine reported in the literature, in 2001 the decision was made to apply intravenous lidocaine as adjuvant therapy in all cases of neurological DCI presenting to the Diving Medical Center of the Royal Netherlands Navy. In the present study, we report on the efficacy of lidocaine in our patients from 2001 to 2011, using an historic cohort as the control group.

Methods

Standardized patient documentation was introduced at our institution in 1996. Adjuvant treatment with lidocaine in all patients with neurological DCI was introduced in June 2001. We reviewed all medical files of patients treated with HBOT from 1996 to 2011 to include patients for our study. Included patients were those with a diagnosis of neurological DCI (neurological DCS or CAGE) who received US Navy Treatment Table 6 as their first HBOT session at our institution within 72 h after start of symptoms

following a dive. Patients who were comatose on arrival were excluded. Since all patient information was handled anonymously, no informed consent was obtained from the patients. From the included patient files we extracted sex, date of birth, weight, length, characteristics of the dive (duration, depth, breathing gas, diving in the preceding 18 h (repetition dive)), time from end of dive to start of symptoms and time from start of symptoms to start of HBOT. Also, the performed dive was compared to the Canadian Defence and Civil Institute of Environmental Medicine dive tables and their guidelines (10, 11) to see if the required decompression stops were adhered to. If not, or if the diving history revealed occurrence of rapid ascent, the dive was categorized as 'unsafe'. Clinical course from start of symptoms to beginning of HBOT was noted as improving, stable or worsening. Neurological symptoms were graded according to the Dick and Massey (DM) scoring system (12) (table 1). We also calculated the severity score as devised by Blatteau (13) (table 1, an adapted version of the original score introduced by Boussuges (14)). Since both these scores are primarily designed for use in spinal cord DCS, we furthermore calculated an adapted version of the DM scale to include symptoms specific for cerebral DCS, vestibular DCS and CAGE (table 1). Diagnosis as established based on history and physical and neurological examination, in accordance with the US Navy Diving Manual (15), was recorded. As for treatment, we noted type and amount of HBOT treatments and whether or not lidocaine was given. Neurological symptoms at the end of the last HBOT session were noted, from which DM score as well as our adapted version of this score were calculated. Outcome after the last HBOT session was also expressed as absence or presence of objective neurological signs. Since follow up data were available in only 14% of patients, we were not able to determine delayed outcome.

Patients suspected of neurological DCI (including those with only subjective symptoms) who are presented to our institution are immediately treated with 100% oxygen followed by neurological examination and initiation of US Navy Treatment Table 6 as soon as possible. This table is extended if necessary as recommended in the US Navy Diving Manual (16).

A. Dick and Massey severity score (total possible score is 10).

Sensory symptoms	1. paresthesia of single limb or area
	2. paresthesia of multiple regions
	3. numbness of single region or limb
	4. numbness of two regions or limbs
	5. numbness of three or more limbs
Motor symptoms	1. paresis of single limb or muscle group
	2. paresis of multiple limbs or muscle groups
	3. paralysis of single limb or muscle group
	4. paralysis of two limbs
	5. paralysis of three or more limbs

B. Adapted version of Dick and Massey severity score (total possible score is 24).

The following items are scored, giving 0 points for absence, 1 point for mild presence and 2 points for severe presence. This score is added to the Dick and Massey score as calculated in panel A.

Deep boring limb/abdominal pain, headache, vertigo, dyspnea, skinbends, visual disturbances, general malaise.

C. Blatteau severity score (total possible score is 22).

Age ≥ 42	no = 0		yes = 1
Back pain	no = 0		yes = 1
Clinical course before recompression	better = 0	stable = 3	worse = 5
Objective sensory deficit	no = 0		yes = 4
Motor impairment	no = 0	paresis = 4	paralysis = 5
Bladder dysfunction	no = 0		yes = 6

Table 1. Severity scores used in the study. A = Dick and Massey severity score. (12) B = adapted version of Dick and Massey severity score. C = Blatteau severity score (13).

Additional treatment tables (US Navy Treatment Table 5 or 6, HBOT at 1.9 atmospheres absolute (190 kPa) for 180 min or HBOT at 1.5 atmospheres absolute (150 kPa) for 90 min, at the diving medical officer's discretion) are prescribed when residual symptoms are present. 24 h intervals are maintained between HBOT sessions. Administration of additional HBOT sessions is stopped when no further improvement is observed or the patient reports symptoms of pulmonary oxygen toxicity. Adjuvant treatment with intravenous lidocaine (implemented in June 2001) consists of an initial bolus of 100 mg at the start of the first HBOT session followed by

continuous administration of 3 mg/min during 8 h.

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL). Differences for nominal variables between control and lidocaine groups were tested using Fisher's exact test for 2x2 tables and Chi-Square test (without continuity correction) for 2x3 tables. Chi-Square test for trend was used for the ordinal variables (DM score, adapted DM score and Blatteau score). The Mann-Whitney U test was used for scale variables since the values of these variables were not normally distributed (tested using Shapiro-Wilk test). Relative risk was calculated using the Mantel-Haenszel method. All tests were performed two-sided and statistical significance was accepted at $p<0.05$.

		control	lidocaine	p value
sex	male	81%	71%	0.685
	female	19%	29%	
age (y)		36 (9.2)	36 (6.8)	0.946
body mass index (kg/m^2)		24 (3.4)	24 (2.8)	0.752
maximum diving depth (m)		24 (12)	30 (15)	0.224
diving time (min)		40 (14)	35 (13)	0.252
breathing gas	air	76%	79%	0.113
	nitrox	24%	7%	
	trimix	0%	14%	
repetition dive		43%	57%	0.500
unsafe dive		62%	14%	0.007[a]
time until start of symptoms (h)		3.7 (7.0)	6.5 (11)	0.906
time until HBOT (h)		16 (12)	22 (17)	0.418
clinical course until HBOT	better	24%	21%	0.985
	stable	48%	50%	
	worse	29%	29%	
diagnosis	DCS	86%	79%	0.664
	CAGE	14%	21%	

Table 2. General and diving parameters. Values between parentheses are standard deviations. Percentages may not add up to 100% due to rounding errors. [a] = $p<0.05$; nitrox = breathing gas containing oxygen and nitrogen, in which the oxygen content is larger than in air; trimix = breathing gas containing oxygen, nitrogen, and helium.

Results

A total number of 140 patients was treated with HBOT in the investigated period. From this total, 37 patients met our inclusion criteria. 2 of these patients were excluded because they were comatose on arrival. The total patient group consisted of 21 patients who were treated between 1996 and 2001 and did not receive lidocaine and 14 patients who were treated between 2001 and 2011 and did receive lidocaine. General parameters of the patients are displayed in table 2.Groups were comparable with regard to gender, age, body mass index, diving depth, diving time, breathing gas, percentage repetitive dives, time until start of symptoms, time until HBOT, clinical course until start of HBOT and percentage of DCS and CAGE. Significantly more patients in the lidocaine group made an unsafe dive (62% vs 14%, p=0.007).

With respect to initial injury severity (table 3), both groups had comparable DM, adapted DM and Blatteau scores. The differences between groups in regard to percentage of patients with objective neurological signs on admission (38% in the control group, 64% in the lidocaine group) was not statistically significant (p=0.176). The number of treatment sessions given was similar in both groups. Treatment reduced DM score from 2.0 ± 1.6 to 0.1 ± 0.5 in the control group and from 2.7 ± 1.7 to 1.4 ± 3.0 in the lidocaine group, differences between groups were not statistically significant. Percentage of patients with objective neurological signs at the end of the last HBOT session was 5% in the control group and 14% in the lidocaine group. DM score, adapted DM score and percentage of patients with objective neurological signs were not significantly different between groups at the end of the last HBOT session. The relative risk for unwanted outcome (objective neurological signs) when receiving lidocaine, corrected for objective neurological signs before first therapy, was 1.8 (95% confidence interval 0.2-16).

	before first HBOT session			after last HBOT session		
	control	lidocaine	p value	control	lidocaine	p value
number of treatment tables	n/a	n/a	n/a	2.0 (1.0)	2.7 (2.3)	0.537
Dick and Massey score	2.0 (1.6)	2.7 (1.7)	0.221	0.1 (0.5)	1.4 (3.0)	0.074
adapted Dick and Massey score	4.2 (2.6)	4.1 (2.6)	0.914	0.5 (1.0)	1.4 (3.0)	0.177
Blatteau score	5.4 (2.8)	7.4 (5.2)	0.152	n/a	n/a	n/a
objective neurological signs	38%	64%	0.176	5%	14%	0.551

Table 3. Treatment and injury severity before and after HBOT. Values between parentheses are standard deviations.

Discussion

In this small retrospective cohort study, we were not able to demonstrate a positive effect of intravenous lidocaine versus no lidocaine on outcome in patients with neurological DCI.

The use of lidocaine in DCI has been the subject of study for decades. Since the first report of a positive effect of this substance in preventing neurological injury in CAGE induced in cats (17), multiple animal and human studies have been performed on this matter. Lidocaine is a sodium channel blocker, which accounts for several of its neuroprotective effects, as reviewed by Mitchell (18, 19). Briefly, in the first place lidocaine is an anesthetic drug that depresses neuronal metabolism when given intravenously, rendering the brain less vulnerable when it is deprived of oxygen and furthermore lowering intracranial pressure. Secondly, lidocaine stabilizes the neuronal membrane, protecting the cell against damage in the case of ischemia. In the third place, its antiarrhythmic effect attenuates the cardiac arrhythmias that often occur in DCI and contribute to adverse outcome. Furthermore, apart from the effects due to sodium channel blocking, lidocaine has anti-inflammatory properties (20), which attenuate the inflammatory response associated with endothelial damage

as occurs in DCI. Several animal studies on lidocaine in CAGE confirmed the positive results of the first investigation, not only when lidocaine was given as pretreatment, but also when given after induction of CAGE (4, 21-23). Animal studies on lidocaine in DCS were less unequivocal, with one study showing a positive effect (3) and other studies being unable to demonstrate better outcome (9, 24). Human studies on lidocaine in DCI are very scarce and limited to a few case reports (25-28) and a small retrospective study, of which unfortunately only an abstract has been published (29). The most interesting data, however, come from four human studies on the use of intravenous lidocaine in cardiac surgery. Patients undergoing heart surgery are known to be at risk for postoperative neurocognitive decline, especially in open chamber surgery, and cerebral air embolization has been suggested as an important contributing factor (30). Therefore, cardiac surgery may have similarities to diving-related CAGE. The first two studies, published in 1999 and 2002, showed a positive effect of lidocaine on postoperative neurocognitive decline in patients undergoing open chamber surgery patients (5) and coronary artery bypass grafting with cardiopulmonary bypass (6). The two other studies, both published in 2009, included mixed groups of patients undergoing open chamber surgery or coronary artery bypass grafting. These studies failed to demonstrate a positive effect (7, 8). In fact, in one of these studies total lidocaine dose was an independent predictor of cognitive decline. All in all, based on these animal and human studies, lidocaine can be regarded as an interesting substance in DCI, but a positive effect has of yet not been proven. The only human studies showing beneficial effects have been performed in cardiac surgical cases, which may have similarities with CAGE but certainly not with other forms of DCI.

For the current study we included all patients who received lidocaine for neurological DCI and compared these patients to an historical cohort. The control group was too small to perform a matched analysis, but the two groups were nevertheless comparable in regard to most confounding factors. Significantly more patients in the control group had made a dive that did not comply with decompression tables and guidelines, and can

therefore be said to have suffered an 'explainable' injury. This was however not reflected in increased disease severity since none of the injury scores showed statistically significant differences between groups. There was a trend toward increased risk of unwanted outcome in the lidocaine group (relative risk 1.8), even after correction for the larger percentage of objective neurological signs before start of therapy in the lidocaine group, but the large confidence interval (0.2-16) precludes any definitive statements. We must therefore conclude that we observed neither a positive nor a negative effect of lidocaine in our study.

Our study is of course limited by its small sample size. Nevertheless, this patient population in our opinion represents the daily practice of the diving physician who faces relatively small numbers of patients with heterogeneous presentations. The heterogeneity is reflected in our study by the varying time until start of symptoms and time until start of HBOT (although we limited our study group to patients receiving HBOT within 72 after symptom onset). Furthermore, we included all diseases that were eligible for adjuvant treatment with lidocaine, being spinal DCS, cerebral DCS, vestibular DCS (together termed neurological DCS) as well as CAGE. One might argue to analyze these categories separately, in order to determine if lidocaine would have a beneficial effect in any of these subgroups. The small size of our population, however, precluded any meaningful subgroup analysis. Furthermore, it is not always possible to reliably distinguish the various forms of neurological DCI and a patient may suffer from various types at the same time.

Symptom severity in our patients was relatively low on average, with DM scores of 2.0±1.6 and 2.7±1.7 (maximum possible score 10) in control and lidocaine group, respectively. We cannot rule out the possibility that more severely affected patients would have benefited from lidocaine, but in our study subgroup analysis was not possible due to the small sample size. Furthermore, HBOT was very effective in our control patients, leaving little room for further improvement due to lidocaine. On the other hand, a positive effect of lidocaine could also have been detectable as a lower number

of HBOT sessions needed in the lidocaine group, which was not the case.

Our study suffers from possible bias since the control and lidocaine patients were not treated in the same period. The control patients were seen from 1996 to 2001, and the lidocaine patients from 2001 to 2011. Therefore, although except for the addition of lidocaine we are not aware of any differences in treatment between the groups, we cannot exclude the effect of time as a confounding factors.

The lidocaine dose used in our patients was in line with the doses used in previous investigations and the advice given by the Undersea & Hyperbaric Medical Society (31). Although we did not obtain plasma levels of lidocaine in our patients, similar infusion strategies used in other studies resulted in lidocaine levels within the desired range (5-8). We infused lidocaine during 8 h, starting at the beginning of the HBOT treatment. The duration of lidocaine administration varies between the four published human studies. Two studies used a 48 h lidocaine infusion (5, 8), one study used 12 h (7) and one study administered lidocaine intraoperatively, without mentioning the exact duration of the infusion (6). There is currently no data that supports any specific duration of lidocaine infusion.

The question remains if there is any future for the use of lidocaine in the treatment of neurological DCI. Our study is presently the most comprehensive investigation available, since larger and/or prospective studies are lacking. Although our study was underpowered to draw definitive conclusions, we have demonstrated that despite data collection over a period of 15 years in a relatively large hyperbaric center, we were not able to demonstrate a positive effect of lidocaine on neurological outcome in DCI. This is mainly caused by the small number of patients and the heterogeneity of the patient population. The current Undersea & Hyperbaric Medical Society best practice guidelines on DCS and AGE discourage the use of lidocaine in DCS and are impartial on its use in AGE, only giving advice on lidocaine dose for those cases in which the physician chooses to use it (31). The data on which these recommendations are based, as

summarized in the present article, are weak and a prospective study on lidocaine in diving accidents is still lacking. We believe the most rational strategies would be to either abandon the use of lidocaine in neurological DCI altogether, or to perform a prospective study. Given the low prevalence of neurological DCI, the heterogeneous population and the fact that DCS and CAGE should be studied separately, this would call for a large multicenter investigation.

References

1. Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. Lancet 2011; 377:153-64.
2. van Hulst RA, Klein J, Lachmann B. Gas embolism: pathophysiology and treatment. Clin Physiol Funct Imaging 2003; 23:237-46.
3. Lyle CB, Jr., Dahl EV. Protection of rapidly decompressed rats by pharmacologic and physical means. Am J Physiol 1961; 201:759-61.
4. Dutka AJ, Mink R, McDermott JJ, Clark JB, Hallenbeck JM. Effect of lidocaine on somatosensory
5. Mitchell SJ, Pellett O, Gorman DF. Cerebral protection by lidocaine during cardiac operations. Ann Thorac Surg 1999; 67:1117-24.
6. Wang D, Wu X, Li J, Xiao F, Liu X, Meng M. The effect of lidocaine on early postoperative cognitive dysfunction after coronary artery bypass surgery. Anesth Analg 2002; 95:1134-41.
7. Mitchell SJ, Merry AF, Frampton C, Davies E, Grieve D, Mills BP et al. Cerebral protection by lidocaine during cardiac operations: a follow-up study. Ann Thorac Surg 2009; 87:820-5.
8. Mathew JP, Mackensen GB, Phillips-Bute B, Grocott HP, Glower DD, Laskowitz DT et al. Randomized, double-blinded, placebo controlled study of neuroprotection with lidocaine in cardiac surgery. Stroke 2009; 40:880-7.
9. Montcalm-Smith EA, Fahlman A, Kayar SR. Pharmacological interventions to decompression sickness in rats: comparison of five agents. Aviat Space Environ Med 2008; 79:7-13.
10. Nishi RY. Revised metric decompression tables for air and surface supplied helium-oxygen diving. Toronto, Canada: DRDC, 2009.
11. Defence and Civil Institute of Environmental Medicine: DCIEM diving manual: air decompression procedures and tables. Richmond, Canada: Univeral Dive Techtronics, 1992.
12. Dick AP, Massey EW. Neurologic presentation of decompression sickness and air embolism in sport divers. Neurology 1985; 35:667-71.
13. Blatteau JE, Gempp E, Simon O, Coulange M, Delafosse B, Souday V et al. Prognostic factors of spinal cord decompression sickness in

recreational diving: retrospective and multicentric analysis of 279 cases. Neurocrit Care 2011; 15:120-7.
14. Boussuges A, Thirion X, Blanc P, Molenat F, Sainty JM. Neurologic decompression illness: a gravity score. Undersea Hyperb Med 1996; 23:151-5.
15. U.S. Department of the Navy, Naval Sea Systems Command: U.S. Navy Diving Manual. Volume 5: Diving Medicine & Recompression Chamber Operations. Washington, D.C.: Naval Sea Systems Command, 2008.
16. U.S. Department of the Navy, Naval Sea Systems Command: U.S. Navy Diving Manual. Volume 1: Diving Principles and Policies. Washington, D.C.: Naval Sea Systems Command, 2008.
17. Evans DE, Kobrine AI, LeGrys DC, Bradley ME. Protective effect of lidocaine in acute cerebral ischemia induced by air embolism. J Neurosurg 1984; 60:257-63.
18. Mitchell SJ. Lidocaine in the treatment of decompression illness: a review of the literature. Undersea Hyperb Med 2001; 28:165-74.
19. Mitchell SJ, Merry AF. Lignocaine: neuro-protective or wishful thinking? J Extra Corpor Technol 2009; 41:37-42.
20. Hollmann MW, Durieux ME. Local anesthetics and the inflammatory response: a new therapeutic indication? Anesthesiology 2000; 93:858-75.
21. Evans DE, Kobrine AI. Reduction of experimental intracranial hypertension by lidocaine. Neurosurgery 1987; 20:542-7.
22. Evans DE, Catron PW, McDermott JJ, Thomas LB, Kobrine AI, Flynn ET. Effect of lidocaine after experimental cerebral ischemia induced by air embolism. J Neurosurg 1989; 70:97-102.
23. McDermott JJ, Dutka AJ, Evans DE, Flynn ET. Treatment of experimental cerebral air embolism with lidocaine and hyperbaric oxygen. Undersea Biomed Res 1990; 17:525-34.
24. Broome JR, Dick EJ, Jr. Neurological decompression illness in swine. Aviat Space Environ Med 1996; 67:207-13.
25. Drewry A, Gorman DF. Lidocaine as an adjunct to hyperbaric therapy in decompression illness: a case report. Undersea Biomed Res 1992; 19:187-90.

26. Cogar WB. Intravenous lidocaine as adjunctive therapy in the treatment of decompression illness. Ann Emerg Med 1997; 29:284-6.
27. Mitchell SJ, Benson M, Vadlamudi L, Miller P. Cerebral arterial gas embolism by helium: an unusual case successfully treated with hyperbaric oxygen and lidocaine. Ann Emerg Med 2000; 35:300-3.
28. Weisher DD. Resolution of neurological DCI after long treatment delays. Undersea Hyperb Med 2008; 35:159-61.
29. Mutzbauer TS, Ermisch J, Tetzlaff K, Frey G, Lampl LA. Low dose lidocaine as adjunct for treatment of decompression illness (DCI) (abstract). Undersea Hyperb Med 1999; 26(suppl):A20.
30. Carrascal Y, Guerrero AL. Neurological damage related to cardiac surgery: pathophysiology, diagnostic tools and prevention strategies. Using actual knowledge for planning the future. Neurologist 2010; 16:152-64.
31. Undersea Hyperbaric Medical Society. UHMS best practice guidelines. Prevention and treatment of decompression sickness and arterial gas embolism. Retreived 1 September 2012 from http://c.ymcdn.com/sites/membership.uhms.org/resource/resmgr/dcsandage_prevandmgt_uhms-fi.pdf.

Acute neurological symptoms during hypobaric exposure: consider cerebral air embolism

8

Weenink RP, Hollmann MW, van Hulst RA
Aviat Space Environ Med 2012; 83:1084-91

Abstract

Cerebral arterial gas embolism (CAGE) is well known as a complication of invasive medical procedures and as a risk in diving and submarine escape. In the underwater environment, CAGE is caused by trapped air, which expands and leads to lung vessel rupture when ambient pressure decreases during ascent. Pressure decrease also occurs during hypobaric activities such as flying and, therefore, CAGE may theoretically be a risk in hypobaric exposure. We reviewed the available literature on this subject. Identified were 12 cases of CAGE due to hypobaric exposure. Based on these cases, we discuss pathophysiology, diagnosis, and treatment of CAGE due to hypobaric exposure. The low and slow pressure decrease during most hypobaric activities (as opposed to diving) account for the low incidence of CAGE during these exposures and suggest that severe air trapping must be present to cause barotrauma. This is also suggested by the large prevalence of air filled cysts in the case reports reviewed. We recommend considering CAGE in all patients presenting with acute central neurological injury during or shortly after pressure decrease such as flying. A CT scan of head and chest should be performed in these patients. Treatment with hyperbaric oxygen therapy should be initiated as soon as possible in cases of proven or probable CAGE.

Introduction

The introduction of air into the cerebral arteries (cerebral arterial gas embolism, CAGE) has been regularly described as a complication of invasive medical procedures. CAGE is a serious disorder not only due to the ischemic injury to the brain caused by lodging of air bubbles, but also because of the damage the air bubbles inflict on the blood-brain barrier. CAGE can cause slight transient neurological symptoms, but many patients experience more severe neurological dysfunction and often suffer permanent deficits (1).

Apart from iatrogenic introduction of air, CAGE is a well-known complication in diving and submarine escape. In these cases, air introduction is thought to result from pulmonary barotrauma due to increasing gas volume as surrounding pressure decreases during ascent. In the presence of pulmonary disease with air trapping, but also in healthy lungs when the subject fails to exhale, this increased pressure leads to lung rupture, with ensuing entry of air into the pulmonary venous system and thence via the heart to the brain.

Every day, millions of people are subjected to pressure change due to activities other than diving, for instance when flying or mountaineering. Although

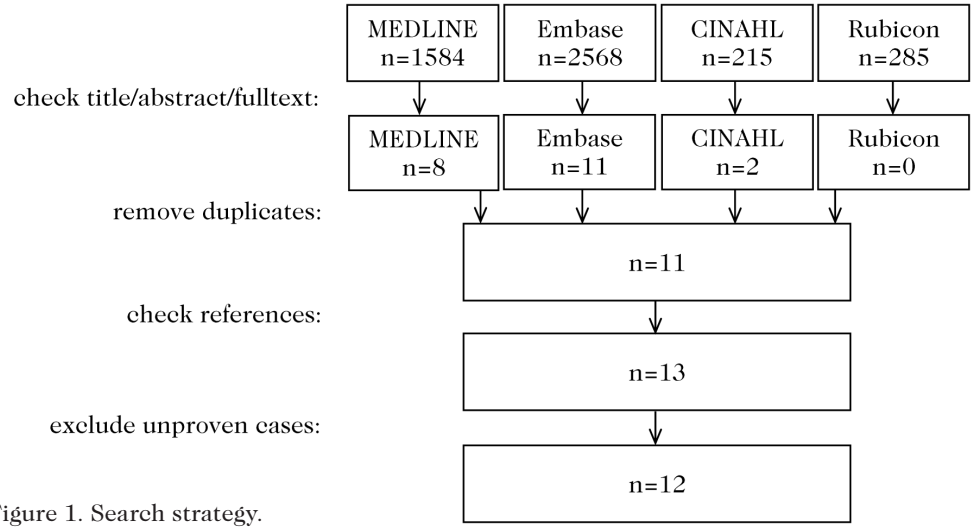


Figure 1. Search strategy.

the amount and speed of pressure change involved in these activities is usually much less than in the underwater environment, the large quantity of persons involved suggests that there should be some incidence of CAGE due to these activities. In this review, we summarize the literature available on CAGE due to hypobaric activities. We investigate the published cases and make recommendations on diagnosis and therapy. This review does not focus on decompression sickness (DCS) in the hypobaric environment. Reviews are available on this topic (2).

Case	Ref	Sex / Age (y)	Medical history	Activity	Onset of symptoms
1	(3)	F 19	healthy	flying	10-15 min after take-off
2	(4)	M 45	healthy except smoker	chamber	1 min after descent to 25000 ft after ascent to 30000 ft at 3000 ft/min
3	(5)	F 40	healthy	flying	10 min after take-off
4	(6)	M 43	previous aeromedical examination normal	chamber	at 18000 ft during ascent to 25000 ft
5	(7)	M 43	asthma, smoker	flying	20 min after take-off
6	(8)	M 71	multiple[a]	flying	shortly after take-off
7	(9)	F 62	not mentioned	flying	20 min after take-off
8	(10)	M 17	not mentioned except non-smoker	flying	15 min after take-off
9	(11)	M 68	1x LOC during flight, pulmonary cyst on CT[b]	flying	30 min after take-off
10	(12)	M 60-69	not mentioned	mountaineering	during ascent from 3392 to 11332 ft at 84 ft/min
11	(13)	F 62	healthy	flying	30 min after take-off
12	(14)	F 52	2x seizure during flight	flying	during flight

Table 1. Age, gender, history, hypobaric activity and onset of symptoms in the 12 relevant cases. [a] anticardiolipin syndrome, deep vein thrombosis, Sneddon syndrome, coronary artery bypass graft, pacemaker for atrial fibrillation; [b] the patient had experienced loss of consciousness (LOC) during a previous flight, which most likely resulted from cardiogenic emboli from a mural thrombus.

Methods

We performed a literature search to identify all articles regarding CAGE due to hypobaric exposure (figure 1). Our MEDLINE search strategy was ("embolism, air"[mesh] AND ("brain"[mesh] OR "arteries"[mesh])) OR ("embolism"[tiab] AND ("brain"[tiab] OR "cerebrum"[tiab] OR "cerebral"[tiab] OR "arterial"[tiab]) AND ("air"[tiab] OR "gas"[tiab])) and adapted versions of this strategy were used for the Embase, CINAHL and Rubicon. The search was performed in July 2011. Search results were limited to articles in English with no constraints made on publication date. All search results were checked manually and irrelevant articles were excluded. Articles that were only available as meeting abstracts were excluded. References of included articles were checked to obtain additional articles. In total, we included 13 papers, which were all case reports, describing a total of 13 patients. We critically evaluated these cases and classified them as proven, probable, possible or unproven CAGE according to our criteria, which will be discussed in detail below:

1. Clinical suspicion: objective neurological signs occurring during or shortly after pressure decrease.
2. Brain findings: cerebral ischemia on imaging/autopsy.
3. Thoracic findings: thoracic abnormalities consistent with air trapping or pulmonary barotrauma on imaging/autopsy.
4. Signs of other air emboli than CAGE, e.g. livedo reticularis, air in the retinal arteries, myocardial ischemia.

Where:

Proven CAGE = air in the cerebral arteries;
Probable CAGE = [1] and at least two of [2], [3], or [4];
Possible CAGE = [1] and one of [2], [3], or [4].

Table 1-4 list the characteristics of all 12 cases that were classified as proven, probable or possible CAGE. The reason for rejection in the case

Case	Chest symptoms	Neurological signs (objective)
1	none	GCS 4-5, myopic unreactive pupils, extensor posturing L
2	none	dysarthria, L hemiparalysis arm/leg/face, L hemisensory deficit
3	chest pain, dyspnea	comatose, seizures, absent brain stem and deep tendon reflexes, bilateral Babinski
4	discomfort, dyspnea	mild disorientation and R homonymous hemianopia
5	dyspnea	unconscious, then GCS 7, L hemiparesis (upper motor neuron signs), decorticate posturing R
6	discomfort R	GCS 3
7	none	GCS 3, clonus L lower leg
8	none	temporary unconscious, on admission bulbar palsy and L hemiparesis
9	none	unconscious, bilateral extensor posturing
10	none	GCS 3, pinpoint pupils
11	none	CGS 5-6, absent gag and deep tendon reflexes, extension L, Babinski L
12	dyspnea	seizure, GCS 5

Table 2. Chest symptoms and neurological signs. GCS = Glasgow Coma Scale.

that was classified as unproven was that no brain or chest imaging was performed and, therefore, no proof of CAGE was present except the clinical presentation of the patient (15). In the following paragraphs we will discuss the considerations regarding pathophysiology, diagnosis, and treatment of CAGE due to hypobaric exposure, based on the findings in the 12 patients in which we deemed CAGE was proven, probable or possible.

Case reports

Of the 12 cases, nine occurred during flight, two occurred during hypobaric chamber training, and one patient experienced symptoms during mountaineering (table 1). All in-flight cases occurred on commercial airplanes and none of the articles report any technical malfunction that may

have resulted in increased or rapid decompression. All of these patients first reported symptoms shortly after takeoff (10-30 min) except in one article which only states that symptoms started 'during flight'. One of the hypobaric chamber related cases occurred after ascent to 30000 ft (9144 m) at 3000 ft/min (914.4 m/min) and subsequent descent to 25000 ft (7620 m), symptoms started after 1 min at 25000 ft. The other hypobaric chamber case occurred during ascent at 1400 ft/min (426.7 m/min) at an altitude of 18000 ft (5486 m). In the mountaineering case the patient was on his way from 3392 ft (1034 m) to 11332 ft (3454 m) in a cable cart at 84 ft/min (25.6 m/min), the altitude at which symptoms started is not mentioned. The pressure difference to which the patients were subjected due to their ascent can, in most cases, only be estimated. In the in-flight cases this estimation is based on the fact that cabin altitude in many modern commercial airplanes is maintained at 8000 ft (2438 m) (16) and the assumption that this cabin altitude is reached after about 20 min of climbing. Thus, the estimated pressure decrease before start of symptoms ranged from 13 to 63 kPa.

Seven patients did not report any chest symptoms, while the other five patients reported chest pain or discomfort and/or dyspnea (table 2). Neurological symptoms were variable, so we report only the objective neurological signs. Of the 12 patients, 11 had at least temporarily decreased consciousness, six of whom were comatose (Glasgow Coma Scale < 8). Two patients had generalized seizures. In four patients cranial nerve abnormalities are reported. In nine patients some abnormality of the neurological examination of the extremities was present. Of note, in six of these patients the defects were strictly left sided, mostly consisting of hemiparesis and hemisensory deficit, and purely right sided abnormalities are not reported.

In all patients some form of cerebral imaging was performed, consisting of CT scan in 11 cases and MRI scan in five cases (table 3). Imaging showed abnormalities in nine patients, of whom six showed air bubbles with or without cerebral ischemia and the other patients had cerebral ischemia

Case	Brain evidence	Thoracic evidence	Other air emboli	Class
1	yes (CT: bilateral edema)	yes (X/postmortem: bulla)	no	probable
2	yes (MRI: ischemia R)	yes (CT: blebs)	no	probable
3	yes (CT/autopsy: bilateral multifocal ischemia)	yes (CT/autopsy: BC)	yes (myocardial ischemia, livedo reticularis)	probable
4	no (CT after 24h normal)	yes (CT/resection: BC)	no	possible
5	no (CT/EEG normal)	yes (CT: bulla)	yes (myocardial ischemia)	probable
6	yes (CT: bilateral air)	yes (CT: BC)	yes (myocardial ischemia)	proven
7	yes (CT: air/edema; MRI: multifocal ischemia)	yes (CT: CCAM)	yes (myocardial ischemia)	proven
8	no (CT/MRI/EEG normal)	yes (CT: CCAM)	yes (myocardial ischemia)	probable
9	yes (CT: air; MRI: multifocal ischemia R>L)	yes (CT: BC)	no	proven
10	yes (CT: air R)	yes (CT: BC, pneumomediastinum, pneumopericardium)	yes (myocardial ischemia)	proven
11	yes (CT: air; MRI: multifocal ischemia R>L)	yes (CT: cystic mass)	no	proven
12	yes (CT: bilateral air)	yes (CT: CB)	yes (myocardial ischemia)	proven

Table 3. Brain and thoracic findings, other signs of extrapulmonary air and classification. X = chest X-ray; BC = bronchogenic cyst; CCAM = congenital cystic adenomatoid malformation.

and/or edema without visible air. Chest imaging was performed using CT in all patients, except in one in whom only chest radiography was performed. All patients had thoracic lesions, namely bronchogenic cyst (six patients), congenital cystic adenomatoid malformation (two cases), unspecified cystic mass (one patient) and bullae and/or blebs (three cases).

One patient had signs of pulmonary barotrauma, namely pneumomediastinum and pneumopericardium. Seven patients had concurrent myocardial infarction which was thought to result from coronary air embolism. One of these patients also had livedo reticularis of the hands and upper thorax as a sign of systemic air embolization.

Five patients were treated with hyperbaric oxygen therapy (HBOT), but complete data regarding the treatment (delay until start, types, and amounts of sessions) are available in only two cases (table 4). The reason for not instituting HBOT was given in four of the seven cases, being that the patient was too ill (two patients), that too much delay had already occurred (one patient) and that the patient had spontaneously recovered (one patient). Six patients died, four patients recovered completely, one patient had severe permanent deficits, and one patient initially recovered but then died of pulmonary embolism.

Case	Hyperbaric oxygen therapy	Outcome
1	no (no reason)	death
2	yes (delay 2.5 h, 3 sessions (1x extended table 6A, 2x 90 min at 2 ATA))	complete recovery
3	no (too ill)	death
4	yes (delay unknown, 4 sessions)	complete recovery
5	yes (delay 48h, 2 sessions (8h HeO2 table starting at 6 ATA))	complete recovery
6	no (too much delay (48-72h))	death
7	no (no reason)	severe disability
8	no (spontaneous improvement at 72h)	complete recovery
9	yes (delay unknown, 1 session)	death
10	no (no reason)	death
11	no (too ill)	moderate recovery, then death (pulmonary embolism)
12	yes (delay >2 h, 1 session?)	death

Table 4. Treatment and outcome. ATA = atmospheres absolute.

Pathophysiology

A number of reviews have addressed the pathophysiology of CAGE (1, 17, 18). We will give a brief summary of this subject with special interest in the differences between hyperbaric and hypobaric origin of CAGE. Noniatrogenic CAGE is best known in diving medicine. In these patients, the etiology of air introduction is pulmonary barotrauma with subsequent flow of air into the pulmonary venous vasculature and thence to the systemic arterial circulation. In order for air to flow from the lungs into the vessels, some degree of air trapping in conjunction with decrease of ambient pressure must be present. Under these circumstances, the trapped air will expand as dictated by Boyle's law, which states that pressure and volume are inversely proportional (19). This expanded air may cause volume increase of the space in which the air is contained, causing stretching of the wall of the structure, which may lead to vessel rupture. Apart from CAGE, escaping air may also cause pneumothorax, pneumomediastinum, and subcutaneous emphysema. While some patients with CAGE may concurrently have one or more of these other signs of lung damage, in many patients CAGE is the sole manifestation of pulmonary barotrauma.

It is important to realize that pulmonary barotrauma can occur even when the trapping of air is not complete (i.e., there is some degree of communication between the trapped air and the rest of the bronchial tree) as long as there is relative air trapping, meaning the escape of air from the contained space cannot keep up with the ambient pressure decrease. CAGE can even occur in healthy persons without thoracic abnormalities when ambient pressure decreases while the subject fails to exhale, due to active breath-holding or, for instance, laryngospasm (20).

The above-mentioned etiology of noniatrogenic CAGE explains why this disease is so much better known in diving medicine than in other types of dysbaric activities such as flying. Arterial gas embolism was the cause of 3% of diving fatalities and 33% of diving accidents resulting

in disabling injury in the 2008 Diver's Alert Network report (21), while our current search on hypobaric CAGE yielded only 12 published cases throughout the literature. There are numerous differences between the underwater and the hypobaric situation that explain the higher incidence in the underwater environment (22). First of all, the amount of pressure difference involved in underwater activities is much higher than during flying. In commercial airliners cabin altitude is required by law to be maintained at a maximum of 8000 ft (2438 m) under normal operating conditions, which corresponds to a pressure of approximately 75 kPa or about 26 kPa difference compared to ground level pressure. During diving, this pressure difference is reached by descending only approximately 10 ft (≈3 m). Nevertheless, the pressure decrease in most hypobaric activities is theoretically large enough to cause barotrauma, given the fact that Malhotra and Wright observed pulmonary damage at transpulmonary pressures of 9.7 kPa (23). This pressure decrease is achieved by ascending to as little as 2700 ft (823 m). In all of the cases described in this review the pressure difference to which the patients were subjected was larger than 9.7 kPa.

The second reason for the low incidence of CAGE due to hypobaric activities is the lower speed of pressure decrease involved when compared to underwater activities. When decompression occurs at a higher rate, even air collections that partly communicate with the bronchial tree may cause pulmonary barotrauma since the pressure decrease occurs so rapidly that the escape of air cannot keep up with its expansion. The slow rate of decompression in, for example, flying may require more profound air trapping in order for barotrauma to occur. Furthermore, in divers and submarine escape trainees, active breath-holding or laryngospasm due to panicking are known to occur, which renders the total lung volume a closed compartment (20) Breath-holding will not be an issue in the hypobaric environment due to the slow pressure decrease. An exception may be rapid decompression, either when trained in a hypobaric chamber or when accidentally sustained during flight. There are cases of suspected CAGE due to rapid decompression during

pressurization of aircraft cabins on the ground (24, 25) and one article describes suspected CAGE after rapid decompression in a hypobaric chamber (15). The articles on decompression on the ground were not included in this review because they did not arise from primarily hypobaric activities and the case of CAGE in a hypobaric chamber (15) was excluded as discussed above.

A third factor that may account for the higher incidence of diving-related CAGE is the fact that immersion causes increased small airway closure and might thus potentiate air trapping. The mechanism responsible is redistribution of venous blood leading to pooling of blood in the lungs due to the smaller effect of gravitation and the usually horizontal position of the diver (26).

In the fourth place, clinical awareness of CAGE is much higher in the diving community than it is in the general population. In any diver who surfaces with neurological symptoms, CAGE will be high in the differential diagnosis. Recent studies show that neurological incidents are responsible for 22-24% of all in-flight medical emergencies requiring telemedical assistance (27, 28). It is possible that some of these patients suffered CAGE but were not diagnosed as such.

The abovementioned arguments suggest that more severe air trapping may be required for a person to sustain hypobaric CAGE than is necessary to develop CAGE in the underwater situation. While Calder reported slight pulmonary abnormalities in many autopsies of divers in whom the suspected cause of death was pulmonary barotrauma (29), the currently reported cases all showed gross thoracic abnormalities consistent with air trapping, for instance bronchogenic cysts. This is not due to our inclusion criteria since taking the requirement for thoracic abnormalities out of our algorithm did not result in more included articles. However, results may be biased due to the fact that clinicians will be more likely to establish a diagnosis of CAGE (and publish the case) if there is an abnormality that can explain the air entrance (sampling bias).

Diagnosis

We consider the patient who presents with acute central nervous system dysfunction. The recognition of CAGE is primarily dependent on clinical suspicion, so it is important for the clinician to elucidate the circumstances in which the complaints occurred. A history of pressure decrease in close temporal relationship with the start of symptoms should trigger the clinician to include CAGE in the differential diagnosis. In these cases, there are mainly three possible diagnoses to consider, as pointed out by Rios-Tejada et al. (4). These are CAGE, neurological DCS, and some other sort of cerebral insult, e.g., transient ischemic attack or cerebrovascular hemorrhage. Distinguishing these diagnoses from each other primarily relies on patient history since in most cases direct evidence pathognomonic for one of the entities will not be present.

A history of risk factors such as hypertension and diabetes may suggest a thrombo-embolic origin, while the presence of pulmonary disease or chest symptoms such as pain or dyspnea may point in the direction of CAGE. During normal commercial flight the risk of DCS is very low, because of the relatively low altitude at which the cabin is maintained. The diagnosis becomes more likely when the patients has had hyperbaric exposure (for instance diving) within 24 h before the flight or when the aircraft has suffered technical malfunctions which lead to higher cabin altitude. There is a known risk of DCS in subjects involved in hypobaric chamber training (30). The time course of the symptoms is also of importance. In CAGE there is usually a close temporal relationship between pressure decrease and start of symptoms, although this may be less evident in hypobaric situations than in diving because of the slower decompression. The onset of symptoms in DCS is usually more delayed (31).

Physical examination is not likely to be able to distinguish between the three diagnostic categories. The neurological examination in CAGE does not differ from that in other central stroke syndromes, as can be seen in the cases reviewed here. There seems to be a preponderance of right

hemispherical ischemia in CAGE, in the literature (32) as well as in the currently reviewed cases. This is hypothesized to be due to the fact that the brachiocephalic artery, which supplies the right hemisphere, is the first branch of the aortic arch and may, therefore, catch the largest amount of air (22). This criterion, however, cannot be used to reliably distinguish CAGE from the other causes of neurological injury. Neurological DCS generally involves the spinal cord, so the presence of spinal cord dysfunction is suggestive of DCS (31). Air emboli that are propelled from the heart can disperse to all of the systemic circulation, so signs of other locations of air embolization (e.g., myocardial infarction, air bubbles in the retinal arteries, or livedo reticularis) can support the diagnosis of CAGE. Signs of myocardial infarction (mostly ECG changes and elevated cardiac enzymes) were present in 7 of 12 cases presented here.

The presence of gas in the cerebral arteries on brain imaging proves CAGE or DCS (33). Of the patients reviewed here, 50% had intracerebral air bubbles on CT. This may again be partly due to sampling bias since a retrospective series on iatrogenic CAGE showed that most patients had no air on brain CT (34). The absence of air on brain imaging, therefore, does not rule out CAGE and DCS. Cerebral imaging is nevertheless important to rule out conditions such as cerebral hemorrhage. CT is the imaging modality of choice, since it can be performed quickly and provides the best way of visualizing cerebrovascular air (14). CT is inferior to MRI in demonstrating early cerebral ischemia, therefore MRI techniques such as diffusion weighted imaging may be necessary to demonstrate ischemia in the first hours (35). In a retrospective study, MRI abnormalities were present in six of eight patients with CAGE but in only two of eight patients with DCS, although it must be noted that all MRI scans were made at least hours after the incident (36). Recently, a case report demonstrated the possible utility of CT perfusion scanning in demonstrating early cerebral ischemia in CAGE (37).

Reviews of diving-related DCS and CAGE discourage the use of brain imaging because negative results do not rule out the diagnosis and the time

necessary to perform the scan delays start of treatment (18). We believe that in cases of suspected hypobaric CAGE cerebral imaging is warranted since the likelihood of other causes of neurological injury is much higher under these circumstances than in the case of a diver surfacing with neurological symptoms.

The likelihood of CAGE is increased when there is evidence of pulmonary air trapping since this provides an explanation for entrance of air into the vasculature. Air trapping can be present in a multitude of pulmonary diseases such as asthma, chronic bronchitis, and bullous and cystic lung diseases (38). However, as discussed above, the case reports reviewed here suggest that relative air trapping such as present in, for example, asthma may not be enough to cause hypobaric CAGE because of the slow pressure decrease involved in most hypobaric exposures. An air filled cyst was present in 9 of 12 cases reviewed here, suggesting that many patients with hypobaric CAGE will have such a cystic thoracic abnormality. Because the diagnosis of CAGE has important therapeutic implications, we advise to perform a chest CT in all patients suffering neurological injury after hypobaric exposure. The presence of an air-filled cyst is highly indicative of CAGE, especially if there is an air-fluid level suggesting recent hemorrhage. Furthermore, chest CT can demonstrate other manifestations of pulmonary barotrauma, such as pneumothorax, which strongly support the diagnosis of CAGE.

It is important to realize that the suggestions regarding diagnosis made in this article are based on the cases reviewed, which were selected based on a predefined algorithm for diagnosis of CAGE. This could result in a kind of spectrum bias, meaning that had we applied other criteria for establishing the diagnosis of CAGE after hypobaric exposure, we might have come to different conclusions. It is therefore important to critically consider our diagnostic criteria. There is no gold standard for establishing the diagnosis of CAGE, so all algorithms including ours are essentially arbitrary. We regard CAGE to be proven when air is seen in the cerebral arteries on brain imaging. Although this criterion may theoretically include cases of

neurological DCS, this is not problematic in practice since treatment for both disorders is the same. In cases where CAGE is suspected but no intravascular air can be seen on cerebral imaging, we require a combination of: 1) evidence of cerebral ischemia; 2) thoracic air trapping or evidence of pulmonary barotrauma; and 3) signs of other air emboli than CAGE. When at least two of these criteria are present, we regard CAGE to be probable, when only one of these criteria are present the patient is diagnosed with possible CAGE. We are aware that these criteria may lead to over- as well as underdiagnosis of CAGE. Patients who suffer, for instance, thromboembolic stroke while flying and who have concurrent pulmonary disease may be diagnosed with CAGE in this algorithm. Underdiagnosis may occur in cases of small thoracic abnormalities and low volumes of air, when brain and chest CT are both negative. However, this situation is not likely to occur since the small thoracic abnormalities that might be missed by CT are not very likely to cause CAGE in the hypobaric environment.

Treatment

Patients suspected of CAGE should receive high flow oxygen, initiated as soon as possible after the insult. High blood oxygen levels provide a favorable gradient for denitrogenation of the air bubbles and supply oxygen to critically perfused cerebral tissue. General hemodynamic supportive measures may be necessary, especially in cases of massive CAGE or concurrent coronary artery air embolism (1). HBOT is the only specific treatment for CAGE. It reduces cerebral injury by compressing the air bubbles in the cerebral arteries, which promotes passage of the bubbles through the capillaries to the venous circulation. Denitrogenation of the bubbles and oxygenation of brain tissue is significantly enhanced when compared to normobaric oxygen therapy (39). It must be stated that there is no level I evidence for the efficacy of HBOT in CAGE but retrospective studies and case reports have been convincing to such an extent that randomized trials will probably never be undertaken (40). Therefore, many questions regarding the optimal application of HBOT remain. One important unan-

swered question is the maximum delay that can be tolerated before HBOT should be initiated. While good recovery has been documented in patients after more than 24 h delay (41), earlier treatment is associated with better outcome (42). A retrospective study showed that patients treated within 7 h have a better outcome than patients treated after 7 h (43). In light of the significant delay that may occur before patients with suspected CAGE report to a hyperbaric facility, especially in case of in-flight neurological disorders, this small therapeutic window calls for a high clinical suspicion of CAGE and expeditious treatment of eligible patients.

We suggest that patients with proven or probable CAGE according to our criteria should be treated with HBOT according to US Navy Treatment Table 6 as soon as possible after the insult. The small therapeutic window may demand that ancillary investigations be limited to CT scanning of the cerebrum and chest, as discussed above. In case of possible CAGE, the decision whether or not to administer HBOT should be made based on factors such as severity of symptoms, delay from start of symptoms and availability of hyperbaric facilities. As can be seen in table 4 not all patients reviewed here were treated with HBOT. The reasons for not giving treatment were mentioned above. Because of the retrospective nature of this study, it is not possible to determine if HBOT was advantageous in the patients reviewed here.

All patients reviewed in this article had a form of pulmonary or airway disease that may have resulted in air trapping, thus providing a mechanism for air entry during hypobaric exposure. As discussed, this high incidence of pulmonary abnormalities may be biased due to the fact that the diagnosis of CAGE is more likely to be made if a patient suffering neurological injury during hypobaric exposure has a disease that provides an explanation for air entry. Nevertheless, discussion of the safety of hypobaric exposure in the presence of thoracic disease with possible air trapping is warranted. In accordance with guidelines on air travel published by the British Thoracic Society (44) and the Aerospace Medical Association (45), we believe that the large majority of patients with respiratory disease can

endure hypobaric exposure as long as they comply to the requirements set out in these guidelines. A review on travel to altitude in patients with lung disease summarized the literature on hypobaric exposure of subjects with bullous lung disorders and concluded that none of the patients suffered worsening pulmonary function or pneumothorax and that these patients can, therefore, safely travel to high altitude (46).

However, since the case reports reviewed in this article suggest that a substantial part of patients who suffered hypobaric CAGE may be diagnosed with bronchogenic cysts or other cystic abnormalities, the presence of such cysts may be less easily compatible with air travel. We believe that patients with cysts that have given rise to CAGE should be denied hypobaric exposure until their cyst has been removed. However, the large majority of patients with bronchogenic cysts are asymptomatic and most cysts are not filled with air (47). The guidelines of the British Thoracic Society recognize that completely encysted air spaces may expand due to ambient pressure decrease and cause CAGE, while cysts that communicate with the airways pose no such risk (44). The Aerospace Medical Association guidelines state that "the presence of lung cysts or bullae is usually not a problem as long as the airways communicate with the abnormal air collection" (45). While the question whether or not an air filled cyst communicates with the bronchial tree is an important one, unfortunately it is often not possible to assess the amount of communication with certainty (5). Furthermore, even cysts that do communicate with the bronchial tree may be hazardous because a 'stopcock valve mechanism' may allow air entry but not air escape (5). Retrospective studies suggest that the majority of people with asymptomatic bronchogenic cysts eventually develop symptoms, mostly due to compression, infection, or hemorrhage within the cyst. The current recommendation is, therefore, to resect all bronchogenic cysts in operable candidates (48, 49). The question is whether or not these asymptomatic patients should be allowed to fly until their cyst has been removed. While there are probably very many people who are unaware of their cyst and who travel by airplane for years without problems, we believe the most prudent approach would be to deny hypobaric exposure to these patients.

Conclusions

According to Van Hulst CAGE should be considered in all patients with central neurological changes when circumstances were such that gas embolism could have occurred (1). This is essentially the case for all acute neurological disorders occurring in flight or during other hypobaric activities. The presence of chest symptoms is an unreliable marker for barotrauma and neurological examination cannot reliably distinguish between CAGE and other central neurological disorders. CT imaging of the brain should be performed in all patients since CT can demonstrate air in the cerebral arteries, which proves the diagnosis of CAGE. If no air is present, cerebral ischemia may be visualized, but results may be false negative in the first hours. Chest CT should be performed to find evidence of pulmonary barotrauma and thoracic abnormalities that may account for air entrance. When CAGE is proven or probable, HBOT must be initiated as soon as possible since it is the only specific treatment for CAGE. We recommend adherence to published guidelines in determining whether or not patients with respiratory disease can be allowed to fly (44, 45). In asymptomatic patients with air filled cysts, the most careful approach is to deny flying until the cyst has been removed.

References

1. van Hulst RA, Klein J, Lachmann B. Gas embolism: pathophysiology and treatment. Clin Physiol Funct Imaging. 2003;23:237-46.
2. Foster PP, Butler BD. Decompression to altitude: assumptions, experimental evidence, and future directions. J Appl Physiol. 2009;106:678-90.
3. Neidhart P, Suter PM. Pulmonary bulla and sudden death in a young aeroplane passenger. Intensive Care Med. 1985;11:45-7.
4. Rios-Tejada F, Azofra-Garcia J, Valle-Garrido J, Pujante EA. Neurological manifestation of arterial gas embolism following standard altitude chamber flight: a case report. Aviat Space Environ Med. 1997;68:1025-8.
5. Zaugg M, Kaplan V, Widmer U, Baumann PC, Russi EW. Fatal air embolism in an airplane passenger with a giant intrapulmonary bronchogenic cyst. Am J Respir Crit Care Med. 1998;157:1686-9.
6. Cable GG, Keeble T, Wilson G. Pulmonary cyst and cerebral arterial gas embolism in a hypobaric chamber: a case report. Aviat Space Environ Med. 2000;71:172-6.
7. Closon M, Vivier E, Breynaert C, Duperret S, Branche P, Coulon A et al. Air embolism during an aircraft flight in a passenger with a pulmonary cyst: a favorable outcome with hyperbaric therapy. Anesthesiology. 2004;101:539-42.
8. Almeida FA, Desouza BX, Meyer T, Gregory S, Greenspon L. Intrapulmonary bronchogenic cyst and cerebral gas embolism in an aircraft flight passenger. Chest. 2006;130:575-7.
9. Almeida NJ, Schuller D. Cerebral air embolism resulting from an airplane flight. Journal of Respiratory Diseases 2007. Retrieved 3 January 2011 from http://www.consultantlive.com/display/article/10162/13252.
10. Belcher E, Lawson MH, Nicholson AG, Davison A, Goldstraw P. Congenital cystic adenomatoid malformation presenting as in-flight systemic air embolisation. Eur Respir J. 2007;30:801-4.
11. Edwardson M, Wurth D, Lacy JM, Fink J, Becker K. Cerebral air embolism resulting in fatal stroke in an airplane passenger with a

pulmonary bronchogenic cyst. Neurocrit Care. 2009;10:218-21.
12. Jung S, Wiest R, Frigerio S, Mattle HP, Hess CW. Cerebral air embolism caused by a bronchogenic cyst. Pract Neurol. 2010;10:164-6.
13. Salameh J. A 62-year-old woman with cerebral artery air embolism during commercial air travel. Neurologist. 2010;16:136-7.
14. Arnaiz J, Marco de LE, Piedra T, Arnaiz Garcia ME, Patel AD, Gutierrez A. In-flight seizures and fatal air embolism: the importance of a chest radiograph. Arch Neurol. 2011;68:661-4.
15. Rudge FW. Altitude-induced arterial gas embolism: a case report. Aviat Space Environ Med. 1992;63:203-5.
16. Pickard JS, Gradwell DP. Respiratory physiology and protection against hypoxia. In: Davis JR, Johnson R, Stepanek J, Fogarty JA, eds. Fundamentals of aerospace medicine. Philadelphia, PA: Lippincott Williams & Wilkins, 2008:20-45.
17. Muth CM, Shank ES. Gas embolism. N Engl J Med. 2000;342:476-82.
18. Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. Lancet. 2011;377:153-64.
19. Neuman TS. Arterial gas embolism and pulmonary barotrauma. In: Brubakk AO, Neuman TS, eds. Bennett and Elliott's physiology and medicine of diving. Philadelphia, PA: Elsevier, 2003:557-577.
20. U.S. Department of the Navy. U.S. Navy Diving Manual. Volume 1: Diving Principles and Policies. Washington, D.C.: Naval Sea Systems Command, 2008.
21. Pollock NW. Annual diving report: 2008 edition. Durham: Divers Alert Network, 2008.
22. Wolf HK, Moon RE, Mitchell PR, Burger PC. Barotrauma and air embolism in hyperbaric oxygen therapy. Am J Forensic Med Pathol. 1990;11:149-53.
23. Malhotra MS, Wright HC. The effects of a raised intrapulmonary pressure on the lungs of fresh unchilled cadavers. J Pathol Bacteriol. 1961;82:198-202.
24. Lee CT. Cerebral arterial gas embolism in air force ground maintenance crew--a report of two cases. Aviat Space Environ Med. 1999;70:698-700.

25. Hickey MJ, Zanetti CL. Delayed-onset cerebral arterial gas embolism in a commercial airline mechanic. Aviat Space Environ Med. 2003;74:977-80.
26. Camporesi EM, Bosco G. Ventilation, gas exchange and exercise under pressure. In: Brubakk AO, Neuman TS, eds. Bennett and Elliott's physiology and medicine of diving. Philadelphia, P.A.: Saunders, 2003:77-114.
27. Urwin A, Ferguson J, McDonald R, Fraser S. A five-year review of ground-to-air emergency medical advice. J Telemed Telecare. 2008;14:157-9.
28. Weinlich M, Nieuwkamp N, Stueben U, Marzi I, Walcher F. Telemedical assistance for in-flight emergencies on intercontinental commercial aircraft. J Telemed Telecare. 2009;15:409-13.
29. Calder IM. Autopsy and experimental observations on factors leading to barotrauma in man. Undersea Biomed Res. 1985;12:165-82.
30. Ryles MT, Pilmanis AA. The initial signs and symptoms of altitude decompression sickness. Aviat Space Environ Med. 1996;67:983-9.
31. Dick AP, Massey EW. Neurologic presentation of decompression sickness and air embolism in sport divers. Neurology. 1985;35:667-71.
32. Griese H, Seifert D, Koerfer R. Cortical infarction following cardiosurgical procedures - air embolism as a probable cause. Eur Neurol. 2009;61:343-9.
33. Ghatge SB, Bhatgadde VL, Nagar AM, Raut AA, Narlawar RS. Paradoxical cerebral arterial gas embolism: Computed tomography findings. Australas Radiol. 2007;51(Suppl):b210-3
34. Benson J, Adkinson C, Collier R. Hyperbaric oxygen therapy of iatrogenic cerebral arterial gas embolism. Undersea Hyperb Med. 2003;30:117-26.
35. Baird AE, Warach S. Magnetic resonance imaging of acute stroke. J Cereb Blood Flow Metab. 1998;18:583-609.
36. Reuter M, Tetzlaff K, Hutzelmann A, Fritsch G, Steffens JC, Bettinghausen E et al. MR imaging of the central nervous system in diving-related decompression illness. Acta Radiol. 1997;38:940-4.
37. Murphy A, Torres C, Lum C, Hogan M, Bussiere M. Whole brain CT

Perfusion after cerebral air embolism. Can J Neurol Sci. 2011;38:522-5.
38. Bove AA. Fitness to dive. In: Brubakk AO, Neuman TS, eds. Bennett and Elliott's physiology and medicine of diving. Philadelphia, P.A.: Saunders, 2003:700-717.
39. Tibbles PM, Edelsberg JS. Hyperbaric-oxygen therapy. N Engl J Med. 1996;334:1642-8.
40. 40. Crit Care Med. 2005;33:909-10.
41. Massey EW, Moon RE, Shelton D, Camporesi EM. Hyperbaric oxygen therapy of iatrogenic air embolism. J Hyperbaric Med. 1990;5:15-21.
42. Ziser A, Adir Y, Lavon H, Shupak A. Hyperbaric oxygen therapy for massive arterial air embolism during cardiac operations. J Thorac Cardiovasc Surg. 1999;117:818-21.
43. Bessereau J, Genotelle N, Chabbaut C, Huon A, Tabah A, Aboab J et al. Long-term outcome of iatrogenic gas embolism. Intensive Care Med. 2010;36:1180-7.
44. Ahmedzai S, Balfour-Lynn IM, Bewick T, Buchdahl R, Coker RK, Cummin AR et al. Managing passengers with stable respiratory disease planning air travel: British Thoracic Society recommendations. Thorax. 2011;66(Suppl 1):i1-30.
45. Aerospace Medical Association Medical Guidelines Task Force. Medical Guidelines for Airline Travel, 2nd ed. Aviat Space Environ Med. 2003;74(Suppl 5):A1-19.
46. Luks AM, Swenson ER. Travel to high altitude with pre-existing lung disease. Eur Respir J. 2007;29:770-92.
47. Fraser RS, Müller NL, Colman N, Paré PD. Developmental anomalies affecting the airway and lung parenchyma. In: Fraser RS, Müller NL, Colman N, Paré PD, eds. Diagnosis of diseases of the chest. Philadelphia, P.A.: Saunders, 1999:597-635.
48. Liu HS, Li SQ, Cao ZL, Zhang ZY, Ren H. Clinical features and treatment of bronchogenic cyst in adults. Chin Med Sci J. 2009;24:60-3.
49. Patel SR, Meeker DP, Biscotti CV, Kirby TJ, Rice TW. Presentation and management of bronchogenic cysts in the adult. Chest. 1994;106:79-85.

Summary and conclusions

Summary

Chapter 2 provides an overview of all published animal models of CAGE. It describes the aspects that should be taken into account when developing or evaluating an animal model for this disease. The most important of these factors are species, location of air administration, amount of air, outcome parameters, and anesthetic regimen. Many of these aspects, in particular species, amount of air, and outcome parameters, depend on the research question under study. Some general considerations of CAGE animal models are also discussed, for example the necessity to introduce the air as close to the cerebral vasculature as possible, which requires extensive knowledge of cerebrovascular anatomy.

In chapter 3, we discuss an important aspect in the use of the pig (and some other animals) in CAGE models, namely the carotid rete. Since this network of finely entangled arterioles precludes access to the cerebral arteries with a microcatheter, it is imperative to know whether introduction of air upstream of the carotid rete results in cerebral embolism. In order to elucidate this matter, we injected air into the external carotid artery (which is known to result in cerebral embolism because of anastomoses between external and internal carotid territories in the pig) and the ascending pharyngeal artery (which feeds the carotid rete and thus the internal carotid circulation). Although intracranial pressure (ICP) and brain oxygen tension ($PbtO_2$) after embolization did not significantly differ between the groups, we found an increased brain lactate level after injection into the ascending pharyngeal artery as compared to the external carotid artery. Furthermore, ICP, $PbtO_2$, and brain lactate correlated significantly after injection into the ascending pharyngeal artery, but not after injection into the external carotid artery. We therefore conclude that air introduced into the ascending pharyngeal artery does indeed pass through the rete into the cerebral arteries, and furthermore that the ascending pharyngeal artery is the most appropriate vessel for air introduction.

The issue addressed in chapter 4 is the use of quantitative electroencephalography (qEEG) for detection of the effects of CAGE on cerebral function. The advantages of EEG are its relatively easy, inexpensive, and non-invasive application, and its high sensitivity for cerebral ischemia. However, traditional qualitative assessment of the signal limits comparison between patients and its use as a research tool. Quantitative assessment of the EEG signal is increasingly used to overcome these disadvantages, and many different methods for analyzing the raw EEG have been developed. In chapter 4, we describe the use of five qEEG features for the acute detection of CAGE, by correlating the values of these qEEG features with the outcome parameters ICP, $PbtO_2$, and brain lactate. The results indicate that mean amplitude and temporal brain symmetry, but not alpha-delta ratio, spectral edge frequency, and spatial brain symmetry index, are useful for the detection of CAGE. This is demonstrated by the good correlation of these two parameters with the other outcome parameters 4 h after air embolism, and the fact that early levels (30 min after CAGE) of mean amplitude and temporal brain symmetry index adequately predict the level of ICP and brain lactate after 4 h. Also, mean amplitude and temporal brain symmetry can distinguish between bad outcome (ICP>20 mmHg) and good outcome (ICP≤20 mmHg), while the other qEEG features cannot.

The study described in chapter 5 further investigates the use of various monitoring modalities to detect CAGE. Apart from qEEG (specifically temporal brain symmetry index), $PbtO_2$, and microdialysis (brain lactate and brain glycerol), which had been used in previous studies, this study introduces measurement of regional cerebral oxygen saturation (rSO_2) using near-infrared spectroscopy as a possible method for detecting the cerebral effects of CAGE. Increasing amounts (0.2, 0.4, 0.8, and 1.6 ml) of air were injected in 12 animals, and the mentioned parameters were continuously recorded. The results showed weak correlation of $PbtO_2$ and rSO_2, but intermediate to good correlation between all outcome parameters were found when results were condensed to one value per embolization using area-under-the-curve. Furthermore, results indicate that rSO_2

and qEEG can detect air boluses almost instantaneously, but with reduced sensitivity as compared to invasive $PbtO_2$ measurement.

Chapter 6 describes a study investigating the maximum delay that can be tolerated after CAGE, before treatment with hyperbaric oxygen therapy (HBOT) must be started. This is an important issue in this disease, since a significant delay is known to occur in many cases of CAGE, and very little data is available on how quickly HBOT must be commenced. Using the knowledge obtained from the studies described in chapters 3 and 4, air was introduced in the ascending pharyngeal artery and titrated to obtain a standardized level of cerebral injury, based on changes in temporal brain symmetry index. Animals were treated with a single session of US Navy Treatment Table 6 after either 2 or 4 h of delay, while control animals were not treated with HBOT. Interestingly, neither of the intervention groups demonstrated improvement of temporal brain symmetry index, ICP, and brain microdialysis values as compared with the control group, despite the fact that significantly higher $PbtO_2$ values were recorded during HBOT in both intervention groups. The results may be due to type II error, since the observed effect size was smaller than expected due to larger variance of the data. The other explanation for our results is that HBOT actually had no positive effect on our animals, which may suggests that the damage inflicted in our model was too severe for a single session of HBOT to be beneficial.

The clinical study presented in chapter 7 regards the use of intravenous lidocaine as a neuroprotective agent in neurological decompression illness (this includes CAGE and neurological decompression sickness). Previous studies have reported conflicting results on this matter, and the study described in this chapter is the first study to investigate the use of intravenous lidocaine in a group of divers with neurological decompression illness. Patient characteristics, injury severity, and neurological outcome were retrospectively analyzed in 14 patients who had received lidocaine, and were compared to a historic cohort of 21 patients who had not received lidocaine. All divers were treated with HBOT according to accepted

protocols. The study failed to demonstrate a positive (or negative) effect of lidocaine on neurological outcome.

Chapter 8 presents a review of a specific category of CAGE, namely CAGE due to hypobaric exposure. While pulmonary barotrauma leading to CAGE is a well-known complication of decompression after initial hyperbaric exposure (e.g., diving), on a theoretical basis CAGE can also be caused by hypobaric activities, such as flying. A literature search resulted in 12 case reports describing this phenomenon, and the pathophysiological, diagnostic, and therapeutic aspects of this form of CAGE are described. The low and slow pressure decrease during most hypobaric activities, among other reasons, accounts for the low incidence of hypobaric CAGE. The diagnosis should be considered in all patients suffering acute central neurological dysfunction during or shortly after pressure decrease. Cerebral and thoracic imaging may reveal findings supporting the diagnosis. The case reports suggest that in a substantial percentage of these patients the mechanism for air entry is rupture of a non-communicating air-filled thoracic cyst during pressure decrease. The review suggests that the most prudent approach in patients with known air-filled thoracic cysts is to deny flying until the cyst is removed.

Conclusions and future research

Diagnosis of CAGE

Our investigations on improving detection of CAGE have focused on non-invasive measurement of cerebral function. Specifically, our attention has been directed towards application of qEEG and measurement of rSO_2 using near-infrared spectroscopy. We have demonstrated that qEEG (in our studies specifically using temporal brain symmetry index) is a promising tool in this regard. Although rSO_2 was less sensitive than $PbtO_2$ and qEEG in detecting the acute effects of CAGE, it should still be regarded as an interesting modality, not in the last place because of its increasing use in anesthesiological practice.

Both qEEG and rSO_2 are most valuable for monitoring patients at risk for CAGE, as opposed to those patients who first come to medical attention after sustaining CAGE. Thus, the findings of our studies are primarily of interest for the detection (and subsequent treatment) of clinical cases of CAGE, for example during cardiac surgery. This may lead one to conclude that the findings of our studies have no relevance for the diving community. That this is not true, however, is demonstrated by the fact that our results on qEEG have already spawned follow-up studies on the application of this technique in divers. In diving medicine, qEEG may not only be used for follow-up and possible prognostication of diving injuries, but also for research on oxygen toxicity and inert gas narcosis.

In the field of clinical CAGE, our results are mostly relevant for the ongoing debate on postoperative stroke and cognitive dysfunction. While cerebral air embolization certainly plays a role in the etiology of this disorders, the extent of its contribution is not yet elucidated. Multimodal monitoring involving qEEG, rSO_2, transcranial Doppler, and other techniques may in the future lead to a better understanding of this multifactorial issue, possibly resulting in more clinical awareness of CAGE as a contributing factor to cerebral complications of surgery.

Treatment of CAGE

Our study on the effect of HBOT after 2 or 4 h delay in CAGE has produced the interesting result that neither of the intervention groups reacted favorably to the hyperbaric treatment. Above all, this calls for further research into this important subject. Since one of the possible causes for our non-significant results may be that our method of air embolization is not specific enough, ongoing effort should be put in the development of more refined animal models of this disease. Currently, our results should not change accepted policies on HBOT in CAGE, but our study indicates that HBOT – despite its universal acceptance as the only specific treatment for CAGE – needs continuing investigation.

In the first controlled study of lidocaine in decompression illness, we

were unable to demonstrate either a positive or a negative effect of this substance on neurological outcome. This study adds to the body of evidence against the use of lidocaine in decompression illness, despite the promising early results in animal and human studies. Only a large multicenter randomized controlled trial would be able to provide definitive conclusions.

Animal models of CAGE

The majority of the studies that form this thesis have been performed in a pig model of CAGE. Also, chapter 2 contains an overview of all animal models used for research of this disease. Our interest in animal models stems from the fact that this type of research plays a significant role in CAGE research, and will probably always continue to do so. This is explained on the one hand by the heterogeneity of the disease and its low prevalence, but also by the fact that HBOT is accepted as the standard treatment modality, despite the lack of level I evidence supporting its use. This situation precludes execution of randomized controlled trials on the use of HBOT in CAGE. Therefore, despite the fact that no single animal model will be able to answer all questions regarding this disease, there should be a continuous effort on the development of more refined animal models. Throughout the research described in this thesis, our own animal model has been continuously updated. This included implementation of a more specific artery for embolization (chapter 3), expanding the modalities to assess cerebral function (chapter 4 and 5), and implementation of more selective application of the air through the use of a balloon catheter (chapter 5 and 6). Despite these efforts and the concurrent increasingly smaller amounts of air used in our studies, one of the main conclusions of our article on HBOT in CAGE (chapter 6) is that the variation of our results is too large. Obviously, future research on this subject should focus on even more selective administration of air, resulting in more standardized effects. A second shortcoming of our studies is the lack of clinical endpoints. No matter how many cerebral parameters the researcher monitors and how elegant they are, none of these can replace clinical examination. Therefore, this should be implemented in future animal models of CAGE.

Military research into CAGE

In the military operational theater, CAGE can occur in various populations. Divers are at risk during their work for salvage diving and mine-countermeasures, pilots and aircrew when rapid decompression occurs during flight, and submarine escapees during disabled submarine disasters. All these populations have in common the lack of nearby decompression facilities and therefore delay in the treatment of decompression sickness and CAGE, which interferes negatively with clinical outcome and operational fitness.

It is of paramount importance to continue animal research on CAGE with the focus on adjuvant therapy, delay of treatment, and the use of other treating gas mixtures, e.g. heliox and trimix. Since investigations on humans are difficult to perform in this uncommon disease, further understanding will have to be based to a large extent on studies in animals.

Samenvatting en conclusies

Samenvatting

Hoofdstuk 2 geeft een overzicht van alle gepubliceerde diermodellen van CAGE. Het beschrijft de aspecten waarmee rekening moet worden gehouden bij het ontwikkelen en evalueren van diermodellen van deze ziekte. De meest belangrijke factoren zijn diersoort, locatie van toediening van de lucht, hoeveelheid lucht, gebruikte uitkomstmaten en anesthesie. Veel van deze aspecten, in het bijzonder diersoort, hoeveelheid lucht en uitkomstmaten, hangen af van de specifieke onderzoeksvraag. Ook worden enkele algemene overwegingen met betrekking tot diermodellen van CAGE besproken. Zo is het bijvoorbeeld van belang de lucht zo dicht mogelijk bij de cerebrale arteriën te introduceren, hetgeen uitgebreide kennis van de cerebrovasculaire anatomie vereist.

In hoofdstuk 3 wordt een aspect bediscussieerd dat van belang is bij het gebruik van varkens (en sommige andere diersoorten) bij CAGE onderzoek, namelijk de rete mirabile. Aangezien dit netwerk van arteriolen de toegang tot het brein met een microcatheter onmogelijk maakt, is het noodzakelijk om te weten of injectie van lucht vóór de rete leidt tot embolisatie van de cerebrale arteriën. Om deze vraag op te helderen injecteerden wij lucht in de a. carotis externa (hetgeen leidt tot cerebrale embolisatie vanwege anastomosen tussen de territoria van carotis externa en interna) en de a. pharyngea ascendens (de voedende arterie van de rete mirabile en daarmee de a. carotis interna). Hoewel intracraniële druk (intracranial pressure, ICP) en breinzuurstofspanning ($PbtO_2$) geen significante verschillen vertoonden tussen de groepen, vonden wij een verhoogde brein lactaat concentratie na embolisatie van de a. pharyngea ascendens in vergelijking met de a. carotis externa. Bovendien was er significante correlatie tussen ICP, $PbtO_2$ en brein lactaat na injectie in de a. pharyngea ascendens, terwijl deze correlaties na injectie in de a. carotis externa niet significant waren. Wij concluderen daarom dat lucht die in de a. pharyngea ascendens geïntroduceerd wordt inderdaad door de rete mirabile naar de cerebrale arteriën stroomt en dat de a. pharyngea ascendens het meest geschikte vat is voor toediening van de lucht.

De kwestie die in hoofdstuk 4 wordt behandeld is het gebruik van kwantitatieve elektro-encefalografie (quantitative electroencephalography, qEEG) voor het detecteren van de effecten van CAGE op de cerebrale functie. De voordelen van EEG zijn het relatief eenvoudige gebruik, het non-invasieve karakter en de hoge gevoeligheid voor cerebrale ischemie. Echter, traditionele kwalitatieve analyse van het signaal maakt vergelijking tussen patiënten en dus het gebruik bij onderzoek lastig. Kwantitatieve analyse van het EEG signaal wordt in toenemende mate gebruikt om deze tekortkomingen te omzeilen en er zijn dan ook veel verschillende methoden ontwikkeld om het EEG signaal te kwantificeren. In hoofdstuk 4 beschrijven we vijf qEEG parameters voor de acute detectie van CAGE, door de waarden van deze parameters te correleren met de uitkomstmaten ICP, $PbtO_2$ en brein lactaat. De resultaten geven aan dat mean amplitude en temporal brain symmetry index gevoelig zijn voor de effecten van CAGE op het brein, in tegenstelling tot alfa-delta ratio, spectral edge frequency en spatial brain symmetry index. Dit wordt aangetoond door de goede correlatie van de eerste twee qEEG parameters met de andere uitkomstmaten 4 u na de embolisatie. Verder waren vroege waarden (30 min na embolisatie) van deze twee qEEG parameters een goede voorspeller van ICP en brein lactaat na 4 u. Bovendien konden mean amplitude en temporal brain symmetry index onderscheid maken tussen slechte uitkomst (ICP>20 mmHg) en goede uitkomst (ICP≤20 mmHg), terwijl de andere qEEG parameters dat niet konden.

De studie die in hoofdstuk 5 wordt beschreven onderzoekt het gebruik van verschillende modaliteiten om CAGE te detecteren. Afgezien van de in de vorige studies gebruikte parameters qEEG (in het bijzonder temporal brain symmetry index), $PBtO_2$ en microdialyse (brein lactaat en brein glycerol), wordt in deze studie gebruik gemaakt van regionale cerebrale zuurstofsaturatie (rSO_2) gemeten met near-infrared spectroscopy als mogelijke methode om CAGE te detecteren. Toenemende hoeveelheden lucht (0,2, 0,4, 0,8 en 1,6 ml) werden geïnjecteerd in 12 dieren, terwijl de genoemde parameters continu geregistreerd werden. De resultaten toonden zwakke correlaties tussen $PbtO_2$ en rSO_2, maar redelijke tot goede correlaties

wanneer de waarden van de uitkomstparameters werden samengevoegd tot één waarde per embolisatie door middel van area-under-the-curve. Bovendien laten de resultaten zien dat rSO_2 en qEEG de luchtbolussen vrijwel direct kunnen detecteren, zij het met verminderde gevoeligheid vergeleken met invasieve $PbtO_2$ meting.

Hoofdstuk 6 beschrijft een studie naar de maximale tijdsduur die kan worden geaccepteerd tussen ontstaan van CAGE en aanvang van hyperbare zuurstoftherapie (hyperbaric oxygen therapy, HBOT). Dit is een belangrijk onderwerp bij dit ziektebeeld, aangezien in veel gevallen van CAGE een aanzienlijke vertraging optreedt en er weinig bekend is over de effecten van een dergelijke vertraging op de effectiviteit van HBOT. Gebruikmakend van de opgedane kennis beschreven in hoofdstuk 3 en 4 werden kleine bolussen lucht geïntroduceerd in de a. pharyngea ascendens tot een bepaald niveau van cerebrale schade was bereikt, hetgeen werd gekwantificeerd middels temporal brain symmetry index. De dieren werden vervolgens behandeld met één sessie van de US Navy Treatment Table 6 na 2 of 4 u vertraging, terwijl de controledieren niet met HBOT werden behandeld. Ondanks het feit dat $PbtO_2$ waarden in de met HBOT behandelde dieren significant hoger waren dan in de controledieren, liet geen van beide interventiegroepen een betere uitkomst zien op het gebied van qEEG, ICP en microdialysewaarden. Dit resultaat wordt mogelijk veroorzaakt door een type II fout, aangezien de effectgrootte kleiner was dan verwacht vanwege grotere variabiliteit van de data. De andere verklaring is dat HBOT in onze dieren daadwerkelijk geen positief effect had, hetgeen suggereert dat de schade die wij in ons model induceerden te groot was voor een enkele sessie HBOT.

De klinische studie die in hoofdstuk 7 wordt beschreven onderzoekt het gebruik van intraveneuze lidocaïne als een neuroprotectieve strategie bij neurologische decompressieziekte. Eerdere studies lieten op dit gebied wisselende resultaten zien en onze studie is de eerste die intraveneuze lidocaïne onderzoekt in een groep duikers met neurologische decompressieziekte. Patiëntkarakteristieken, ernst van het ziektebeeld en neurolo-

gische uitkomst werden retrospectief geanalyseerd in 14 patiënten die met lidocaïne waren behandeld en vergeleken met een historisch cohort van 21 patiënten die geen lidocaïne hadden gekregen. Alle duikers werden volgens algemeen geaccepteerde protocollen met HBOT behandeld. De studie laat een positief noch negatief effect zien van lidocaïne op de neurologische uitkomst.

Hoofdstuk 8 geeft een overzicht van een specifieke categorie van CAGE, namelijk CAGE ten gevolge van hypobare expositie. Longoverdrukletsel leidend tot CAGE is een bekende complicatie van decompressie na initiële hyperbare expositie (bijvoorbeeld duiken), maar op theoretische gronden kan CAGE ook ontstaan bij primair hypobare activiteiten zoals vliegen. Een zoekopdracht in de literatuur resulteerde in 12 casus die dit fenomeen beschrijven. Aan de hand van deze casus worden pathofysiologie, diagnostiek en therapeutische aspecten van deze vorm van CAGE besproken. De lage incidentie wordt verklaard door de geringe en langzaam afname van de omgevingsdruk die bij de meeste hypobare activiteiten plaatsvindt. De diagnose dient overwogen te worden bij alle patiënten die zich presenteren met acute centraal neurologische afwijkingen tijdens of kort na ondergaan van drukverlaging. Cerebrale en thoracale beeldvorming kan de diagnose ondersteunen. De casusbeschrijvingen suggereren dat de luchtembolieën bij een aanzienlijk deel van deze patiënten worden veroorzaakt door ruptureren van een niet-communicerende met lucht gevulde cyste in de thorax. Het artikel suggereert dat bij patiënten die bekend zijn met luchthoudende thoracale cystes, het meest verstandige beleid is hen het vliegen te verbieden totdat de cyste is verwijderd.

Conclusies en toekomstig onderzoek

Diagnostiek van CAGE

Onze studies naar het verbeteren van detectie van CAGE hebben zich toegespitst op non-invasieve bepalingen van de cerebrale functie. In het bijzonder hebben wij de aandacht gericht op het toepassen van qEEG en

rSO_2 gemeten met near-infrared spectroscopy. We hebben laten zien dat qEEG (in het bijzonder temporal brain symmetry index) in dit verband een veelbelovend instrument is. Hoewel rSO_2 minder sensitief was dan $PbtO_2$ en qEEG voor het detecteren van de acute effecten van CAGE, moet dit toch als een interessante modaliteit worden gezien, niet in de laatste plaats vanwege het toenemend gebruik van rSO_2 in de anesthesiologische praktijk.

Zowel qEEG als rSO_2 zijn vooral van waarde voor het bewaken van patiënten die risico lopen op CAGE en niet voor patiënten die pas onder medische aandacht komen nadat ze CAGE hebben opgelopen. Onder resultaten zijn daarom vooral interessant voor het detecteren (en vervolgens behandelen) van klinische gevallen van CAGE, zoals zich voordoen tijdens bijvoorbeeld cardiochirurgie. Hieruit zou men de conclusie kunnen trekken dat onze studies voor de duikpopulatie geen relevantie hebben. Dat dit niet het geval is blijkt uit het feit dat onze resultaten met betrekking tot qEEG reeds vervolgstudies bij duikers hebben opgeleverd. In de duikgeneeskunde kan qEEG niet alleen interessant zijn voor het volgen en mogelijk prognosticeren van duikongevallen, maar ook bij onderzoek naar zuurstoftoxiciteit en inert gas narcose.

Op het gebied van klinische CAGE zijn onze resultaten vooral relevant voor de discussie over postoperatieve cerebrale infarcten en cognitieve disfunctie. Hoewel gasembolieën zeker een rol spelen bij het ontstaan van deze ziektebeelden, is de omvang van hun bijdrage nog niet opgehelderd. Multimodale bewaking met qEEG, rSO_2, transcraniële Doppler en andere technieken kan in de toekomst mogelijk leiden tot een beter begrip van dit multifactoriële probleem, hetgeen kan leiden tot meer klinische aandacht voor CAGE als bijdragende factor aan cerebrale complicaties van chirurgie.

Behandeling van CAGE

Onze studie naar het effect van HBOT bij CAGE na 2 of 4 uur vertraging leverde het interessante resultaat op dat geen van beide interventiegroe-

pen een gunstig effect van de hyperbare behandeling liet zien. Dit vraagt in de eerste plaats om meer onderzoek naar dit belangrijke probleem. Aangezien een van de mogelijke oorzaken van onze resultaten een te aspecifieke embolisatietechniek is, blijft het van belang te streven naar verfijndere diermodellen van CAGE. Op dit moment zouden onze resultaten niet moeten leiden tot een ander beleid ten aanzien van HBOT bij CAGE, maar onze studie laat zien dat HBOT – ondanks het feit dat het universeel geaccepteerd wordt als de enige specifieke behandeling van CAGE – voortdurend onderzoek vereist.

In de eerste gecontroleerde studie naar het gebruik van lidocaïne bij decompressieziekte konden wij een positief noch negatief effect van lidocaïne op neurologische uitkomst aantonen. Deze studie draagt bij aan het toenemende bewijs tegen het gebruik van lidocaïne bij decompressieziekte, ondanks de veelbelovende resultaten van de eerste dierstudies en humane onderzoeken. Alleen een groot gerandomiseerd gecontroleerd onderzoek, waaraan meerdere centra zouden moeten deelnemen, kan op dit gebied tot een definitieve conclusie leiden.

Diermodellen van CAGE

De meerderheid van de studies die in dit proefschrift worden beschreven zijn uitgevoerd in een varkensmodel van CAGE. Bovendien bevat hoofdstuk 2 een overzicht van alle diermodellen die ooit bij onderzoek naar dit ziektebeeld zijn gebruikt. Onze interesse in diermodellen komt voort uit het feit dat dit type onderzoek een belangrijke rol speelt bij onderzoek naar CAGE en waarschijnlijk altijd een belangrijke rol zal blijven spelen. Dit komt enerzijds door de lage incidentie en de heterogeniteit van het ziektebeeld en anderzijds uit het feit dat HBOT algemeen geaccepteerd is voor de behandeling van CAGE, ondanks het feit dat hier geen niveau I bewijs voor bestaat. Dit zorgt ervoor dat er feitelijk geen gerandomiseerde gecontroleerde studies kunnen worden uitgevoerd naar de effectiviteit van HBOT bij CAGE. Om deze reden is het van belang om aandacht te blijven besteden aan het verbeteren van diermodellen, ondanks het feit dat uiteraard geen enkel model antwoord zal kunnen geven op alle bestaande

vragen omtrent CAGE. Gedurende het onderzoek dat in dit proefschrift wordt beschreven is ook ons eigen model continu verbeterd. Zo is de locatie van embolisatie verbeterd door een specifiekere arterie te gebruiken (hoofdstuk 3), is het aantal methoden om de cerebrale functie te bepalen uitgebreid (hoofdstuk 4 en 5) en is de embolisatie selectiever gemaakt door implementatie van een balloncatheter (hoofdstuk 5 en 6). Ondanks deze inspanningen en de steeds kleinere hoeveelheid lucht die werd geïnjecteerd, is één van de belangrijkste resultaten van ons onderzoek naar HBOT in CAGE (hoofdstuk 6) dat onze resultaten te variabel zijn. Hieruit kan worden afgeleid dat toekomstig onderzoek zich onder ander moet richten op nog selectievere embolisatietechnieken, leidend tot meer gestandaardiseerde effecten. Een tweede tekortkoming van onze studies is het gebrek aan klinische eindpunten. Het maakt niet uit hoeveel cerebrale parameters de onderzoeker bepaalt en hoe elegant deze methoden zijn, geen van allen kunnen ze het klinische onderzoek geheel vervangen. Om deze reden zullen klinische eindpunten in toekomstige modellen van CAGE moeten worden opgenomen.

Militair onderzoek naar CAGE

In de militair operationele wereld kan CAGE bij verschillende groepen personeel voorkomen. Duikers lopen risico bij bergings- en mijnbestrijdingsoperaties, vliegers bij snelle decompressie tijdens de vlucht en onderzeebootbemanningen bij ontsnappingen uit gestrande onderzeeboten. Al deze groepen hebben gemeen dat er geen decompressiefaciliteiten in de nabijheid aanwezig zijn en dat er dus onherroepelijk vertraging optreedt bij de behandeling van decompressieziekte en CAGE, hetgeen een negatief effect heeft op klinische uitkomt en operationele geschiktheid.

Het is van groot belang om het dieronderzoek naar CAGE voort te zetten, waarbij de nadruk dient te liggen op adjuvante therapie, het effect van vertraging op het effect van behandeling en het gebruik van alternatieve ademhalingsmengsels zoals heliox en trimix. Aangezien onderzoek met mensen moeilijk uitvoerbaar is bij dit zeldzame ziektebeeld, zal toekomstige kennis in grote mate gebaseerd zijn op dierstudies.

Dankwoord

Uiteraard is dit proefschrift het resultaat van de samenwerking tussen velen.

Traditiegetrouw bedank ik ten eerste mijn promotores, Prof. Dr. Dr. Hollmann en Prof. Dr. Van Gulik. Beste Markus, ik wil u bedanken voor het onder uw wetenschappelijke hoede nemen van deze buitenstaander. U heeft vanaf het begin in het project geloofd en dat waardeer ik. Meermaals heb ik mij verbaasd hoe u binnen twee zinnen tot de kern van een probleem kunt doordringen, zelfs als we elkaar lange tijd niet gesproken hadden. Ik hoop dat deze onderzoekslijn onder uw supervisie een mooie toekomst zal kennen; aan u zal het niet liggen. Beste Thomas, mijn enorme dank voor de fantastische faciliteiten die u aan deze vreemde vogel heeft verstrekt. Het heeft mij qua ondersteuning door u en uw afdeling aan niets ontbroken.

Mijn grootste dank gaat uit naar mijn co-promotor en begeleider, KTZAR Van Hulst. Beste Rob, ik heb het een eer gevonden om dit onderzoek te mogen doen en wil je danken voor het vertrouwen dat je in mij hebt gesteld. Ondanks jouw fenomenale kennis van de duikgeneeskunde heb je het toegestaan dat een 'jonge collega' jouw vakgebied binnentrad en heb je me binnen een aantal maanden de vrijheid gegeven om mijn eigen invulling aan het onderzoek te geven. Ondanks vele noodzakelijke aanpassingen aan het oorspronkelijke plan denk ik dat we een mooi product hebben neergezet. Ik kan alleen maar hopen dat alle betrokken partijen dit ook zo zien en wens jou dan ook zowel privé als op professioneel gebied niets dan het beste.

Dank eveneens aan mijn tweede co-promotor, Markus Stevens. Jouw kritische kijk op het onderzoek en mijn manuscripten heb ik als enorm waardevol ervaren.

De leden van mijn promotiecommissie, Prof. Dr. Knape, Prof. Dr. De Mol, Prof. Dr. Schlack, Prof Dr. Schultz, dank dat u de moeite heeft willen nemen om het manuscript kritisch te beschouwen. Ik ervaar het als een

eer mijn proefschrift tegenover u te mogen verdedigen. Professor Moon and Professor Radermacher, it is truly an honor to have experts with your exceptional qualities and experience on my committee.

Sander en Janine, fantastisch dat jullie mijn paranymfen willen zijn en op deze manier samen met mij mijn promotietraject willen afsluiten. Sander, ik ben blij dat je eindelijk je droom mag verwezenlijken nu je bent begonnen in je eerste baan als vlieger. Volgende doel: captain op de triple seven… Janine, jouw eigen onderzoek nadert de ontknoping en over enige tijd mag ook jij je proefschrift verdedigen. Volgende doel: medisch oncoloog…

Ik realiseer mij dat ik de afgelopen jaren een bijzondere positie binnen de groep marineartsen heb mogen innemen. Het gebeurt niet vaak dat een marinearts in de gelegenheid wordt gesteld om op zo'n uitgebreide schaal wetenschappelijk onderzoek te doen. Mijn dank voor deze mogelijkheid gaat daarom uit naar de (voormalig) hoofden Geneeskundige en Personele Zorg en hun plaatsvervangers, KTZAR Blom, KTZAR Bos, KTZAR Hoogland en KLTZAR Terlouw.

Hoewel dit onderzoek formeel onder de Experimentele Anesthesiologie was geschaard zouden we geen enkele stap hebben kunnen zetten zonder de fantastische ondersteuning van de afdeling Experimentele Chirurgie onder leiding van Prof. Dr. Van Gulik. Binnen deze afdeling gaat mijn onuitsprekelijk grote dank uit naar twee dames voor wie woorden te kort schieten. Goos en Lindy, steun en toeverlaat in de breedste zin des woords. Goos, je bent met recht de Moeder Overste geweest voor deze Mini Generaal. Jouw kennis van varkens is fabelachtig. Uiteraard was ik eigenwijs genoeg om je raad regelmatig in de wind te slaan, maar achteraf had je eigenlijk altijd gelijk. Of, zoals je zelf zegt: "Ik heb gesproken, wijze woorden.". Ik hoop dat je nog vele jaren op jouw vertrouwde afdeling je experimenteel chirurgische werk kunt voortzetten. Lindy, toen ik begin 2008 binnenkwam was je nog een jonkie en bezorgde elk infuus en elke intubatie je nachtmerries. En nu, vijf jaar later, is dat nog steeds

zo. Nee, zonder grappen, ik ben er trots op dat mijn onderzoek mede de leerschool mocht zijn die jou tot een fantastische allround biotechnicus heeft gesmeed. Ik heb genoten van je aanwezigheid, je muziekkeuze en de vele sessies op 18 meter waterdiepte.

Xavier, je kwam binnen voor een afstudeerproject en bent eigenlijk niet meer weggegaan. Jouw EEG metingen hebben een prominente plaats in ons model ingenomen en dat is allemaal te danken aan jouw technisch enthousiasme. Dank voor al je hulp bij onze experimenten. Ik wens je een schitterende carrière.

De andere medewerkers van de Experimentele Chirurgie wil ik bedanken voor hun assistentie op een veelheid aan fronten. Albert, je bent een held, dank voor al het ad hoc werk als ik weer eens op het laatste moment een bestelling had of wanneer op een totaal verkeerd moment de zekeringen van de apparatuur sprongen. Andrea, Esther en Adrie, dank voor al jullie hulp.

Uiteraard dank aan alle mede-onderzoekers die tegelijk met mij op het lab hebben gewerkt. Van de Experimentele Chirurgie Arvid, Floor, Geert, Lisette, Ivo, Marcel, Mans, Matthanja, Megan, Michal, Nienke, Rowan, Ruurdtje en Thony. Van de Experimentele Anesthesiologie (al was ik er nooit, behalve tijdens wintersport) met name Charlotte, Coert, Daniël, Djai, Friso, Gezina, Hamid, Ilse, Kirsten, Koen, Nina en Sabrine. Een speciaal woord van dank aan de leden van het Apple eskader, mijn roomies van het eerste uur, Ivo en Marcel. Al zijn jullie discussies meestal voor ieder ander (inclusief mijzelf) onbegrijpelijk, ik heb dankzij jullie een fantastische tijd gehad op het lab en wens jullie het allerbeste in jullie klinische en wetenschappelijke carrières.

De experimenten in de hyperbare kamer van het AMC waren op zijn zachtst gezegd een technische en logistieke uitdaging. Zonder de assistentie van het onvolprezen duo Arjen en Stephen en de hulp van het tankpersoneel Max, Judith en Shaida en hun studenten had ik niet geweten waar ik moest beginnen. Dank jullie.

Toen ik begon met dit onderzoek dacht ik in een gespreid bedje terecht te komen. Gelukkig wist Rob me er al snel van te overtuigen dat ik dat idee maar uit mijn hoofd moest zetten. En inderdaad, er zijn genoeg 'knak-momentjes' geweest de afgelopen vier jaar. Op die momenten was het goed dat er meer was dan alleen maar werk. Ik wil dan ook al mijn vrienden bedanken, gewoon voor het feit dat ze er waren. In het bijzonder Erik en Jeroen. Thnx guys!

Pap, Sander, Ria, Robert en Susan: op een mooie toekomst voor jullie allemaal! Pap, jij bent er altijd voor mij en helpt me de dingen op een rijtje te zetten als ik er even niet meer uitkom. Blijf lekker werken, klussen en wat al niet, maar geniet op z'n tijd ook van je rust. Probeer het ten minste!

Als laatste. Lieve mama, dertien jaar alweer. Onvoorstelbaar. Ik mis je, deze is voor jou.

Portfolio

PhD periode: maart 2009 t/m april 2012.

Curriculum vitae

Robert Paul Weenink werd op 28 oktober 1982 geboren te Amsterdam. Zijn middelbare schooltijd bracht hij door op de Katholieke Scholengemeenschap Hoofddorp, alwaar hij in 2000 cum laude slaagde voor het Gymnasium. In datzelfde jaar startte hij met de studie Geneeskunde aan de Vrije Universiteit te Amsterdam. Zijn affiniteit met de neurowetenschappen kwam tot uitdrukking tijdens keuzevakken en stages, waaronder een wetenschappelijke stage bij de afdeling neurochirurgie van het VUmc (begeleider: Prof. Dr. Dirven). Na het artsexamen in 2007 volgde een jaar als assistent-niet-in-opleiding bij deze afdeling (hoofd: Prof. Dr. Vandertop). Hierna maakte hij de overstap naar de Koninklijke Marine, waar zich eind 2008 de mogelijkheid voordeed om promotieonderzoek te doen op het grensgebied van duikgeneeskunde, anesthesiologie en neurowetenschappen. Dit onderzoek heeft geleid tot het onderhavige proefschrift. Naast de activiteiten als onderzoeker is hij de afgelopen jaren werkzaam geweest in de eerstelijns curatieve en bedrijfsgeneeskundige zorg voor het Marinepersoneel alsmede op het gebied van de luchtvaartgeneeskunde. Begin 2013 heeft hij de opleiding tot vliegerarts afgerond op Naval Air Station Pensacola, Florida. Zijn vrije tijd besteed Robert grotendeels aan Scouting en zeilen.

Publicaties

1. Weenink RP, Hollmann MW, Stevens MF, van Lienden KP, Ghazi-Hosseini E, van Gulik TM, van Hulst RA. Cerebral arterial gas embolism in swine. Comparison of two sites for air injection. J Neurosci Methods 2011; 194:336-41.
2. Weenink RP, Vrijdag XC, Hollmann MW, van Putten MJ, Stevens MF, van Gulik TM, van Hulst RA. Quantitative electroencephalography in

a swine model of cerebral arterial gas embolism. Clin Neurophysiol 2012; 132:411-7.
3. Weenink RP, Hollmann MW, van Hulst RA. Animal models of cerebral arterial gas embolism. J Neurosci Methods 2012; 205:233-45.
4. Weenink RP, Hollmann MW, van Hulst RA. Acute neurological symptoms during hypobaric exposure: consider cerebral air embolism. Aviat Space Environ Med 2012; 83:1084-91.
5. Post IC, Weenink RP, van Wijk AC, Heger M, Boing AN, van Hulst RA, van Gulik TM. Characterization and quantification of porcine circulating endothelial cells. Xenotransplantation 2013; 20:18-26.
6. Weenink RP, Hollmann MW, Vrijdag XC, Van Lienden KP, De Boo DW, Stevens MF, Van Gulik TM, Van Hulst RA. Hyperbaric oxygen does not improve cerebral function when started two or four hours after cerebral arterial gas embolism in swine. Crit Care Med 2013; 41:1719-27.
7. Weenink RP, Hollman MW, Van Hulst RA. A retrospective cohort study of lidocaine in divers with neurological decompression illness. Undersea Hyperb Med, geaccepteerd voor publicatie.
8. Weenink RP, Hollmann MW, Van Hulst RA. Detection of cerebral arterial gas embolism using regional cerebral oxygen saturation, quantitative electroencephalography, and brain oxygen tension in the swine. Ingestuurd voor publicatie.
9. Weenink RP, Hollmann MW, Van Hulst RA. Cerebrale arteriële gasembolieën: anesthesiologische aspecten van een duikmedisch ziektebeeld. Ingestuurd voor publicatie.

Mondelinge presentaties

1. Weenink RP, Vrijdag XC, Hollmann MW, van Putten MJ, Stevens MF, van Gulik TM, van Hulst RA (2011). Quantitative electroencephalography in a swine model of cerebral arterial gas embolism. 44th Annual Scientific Meeting of the Undersea &

Hyperbaric Medical Society, 15-18 juni 2011, Fort Worth, Texas, Verenigde Staten.

Poster presentaties

1. Weenink RP, Vrijdag XC, Hollmann MW, van Putten MJ, Stevens MF, van Gulik TM, van Hulst RA (2011). Quantitative electroencephalography in a swine model of cerebral arterial gas embolism. 44th Annual Scientific Meeting of the Undersea & Hyperbaric Medical Society, 15-18 juni 2011, Fort Worth, Texas, Verenigde Staten.
2. Van Hulst RA, van Ooij PJ, Zomervrucht A, Weenink RP (2011). Lidocaine in decompression illness, a retrospective controlled study in 17 divers. 44th Annual Scientific Meeting of the Undersea & Hyperbaric Medical Society, 15-18 juni 2011, Fort Worth, Texas, Verenigde Staten.

Congressen

1. Aerospace Medical Association, 81st Annual Scientific Meeting, 9-13 mei 2010, Phoenix, Arizona,Verenigde Staten.
2. European Society of Anaesthesiology, Euroanaesthesia 2011, 11-14 juni 2011, Amsterdam.
3. Undersea & Hyperbaric Medical Society, 44th Annual Scientific Meeting, 15-18 juni 2011, Fort Worth, Texas, Verenigde Staten.
4. United States Naval Aeromedical Conference, 14-17 januari 2013, Pensacola, Verenigde Staten.

Cursussen

1. Cursus Proefdierkunde. 2009, Academisch Medisch Centrum, Amsterdam.

Onderscheidingen

1. Undersea & Hyperbaric Medical Society, 44th Annual Scientific Meeting, 15-18 juni 2011, Fort Worth, Texas, Verenigde Staten. Prijs voor beste poster in de categorie trainee/resident.