Microbubble damage to the blood-brain barrier: relevance to decompression sickness

B. A. HILLS and P. B. JAMES

Department of Physiology, The University of New England, Armidale, N.S.W., Australia, and Wolfson Institute of Occupational Health, Department of Community Medicine, The University of Dundee, Dundee, Scotland

Hills BA, James PB. Microbubble damage to the blood-brain barrier: relevance to decompression sickness. Undersea Biomed Res 1991; 18(2):111–116.—Decompression sickness affecting the nervous system is still a serious problem in diving, but the mechanisms involved are in dispute. Although microbubbles can be detected in the pulmonary artery on decompression using ultrasound, mammalian lungs are competent filters for microbubbles larger than 20 μm in diameter. It has been assumed that smaller bubbles released by the lungs are harmless, because there is evidence that they do not arrest in the cerebral circulation. We injected 15 ± 5 μm diameter microbubbles in 5 ml of plasma slowly into the right carotid artery of anesthetized guinea pigs. At intervals of 1, 2, or 3 h postinjection, 2% trypan blue in 2 ml of plasma was injected into the same artery or the contralateral carotid artery. A control animal for each experiment was injected with 5 ml of plasma only, followed by the injection of dye at the same interval. After the animals were killed, the brains were examined for evidence of blood-brain barrier dysfunction. All animals at 1 h, and 9 out of 10 animals at 2 h after the injection of microbubbles, showed extravasation of the albumin-binding dye in the ipsilateral hemisphere, indicating gross blood-brain barrier dysfunction. In each of the matched controls, the barrier in the neocortex remained intact. At Hour 3 the barrier was impermeable to the trypan blue in both experimental and control animals. These experiments demonstrate that microbubbles impair the blood-brain barrier integrity to protein, causing focal edema. The mechanism can account for the cerebral features of decompression sickness which are revealed by isotope scanning, changes in the cerebral vessels of divers, and focal myelin and axon damage in the spinal cord. A similar, generalized increase in vascular permeability may be responsible for the extravasation of plasma in shock associated with fulminating decompression sickness.

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Neurologic decompression sickness is still a major problem in both amateur and commercial diving using compressed air. Myelin degeneration has been found in the spinal cords of commercial divers with no recorded incidents of neurologic decompression sickness (DCS) (1) and in a symptomless diver after recovery from an episode of neurologic decompression sickness (2). Cerebral symptoms such as dizziness, vertigo, and disturbances of vision are still commonly reported by air

divers, but they are usually transient, and most residual disability relates to lesions in the spinal cord. Recent studies using isotope scanning (3) and the demonstration of cerebrovascular changes (4) suggest that the brain may be extensively involved in DCS. Early investigators reported loss of consciousness and a variety of cranial nerve disorders resulting from DCS in divers, and at necropsy found disseminated petechial hemorrhages in the brain (5). Clinical evidence of cerebral damage is unusual, but nystagmus is not uncommon, indicating persisting vestibular damage. Subtle changes in the central control of oculomotor function have also been detected in commercial divers (6), together with evidence of neuropsychiatric deficits (7). Preliminary studies using magnetic resonance imaging and visual evoked potentials, which are sensitive to demyelination, also suggest that permanent structural changes may occur in the brains of divers (8).

Silent arterial bubbles have been detected using decompression from very deep dives on helium and oxygen mixtures (9, 10). Although the same monitoring has not yet been undertaken in air divers, systemic arterial bubbles have been found in experimental animals during decompression breathing air (11). Arterial embolism, in situ gas formation, and vertebral venous occlusion have all been proposed as mechanisms in neurologic DCS. The major argument against an arterial mechanism has been the dominance of cerebral rather than spinal cord effects when gross arterial gas embolism results from pulmonary barotrauma, and in embolism associated with heart disease and atheroma. Air embolism may affect the cord (12), and in view of the loss of consciousness which typifies major cases it may be involved more frequently than is recognized, but residual neurologic sequelae are rare.

It has been established that for gaseous emboli to arrest in the cortical arteries of the mammalian brain they must be in excess of 200 μm in diameter (13). At this size, the pressure drives the bubble into the vasculature until the surface tension produced by the elongation of the bolus is sufficient to arrest flow. This occurs in arterioles of about 40 μm in diameter (13). Bubbles arising during decompression are about 20 μm measured at normal atmospheric pressure (14), again appearing to rule out an arterial mechanism. However, as with erythrocytes, they are more likely to be universally distributed in the blood and may arrest at the capillary level. The use of contrast in ultrasonic studies has confirmed that bubbles present in the systemic venous return can be released into the arterial circulation by transpulmonary passage (15) and has demonstrated intracardiac shunting in 24% of normal amateur divers (16). When injections are made directly into the left ventricle, neurologic complications occasionally arise, but again involve the brain rather than the spinal cord (17). Chryssanthou et al. (18) were the first to observe experimentally a reversible increase in the permeability of the blood-brain barrier after decompression. Lehtosalo et al. (19) demonstrated the involvement of the spinal cord in addition to the brain, using horseradish peroxidase as a tracer in rats decompressed from an exposure to 6.2 atm abs for 90 min over 1 min. In neither case was it possible to relate the effects to bubble formation.

In view of these factors, it was decided to study the effect of the intracarotid injection of sized microbubbles on the integrity of the blood-brain barrier. The size range used, from 10 to 20 μm in diameter, is known to escape pulmonary filtration (20).

MATERIALS AND METHODS

Adult guinea pigs of either sex and 850 ± 50 g were anesthetized by the intraperitoneal injection of sodium nembutal and randomly allocated to test and control groups.

The test group received an injection of 5 ml of plasma, containing microbubbles subjected to ultrasonic dispersion, into the right carotid artery. In the control animals, only plasma was injected. In all cases the plasma was obtained by centrifuging a sample of the animal's own blood 2 days before the experiment. Anesthesia was maintained intravenously with sodium nembutal, and the animals were placed on a rodent ventilator. The injections were given slowly over a 2-min period to avoid elevations of perfusion pressure, which may disturb the blood-brain barrier.

After an elapsed time of 1, 2, or 3 h, 2 ml of trypan blue (2%) was injected into the same carotid artery in both the test and control groups. After a further 15 min the animals were given a lethal dose of sodium nembutal and potassium chloride, i.v. The brains were excised and examined by serial section. The experiment was repeated in 4 more animals using the same procedure. A second experimental series using 5 more animals was then undertaken, but with the dye injected into the left carotid artery. This made a total of 10 experiments for each elapsed time. Analysis of the microbubble suspensions after ultrasonic dispersion, using a Coulter counter, showed the size range to be 15 ± 5 μm.

RESULTS

The results in 30 test animals and 30 controls, in total 60 brains, are given in Table 1. Each square represents 1 animal and 1 experiment. There is a very clear division between the brains subjected to the microbubble embolism, which at 1 h showed unequivocal evidence of blood-brain barrier breakdown, and the controls. At 2 h, 9 of the 10 test animals also showed gross barrier breakdown. Only 1 control showed very slight staining, just in the brainstem. The test animals also served as their own controls, as the breakdown was not seen in the contralateral hemisphere even when the trypan blue was injected into the carotid artery on that side. It has also been shown that the cortical blood-brain barrier disturbance induced by the microbubbles

TABLE 1
EFFECT OF AIR MICROBUBBLES ON THE BLOOD-BRAIN BARRIER

Time Elapsed		1 h		2 h		3 h	
Test/Control	No.	T	C	T	C	T	C
Dye	1	■	□	■	□	□	□
Injected	2	■	□	■	□	□	□
Same	3	■	□	■	□	□	□
Carotid	4	■	□	■	□	◪	□
Artery	5	■	□	■	□	□	□
Dye	6	■	□	■	□	□	□
Injected	7	■	◪	■	□	□	□
Other	8	■	□	◪	□	□	□
Carotid	9	■	□	■	□	□	□
Artery	10	■	□	■	□	□	□

Key: ■ = Permeable, ◪ = permeable in brainstem only, □ = impermeable.

is reversible; permeability, at least as indicated by trypan blue, returned to normal between Hours 2 and 3.

DISCUSSION

A disturbance of vascular permeability due to the passage of microbubbles can explain many well-established clinical features of DCS that are difficult to reconcile with simple vascular occlusion. For example, in severe DCS the extravasation of plasma has led to severe hemoconcentration and shock (21). Although embolism is generally assumed to cause sudden changes, microembolism, by disturbing the blood-brain barrier, may also cause the more gradual onset of neurologic symptoms. In mild cases of neurologic DCS there is often a biphasic presentation, the initial symptoms resolving only to reappear several hours later. This is consistent with the first presentation being due to the passage of bubbles, and the delayed symptoms being due to the gradual development of edema in the same area. Support for this suggestion can also be derived from the effect of therapy. Pressure is effective if used immediately, but if therapy is delayed, hyperbaric oxygen is likely to be more successful than higher pressures (22).

Although microembolism can explain the transient cerebral symptoms experienced by divers, it is still necessary to account for the involvement of the spinal cord in DCS. There is good evidence demonstrating that the same blood-brain barrier dysfunction found in the brain also occurs in the cord in DCS (19, 23), but the absence of spinal cord symptoms from the microbubbles of ultrasonic contrast must be explained. The major difference in divers undergoing decompression may be the presence of high nitrogen tensions in the spinal cord. Arterial emboli may arrest and grow in the cord, but microemboli, in triggering edema and mechanically compromising blood flow (24), may also predispose to autochthonous gas formation (25). The vessels principally affected by the blood-brain barrier disturbance are veins, and this may contribute to petechial hemorrhages, endothelial damage (26), and venous stasis in spinal cord DCS (23), together with the damage to myelin (2). As the bubbles in these experiments do not arrest in the cerebral cortex, the effect is not due to hypoxia or ischemia. Using unsized bubbles, others have shown that the barrier breakdown occurs in seconds (27), in contrast to delays of up to 6 h found after major arterial occlusion and even after death (28). The small size of the bubbles used in these experiments clearly prevented the development of global hypoxic cerebral edema, because the contralateral hemisphere did not show evidence of barrier disturbance, even in animals injected with trypan blue 3 h after the injection of the emboli. Nevertheless, as trypan blue binds to serum albumin, the increase in permeability is of significant proportions, and where this degree of breakdown has been demonstrated it is unusual for a more sensitive marker, such as horseradish peroxidase, to show evidence of continued dysfunction over a much longer period. Vise et al. (29), using 15-μm solid microspheres in the cat, found that cerebral blood flow actually increased after microembolism and there was a rise in venous oxygen content. Zulch and Tzonos (30) also found damage to the venous blood-brain barrier and demyelination from the transit of solid microemboli and termed the effect the "perivenous syndrome."

The reason for the gross increase in blood-brain barrier permeability is still unknown but may involve the inflammatory response. Histamine (31) and serotonin

and bradykinin (32) may transiently open the barrier, and the activation of complement may be involved in demyelination (33, 34). There is evidence that membrane effects conferring hydrophobicity to the blood-brain barrier may be due to an adsorbed layer of phospholipid (35). Phospholipids have been shown to migrate onto the surface of microbubbles (36). Recent studies using a refined fixation procedure have demonstrated a phospholipid lining consisting of 8–10 lamellae adjacent to the bilayer of the endothelium of cerebral vessels (37). This may be a physical component of the blood-brain barrier and it is possible that in transit microemboli remove this adsorbed layer of hydrophobic phospholipid, and therefore destabilize the barrier. A physicochemical effect may be a reasonable explanation for the breakdown because it occurs within seconds in the absence of ischemia or hypoxia and has now been shown to be reversible.

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