Interaction of ethanol and the high pressure nervous syndrome in rats

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García-Cabrera I, Berge O.-G. Interaction of ethanol and the high pressure nervous syndrome in rats. Undersea Biomed Res 1990; 17(5):375–382.—The aim of this study was to determine whether administration of ethanol protects rats against the preconvulsive symptoms of high pressure nervous syndrome (HPNS). Male Sprague-Dawley rats were given either saline or 0.5, 1.5, or 2.5 g/kg ethanol i.p. After injection, the animals were individually exposed to helium-oxygen at 60 atmospheres absolute (atm abs) pressure. The chamber temperature was adjusted to counteract ethanol- and helium-induced hypothermia. Several behavioral parameters were scored continuously during the first 64 min after injection. The time spent in tremor at high pressure was significantly less in the 1.5 and 2.5 g/kg ethanol-treated groups. The number of jerks was significantly lower in the 2.5 g/kg ethanol-treated group. The two highest doses of ethanol induced a characteristic pattern of unsteady locomotion, which was returned to normal in the 1.5 g/kg group at 60 atm abs. Other behavioral effects of ethanol, such as depression of total motor activity, were also reduced. These results indicate that ethanol can significantly ameliorate some of the adverse symptoms of HPNS in freely moving rats.

The high pressure nervous syndrome (HPNS) in animals is characterized by tremor, myoclonic jerks, alterations in the electroencephalogram, and generalized convulsions followed by coma (1, 2). It has long been known that anesthetics can ameliorate the adverse effects of hyperbaric exposure. However, this is not a universal property because some anesthetics do not affect or may exacerbate the HPNS symptoms. Furthermore, there is no correlation between pressure reversal of the action of an anesthetic and the potency of the anesthetic in opposing adverse effects of pressure (2, 3).

The action of ethanol on the adverse effects of high pressure has been little studied in freely moving animals. In our previous work we demonstrated pressure reversal of the depressant effects of a moderate dose of ethanol on spontaneous behavior in rats (4). Although the effects of a narcotic dose of ethanol were not significantly reversed at 72 atmospheres absolute (atm abs), it was noticed that the compound
seemed to protect rats against convulsions induced by hyperbaric exposure (5). The aim of this study was to determine whether ethanol protects rats against the preconvulsive symptoms of HPNS.

METHODS

Animals

Thirty-two, drug-naive, male Sprague-Dawley rats (Møllegård, Denmark), weighing 225–275 g at the beginning of the experiment, were used. The animals were maintained at an ambient temperature of 22°–23°C, under a 12/12 h light cycle with lights on at 0600 h. All experiments took place between 0800 and 1700 h and the various treatment groups were tested in balanced order across days and with regard to time of day.

Pressure chamber

The experiments were done in a 24.5-liter steel chamber, which was equipped with temperature- and pressure-monitoring systems, fan, CO₂ scrubber, and heating system as described previously (4). A window at one end of the chamber permitted videotaping of the animals’ behavior. To facilitate observation, a mirror was positioned at a 45° angle at the back of the enclosure. On the basis of previous results (6–8), the chamber temperature was adjusted to 34° ± 1°C to offset ethanol- and helium-induced hypothermia. The partial pressure of oxygen was maintained at between 0.2 and 0.4 atm abs.

Procedure

Rats were injected intraperitoneally with 0.5, 1.5, or 2.5 g/kg ethanol [21 ml/kg of, respectively, 3, 9, and 18% (vol/vol) ethanol-isotonic saline solutions] or with a corresponding volume of isotonic saline. Immediately after injection the animals were individually placed in the chamber and exposed to 60 atm abs. The chamber was first purged for 2 min with an 80% helium: 20% oxygen mixture (heliox) to remove nitrogen from the breathing gas. Compression with helium (3 atm abs/min) started 4 min 20 sec after injection, and all groups reached the target pressure 24 min after ethanol administration. All animals remained at the target pressure for 40 min. After completion of the experiment, the rats were anesthetized with N₂O and killed by rapid decompression. 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 21 × 22 cm. Behavioral scoring was performed twice with the aid of a computer program. In the first scoring session the duration of all observable motor activity was recorded, as previously described (4). This measure of total motor activity was expressed as a percentage of the available time in each observation period. Staggering (uncoordinated locomotion),
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which was found to be a very sensitive measure of the ethanol intoxication (4), was presented as a percentage of total locomotion (the sum of normal locomotion and staggering). In the second scoring session, the following were recorded: a) The cumulative duration of tremor in face and/or forelimbs or in the whole body; b) the number of occurrences of myoclonic jerks. The pressure at which the first tremor was observed was considered the pressure threshold for tremor.

Statistics

Analysis of variance (ANOVA) was employed as detailed in Results. Subsequent paired comparisons of group means were performed by Scheffe’s test. Data on staggering were analyzed by Student’s t test (two-tailed) as analysis was restricted to data from only 2 groups. Nonparametric Kruskal-Wallis ANOVA by ranks and the Mann-Whitney U test were used for analysis of myoclonic jerks.

RESULTS

Total motor activity

The results for the four observation periods (shown in Fig. 1) are presented in Table 1. The saline- and 0.5 g/kg ethanol-treated groups maintained a high level of

<table>
<thead>
<tr>
<th>Period</th>
<th>$P$</th>
<th>Saline</th>
<th>Ethanol, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>84.8±1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>I</td>
<td>&lt; 0.005</td>
<td>81.1±1.4</td>
<td>78.4±2.9</td>
</tr>
<tr>
<td>II</td>
<td>&lt; 0.0005</td>
<td>90.7±1.4</td>
<td>60.3±6.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>N.S.</td>
<td>82.8±5.2</td>
<td>72.8±3.2</td>
</tr>
<tr>
<td>IV</td>
<td>&lt; 0.05</td>
<td>85.9±4.2</td>
<td>62.2±7.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data given as mean ± SEM ($n = 8$ in each group). See Fig. 1 for definition of observation periods. $P$ values refer to one-way ANOVA across treatment groups.

<sup>b</sup>$P < 0.005$ significantly different from saline-treated group (Scheffe’s test subsequent to ANOVA).
motor activity throughout the experiment. The 1.5 and 2.5 g/kg ethanol-treated animals showed a significantly lower level of activity during the compression phase and subsequently increased their motor activity at stable pressure, especially during the first 20 min. One-way ANOVA demonstrated a significant difference between groups during the last observation period, but subsequent application of Scheffe's test demonstrated only nonsignificant tendencies toward differences between the 1.5 and 0.5 g/kg ethanol-treated groups (0.05 < P < 0.10).

Staggering

Virtually no staggering was observed in the saline- and the 0.5 g/kg ethanol-treated animals throughout the experiment. The results of the 2 groups injected with 1.5 and 2.5 g/kg ethanol, respectively, are presented in Fig. 2. The rats that had received 1.5 g/kg ethanol showed hardly any staggering at stable pressure.

Tremor

No tremor was registered during the precompression period. The mean pressure thresholds for tremor were 25.7 ± 2.7, 23.4 ± 2.4, 24.3 ± 4.9, and 34.1 ± 5.0 in the saline- and the 0.5, 1.5, and 2.5 g/kg ethanol-treated groups, respectively. There was no significant difference between the groups in the mean pressure thresholds for tremor (ANOVA).

Fig. 2. Staggering in rats administered ethanol as percentage of total locomotor activity. Results are expressed as the mean ± SEM. $n = 8$ in each group. *P < 0.005, significantly different from 1.5 g/kg ethanol-treated group (t test).
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The results for the compression and postcompression periods are shown in Fig. 3. During observation period II (compression phase) there was a significant difference between the groups, $F(3,28) = 7.44, P < 0.005$. Subsequent application of Scheffe’s test showed that the 2.5 g/kg ethanol-treated group spent significantly less time in tremor than either the saline group ($P < 0.005$) or the 0.5 g/kg ethanol-treated group ($P < 0.05$).

During observation periods III and IV at stable pressure the saline- and 0.5 g/kg ethanol-treated groups had tremor about half of the time, whereas the groups that had received the higher doses of ethanol showed significantly less tremor. ANOVA demonstrated highly significant differences between groups in both periods, $F(3,28) = 24.2, P < 0.00001$ (period III); $F(3,28) = 22.7, P < 0.00001$ (period IV). Subsequent application of Scheffe’s test demonstrated that the 1.5 and 2.5 g/kg ethanol-treated groups were significantly different from both the saline- and the 0.5 g/kg ethanol-treated groups. There were no statistical differences between the saline- and the 0.5 g/kg ethanol-treated groups in any of the periods.

Myoclonic jerks

Jerks occurred in most animals for the first time during compression except in the 2.5 g/kg ethanol-treated group, in which most animals showed the first jerk during the stable-pressure period (Table 2). Kruskal-Wallis ANOVA demonstrated nonsignificant tendencies toward group differences ($0.05 < P < 0.10$) in the number of jerks during the compression period. Scores for the postcompression periods were pooled. Animals that had received the highest dose of ethanol showed considerably fewer myoclonic jerks than the others at stable pressure; there was a significant difference between groups ($P < 0.005$, Kruskal-Wallis ANOVA). Subsequent analysis showed that the 2.5 g/kg ethanol-treated group had significantly fewer jerks than the other groups.

DISCUSSION

These results show that ethanol can ameliorate the preconvulsive symptoms of HPNS in freely moving rats. The highest dose employed (2.5 g/kg) was the most

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Fig. 3. Tremor as percentage of total available time. Results are expressed as the mean ± SEM. $n = 8$ in each group. $^*P < 0.005$, $^{**}P < 0.001$, $^{***}P < 0.0001$ significantly different from saline-treated group (Scheffe’s test subsequent to ANOVA).
TABLE 2
NUMBER OF JERKS\textsuperscript{a}

<table>
<thead>
<tr>
<th>Period</th>
<th>Saline \ (0-5)</th>
<th>Ethanol, g/kg</th>
<th>0.5 \ (0-4)</th>
<th>1.5 \ (0-2)</th>
<th>2.5 \ (0-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>III+IV</td>
<td>14</td>
<td>21</td>
<td>12</td>
<td>4.5\textsuperscript{c}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(10-53)</td>
<td>(9-78)</td>
<td>(7-58)</td>
<td>(2-13)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data given as medians, with extreme scores in parentheses. \( n \) = number of animals showing jerks out of a total number of 8 in each group.

\textsuperscript{b} \( P < 0.05 \) significantly different from saline-treated group.

\textsuperscript{c} \( P < 0.005 \) significantly different from saline- and 0.5 g/kg ethanol-treated groups; \( P < 0.01 \) significantly different from the 1.5 g/kg ethanol-treated group (Mann-Whitney U test).

effective because it reduced significantly the time spent in tremor and the number of myoclonic jerks, both during compression and at stable pressure. Administration of 1.5 g/kg ethanol significantly reduced the time spent in tremor at stable pressure. None of the doses of ethanol used had any significant effect on the pressure threshold of tremor.

In previous studies (4, 5), brain and blood concentrations of ethanol using different doses and levels of pressure were not altered in relation to control animals. On this basis it seems reasonable to assume that exposure to 60 atm abs in the present study did not significantly alter the elimination or distribution of ethanol in the rats.

High pressure usually exerts antagonistic effects on anesthesia, although synergistic effects have also been reported (2). With regard to ethanol, pressure reversal of its depressant effects have been demonstrated in luminous bacteria (9), tadpoles (10, 11), mice (12-15), and rats (4). In this study, exposure to 60 atm abs antagonized the depressant effects of a subhypnotic dose of ethanol (1.5 g/kg) in rats, confirming previous findings (4).

Another interesting aspect of the interaction of anesthetics with high pressure is the protection that several, but not all, of these agents provide against the symptoms of HPNS. Relatively few behavioral studies have addressed the role of ethanol as a protective agent against the adverse effects of high pressure. Ethanol provides a certain degree of protection against pressure-induced paralysis in tadpoles (11). A narcotic dose of ethanol also protects rats against pressure-induced convulsions (5). In this study the two highest doses of ethanol employed were, to different extents, effective in ameliorating HPNS.

Under normobaric conditions different doses of ethanol depress total motor activity in rats (4, 5). Thus, the high level of activity which we observed in the 2.5 g/kg ethanol-treated group suggests that hyperbaric exposure exerted some antagonism. However, the depressant effects of this dose of ethanol were not completely reversed by high pressure, since these animals did not show a significant decrease in the
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amount of staggering. On the other hand, the 1.5 g/kg group was not only active but also showed a negligible amount of staggering at high pressure. It is clear that a moderate dose of ethanol whose depressant effects were completely counteracted by high pressure would be preferable to a larger dose that was more efficient in ameliorating HPNS symptoms.

The mechanism of action of ethanol in reducing HPNS signs is a complex problem. The precise mechanisms that cause HPNS remain elusive. High pressure affects a number of transmitter systems but no particular transmitter system malfunction has been shown to produce HPNS (2, 3). However, interference with γ-aminobutyric acid (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 (16). One of these drugs, sodium valproate, has recently been reported as ameliorating HPNS symptoms in freely moving baboons (17). Other investigators, however, have suggested that excitatory transmitters may be more important than GABA in contributing to the behavioral aspects of HPNS (18).

Ethanol presumably affects most transmitter systems to various degrees (19). However, several studies suggest that ethanol may bring about most of its effects by enhancing GABA-ergic transmission (20). It is therefore possible that the protective effects of ethanol against HPNS are partly mediated through its enhancement of GABA-ergic transmission, although the neurotransmitter perturbations induced by the combination of ethanol and pressure are unlikely to be limited to the GABA-ergic system.

In conclusion, these results indicate that ethanol can ameliorate preconvulsive HPNS symptoms in freely moving rats. Assessment of the effects of ethanol in other species using a wide range of compression rates will be necessary before these data can be extrapolated to human diving conditions.

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