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Genetic Differences in Tolerance to Ethanol: A Study in UChA and UChB Rats[1]

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TAMPIER, L., M. E. QUINTANILLA AND J. MARDONES. *Genetic differences in tolerance to ethanol: A study in UChA and UChB rats*. PHARMAC. BIOCHEM. BEHAV. 14(2) 165–168, 1981.—The development of tolerance to ethanol was studied in two strains of rats, UChA (low ethanol consumer) and UChB (high ethanol consumer), by the sleeping time test. Marked tolerance (perhaps both metabolic and tissue) appeared in UChA rats, while only a slight tissue tolerance and not a metabolic one appeared in the UChB rats, when they were offered 10% ethanol as the sole fluid during 31 days ($p<0.001$). In UChA, but not in the UChB rats, the chronic treatment with ethanol induced tolerance to pentobarbital as shown by the same test.

Ethanol Tolerance Rats Metabolic tolerance Tissue tolerance Genetic difference

SPECIES, strain and individual differences in the voluntary alcohol consumption of laboratory animals have often been ascribed to differences in the metabolism of this drug. It has been reported [11] that a C57BL/Crgl mice, which exhibit a high preference for alcohol, develop a significantly lower level of blood acetaldehyde after ethanol administration than DBA/Crgl mice, which have a low alcohol preference. Furthermore, the high voluntary alcohol intake rats (ALKO's AA strain) exhibit a lower acetaldehyde level in peripheral blood after alcohol administration than the low preference alcohol rats (ALKO's ANA strain) after receiving the same dose of ethanol [2]. Experiments in our laboratory [12] studying ethanol and methanol metabolism in the brain of rats of our strains UChA (low ethanol consumer) and UChB (high ethanol consumer) showed also a significantly lower level of acetaldehyde or formaldehyde, respectively, when incubated with ethanol or methanol cortex-diencephalon homogenates from UChB rats as compared with those of UChA rats. However, there are some discrepancies, since results obtained using 4-methylpyrazole in order to reduce acetaldehyde formation in Long Evans, AA and ANA rats, have shown [6] that high acetaldehyde levels are not needed for maintaining a low ethanol consumption. On the other hand, no difference in the rate of elimination of labeled carbon dioxide from labeled ethanol was found between UChB and UChA rats [9]. Also, no difference between AA strain and ANA strain was found in blood ethanol level after alcohol administration [2].

It is well known that prolonged ethanol consumption induces tolerance to the intoxicating effects of this drug, but there is no agreement concerning whether it is metabolic or tissue tolerance or both [4, 8, 14].

Since tolerance to the effect of ethanol is an important feature of the effect of this drug in the CNS, we have studied whether the rats of our UChA and UChB strains, low and high ethanol consumers, also differ in the development of tolerance to the effect of ethanol.

METHOD

Animals

Adult rats of both sexes weighing 147 to 232 g, belonging to the strains developed by genetic selection in our laboratory [10] for high (UChB) or low (UChA) ethanol consumption, were used. The strain UChA was obtained by inbreeding of rats drinking less than 1.6 g ethanol per kg of body weight per day and the UChB one by inbreeding of rats which drank more than 2.4 g ethanol per kg body weight per day. In the experiments here reported, we have used UChA rats of the F51 generation of inbreeding. The rats of this generation consumed, in general, less than 1.2 g ethanol per kg body weight daily. The mean daily ethanol consumption of the rats used in the experiments was 0.78 g/kg (range 0.4–1.1). The UChB strain we have employed were rats of the F42 generation of inbreeding. In this strain the voluntary consumption of ethanol exhibits a wider range. In fact, about one third of them drink daily less than 1.6 g ethanol per kg. In these experiments we have used only UChB rats exhibiting a regular daily consumption of ethanol higher than 2.8 g/kg. The mean consumption was 4.03 g/kg (range 3.0–6.6).

The animals were housed in individual cages and fed ad lib on standard laboratory diet (consumption was not measured).

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TABLE 1

SLEEPING TIME INDUCED BY ETHANOL (60 mmole/kg IP) IN RATS UChA AND UChB OF BOTH SEXES UNDER DIFFERENT CONDITIONS OF ETHANOL SOLUTION AND WATER INTAKE (MEAN VALUES ± SEM)

Strain	Daily Intake of Fluid 10% Ethanol ml/kg	Water ml/kg	Weight Changes* g	Proportion that Lost Righting Reflex	Latency Time† min	Sleeping Time† min
UChA	9.8 ± 1.0	99.5 ± 5.3	+39.9 ± 8.8	22/22	3.4 ± 0.2	61.4 ± 5.1
UChA	89.4 ± 3.0	—	+49.3 ± 8.1	8/15	8.6 ± 1.3	6.0 ± 1.5
UChB	51.2 ± 5.0	58.9 ± 7.2	+38.7 ± 7.2	15/15	3.7 ± 0.2	49.3 ± 6.8
UChB	110.2 ± 8.6	—	+42.7 ± 6.7	9/10	5.5 ± 0.6	51.3 ± 5.0

*During experimental period.
†For rats that lost righting reflex.

Chronic Ethanol Intake

Rats of each strain were divided into two groups: (a) forced ethanol intake, receiving only 10% v/v ethanol as drinking fluid (experimental), and (b) rats provided ad lib ethanol 10% and distilled water (controls). The consumption of both fluids was measured daily. The weight gain during the experimental period was similar in the four groups. The consumption of total fluid was also similar, with the exception of the UChA (a) group which exhibited a consumption about 20% less than the respective control.

After 21 days, the experimental and control rats were tested for tolerance to the depressant effect of ethanol, assessed by measuring the duration of "sleeping time" induced by a similar dose of ethanol (60 mmole/kg IP). Sleeping time was defined as the interval between the loss and return of the righting reflex. Also the sleeping time induced by sodium pentobarbital (0.1 mmole/kg IP) was tested in equivalent groups of rats.

Blood Ethanol Level

Blood ethanol levels were measured in some of the rats of each group 30 minutes after the administration of standard dose of 60 mmole kg IP of ethanol as 25% v/v solution. The ethanol level was measured in a sample of 0.2 ml of blood obtained from the heart and transferred into a flask containing 1.8 ml of a 40.2 mg/ml *n*-propanol solution, and heated during 15 minutes at 60°C. Ethanol was measured by a Perkin Elmer Gas Chromatographer using the standard head space method described by Eriksson *et al.* [1].

Tolerance

When blood levels and effects are considered together, two kinds of tolerance developed after repeated administration of a drug can be operationally distinguished: (a) *metabolic tolerance* revealed by lower ethanol blood levels observed after the same doses, and (b) *tissue tolerance*, revealed by lower effects observed with the same blood level, or the same effects with higher blood levels. Since the blood level rapidly increases after IP injection of ethanol, a longer latency time reveals that the effect appears with higher blood level, and thus it can be considered operationally as revealing tissue tolerance.

In the present paper the effect of ethanol is considered to be a narcosis as well as its latency time and duration.

Data Analysis

Results are expressed as mean values ± SEM. The statistical comparisons were made with two-tailed Student's *t* tests.

RESULTS AND DISCUSSION

The sleeping time induced by a standard dose of ethanol (60 mmole kg IP) was not sigificantly different in UChA and UChB rats drinking ethanol ad lib. UChA rats forced to drink ethanol chronically, exhibited after 21 days of treatment a marked tolerance to the depressant action of ethanol, since the sleeping time was drastically reduced as compared with UChA ad lib controls (Table 1, Fig. 1). In fact, tolerance was noticeable at 7 days of forced ethanol intake, reached the maximum at 21 days and remained constant over the 31 days of observation. Afterwards the ethanol bottle was removed and replaced by a water one. During this last period, sleeping time slowly returned to the base level (Fig. 1). Time course of acquisition of tolerance was similar to that reported by other authors in other strains of rats [4,14].

UChB rats under the same conditions of forced ethanol intake did not develop such tolerance, in spite of the fact that their ethanol intake was about twice that exhibited by the same rats which received ethanol and water ad lib (Table 1; $p<0.001$), and it was also higher (Table 1, $p<0.05$) than that of the UChA rats under forced ethanol intake. The voluntary ethanol intake of UChB rats did not seem to induce tolerance, since their ethanol sleeping time was not significantly different to that of UChA rats controls (49.3 ± 6.8 vs 61.4 ± 5.1; N.S.).

Latency time, defined as the interval between injection of ethanol and loss of the righting reflex, was significantly higher in the experimental UChA rats as compared with ad lib controls (Table 1, $p<0.001$). A slight increase of latency time was also observed in experimental UChB rats as compared with their ad lib controls (Table 1, $p<0.01$). The observed increase of latency time is an indication that a tissue tolerance was induced. This tolerance appeared to be higher in UChA rats, since the latency time of the experimental rats of this strain which lost the righting reflex was significantly higher than in UChB rats (Table 1, $p<0.05$).

Ethanol blood level measured 30 minutes after IP administration of ethanol was not different in control rats of both

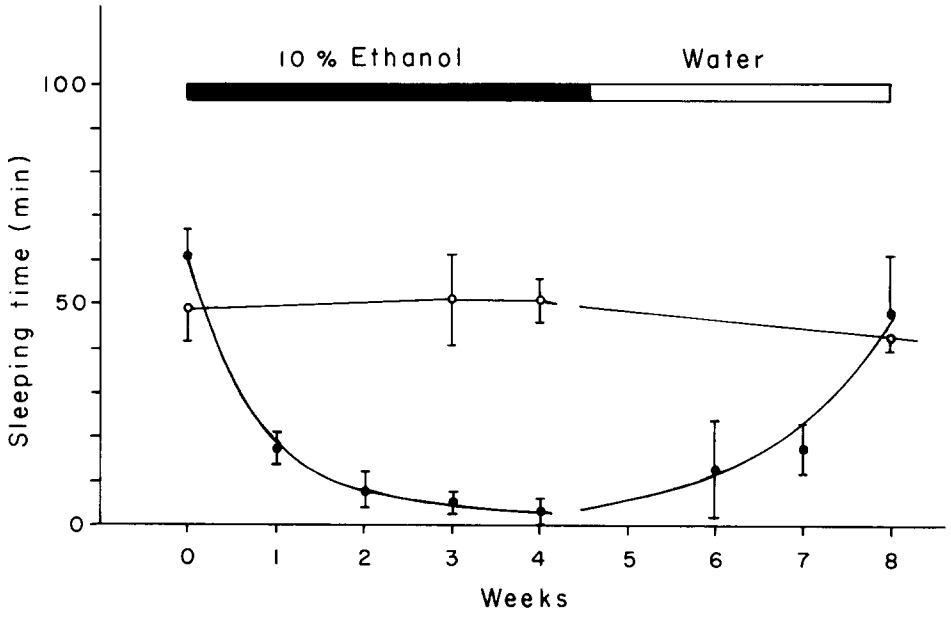

FIG. 1. Influence of forced ethanol intake in the tolerance to the depressant effect of ethanol (60 mmole/kg IP) in UChA (●) and UChB (○) rats.

strains (Table 2). This result is in agreement with those reported in AA and ANA rats [2]. But a significant lower ethanol blood level was measured in UChA rats with forced alcohol as compared with that in UChA aid lib controls, while no difference in the blood ethanol level between experimental and control UChB rats was observed. These results suggest that UChA, but not UChB rats, develop a metabolic tolerance when they were chronically forced to drink ethanol. It has been reported by many observers (reviewed in [7]) that chronic ethanol administration results in increased ethanol metabolism, both in humans and experimental animals, but the mechanism for this effect is controversial [5,13].

The data concerning sleeping times induced by sodium pentobarbital in rats of both strains under ad lib or forced ethanol intake appear in Table 3. Sodium pentobarbital sleeping time was also reduced significantly in the UChA rats chronically treated with ethanol as compared with their ad lib controls, i.e., chronic ethanol ingestion appeared to induce cross tolerance between ethanol and a barbiturate. No such effect was observed in UChB rats chronically forced to drink ethanol. Studies of Khanna *et al.* [3] suggest that changes in barbiturate sleeping time of rats made tolerant to ethanol are the result of alterations in CNS sensitivity rather than a modification of drug disposal.

In summary, our experiments demonstrate the presence of genetic difference in the ability to develop tolerance to the depressant effect of ethanol, since a clear tolerance (may be metabolic and tissue tolerance) appeared in UChA rats when they were chronically forced to drink ethanol, while only a slight tissue tolerance, but not a metabolic one, was observed in UChB rats under the same conditions.

TABLE 2

BLOOD ETHANOL LEVEL 30 MINUTES AFTER 60 mmole/kg IP, IN RATS OF THE STRAINS UChA AND UChB UNDER DIFFERENT CONDITIONS OF ETHANOL AND WATER INTAKE (MEAN VALUES ± SEM)

Strain	Drinking Fluid Ethanol 10%	Drinking Fluid Water	Number of Rats	Blood Ethanol mg/dl	Significance $2p$
UChA	+	+	6	342 ± 9	<0.005
UChA	+	−	6	259 ± 21	
UChB	+	+	4	354 ± 20	N.S.
UChB	+	−	4	368 ± 7	

TABLE 3

SLEEPING TIME INDUCED BY SODIUM PENTOBARBITAL (0.1 mmole/kg IP) IN UChA AND UChB RATS UNDER DIFFERENT CONDITIONS OF ETHANOL AND WATER INTAKE (MEAN VALUES ± SEM)

Strain	Drinking Fluid Ethanol 10%	Drinking Fluid Water	Number of Rats	Latency Time min	Sleeping Time min	Significance 2 p
UChA	+	+	4	6.5 ± 1.1	134 ± 15	<0.01
UChA	+	−	4	6.0 ± 0.7	71 ± 4	
UChB	+	+	4	5.8 ± 0.3	137 ± 16	N.S.
UChB	+	−	4	5.5 ± 0.3	136 ± 19	

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