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BRIEF COMMUNICATION

Interaction of High Pressure and a Narcotic Dose of Ethanol on Spontaneous Behavior in Rats

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GARCIA-CABRERA, I. AND O.-G. BERGE. *Interaction of high pressure and a narcotic dose of ethanol on spontaneous behavior in rats*. PHARMACOL BIOCHEM BEHAV **37**(3) 577–581, 1990.—This study analyses the spontaneous motor activity of rats that had received a narcotic dose of ethanol (3.5 g/kg) and were then exposed to 1 atmosphere absolute pressure (ATA) air or to 1 or 72 ATA of helium-oxygen (heliox). The ambient temperature was adjusted to offset ethanol- and helium-induced hypothermia. Ethanol administration prevented the occurrence of convulsions but did not alter the total number of myoclonic jerks at stable pressure. The ethanol-intoxicated animals exposed to high pressure did not exhibit normal locomotion but showed a trend towards increased activity during the last observation period. Similar blood and brain concentrations of ethanol were found in the 1 and 72 ATA groups. These results show that exposure to 72 ATA for 40 min started to exert some antagonistic effects, and they suggest that exposure to higher pressures or for a longer period of time may be sufficient to significantly offset the depressant effects of a narcotic dose of ethanol on spontaneous behavior in rats. At the same time, ethanol seems to protect against some aversive effects of high pressure.

Hyperbaric environment Pressure reversal Rats Ethanol intoxication Convulsions Blood and brain ethanol

THE reversal of anesthesia by high pressure was first described in luminescent bacteria and tadpoles (15,16), and these results were confirmed and extended (13). It was later demonstrated that a variety of intravenous anesthetics must be given in higher doses in order to maintain anesthesia in rats under high pressure (4,14).

Exposure to high pressure induces a number of neurological symptoms known as the high pressure nervous syndrome (HPNS); in animals, this syndrome is characterized by tremor, myoclonic jerks and alterations in the electroencephalogram. If pressure is further increased, generalized convulsions occur (12). While it seems that high pressure usually opposes the sedative actions of anesthetics, not all of the anesthetics tested counteract the effects of high pressure (12,23). Furthermore, there is no correlation between pressure reversal of an anesthetic action and the potency of the anesthetic in opposing pressure effects.

Recently, interest in the ameliorative effects of hyperbaric exposure on acute ethanol intoxication has increased. It has been reported that moderate increases in ambient pressure (4 to 12 ATA) antagonize the acute depressant effects of ethanol on the righting reflex in mice (1–3). Exposure to 12 ATA also antagonizes the decrease in locomotor activity induced by ethanol (20). Our previous work has shown that the depressant effects of a moderate dose of ethanol (1.5 g/kg) on spontaneous behavior could be counteracted by exposure to 48 ATA (10). On the other hand, the effects of a narcotic dose of ethanol (3.5 g/kg) could not be counteracted by the same hyperbaric exposure (9). We wanted to further clarify whether these effects are dose- and/or pressure-dependent. In this study, therefore, we used a narcotic dose of ethanol and exposure to a higher level of pressure, 72 ATA. The possible protective action of ethanol against the adverse effects of high pressure was also examined. The effects of ethanol were assessed by scoring spontaneous motor activity. Blood and brain concentrations of ethanol were measured in order to check for alterations in ethanol distribution or elimination following exposure to hyperbaric heliox.

METHOD

Subjects

A total of forty-two drug-naive male Sprague-Dawley rats (Møllegård, Denmark), weighing 290–340 g at the beginning of the experiment, were used. The animals were maintained at an

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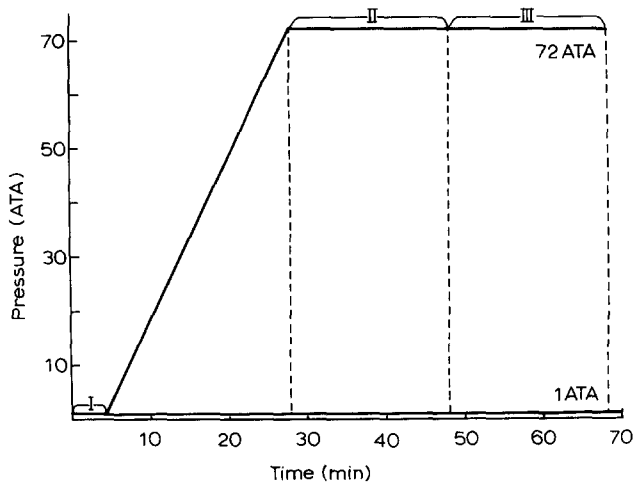

FIG. 1. Pressure profiles. Time of injection = 0 min. Compression started at 4 min 20 sec and target pressure was reached 28 min after injection in the 72 ATA groups. I = Observation period I (precompression period, 0 to 4 min 20 sec). II = Observation period II (28 to 48 min). III = Observation period III (48 to 68 min).

ambient temperature of 22–23°C, under a 12:12-hr light cycle with lights on at 06:00. All experiments took place between 08:30 and 15:00 and the groups were systematically rotated across days and with regard to time of day.

The Hyperbaric Chamber

The experiments were carried out in a 24.5-liter steel chamber, which was equipped with temperature- and pressure-monitoring systems, fan, CO_2 scrubber and heating system as described previously (10). A window at one end of the chamber permitted videotaping of the animals' behaviour. The chamber temperature was adjusted to offset ethanol- and helium-induced hypothermia on the basis of previous results (6, 18, 19). The temperature settings were: 26 ± 1°C for 1 ATA air, 30 ± 1°C for 1 ATA heliox and 34 ± 1°C for the groups under pressure. The partial pressure of oxygen was maintained at between 0.2 and 0.4 ATA.

Procedure

Rats were injected intraperitoneally with 3.5 g/kg ethanol [21 ml/kg of a 21% (v/v) ethanol/isotonic saline solution]. Immediately after injection, the animals were placed in the chamber and exposed to 1 ATA air or to 1 or 72 ATA of a 80% helium/20% oxygen mixture (heliox). An additional control group of saline-injected rats was pressurized to 72 ATA. Saline control groups for 1 ATA air or 1 ATA heliox were not included since data from a previous study were available (10). The chamber was initially purged for 140 sec with either air or heliox. Compression (3 ATA/min) started 4 min 20 sec after the beginning of each experiment. The 72 ATA groups thus reached the target pressure 28 min after injection. All animals remained at the target pressure for 40 min. Figure 1 shows the compression profile and the observation periods for the behavioral analysis.

Behavioral Observations

In the chamber, the rats were free to move within an area of 210×220 mm. Behavioral analysis was performed blind with the aid of a computer programme. The number of myoclonic jerks and the duration of all additional motor activity were recorded. The total motor activity (accumulated time) consisted of the following discrete and mutually exclusive categories: 1) Movements restricted to the head. 2) Moving—movements of other parts of the body not included in categories 3 to 7. 3) Normal locomotor activity—normal walking or running. 4) Staggering—uncoordinated locomotor activity. 5) Grooming—licking or rubbing the fur. 6) "Wet-dog" shaking. 7) Rearing—front part of the body raised off the floor.

The total motor activity was expressed in percent of the available time in each observation period. Since physical manipulation of the animals in the chamber was not possible, the time of recovery or "sleeping time" was estimated by measuring the time from complete cessation of motor activity until the first signs of activity occurred in the intoxicated animals. If the animals convulsed, the experiment was immediately stopped and the data were excluded from the behavioral analysis.

Measurement of Blood and Brain Concentration of Ethanol

Sixty-eight minutes after the beginning of the experiment, the ethanol-injected rats were anesthetized by admitting N_2O to the pressure chamber, and then rapidly decompressed. They were removed from the chamber and decapitated. Brain and blood samples were collected 80 min after injection. Ethanol concentrations were determined enzymatically using the ADH/NAD technique (Boehringer/Mannheim, F.R.G.).

Statistics

Analysis of variance was used to analyze the behavioral activity data and the blood and brain concentrations of ethanol. Student's *t*-test (two-tailed) was performed when the analysis was restricted to the data obtained from the two groups exposed to high pressure. Nonparametric Kruskal-Wallis ANOVA by ranks and the Mann-Whitney U-test were used when the variance was not homogeneous. The Fisher exact probability test was used to analyze the occurrence of convulsions.

RESULTS

Motor Activity

Doses of 3.5 g/kg ethanol caused a pronounced reduction in total motor activity in all the experimental groups, particularly during the last two observation periods. In the precompression period the saline-injected animals spent $86.0 \pm 0.8\%$ of the available time in activity, while the corresponding scores of the ethanol-treated animals ranged from $35.8 \pm 3.1\%$ to $43.3 \pm 3.9\%$ (Fig. 2).

All ethanol-treated groups showed very low levels of activity during observation period II (corresponding to the first 20 min at final pressure) ranging from $1.6 \pm 0.9\%$ to $3.6 \pm 2.4\%$ of the available time. Half of the animals from each 1 ATA group and five from the 72 ATA group did not show any motor activity. Analysis of the results did not indicate a significant difference between the ethanol-treated groups. However, there was a highly significant difference, $t(14) = 9.93$, $p < 0.0001$, between the ethanol- and saline-treated rats exposed to 72 ATA. Results from a previous experiment (10) showed that, under similar conditions, saline-injected rats exposed to 1 ATA air or to 1 ATA heliox were active for $37.6 \pm 9.8\%$ and $15.5 \pm 3.5\%$ of the available time respectively.

Similarly, during observation period III (the last period of 20 min at final pressure), the mean level of activity remained low in

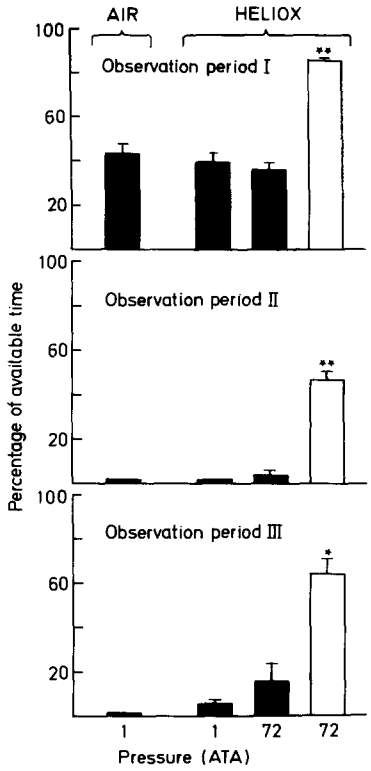

FIG. 2. Total motor activity. Open bars represent the saline control group; closed bars the groups administered 3.5 g/kg ethanol. See Fig. 1 for definition of the observation periods. Results are expressed as the mean ± S.E.M., N = 8 in each group. *$p<0.001$, **$p<0.0001$, significantly different from corresponding ethanol-treated group (t-test).

the ethanol-treated animals, especially in the 1 ATA groups (Fig. 2). In this period only two subjects from each group remained completely inactive. Although the ethanol-treated animals at 72 ATA showed a higher mean level of overall activity in comparison to the corresponding values in the 1 ATA air and 1 ATA heliox groups, Kruskal-Wallis ANOVA demonstrated only a nonsignificant tendency towards differences between the groups ($0.05<p<0.10$). During this observation period, a statistically significant difference was also found between the ethanol- and saline-injected groups exposed to 72 ATA, $t(14)=4.58$, $p<0.001$. Previous results for the corresponding period of time in saline-injected rats exposed to 1 ATA air or to 1 ATA heliox showed mean durations of activity of 2.4 ± 0.5% and 3.4 ± 1.2% of the available time respectively.

With regard to the "sleeping time," two ethanol-treated animals in the 1 ATA group and one animal in the 72 ATA group did not show any signs of recovery of motor activity during the experiment and were assigned a weak-up time of 68 min. The mean "sleeping times" in the animals exposed to 1 ATA air or to 1 or 72 ATA heliox were 41.2 ± 8.0, 37.5 ± 6.8 and 37.2 ± 7.5 min respectively. No statistically significant differences were found, $F(2,21)=0.09$, one-way ANOVA.

Table 1 shows the behavioral categories that contributed most to the total motor activity during the observation periods at stable pressure. The ethanol-treated animals did not show any locomotion and their activity was basically restricted to movements of the head and the rest of the body. On the other hand, the relative contribution of locomotion to the total motor activity in the saline-treated animals was only about 11%.

TABLE 1

PERCENTAGE OF TOTAL MOTOR ACTIVITY IN EACH BEHAVIORAL CATEGORY AT STABLE PRESSURE

Period	Pressure (ATA)		Head Movements	Moving	Normal Locomotion	Animals Showing Activity
II	Air					
	1	Ethanol	71.2 ± 16.9	28.8 ± 16.9	0.0 ± 0.0	4
	Heliox					
	1	Ethanol	78.0 ± 10.1	22.0 ± 10.1	0.0 ± 0.0	4
	72	Ethanol	73.4 ± 9.3	26.6 ± 9.3	0.0 ± 0.0	3
	72	Saline	80.6 ± 5.0	7.6 ± 2.1	11.1 ± 3.5	8
III	Air					
	1	Ethanol	79.2 ± 11.1	20.8 ± 11.1	0.0 ± 0.0	6
	Heliox					
	1	Ethanol	61.9 ± 9.5	38.1 ± 9.5	0.0 ± 0.0	8
	72	Ethanol	52.6 ± 14.3	46.8 ± 14.3	0.0 ± 0.0	6
	72	Saline	83.2 ± 4.6	3.6 ± 1.0	11.9 ± 3.9	8

Data given as mean ± S.E.M. on basis of scores calculated for each rat as the percentage of total motor activity spent in each category. See Fig. 1 for definitions of the observation periods. Data from the animals that remained inactive are not included in these figures.

Myoclonic Jerks and Convulsions

The rats exposed to 1 ATA did not show any myoclonic jerks. During the compression phase, very few myoclonic jerks were observed. At stable pressure, all of the saline-injected rats, but only five rats in the ethanol group, had myoclonic jerks. The median numbers (with ranges) of jerks during the first 20 min period at stable pressure were 1.5 (0–6) and 1.0 (0–7) for the saline and ethanol groups respectively. The corresponding results for the last observation period were 3.5 (1–18) and 3.0 (0–150). No significant statistical differences were found in either observation period (Mann-Whitney U-test).

Ten of the eighteen animals included in the initial saline control group convulsed between 64.3 and 72 ATA. In these cases the experiment was stopped immediately and the animals were excluded from behavioral analysis. None of the ethanol-injected rats showed convulsions. The Fisher exact probability test revealed a statistical difference ($p<0.01$).

Blood and Brain Ethanol Levels After Injection of 3.5 g/kg

Similar levels of blood and brain ethanol were found in the 1 ATA air and the 1 and 72 ATA heliox groups (Table 2). No statistically significant differences were found.

TABLE 2

ETHANOL LEVELS IN BLOOD AND BRAIN AFTER INJECTION OF 3.5 g/kg ETHANOL

	1 ATA Air	1 ATA He-O	72 ATA He-O
Blood (mg/ml)	3.49 ± 0.24	3.24 ± 0.22	3.71 ± 0.08
Brain (mg/g)	3.78 ± 0.18	3.46 ± 0.10	3.67 ± 0.11

Data given as mean ± S.E.M. N = 7–8 animals in each group.

DISCUSSION

This study shows that a high dose of ethanol may protect rats against pressure-induced convulsions. On the other hand, exposure to 72 ATA did not significantly counteract the narcotic effects of ethanol. The intoxicated animals exposed to high pressure were not significantly more active than those exposed to 1 ATA air or 1 ATA heliox. Besides, highly significant differences could be detected between the activity level of the intoxicated animals and that of the saline-injected animals exposed to the same high pressure. However, during the third observation period (from min 48 to min 68), exposure to high pressure tended to increase the total motor activity in most of the ethanol-treated animals (the animals that woke up under high pressure tended to be more active than the 1 ATA controls). This suggests that hyperbaric exposure did exert some antagonistic effect which may have become significant with a longer exposure.

None of the ethanol-treated rats in this study walked after the narcosis period and it can be argued that the limited space available may have prevented locomotion. This seems unlikely since locomotion contributed about 11% of the total motor activity in the saline-treated animals exposed to 72 ATA. Furthermore, in a previous study using the same apparatus (10), rats that had received moderate doses of ethanol and which were subsequently exposed to 48 ATA were not only more active than control animals at 1 ATA, but also showed a high percentage of normal locomotion. In addition, a significant increase in locomotor activity in ethanol-treated mice exposed to 12 ATA has recently been observed (20). It is therefore likely that a significant reversal of ethanol intoxication in our rats would have resulted in some normal locomotion.

These results may appear to conflict with previous reports that exposing mice to hyperbaric heliox in the range of 4 to 12 ATA is sufficient to antagonize the depressant effect of ethanol in mice (1–3). In these studies, "sleep time," as defined by recovery of the righting reflex, was used as the criterion of the depressant effects; the doses of ethanol employed were in the same range as ours. Differences in the behavioral assessments of the depressant effects of ethanol may partly explain this divergence, although species differences may be a contributing factor. However, there are two common findings in the studies that deal with the interaction of high pressure and ethanol. The degree of antagonism produced by a given pressure decreases as the dose of ethanol increases and, second, the antagonism for a given dose is pressure-dependent (1, 10, 13). In the present study, pressure was limited to 72 ATA. It is possible that higher pressures might counteract the depressant effects of a narcotic dose of ethanol in rats.

Brain and blood concentrations of ethanol were not altered by exposure to 72 ATA. On the basis of this and our previous results using different doses of ethanol and level of pressure (9,10), it seems reasonable to assume that hyperbaric treatment does not alter the elimination or distribution of ethanol in rats.

The role of ethanol as a protective agent against the adverse effects of high pressure is not clear. None of our ethanol-injected animals showed convulsions and this indicates that there is a protective effect. Ethanol and other anesthetics ameliorate pressure-induced paralysis in tadpoles (13). Some anesthetics also raise the pressure at which mice have convulsions, at both slow and fast compression rates (5). Likewise, certain gaseous and intravenous anesthetic agents are effective in ameliorating the effects of pressure in rats (4, 8, 11, 24). However, protection against pressure-induced effects does not seem to be a general property of anesthetics (11, 12, 23, 24) and the basis of protection against HPNS afforded by some anesthetics is not fully understood.

The interaction between ethanol and pressure is a complex problem. Ethanol presumably affects most transmitter systems to various extents (17). However, it is worth noting that several pharmacological effects of ethanol can be related to its enhancing effects on GABA-ergic transmission (21). High pressure interferes with a number of transmitter systems (12,23), but no particular transmitter system malfunction has been shown to produce HPNS (23). However, interference with GABA-ergic transmission may contribute to the adverse effects of high pressure. Drugs that increase GABA-ergic transmission elevated the threshold pressures for HPNS tremor and convulsions and these effects correlated with the ability of the drugs to raise the threshold concentration at which the GABA receptor antagonist bicuculline caused convulsions (7). Ethanol possesses marked anticonvulsant properties in a number of seizure paradigms including those caused by bicuculline (22). It is therefore possible that the protective effects of ethanol against HPNS convulsions are mediated through its enhancement of GABA-ergic transmission.

In conclusion, although our results did not show a statististically significant reversal of the narcotic effects of ethanol, hyperbaric exposure to 72 ATA seemed to exert some antagonistic effects which might have become significant with exposure to higher pressures or for a longer period. At the same time, ethanol appears to protect rats against convulsions induced by high pressure.

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