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ATTENUATION OF A RADIATION-INDUCED CONDITIONED TASTE AVERSION AFTER THE DEVELOPMENT OF ETHANOL TOLERANCE

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Summary

An attempt to reduce a radiation-induced conditioned taste aversion (CTA) was undertaken by rendering animals tolerant to ethanol. Ethanol tolerance, developed over 5 days, was sufficient to block a radiation-induced taste aversion, as well as an ethanol-induced CTA. Several intermittent doses of ethanol, which did not induce tolerance but removed the novelty of the conditioning stimulus, blocked an ethanol-induced CTA but not the radiation-induced CTA. A CTA induced by doses of radiation up to 500 rads was attenuated. These data suggest that radioprotection developing in association with ethanol tolerance is a result of a physiological response to the chronic presence of ethanol not to the ethanol itself.

Animals have developed over the course of evolution mechanisms to help prevent accidental poisoning. In addition to emesis during which presumably tainted food is expelled from the stomach, animals are also capable of avoiding potentially toxic substances after a single ingestion of quantities less toxic than those required to induce vomiting. This is done through a process called the conditioned taste aversion (CTA). A CTA develops when the animal associates the taste of novel tasting food with a physiological response, possibly illness, and then subsequently avoids further ingestion of that food. In a laboratory setting, a CTA is typically induced by pairing a normally preferred but novel tasting fluid with exposure to a toxin. The animal will then avoid drinking the fluid.

The CTA has been extensively studied and a specific nucleus of the brain stem, the area postrema, has been demonstrated to play an important role in the development of a CTA to a broad range of unrelated toxins. These toxins include ionizing radiation, lithium chloride, copper sulfate, paraquat, amphetamine, and ethiofos (WR-2721). The area postrema is sufficiently important that if lesions are placed in this nucleus, the development of a CTA to these toxins is blocked (1-6). Not all toxin-induced CTAs are mediated by the area postrema. For example, CTAs induced by ethanol and morphine are not blocked by lesions of the area postrema (7).

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The biological mechanisms by which toxins induce a CTA mediated by the area postrema are not well understood. Toxins generally are not likely to act directly on specific receptors in this area of the brain, since toxins are usually foreign substances, and specific receptors for each possible toxin are not expected to have evolved. The area postrema is a highly vascularized nucleus in the brain stem and has a poorly developed blood-brain barrier (8). With this characteristic, it can monitor the blood for the presence of toxins (9). However, in order to effectively detect most toxins, an intermediary mechanism in the induction of CTAs involving one or more secondary mediators has been postulated (10,11). Basically, the theory suggests that a toxin primarily acts peripherally, rather than centrally, to stimulate the release of such a mediator into the blood, which, in turn, circulates to the area postrema, where it interacts with a specific receptor. If this interaction occurs following consumption of a novel tasting fluid, a CTA is then initiated.

Several approaches have been taken to block the development of a CTA. One approach involves preexposure to a toxin different from the one used to induce a CTA. If both toxins act by similar mechanisms, then the novelty of the CTA-inducing stimulus will be lost and a CTA will not develop, a process of behavioral conditioning. A recent study from our laboratory has employed this approach (12). However, the results provided mixed results. Preexposure to lithium chloride could block a CTA induced by ionizing radiation or ethanol, but preexposure with the latter toxins could not block a lithium chloride-induced CTA.

Another approach is to intervene in the interaction of the toxin or secondary mediator with receptors in the neural circuitry involved with the acquisition of a CTA. These receptors could be located in the area postrema or other parts of the brain. Biogenic amines have been the primary focus of the available studies. The results obtained using drugs that modify transmitter-receptor interactions or placing lesions in relevant areas of the brain have depended on the toxin used but have produced variable results. For example, although catecholamines appear to be involved in amphetamine-induced CTAs, they are not involved in lithium chloride- nor radiation-induced CTAs (see review of Rabin and Hunt (11) for details).

A third approach to block a CTA might be to interrupt the physiological consequences of stimulating receptors by a toxin or secondary mediator, rather than attempting to block the action of a toxin directly at receptors. Many mechanisms are activated after receptors are stimulated and if these mechanisms could be desensitized so that their responsiveness to the presence of a toxin is reduced, the behavioral consequences of exposure to the toxin might also be reduced. The present experiments were undertaken to test this possibility.

The procedure selected to desensitize neurons was to render them tolerant to the nonspecific toxin ethanol. The use of ethanol tolerance has one major advantage over the use of tolerance induced by other drugs. The induction of ethanol tolerance can reduce nonspecifically the sensitivity to a variety of different classes of drugs. Chronic ethanol administration is known not only to induce cross-tolerance to other depressants, such as gaseous anesthetics, barbiturates, and benzodiazepines, but also to reduce the sensitivity to other drug classes, such as dopamine agonists and antagonists (13-16), γ-aminobutyric acid agonists and antagonists (17), and in some cases opiates (18-19). With this relatively nonselective desensitization in the presence of ethanol tolerance to the effects of a number of drug types, the probability of reducing toxin-induced CTAs might be improved.

In these experiments ionizing radiation was used as the toxin to avoid drug interactions with ethanol. Also, both toxins exert similar effects on a number of neural mechanisms, increasing the likelihood of developing cross-tolerance. For example, radiation and ethanol induce hypothermia (20,21), stimulate striatal potassium-stimulated dopamine release in vitro (22,23), reduce the concentrations of striatal dopamine metabolites (24,25), and inhibit voltage-dependent sodium and calcium channels (26-29). Since ethanol itself can induce a CTA (30,31), the effect of tolerance on this end-point was assessed for comparison with the radiation-induced CTA.

Methods

Male Sprague-Dawley Crl:CD(SD)BR rats (Charles River Breeding Laboratories, Kingston, NY) weighing 200-300 g were used in these experiments. Rats were quarantined on arrival and screened for evidence of disease by serology and histopathology before being released from quarantine. The rats were housed individually in polycarbonate isolator cages (Lab Products, Maywood, NJ) on autoclaved hardwood contact bedding ('Beta Chip' Northeastern Products Corp., Warrensburg, NY) and were provided commercial rodent chow ('Wayne Rodent Blok' Continental Grain Co., Chicago IL) and acidified water (pH 2.5 using HCl) ad libitum to reduce Pseudomonas infections. Animal holding rooms were kept at $21 \pm 1^{\circ}$ C with $50 \pm 10\%$ relative humidity on a 12-hr, light:dark lighting cycle with no twilight (lights on at 7 AM; off at 7 PM).

The animals were rendered ethanol-tolerant by giving them doses of ethanol twice daily (morning and afternoon) for 5 days. Ethanol was administered orally as a 20% (w/v) aqueous solution using a pediatric feeding tube. The initial dose was 4 g/kg and increased toward the end of the 5-day period to 6 g/kg, as the responsiveness to a dose of ethanol decreased. The second dose each day was determined based on the degree of intoxication as described by Majchrowicz (32). The higher the degree of intoxication, the lower the dose of ethanol administered. Control groups were given an amount of water equivalent to that received by the experimental groups. Tolerance was demonstrated 5 days after the last dose of ethanol by comparing sleep-times induced by a single dose (3-g/kg, ip) of ethanol obtained from the ethanol-tolerant and control groups. The sleep-time was the period during which the animal lost its righting reflex.

After the last dose of ethanol, the animals were deprived of water for 23.5 hr per day for five days. On each of those days, they were given water for only a 30-min period during the early light phase of the diurnal cycle (8-11 AM). On the fifth day after the last dose of ethanol (the conditioning day), the animals were presented with a 10% sucrose solution. After they were permitted to consume the fluid for 30 min and the amount consumed recorded, the animals were exposed to varying doses of ionizing radiation or to a 4-g/kg, oral dose (via intubation) of ethanol. Two days later on the test day, the 10% sucrose solution was presented again for a 30-min period, and the amount of fluid consumed was recorded. A CTA was defined as a statistically significant reduction in the intake of the 10% sucrose solution on the test day.

Irradiated animals were exposed to a single bilateral dose of gamma radiation from a ^{60}Co source at a rate of 40 rads/min. Radiation dosimetry was performed using paired, 50-ml ion chambers. Delivered dose was expressed as a ratio of the dose measured in a tissue-equivalent plastic phantom enclosed in a restraining tube, to that measured free in air.

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Results

Exposure to either ionizing radiation (100 rads) or ethanol (4 g/kg, po) resulted in the development of a CTA in control animals (Fig. 1). As expected, when animals were rendered tolerant to ethanol (as demonstrated by reduced sleep-times in Table 1), they were resistant to the acquisition of an ethanol-induced CTA. However, ethanol-tolerant animals were also resistant to a radiation-induced CTA as well (Fig. 1). No significant difference in sucrose consumption was found on the test day for either toxin.

The attenuation of a radiation-induced CTA in ethanol-tolerant rats might suggest that radiation and ethanol are experienced as similar stimuli by the animal. Consequently, this attenuation could be due to the lack of novelty of the experience of the toxin, and such a lack in itself would block the acquisition of a CTA. To test this possibility, animals were given single doses of ethanol on 3 days prior to the conditioning day on which they were exposed to either ethanol or ionizing radiation. Single doses of ethanol were administered 2, 5, and 8 days before the conditioning day. This procedure was not sufficient to induce tolerance (Table 1). Under these experimental conditions, although several doses of ethanol were sufficient to block an ethanol-induced CTA, they were not sufficient to block a radiation-induced CTA (Fig. 2) (cf. ethanol/rad groups in Fig. 1 with ethanol/rad groups in Fig. 2).

In another set of experiments to determine if ethanol tolerance could block the CTA induced by higher doses of radiation, animals were exposed to 100-, 300-, or 500-rad doses of radiation and the presence of a CTA was assessed in the manner described above. Increasing doses of radiation as expected intensified the CTA in a dose-dependent manner in nontolerant animals with the maximum CTA being observed after a dose of 500 rads (Fig. 3). When animals were rendered ethanol-tolerant, a similar dose-response effect was observed, but revealed a reduced sensitivity to radiation at all doses. The degree to which ethanol-tolerance could confer radioprotection depended somewhat on the intensity of the CTA. Although the dose required to reduce the consumption of the 10% sucrose solution by 50% (ED_{50}) was 2.5 times greater for the ethanol-tolerant rats, it was 3.4 times greater for the ED_{40} but only 2.0 times greater for the ED_{70} (Fig. 3). In effect, ethanol tolerance had the most beneficial effects after exposure to the lower doses of radiation.

Discussion

The present results indicate that short-term chronic administration of ethanol sufficient to render animals tolerant to the depressant effects of ethanol can attenuate a radiation-induced CTA. This attenuation could be observed over a range of doses from 100 to 500 rads.

There are three possible mechanisms by which ethanol treatment could have a radioprotectant effect. One mechanism could involve a competitive interaction between ethanol and radiation (possibly via a secondary mediator) on the sites in the brain where CTAs are initiated. This possibility appears unlikely. Although radiation and ethanol have some actions in common, as discussed earlier, radiation acts, at least indirectly, on the important center of CTA induction, the area postrema (2,3), while ethanol does not (7). Thus, the radioprotectant effect apparently does not result from a direct action of ethanol per se on the systems responsible for the induction of CTAs, but rather from the response of the animal to the continuous presence of ethanol.

TABLE 1

SLEEP-TIMES (MIN) AS A FUNCTION OF ETHANOL TOLERANCE AND PREEXPOSURE PROCEDURES

	N	PRETREATMENT	POSTTREATMENT
TOLERANCE			
Ethanol	15	100.07 ± 8.06*	61.40 ± 5.15**
Water	14	105.64 ± 7.88	124.57 ± 10.26**
PREEXPOSURE			
Ethanol	12	114.83 ± 8.26	100.58 ± 11.67
Water	11	110.73 ± 10.68	112.45 ± 17.40

* Mean ± standard error of the mean.
** Significantly different from pretreatment mean, $p < 0.05$, based on Student's t-tests.

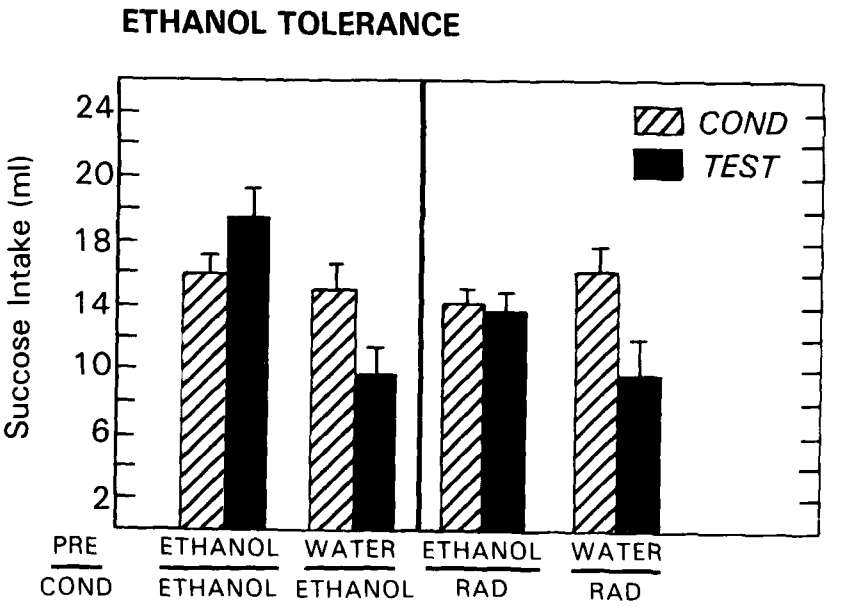

FIG. 1

The effect of ethanol tolerance on an ethanol and radiation-induced CTA. Each pair of bars represents the sucrose intake on the conditioning and test days under different experimental conditions. Animals were pretreated chronically with either ethanol or water for 5 days. A 100-rad dose of ionizing radiation or a 4-g/kg dose of ethanol could induce a CTA in non-ethanol-tolerant animals but not in tolerant ones. Statistical analyses were performed using the paired t-test; 0.05 was the level of significance. No CTA developed when water was substituted for sucrose or when animals were sham-irradiated (data not shown).

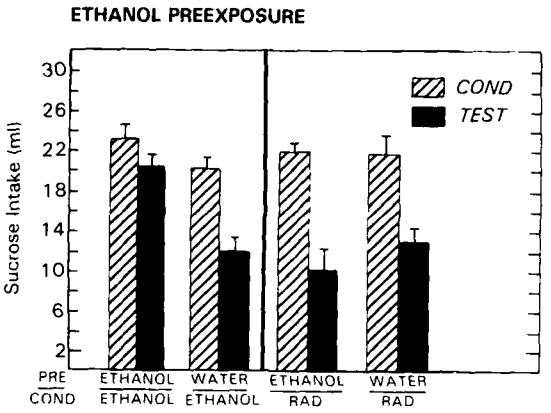

FIG. 2

The effect of ethanol preexposure on an ethanol and radiation-induced CTA. Each pair of bars represents the sucrose intake on the conditioning and test days under different experimental conditions. Animals were administered a single dose of ethanol on 3 separate days. Preexposure to ethanol blocked an ethanol-induced CTA but not a radiation-induced CTA. Statistical analyses were performed using the paired t-test; 0.05 was the level of significance. No CTA developed when water was substituted for sucrose or when animals were sham-irradiated (data not shown). In these experiments, the animals were water deprived for 10 days prior to the conditioning day to maximize the chance of finding an effect of preexposure. As a result, they consumed more fluid on the conditioning day than the ethanol-tolerant animals deprived of water for only 5 days.

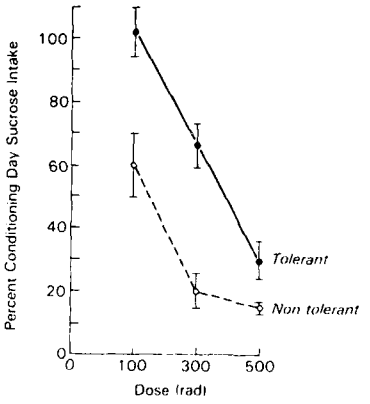

FIG. 3

The effect of increasing doses of radiation on sucrose intake in ethanol-tolerant and non-tolerant animals expressed as a percentage calculated by dividing the intake on the test day by the intake on the conditioning day. Ethanol-tolerant animals were less sensitive to the induction of a radiation-induced CTA at all doses examined ($p < 0.001$, based on an analysis of variance, $F = 32.0$, $df = 1,56$).

If the stimuli underlying the acquisition of radiation- and ethanol-induced CTAs were similar, the novelty of the radiation stimulus would be reduced by prior experience with an ethanol stimulus and, consequently, the intensity of a radiation-induced CTA would be attenuated. The lack of involvement of the area postrema in the induction of both CTAs and the results in Fig. 2 argue against this possibility of a preexposure effect. The administration of several doses of ethanol prior to the pairing of the sucrose with radiation was not sufficient to block the subsequent radiation-induced CTA. Also, in a more extensive study ethanol preexposure was unable to block a CTA induced by lithium chloride nor amphetamine (12). Only an ethanol-induced CTA could be blocked by ethanol preexposure.

The development of tolerance to ethanol appears to be the best explanation of the radioprotectant effect observed. Tolerance to a drug is defined as the progressive decline in the effect of a given dose to induce a given response or as the need to increase the dose to maintain the response. There are several types of ethanol tolerance that can be studied in experimental animals, including environmentally conditioned, metabolic, and cellular tolerance. However, the first two types of tolerance are not pertinent to the present experiments. Environmentally conditioned tolerance can occur when animals are tested for sensitivity and tolerance to ethanol and chronically treated under the same experimental and environmental conditions (33). They are, in effect, conditioned by environmental cues to compensate for the depressant effect of ethanol. Since sleep-times in our studies were performed after a different route of administration of ethanol than was used to induce tolerance, and since irradiations were performed in a *different environment*, *environmentally* conditioned tolerance would not play a role in the results obtained. Metabolic tolerance is a result of an increased rate of ethanol metabolism, a process not relevant to radiation exposure. Cellular tolerance is expressed as a reduced responsiveness of physiological processes to the presence of ethanol and correlates with the development of behavioral tolerance. Since behavioral and cellular tolerance to ethanol results from a reduction in the ability of ethanol to disrupt the structure and function of cellular membranes (34,35) and affected mechanisms become cross-tolerant to the actions of other drugs, the responsiveness of these mechanisms may also be reduced to the actions of a toxin not a chemical substance, such as ionizing radiation. This process may be the basis of the radioprotective effect of ethanol tolerance.

The induction of cellular tolerance to ethanol is a general way to desensitize a variety of systems, presumably nonspecifically. As pointed out earlier, the actions of a number of unrelated classes of drugs are less effective in ethanol-tolerant animals. It would appear from the present results that the ability to induce a CTA after exposure to ionizing radiation is also reduced in ethanol-tolerant animals. The usefulness of this approach to block the development of a CTA might depend on its ability to block CTAs induced by other toxins. Preliminary results indicate that ethanol tolerance also attenuates a CTA induced by lithium chloride. Further research is in progress.

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