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Pressure Reversal of the Depressant Effect of Ethanol on Spontaneous Behavior in Rats

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GARCIA-CABRERA, I. AND O.-G. BERGE. *Pressure reversal of the depressant effect of ethanol on spontaneous behavior in rats*. PHARMACOL BIOCHEM BEHAV 29(1) 133–141, 1988.—This study deals with the interaction between high pressure and a sub-hypnotic dose of ethanol in rats. Male Sprague-Dawley rats were given either ethanol 1.5 g/kg or saline IP and subsequently exposed to 1 atmosphere absolute pressure (ATA) air or to 1, 12, 24 or 48 ATA of helium-oxygen (heliox). The gas temperature was adjusted to offset ethanol and helium-induced hypothermia. Ethanol induced a characteristic unsteady pattern of locomotion which was completely reversed at 48 ATA, partially reversed at 24 ATA, but not affected at 12 ATA. Other behavioral effects of ethanol such as depression of total motor activity and rearing were similarly affected. Blood and brain concentrations of ethanol in the pressure groups did not differ significantly from concentrations measured in the 1 ATA groups. A similar pattern of reversal was observed whether the compression was initiated 4, 10 or 16 min after injection. These results show that hyperbaric exposure antagonizes the depressant effect of ethanol on spontaneous behavior in rats. This antagonism does not appear to be due to changes in ethanol distribution or elimination.

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

HYDROSTATIC pressure restores the luminosity of bacteria exposed to a number of narcotics including ethanol [9]. Pressure in the range of 2000–5000 psi (136–340 absolute atmospheres, ATA) reverse ethanol inhibition of swimming in tadpoles [10]. These findings were later confirmed and extended to a variety of anesthetics and other drugs [8]. In these experiments, a complete reversal of narcosis in tadpoles induced by ethanol (0.43 M) was observed at 68 ATA.

Only sparse information is available regarding the effect of hyperbaric exposure on ethanol intoxication in terrestrial animals. In rats, no significant change in the mean lethal dose of ethanol was found at 19.4 ATA helium-oxygen (heliox), suggesting that the acute toxicity of ethanol is unaltered by exposure of animals to a hyperbaric helium environment [24]. On the other hand, exposure to 1 or 12 ATA heliox in mice reduced the lethality of ethanol given alone or in combination with pentobarbital [17].

Moderate increases in ambient pressure antagonized the acute depressant effects of ethanol on the righting reflex in mice [2–5, 16]. Hyperbaric exposure to heliox also precipitated and exacerbated withdrawal symptoms in ethanol-dependent mice [1].

In the studies referred to above that dealt with interactions between high pressure and acute effects of ethanol in mice, the only behavioral parameter scored was the "sleep-time." This study investigates the effects of hyperbaric conditions on the acute administration of ethanol in a different species, the rat, and analyzes a wider range of behavior. Blood and brain concentrations of ethanol were measured in order to detect whether ethanol distribution or elimination had been modified as a consequence of exposure to hyperbaric heliox.

In this type of experiment it is virtually impossible to introduce a control condition which differs from the experimental condition only with regard to level of pressure since the compression as such, as well as the required modifications of ambient temperature and gas composition, may influence the results. Thus, two different normobaric conditions (air and heliox) and three pressure conditions (12, 24, 48 ATA heliox) were employed.

Some of these data were presented in preliminary form at the XII EUBS meeting in Rotterdam, 1986.

GENERAL METHOD

Subjects

Drug-naive male Sprague-Dawley rats (Møllegård, Denmark) weighing 240–310 g at the begining of the experiment were housed three to a cage. In order to stabilize body weight during the experimental period, food was limited to 15 g of pellets per animal per day; there was free access to water. The light phase lasted from 8:00 to 20:00 hours and ambient temperature was 22–23°C. All experiments took place between 8:30 and 15:00 and the various treatment groups were tested in random order across days with regard to time of the day. Food but not water was removed one hour before testing.

The Hyperbaric Chamber

Experiments were carried out in a steel hyperbaric

chamber. The internal configuration of the chamber was a cylinder 50 cm long by 25 cm in diameter. The chamber was fitted with an 8 cm diameter acrylic window. The end wall of the chamber was provided with a number of penetrators for gas supply, electricity and instruments. Temperature was controlled by circulating water through a copper coil wound around the outside of the chamber. Chamber temperature was monitored by means of a thermistor probe connected to a computer interface. Pressure was measured with a gauge connected directly to the inside of the chamber and the information fed to a computer interface. Temperature and pressure data were continuously updated on the computer monitor and the median values for each minute were stored. An electric fan ensure proper mixing of the gases inside the chamber and forced the gases through a cartridge containing soda lime to prevent CO_2 accumulation.

Behavioral Observations

In the chamber, the rats were free to move within an area measuring 210×220 mm. Videotape recordings were made during the 60 minutes following injection by means of a video camera pointing through the end window. Analysis of the behavior was performed blind. Duration of all observable motor activity was recorded. This measure of total motor activity was divided into the following discrete and mutually exclusive categories: (1) Scan—side to side movements of the head. (2) Movements of the forelimbs without locomotion. (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 from the ground. In addition to the cumulative time of these categories, the number of rearings was registered.

The total motor activity was expressed in percent of the available time in each observation period. Since locomotion was either scored as "normal" or "uncoordinated," total locomotor activity was the sum of normal locomotion and staggering. Staggering in percent of total locomotor activity was therefore calculated as a measure of the extent to which the behavior was affected by ethanol.

Statistics

Behavioral activity data were analyzed by analysis of variance as detailed in the results. Subsequent paired comparisons of group means were performed by Scheffe's test. Non-parametric Kruskal-Wallis ANOVA by ranks and the Mann-Whitney tests were used when appropriate. Blood and brain concentrations of ethanol were analyzed using one-factor randomized analysis of variance with subsequent application of Scheffe's test.

EXPERIMENT 1

Procedure

Rats were injected intraperitoneally either with 1.5 g/kg ethanol (21 ml/kg of a 9% (v/v) ethanol/isotonic saline solution) or with a corresponding volume of isotonic saline. Immediately after injection, the animals were individually placed in the pressure chamber. From 2 min after injection until the start of compression, the chamber was flushed with a mixture of 80% helium and 20% oxygen in order to remove nitrogen from the breathing gas. When animals belonging to the 1 ATA groups were to be tested, the chamber was flushed with either air or heliox according to the same schedule as the 12 ATA group. Compression was started at different times after injection so that all animals reached final pressure exactly 20 min after ethanol administration (Fig. 1).

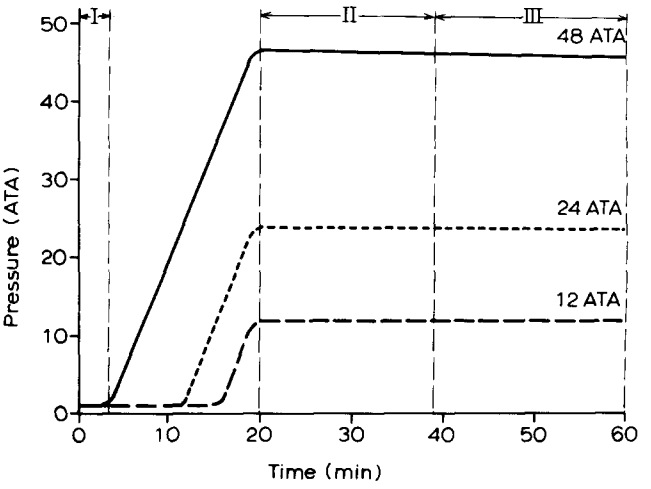

FIG. 1. Experiment 1: Pressure profiles. Time of injection=0 min. Compression started at 4 min 20 sec, 12 min 20 sec and 16 min 20 sec for the 48, 24, and 12 ATA groups. Target pressure was reached 20 min after injection in all groups. I=Observation period I (pre-compression period, 0 to 4 min 20 sec). II=Observation period II (20 to 40 min). III=Observation period III (40 to 60 min).

The rats were exposed to one atmosphere absolute pressure (ATA) of air or to 1, 12, 24 or 48 ATA of heliox. Oxygen partial pressure was maintained between 0.2 and 0.4 ATA. Compressions were carried out at a rate of 3 ATA/min and the duration of each experiment was 60 min. During compression and during the first and last 20 min periods at pressure the mean recorded pressure for any animal deviated by less than 1% of the predefined values.

The temperature of the chamber was adjusted to offset ethanol- and helium-induced hypothermia, on the basis of previous results ([16,26], Berge and Garcia-Cabrera, in preparation). The temperature settings were: 26±1°C for 1 ATA air, 30±1°C for 1 ATA heliox and 34±1°C for all groups under pressure. The mean ambient temperatures recorded during the experiments did not deviate from the predefined values. There were no overall differences between saline- and ethanol-treated groups under any pressure condition.

Measurement of Blood and Brain Concentrations of Ethanol

Sixty min after the beginning of the experiment, the rats were anesthetized by admitting N_2O to the pressure chamber, and then rapidly decompressed. They were removed from the chamber and the ethanol-treated animals were decapitated (65 min after injection) and brain and blood samples were collected. The samples were diluted 8:1 with 0.33 M perchloric acid (volume:volume for blood, volume:weight for brain). Brain samples were homogenized and centrifuged at 3000 rpm for 10 min. Blood samples were vortexed and centrifuged at 3000 rpm for 5 min. Ethanol concentrations were determined enzymatically in the supernatant using the ADH/NAD technique (Boehringer/Manneheim, F.R.G.).

RESULTS

Total Motor Activity

In the pre-compression period, time in activity ranged from 80±3% to 89±2% of the available time in the groups of

TABLE 1

TOTAL MOTOR ACTIVITY DIVIDED INTO CATEGORIES

Period		Air 1 ATA	He-O 1 ATA	He-O 12 ATA	He-O 24 ATA	He-O 48 ATA
			Scan			
I	Saline	20.7 ± 4.2	11.5 ± 3.6	16.3 ± 6.3	20.1 ± 6.6	13.4 ± 4.7
	Ethanol	41.1 ± 10.6	21.7 ± 4.7	23.7 ± 3.0	14.4 ± 2.5	22.7 ± 5.6
II	Saline	37.5 ± 8.6	11.3 ± 3.1	81.7 ± 14.9	49.7 ± 20.9	39.6 ± 14.5
	Ethanol	42.1 ± 13.9	18.3 ± 10.2	24.3 ± 8.6	20.0 ± 6.4	55.3 ± 10.7
III	Saline	5.3 ± 1.4	3.5 ± 1.1	45.9 ± 13.1	21.0 ± 8.1	26.5 ± 10.1
	Ethanol	17.8 ± 2.9	19.3 ± 5.7	21.8 ± 6.4	34.4 ± 13.0	38.3 ± 5.8
			Movements of Forelimbs			
I	Saline	58.9 ± 4.0	51.4 ± 6.9	70.2 ± 5.5	64.9 ± 6.1	59.8 ± 7.4
	Ethanol	37.6 ± 7.4	41.8 ± 7.1	35.1 ± 3.5	42.7 ± 8.2	41.2 ± 8.1
II	Saline	115.2 ± 32.9	59.5 ± 24.7	144.8 ± 40.5	155.5 ± 20.6	173.7 ± 16.6
	Ethanol	44.4 ± 16.8	14.1 ± 4.7	32.0 ± 12.0	29.4 ± 7.8	57.3 ± 18.5
III	Saline	14.6 ± 3.2	15.8 ± 6.1	66.8 ± 9.3	100.1 ± 34.5	233.4 ± 38.3
	Ethanol	34.1 ± 8.3	59.9 ± 21.3	38.0 ± 13.1	51.3 ± 15.4	89.3 ± 33.1
			Normal Locomotor Activity			
I	Saline	60.9 ± 6.1	73.4 ± 5.0	75.8 ± 8.5	67.7 ± 5.2	67.2 ± 5.7
	Ethanol	35.4 ± 2.5	36.4 ± 5.5	47.0 ± 8.3	41.5 ± 6.1	41.9 ± 10.0
II	Saline	32.3 ± 10.7	15.4 ± 3.5	102.3 ± 16.7	206.3 ± 26.9	92.4 ± 27.2
	Ethanol	4.6 ± 1.8	0.2 ± 0.2	18.8 ± 10.7	29.1 ± 9.1	128.0 ± 26.1
III	Saline	4.1 ± 1.6	5.9 ± 2.1	85.4 ± 21.5	99.3 ± 14.9	88.2 ± 13.2
	Ethanol	5.5 ± 2.0	0.9 ± 0.5	8.7 ± 5.4	19.0 ± 11.8	84.6 ± 19.4
			Staggering			
I	Ethanol	23.2 ± 4.1	28.3 ± 7.2	24.4 ± 6.0	25.3 ± 6.8	25.8 ± 8.5
II	Ethanol	10.4 ± 3.4	3.9 ± 2.5	14.8 ± 6.8	5.5 ± 4.3	0.4 ± 0.2
III	Ethanol	3.4 ± 1.1	7.6 ± 4.2	21.1 ± 9.6	12.9 ± 8.8	0.0 ± 0.0
			Grooming			
I	Saline	13.2 ± 5.0	14.5 ± 5.1	14.0 ± 4.5	18.2 ± 8.8	15.0 ± 8.8
	Ethanol	2.6 ± 2.0	4.7 ± 2.9	4.5 ± 4.2	3.5 ± 2.2	2.4 ± 2.3
II	Saline	206.4 ± 63.1	72.0 ± 24.0	221.2 ± 53.0	101.9 ± 23.8	7.1 ± 3.0
	Ethanol	29.6 ± 10.1	37.9 ± 20.3	161.7 ± 118.3	147.5 ± 92.3	40.9 ± 11.3
III	Saline	4.8 ± 3.1	15.8 ± 8.4	101.7 ± 28.5	120.4 ± 27.6	13.5 ± 3.2
	Ethanol	54.5 ± 20.4	51.7 ± 23.7	45.6 ± 13.2	105.8 ± 44.6	84.1 ± 27.2
			Wet-Dog Shaking			
I	Saline	4.8 ± 1.6	3.5 ± 1.0	1.7 ± 0.4	1.9 ± 0.4	2.9 ± 1.3
	Ethanol	0.8 ± 0.5	2.0 ± 1.1	2.1 ± 1.5	1.0 ± 0.4	1.9 ± 0.8
II	Saline	0.7 ± 0.4	0.1 ± 0.1	4.2 ± 2.4	0.4 ± 0.4	1.3 ± 1.0
	Ethanol	0.8 ± 0.7	0.0 ± 0.0	4.4 ± 2.0	2.8 ± 2.5	1.4 ± 0.6
III	Saline	0.0 ± 0.0	0.1 ± 0.1	0.6 ± 0.3	0.3 ± 0.2	2.0 ± 1.4
	Ethanol	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.2	1.0 ± 0.7	0.8 ± 0.4
			Rearing			
I	Saline	48.1 ± 3.5	61.5 ± 5.1	51.4 ± 4.6	50.9 ± 6.9	63.2 ± 5.6
	Ethanol	20.1 ± 4.1	22.4 ± 5.2	20.3 ± 5.3	25.2 ± 5.2	23.3 ± 5.0
II	Saline	57.7 ± 20.0	27.7 ± 13.3	10.6 ± 4.7	22.8 ± 7.0	5.9 ± 2.5
	Ethanol	0.0 ± 0.0	0.7 ± 0.7	0.3 ± 0.2	2.9 ± 2.1	47.1 ± 31.1
III	Saline	0.5 ± 0.5	0.0 ± 0.0	9.5 ± 2.0	21.6 ± 7.1	9.2 ± 2.7
	Ethanol	1.8 ± 0.8	0.9 ± 0.6	1.5 ± 1.5	18.4 ± 17.5	35.7 ± 22.7

Data given as mean ± S.E.M. (N=7–8 in each group) of cumulative time (sec) in each category. See Fig. 1 for definitions of the observation periods.

TABLE 2

PERCENTAGE OF TOTAL MOTOR ACTIVITY IN EACH BEHAVIORAL CATEGORY

Period		Air 1 ATA	He-O 1 ATA	He-O 12 ATA	He-O 24 ATA	He-O 48 ATA
			Scan			
I	Saline	9.8 ± 1.8	5.3 ± 1.6	7.3 ± 2.9	9.0 ± 3.0	6.1 ± 2.1
	Ethanol	24.7 ± 6.0	14.1 ± 2.9	15.5 ± 2.2	10.2 ± 2.2	14.6 ± 3.5
II	Saline	20.7 ± 11.5	11.0 ± 4.8	16.1 ± 3.2	8.8 ± 3.0	10.9 ± 3.1
	Ethanol	33.4 ± 8.8	24.7 ± 6.1	15.9 ± 6.5	10.1 ± 1.9	19.0 ± 4.2
III	Saline	22.5 ± 5.5	12.4 ± 5.1	16.2 ± 4.3	4.6 ± 1.6	7.7 ± 3.1
	Ethanol	23.1 ± 5.5	27.2 ± 11.1	14.3 ± 4.1	18.6 ± 6.3	15.2 ± 2.5
			Movements of Forelimbs			
I	Saline	28.9 ± 2.4	23.8 ± 3.2	30.6 ± 2.3	28.9 ± 2.6	27.1 ± 3.3
	Ethanol	23.5 ± 4.3	25.9 ± 3.1	22.5 ± 2.3	27.1 ± 3.3	24.5 ± 3.3
II	Saline	22.6 ± 5.0	28.1 ± 5.1	24.8 ± 3.8	29.8 ± 3.6	58.0 ± 8.6
	Ethanol	32.0 ± 7.7	26.8 ± 7.2	17.9 ± 4.8	22.2 ± 6.2	16.7 ± 3.2
III	Saline	50.9 ± 6.7	36.3 ± 6.1	22.5 ± 2.7	24.5 ± 4.6	61.7 ± 4.7
	Ethanol	28.9 ± 5.5	39.1 ± 6.5	27.8 ± 6.8	27.6 ± 9.8	24.8 ± 5.0
			Normal Locomotor Activity			
I	Saline	29.2 ± 2.3	33.9 ± 2.1	32.7 ± 3.1	30.1 ± 2.0	30.4 ± 2.5
	Ethanol	22.4 ± 2.0	22.9 ± 2.7	29.1 ± 3.5	26.5 ± 2.0	25.7 ± 4.5
II	Saline	6.0 ± 1.6	8.2 ± 1.8	20.7 ± 4.3	38.9 ± 4.5	26.7 ± 6.8
	Ethanol	4.9 ± 2.4	0.5 ± 0.5	17.9 ± 10.4	20.4 ± 6.3	41.5 ± 6.3
III	Saline	13.7 ± 5.4	20.2 ± 6.5	27.0 ± 4.7	32.0 ± 4.6	24.0 ± 2.8
	Ethanol	9.7 ± 5.5	0.6 ± 0.3	6.5 ± 4.4	6.2 ± 1.8	28.6 ± 4.3
			Staggering			
I	Ethanol	14.7 ± 2.7	18.8 ± 4.6	16.4 ± 4.2	17.7 ± 4.3	18.3 ± 0.6
II	Ethanol	9.9 ± 3.8	5.7 ± 1.7	6.3 ± 2.8	2.5 ± 1.5	0.1 ± 0.0
III	Ethanol	3.7 ± 1.4	5.0 ± 2.1	12.0 ± 5.1	5.1 ± 3.8	0.0 ± 0.0
			Grooming			
I	Saline	6.2 ± 2.4	6.8 ± 2.4	6.0 ± 1.9	8.0 ± 3.9	6.6 ± 3.8
	Ethanol	1.6 ± 1.3	3.2 ± 2.0	2.8 ± 2.6	1.8 ± 1.1	1.1 ± 1.1
II	Saline	41.0 ± 9.2	37.2 ± 12.0	36.2 ± 6.9	18.8 ± 3.9	2.1 ± 0.8
	Ethanol	19.4 ± 4.7	39.4 ± 9.9	39.7 ± 12.5	42.1 ± 9.9	13.1 ± 4.1
III	Saline	10.8 ± 6.6	30.8 ± 11.8	30.6 ± 7.3	34.1 ± 4.7	3.8 ± 0.9
	Ethanol	33.3 ± 9.7	27.6 ± 8.5	34.3 ± 7.0	38.7 ± 12.4	24.7 ± 6.5
			Wet-Dog Shaking			
I	Saline	2.2 ± 0.7	1.7 ± 0.5	0.8 ± 0.2	0.8 ± 0.2	1.3 ± 0.6
	Ethanol	0.5 ± 0.3	1.3 ± 0.8	1.3 ± 0.9	0.6 ± 0.3	1.1 ± 0.5
II	Saline	0.1 ± 0.1	0.1 ± 0.0	0.6 ± 0.3	0.1 ± 0.1	0.3 ± 0.2
	Ethanol	0.4 ± 0.4	0.0 ± 0.0	2.4 ± 1.3	0.6 ± 0.3	0.5 ± 0.2
III	Saline	0.0 ± 0.0	0.3 ± 0.3	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.4
	Ethanol	0.0 ± 0.0	0.1 ± 0.1	3.1 ± 3.0	0.2 ± 0.2	0.5 ± 0.3
			Rearing			
I	Saline	23.4 ± 1.8	28.5 ± 2.3	22.5 ± 2.1	22.9 ± 3.2	28.6 ± 2.7
	Ethanol	12.5 ± 2.2	13.9 ± 2.5	12.4 ± 2.7	16.1 ± 2.6	14.7 ± 2.9
II	Saline	9.6 ± 2.8	15.5 ± 6.1	1.6 ± 0.6	3.6 ± 1.1	2.0 ± 0.9
	Ethanol	0.0 ± 0.0	2.9 ± 2.9	0.0 ± 0.0	2.1 ± 1.8	9.1 ± 6.1
III	Saline	2.2 ± 2.2	0.0 ± 0.0	3.3 ± 0.7	4.6 ± 1.2	2.3 ± 0.7
	Ethanol	1.3 ± 0.4	0.4 ± 0.3	2.1 ± 2.1	3.4 ± 2.8	6.1 ± 3.6

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

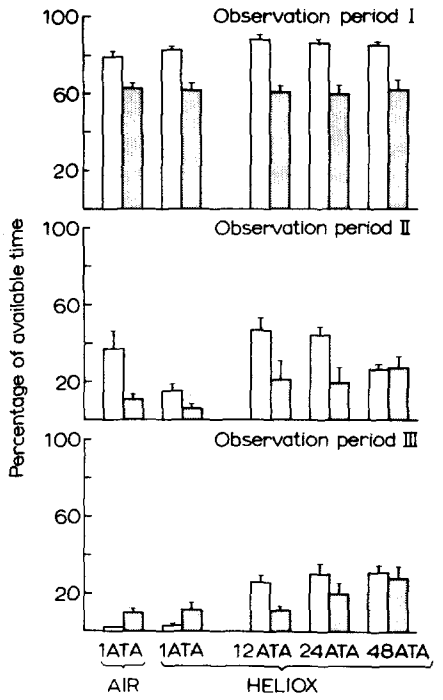

FIG. 2. Experiment 1: Total motor activity. Open bars represent the saline control groups; shaded bars the groups administered 1.5 g/kg ethanol. Results are expressed as the mean±S.E.M., N=7–8 in each group. See Fig. 1 for definitions of the observation periods.

saline-treated rats and from 60±5% to 63±2% in the ethanol-treated animals (Fig. 2). The reduction in total activity in the ethanol-treated groups was largely due to less time being spent in movements of the forelimbs, grooming and rearing (Table 1). ANOVA (2×5 design; factor 1: saline or ethanol treatment, factor 2: assignment to the various 1 ATA and pressure conditions, hereafter referred to as pressure conditions) demonstrated that the difference in total activity between the ethanol and saline treatments was highly significant, $F(1,69)=127.49$, $p<0.0001$, whereas there was no effect of assignment to pressure conditions and no interaction between the two factors.

All groups showed less activity during the second observation period, which consisted of the first 20 min of stable hyperbaric conditions in the pressure groups (Fig. 2). The reduction in total activity was accompanied by an overall shift in the response pattern with a higher percentage of the activity being grooming (except in the 48 ATA saline-treated group) and less being rearing (Table 2). The contribution of locomotion to the total activity was reduced in the 1 and 12 ATA groups. At 48 ATA, the saline- and ethanol-treated animals showed similar amounts of total activity, but the ethanol group spent relatively more time in locomotion, rearing and grooming. At lower pressures, the ethanol-treated rats showed less overall activity than the saline-treated animals (Fig. 2) and consistently spent less time in rearing and locomotion. However, in terms of percentage of total activity, only rearing was consistently affected. The ethanol-treated rats exposed to 24 ATA showed relatively more activity as grooming than the corresponding saline-treated animals. The groups at hyperbaric pressures were more active than the groups at 1 ATA heliox but the increase in activity was not correlated with the level of pressure. As during the first period, ANOVA of the total activity scores

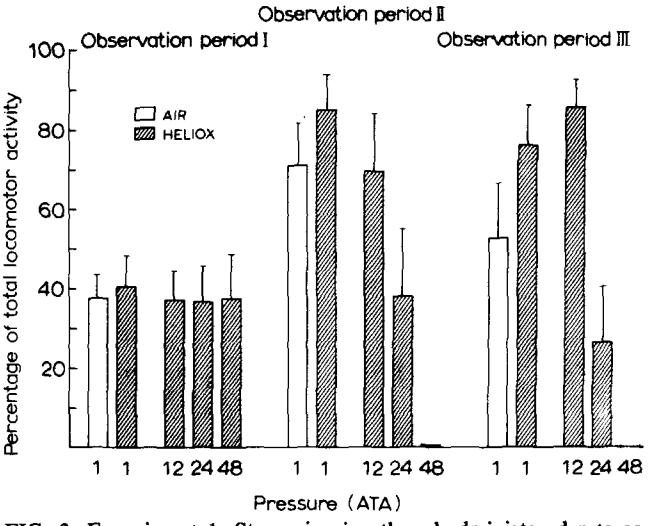

FIG. 3. Experiment 1: Staggering in ethanol-administered rats as percentage of total locomotion. Results are expressed as the mean±S.E.M., N=7–8. See Fig. 1 for definitions of the observation periods. Saline groups, which showed no staggering, are not represented.

(2×5 design) demonstrated a significant difference between the ethanol- and saline-treated groups, $F(1,69)=18.39$, $p<0.0001$. There was also a significant difference between the pressure conditions, $F(4,69)=4.23$, $p<0.005$, but no significant interaction.

During the third observation period, the ethanol-treated animals at 1 ATA showed somewhat more overall activity than the rats which received saline (Fig. 2). At 12 and 24 ATA, the ethanol-treated rats were less active than the corresponding groups of saline-treated animals and less of the activity was scored as locomotion. Again, the two groups at 48 ATA had nearly identical levels of total activity with the ethanol-treated animals spending more time grooming and rearing but showing less movements restricted to the forelimbs. ANOVA of the total activity (2 drug levels × 5 pressure conditions) revealed no significant difference between the ethanol- and saline-treated groups, while there was a highly significant effect of pressure conditions, $F(4,69)=12.75$, $p<0.0001$, and a significant interaction between the two factors, $F(4,69)=3.19$, $p<0.02$.

Staggering

No staggering was observed in the saline-treated rats. The results of the ethanol-injected animals are presented in Fig. 3.

In the pre-compression period, the amount of staggering for each group was within the range of 35±7% to 40±8% of total locomotion. There was no statistical difference between the groups (one-way ANOVA).

During the second and third periods, there was a tendency to higher staggering scores in the 1 ATA heliox group than in the 1 ATA air group. The scores of the 24 ATA group were somewhat lower than either of the 1 ATA groups while the 48 ATA group hardly showed any staggering.

Statistical analysis of the results from the second period showed a significant difference between groups, $F(4,34)=8.66$, $p<0.0001$; one-way ANOVA. Subsequent application of Scheffe's test showed that the 48 ATA group was significantly different from both the 1 ATA groups and the 12 ATA group ($p<0.01$), but not from the 24 ATA group.

TABLE 3
NUMBER OF REARINGS, EXPERIMENT 1

Treatment	Air 1 ATA	He-O 1 ATA	He-O 12 ATA	He-O 24 ATA	He-O 48 ATA
			Period I		
Saline	23.5 (21–31)	28.5 (16–38)	23.0 (17–32)	23.0 (15–32)	24.0 (21–39)
N	8/8	8/8	8/8	8/8	7/7
Ethanol	15.0† (4–28)	10.5† (4–26)	13.0* (5–28)	15.0† (7–29)	17.0‡ (7–22)
N	8/8	8/8	7/7	8/8	8/8
			Period II+III		
Saline	12.0 (0–26)	1.5 (0–12)	5.5 (3–15)	12.0 (1–34)	3.0 (0–13)
N	6/8	5/8	8/8	8/8	6/7
Ethanol	1.0* (0–1)	0.0 (0–3)	0.0‡ (0–4)	0.0* (0–39)	3.0 (0–109)
N	5/8	3/8	2/7	3/8	7/8

Data given as medians with extreme scores in parentheses. N=number of animals showing rearing/number of animals in the group. *$p<0.05$, †$p<0.01$, ‡$p<0.005$, significantly different from corresponding saline-treated group (Mann-Whitney U-test).

There were no significant differences between any of the other means.

Similarly, the difference between groups during the third period was significant, $F(4,34)=11.29$, $p<0.0001$; one-way ANOVA. Scheffe's test showed that the 48 ATA group was significantly different from the 1 ATA air group ($p<0.05$) and from the 1 ATA and 12 ATA heliox groups ($p<0.001$), but not from the 24 ATA group. The 24 ATA group was different from the 1 ATA and 12 ATA heliox groups ($p<0.05$ in each case) but not from the 1 ATA air group. There were no significant differences between the 1 and 12 ATA groups.

Rearing

During the pre-compression period, the ethanol-treated animals showed considerably less rearing than the saline-treated rats (Table 3). There was no significant difference between age groups that received saline ($p>0.70$, Kruskal-Wallis ANOVA) or between the groups that received ethanol ($p>0.95$, Kruskal-Wallis ANOVA).

The scores for the post-compression periods were pooled in view of the low level of rearing activity observed. Again, there was a tendency to less rearing in the groups that received ethanol, but the difference was not significant at 1 and 48 ATA heliox. In the latter case, some of the scores in the ethanol group were considerably higher than in any of the other groups. Kruskal-Wallis ANOVA demonstrated non-significant tendencies towards differences between the saline-treated groups ($0.10<p<0.15$) and between the groups that received ethanol ($0.05<p<0.10$).

Ethanol Levels in Blood and Brain

The mean levels of ethanol in samples of blood obtained immediately after decompression were within the range of 1.32 to 1.35 mg/ml (Table 4), with no significant difference between the groups (one-way ANOVA). The mean brain concentrations were in the range of 1.84 to 1.87 mg/g, with the exception of the 12 ATA heliox group which had somewhat lower values. One-way ANOVA demonstrated a significant difference between groups, $F(4,34)=3.01$, $p<0.05$, but subsequent application of Scheffe's test did not demonstrate a difference between any two means although the 12 ATA group tended to be different from each of the other groups ($0.05<p<0.10$).

EXPERIMENT 2

In this experiment, three groups of animals were tested at 48 ATA in order to eliminate any effects of the different delays between injection and start of compression in the previous experiment. Two different delays which covered the time span of those used for the groups pressurized to 12 and 24 ATA in Experiment 1 were introduced.

Procedure

The rats were randomly assigned to three groups (7–8 rats in each). All rats were given ethanol 1.5 g/kg IP (21 ml/kg of a 9% (v/v) ethanol/isotonic saline solution). Immediately after injection, the animals were placed in the pressure chamber for individual testing. From 2 min after injection until the start of compression the chamber was flushed with a mixture of helium (80%) and oxygen (20%). Compression started at the following times after ethanol injection: group 1: 4 min 20 sec, group 2: 10 min 20 sec, group 3: 16 min 20 sec. All groups were compressed to 48 ATA. As in Experiment 1, all pressures recorded were within ±1% of the predefined values. The compression rate, the chamber temperature and the oxygen partial pressure were the same as in Experiment 1. The mean ambient temperatures recorded during the experiments did not deviate from the predefined values and there were no significant differences between the groups during or after compression. The experiment was terminated 60 min after ethanol administration.

RESULTS

Total Motor Activity

Time in activity was calculated for the pre-compression period, for each 2 min period during compression and for the period 40–60 min after ethanol administration. In the pre-compression period, the mean times in activity for Groups 1, 2 and 3 were 57±7%, 62±5% and 65±5%. During the period 40–60 min after injection, the corresponding values were 35±5%, 29±5% and 35±4%. These values were similar to the scores of the 48 ATA/ethanol group in Experiment 1 (Fig. 2). ANOVA revealed no difference between the groups during any period.

Staggering

Staggering was analyzed in the same way as the total activity. During the pre-compression period, the percentage of locomotion scored as staggering was 59±8%, 64±3% and 60±7% for groups 1, 2 and 3 respectively. These scores were somewhat higher than the scores observed in Experiment 1. As the case was with the 48 ATA group in Experiment 1, no staggering was observed in any of the groups after the completion of compression.

The results for the compression period are shown in Fig. 4. Comparison of the scores obtained during the 2 min period

TABLE 4

ETHANOL LEVELS IN BLOOD AND BRAIN AFTER 1.5 g/kg ETHANOL GIVEN IP

Tissue		Air 1 ATA	He-O 1 ATA	He-O 12 ATA	He-O 24 ATA	He-O 48 ATA
Blood (mg/ml)	Mean	1.35	1.35	1.34	1.32	1.32
	S.E.M.	0.04	0.04	0.08	0.04	0.04
Brain (mg/g)	Mean	1.84	1.87	1.65	1.87	1.86
	S.E.M.	0.07	0.06	0.06	0.03	0.03

N=7–8 animals in each group.

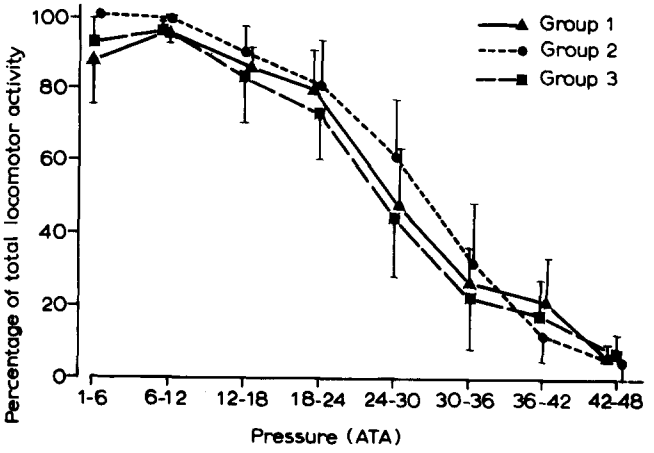

FIG. 4. Experiment 2: Staggering as percentage of total locomotion during compression. Results are expressed as mean±S.E.M. Each group consisted of 7–8 animals. Group 1 (▲), group 2 (●), group 3 (■).

immediately prior to and during compression (3 groups × 9 pressures ANOVA) demonstrated a significant pressure effect, $F(8,160)=53.00$, $p<0.00001$, but no group effect, $F(2,20)<1$, or interaction, $F(16,160)<1$. Subsequent comparison of the results obtained prior to compression with the scores of each pressure segment demonstrated a non-significant tendency to effect at 18–24 ATA ($0.05<p<0.10$) and reliable effects at higher pressures ($p<0.0005$).

Rearing

During the pre-compression period, the median numbers of rearings were 11.5, 14.5 and 15 for groups 1, 2 and 3 respectively, similar to the values observed in Experiment 1. During the period at final pressure, all rats in group 1 (median=9.5), four out of seven rats in group 2 (median=3), and seven out of eight in group 3 (median=3) showed some rearing activity (note that the observation period differed for the three groups since the experiments were completed 60 min after injection of ethanol, regardless of when compression was started). There were no statistically significant differences between the groups in any period (Kruskal-Wallis ANOVA by ranks).

GENERAL DISCUSSION

This study demonstrates pressure reversal of the effects of ethanol on spontaneous behavior in rats. The reversal appears to be pressure-dependent, with virtual disappearance of the pronounced motor uncoordination (staggering) induced by ethanol at 48 ATA. In addition, at 48 ATA no differences could be detected between saline- and ethanol-injected rats with regard to other depressant effects of ethanol such as reduction in total motor activity and reduction in the number of rearings. Differences in these parameters were present under normobaric conditions and after compression to 12 and 24 ATA. Exposure to the various compression schedules did not alter the blood and brain concentrations of ethanol, as measured 65 min after injection.

Both helium and ethanol may compromise the ability of the animals to regulate body temperature [12, 15, 16, 20, 23, 26] and changes in body temperature may alter the metabolism of ethanol [21] and affect behavioral responses to the compound [22]. It is not likely that significant changes in body temperature occurred in these experiments. Data obtained from mice [16] suggest that ambient temperatures between 34 and 35°C prevent the combined hypothermic effects of ethanol and hyperbaric helium. Similar results have been found with restrained rats using the same experimental setup and chamber temperature ranges as in this study (Berge and Garcia-Cabrera, in preparation). Furthermore, we found no differences in brain and blood concentrations that would suggest any difference in metabolism or distribution of ethanol between the various pressure conditions.

In these experiments, saline-treated rats under pressure tended to show more overall motor activity than the rats in a 1 ATA air or 1 ATA heliox atmosphere, particularly during the last 20 minutes of observation, suggesting that the animals adapted more slowly to the chamber under hyperbaric conditions. The effect was not directly correlated with the level of pressure, which may imply that other factors than pressure *per se* may be important, e.g., noise and discomfort during compression or reactions to the thermal properties of the hyperbaric helium atmosphere.

There are few quantitative studies about the effect of high pressure on spontaneous motor activity in mammals. Species differences with regard to adverse pressure effects have been described [6]. In contrast to the present study, hyperbaric exposure has been found to reduce locomotor activity in mice [27]. Although experimental methods and assessment procedures may account for the differences, the possibility of species-related variations in the responses to high pressure should be noted. However, the observed increase in overall activity in the control groups under pressure in the present study provided a suitable background for further analysis of behavior. In addition, it is noteworthy that the general depressant effect of ethanol on this parameter was not detectable in the 48 ATA heliox group.

The staggering parameter provides a more direct assess-

ment of ethanol effects. Since the criterion is applied in a qualitative way, that is, scored whenever uncoordinated forward locomotion is observed, reversal of ethanol intoxication would only be recorded as far as the animal was able to show apparently normal locomotion. Thus, lack of staggering implies a relatively complete reversal of intoxication and it is likely that a partial reversal occurred at lower pressures than 48 ATA in these experiments. In fact, a tendency towards reduced effects of ethanol was found in the rats exposed to 24 ATA. These data together with the data from Experiment 2 suggest that the antagonism of the staggering behavior is pressure-related.

The number of rearings showed the same pattern as the motor activity and staggering scores with the general depressant effect of ethanol not detectable at 48 ATA. A relatively high level of rearing activity was also found in the second experiment at 48 ATA. However, the two groups which were exposed to 1 ATA heliox during the last 40 minutes of the experiment showed very little rearing and no statistical difference could be demonstrated. The low amount of rearing in the saline group may have been caused by exposure to heliox *per se* or to the associated high temperature necessary to avoid hypothermia in a heliox atmosphere.

Previous work has shown that compression to between 6 and 12 ATA in a heliox atmosphere is sufficient to reverse the depressant effects of ethanol in mice using "sleep time" (absence of righting reflex) as a criterion [2–5, 16]. The different criteria used may at least partly explain the difference between the ranges of pressure needed for antagonism in mice and rats, assuming that the righting reflex may be performed by animals too intoxicated to display completely normal locomotion. On the basis of the available data, however, it is not possible to exclude species differences as a contributing factor.

Although the first experiment demonstrated a consistent reversal of ethanol intoxication in the 48 ATA group, the possible effect of the variable delay between ethanol administration and start of compression (16 min 20 sec in the 12 ATA group, 4 min 20 sec in the 48 ATA group) had to be considered. The groups probably had different blood levels of ethanol at the start of compression [29], and the lack of behavioral effects of ethanol in the 48 ATA group in the first experiment could have been due to prevention rather than reversal of intoxication. However, the delays employed in the second experiment covered the same time span. The fact that similar results were obtained from the three groups on all parameters in this experiment precludes an important contribution of delay-related factors. Furthermore, all groups showed complete reversal at 48 ATA. These results support the findings of other workers that there is no critical time period after ethanol administration during which hyperbaric treatment must be initiated in order to achieve maximal antagonism [5].

Relatively few behavioral studies have addressed the problem of interaction between high pressure and ethanol-induced effects. Hyperbaric air (7.1 ATA) reduced the effects of ethanol in rats tested using operant conditioning techniques [28]. On the other hand, in human subjects, moderate doses of ethanol were found to have a significant potentiating action on the increase in body sway induced by acute exposure to compressed air [11]. The subjects were exposed to 4 and 6 ATA, and it is likely that the performance decrement was a consequence of the interaction between inert gas narcosis and ethanol intoxication.

The mechanism by which hyperbaric exposure antagonizes the depressant effects of ethanol and other anesthetics is still unclear. Several *in vitro* studies have shown antagonistic effects of ethanol and high pressures. High pressure restored the amplitude of the action potential in the squid axon exposed to ethanol [25]. Ethanol and other anesthetics inhibit pressure-induced repetitive nerve activity without blocking conduction in the nerve [13]. Ethanol produces an increase in nerve membrane fluidity whereas high pressure decreases fluidity [18].

It has been argued that the antagonism occurs because the reduction in membrane volume produced by an effective pressure balances the membrane expansion which causes anesthesia [14,19]. However, additive or synergistic effects of pressure and certain anesthetics have also been reported [7], suggesting that other mechanisms may be involved in pressure-anesthetics interactions. Further work is clearly needed to establish the nature of pressure reversal of anesthetics' effects.

In conclusion, the present study of spontaneous behavior in rats is in agreement with previous work demonstrating pressure reversal of ethanol narcosis in intact animals. The results demonstrate a pressure-related antagonism of the depressant effects of a moderate dose of ethanol with complete reversal at 48 ATA.

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