Adaptation to ethanol in rats with special reference to brain tissue respiration

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THE respiration rate of brain cortex tissue stimulated electrically or with potassium chloride is reduced by ethanol[1] and by other depressants such as barbiturates.[2] Stimulated cortex slices are much more sensitive to the effect of these agents than are unstimulated ones.

It is well established that prolonged use of alcoholic beverages or other intoxicants will result in increased tolerance to these substances. Takemori[3] has demonstrated a tissue adaptation to morphine in rats, employing potassium-stimulation of brain cortex slices from animals given a course of morphine treatment. The present experiment was an attempt to discover whether adaptation to ethanol would be reflected in a reduction of the effect of this substance on electrically stimulated cortex tissue.

The experimental animals used were two groups of 20 six-month-old male rats (weight 300–400 g) from the laboratory stock. The rats were made to fast overnight. For measuring alcohol intoxication, the tilted plane technique[4] was used. First, the animals were tested when sober, and then each animal received 4 mg/g ethanol *per os* as a 25% (w/v) solution in tap water, and was tested at 20-min intervals for 100 min. The means of the lowest values observed for each individual are shown in Fig. 1 (A). The performance of the groups was similar. Then the experimental group was given 5 mg/g ethanol (without previous fasting) every other day for 20 days. The control animals received corresponding amounts of water. After the termination of this treatment, the effect of 4 mg/g ethanol after fasting overnight was tested again (Fig. 1 (B)). The difference between the groups was quite clear ($P < 0{\cdot}001$, calculated by means of Student's t-test). Before the repeated treatment with ethanol the performance of the experimental group was reduced by 31·9 per cent, after it by only 16·7 per cent. The animals had acquired a tolerance to alcohol, whereas the performance of the control group was virtually unchanged.

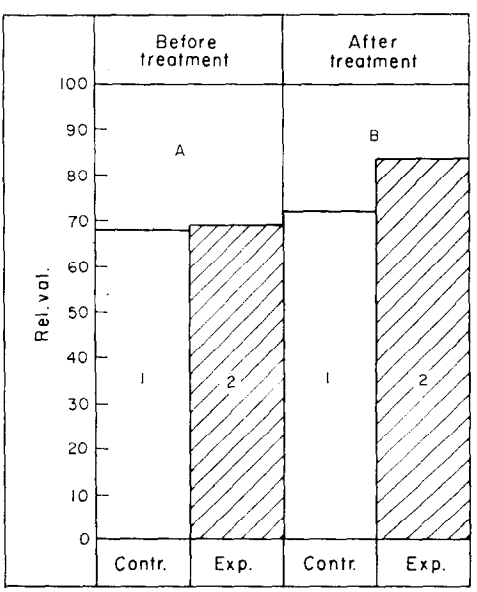

FIG. 1. The performance in the tilted plane test of two groups of 20 rats after administration of 4 mg/g ethanol. A, initial values; B, after repeated administration of water (control group) and 5 mg/g ethanol (experimental group). The performance of the same animals when not intoxicated is taken as 100 in both cases. The difference in the performances of groups 1 and 2 in B during intoxication was significant. ($P < 0{\cdot}001$, s.d. ± 7·7 per cent in group 1; ± 8·5 per cent in group 2).

The administration of ethanol to the experimental animals was continued every second or third day in order to maintain their state of adaptation, until the animals were decapitated and slices prepared from the cerebral hemispheres for assay of the oxygen consumption. The method was as described earlier,[1] except that an electronic pulse generator was used for stimulation. The pulses were alternating condenser discharges, the frequency being 50 Hz, the peak voltage 12 v (10 mm distance between electrodes), and the time constant 0·6 msec. Stimulation was observed with an oscilloscope. As regards the magnitude and stability of the response, and the effect of ethanol, the results obtained with this apparatus are entirely comparable with those obtained with the earlier one.[1]

Three slices (60–80 mg) from one cerebral lobe were used as controls and the corresponding slices from the other lobe incubated in the presence of 0·130 M (0·6%) ethanol, the right and left lobes being used alternately as controls. Respiration during 60 min after an initial equilibration period of 18 min served as the measure of stimulation. Since controls and experimental slices were from the same brain, the *t*-test for correlated measures was used in the statistical evaluation of the results.

TABLE 1. DEPRESSING ACTION OF 130 MM (0·6%) ETHANOL ON THE RESPIRATION OF STIMULATED BRAIN TISSUE OF RATS AFTER REPEATED ADMINISTRATION OF WATER (CONTROL) AND 5 MG/G ETHANOL (EXPERIMENTAL)

Group	No. of animals	Stimulated oxygen uptake μmoles/g fresh weight/hr		Effect of ethanol $P <$	Percent inhibition
		No ethanol	130 mM ethanol		
Control	13	191·8±12·5	169·3±21·5	0·005	11·7
Experimental	18	189·2±17·5	171·4±18·7	0·01	9·4

No significant difference between the groups in respect of the action of ethanol was observed.

As shown in Table 1, the respiration of both the tissue from the control animals and that from the rats treated with ethanol was clearly depressed by ethanol. The slight numerical difference in the effect of ethanol on the controls (reduction 11·7 per cent) and on the experimental group (reduction 9·4 per cent) after this fairly short treatment was far from significant, however. That the acquired overall physiological tolerance to the effects of ethanol was not reflected in the experiments with brain slices may be due to the roughness of the method. It is thus felt that the present experiment still does not exclude the possibility of changes in cerebral cellular characteristics due to repeated treatment with ethanol.

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Note added in proof: After this communication had gone to press, the authors found that A. F. HOGANS, O. M. MORENO and D. A. BRODIE (*Amer. J. Physiol.* **204**, 434, (1961)). report behavioural tolerance to alcohol in monkeys after as little as 4 times of intravenous administration (2 g/kg). This tolerance was not parallelled by diminished effects on EEG-patterns recorded with chronic electrodes from the cortex, suggesting that subcortical centers may be involved in the early stages of nervous adaptation to ethyl alcohol.