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BRIEF COMMUNICATION

Conditioned Tolerance Provides Protection Against Ethanol Lethality

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MELCHIOR, C. L. *Conditioned tolerance provides protection against ethanol lethality.* PHARMACOL BIOCHEM BEHAV **37**(1) 205–206, 1990.—To produce conditioned drug tolerance, mice were given twice daily injections of 3.5 g/kg of ethanol for four days and were tested on the fifth day in the same environment or in a novel environment. A range of doses of ethanol were utilized on the test day to assess lethality. The LD_{50} for ethanol was higher in mice tested in the environment previously associated with the administration of ethanol than in those tested in a novel environment. Therefore, conditioned tolerance can provide protection against ethanol lethality.

Ethanol Tolerance Pavlovian conditioning LD_{50}

USING a Pavlovian conditioning paradigm, tolerance has been shown to develop to a variety of effects of several different drugs (8,9). The tolerance which is developed in this paradigm is sensitive to environmental cues, i.e., tolerance can be demonstrated in the presence of cues previously associated with the administration of the drug and not in the absence of such cues.

Protection against the lethal effects of drugs due to this type of tolerance has been observed for opiates (9,10) and pentobarbital (12). While the hypothermic response to ethanol has been used to demonstrate various aspects of Pavlovian conditioned tolerance (1, 3, 5), there have been no investigations of the effectiveness of this type of tolerance in protecting against an overdose of ethanol. Very little change has been reported in the LD_{50} for ethanol due simply to previous exposure to the drug (2). The purpose of the present experiment, therefore, was to determine the effect of conditioned tolerance on the lethal effect of ethanol.

METHOD

Male Balb/c mice weighing 22–28 g were housed in groups of five per cage and maintained under a 12-hour light/dark cycle at an ambient temperature of 23 ± 1°C. Purina Lab Chow and water were continuously available to the animals.

Twice per day at 0800 and 1500 hours, the animals' cages were taken from their racks and placed on a table in an adjoining room in which a radio was playing. The mice were weighed and injected with either 3.5 g/kg ethanol or an equal volume of saline. The ethanol was prepared as a 20% w/v solution. Following the morning injection the animals were monitored for the duration of loss of righting reflex and change in body temperature. Body temperature was assessed with a rectal probe prior to and 30 minutes after injection. Changes in these parameters over days assured that tolerance developed as previously reported for C57BL/6 mice (6).

The mice received the twice daily injections of either ethanol or saline solutions for 4 days in the animal housing facilities described above. These facilities will be referred to as the "cued" environment. On Day 5, the day of testing, all the animals were given an injection of ethanol either in the cued environment or in a novel environment. The novel ("uncued") environment was a section of a chemistry laboratory which differed from the cued environment in olfactory, acoustic and lighting conditions. Room temperature in both environments was 23 ± 1°C. Four groups of experimental animals were therefore generated: 1) mice injected with ethanol for 4 days and tested with ethanol in the same environment on the 5th day (ethanol/cued), 2) mice injected with ethanol for 4 days and tested with ethanol in a different environment on the 5th day (ethanol/uncued), 3) mice injected with saline for 4 days and tested with ethanol in the same environment on the 5th day (saline/cued), and 4) mice injected with saline for 4 days and tested with ethanol in a different environment on the 5th day (saline/uncued).

The LD_{50} for ethanol was determined for each of the four groups of animals by injecting doses of ethanol ranging from 4.5 to 7.0 g/kg and counting the number of mice that had died at 24 hours after injection. At least 5 different doses were tested for each

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group, with at least 10 mice tested at each dose. Different groups of mice were used for different test doses of ethanol. The data were analyzed by the method of Litchfield and Wilcoxon (4).

RESULTS

In a representative group of 30 mice, sleep time decreased from 41.0 ± 0.5 minutes (mean $\pm$ SEM) on the first day of chronic injections of 3.5 g/kg ethanol to 28.9 ± 0.3 minutes on the fourth day. Similarly, the amount of change in body temperature decreased from 4.7 ± 0.1°C on the first day to 3.5 ± 0.1°C on the fourth day. These data indicate that tolerance developed on these measures.

Figure 1 shows the LD_{50} obtained for each group. The LD_{50} for the ethanol/cued group was significantly higher than any of the other groups. The potency ratios for the ethanol/cued group to the ethanol/uncued, saline/uncued, and saline/cued groups were 1.17, 1.21, and 1.26, respectively. Analysis of the dose-response curves showed that the data were not significantly heterogeneous (i.e., the lines were good fits) and the slopes were parallel. At a dose of 5.5 g/kg, 100% of the saline/cued, 80% of the saline/uncued, and 60% of the ethanol/uncued mice died, whereas none of the ethanol/cued mice died at this dose.

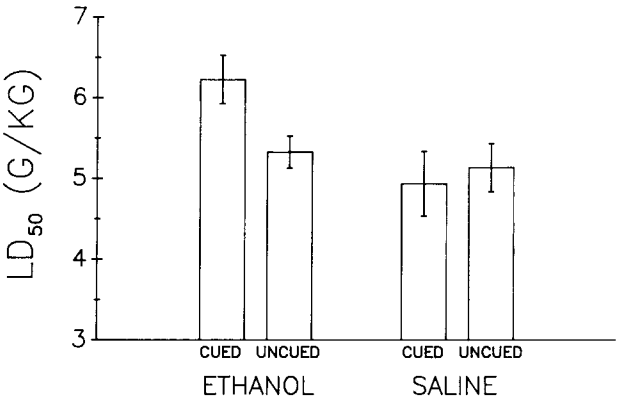

FIG. 1. The LD_{50} and 0.05 level confidence intervals for ethanol were determined in mice that were given 3.5 g/kg ethanol or saline twice per day for four days and tested with a range of doses of ethanol on the fifth day in the same (Ethanol/Cued or Saline/Cued) or a different (Ethanol/Uncued or Saline/Uncued) environment.

DISCUSSION

The LD_{50} for ethanol is significantly higher in an environment previously associated with the administration of the drug than in a novel environment. This pattern of response is consistent with a Pavlovian conditioning analysis of drug tolerance (8,9).

Pharmacologically, tolerance is defined as a shift to the right in the dose-response curve (2). However, the data provided in this study are somewhat unique in demonstrating a shift in a dose response curve, since studies of conditioned tolerance have generally utilized only one test dose.

In examining the role of conditioned tolerance in heroin overdose deaths, Siegel (9) described the situation as one in which a "failure of tolerance" was a critical feature. Conditioned tolerance is usually blocked, or made to fail, simply by administering the drug in the absence of cues previously associated with its presentation. However, it has also been shown that the presence of novel stimuli whether external, such as a flashing light (11), or internal, such as the presence (or absence) of another drug (7), can also cause tolerance to fail. In considering deaths in the presence of high levels of alcohol in situations such as accidents or the unaccustomed addition of another drug, the occurrence of these novel stimuli may contribute to causing death by blocking the expression of tolerance.

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