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Acute Tolerance to Ethanol in the Rat

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Abstract. Male Wistar rats were examined on the moving belt test at approximately 10, 30 and 60 min after administration of ethanol in doses ranging from 1.0–2.8 g/kg. Immediately after the test, each animal was sacrificed and ethanol concentrations were measured. The regression line of impairment as a function of brain alcohol concentration showed a progressive shift toward higher brain levels with increasing time after alcohol administration. The results confirm the existence of acute tolerance to ethanol, as defined by reduced impairment of function for a given blood level on the falling versus the rising arm of the blood alcohol curve. Confusion of interpretation due to arterio-venous differences in alcohol concentration was ruled out by simultaneous measurements in arterial blood and brain. Practice effects were ruled out by testing each animal only once.

Key words: Acute Tolerance – Brain – Ethanol – Moving Belt Test – Rats.

The concept of acute tolerance to ethanol was first proposed by Mellanby (1919), who observed a greater degree of impairment at a given concentration of ethanol during the rising portion of the blood alcohol curve than at the same concentration in the descending part of the curve. This has been confirmed repeatedly, with a wide range of physiological and behavioral measures of alcohol effect, including positional alcohol nystagmus (Aschan *et al.*, 1956), electroencephalographic changes (Caspers and Abele, 1956; Story *et al.*, 1961), synergism with hexobarbital-induced EEG changes (Wahlström and Widerlöv, 1971), ear-drop and nystagmus (Mirsky *et al.*, 1941), diuresis (Eggleton, 1942), cutaneous vasodilatation (Heidelmann *et al.*, 1952) and various psychomotor performance tests (Goldberg, 1943). These findings have been interpreted as evidence that the central nervous system can make adaptive or compensatory changes during the period of a single exposure to ethanol. If this is true, the time base of alcohol tolerance would be measured in hours rather than in days or weeks.

Two objections have been raised to this interpretation of the Mellanby effect. The first (Harger and Forney, 1963) is that ethanol is not distributed from the blood into all organs and tissues at the same rate. Equilibration with brain is rapid, while in skeletal muscle and skin it is slow. The measurement of ethanol in antecubital venous blood does not, therefore, reflect the level in the brain during the time in which the alcoholemia is rapidly rising after the ingestion of a single dose (Gostomzyk *et al.*, 1969). In contrast, ethanol concentrations in venous blood and brain agree closely after the bulk of absorption is finished and distribution is essentially complete, as during the falling phase of the alcohol curve.

The second objection is that repeated testing of performance at different times during the rise and fall of blood alcohol level provides the opportunity for continued practice in compensating for the effect of alcohol on performance of the task. Therefore, the improved performance during the descending part of the blood alcohol curve may simply reflect a greater learning than would have been possible during the early tests in the rising phase (Jones and Vega, 1972).

The present paper reports the results of an experiment in which both of these possible explanations have been eliminated. Alcohol concentration was measured in the brain directly as well as in the blood, and each animal was tested only once following the administration of the ethanol. The results confirm the existence of acute tolerance as a true phenomenon.

Methods

Animals. Sixty male Wistar rats, whose mean initial body weight was 300 g, were housed singly and fed standard laboratory chow and water *ad lib.* Body

weight was held constant by appropriate rationing of the chow.

Procedure. The test employed was the moving belt test described previously (Gibbins, Kalant, and LeBlanc, 1968). Animals are obliged to remain on a motor-driven belt which moves continuously over a shock-grid. If the animal puts one or more paws on the grid it receives shock and activates a cumulative timer. The effects of ethanol, pentobarbital and other drugs are seen as monotonic dose-dependent increases in time off belt. Slight modifications of the apparatus[1], for convenience of training and maintenance, had no significant effect on the dose-response or blood level-response curves. The animals were trained to a criterion of no more than 1% error on each run (LeBlanc *et al.*, 1969). The training procedure occupied several weeks, and led to a highly stable level of performance.

For the experiment proper, each rat was given a dose of ethanol (8% w/v in physiological saline) by intraperitoneal injection, and was run for a single two-minute trial on the moving belt test, commencing 9, 29 or 59 min after the injection. During the waiting period between alcohol injection and testing, each animal was kept in a small confining box (20 cm long × 10 cm wide × 10 cm high) to minimize the possibility of practising motor coordination under the effect of the alcohol. Even a non-drugged animal is ordinarily quiescent in such circumstances. Immediately on completion of the test run, the animal was decapitated and slices of cerebral cortex were removed as rapidly as possible from both hemispheres (Israel, Kalant, and LeBlanc, 1966) for analysis of ethanol content.

Analysis of Brain Ethanol Content. Ethanol concentration in the brain samples was measured by a modification of the butanol internal standard method of gas liquid chromatography as used previously for blood (LeBlanc, 1968). Several preliminary studies were carried out to validate the modification. In the first, the water content of brain cortex slices was determined by difference between fresh and constant dry weight, and was found to be 82.1 ± 1.7%, with a variation of less than 0.25% between hemispheres. This agrees very closely with values in the literature (McIlwain, 1963). Since the water content of blood is 80%, it was permissible to use brain samples of the same size as those previously used for blood.

In another preliminary experiment, it was found that the presence of brain tissue in concentrations of up to 25% of the final volume did not alter the measured ratios of ethanol and butanol in standard solutions. It was therefore permissible to use the butanol internal standard method of gas-liquid chromatography for analysis of the brain samples.

The third preliminary study dealt with the completeness of extraction of ethanol from brain samples. Homogenization of the tissue in deproteinizing solution occasionally resulted in problems of emulsification. It was therefore desirable to extract the ethanol without homogenization, and for this purpose the speed of simple diffusion of ethanol out of whole cortex slices into the deproteinizing solution was examined. Animals were sacrificed at 30 min after an injection of ethanol, 1.9 g/kg i.p., and cortical slices were cut and placed in the customary volume of deproteinizing solution. Serial ethanol measurements on aliquots of the latter indicated that a 15-min period was ample for complete equilibration of the ethanol between the tissue and the bulk of the sample.

Comparison of Brain and Blood Alcohol Content. Ten animals were each given a single injection of ethanol (1.9 g/kg i.p.) and were decapitated 30 min later. Samples (50 µl) of arterial blood spurting from the cut end of the carotid artery were caught in micropipettes and transferred immediately to the laking and deproteinizing solution. Samples of cortex were then obtained from each cerebral hemisphere as described. Ethanol concentration was measured in blood and brain samples from each animal.

Results

Comparisons between carotid arterial blood and brain samples taken at the same time in 10 animals showed mean ± S.E. ethanol concentrations of 2.59 ± 0.081 mg/g of water content in arterial blood and 2.71 ± 0.073 mg/g in brain. This difference was not significant ($P > 0.10$ by t-test for paired data). As expected, therefore, the correlation between blood and brain levels of alcohol was very high ($r = 0.92$). Samples taken from the two hemispheres within the same animal differed in ethanol concentration by less than 5%. The precision and reliability of brain ethanol measurements were identical with those found previously for blood (LeBlanc, 1968).

In order to produce comparable degrees of impairment on the moving belt test in all three groups of animals, as well as to provide significant differences in brain alcohol level at the time of the test, the administered doses were varied according to the time interval between administration and testing. The dose ranges used were 1.0–2.0, 1.4–2.4, and 1.8–2.8 g/kg for the animals tested at 10, 30 and 60 min respectively. Each dose range was divided into 10 equally spaced levels, with two animals at each level. Consequently, the ethanol concentrations in the brain ranged from approximately 100 to 350 mg/100 g fresh weight of brain in the three groups taken together.

This experimental design confounds the effects of dose and time in the production of different brain alcohol levels, but this should not have any importance with respect to the correlation between brain alcohol level and degree of impairment. The design also introduces differences in the volume of fluid injected, but preliminary studies showed that injection of saline in a volume three times as great as the largest volume used here had no effect on test performance. Fig. 1 shows the regression lines for time off belt (error score) on brain ethanol concentration, calculated separately for each of the three groups of animals. The three regression lines did not differ significantly from

[1] Details of these changes are available in the form of an internal substudy which may be requested from the authors.

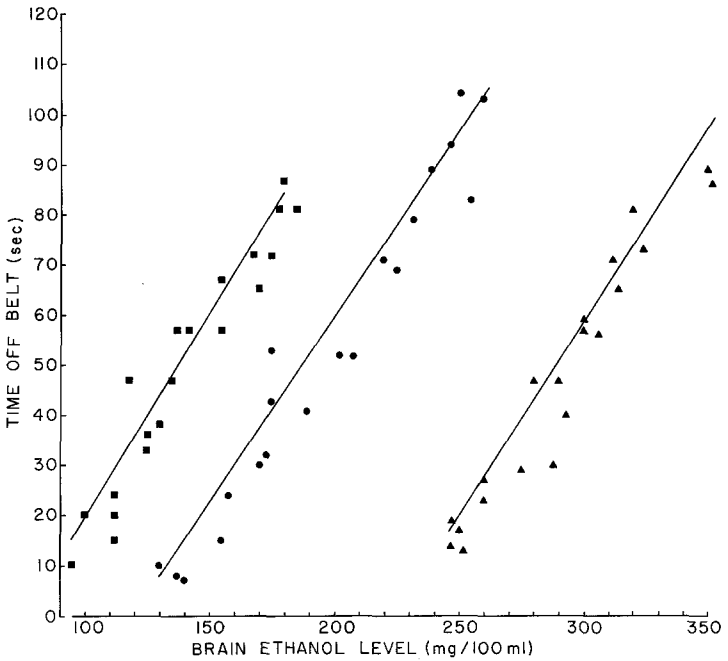

Fig. 1. Error score on the moving belt test, as a function of brain ethanol concentration. Measurements were made at 9–11 (□), 29–31 (○) or 59–61 (△) min after i.p. injection of ethanol. The slopes of the calculated regression lines are 0.805, 0.741 and 0.778 respectively

linearity. A regression analysis indicated that the three lines are parallel, since the slopes were not significantly different ($F = 0.49$; *df* 2, 56), but that there was a significant treatment effect ($F = 406.6$; *df* 2, 56; $P < 0.01$). *A posteriori* tests were therefore carried out to assess the significance of the differences between individual treatment groups. The smallest difference was that between the 10-min and 30-min groups (Fig. 1) and this proved to be quite significant ($F = 279.4$; *df* 1, 56; $P < 0.01$).

Discussion

This study demonstrates the feasibility of determining brain–level response curves, analogous to dose response and blood-level response curves. This type of experiment is extremely expensive in terms of preparation time, yielding only a single measurement on each trained animal. However, it was necessary in the present case to establish clearly that differences of distribution of ethanol between blood and brain did not account for the differences in performance among the three groups.

The close agreement between alcohol concentrations in arterial blood and both cerebral hemispheres is entirely consistent with the well-known distribution of ethanol throughout body water, and its rapid diffusion across the capillary walls in the brain (Gostomzyk *et al.*, 1969). It would therefore be legitimate, if repeated analyses were required in the same animal, to use arterial blood samples in lieu of brain.

The present results provide strong support for the concept of acute development of tolerance within a single alcohol exposure. Many previous experiments in our laboratory have shown that after i.p. injection of ethanol the 10 min value of alcoholemia lies on the ascending limb or at the peak of the curve, while the 30 and 60 min values lie on the descending limb (Czaja and Kalant, 1961). The present results therefore constitute a verification of the Mellanby effect.

The parallel shift of regression lines (Fig. 1) constitutes by definition the evidence of tolerance (Kalant, LeBlanc and Gibbins, 1971). In addition, the use of each animal once only following administration of ethanol eliminates the possibility that practice on repeated trials could contribute to the development of apparent acute tolerance.

These results are consistent with those reported in the literature for studies with other drugs. Maynert and Klingman (1960) have reported acute tolerance to paraldehyde, thiopental, pentobarbital and trichloroethanol, as well as ethanol, when duration of anaesthesia was compared with the drug concentration in the jugular venous blood at the time of awakening.

Two questions remain unanswered by the present study. The first is whether or not the difference in impairment produced at a given blood alcohol level on the rising and falling limbs of the curve depends in any way upon the direction from which that level is approached, rather than the magnitude of the concentration itself. The second question is the relation, if any, between acute tolerance and the more conventionally recognized tolerance which develops during chronic administration of alcohol or other drugs.

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