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Ethanol-Induced Taste Aversions: Lack of Involvement of Acetaldehyde and the Area Postrema

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HUNT, W. A., B. M. RABIN AND J. LEE. *Ethanol-induced taste aversions: Lack of involvement of acetaldehyde and the area postrema.* ALCOHOL 4(3) 169–173, 1987.—Two experiments were run to evaluate the role of acetaldehyde and the area postrema in the acquisition of an ethanol-induced conditioned taste aversion. An ethanol-induced taste aversion was observed in male Sprague-Dawley rats with a dose of 4 g/kg, PO, but not after doses of 1 or 2 g/kg. Pretreatment with 4-methylpyrazole (8 mg/kg, IP), which itself did not induce an aversion as compared to pyrazole (68 mg/kg, IP) that did, and/or prior application of lesions of the area postrema had no influence on the development of an ethanol-induced taste aversion. The results indicate that ethanol-induced taste aversion learning does not result from the metabolism of ethanol to acetaldehyde and does not, like other toxins, involve the mediation of the area postrema.

Conditioned taste aversion / Ethanol / Area postrema / Pyrazole / 4-Methylpyrazole

IT is well known that under certain conditions ethanol has aversive properties. The most notable examples of these properties are found when alcoholics drink ethanol while being treated with disulfiram (Antabuse), and when some Orientals exhibit a "flushing response" after drinking ethanol [26]. These responses, characterized by facial flushing, vasodilitation, nausea, and vomiting, are presumed to be due to an increase in the concentration of acetaldehyde in the blood [8,14]. Normally, acetaldehyde concentrations are low. But, disulfiram treatment, which inhibits the conversion of acetaldehyde to acetate, or the lack of the appropriate enzyme (an isozyme of aldehyde dehydrogenase) in some Orientals, results in the accumulation of acetaldehyde after ethanol consumption.

Experimental animals have been shown to alter their preference for ethanol after being treated with drugs that modify the metabolism of ethanol and acetaldehyde. For example, after treatment with calcium cyanamide, animals have been reported to consume less ethanol [8,22]. This effect could not be attenuated when animals were pretreated with 4-methylpyrazole (4-MP), an alcohol dehydrogenase inhibitor, which alone also had no effect on ethanol preference [22]. In addition, different strains of rodents exhibit different preferences for ethanol depending on the activity of aldehyde dehydrogenase, the enzyme that converts acetaldehyde to acetate. Rodents with high preference reportedly have higher hepatic aldehyde dehydrogenase activity than those with low preference [11,21]. When C57 mice were examined for the ability of 4-MP to reduce ethanol consumption, the results depended on the level of consumption prior to 4-MP administration [9]. The high preference animals consumed less ethanol, whereas, the low preference animals increased their alcohol consumption.

Another way to study the aversive effects of ethanol is by using the conditioned taste aversion (CTA) paradigm. A CTA is acquired when a novel tasting solution is paired with an aversive unconditioned stimulus, such that the organism will avoid ingestion of that solution at a subsequent presentation. In common with many drugs that animals will self-administer [3, 7, 10], ethanol will induce a CTA [4,12]. To date, the mechanisms underlying the acquisition of taste aversions produced by self-administered drugs, such as ethanol, and how they are aversive have not been clearly defined.

The present experiments were designed to address two issues about the mechanisms by which ethanol might produce a CTA. The first is whether the acquisition of a CTA is a direct effect of ethanol on a target site or whether the learning depends upon the actions of an intermediary substance, possibly resulting from the metabolism of ethanol. Amit and his collaborators have provided evidence that peripherally administered, but not centrally administered, acetaldehyde, the primary metabolite of ethanol, can function as an unconditioned stimulus for CTA learning [6]. In other experiments, a possible interaction between ethanol and peripheral acetaldehyde was explored by determining whether they are similar enough stimuli that the animals experience the same unconditioned stimulus. For a CTA to develop, a novel unconditioned stimulus must normally be paired with the drinking solution. Prior experience of the

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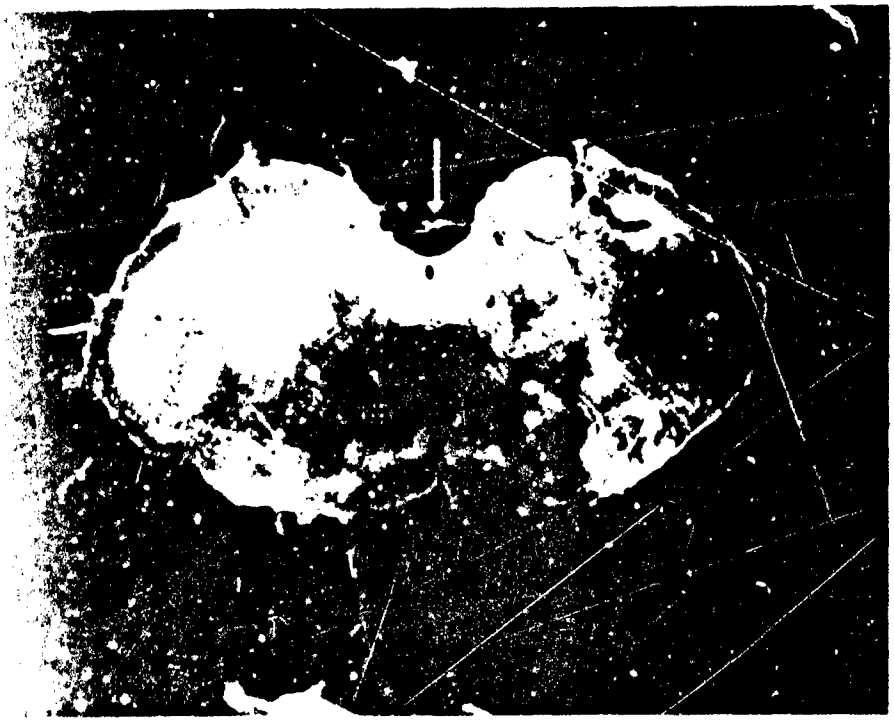

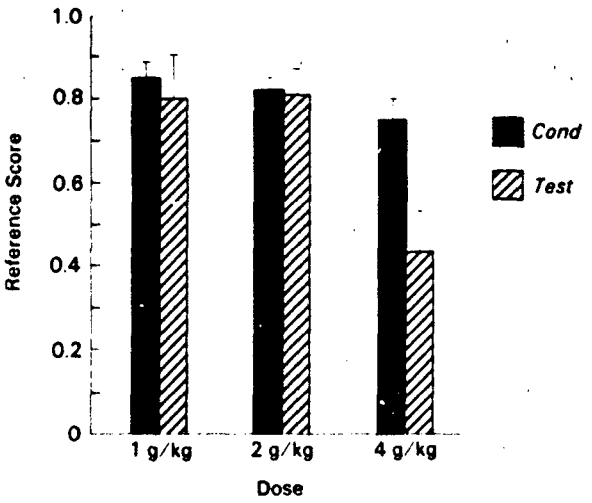

FIG. 2. Preference scores after different doses of ethanol. Means±SE were derived from 11 animals/group.

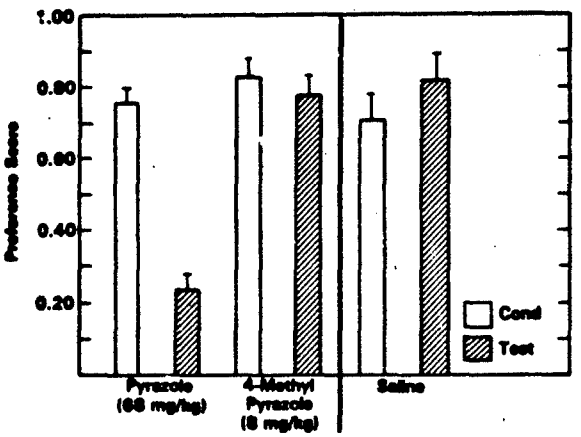

FIG. 3. Preference scores after pyrazole or 4-MP administration. Means±SE were derived from 9–12 animals/group.

unconditioned stimulus without pairing generally results in no CTA developing when the stimulus and solution are paired. When acetaldehyde was administered for several days before pairing it with the novel drinking solution, the acquisition of an ethanol-induced CTA was blocked, while preexposure to ethanol disrupted the acquisition of a CTA produced by low, but not high, doses of acetaldehyde [1]. These findings suggest that ethanol and acetaldehyde might have common effects, when administered peripherally. As a result, Amit has proposed that the metabolism of ethanol to acetaldehyde is responsible for the aversive consequences of ethanol intake. However, if acetaldehyde does function to mediate the aversive consequences of ethanol ingestion, then it should be possible to block the acquisition of a CTA by pretreatment with compounds that block the conversion of ethanol to acetaldehyde.

The second question is whether the area postrema (AP), a nucleus in the brain stem, plays any role in the acquisition of an ethanol-induced CTA. The AP has been shown to be involved in CTA learning after exposure to a variety of unrelated toxins, such as ionizing radiation and lithium chloride [17], and has no blood-brain barrier, so that the blood can be monitored for the presence of the toxins. An action of ethanol or acetaldehyde on the AP might also be postulated, especially since acetaldehyde does not produce a CTA when administered centrally, but must be administered peripherally to be effective [6]. This finding is similar to the results obtained under similar experimental conditions for lithium chloride [23], whose CTA is AP-mediated [18,20], and suggests that the peripheral effects of ethanol or acetaldehyde are essential for the acquisition of an ethanol-induced aversion. If ethanol or acetaldehyde acts on the AP to induce a CTA, placing lesions in this area of the brain should block its development.

METHOD

For these studies 104 male Sprague-Dawley Crl:CD (SD)BR rats (Charles River Breeding Laboratories, Kingston, NY) weighing 250–350 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 acidified water (pH 2.5 using HCl) ad lib. Animal holding rooms were kept at 21±1°C with 50±10% relative humidity on a 12 hr light:dark lighting cycle with no twilight. Food and water were continually available except as required by the experimental protocol.

Taste aversions were produced by placing the rats on a 23.5-hr water deprivation schedule for 10 days during which water was available for only 30 min during the early light phase of the diurnal cycle. On the conditioning day (day 10), the rats were presented with two calibrated drinking tubes, one containing 10% sucrose and the other containing tap water, after which the intake of each fluid was recorded. Immediately following the drinking period, rats were injected with either pyrazole (68 mg/kg, IP), or 4-MP (8 mg/kg,

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FIG. 1. Representative sections through the brain stem. (A) Section from a sham-lesioned animal. (B) Section from an animal with an area postrema lesion.

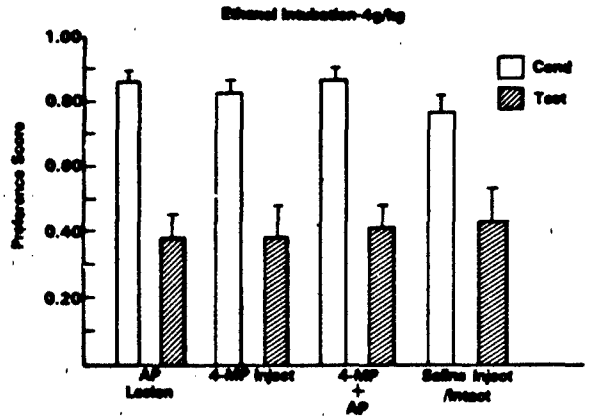

FIG. 4. Preference scores after ethanol administration of animals with area postrema lesions and/or pretreated with 4-MP (administered 30 min before the ethanol). Means±SE were derived from 10 animals/group.

IP), alcohol dehydrogenase inhibitors [24], ethanol (4 g/kg, a 20% (w:v) solution administered through an infant feeding tube), combinations as indicated (4-MP was administered 30 min before the ethanol), or an IP injection of isotonic saline as a control for handling and injection procedures. (Saline-treated controls consumed the same amount of sucrose as untreated controls). On the test day (day 11), the rats were again presented with the two drinking tubes containing sucrose solution and tap water, and their fluid intakes recorded.

Histologically verified lesions were made in the AP of rats anesthetized with sodium pentobarbital (35 mg/kg, IP) using a thermal cautery probe under direct visual control [18]. After a three-week recovery period, the rats were divided into 4 groups: AP lesions, 4-MP pretreatment, AP lesions and pretreatment with 4-MP, and control animals that were sham-lesioned and saline-injected.

At the conclusion of the experiment, the animals with AP lesions were euthanized with an overdose of sodium pentobarbital (80 mg/kg), perfused with isotonic saline and 10% formalin-saline, and the brains fixed in 10% formalin-saline. Fifty-micron sections were cut through the brainstem at the level of the AP and stained with thionin. Photomicrographs showing the AP of a control animal and one with a lesion are presented in Fig. 1. For the most part, the lesions were restricted to the AP, but in some cases extended beyond the AP to include parts of the nucleus of the solitary tract.

The intake data were transformed to preference scores: sucrose intake divided by total fluid intake. No differences in total fluid consumption were observed after any of the experimental manipulations. For statistical analyses (as indicated in the test), all preference scores were subjected to arcsin transformations to normal distributions [25]. A preference score less than 0.50 indicates an avoidance of the normally preferred sucrose solution and the presence of a CTA.

RESULTS

Initial experiments were designed to find the appropriate dose of ethanol to use for subsequent experiments and whether pyrazole or 4-MP in themselves induce a CTA. Figure 2 shows the preference for sucrose as a function of the dose of ethanol. The results indicate that a dose of ethanol of 4 g/kg, PO, is required to induce a CTA. Doses of 1 or 2 g/kg had no effect.

The results of the experiment to determine if pyrazole or 4-MP induces a CTA are found in Fig. 3. The data indicate that the injection of pyrazole produces a CTA, while injection 4-MP does not. Statistical analysis of the results using individual t-tests for paired samples showed that treatment with pyrazole produced a significant reduction in test-day sucrose preference, $t(9)=9.85$, $p<0.001$. Injection of either 4-MP, $t(9)=0.52$, $p>0.10$, or saline, $t(9)=-1.12$, $p>0.10$, in contrast, had no effect on test-day sucrose preference compared to the conditioning day preference. These data, which show that treatment with pyrazole produces a CTA, while treatment with 4-MP does not, are consistent with other data indicating that pyrazole is more toxic than 4-MP [13]. Because these data show that injection with 4-MP does not produce a CTA by itself, they indicate that pretreatment with 4-MP is a better alcohol dehydrogenase inhibitor to use than pyrazole to study the role of acetaldehyde in the acquisition of an ethanol-induced CTA.

The effects of pretreatment with 4-MP and/or lesion of the AP on the acquisition of an ethanol-induced CTA are summarized in Fig. 4. The data indicate that neither 4-MP nor AP lesions, singly or in combination, had any effect on the acquisition of an ethanol-induced CTA. The data were analyzed using a 2-way analysis of variance with one repeated measure (conditioning and test days). The analysis of variance showed that the comparison between conditioning-day and test-day preference was highly significant, $F(1,38)=72.95$, $p<0.001$. Neither the main effect for the comparison between the four treatment conditions, $F(3,38)=0.24$, $p>0.10$, nor the treatment by day interaction, $F(3,38)=0.29$, $p>0.10$, was significant. This analysis indicates that there was a consistent decrease in sucrose preference from the conditioning day to the test day in all four experimental groups.

DISCUSSION

These results do not support the possible involvement of either acetaldehyde or the AP in the acquisition of a CTA following treatment with ethanol and are not consistent with the proposal that the conversion of ethanol to acetaldehyde is the basis for the CTA induced after ethanol ingestion [1, 2, 6]. While it has been shown that acetaldehyde can produce a CTA [6], this observation by itself does not provide direct evidence to support the hypothesis that the metabolism of ethanol to acetaldehyde is the proximal unconditioned stimulus for the acquisition of the CTA. In the present experiments, despite the fact that the metabolism of ethanol was blocked by pretreating the animals with 4-MP, the animals still acquired a CTA that was similar to the one acquired by the untreated animals. Therefore, peripheral acetaldehyde resulting from the metabolism of the ethanol treatment could have played no part in the acquisition of the CTA.

It has also been proposed that acetaldehyde synthesized in the brain may mediate CTA learning following treatment with ethanol. Disrupting the central synthesis of acetaldehyde from ethanol by pretreating rats with 3-amino-1,2,4-triazole, a catalase inhibitor, administered centrally, will block the acquisition of an ethanol-induced CTA [2]. However, it is not clear what this finding means because previous work from the same laboratory has established that

centrally administered acetaldehyde does not produce a CTA [6].

Our results would indicate that the conversion of ethanol to acetaldehyde is not a necessary prerequisite to produce an ethanol-induced CTA. It may be, as has been suggested for radiation and lithium chloride [17], that the peripheral administration of both compounds causes the release of some endogenous factor, or that both compounds produce similar internal states within the organism, which can serve as the proximal unconditioned stimulus leading to the acquisition of the CTA.

Given that high doses of ethanol are toxic, it is not clear why AP lesions produced no attenuation of the ethanol-induced CTA. Because the AP functions as part of the neural system regulating the emetic response to poisons [5], lesions of the AP may not attenuate the ethanol-induced CTA because ethanol does not normally induce emesis, except as a result of direct irritation of the stomach. With ethanol, as with amphetamine [10], a severe toxic reaction may not be the primary response and, therefore, the acquisition of a CTA would not depend upon the integrity of the AP.

In summary, the present results provide no support for the hypothesis that the aversive consequences of ethanol result from the metabolism of ethanol to acetaldehyde. They also provide no support for the hypothesis that an ethanol-induced CTA involves the mediation of the AP, as with other toxins. The mechanisms by which ingestion of ethanol can lead to the acquisition of a CTA remain to be established. There is, however, some research suggesting that ethanol-induced CTAs are mediated by the endogenous opioid system [15,16]. Whether common mechanisms involving opiate receptors underlie taste aversions produced by both ethanol and morphine will require additional research.

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