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Conditioned stimulus preference after acetaldehyde but not ethanol injections

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Abstract

Acetaldehyde, the first ethanol metabolite, has been suggested to mediate some of the behavioral effects of ethanol and particularly its reinforcing properties, although this later hypothesis remains extremely controversial. While several studies demonstrated the reinforcing effects of brain acetaldehyde, blood acetaldehyde accumulation is believed to be primarily aversive. In the present study, a conditioned reinforcement procedure has been used to investigate the reinforcing and/or aversive effects of intraperitoneal injections of both acetaldehyde and ethanol in Wistar rats. An olfactory stimulus was paired with daily injections of either ethanol (0, 0.25, 0.5, 1 and 2 g/kg) or acetaldehyde (0, 10, 20, 100 and 150 mg/kg). After eight conditioning sessions, all rats were tested for their stimulus preference or aversion. The results show that conditioning with small, 0.25 and 0.5 g/kg, ethanol doses induced neither preference nor aversion for the olfactory cue. In contrast, higher ethanol doses (1.0 and 2.0 g/kg) resulted in significant stimulus aversions. Acetaldehyde conditioning led to a biphasic stimulus preference, with a maximal preference around 20 mg/kg acetaldehyde. No evidence of aversive effects was found with increasing doses of acetaldehyde, even with concentrations close to the lethal limit. The present study clearly shows that systemic acetaldehyde injections induced significant stimulus preferences. This suggests that acetaldehyde may be, at least in part, responsible for the reinforcing effects of alcohol intake.

Keywords: Ethanol; Acetaldehyde; Place conditioning; Reinforcement

1. Introduction

Although considerable amounts of studies have investigated the behavioral and neurochemical factors that affect alcohol consumption and abuse, several crucial issues remain unresolved. For example, the respective roles of ethanol and its first metabolite acetaldehyde in mediating the pharmacological effects of alcohol consumption have not yet been clearly established. Recent studies have suggested that acetaldehyde mediates some of the behavioral effects of ethanol, such as narcosis (Aragon et al., 1991), conditioned taste aversion (Aragon et al., 1985) and increased locomotor activity (Aragon et al., 1989). Acetaldehyde has also been suggested to mediate the reinforcing properties of ethanol, thereby regulating alcohol consumption (see review in Smith et al., 1997). However, this later hypothesis remains extremely controversial.

On one hand, several studies reported evidence that acetaldehyde exerts reinforcing properties. Acetaldehyde is easily self-administered by rats through several routes of administration (Brown et al., 1980; Myers et al., 1984a,b). Rats have also been shown to display a place preference for an environment in which they previously received intracerebroventricular infusions of acetaldehyde (Smith et al., 1984). Furthermore, drugs, which reduce brain acetaldehyde concentrations, have been shown to decrease alcohol consumption (Aragon and Amit, 1992; Koechling and Amit, 1994).

On the other hand, elevated blood acetaldehyde concentrations have long been considered aversive and are the basis for treating alcoholics with disulfiram. Additional evidence of the aversive effects of acetaldehyde has been reported in the literature. Drugs, which increase blood acetaldehyde concentrations after ethanol intake, have been shown to reduce voluntary ethanol consumption (Eriksson and Deitrich, 1980; Sinclair et al., 1980), while direct peripheral administrations of acetaldehyde induced a strong conditioned taste aversion (Brown et al., 1978). Further-

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more, ethanol administrations result in higher blood acetaldehyde concentrations in ethanol-avoiding ANA rat lines, and this has been considered as a basis for their ethanol avoidance (Koivisto and Eriksson, 1994). In some humans, a point mutation in the encoding gene for aldehyde dehydrogenase (ALDH) 2, the ALDH isoenzyme, mainly responsible for acetaldehyde catabolism, results in a decreased capacity to oxidize acetaldehyde and leads to its blood accumulation after ethanol intake (Yoshida et al., 1983). The ALDH2 deficient people have been shown to consume less alcohol, probably because of their adverse physiological reactions to ethanol (Higuchi et al., 1992). All these observations strongly suggest that blood acetaldehyde accumulation produces aversive physiological reactions and, therefore, prevents alcohol consumption.

An important factor to determine whether acetaldehyde exerts either reinforcing or aversive effects may be the localization of its accumulation. Indeed, the majority of studies, which attributed reinforcing properties to acetaldehyde, investigated its effects within the brain, either by direct intracerebral infusion of acetaldehyde (Brown et al., 1980; Smith et al., 1984) or by the use of drugs, which alter its brain concentration (e.g., Aragon and Amit, 1992; Koechling and Amit, 1994). In contrast, the peripheral accumulation of acetaldehyde generally produced strong aversive effects (Brown et al., 1978; Eriksson and Deitrich, 1980; Higuchi et al., 1992; Sinclair et al., 1980). Therefore, it could be hypothesized that peripheral acetaldehyde is primarily aversive, while its local accumulation within the brain may be reinforcing. Another factor to be considered is acetaldehyde concentration. It has been suggested that a narrow range of acetaldehyde concentrations may exert reinforcing effects (Hunt, 1996). Once an upper threshold of acetaldehyde concentration has been attained, its aversive effects would be predominant.

In the present study, we have investigated the reinforcing and/or aversive effects of peripheral administrations of various acetaldehyde and ethanol doses in male Wistar rats using a conditioned reinforcement procedure. This procedure consisted of a modification of the classical place conditioning methodology, which has been widely used to investigate the reinforcing and aversive properties of many addictive drugs (Mucha et al., 1982), including alcohol (e.g., Asin et al., 1985; Cunningham, 1981; Van der Kooy et al., 1983).

2. Materials and methods

2.1. Subjects

One hundred male Wistar rats, weighing 250–300 g, were individually housed in plastic cages in a room maintained on a reversed 12-h light/dark cycle (lights on at 6:00 p.m.) and kept at a constant temperature. All habituation, conditioning and testing sessions were carried out during the dark portion of the light/dark cycle under red lights. Food and water were continuously available in the home cages during all the experiment.

The rats were randomly divided into 10 groups. Each of the groups was assigned a specific dose of either ethanol (0, 0.25, 0.5, 1.0 or 2.0 g/kg) or acetaldehyde (0, 10, 20, 100 or 150 mg/kg) for daily conditioning. At 200 mg/kg acetaldehyde, severe toxicity and death were observed during conditioning, and this dosage was therefore withdrawn.

2.2. Apparatus

The apparatus consisted of a box with two compartments separated by a connecting passage $12 \times 12 \times 12$ cm^3 in gray plastic. It was possible to separate this passage from the two compartments by guillotine doors. Each compartment, measured 34 cm long, 26 cm wide and 30 cm deep, was in gray plastic except the front wall, which was transparent plexiglas, and had a grid floor. The entire apparatus was contained within a ventilated, sound-attenuating and red light-illuminated room.

2.3. Procedure

This procedure consisted of a modification of the classical place conditioning procedure. Instead of associating a specific environment, i.e., a box compartment, with drug administrations, the present studies used a more discrete olfactory stimulus, which was repeatedly associated with either ethanol or acetaldehyde injections (Quertemont et al., 1998).

During the 8-day conditioning period, all rats received two daily sessions. In the morning, a habituation session was conducted during which time each animal was allowed to explore freely the entire apparatus with the guillotine doors removed for a 30-min period. The conditioning session itself started in the afternoon between 2:00 and 5:00 p.m. After an initial period of 20 min, when the rats were allowed to run freely in the entire apparatus, each rat was injected intraperitoneally with either ethanol (0, 0.25, 0.5, 1.0 or 2.0 g/kg) or acetaldehyde (0, 10, 20, 100 or 150 mg/kg). The rat was then confined in one side of the box with a specific olfactory stimulus for the next 20 min to allow the association between the acute effects of the conditioning drug and the olfactory stimulus. The olfactory stimulus consisted of 5 ml of an acetic acid solution (5% in water) in a jar containing tissue paper, which totally absorbed the acetic acid solution. The position of the olfactory stimulus was daily alternated between the left and right sides, such that each rat was confined with the cue for equal amounts of time on either side of the apparatus during the conditioning time period. This was entailed to make sure that the rats do not associate the effects of the drug with a particular compartment but only with the olfactory stimulus.

The testing session was conducted on the day after the last conditioning session between 2:00 and 5:00 p.m. The

rats were allowed to run freely in the entire apparatus with the guillotine doors removed during the first 20 min, and the time spent in each side was recorded under a blind procedure, i.e., the observer did not know the experimental procedure undertaken on the rat under investigation. This data served as a measure of each animal's basal preference for the left or right side. The olfactory stimulus associated with either ethanol or acetaldehyde intraperitoneal injections during the conditioning procedure was then introduced into the side of the box where each rat had spent the less time. After the olfactory stimulus introduction, the time spent in each side was recorded for another 20-min period to assess the changes in place preference induced by the presence of the olfactory cue.

All experiments and procedures were carried out according to the European Communities Council Directive (86/609/EEC) for care and use of laboratory animals and in agreement with the UK Animals Scientific Procedures Act 1986.

2.4. Drugs

Acetaldehyde, purchased from Sigma (St Louis, MO), was mixed in 0.9% saline, concentration 2% v/v. Ethanol solutions, concentration 15% v/v, were prepared by mixing absolute ethanol (Merck, Darmstadt, Germany) with 0.9% saline.

2.5. Data analysis

Any animal, which spent more than 80% of the time in its "preferred" side before the introduction of the olfactory stimulus, was eliminated and replaced to obtain 10 rats in each group. This procedure was required to eliminate animals with a too strong side preference that could interfere with drug-conditioned stimulus preference. Only five rats were replaced in the entire study.

Changes in side preference were calculated by measuring the time spent by each rat on the olfactory stimulus side and subtracting from this the amount of time spent on that side during the 20-min before the stimulus introduction. A score of zero indicated that there was no change in place preference after the olfactory stimulus introduction, while positive and negative scores indicate, respectively, preferences and aversions for the olfactory cue.

The changes in side preference for both acetaldehyde and ethanol groups were compared with one-way analysis of variance (ANOVA) followed by the least-significant difference test of multiple comparisons (Fisher's test) to determine statistical significances between groups (Statview v. 4.5, SAS Institute).

2.6. Unconditioned preference for the olfactory stimulus

A separate experiment was carried out to assess the unconditioned preference or aversion of naive rats for the olfactory stimulus, together with the effects of habituation on such a preference or aversion. Eight naive rats were first tested for their preference or aversion for the olfactory stimulus following the same procedure as described above. Briefly, the time spent by each rat in either side of the box was recorded for the first 20 min. The olfactory stimulus was then introduced into the side of the box where each rat had spent the less time, and the time spent in each side was recorded for another 20-min period.

One week after this testing, all rats started the conditioning procedure as described above, except that they did not receive any injection before the introduction of the olfactory stimulus. At the end of the conditioning procedure, the preference or aversion of the rats for the stimulus was tested again following the same procedure to measure the effects of habituation to the olfactory cue and experimental procedures.

3. Results

3.1. Unconditioned preference for the olfactory stimulus

Before introduction of the olfactory stimulus, naive rats spent an almost equal amount of time in both the left and right sides, showing no unconditioned preference for either side of the box. They spent an average of 637 ± 180 s (mean ± S.D.) in the right side, which was not significantly different from half of the total 20-min period [$t(7) = 0.59$, $P = .57$]. Furthermore, naive rats showed no unconditioned preference or aversion for the olfactory stimulus. A paired t-test indicated that there was no significant changes in place preference after the introduction of the olfactory stimulus [$t(7) = 1.02$, $P = .34$].

Habituation to the olfactory stimulus and experimental procedures without conditioning with drug injections did not affect the time spent by rats with the olfactory stimulus. There was still no evidence of significant preference or aversion for the olfactory stimulus [$t(7) = 0.36$, $P = .73$].

3.2. Effects of ethanol and acetaldehyde conditionings on stimulus preference

There were no statistical differences between experimental groups in the initial side preferences before stimulus introduction. Rats from ethanol groups spent an average of 431 ± 86 s (mean ± S.D.) in their nonpreferred side of the box [$F(4,45) = 0.47$, $P = .76$]. In the acetaldehyde groups, rats spent 416 ± 106 s (mean ± S.D.) in their nonpreferred side of the box [$F(4,45) = 0.23$, $P = .92$].

Fig. 1 shows the effects of conditioning with the different ethanol doses on preferences and aversions for the ethanol-associated stimulus. The ANOVA indicates a significant effect of ethanol dosage [$F(4,45) = 8.137$, $P < .0001$]. Conditioning with small ethanol doses (0.25 and 0.5 g/kg) induced neither preference nor aversion for the olfactory

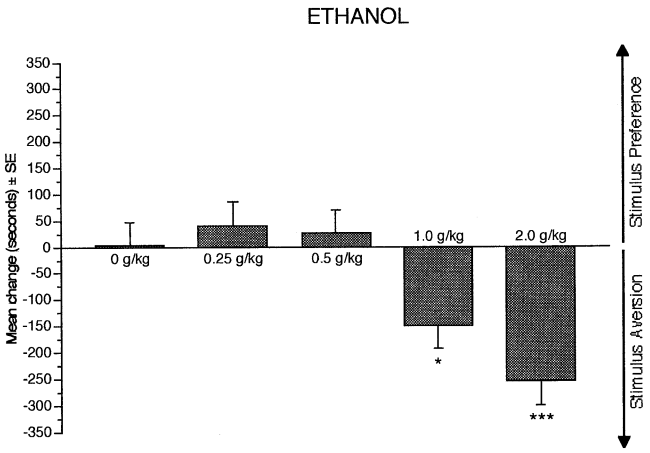

Fig. 1. Mean changes in seconds (±S.E.M.) for the time spent in the nonpreferred side after introduction of the olfactory stimulus, which was previously associated with either 0 (saline), 0.25, 0.5, 1.0 or 2.0 g/kg ethanol injections. Positive scores indicate a preference for the stimulus, while negative scores indicate a stimulus aversion. * $P<.05$ relative to the vehicle control group (0 g/kg ethanol). * * * $P<.001$ relative to the vehicle control group (0 g/kg ethanol).

cue (Fig. 1). In contrast, higher ethanol doses (1.0 and 2.0 g/kg) resulted in significant stimulus aversions as compared with the vehicle group.

The effects of conditioning with various acetaldehyde doses on preferences and aversions for the olfactory cue are shown in Fig. 2. The ANOVA yielded a significant effect of acetaldehyde dosage [$F(4,45)=5.917$, $P<.001$]. Acetaldehyde conditioning led to a biphasic inverted U-shaped effect on stimulus preference, with a maximal preference around 20 mg/kg acetaldehyde (Fig. 2). While 10 and 20 mg/kg acetaldehyde doses induced significant preferences for the olfactory cue, the results for 100 mg/kg acetaldehyde did not meet the $P<.05$ level for significant preference ($P=.061$). With the higher 150 mg/kg acetaldehyde doses, signs of adverse physiological reactions were apparent in rats after injection, although the results show no significant aversion for the acetaldehyde-associated stimulus. We also tried 200 mg/kg acetaldehyde, but this dosage was lethal for a significant number of animals, and the others showed severe sickness. Therefore, experiments with this acetaldehyde dosage were discontinued before the end of conditioning.

4. Discussion

Peripheral accumulation of acetaldehyde is generally assumed to be strongly aversive, such that its occurrence prevents ethanol consumption. It has also been suggested that acetaldehyde has a biphasic effect. At low blood concentrations, it might exert reinforcing effects, while further acetaldehyde accumulation, above a specific upper limit, would be predominantly aversive (Hunt, 1996). The present results contradict these two common assumptions. Peripheral intraperitoneal acetaldehyde injections were not only found primarily reinforcing, but no evidence of aversive effects were observed with increasing doses of acetaldehyde even with concentrations close to the lethal limit. In contrast, intraperitoneal ethanol injections mainly induced aversive effects with doses above 1.0 g/kg, while

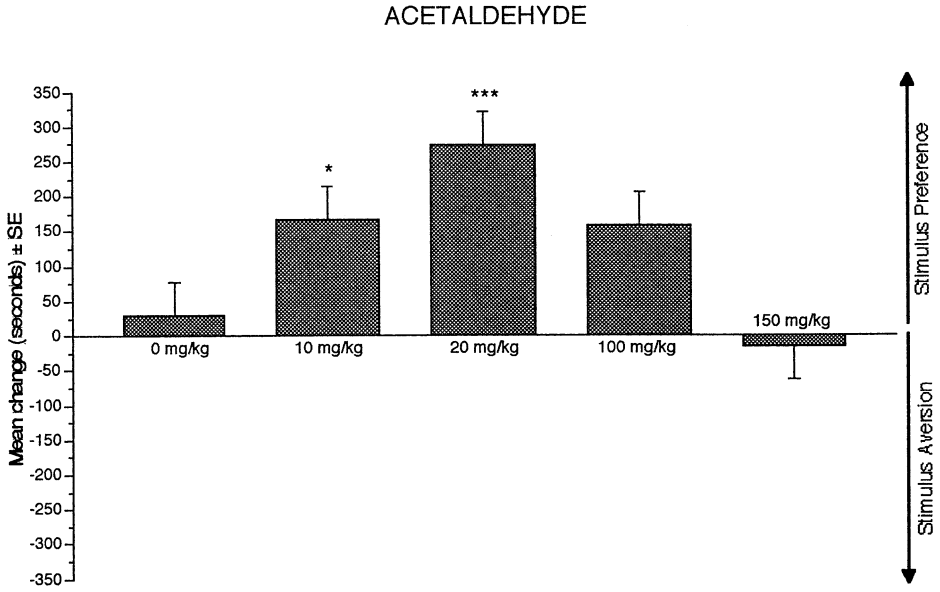

Fig. 2. Mean changes in seconds (±S.E.M.) for the time spent in the nonpreferred side after introduction of the olfactory stimulus, which was previously associated with either 0 (saline), 10, 20, 100 or 150 mg/kg acetaldehyde injections. Positive scores indicate a preference for the stimulus, while negative scores indicate a stimulus aversion. * $P<.05$ relative to the vehicle control group (0 mg/kg acetaldehyde). * * * $P<.001$ relative to the vehicle control group (0 mg/kg acetaldehyde).

no reinforcing effects were observed for lower 0.25 and 0.5 g/kg ethanol doses. These latter results are in agreement with the majority of place conditioning studies, which generally failed to demonstrate the reinforcing effects of intraperitoneal ethanol injections, but, instead, consistently observed place aversions for ethanol doses of 1.0 g/kg and higher (Asin et al., 1985; Cunningham, 1981; Van der Kooy et al., 1983). Only chronic exposure to ethanol over long period of time prior to conditioning with low ethanol doses elicited consistent ethanol-induced place preferences (Reid et al., 1985). It is generally argued that such a chronic exposure is required for the development of tolerance to the initial aversive effects of ethanol in naive animals. The present results further suggest that these initial aversive effects of ethanol may mask the reinforcing action of its first metabolite acetaldehyde, although such a hypothesis clearly requires further investigation. There is also ample evidence that after chronic alcohol consumption, ethanol intake results in higher blood acetaldehyde concentrations (Nuutinen et al., 1983; Pikkarainen et al., 1981). Therefore, an alternative explanation would be that chronic ethanol exposure increases the reinforcing action of acetaldehyde.

Although evidence of the reinforcing properties of acetaldehyde has accumulated during the last decades (see review in Smith et al., 1997), its implication in the reinforcing effects of ethanol remains highly controversial. The most problematic question regarding the role of acetaldehyde is its concentration within the brain after ethanol intake. It is generally argued that acetaldehyde cannot be present in the brain in pharmacologically relevant concentrations, such that it could not play a significant role in mediating some of the central effects of ethanol. Indeed, peripherally produced acetaldehyde hardly penetrates into the brain due to the metabolic barrier, which is created by the presence of ALDH in the microvasculature of the brain (Eriksson and Sippel, 1977). Therefore, very high blood levels of acetaldehyde (>250 μM) are required before it can be detected in brain tissues (Sippel, 1974). Such blood concentrations of acetaldehyde are virtually never obtained with ethanol intake, except after the pharmacological inhibition of ALDH, the enzyme responsible for the degradation of acetaldehyde (Eriksson and Fukunaga, 1993). However, more recent studies have challenged these previous results and found significant brain acetaldehyde concentrations after the intraperitoneal administration of acetaldehyde that produced largely smaller blood acetaldehyde concentrations (Heap et al., 1995; Ward et al., 1997). The intraperitoneal injection of acetaldehyde doses in the same range as those used in the present studies led to detectable amounts of acetaldehyde within the brain 10 min after the injection (Ward et al., 1997). It is therefore likely that acetaldehyde is able to partially cross the blood–brain barrier, such that the present results of acetaldehyde-conditioned stimulus preference most likely resulted from central effects of acetaldehyde. In addition to peripheral acetaldehyde, which possibly reaches the brain, several studies demonstrated that pharmacologically significant amounts of acetaldehyde are produced directly within the brain mainly via ethanol oxidation by the enzyme catalase (Gill et al., 1992). Consequently, brain acetaldehyde probably reaches local significant concentrations after alcohol intake, such that the reinforcing effects of exogenously applied acetaldehyde would be relevant for understanding the in vivo effects of ethanol.

The most surprising result of the present studies is the failure to obtain stimulus aversion with the higher doses of acetaldehyde. This is in apparent contradiction with studies showing that peripheral acetaldehyde accumulation induced ethanol aversion (Eriksson and Deitrich, 1980; Higuchi et al., 1992; Sinclair et al., 1980) and conditioned taste aversion (Brown et al., 1978). However, ingestive behaviors seem to be predisposed to aversive conditioning. There is numerous studies in which drugs induced both conditioned place preferences and conditioned taste aversions at the same doses (e.g., Turenne et al., 1996). Even drugs with strong abuse potentials, such as cocaine and morphine, induce conditioned taste aversion (Grigson, 1997). Although the present results illustrate the reinforcing properties of acetaldehyde, it may be effective in reducing ethanol consumption at high concentrations. This can be related to some unexpected effects of disulfiram and calcium carbimide treatments in alcoholics. These two ALDH inhibitors induce a dramatic increase in blood acetaldehyde concentration after ethanol consumption, which leads to an adverse toxic reaction. However, there has been reports of patients who actually enjoyed taking low doses of alcohol when under disulfiram (Chevens, 1953). Furthermore, both disulfiram and calcium carbimide have been shown to potentiate the euphoric and pleasurable effects of low doses of alcohol (Brown et al., 1983; Peachey et al., 1980).

In conclusion, the present study clearly shows that systemic acetaldehyde injections induced significant stimulus preferences. This suggests that acetaldehyde may be, at least in part, responsible for the reinforcing effects of alcohol intake. However, several questions regarding the role of acetaldehyde in ethanol consumption remain unresolved and clearly require further studies. For example, it is unknown whether chronic alcohol consumption alters acetaldehyde reinforcing effects. Furthermore, the molecular mechanisms underlying acetaldehyde reinforcing effects remain largely undefined, although an interaction with brain catecholamines has been suggested (Heap et al., 1995; Ward et al., 1997).

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