The effect of high pressure on the kinetics of monoamine transmitters in the rat brain

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Aanderud L. Broch O J. The effect of high pressure on the kinetics of monoamine transmitters in the rat brain. Undersea Biomed Res 1987;14(2):85-91.—The effects of 71 ATA pressure on the turnover of serotonin (5-HT), noradrenaline (NA), and dopamine (DA) were studied. High pressure increased the concentration of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) by 40%. A concomitant increase in 5-HT synthesis (measured by the accumulation of 5-hydroxytryptophan after inhibition of the decarboxylase) of 25% was found. The changes in NA and DA metabolites and synthesis were smaller and not statistically significant. The increase in 5-HT synthesis was not a result of changes in the neuronal uptake of transmitter. The observed change might be the result of a compensatory increase in the activity of the serotoninergic neurons at high pressure. Among the three monoamine transmitter systems, that mediated by 5-HT may be most important in the pathogenesis of the high pressure neurologic syndrome.

monoamines
rat brain
high pressure neurologic syndrome

Exposure of man and animals to increased ambient pressure gives rise to a series of manifestations known as the hyperbaric pressure neurologic syndrome (HPNS). This syndrome is characterized by muscular tremor, incoordination, myoclonic jerks, and eventually convulsions (1). Bouts of microsleep are also experienced in humans exposed to high pressure (2). Characteristic changes in the EEG are increase in the slow wave activity and depression of the alpha activity (3, 4). The emergence of HPNS is also modified by the rate of compression (5). The cause and underlying mechanisms of HPNS are unknown and it is not certain whether further exposure to high pressure would reduce the symptoms.

Another peculiar phenomenon occurring at high pressure is the decreased narcotic effect of most anesthetic agents (6). These alterations in the functions of the central nervous system may be due to changes in the synaptic activity induced by the high pressure and there may be a connection between this phenomenon and HPNS.
Studies on the involvement of transmitters in the symptomatology of HPNS have concentrated on the monoamines. Koblin et al. (7) found a 30–40% increase in the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brains of mice exposed to 100 ATA, but no changes in the concentrations of serotonin (5-HT), noradrenaline (NA), or dopamine (DA).

Halsey et al. (6) reported that the anesthetic drug ketamine protected rats from convulsions induced by high pressure. The agent decreased 5-HT synthesis (8), probably secondary to the reported inhibition of neuronal uptake of 5-HT (9), with a resulting increase in the activity of the 5-HT neurons. Depletion of 5-HT, NA, and DA with reserpine decreased the threshold of HPNS (10). Koblin et al. (7) confirmed the results from the reserpine study, but 5-HT depletion by p-chlorophenylalanine or inhibition of NA and DA synthesis by α-methyl-p-tyrosine did not affect the response of animals to pressure. However, a depletion of NA only, by means of FLA-63, lowered the threshold to some extent. According to this, depletion of NA, and to a lesser degree of DA, by intraventricular injection of 6-hydroxydopamine had some modifying influence on HPNS (11).

A summary of the effects of various drugs on the monoamines in the brain and the connection with the HPNS is given in Table 1. The purpose of the present investigation was to study the influence of 71 ATA He-O₂ on the synthesis and metabolism of the monoamines in the rat brain. The observed increase in 5-HIAA could result from (a) an increased 5-HT synthesis possibly secondary to changes in the neuronal uptake of the amine; or (b) a reduction in the transport of 5-HIAA out of the brain.

Moreover, the effect of high pressure on the DA and NA metabolites has not been studied to our knowledge.

MATERIALS AND METHODS

Male rats (mol: Wistar) weighing 230–300 g were used. On the day before the experiments the animals were anesthetized with 1 ml/kg Hypnorm R, i.p. (fluanisone 10 mg and fentanyl 0.315 mg/ml, Mekos, Helsingborg, Sweden). A PE 50 polyethylene catheter (Intramedic, Clay Adams, Parsippany, NJ) was then inserted into the femoral

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percent of Values in Untreated Animals (7)</th>
<th>Convulsions at Relative Pressure, % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-HT DA NA</td>
<td></td>
</tr>
<tr>
<td>Reserpine</td>
<td>40 35 40</td>
<td>47</td>
</tr>
<tr>
<td>p-Chlorophenylalanine</td>
<td>60 90 120</td>
<td>95</td>
</tr>
<tr>
<td>α-Methyl-p-tyrosine</td>
<td>110 60 35</td>
<td>120</td>
</tr>
<tr>
<td>FLA-63</td>
<td>100 115 45</td>
<td>80</td>
</tr>
<tr>
<td>6-Hydroxydopamine (11)**</td>
<td>100 55 30</td>
<td>75</td>
</tr>
</tbody>
</table>

*Data taken from literature (7, 11).  **5-HT and NA were not measured in this paper but have been estimated from other works dealing with the action of intracerebral injections of 6-hydroxydopamine.
HIGH PRESSURE AND BRAIN MONOAMINE KINETICS

vein, led subcutaneously to a pocket under the skin on the back, filled with heparinized saline, and sealed. Bilateral paracentesis was performed to reduce possible ear pain during the pressure experiments.

At the start of the experiment the wound on the back was opened, the catheter exposed, and the rat restrained in a Plexiglas tube which was introduced into a 4-liter pressure chamber. The catheter was connected to an injection system described elsewhere (12). In brief, the chamber was equipped with atmospheric and rectal temperature, monitoring systems, CO₂ and O₂ monitors, and gas mixing, scrubber, and heating equipment. The injections through the intravenous catheter were performed from outside of the chamber by means of a high performance liquid chromatography (HPLC) injector. The period of restraint in the chamber was similar for all groups of animals, 4.5 h.

Chamber procedures

Rats were exposed singly to the experimental procedure. At 1 ATA, the sealed chamber was flushed with oxygen to produce 0.5 ATA O₂ in nitrogen. At 71 ATA, the chamber was sealed and flushed with oxygen until a partial pressure of 0.5 ATA was reached. The compression was then started with pure helium at a rate of 0.3 ATA/min. The atmosphere was continually monitored by means of mass spectrometry (Hewlett Packard) to keep the partial pressure of oxygen between 0.4 and 0.6 ATA and the CO₂ concentration below 0.01 vol/vol (5.6 Torr).

The chamber temperature was kept between 32 and 35°C, and the animals maintained a rectal temperature between 37 and 38°C during the period of exposure.

After the experiments the animals were killed by rapid decompression (less than a minute). Animals that had not been exposed to pressure were killed with a rapid i.v. injection of KCl.

Synthesis experiments

Monoamine synthesis was studied by measurement of the accumulation of monoamine precursors [5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (DOPA)] in the brain after inhibition of the aromatic amino acid decarboxylase (13).

After 4.5 h at ambient pressure or after 3.5 h of compression and 1 h at 71 ATA, the decarboxylase inhibitor m-hydroxybenzylhydrazine hydrochloride (75 mg/kg) was infused i.v. during 3 min. After 30 min the animals were killed.

Tissue preparation

Immediately after the pressure exposure the brains of exposed animals were removed, rinsed with isotonic saline, blotted, and kept frozen at −80°C until analysis.

Chemical analyses

The monoamine metabolites and the amino acids were determined by means of isocratic HPLC with a 20-cm Supersil ODS 10 μm reversed-phase column. The detector was a BAS electrochemical detector with a glassy carbon electrode. The potential was set at +0.7 V (sensitivity range 200 nA, reference electrode AgCl).
Determination of monoamine metabolites

Homovanillic acid (HVA), 5-HIAA, and total (free and conjugated) 3-methoxy, 4-hydroxy-phenylglycol (MOPEG) were determined in one hemisphere which was homogenized in 6 ml 1 M HCl/g tissue.

HVA and 5-HIAA were measured in 1.5 ml of the homogenate, after the addition of 2.5 mg sodium bisulfite and 50 µl concentrated perchloric acid. The extract was centrifuged, neutralized with 5 M KOH, and centrifuged again. Twenty microliter of the supernatant fluid was injected into the column.

MOPEG was determined in 1 ml of the homogenate, which was centrifuged after the addition of 0.25 mg ascorbic acid. The pH of the supernatant was adjusted to 4.0 with 5 M KOH and it was incubated overnight at 37°C with 1 ml of 0.2 M acetate buffer, pH 4.8, containing 1 µl Glusulase (Endo Laboratories, NY) and 0.1 mg saccharic acid (1,4) lactone (inhibiting the glucuronidase to avoid an interfering peak in the chromatogram). After incubation, the sample was extracted twice with 10 ml ethyl ether. The ether was evaporated under a stream of nitrogen before injection into the column. The mobile phase for the metabolites was 0.1 M Na acetate buffer with 1 mM EDTA and 5% methanol, flow rate 1.5 ml/min. Recovery for MOPEG added to a tissue sample was 60%.

Determinations of amino acids

5-HTP and DOPA were extracted from the forebrains by homogenization in 10 vol of n-butanol containing 0.85 ml concentrated HCl/liter. After centrifugation, 0.5 ml of the butanol extract was added to 1 ml ethylbutyl ketone containing 5 mg sodium tetrathyleneboron (Kalinows, Merck, Darmstadt, W. Germany), and the amino acids were then extracted into 0.2 ml phosphate buffer 0.1 M, pH 7.0. The buffer (20 µl) was injected into the column. The mobile phase was a 0.05 M Na acetate buffer with pH 4.6 containing 1 mM EDTA. Kalinows removed noradrenaline which would otherwise give rise to a peak in the chromatogram interfering with DOPA. The recovery for DOPA was 90% and for 5-HTP 72% for standards added to homogenates from untreated animals.

Monoamine uptake in vitro

The forebrain from an untreated rat was homogenized in 10 vol of 0.32 M sucrose. A crude synaptosomal-mitochondrial fraction was prepared by centrifugation for 10 min at 1000 g. A sample of the supernatant was incubated in Krebs' solution containing 0.14 mM pargyline (monoamine oxidase inhibitor) and radioactive NA, DA, or 5-HT at 30°C for 15 min in a high pressure chamber with continuous shaking. A pressure of 71 ATA was reached within 1 min, and the temperature in the chamber was kept between 29.5 and 30.5°C during the incubation. After rapid decompression, the samples were cooled immediately to 0°C and centrifuged in the cold at 2500 g. After removal of the supernatant and rinsing, the radioactivity of the pellets was counted and compared to the protein contents. A similar procedure was followed for the control incubations at 1 ATA. The integrity of synaptosomal preparations was controlled with electron microscopy and measurements of lactic acid dehydrogenase
leakage from pellets. No differences were observed between the controls and the preparations exposed to pressure.

RESULTS

At 71 ATA 5-HIAA concentration increased by approximately 40% whereas only small increases were found for the HVA (17%) and total MOPEG (13%). Only the values for the increase in 5-HIAA were significant (Table 2).

At high pressure, 5-HT synthesis also increased, as may be seen from the accumulation of 5-HTP after decarboxylase inhibition. Synthesis was about 25% higher than at atmospheric pressure. The increase in DOPA accumulation was smaller and again nonsignificant (Table 3). High pressure had no effect on the uptake of noradrenaline, serotonin, or dopamine (results not shown).

DISCUSSION

The most striking change in the present study was the increase in 5-HIAA at pressure. This has also been found by other authors (7, 11). The 40% increase in the metabolite observed in this study could well be due to an observed 25% increase in 5-HT synthesis, an observation that has not been published before to our knowledge. We also showed that there was little or no change in DA and NA metabolism. Rapid decompression destroyed the brains and made them unsuitable for accurate dissection. Otherwise regions with mostly dopaminergic or noradrenergic neurons could

| **TABLE 2** |
| MONOAMINE METABOLITES IN THE RAT BRAIN AT 1 AND 71 ATA |
| Levels, nmol/g (means ± SEM) |
| 1 ATA (n = 10) | 71 ATA (n = 14) |
| 5-HIAA | 1.58 ± 0.10 | 2.21* ± 0.11 |
| HVA | 0.59 ± 0.13 | 0.69 ± 0.12 |
| MOPEG** | 0.24 ± 0.03 | 0.27 ± 0.02 |

*Significant change (P < 0.01, t test). **Free and conjugated (total MOPEG).

| **TABLE 3** |
| MONOAMINE PRECURSORS IN THE RAT BRAIN AT 1 AND 71 ATA |
| AFTER DECARBOXYLASE INHIBITION |
| Levels, nmol/g (means ± SEM) |
| 1 ATA (n = 5) | 71 ATA (n = 5) |
| 5-HTP | 0.41 ± 0.02 | 0.52 ± 0.02* |
| DOPA | 0.82 ± 0.06 | 0.99 ± 0.07 |

*Significant change (P < 0.01, t test).
have been isolated and made possible a separate determination of DA and NA synthesis.

Since no change was found in the 5-HT concentration at high pressure (7), it is conceivable that the increase in 5-HIAA was due to an increase in the activity of 5-HT neurons with a resulting increase in the transmitter synthesis and turnover. Bearing in mind that ketamine increased the threshold for HPNS (1) and that monoamine depletion lowered it (7, 10), it is possible that the increase in synthesis represents a compensatory mechanism counteracting the deleterious effects of high pressure (10). 5-HT has been implicated in the mechanism of sleep, but increased (14, 15) as well as decreased (16, 17) serotonergic activity have been reported. Microsleep is a well-known feature of HPNS.

Changes in metabolism might be the result of altered reuptake of the monoamines. However, no such alteration was found in the in vitro experiment. Although not fully representative, the experiment gave some indication that the reuptake was not changed.

It was considered unlikely that the excretion of 5-HIAA from the brain was reduced during exposure to high pressure. Increased brain uptake of dye and tetracycline has been reported in rabbits after hyperbaric excursions (18, 19). The brain uptake of thiopental and diazepam in the early distribution phase after i.v. injection in the rat was decreased at 71 ATA (20). However, transport of these substances into the brain is mediated by mechanisms different from the excretion of the monoamine metabolites (21). Penicillin is excreted from the brain by means of an energy-requiring, probenecid-sensitive mechanism (22) as is 5-HIAA (23), HVA (24), and MOPEG sulphate (25). Studies with penicillin infusions in rats exposed to 71 ATA He-O2 have shown that the blood:brain penicillin ratio remains unaltered (26).

In conclusion, the present results indicate an increased 5-HT turnover which may represent an adaptive inhibitory mechanism in the brain of rats exposed to 71 ATA of pressure.

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monoamines
cerveau du rat
syndrome neurologique des hautes pressions

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HIGH PRESSURE AND BRAIN MONOAMINE KINETICS


