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# Hyperbaric Ethanol Antagonism: Role of Temperature, Blood and Brain Ethanol Concentrations

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MALCOLM, R. D. AND R. L. ALKANA. *Hyperbaric ethanol antagonism: Role of temperature, blood and brain ethanol concentrations.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 341–346, 1982.—Three studies investigated the mechanism of hyperbaric ethanol antagonism. C57 Bl/6j mice were injected intraperitoneally with 3.6 g/kg ethanol and were exposed to 1–12 atmospheres absolute (ATA) helium-oxygen or to 1 ATA air at temperatures that offset the cooling effects of helium or helium and ethanol. Hyperbaric treatment significantly reduced sleep-time and increased wake-up brain ethanol concentrations compared to temperature matched controls. Treatment with 12 ATA helium-oxygen increased the brain ethanol concentration and the brain-blood ethanol ratio at 150 minutes after ethanol administration. These results exclude helium or pressure-induced changes in body temperature, rate of blood ethanol decline or distribution to the brain as mechanisms mediating the antagonism. The data support a membrane site of antagonism.

Blood and brain ethanol  Ethanol  Ethanol antagonists  Ethanol metabolism  Ethanol narcosis
Hyperbaric pressure  Theories of anesthesia

THE antagonistic effect of high hydrostatic pressures on general anesthetics was first described over 35 years ago [11]. Several laboratories have since extended this finding to demonstrate that hyperbaric or hydrostatic environments of 34–300 atmospheres absolute (ATA) reverse the anesthesia from a variety of drugs including non-inhalational anesthetics such as ethanol [8, 12, 13, 18]. More recent studies indicate that non-inhalational anesthetics may show a greater susceptibility to pressure antagonism than do gaseous agents [5, 9, 19]. Furthermore, comparatively small increases in environmental pressure (<10 ATA) have also been shown to alter the effects of several centrally active drugs such as chlordiazepoxide, tetrahydrocannabinol and amphetamine [26, 27, 29].

Based in part on these reported interactions between pressure and centrally active drugs, our laboratory undertook an investigation of the effects of relatively small increases (≤12 ATA) in hyperbaric pressure on ethanol depression. We found that sleep-time in mice given 3.2 g/kg ethanol was significantly reduced by pressures as low as 3 ATA 100% oxygen [4] and 6 ATA 80% helium-20% oxygen [1]. Subsequent studies demonstrated that the antagonism occurred when the oxygen partial pressure was kept at 0.2 ATA [2]. Higher wake-up blood ethanol concentrations in the hyperbaric mice suggested that the antagonism was not mediated by enhanced ethanol elimination. Furthermore, the degree of ethanol antagonism appeared to be inversely related to the ethanol dose and directly related to the amount of pressure applied [3]. These studies convincingly eliminated increased oxygen partial pressure as a critical factor in mediating the antagonism and suggested that the antagonism resulted from the increased atmospheric pressure *per se* rather than other actions of the hyperbaric helium-oxygen environment.

However, these experiments did not eliminate other possible mechanisms. A recent study demonstrating the importance of body temperature changes in altering ethanol depression [16] suggested that the antagonism could reflect a hypothermic effect of helium resulting from its higher heat conductivity with respect to nitrogen [17,25]. The antagonism could also be mediated by a helium or pressure-induced increase in the rate of ethanol elimination or decrease in brain ethanol concentration secondary to a change in ethanol distribution. The present study investigated the importance of these factors in mediating hyperbaric ethanol antagonism.

## METHOD

Three experiments were conducted. General information regarding the subjects, overall protocol and techniques are described below before the detailed procedures for each experiment.

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*Subjects and Protocol*

Drug naive male C57 Bl/6j mice were housed five per cage on a 12 hour light-dark cycle (0700 on) for at least one week before testing. During testing, all animals were injected intraperitoneally (IP) with 3.6 g/kg ethanol (20% w/v in freshly made normal saline) between 1200 and 1700 hours and were immediately placed on a vertical wire mesh screen suspended above a foam rubber pad. The time from injection until the mouse fell from the screen was measured. This constituted the "drop-time" and began the time of righting reflex loss (sleep-time). Mice having drop times greater than 180 seconds were eliminated from further experimentation. After dropping, sleep-time mice were quickly placed on their backs on a v-shaped tray mounted inside a hyperbaric chamber consisting of a modified transparent ultrafiltration cell (Amicon model 402, Amicon Corp., Lexington, MA). The atmospheric pressure was adjusted from 1–12 ATA with cylinders of compressed helium-oxygen according to a procedure that assured adequate oxygenation during compression and set the final oxygen partial pressure at approximately 0.2 ATA [3]. Control animals were treated similarly and exposed to a chamber environment of 1 ATA air. The chamber temperature was adjusted to the desired level using the technique described below. Upon return of the righting reflex, the chamber was rapidly decompressed. The animals were immediately killed by cervical dislocation, decapitated and the brain was prepared for ethanol determination. The sequence of treatment conditions was counterbalanced across experimental days to minimize order effects or bias introduced by day-to-day variability. Helium was selected as a substitute for the nitrogen in air since, unlike nitrogen [6], helium does not exert depressant effects within the pressure range examined in these studies [15,17]. Helium-oxygen gas mixtures were certified standards (Matheson Gas Co., Cucamonga, CA).

*Environmental Temperature*

Environmental temperatures greater than room temperature were achieved by totally immersing the chamber in a constant temperature water bath. Compartment temperature was continually monitored via a temperature probe (Yellow Springs Instrument Co., Yellow Springs, OH, model 403) mounted within the chamber and connected to a Telethermometer (Yellow Springs Instrument Co., model 41TD). This technique maintained compartment temperature within $\pm 0.5$°C of the desired value. Room temperature varied between 21 and 25°C due to constraints placed on the laboratory air-conditioning system by the physical plant.

*Rectal Temperature*

Rectal temperatures were measured in separate groups of mice not utilized for sleep-time determinations in order to avoid the possible confounding effects from the rectal probe. Prior to ethanol, animals were placed in a small animal restrainer. A rectal probe (Yellow Springs Instrument Co., model 402) was lubricated with glycerol and inserted 2.5 cm into the rectum [7,22]. After a 30 second stabilization period, the rectal temperature was read. Following ethanol injection, the mouse was placed in the hyperbaric chamber. A rectal temperature probe mounted inside the chamber was lubricated with glycerol and inserted into the rectum. The temperature probe was secured in place for the post-ethanol monitoring period by taping it lightly to the animal's tail. The chamber was then sealed and the desired environmental conditions were attained using the procedures previously described.

*Brain and Blood Ethanol Concentration*

Mice were killed by cervical dislocation, then decapitated. The whole brain was rapidly removed, rinsed in ice cold saline, blotted lightly, weighed (mean$\pm$S.D.$=430\pm20$ mg) and then homogenized in 2.0 ml of ice cold 0.6 N perchloric acid solution containing 25 mM thiourea and 10 mM n-propanol [24]. The cold homogenate was centrifuged at 1500 rpm for 10 minutes. A 200 $\mu$l sample of the supernate was transferred to a serum vial and frozen at $-20$°C. Blood samples (20 $\mu$l) were deproteinized in 200 $\mu$l of a 0.6 N perchloric acid solution containing 25 mM thiourea and 4 mM n-propanol and frozen at $-20$°C.

Both blood and brain ethanol concentrations were determined on a "blind" basis. Samples were heated for 30 minutes at 60°C. A 400 ml head space aliquot was then injected into a Hewlett-Packard model 5721A gas chromatograph operated under the following conditions: column oven, 90°C; detector, 130°C; nitrogen (carrier gas), 20 ml/minute; hydrogen, 30 ml/minute; air, 300 ml/minute. Analysis was done on a 6′×1/4″ o.d.×2 mm i.d. glass column packed with 15% Carbowax 20 M coated on Chromosorb W 60/80 mesh (Applied Sciences, Inc., State College, PA). Under these conditions, the retention times for ethanol and n-propanol were 1.4 and 2.3 minutes, respectively.

*Data Analysis*

All data was analyzed by computer using SPSS statistical programs. Mean sleep-time, blood and brain ethanol concentrations and rectal temperatures were calculated. Initial comparisons between control and experimental values were made using one-way analysis of variance. If warranted, further statistical comparisons between control and experimental groups were made using Duncan's new multiple range procedure. $p$ values of $<0.05$ were taken as significant in all statistical analysis.

*Experiment 1 – Normal Hypothermia*

This experiment investigated the effectiveness of hyperbaric environments in antagonizing ethanol sleep-time when the hypothermic effect of helium was eliminated.

*Subjects and protocol.* Rectal Temperature—Mice, 51–61 days old and 19 to 28 g in weight (mean$\pm$S.D.$=22.7\pm1.9$ g), were injected with ethanol (drop times $101\pm30$ sec) and then exposed to environments of 1 ATA air at room temperature, 1 ATA air or 1, 6 or 12 ATA helium-oxygen at 30°C. Rectal temperatures were recorded immediately before ethanol and at 15-minute intervals for 90 minutes after injection.

Sleep-Time—Mice, 50 to 64 days old and 21 to 30 g in weight (mean$\pm$S.D.$=24.8\pm1.3$ g), were injected with ethanol (drop time $104\pm24$ sec) and exposed to 1, 6, 8 or 10 ATA helium-oxygen at 30°C. Control mice were kept in 1 ATA air at room temperature. Another group of mice was injected with ethanol and kept in 1 ATA air at 30°C for comparative purposes.

*Experiment 2 – No Hypothermia*

This experiment investigated the effectiveness of hyperbaric environments in antagonizing sleep-time when the hypothermic effects of ethanol and helium were offset.

*Subjects and protocol.* Rectal Temperature—Mice, 61–68 days old and 18 to 25 g in weight (mean±S.D.=20.6±1.8 g), were injected with ethanol (drop times 103±31 sec) and placed in environments of 1 ATA air at 33.5°C, or 1 or 6 ATA helium-oxygen at 34.5°C. Rectal temperatures were recorded immediately before ethanol and at 15-minute intervals for 90 minutes after injection.

Sleep-Time—Mice, 44 to 59 days old and 16 to 26 g in weight (mean±S.D.=21.5±2.4 g), were injected with ethanol (drop times 100±23 sec) and exposed to environments of 1 ATA air at 33.5°C or to 1, 6, 8, 10 or 12 ATA helium-oxygen at 34.5°C. An additional group was injected with ethanol and kept in 1 ATA air at room temperature for comparative purposes.

### *Experiment 3–Blood Ethanol Concentration and Brain-Blood Ethanol Ratio*

This experiment was conducted to determine whether hyperbaric environments alter the rate of the blood ethanol decline or change the distribution of ethanol between blood and brain.

*Subjects and protocol.* Mice, 46 to 54 days old and 15 to 24 g in weight (mean±S.D.=19.7±2.0 g), were injected between 1100 and 1430 hours with 3.6 g/kg ethanol (drop times 80±26 sec). After loss of their righting reflex, they were placed in a holding cage. Sixty minutes post-injection, a 20 μl blood sample was taken from the ophthalmic venous plexus [21]. The mice were then placed in the hyperbaric chambers and exposed to environments of 1 ATA air at 33.5°C or to 1 or 12 ATA helium-oxygen at 34.5°C. At 150 minutes post-injection, the chamber was rapidly decompressed and the mice were removed. A second blood sample was taken. The mice were immediately sacrificed by cervical dislocation and decapitated. Whole brains were removed and prepared for gas chromatographic ethanol determination.

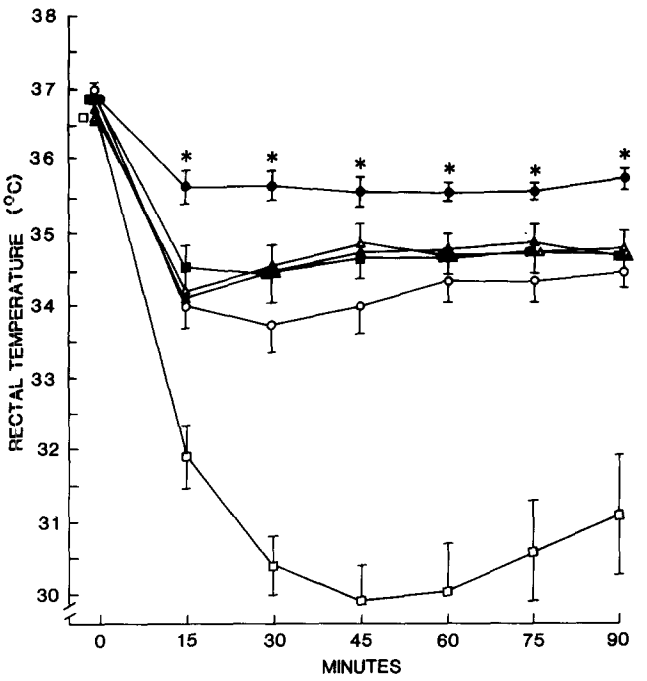


FIG. 1. Rectal temperatures of mice administered 3.6 g/kg of ethanol and exposed to environments of 1 ATA air at room temperature (○), 1 ATA air at 30°C (●), or to 1 ATA (△), 6 ATA (▲) or 12 ATA (■) helium-oxygen at 30°C. Data from a group of mice injected with 3.6 g/kg ethanol and exposed to 1 ATA helium-oxygen at room temperature (□) are included for comparative purposes. Shown are the mean±S.E. at each time point. The N=8 mice for all treatments. The * indicates a statistically significant change from the group exposed to 1 ATA air at room temperature.

## RESULTS

### *Experiment 1 – Normal Hypothermia*

The rectal temperatures of ethanol-injected mice exposed to air at room temperature or 30°C, or to helium-oxygen at 30°C are shown in Fig. 1. Analysis of variance indicated a significant effect of treatments at all time points after ethanol administration, $F(4,35)=4.08$–$7.91$, $p<0.001$. Further comparisons demonstrated that the rectal temperature of intoxicated mice exposed to 1 ATA air at 30°C were significantly higher than those of mice kept in air at room temperature. The rectal temperatures of mice exposed to helium-oxygen environments at 30°C did not differ from those of the 1 ATA air group kept at room temperature.

Treatment with hyperbaric helium-oxygen reduced sleep-times in mice experiencing the normal hypothermic effect of ethanol (Fig. 2A). Analysis of variance indicated a significant effect of treatments on sleep-time, $F(5,47)=6.49$, $p<0.001$. Statistical comparisons made against the 1 ATA air controls (room temperature) demonstrated a significant reduction in sleep-time in the 6 ATA helium-oxygen group and a trend towards a reduced sleep-time at 10 ATA ($p<0.1$). Mice exposed to 1 ATA air at 30°C slept significantly longer than the 1 ATA air group at room temperature. Mean brain ethanol at wake-up was higher in the hyperbaric groups than in the 1 ATA air and 1 ATA helium-oxygen controls (Fig. 2B), but these differences did not reach statistical significance, $F(5,47)=1.14$, $p>0.1$.

### *Experiment 2 – No Hypothermia*

As shown in Fig. 3, the rectal temperatures of ethanol-injected mice exposed to air at 33.5°C or helium-oxygen at 34.5°C were maintained within ±1°C of the pre-ethanol baseline. Analysis of variance indicated that there were no significant differences between these three groups at any time point, $F(2,21)=0.05$–$1.74$, $p>0.2$.

Treatment with hyperbaric helium-oxygen reduced sleep-times (Fig. 4A) and increased wake-up brain ethanol concentrations (Fig. 4B) in mice kept at environmental temperatures which offset ethanol or ethanol and helium hypothermia. Analysis of variance indicated a significant effect of treatments on sleep-time, $F(6,75)=3.84$, $p<0.003$, and brain ethanol at wake-up, $F(6,75)=4.96$, $p<0.001$. Comparisons made against the control mice treated with 1 ATA air demonstrated a significant reduction in sleep-time for mice treated with 6, 8, 10 and 12 ATA helium-oxygen. Wake-up brain ethanol concentrations were significantly higher in all four hyperbaric groups (6–12 ATA) when compared to the 1 ATA air controls. Similar significant differences in sleep-time and wake-up brain ethanol concentrations were also seen when hyperbaric groups (6–12 ATA) were compared to the 1 ATA helium-oxygen group. Mice kept in 1 ATA air at room temperature had significantly shorter sleep-times and higher wake-up brain ethanol concentrations than the 1 ATA air group kept at 33.5°C.

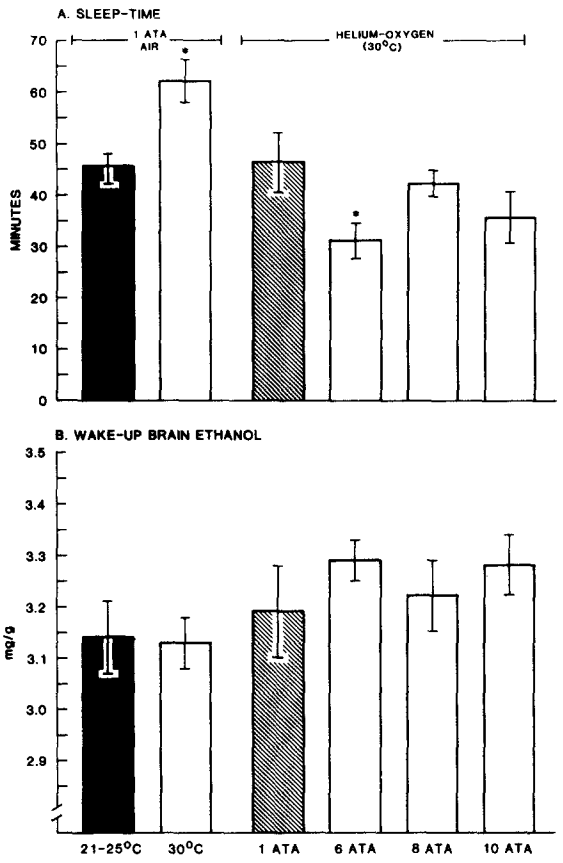


FIG. 2. The effect of 1–10 ATA helium-oxygen on sleep-time (A) and wake-up brain ethanol concentration (B) in mice with acute ethanol hypothermia. Shown are the mean±S.E. for each treatment. The N=9 mice for all groups except 6 ATA helium-oxygen, where the N=8. The * indicates a statistically significant change ($p<0.05$) from the air controls kept at room temperature.

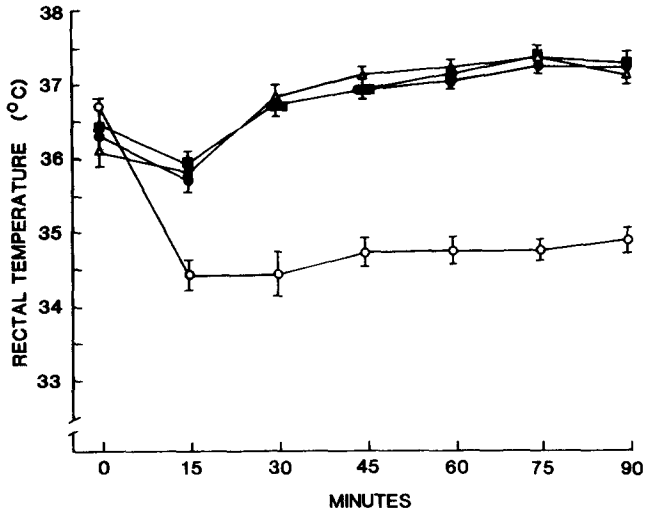


FIG. 3. Rectal temperatures of mice administered 3.6 g/kg of ethanol and exposed to environments of 1 ATA air at 33.5°C (●), or to 1 ATA (△) or 6 ATA (■) helium-oxygen at 34.5°C. Data from a group of mice injected with 3.6 g/kg ethanol and exposed to 1 ATA air at room temperature (○) are included for comparative purposes. Shown are the mean±S.E. at each time point. The N=8 mice for each group.

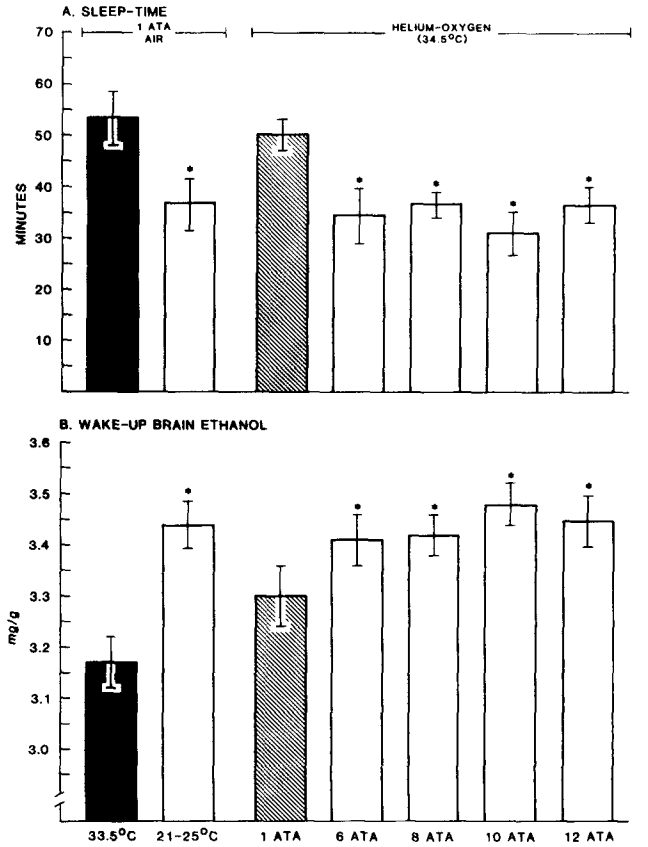


FIG. 4. The effect of 1–12 ATA helium-oxygen on sleep-time (A) and wake-up brain ethanol concentration (B) in mice that do not have ethanol hypothermia. Shown are the mean±S.E. for each treatment group. The N=12 mice for all groups except 1 and 8 ATA helium-oxygen, where the N=11 animals. The * indicates a statistically significant change ($p<0.05$) from the air controls kept at 33.5°C.

*Experiment 3 – Rate of Ethanol Elimination and Brain-Blood Ethanol Ratio*

Table 1 shows that treatment with 1 or 12 ATA helium-oxygen did not alter the rate of blood ethanol decline. On the other hand, animals exposed to 12 ATA had significantly higher brain ethanol concentrations and brain-blood ratios at 150 minutes after ethanol.

## DISCUSSION

The present study confirms that hyperbaric environments antagonize ethanol narcosis and extends earlier reports [1–3] to show that the antagonism is not mediated by helium-induced hypothermia, an increased rate of blood ethanol decline or altered distribution to the brain.

Recent studies demonstrating the influence of body temperature on ethanol depression [16,20] suggested that the antagonistic effect of hyperbaric helium-oxygen could reflect a hypothermia induced by helium. It has a higher thermal conductivity than nitrogen [17,25] and would be expected to cause a greater decrease in the body temperature of mice whose thermoregulatory capacity had been impaired by

TABLE 1

THE EFFECT OF HYPERBARIC HELIUM-OXYGEN ON BLOOD AND BRAIN ETHANOL CONCENTRATION AND BRAIN-BLOOD RATIO FOLLOWING 3.6 g/kg ETHANOL

| Treatment | Blood Ethanol Concentration (mg/dl) | | Brain Ethanol Concentration (mg/g) | Brain-Blood Ratio |
|---|---|---|---|---|
| | 60 min | 150 min | 150 min | 150 min |
| 1 ATA air | 406 ± 6 | 260 ± 2 | 1.90 ± 0.05 | 0.73 ± 0.01 |
| 1 ATA helium-oxygen | 402 ± 5 | 271 ± 5 | 1.97 ± 0.04 | 0.73 ± 0.01 |
| 12 ATA helium-oxygen | 407 ± 6 | 267 ± 7 | 2.08 ± 0.05* | 0.77 ± 0.01* |

Shown are the mean ± S.E. for each parameter. The N=18 animals for each group.
*Significantly different from 1 ATA air controls, $p<0.05$.

ethanol [14,16]. A comparison of the rectal temperatures of intoxicated mice kept in air or helium-oxygen at room temperature (Fig. 1) illustrates the magnitude of the extra hypothermic effect of helium. However, treatment with hyperbaric helium-oxygen significantly reduced sleep-time in mice exposed to heated environments which offset the cooling effect of helium yet permitted the normal degree of ethanol-induced hypothermia to occur (Fig. 2). In addition, sleep-time reduction and elevation of wake-up brain ethanol concentrations also occurred with hyperbaric treatment when the hypothermic effects of both helium and ethanol were eliminated and body temperature was maintained within normal limits (Figs. 3 and 4). These results show that the antagonistic effect of hyperbaric environments is not mediated by helium-induced changes in body temperature.

Interestingly, the degree of antagonism induced by hyperbaric treatment (with respect to rectal temperature matched controls) was lower and less consistent when hypothermia was allowed to occur (Fig. 2) than when hypothermia was offset (Fig. 4). This suggests a possible interaction between the antagonistic efficacy of pressure and body temperature. However, it may also reflect differences in group size or other variables since conditions were not counterbalanced across experiments.

Pharmacokinetic alterations do not appear to contribute to the antagonism. In concert with the reduced sleep-times, hyperbaric treated mice woke up at higher brain ethanol concentrations than controls (Fig. 4). This result parallels the higher wake-up blood ethanol concentrations previously reported for hyperbaric treated intoxicated mice [1–3]. Furthermore, the absence of a hyperbaric effect on the rate of blood ethanol decline and the higher brain ethanol concentration and brain-blood ratio in the 12 ATA group (Table 1) indicate that neither an increased rate of elimination, an alteration in the distribution of ethanol between blood and brain nor a reduced brain ethanol concentration can explain the hyperbaric sleep-time reduction. Rather, these results are consistent with a pharmacodynamic mechanism of antagonism and support the hypothesis that treatment with hyperbaric helium-oxygen reduces the sensitivity of the brain to ethanol.

The precise mechanism for the antagonistic effect of hyperbaric helium-oxygen remains unclear. Exposure to 1 or 2 ATA helium-oxygen has been observed to reduce the anesthetic effects of ethanol, α-chloralose and thiopental [3, 19, 28]. However, the antagonism in the present studies cannot be explained by an action of helium at normal pressure. Exposing ethanol-injected mice to 1 ATA helium-oxygen did not significantly alter sleep-time or wake-up brain ethanol under either thermally controlled condition. Furthermore, hyperbaric treatment significantly reduced ethanol sleep-time when compared to either the 1 ATA air or the 1 ATA helium-oxygen controls.

The antagonism could reflect a direct effect of pressure on ethanol's action via a mechanism similar to that suggested for the reversal of anesthesia induced by high hyperbaric and hydrostatic pressures [8, 10, 12, 13]. Such a mechanism would be consistent with the present results and with earlier studies demonstrating that the antagonism does not result from increased oxygen partial pressures [2]. A mechanism involving a direct effect of pressure would also agree with membrane expansion hypotheses of anesthesia [10, 18, 23]. These hypotheses suggest that the antagonistic effect of high pressures occurs when the pressure induced contraction of the membrane offsets the expansion thought to cause the anesthesia.

Hyperbaric environments represent a potential means of reducing acute ethanol toxicity and may provide an antagonist of ethanol which functions at the molecular level. Further studies are necessary to clarify the precise mechanism of the antagonism and to determine its usefulness as a research and clinical tool.

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