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Total Avoidance of Saccharin Consumption by Rats after Repeatedly Paired Injections of Ethanol or LiCl

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KULKOSKY, P. J., J. L. SICKEL AND A. L. RILEY. *Total avoidance of saccharin consumption by rats after repeatedly paired injections of ethanol or LiCl.* PHARMAC. BIOCHEM. BEHAV. 13(1) 77–80, 1980.—Rats injected with ethanol or LiCl following consumption of novel saccharin solution drank less saccharin than non-poisoned controls on a subsequent exposure with degree of aversion positively related to dose of ethanol (2–5 g/kg). While a single pairing of saccharin with ethanol or LiCl resulted in partial avoidance of saccharin solution, repeated conditioning trials led to total avoidance of saccharin consumption by animals injected with the higher doses of ethanol or with LiCl. These results, characteristic of emetic-induced aversions, support the explanation of the limited consumption of ethanol by rats under ad lib, free-choice conditions as a result of acquired aversion to the oronasal sensory stimuli of ethanol after association with pharmacologically aversive aftereffects of consumed ethanol.

Ethanol LiCl Saccharin Conditioned taste aversion Rat Ethanol dose-response Repeated aversion conditioning trials Animal models of alcoholism

RECENTLY, ethanol-induced conditioned aversions have been invoked to explain the rat's failure to consume addictive amounts of ethanol in non-deprived, free-choice situations [3, 4, 8, 9, 10, 11, 12, 13, 23, 25, 26, 29, 34, 35]. According to this explanation, when the rat consumes ethanol, oronasal stimuli of ethanol become associated with its aversive post-ingestional consequences. This association results in aversion to preabsorptive sensory stimuli of ethanol and limits subsequent free-choice ethanol intake below induction of physical dependence.

That ethanol can condition such an aversion in free-choice situations is only partially supported by assessments of the efficacy of ethanol in conditioned taste aversion designs in which animals are administered ethanol following consumption of a novel solution [32]. The results from these procedures have been equivocal.

While it is reported that ethanol conditions aversions [1, 3, 13, 14, 15, 27, 28, 29, 38], little or no effect following a single taste-ethanol pairing is also reported [2, 5, 7, 8, 16]. These aversions are generally weak and incomplete, i.e., complete suppression of consumption seldom occurs, even with repeated conditioning trials [2, 3, 5, 7, 8, 13, 14, 15, 16, 28]. Such weak and incomplete aversions contrast with the rapidly acquired, robust aversions induced by emetics such as LiCl [30,36]. Ethanol-induced aversions are more similar to the variable aversions induced by other self-administered psychoactive drugs, e.g., morphine, Δ^9-THC and cocaine [17, 18, 19, 21, 37, 39].

These differences in efficacy of self-administered psychotropic drugs and non-self-administered emetics to condition aversions have been cited to support the hypothesis of different physiological substrates underlying the two types of aversion. It has been suggested [1] that emetics induce aversions via peripheral actions but self-administered drugs directly activate central loci to induce aversions. This direct central activation in part accounts for the paradoxical rewarding and punishing effects of psychoactive drugs [1,38].

However, the aforementioned properties of ethanol-induced conditioned aversions might rather reflect specific parameters of studies assessing ethanol-induced aversions. For example, variability across studies may be a function of ethanol concentration and volume, motivational state, or route of administration differences. Also, that ethanol-induced aversions are weak after one conditioning trial and

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incomplete following repeated conditioning trials may simply reflect the range of doses examined. In typical taste aversion designs in which emetics are the aversive agents, aversions are directly dose-dependent. In many of the above studies, the largest dose of ethanol injected was only 1.2 g/kg.

In the single study which assessed ethanol-induced aversions at doses greater than 1.2 g/kg and included an emetic-induced aversion for comparison, ethanol at 4.42 g/kg produced robust aversions equal to those induced by a standard effective dose of LiCl [27]. Given the variability of ethanol-induced conditioned aversions across studies, and since rats under ad lib, free-choice conditions have opportunity for sensory stimuli of ethanol to be repeatedly paired with ethanol's aversive properties, the present paper examined the efficacy of high doses of ethanol and LiCl to condition aversion to saccharin solution with repeated taste-injection pairings.

METHOD

Animals

Animals were 36 experimentally naive female rats of Long-Evans descent (outbred, Charles River Crl:COBS (LE)BR), approximately 90 days of age at the beginning of the experiment. The rats were maintained on a 12:12 L:D lighting cycle (8 a.m.–8 p.m. light) and had ad lib access to Purina Rat Chow throughout the experiment.

Apparatus

Rats were housed in individual wire-mesh cages as previously described [24]. In front of each cage were two openings into which calibrated 50 ml Nalgene tubes fitted with valveless stainless steel spouts were placed for measurement of tap water or 0.1% w/v sodium saccharin (Fisher purified) consumption to the nearest 0.25 ml.

Procedure

Initially, all rats were deprived of water and given ad lib access to food. On the next day, all rats were given 20-min access to water. This procedure was repeated for 32 consecutive days at which point all rats were approaching and drinking from the tube within two sec of presentation. Differential training was then administered to five groups of randomly-selected animals. On Day 33, all groups were given 20-min access to a novel 0.1% saccharin solution followed 15 min later by an intraperitoneal (IP) injection. Groups A2, A3.5, and A5 (N=7 for each group) were given IP injections of 2.0, 3.5, and 5.0 g/kg ethanol (15% w/v from U.S.P. 95%), respectively. Group L (N=7) was given an IP injection of 1.8 mEq/kg of LiCl (0.15 M, Mallinckrodt reagent, 12 ml/kg). Finally, Group W (N=8) was given an IP injection of distilled water in a volume equivalent to the highest dose of ethanol. Following this differential treatment, all groups were given 20-min access to water on each of three consecutive water-recovery days.

This procedure of alternating conditioning trial—water recovery period was continued until all animals had received three complete cycles. On the day following the last water-recovery session, all rats were given 20-min access to saccharin in a final test of the aversion induced by ethanol or LiCl.

Data were analyzed with 1-way analysis of variance and correlated and two-sample t-tests (2-tailed), with $p<0.05$ as significant.

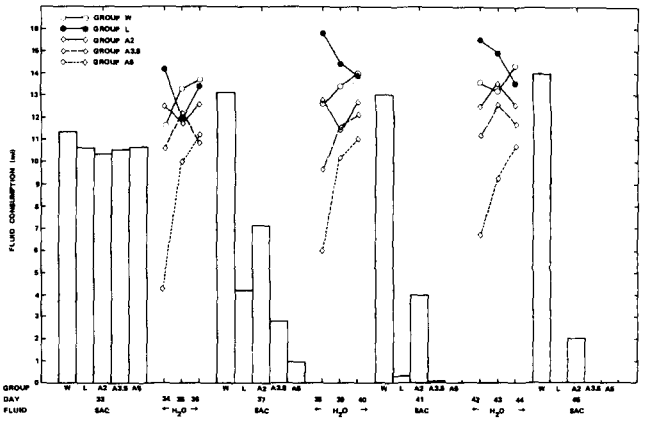

FIG. 1. Mean consumption (ml) of saccharin solution (SAC) over repeated conditioning trials and of water (H_2O) over repeated water recovery sessions for rats receiving injections of distilled water (Group W), LiCl (Group L), or alcohol at 2 g/kg (Group A2), 3.5 g/kg (Group A3.5), and 5 g/kg (Group A5).

RESULTS

One rat in Group A5 died following ethanol administration, reducing the number of animals in this group to six. The data for this rat were omitted from all analyses.

Rats gradually increased consumption of water over adaptation days, drinking an average of 14 ml on the final day of water exposure. When saccharin replaced water on Day 33, all rats significantly decreased consumption below the amount consumed on the preceding day of water adaptation, $t(33)=6.00, p<0.05$, a decrease reflecting the rat's neophobic response to novel solutions [6]. There were no significant differences among groups in amount of saccharin consumed on this initial exposure to saccharin, $F(4,30)=0.11, p>0.05$.

On the second exposure to saccharin, a significant difference emerged among the groups, $F(4,30)=28.53$, $p<0.05$. Group W, rats injected with distilled water following saccharin consumption, showed a slight, nonsignificant increase in consumption of saccharin, $t(7)=1.10$, $p>0.05$. However, all drug-injected groups except Group A2, $t(6)=2.11, p>0.05$, significantly decreased saccharin consumption below the amount consumed on the initial exposure, all ts>3.94, dfs$=6$ or 5(Group A5), ps<0.05 (see Fig. 1, Days 33 and 37).

While all rats injected with ethanol decreased saccharin consumption, the amount consumed on the second exposure was a function of dose of ethanol, i.e., while Groups A3.5 and A5 did not differ in amount of saccharin consumed, both of these groups drank significantly less than Group A2, $t(12)=3.18$, and $t(11)=4.86$, ps<0.05 for Groups A3.5 and A5, respectively. Group L, rats injected with LiCl following saccharin consumption, while not differing from Groups A2 and A3.5, drank significantly more saccharin than Group A5, $t(11)=2.61$, $p<0.05$. All drug-injected animals drank significantly less saccharin than water-injected animals, all ts>4.28, dfs$=13$ or 12(Group A5), ps<0.05.

With repeated exposures to saccharin, each followed by distilled water injection, Group W maintained its high level of saccharin consumption, drinking approximately 14 ml on the final 1-bottle test. Rats injected with ethanol following saccharin exposure further decreased saccharin consumption over repeated conditioning trials with Group A5 consuming no saccharin at all on both the second and final aver-

sion tests. Group A3.5 also drank no recordable amount on the final test, while Group A2 drank only about 2 ml. Group L further decreased saccharin intake with repeated saccharin-LiCl pairings, and also drank no saccharin on the final aversion test. All drug-injected rats drank significantly less saccharin than Group W on the final exposure, all $ts>6.95$, $dfs=12$ or 11(Group A5), $ps<0.05$, with no significant differences among drug-injected groups, all $ts<1.75$, $dfs=12$ or 11 (Group A5), $ps>0.05$.

Figure 1 also illustrates consumption of water over recovery days following each saccharin-injection pairing. There were large differences in amount of water consumed by the groups during these recovery periods. When compared to the last day of water baseline (Day 32), the highest dose of ethanol (5 g/kg) markedly suppressed water consumption on the first day of water recovery after each ethanol treatment, all $ts(5)>4.35$, $ps<0.05$. While water intake was decreased on the first water recovery session, consumption of water returned to its baseline over the recovery period. A similar, but weaker suppression on the first days following injection was also evident for Group A3.5, all $ts(6)>2.87$, $ps<0.05$. None of the remaining groups showed any significant changes in water consumption from their respective pre-injection water baselines of Day 32.

DISCUSSION

As is clear from the data, when high doses of ethanol were administered to rats following consumption of a novel saccharin solution, animals markedly decreased subsequent saccharin consumption. At the highest dose of ethanol, conditioned aversions were more rapidly acquired and robust than the aversions induced by a high dose of LiCl. However, it should be pointed out that on a molar basis, LiCl effects total avoidance at much lower doses than ethanol. The strength of ethanol-induced aversions was a direct function of dose of ethanol administered. This dose-response effect is also characteristic of emetic-induced aversions [30,31]. On the other hand, such dose-response relationships are not characteristic of many self-administered psychoactive drugs such as morphine [17,37], methylphenidate [33] or l-alpha acetyl methadol [40] in which aversions weaken or show no change with increases in dose.

It is also clear that when rats were given repeated taste-ethanol pairings, saccharin consumption gradually decreased over trials. While complete suppression was not evident at a dose of 2 g/kg, total avoidance of saccharin consumption occurred at the two higher doses of ethanol. The incomplete suppression at 2 g/kg is consistent with the aforementioned work on the effects of repeated taste-ethanol pairings at lower doses of ethanol [2, 3, 5, 7, 8, 13, 14, 15, 16, 28]. The total avoidance observed at the higher doses provides verification that the previously reported weak and incomplete ethanol-induced aversions produced by single and repeated conditioning trials may be a function of the range of low doses examined. That ethanol totally eliminated saccharin consumption over repeated conditioning trials is also consistent with the effects of repeated trials when emetics are used as aversive agents ([36], Group L, the present study). Some other self-administered drugs such as morphine [17,37] and chlordiazepoxide [5], on the other hand, fail to suppress consumption even though multiple conditioning trials with extremely high doses of the drug are given.

The earlier described variability among individual studies in the efficacy of ethanol to condition aversions could result from parametric differences among studies or from individual or strain [20] differences among animals in response to ethanol. In the present study, there was little or no variability in degree of aversion within groups receiving the higher doses of ethanol, suggesting that at these doses the animals are similar in response to the ethanol challenge. At 2 g/kg, the range of consumption was again small, indicating that even with lower doses, the variability does not approach the levels seen with drugs such as morphine [37,39]. Since individual subjects of a single strain are not highly variable in their responsivity to the aversive components of injected high doses of ethanol, differences among studies more likely result from parametric differences such as dose, strain of animal, ethanol concentration and injection volume [28], route of administration, conditioned stimulus—unconditioned stimulus interval, or motivational state.

These results indicate that ethanol is a very effective agent in a conditioned taste aversion design. The aversive effects of high doses of ethanol are sufficient to condition total avoidance of an associated solution following repeated aversion trials. Consistent with the present data is the observation that rats regulate maximized free-choice ethanol intakes within approximately 2 g/kg of their ethanol metabolic capacity [23], and that rats and hamsters exhibit distinctly different levels of maximized voluntary ethanol intake in proportion to their differences in ethanol metabolic capacity [22, 23, 25]. These species-specific ethanol metabolic capacities, or total abilities to oxidize and utilize or excrete ethanol, have recently been shown to correspond positively to species differences in both blood ethanol elimination rates and liver alcohol dehydrogenase activities [25]. In the case of deprivation-induced drinking, the formation of conditioned taste aversion to solutions paired with ethanol has been shown to relate directly to the resultant blood ethanol levels [14, 15, 28]. Since repeated consumption of ethanol at rates beyond ethanol eliminative capacity results in increasing blood ethanol levels, the total ability to breakdown and remove ethanol from the blood should effectively impose an upper limit on voluntary ethanol intake. Thus, it was suggested that the ability of rats and hamsters to avoid repeated free consumption of ethanol beyond their respective ethanol metabolic capacities reflected these species' adaptive ability to avoid solutions previously paired with high blood ethanol levels via conditioned aversion [23,25]. That optimized ad lib consumption of ethanol continues at levels about ethanol eliminative capacity suggests an interaction of approach to and avoidance of ethanol in the regulation of maximized free-choice ethanol intake. A similar approach-avoidance interaction has recently been proposed to explain the relative insensitivity of schedule-induced polydipsia to conditioned taste aversions, as compared to water-deprivation-induced or meal-associated drinking [34,35].

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