Pharmacology Biochemistry and Behavior, Vol. 2, pp. 549–551. ANKHO International Inc., 1974. Printed in the U.S.A.

BRIEF COMMUNICATION

Neural Tolerance in High and Low Ethanol Selecting Mouse Strains

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(Received 10 December 1973)

SCHNEIDER, C. W., P. TRZIL AND R. D'ANDREA. *Neural tolerance in high and low ethanol selecting mouse strains.* PHARMAC. BIOCHEM. BEHAV. 2(4) 549–551, 1974. – Determinations were made of neural tolerance in high and low ethanol selecting mouse strains. Neural tolerance was assessed by infusing a 10% ethanol solution intraperitoneally while stimulating and recording the amplitude of the jaw-jerk reflex. The high ethanol selecting C57BL/6j strain required twice as much infusion time as the low ethanol selecting BALB and CBA/j strains to depress the jaw-jerk amplitude to 50% of the pre-infusion level. These results and previous findings suggest that neural tolerance may play a role in ethanol selection.

Mice Alcohol preference Jaw-jerk reflex Neural tolerance

IN A SERIES of experiments, Schneider *et al.* [10] found that mice of C57BL/6j (high ethanol self-selecting) strain have a much higher neural and behavioral tolerance to ethanol than the DBA/2j (low ethanol self-selecting) strain. In those experiments, neural tolerance was determined by measuring depression of the jaw-jerk reflex while infusing 10% ethanol at a rate rapid enought to overcome any differences in metabolic capacity. Attenuation of the response strength to 50% of the pre-infusion amplitude required twice as long in the high ethanol selecting C57BL strain. Recently, genetic differences in the neural sensitivity to ethanol of these two strains has been demonstrated by MacInnes and Uphouse [6].

On the basis of their findings, Schneider *et al.* [10] have suggested that neural tolerance may play a part in determining levels of ethanol self-selection. Further support for this hypothesis comes from the finding that the selection of 1,2 propanediol (propylene glycol) by these two strains is essentially in the same direction and order of magnitude as ethanol. This is of particular interest since 1,2 propanediol is an alochol that produces CNS depression [4,8] but without the complication of toxic metabolites that may also influence selection.

The purpose of the present investigation was to test further this hypothesis by determining whether or not the observed parallel between high or low neural tolerance and high or low ethanol self-selection is a general phenomenon or one unique to the C57BL and DBA strains. In order to accomplish this, neural tolerance was assessed as in the previous investigation by measuring the depression of the jaw-jerk reflex by ethanol in other strains for which selection tendencies are shown.

METHOD

Animals

Three groups of 20 male mice from three strains (C57BL/6j, BALB/cJ and CBA/J) were used. All of the animals were obtained from the Jackson Laboratory, Bar Harbor, Maine and were between 70 and 90 days of age at the time of testing. The C57BL strain are high ethanol self-selecting mice, while the BALB and particularly the CBA strain almost totally avoid a 10% ethanol solution.

Apparatus

The strength of the jaw-jerk response was measured with a Microdisplacement Myograph Transducer F-50 that fed into a Physiograph (DMP-4B), Narco Bio-Systems, Inc., where it was amplified and recorded on a constant speed chart recorder.

Electrical stimulation was provided by a Grass S88 stimulator and delivered with a flat ended bipolar stainless steel electrode that was insulated with Epoxylite 6001-M cement. Ethanol infusion was accomplished with a syringe pump, Model 341, Sage Instrument.

Procedure

Each strain was divided into 15 experimental and 5 control animals. The experimental animals were infused with a 10% V/V solution of 95% ethanol plus physiological saline during stimulation of the reflex, and the controls were infused with physiological saline alone.

Each animal was anesthetized with 0.01 ml/g body weight of 6% sodium barbital solution administered subcutaneously on the back. Upon reaching anesthetic level (45–60 min after injection) the mice were restrained on their backs, a hypodermic needle attached to the syringe pump was inserted intraperitoneally for infusion, at a rate of 10 g/kg/hr, and the electrode was placed on the anterior area of the hard palate for stimulation of the jaw-jerk. The lower jaw was held slightly open by a piece of tough Irish linen thread that was attached to the myograph transducer on one end and looped around the lower incisors on the other end.

Stimulation progressed at a rate of 6 ppm with a 5 msec square wave at a voltage level that produced a maximal response (20–30 V). A vigorous response was easily observed and recorded. Prior to infusion of the ethanol solution or saline alone the jaw-jerk response was elicited and recorded for 6 min in order to obtain a pretreatment response amplitude. Infusion of ethanol was maintained until the response dropped considerably below 50% of the pretreatment level. Treatment of the control group was identical to the other animals with the exception that they were infused with saline alone for a standard period of 20 min.

RESULTS

Figure 1 contains the three curves for the mouse strains showing the pattern of attenuation of the jaw-jerk response amplitude as a function of infusion time. The curves were derived by dividing the average pre-ethanol amplitude based on 3 min (18 responses) immediately preceding infusion into each average amplitude for the six responses per min. It is clear that the C57BL/6j strain required a much longer time to depress the jaw-jerk amplitude to the 50% level than either CBA or BALB strains. The average time to drop below the 50% level was 15.33 min for the C57BL, 6.86 min for the CBA and 6.60 min for the BALB. While the CBA and BALB strains did not differ statistically they both crossed the 50% level significantly sooner ($t = 10.08$, $p<0.0005$ and $t = 9.48$, $p<0.0005$, respectively) than the C57BL/6j. The five saline controls of each strain showed little if any decline in the jaw-jerk amplitude after 20 min of infusion with saline. Compared to pre-infusion levels the % of amplitude for the three strains during the 20th minute was C57BL = 98%, BALB = 103%, CBA = 96%.

DISCUSSION

The findings of this investigation indicate that the high ethanol selecting C57BL strain possess a greater neural tolerance than the low ethanol selecting CBA and BALB strains. These results are almost identical to those obtained by Schneider *et al.* [10] when comparing the C57BL and DBA strains. Furthermore, the results obtained in the comparison of the C57BL and BALB strains are consistent with the findings of Kakihana *et al.* [3] who used sleep time to demonstrate greater brain sensitivity to ethanol in the low selecting BALB strain.

Thus far three low ethanol selecting strains have been compared to the high ethanol selecting C57BL strain, and all of the low selection strains have required half as much ethanol to depress their jaw-jerk reflex as the high selection strain. Metabolic differences, if they do exist, cannot account for this difference in neural tolerance since ethanol was infused at a rate that was far in excess of the metabolic capacity of any of the strains. This consistently positive

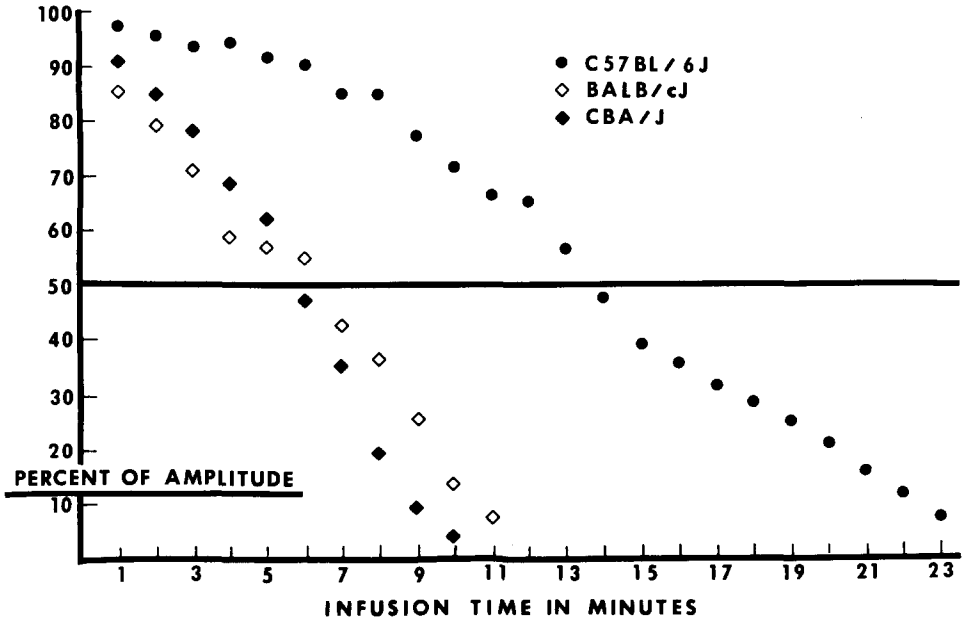

FIG.. 1. Decline in amplitude of the jaw-jerk reflex in three mouse strains during infusion with a 10% ethanol solution.

relationship between neural tolerance to and selection of ethanol suggests that this factor may play some part in determining acceptance or rejection of the substance.

A metabolic factor, particularly oxidation of the toxic metabolite acetaldehyde, has also been implicated in determining ethanol self-selection [9,11]. Presumably, this could occur through the formation of a conditioned aversion induced by a toxic reaction resulting from the accumulation of acetaldehyde that would occur if a strain did not metabolize it rapidly enough [7]. However, there are certain problems with this hypothesis. It is well established that acetaldehyde is eliminated at an extremely rapid rate [2,5], and while the DBA strain apparently metabolizes it more slowly than the C57 strain it is highly unlikely that they could accumulate enough in the blood to produce illness followed by conditioned aversion for two reasons. First, is the fact that in a two choice situation, the licking rate on the ethanol bottle in the initial 24 hr exposure is incredibly low in the DBA mice [10]. Metabolic studies typically use anesthetic levels of ethanol that tend to exaggerate differences in capacity, and while nevertheless indicative of real differences it must be realized that these differences may well be inconsequential at the consumption levels found in a natural situation where the animal constantly regulates its own intake. No one has yet demonstrated that there is a differential accumulation of acetaldehyde in high and low selecting strains following actual drinking. Secondly, the amount of a 10% ethanol solution consumed in the first 24 hr by low self-selection strains is less than a ml, and it is well known that quite high levels of ethanol in the blood are required to produce acetaldehyde levels resulting in even a mild pharmacological effect [1].

At this time there is no evidence that unequivocally indicates what factors are involved in the selection of ethanol by different mouse strains. Neural tolerance has not received a great deal of attention in this regard, and it seems like it might be a fruitful avenue to pursue. It is suggested that one way of gaining insight into this problem might be through the investigation of other alcohols.

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