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Brain Research 1056 (2005) 36 – 42

BRAIN RESEARCH

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Research Report

Effects of repeated hyperbaric nitrogen–oxygen exposures on the striatal dopamine release and on motor disturbances in rats

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Accepted 10 July 2005
Available online 19 August 2005

Abstract

Previous studies have demonstrated disruptions of motor activities and a decrease of extracellular dopamine level in the striatum of rats exposed to high pressure of nitrogen. Men exposed to nitrogen pressure develop also motor and cognitive disturbances related to inert gas narcosis. After repetitive exposures, adaptation to narcosis was subjectively reported. To study the effects of repetitive exposures to hyperbaric nitrogen–oxygen, male Sprague–Dawley rats were implanted in the striatum with multifiber carbon dopamine-sensitive electrodes. After recovery from surgery, free-moving rats were exposed for 2 h up to 3 MPa of nitrogen–oxygen mixture before and after one daily exposure to 1 MPa of nitrogen–oxygen, for 5 consecutive days. Dopamine release was measured by differential pulse voltammetry and motor activities were quantified using piezo-electric captor. At the first exposure to 3 MPa, the striatal dopamine level decreased during the compression (−15%) to reach −20% during the stay at 3 MPa. Motor activities were increased during compression (+15%) and the first 60 min at constant pressure (+10%). In contrast, at the second exposure to 3 MPa, an increase of dopamine of +15% was obtained during the whole exposure. However, total motor activities remained unchanged as compared to the first exposure. Our results confirm that nitrogen exposure at 3 MPa led to a decreased striatal dopamine release and increased motor disturbances in naïve rats. Repetitive exposures to 1 MPa of nitrogen induced a reversal effect on the dopamine release which suggests a neurochemical change at the level of the neurotransmitter regulation processes of the basal ganglia. In contrast, motor activity remained quantitatively unchanged, thus suggesting that dopamine is not involved alone in modulating these motor disturbances.

Theme: Neural basis of behavior
Topic: Monoamines and behavior

Keywords: Basal ganglia; Monoamine; Inert gas narcosis; Voltammetry; Actimetry; Neurotoxicity; Adaptation

1. Introduction

Previous studies have demonstrated disruptions of motor activities and a decrease of extracellular dopamine level in the striatum of rats exposed to high pressure of nitrogen [2,6,25]. Men exposed to increase pressure of nitrogen develop also from 0.3 MPa, motor and cognitive disturbances such as impaired judgement, loss of memory, spatial disorientation and impaired neuromuscular coordination [9], leading ultimately to a loss of consciousness at 1 MPa related to inert gas narcosis [12]. However, adaptation to signs and symptoms of narcosis were subjectively reported after repetitive exposures to nitrogen narcosis [9].

A link between narcotic potency, anaesthetic power and lipid solubility of nitrogen, and more generally of inert gases, has been established [for review [18]. Narcotic effects would be directly related to the increase of partial pressure of the inert gas [8]. Under pressure, nitrogen would dissolve

Abbreviations: DA, dopamine; NMDA, *N*-methyl-D-aspartate; MPa, Megapascal

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doi:10.1016/j.brainres.2005.07.004

in the phospholipidic membrane of the neural cell and expand its volume [19]. This volume increase could disrupt the membrane environment of some receptors and thus modify their responsiveness. Moreover, recent researches have suggested a binding between gas and protein of the receptor which induced changes in their activities [1,13].

In rats, first narcotic symptoms appear around 1 MPa and loss of consciousness, as measured by the loss of the righting reflex, at 4 MPa [1]. Several studies on both the behavioral [5] and neurochemical effects [2,6,11] of hyperbaric nitrogen at 3 MPa, which corresponds to 75% of its anaesthetic pressure in rats, indicated an increased of motor activity disturbances and a decreased dopamine (DA) release in the striatum, a structure involved in the regulation of extrapyramidal motricity.

However, there are no basic studies and no statistical data about the effects of successive exposures to pressure that induces narcosis in humans. Some studies failed to detect an improvement of motor disturbances, following 5 successive exposures to relative pressure of 0.55 MPa [17]).

In order to study the effects of repetitive exposures to nitrogen pressure, actimetric measurements of locomotor and motor activities and electrochemical analysis of the extracellular striatal dopamine release at 3 MPa, equivalent to a narcosis level in man at 0.7 to 0.8 MPa, were performed in rats, before and after five successive dives at 1 MPa, equivalent to a narcosis level in man at 0.3–0.4 MPa.

2. Materials and methods

2.1. Animals preparation and surgery

Male Sprague–Dawley rats ($n = 11$) weighting 300 ± 20 g at the time of the surgery were housed at 22 ± 0.5 °C in individual home cages under a 12:12 h light–dark cycle (with lights on from 07:00 to 19:00 h) with free access to food and water.

Under general anesthesia (halothane, pentobarbital sodium 30 mg/kg i.p. and ketamine 100 mg/kg i.m.), rats were stereotaxically implanted with DA sensitive electrodes in the dorsal striatum (A: 10.2, L: 2.3, H: 4.8) according to the rat atlas of Paxinos and Watson [20]. Reference and auxiliary electrodes (stainless steel screws; copped wire) were fixed on the bone. The electrodes were attached to a miniconnector, and the whole assembly was held in place with dental cement (Unifast Trade).

2.2. Electrochemical measurements of the striatal DA release

Differential pulse voltammetric (DPV) measurements were performed in vivo on unrestrained awake animals using a PRG5 polarograph (Taccussel, France), and a classical three electrodes potentiostatic system with reference, auxiliary and working carbon electrodes [14].

Multifiber working carbon electrodes were made from a rigid rod of 10^4 carbon fibers sharpened at one extremity to reduce the external diameter of the electrode from 1 mm to 50 μm at the tip. The surface of the largest extremity was covered with conducting epoxy resin infused with silver (Elecolit). The entire electrode was encased in an insulating resin (Araldite standard) and the tip was exposed using an abrasive disc to shape the active surface of the electrode. Before use, working electrodes were electrochemically treated, by applying a triangular wave potential of 0–3 V, 70 Hz, 15 s, with 10 dB attenuation, to increase their sensitivity to DA [14]. As previously reported, no detectable signal was obtained in 3,4 dihydroxyphenylacetic (DOPAC), ascorbic acid (AA), uric acid (UA) or homovanilic acid (HVA) solutions of 10^{-6} to 10^{-3} M [23].

After surgery, animals were allowed to recover for 1 week before being submitted to recordings at atmospheric pressure in order to permit the stabilization of electrodes. 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 (×10) and recorded every 3 min. A computer piloted the polarograph and DA release was quantified automatically by measuring the amplitude of the DA oxidation peak, which is related to its extracellular concentration, using a computerized device. Electrochemical responses were obtained around 180 mV, corresponding from in vitro calibration to extracellular DA concentrations ranging from 5.10^{-9} to 5.10^{-8} M.

2.3. Pressure exposure

During pressure exposure, the 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.

After stabilization of the working electrode, measurements of extracellular dopamine concentration at atmospheric pressure were performed before exposures to nitrogen to control the variability of dopamine level. After the first exposure to nitrogen–oxygen mixture at a pressure of 3 MPa (which correspond to 75% of the threshold pressure of anesthesia in rat and is equivalent to a narcosis level in man at 0.7 to 0.8 MPa, (70–80 m of sea water), animals were submitted for 5 consecutive days to one daily exposure at 1 MPa of nitrogen–oxygen mixture, corresponding to a level of narcosis recorded at 0.3–0.4 MPa (30–40 m) in man. Following this protocol of repetitive nitrogen exposures, a second exposure to 3 MPa permitted to evaluate the eventual changes induced by successive dives at 1 MPa.

Compression rates were of 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. As classically described, oxygen partial