Post effect of repetitive exposures to pressure nitrogen-induced narcosis on the dopaminergic activity at atmospheric pressure.

C. LAVOUTE, M. WEISS, JM SAINTY, JJ RISSO, JC ROSTAIN

Université de la Méditerranée et IMNSSA (Institut de Médecine Navale du Service de Santé des Armées), EA 3280, Physiopathologie et Action Thérapeutique des Gaz Sous Pression, IFR Jean-Roche, Faculté de Médecine Nord, 13015 Marseille, France.

Lavoute C, Weiss M, Sainty JM, Risso JJ, Rostain JC. Papers from IHPB Meeting, 2007. Undersea Hyperb Med 2008; 35(1):21-25. Nitrogen at pressure produces a neurological syndrome called nitrogen narcosis. Neurochemical experiments indicated that a single exposure to 3 MPa of nitrogen reduced the concentration of dopamine by 20% in the striatum, a structure involved in the control of extrapyramidal motor activity. This effect of nitrogen was explained by enhanced GABAergic neurotransmission through $GABA_A$ receptors and, to a lesser extent, by a decreased glutamatergic input to DA cells through NMDA receptors. The aim of this study was to study, under normobaric conditions, possible alterations of NMDA receptor activity in the substantia nigra pars compacta (SNc) induced by repetitive exposures to nitrogen pressure. Under general anesthesia, male Sprague-Dawley rats were implanted in the striatum with multifiber carbon dopamine-sensitive electrodes and in the SNc with guide cannulae for drug injections. After recovery from surgery, the striatal dopamine level was recorded by voltammetry in freely-moving rats, in normobaric conditions, before and after 5 repetitive exposures to 1MPa of nitrogen (threshold of nitrogen narcosis occurrence in rat). The effect of NMDA receptor activity on DA concentration was investigated using agonist (NMDA) and specific antagonist (AP7) SNc administration. Following repetitive nitrogen exposures, the ability of NMDA to elevate DA concentrations was enhanced. In contrast, after nitrogen exposure AP7 produced a paradoxical increase in DA concentration compared to its inhibitory effect before any exposure. Similar responses were obtained after a single exposure to 3MPa nitrogen. Thus, repetitive exposures to nitrogen narcosis produced a sensitization of postsynaptic NMDA receptors on DA cells, related to a decreased glutamatergic input in SNc. Consequently, successive nitrogen narcosis exposures disrupted ion-channel receptor activity revealing a persistent nitrogen-induced neurochemical change underlying the pathologic process.

INTRODUCTION

Humans exposed to hyperbaric nitrogen-oxygen breathing mixtures develop a neurological syndrome called nitrogen narcosis. Nitrogen may cause its sedative action by altering different neurotransmitter systems in the central nervous system, such as the dopaminergic pathway. In rats, neurochemical studies demonstrated a depressant action of 3MPa nitrogen exposure on dopaminergic activity, resulting in decreased dopamine release in the striatum, a structure involved in the control of motor function (Bennett & Rostain, 2003).

At the level of the substantia nigra pars compacta (SNc), dopaminergic neurons receive excitatory glutamatergic input, originating from the cortical mantle, which modulate the

striatal dopamine release, through postsynaptic NMDA receptors (Christoffersen *et al*, 1995).

A single exposure to nitrogen has been demonstrated to disturb the glutamatergic input through a pre-synaptic action by decreasing glutamate release, without affecting NMDA receptors (Lavoute *et al*, 2006, 2007).

Repetitive exposures to nitrogen produced a reversed dopaminergic response to 3MPa nitrogen leading to an increase in the dopamine concentration by 10% (Lavoute *et al*, 2005). Such changes could suggest a disturbance in the dopaminergic control by excitatory afferents.

The aim of this study was to investigate the post-synaptic effect of repetitive exposures to nitrogen on NMDA receptor function under normobaric conditions and to compare these to the change obtained after a single exposure to 3MPa nitrogen.

MATERIALS AND METHODS

Animal preparation and surgery

Animal experiments were carried out in accordance with the guidelines laid down by the Institute's Ethics Committee for Care and Use of Animals in Experimental Work.

Male Sprague-Dawley rats (n=50) weighing 300±20g at the time of the surgery were housed at 22±0.5°C in individual home cages under a 12:12 hr light-dark cycle (with lights on from 07:00 to 19:00 h) with free access to food and water. Under general anaesthesia (pentobarbital sodium 30 mg/kg i.p. and ketamine 100 mg/kg i.m.), rats were stereotaxically and bilaterally implanted with DA-sensitive carbon electrodes in the dorsal striatum (A: 10.2, L: 2.3, H: 4.8) and cannulae in the substantia nigra pars compacta (A: +3.4; L: +1.6; H: +2), according to the rat atlas of Paxinos and Watson (1986). Reference and auxiliary electrodes (stainless steel screws; coppered wire) were fixed on the bone. The electrodes were attached to a mini-connector, and the whole assembly was held in place with dental cement (Unifast Trade).

Electrochemical measurements of the striatal DA release

Differential pulse voltammetric (DPV) measurements were performed *in vivo* on freely moving animals using a PRG5 polarograph (Taccussel, France), and a classical three-electrode potentiostatic system (Forni & Nieoullon, 1984). Multifiber working carbon electrodes were made according to the method described elsewhere (Forni 1982, Lavoute *et al*. 2006). One week after surgery, recordings at atmospheric pressure were made in order to stabilize the electrode's basal response to dopamine. The animals were connected to the polarograph through a flexible cable and a swivel connector, and the polarograph was set to the following parameters: scan rate 20 mV/s; voltage range 0-1000 mV; pulse modulation amplitude 50 mV; pulse modulation duration 48 ms; pulse period 0.2 s. Electrochemical signals were amplified (x10) and recorded every 3 min, a period chosen to avoid exhausting the medium. A computer controlled the polarograph and automatically quantified the dopamine release by measuring the amplitude of the DA oxidation peak, which is related to its extracellular concentration. Electrochemical responses were obtained around 180 mV, corresponding to *in vitro* calibration to extracellular DA concentrations ranging from 5×10^{-9} to 5×10^{-8} M.

Pressure exposure

Freely moving rats were placed in separate altuglass cylinders in a 50-L pressure chamber in which a 12-12 h light-dark regime was maintained. Basal values of extracellular dopamine concentration were performed during a 60 min period at atmospheric pressure, before treatment or exposures to nitrogen. Nitrogen

narcosis was studied at 3 MPa (75% of the threshold anesthetic pressure with nitrogen in rats, Abraini *et al*, 1998). This relative pressure would correspond to a narcotic level equivalent in men to 0.8-0.9 MPa as reported by the loss of consciousness around 1.2 MPa when the compression is not too rapid to permit the critical concentration of nitrogen necessary for narcosis to accumulate in the brain (Hill *et al*, 1933, for review Bennett and Rostain, 2003). Moreover, at this pressure, nitrogen narcosis symptoms are well established, as well as changes in striatal dopamine release. After 2 resting days, animals were exposed for 5 consecutive days to one daily exposures to 1 MPa of nitrogen-oxygen mixture (25% of the threshold anesthetic pressure in rat; level of narcosis recorded at 0.3-0.4 MPa in men). This protocol requires a night rest for the animals since a safe decompression lasts 3-4h. Compression rates were 0.01 MPa/min up to 0.1 MPa for the first 10 min, then at a rate of 0.1 MPa/min up to 1 or 3 MPa. Oxygen partial pressure was controlled and maintained at a constant partial pressure of 0.04 MPa (400 mbar) whereas carbon dioxide was kept at a value less than 0.0003 %, humidity was controlled between 40% and 60 % and the temperature was progressively increased (27-28 °C) to maintain the thermal comfort of the animals. Animals remained 3 MPa and 1 MPa for 2 h and 40 min, respectively. Animals were decompressed at a rate of 0.005 MPa/min with an oxygen partial pressure of 0.05 MPa.

Pharmacological protocol and drugs

Drugs were delivered in 0.5 µl PBS (calcium- and magnesium- free) at a rate of 0.1 µl/min, through a pre-implanted stainless steel cannula connected via a catheter to a Hamilton micro-syringe (25µl) driven by a microdrive unit. The following drugs (Sigma RBI) were used and injected bilaterally through each cannula in the SNc: 0.5 nmol for N-methyl-D-aspartate (NMDA receptor agonist); 1 nmol for 2-amino-7-phosphonoheptanoic acid (AP7; NMDA receptor antagonist). Control animals received the vehicle PBS alone. Drug effects were analysed for a period of 2h.

Statistical Analysis

Neurochemical changes were expressed as a percentage of the basal dopamine release recorded during a 60 min control period before drug injection and/or nitrogen exposure. Statistical comparisons of dopamine concentrations between groups pre-treated with drugs under normobaric conditions before and after repetitive exposure to 1 MPa nitrogen or exposed to 3MPa nitrogen were performed using the H Kruskal-Wallis analysis of variance by rank; given a significant H value, post-hoc comparisons were made using the Mann-Whitney Rank Sum test (U-test).

RESULTS

Normobaric conditions (Table 1)

NMDA administration: At atmospheric pressure before any nitrogen exposure (atmospheric 1), administration of 0.5 nmol of NMDA produced a modest increase in dopamine concentration (5%) as compared to the basal value. After repetitive exposures to nitrogen narcosis (atmospheric 2), a similar dose of NMDA produced a greater increase ($p<0.01$) in dopamine concentration (+20%).

AP7 administration: At atmospheric pressure before any nitrogen exposure (atmospheric 1), administration of 1 nmol AP7, a specific antagonist at NMDA receptors, produced a significant decrease in striatal dopamine release (-20%), compared to basal value. In contrast, after repetitive exposures to nitrogen (atmospheric 2), AP7 induced a significant ($p<0.05$) increase (13%) in striatal dopamine release.

Treatment	DA response to drug (%)	n
Atmospheric 1		
+ *PBS*	No effect	7
+ *NMDA*	+ 5 ±0.1*	5
+ *AP7*	- 18.9 ±3.6**	4
Atmospheric 2		
+ PBS	No effect	5
+ NMDA	+ 23.6 ±2.4**	5
+ AP7	+ 13.5 ±2.8*	4

Table 1. DA response to drug injections in normobaric condition before (atmospheric 1) and after (atmospheric 2) repetitive exposures. Results are expressed as percentage compared to basal value. $^{*}p<0.05$; $^{**}p<0.01$

Nitrogen exposures to 3MPa (Table 2)

NMDA administration: Under a single exposure to nitrogen pressure (3MPa), administration of 0.5 nmol of NMDA reversed the nitrogen-induced decrease in dopamine concentration. Dopamine release following administration of NMDA was significantly ($p<0.05$) enhanced (+20%) under nitrogen exposure compared to that obtained in the presence of 3 MPa nitrogen.

AP7 administration: Under a single exposure to nitrogen pressure (3MPa), administration of 1 nmol AP7 reversed the nitrogen-induced decrease in dopamine release, resulting in an increase of 10%. Thus, in contrast to its decreasing effect at atmospheric pressure before any exposure to nitrogen, AP7 induced an increase of the striatal dopamine level. The effect of the drug was significantly different to the nitrogen effect alone.

Treatment	DA response (%)	Amplitude of DA response compared to nitrogen	n
Nitrogen	-17.4 ±5.3***		10
+ *PBS*	-18.5 ±3.1***	- 1.1	7
+ *NMDA*	1.3 % ±2.1***	+ 18.7	4
+ *AP7*	12.5 % ±4.5***	+ 29.9	4

Table 2. DA response under nitrogen exposure to 3MPa, with or without pre-treatment to drugs. In column 2, results are expressed as percentage compared to basal value in normobaric condition. In column 3, results are expressed as the difference compared to the nitrogen effect.

DISCUSSION

The present study provides a pharmacological analysis of NMDA receptor function at atmospheric pressure, before and after repetitive nitrogen pressure (1MPa) exposure as compared to NMDA function under a single exposure to 3MPa nitrogen. Our data indicate that, in the substantia nigra pars compacta, there is a reduced glutamatergic input to DA cells while postsynaptic NMDA receptors were sensitized to agonist.

NMDA receptor functions

At atmospheric pressure, specific agonists and antagonists would act on postsynaptic NMDA receptors located on dopaminergic cells. In accordance with the literature, activation or blockade of these receptors produced, respectively, an increase and a decrease of striatal dopamine release.

Under a single exposure to nitrogen pressure at 3MPa, we observe an increased sensitivity of NMDA receptors to the agonist (+ 20% as compared to +5% in atmospheric pressure), revealing functional NMDA receptors (Lavoute *et al*, 2006). Such hyperexcitability of NMDA receptors is described under high pressure helium exposure attributed to an effect of the pressure *per se* (Mor and Grossman,

2007). In contrast to its decreasing effect (-20%) at atmospheric pressure, AP7 produce a significant increase in DA release (+10%), previously attributed to the inefficiency of the drug at blocking the glutamatergic input, perhaps due to a depressant action of nitrogen, at the presynaptic site, on the glutamate release in SNc (Lavoute *et al*, 2007).

Interestingly, following successive exposures to nitrogen narcosis, the enhancing effect on NMDA activation by the agonist is maintained under atmospheric pressure, as well as the reversal effect of AP7. Thus, nitrogen may profoundly disrupt the glutamatergic input in SNc by inducing a decrease in glutamate release, which may cause up-regulation of NMDA receptors following repetitive exposures.

In conclusion, repetitive nitrogen pressure exposures induced neurochemical changes involving NMDA receptor function, leading to a persistent decrease in glutamate release and NMDA hyper-excitability in normobaric conditions. Thus, basal dopaminergic pathway activity could be strongly disturbed. Further experiments are necessary to investigate the long-term effects of nitrogen exposures as well as the involvement of pressure *per se*.

ACKNOWLEDGMENTS

This study was supported by PEA n°010809, Research grant from the Délégation Générale de l'Armement, Paris, France. Cécile Lavoute was supported by the Society of Physiology and Hyperbaric and Subaquatic Medicine of French language.

REFERENCES

1. Abraini JH, Rostain JC, Kriem B (1998) Sigmoidal compression rate-dependence of inert gas narcotic potency in rats: implication for lipid vs. protein theories of inert gas action in the central nervous system. *Brain Res* 19: 300-304.
2. Bennett PB, Rostain JC (2003) Inert Gas Narcosis. In Brubakk AO and Neuman TS (eds) Bennett and Elliott's Physiology and Medecine of Diving. Saunders Compagny Ltd, London, pp 300-322.
3. Christoffersen CL, Meltzer LT (1995) Evidence for N-methyl-D-aspartate and AMPA subtypes of the glutamate receptor on substantia nigra dopamine neurons: possible preferential role for N-methyl-D-aspartate receptors. *Neuroscience* 67:373-381.
4. Forni C (1982) Realization of a new multifiber electrochemical device allowing continuous in vivo measurements of neuromediators. *J Neurosci Methods*, 5, 167-171.
5. Hill L, Davis RH, Selby RP, Pridham, A., Malone, A.E., 1933. Deep diving and ordinary diving. In: report of a Committee Appointed by British Admiralty.
6. Lavoute C, Weiss M, Rostain JC (2005) Effects of repeated hyperbaric nitrogen-oxygen exposures on the striatal dopamine release and on motor disturbances in rats. *Brain Res*. 1056, 36-42.
7. Lavoute C, Weiss M, Rostain JC (2006) Effects of NMDA administration in the substantia nigra pars compacta on the striatal dopamine release before and after repetitive exposures to nitrogen narcosis in rats. *Undersea Hyperb Med.* 33, 175-179.
8. Lavoute C, Weiss M, Rostain JC (2007) The role of NMDA and GABAA receptors in the inhibiting effect of 3MPa nitrogen on striatal dopamine level. *Brain Res* 1176: 22-27.
9. Mor A, Grossman Y (2007) High pressure modulation of NMDA receptor dependent excitability. *Eur J Neurosci.* 25: 2045-2052.
10. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic press, New York.