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**Catalase Inhibition Attenuates the Acquisition and Maintenance of Ethanol
and Saccharin-Quinine Consumption in Laboratory Rats**

Susan Rotzinger

A Thesis

in

The Department

of

Psychology

**Presented in Partial Fulfilment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montreal, Quebec, Canada**

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ABSTRACT

Catalase Inhibition Attenuates the Acquisition and Maintenance of Ethanol and Saccharin-Quinine Consumption in Laboratory Rats

Susan Rotzinger

The central production and elimination of acetaldehyde has long been thought to mediate many of the behavioral and rewarding effects of ethanol. The enzyme catalase is thought to play an important role in the central production of acetaldehyde, and hence in many of ethanol's effects. The objective of the present work was to examine the role of catalase in the acquisition and maintenance of voluntary ethanol consumption in rats using the catalase inhibitor 3-amino-1,2,4-triazole (AT). In the first experiment, AT (0.5 g/kg) significantly attenuated consumption of novel ethanol and saccharin-quinine (SQ) solutions throughout an acquisition period and a subsequent maintenance period during which no injections were administered. This finding suggested that AT had a non-specific suppressive effect on the consumption of a novel flavored fluid. In a second experiment, the consumption of familiar ethanol or SQ solutions was examined following two doses of AT. Both AT doses decreased ethanol consumption, supporting the notion that catalase activity is involved in the modulation of ethanol intake. However, the higher dose of AT also decreased SQ consumption, suggesting that some other mechanism may have been involved in the decreased consumption. Therefore, the possibility that AT induced a conditioned taste aversion (CTA) was assessed in a final experiment. The results suggested that a CTA was not likely the sole cause of the decreased consumption, and suggested the need to further examine the effects of AT on the consumption of flavored fluids.

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INTRODUCTION

Determining the biological factors that control ethanol intake is an important problem on an applied level in terms of the obvious health and social benefits, but is also important on a more basic level in terms of providing insight into the neurobiological mechanisms which regulate ethanol intake. Arguments have been presented to suggest that the basis of ethanol consumption and addiction is its reinforcing properties, and is not a result of physical dependence, stress reduction, or nutritional deficiencies (Amit & Sutherland, 1987; Amit, Sutherland, & White, 1976). However, the biochemical substrate of this reinforcement has not been adequately identified. Several neurotransmitter systems have been proposed to play a role in ethanol's reinforcing and behavioral effects; however, regardless of which transmitter systems are ultimately found to mediate ethanol consumption, the critical question which remains to be answered is how ethanol or its metabolites initiate these changes. The first step in any of ethanol's effects is likely to be the direct action of either ethanol or one of its metabolites.

Acetaldehyde is the first metabolic product of ethanol metabolism, and it has been implicated in many of ethanol's toxic (Raskin, 1975; Raskin & Sokoloff, 1972), behavioral, and rewarding properties (Amir, Brown & Amit, 1980; Amit & Smith, 1989). The theoretical basis of the present thesis is that the rate of central acetaldehyde formation and degradation is critical in the mediation of ethanol's effects, and that it is the enzyme catalase which is critical in central acetaldehyde formation (e.g. Aragon & Amit, 1985). The present work will be concerned with demonstrating the importance of catalase activity in mediating voluntary ethanol consumption in rats.

The first part of this introduction will provide a brief background into the metabolism of ethanol, and some of the studies implicating differences in ethanol metabolism as underlying differences in response to, and consumption of, ethanol. Human studies of differences in ethanol and acetaldehyde metabolism will be briefly examined to illustrate that although acetaldehyde has well known aversive properties, it also has potentially reinforcing properties. Next, a detailed examination of experimental studies of the reinforcing and behavioral effects of acetaldehyde will be presented. Finally, the putative role of the enzyme catalase in central acetaldehyde formation, and the behavioral significance of this presumed pathway, will be examined in detail, and will conclude with the rationale and purpose of the present work.

Enzymes of Peripheral Ethanol Metabolism

Ethanol is thought to be oxidized to acetaldehyde in the liver primarily by alcohol dehydrogenase (ADH) (Koop, 1989). There are also several minor pathways in the liver through which ethanol oxidation takes place. Two of the more important of these minor pathways are the cytochrome P450 system, and the hydrogen peroxide (H₂O₂) dependent catalase pathway (Koop, 1989). Following its formation, acetaldehyde is then rapidly oxidized to acetate by the NAD⁺-dependent aldehyde dehydrogenase (ALDH) (Agarwal & Goedde, 1989). Several forms of human ALDH exist, the two major ones being the cytoplasmic ALDH1, and the mitochondrial high affinity (low K_m) ALDH2 (Agarwal & Goedde, 1989).

Human Studies

Studies of the drinking patterns of certain racial and ethnic groups suggest that biological factors controlling acetaldehyde production and elimination may play an important role in the regulation of alcohol intake. A

large percentage of Orientals experience the alcohol sensitivity symptoms of facial flushing and increased heart rate in response to alcohol consumption (Agarwal & Goedde, 1989). This effect is associated with the absence of the low K_m ALDH isozyme in these individuals (vonWartburg & Buhler, 1984; Agarwal & Goedde, 1989), and is attributed to an increase in blood acetaldehyde levels (Mizoi et al., 1979). These higher blood acetaldehyde levels, and the concomitant aversive affects, are thought to be responsible for the lower alcohol consumption seen in many segments of the Oriental population (Higuchi et al., 1992; Thomasson, Crabb, Edenberg, & Li, 1993)

In a somewhat related vein, a drug therapy for alcoholism was initiated based on the finding that peripheral acetaldehyde accumulation has aversive effects (Hald & Jacobsen, 1948). Inhibitors of ALDH were administered so that when alcohol was consumed, the metabolism of acetaldehyde would be inhibited, leading to illness which would curtail further drinking (Peachey, 1989). However, it was soon discovered that at low levels, acetaldehyde could produce euphoric effects, and could actually enhance ethanol's effects. Non-alcoholic human volunteers given the ALDH inhibitors disulfiram or calcium carbimide, and small quantities of ethanol, reported feeling less sober, happier, more clear thinking, alert, relaxed, and more intoxicated and euphoric than controls (Brown, Amit, Smith, Sutherland, & Selvaggi, 1983). Other data also suggested that the reaction to calcium carbimide or disulfiram and ethanol is highly variable between and within individuals, and may actually have positive subjective effects in some (Peachey, 1989). These findings support the notion that acetaldehyde may have positively reinforcing properties in humans.

Animal Studies

The production and elimination of acetaldehyde has been studied in animals with respect to its role in regulating ethanol intake. Blocking the production of acetaldehyde with ADH inhibitors decreased voluntary ethanol consumption in rats (Carr, Brown, Rockman, & Amit, 1980), which suggested that acetaldehyde production may be important in mediating some aspects of alcohol consumption.

Rapid elimination of acetaldehyde also appears to be involved in the mediation of alcohol consumption, since a positive correlation between ALDH activity and ethanol consumption has been established (Amir, 1977; Sinclair & Lindros, 1981; Socaransky, Aragon, & Amit, 1985; Socaransky, Aragon, Amit, & Blander, 1984). Furthermore, a pharmacological manipulation which decreased the activity of ALDH (cyanamide) also decreased voluntary ethanol consumption in rats in a 24 hour free access paradigm (Sinclair & Lindros, 1981; Aragon, Spivak, Smith & Amit, 1993). An important extension of this finding was that when cyanamide was co-administered with 4-methylpyrazole (an ADH inhibitor), to prevent peripheral acetaldehyde accumulation, the suppression of drinking was not removed (Sinclair & Lindros, 1981). This suggested that the effects of ALDH inhibition on ethanol consumption were centrally mediated, and were not due to the aversive effects of peripheral acetaldehyde accumulation.

The enzymes of ethanol and acetaldehyde metabolism appear to play a significant role also in the behavioral effects of ethanol. Inhibition of brain ALDH attenuated ethanol-induced locomotor behavior (Spivak, Aragon, & Amit, 1987b), which suggested that the rapid elimination of central acetaldehyde was important in the expression of this effect.

When ethanol is administered following the consumption of a novel flavored saccharin solution, it will cause a decrease in the consumption of that fluid on subsequent presentations, an effect known as a conditioned taste aversion (CTA) (Kulkosky, Sickel & Riley, 1980; Lester, Nachman & LeMagnen, 1970). All self-administered drugs are also capable of producing a CTA, which is thought to reflect an aversion to the novelty of the psychopharmacological effects of the drug (Hunt & Amit, 1987; Sklar & Amit, 1977). Therefore, it can be inferred that a manipulation which attenuates the CTA to ethanol may have attenuated ethanol's psychopharmacological effects. This logic was applied in a study of the effects of ALDH inhibition on an ethanol-induced CTA (Spivak, Aragon & Amit, 1987a). Low ethanol doses (0.4 g/kg), which alone were too small to produce a CTA, were capable of producing a CTA when administered in combination with the ALDH inhibitor cyanamide. This result may have been due to an enhancement of acetaldehyde's central effects via ALDH inhibition, similar to the effect seen in humans when low quantities of ethanol were consumed following the administration of ALDH inhibitors (Brown et al., 1983). However, at the highest ethanol doses tested (1.2g/kg), cyanamide decreased an ethanol CTA. At first, this result seemed to suggest that ALDH inhibition attenuated some of the psychopharmacological properties of ethanol, which resulted in the attenuated CTA. This interpretation would be consistent with the findings that ALDH inhibition decreased unrestricted voluntary ethanol consumption (Sinclair & Lindros, 1981), and that an increase in ALDH activity was associated with an increase in ethanol drinking (Amir, 1977; Sinclair & Lindros, 1981; Socaransky et al., 1984;1985). However, the CTA results must be viewed with caution in light of recent evidence that cyanamide may have a general effect of increasing fluid consumption in a restricted access paradigm

(Aragon et al., 1993; Spivak & Amit, 1987). Thus, the attenuation in the CTA to the high dose of ethanol by cyanamide may have been secondary to cyanamide's effect of increasing fluid consumption in general.

Reinforcing Properties of Acetaldehyde

Although the studies cited above indirectly implicated acetaldehyde in ethanol reinforcement, more direct evidence of acetaldehyde's reinforcing properties has also been obtained. Centrally administered acetaldehyde produced a conditioned place preference in rats (Smith, Amit, & Splawinsky, 1984), an effect taken as evidence of reinforcement (Mucha, van der Kooy, O'Shaughnessy & Bucenicks, 1982). Rats also performed an operant response to receive intracerebroventricular (Brown, Amit, & Rockman, 1979) or intravenous (Myers, Ng, & Singer, 1982) infusions of acetaldehyde. Furthermore, the rate at which rats self-administered acetaldehyde centrally was correlated with subsequent levels of voluntary ethanol intake (Brown, Amit & Smith, 1980). This finding provided evidence not only that central acetaldehyde could support reinforcement, but also that ethanol intake was related to acetaldehyde intake, which implied a possible common regulatory mechanism.

Further support that ethanol and acetaldehyde have similar psychopharmacological properties came from a report that ethanol pre-exposure blocked a CTA to a low dose of acetaldehyde, and a low dose of acetaldehyde blocked an ethanol-induced CTA (Aragon, Abitbol, & Amit, 1986). Since pre-exposure to a conditioning agent reduces the subsequent ability of that agent to induce a CTA, presumably through latent inhibition (Logue, 1979), pre-exposure disruption of a CTA can be taken as evidence of a pharmacological similarity between the drug given during pre-exposure and the drug used in CTA conditioning (Aragon et al., 1986).

Thus, several lines of evidence indicate that acetaldehyde has reinforcing properties, and that the rate of acetaldehyde formation and degradation may be important in mediating these reinforcing effects. However, the mechanism by which acetaldehyde is formed, and subsequently mediates these effects, is not currently known.

Catalase

Catalase-mediated acetaldehyde formation

Although considerable evidence supported the role of acetaldehyde in many of ethanol's effects, it was still unclear how acetaldehyde mediated these effects in the brain, since acetaldehyde was not readily detectable in the brain during ethanol intoxication (Eriksson & Sippel, 1977; Sippel, 1974; Westcott, Weiner, Shultz, & Myers, 1980). Acetaldehyde was unlikely to be carried by the blood to the brain in appreciable quantities, since erythrocytes rapidly oxidize acetaldehyde, and since during normal ethanol intake blood acetaldehyde levels are very low for this reason (Lindros, 1989). Furthermore, blood acetaldehyde levels do not reflect those found in the brain (Tabakoff, Anderson, & Ritzman, 1976), and appear to reflect artefactual non-enzymatic acetaldehyde production (Eriksson, 1980; Eriksson & Fukunaga, 1993). However, acetaldehyde could still be postulated to mediate ethanol's behavioral and rewarding effects if a mechanism for the production of acetaldehyde in the brain itself was identified.

The first demonstration of ethanol oxidation by rat brain in vivo involved the action of the enzyme catalase in conjunction with endogenous H_2O_2 (Cohen, Sinet & Heikkila, 1980). The presence of catalase in brain had already been demonstrated by biochemical (Gaunt & DeDuve, 1976), and histochemical (McKenna, Arnold & Holtzman, 1976) techniques. As well, evidence for H_2O_2 production by rat brain in vivo had also been obtained

(Sinet, Heikkila & Cohen, 1980). Thus, all that remained was to demonstrate that ethanol served as a substrate for catalase activity *in vivo*.

Catalase reacts with H₂O₂ to form a compound known as compound I (Cohen et al., 1980) (see Table 1, reaction 1). Under normal physiological conditions, catalase decomposes H₂O₂ via the formation of the catalase-H₂O₂ compound I, which is followed by a reaction between compound I and another molecule of H₂O₂ to form oxygen, water, and free catalase (see Table 1, reaction 2). Compound I can also react with the drug 3-amino-1,2,4-triazole (AT) to irreversibly inhibit catalase activity (Margoliash, Novogrodsky, & Schejter, 1960) (see Table 1, reaction 3). Catalase also reacts with ethanol to produce acetaldehyde and free catalase (Keilin & Nicholls, 1958) (Table 1, reaction 4). The reactions of ethanol and AT with compound I are competitive (Table 1, reactions 3 and 4).

Table 1
Reactions of catalase and compound I

1. $\text{catalase} + \text{H}_2\text{O}_2 \rightarrow [\text{catalase-H}_2\text{O}_2]$
 compound I
 2. $\text{compound I} + \text{H}_2\text{O}_2 \rightarrow \text{catalase} + \text{O}_2 + 2\text{H}_2\text{O}$
 3. $\text{compound I} + \text{AT} \rightarrow \text{irreversibly inhibited}$
 catalase
 4. $\text{compound I} + \text{ethanol} \rightarrow \text{catalase} +$
 acetaldehyde
-

Cohen et al. (1980) reasoned that if ethanol prevents the inhibition of catalase by AT, then it can be inferred that ethanol has served as a substrate for catalase oxidation. In a study examining this question, rats were injected with ethanol followed 30 minutes later by AT. Inhibition of catalase by AT

was completely blocked by ethanol, whereas ethanol by itself had no effect on brain catalase activity. This finding represented the first demonstration of ethanol oxidation by rat brain *in vivo* (Cohen et al., 1980). This finding was extended in a subsequent study which showed that ethanol protects catalase from inhibition by cyanamide and 4-hydroxypyrazole dose dependently *in vitro* and *in vivo*, again providing evidence that ethanol is a substrate for catalase (Aragon, Stotland, & Amit, 1991). Further evidence of ethanol oxidation by the catalase pathway came from studies of acetaldehyde production *in vitro* by rat brain homogenates incubated with ethanol. The production of acetaldehyde was increased by the addition to the incubation medium of glucose oxidase, a known hydrogen peroxide generator (Aragon, Rogan, & Amit, 1992; Gill, Menez, Lucas, & Deitrich, 1992; Tampier, Quantanilla, Letelier & Mardones, 1988). However, the same studies showed that acetaldehyde production was unaffected by metyrapone, an inhibitor of cytochrome P450, or by pyrazole, an alcohol dehydrogenase inhibitor. These findings demonstrated that the cytochrome P450 pathway and the ADH pathways were not playing a significant role in acetaldehyde production.

Further evidence for the role of catalase in acetaldehyde production *in vivo* came from studies of normal and acatalasemic mice. The C3H-N and C3H-A mouse strains are similar in every respect except that the C3H-A strain is genetically acatalasemic (Feinstein, Howard, Braun, & Seaholm, 1966; Feinstein, Seaholm, Howard, & Russell, 1964). Mean brain catalase activity in the C3H-A mice is approximately half of that in the normal C3H-N mice (Aragon & Amit, 1993). When normal C3H-N brain homogenates were incubated with increasing concentrations of ethanol, there was a dose-dependent increase in acetaldehyde production (Aragon & Amit, 1993). However, when acatalasemic C3H-A brains were incubated with increasing

ethanol concentrations, there was a negligible increase in acetaldehyde production. Moreover, the amount of acetaldehyde recovered following ethanol incubation with C3H-A brains was significantly less than was recovered following incubation with the normal C3H-N brains (Aragon & Amit, 1993). Thus, this study demonstrated a difference in acetaldehyde production from brain homogenates differing only in their amount of catalase activity, and provided further support for the importance of the catalase pathway in acetaldehyde production.

Other evidence supporting the importance of catalase activity in ethanol metabolism comes from studies of alcohol dehydrogenase deficient (ADH⁻) deer mice. The ADH inhibitor 4-methylpyrazole (4-MP) significantly decreased ethanol metabolism in ADH⁻ mice, suggesting that 4-MP must have affected ethanol metabolism by a pathway other than the ADH pathway (Bradford, Forman, and Thurman, 1993). Since 4-MP also inhibits the availability of H₂O₂, this study suggested that the catalase pathway may play a larger role in ethanol metabolism than was previously thought. It has been estimated that the catalase pathway accounts for approximately 75% of ethanol metabolism in ADH⁻ mice, and approximately 50% in ADH⁺ mice (Bradford, Seed, Handler, Forman & Thurman, 1993).

Catalase and the behavioral effects of ethanol consumption

Although catalase had been demonstrated to oxidize ethanol in vivo in rat brain (Cohen et al., 1980), the neurobehavioral significance of this finding still remained to be demonstrated. Therefore, the drug 3-amino-1,2,4-triazole (AT), which has been shown to attenuate catalase activity dose and time dependently in vivo (Aragon, Rogan & Amit, 1991), was used in a number of studies on ethanol-induced behavioral effects in an attempt to demonstrate

the importance of catalase-mediated acetaldehyde production in the expression of these behaviors.

AT pretreatment was found to attenuate locomotor depression induced by a dose of 2.0 g/kg of ethanol in rats (Aragon, Spivak, & Amit, 1989). AT alone had no effect on locomotor behavior, which suggested that it was the interaction of AT and ethanol which was critical in the mediation of the attenuated locomotor depression. Also, there were no differences in blood ethanol levels between the AT and saline-treated animals, which argued against the possibility that the differences in locomotor behavior were due to differences in overall rate of ethanol metabolism and availability.

In mice, low doses of ethanol induce locomotor excitation, whereas higher ethanol doses induce locomotor depression (Frye & Breese, 1981). These effects of ethanol were found to be attenuated in genetically acatalasemic mice (Aragon, Pesold & Amit, 1992), and also attenuated in both normal and acatalasemic mice following AT administration (Aragon & Amit, 1993). These findings are consistent with the hypothesis that catalase-mediated acetaldehyde production is important in the expression of ethanol-induced locomotion. Cocaine-induced locomotion was not affected by AT in normal or acatalasemic mice, ascribing the specificity of AT's effect to an interaction with ethanol (Aragon & Amit, 1993). Blood ethanol levels did not differ between AT treated animals and controls in these studies, suggesting that the acatalasemic mice do not differ from normals in peripheral ethanol metabolism, and that the effects of AT on locomotion are likely to be centrally mediated.

Pre-treatment with AT has also been found to attenuate ethanol-induced narcosis and lethality (Aragon, Spivak, & Amit, 1991, Tampier et al., 1988). This effect was observed with several doses of ethanol (Tampier et al.,

1988), and AT (Aragon, Spivak & Amit, 1991). Studies with normal and acatalasemic mice have also revealed differences in the duration of ethanol-induced narcosis (Aragon & Amit, 1993). However, it was found that the acatalasemic mice had longer ethanol-induced sleep durations than those observed in normal mice. This at first seems contrary to expectations, since catalase depletions with AT attenuated sleep times in rats. However, when AT was administered to rats following ethanol injections, rather than preceding ethanol injections, an increase in narcosis time was also noted (Tampier et al., 1988). Therefore, the effects of catalase depletion via AT, and low catalase activity in genetically acatalasemic mice, may not be directly comparable in this instance. However, the important point is that there are differences observed in ethanol-induced narcosis and lethality as a function of catalase activity.

The C57BL/6 and DBA/2 mouse strains were also found to differ in catalase activity, with C57BL/6 mice having approximately 35% less catalase than the DBA/2 (Aragon & Amit, 1987). It is of interest that there are also differences in response to ethanol between these two strains which closely reflect the differences seen between AT treated rats and control rats in response to ethanol (Aragon & Amit, 1987). For example, the C57BL/6 strain has less catalase activity, and showed an attenuated ethanol-induced CTA to saccharin, similar to the effect seen in AT-treated rats (Aragon & Amit, 1987). The C57BL/6 strain also differed from the DBA/2 strain in ethanol-induced changes in rectal temperature, regaining of the righting reflex, and locomotor activity, all in the same direction as seen in rats administered AT (Aragon & Amit, 1987).

The psychopharmacological significance of central acetaldehyde production via the catalase pathway was investigated using an ethanol-

induced CTA paradigm (Aragon, Spivak, & Amit, 1985). Pre-treatment with AT, and thus a decrease in catalase activity, blocked an ethanol-induced CTA to a saccharin solution, suggesting that catalase-mediated acetaldehyde formation may play a role in some of the psychopharmacological effects of ethanol. AT pretreatment did not affect a lithium chloride or morphine induced CTA, and did not induce a CTA in control animals, demonstrating the specificity of AT to ethanol-induced effects. The effect of AT was also not likely to be due to peripheral effects on ethanol metabolism, since blood ethanol levels did not differ between AT treated and control animals (Aragon, Spivak & Amit, 1985).

Catalase and Ethanol Consumption

In addition to the interaction of catalase and ethanol-induced behaviors, brain catalase activity has also been implicated in voluntary ethanol consumption in mice and rats. Normal (C3H-N) and acatalasemic (C3H-A) mice differ from each other in their patterns of ethanol intake (Aragon & Amit, 1993). Acatalasemic mice drink more ethanol than normals at concentrations of 13-18% ethanol (v/v), and drink equivalent volumes at concentrations of 2-12% and 19-22%. Similarly, the C57BL/6 mice have lower catalase activity than the DBA/2 mice (Aragon & Amit, 1987), yet are known to be a high alcohol consuming strain (Whitney & Horowitz, 1978). It is not presently clear why lower catalase activity is associated with higher ethanol consumption in mice, whereas the inverse is true for rats (see below). However, one obvious possibility is that there are species differences in sensitivity to ethanol, an assertion which is supported by the well known differences between rats and mice in ethanol induced locomotion (Frye & Breese, 1980).

Studies of catalase activity in rats have indicated a positive correlation between brain and blood catalase activity and ethanol intake. Brain catalase activity in rats was analyzed after a period of 25 days of voluntary ethanol consumption, and significant positive correlations between ethanol intake and catalase activity in individual rats were obtained (Aragon, Sternklar, & Amit, 1985). This effect was not likely to have been due to the immediate effects of ethanol consumption on brain catalase activity because the correlations between catalase activity and ethanol consumption were significant whether the rats were sacrificed immediately, or 15 days following ethanol withdrawal. The possibility that catalase activity was induced by ethanol consumption, and therefore accounted for the correlations, was further tested by comparing catalase activity in rats consuming only water with catalase activity in rats consuming only ethanol (Aragon, Sternklar & Amit, 1985). Mean brain catalase activity did not differ between the groups, indicating again that catalase activity was not induced by ethanol consumption. The possibility that catalase activity may be an inherent predisposing factor for ethanol consumption was still further tested in a subsequent study in which blood catalase activity was measured in rats naive to alcohol (Amit & Aragon, 1988). These rats were then given access to ethanol in free choice with water for 30 days, following which they were sacrificed and the brains were assayed for catalase activity. Blood catalase activity measured before ethanol consumption was significantly correlated with stable levels of ethanol intake, indicating that catalase activity was an inherent predisposing factor. Furthermore, blood catalase activity was significantly correlated with brain catalase activity, indicating that blood catalase is a reliable and valid indicator of brain catalase activity.

The possibility that catalase activity represents a biological marker of the propensity to consume alcohol in humans was tested in a recent study in which subjects were asked to complete a number of questionnaires on their typical alcohol and drug use, and to provide a blood sample from which catalase activity could be determined (Koechling & Amit, 1992). Blood catalase activity was significantly correlated with typical levels of self-reported alcohol consumption. When drug users were excluded from the analysis so that only alcohol consumption was reflected, the correlation was further improved. In fact, of all the predictor variables included in the analysis, catalase activity explained the largest portion of the variance in alcohol consumption. This study provided evidence that catalase activity represents a possible biological marker system of alcohol intake.

Although many correlations between catalase activity and ethanol consumption had been demonstrated, it was important to try to demonstrate a causal relationship in which manipulations of catalase activity resulted in changes in ethanol intake. Recent findings indicated that catalase inhibition via AT administration resulted in a dose dependent decrease in an established pattern of alcohol consumption in rats (Aragon & Amit, 1992), and mice (Koechling & Amit, 1993).

The main objective of the present work was to further examine the effects of catalase inhibition on voluntary ethanol consumption in rats. In addition to studying the effects of catalase inhibition on stable ethanol intake, the effects of catalase inhibition on the acquisition of ethanol intake were also studied. Presumably, the acquisition period is when rats learn about the reinforcing properties of ethanol consumption, and associate these effects with the taste and smell of alcohol. If catalase-mediated acetaldehyde

production is important in this process, then attenuating catalase activity should attenuate the acquisition of ethanol consumption.

A second objective of the present work was to assess the specificity of AT's effect on ethanol consumption. Several pharmacological manipulations which have been shown to attenuate ethanol consumption have later been discovered to attenuate the consumption of other flavored fluids, reflecting a non-specific effect of drug treatment on alcohol consumption. Two examples are the dopamine-B-hydroxylase inhibitor FLA-57 (Amit, Gill & Ng Cheong Ton, 1991; Sirota & Boland, 1987), and the serotonin reuptake inhibitor zimeldine (Gill, Amit & Ogren, 1985; Gill, Shatz, Amit & Ogren, 1986).

Therefore, the purpose of the present thesis was to further examine the role of catalase-mediated acetaldehyde formation in the acquisition and maintenance of voluntary consumption of ethanol, and to assess the specificity of the catalase inhibitor AT on ethanol consumption.

Experiment 1A

The purpose of this experiment was to further explore the relationship between catalase activity and voluntary ethanol consumption in rats by examining the effect of AT on the acquisition of ethanol consumption. It has been suggested that the acquisition of ethanol consumption may be affected by factors other than those involved in the maintenance of ethanol consumption (Ng Cheong Ton, Brown, Michalakeas & Amit, 1983). Although it has been shown that AT attenuated maintained ethanol consumption (Aragon & Amit, 1992), implying a role for catalase in supporting maintained ethanol consumption, it was nevertheless not known if catalase was important in the acquisition of stable ethanol consumption.

It is likely that during the acquisition of high, stable levels of voluntary ethanol intake, both taste and post-absorptive factors play a role. By comparing ethanol intake acquisition with the intake acquisition of a saccharin-quinine solution, it might be possible to separate the effects of drug treatment on the consumption of a novel flavor from the pharmacological effects associated with ethanol consumption. If catalase-mediated central acetaldehyde production is important for learning about ethanol's reinforcing, postabsorptive effects, then blocking acetaldehyde production with AT should decrease ethanol intake relative to controls. However, if AT administration in some way interferes with the propensity to consume novel flavors, then drinking in both the ethanol and saccharin-quinine conditions should be decreased below control levels.

METHOD

Subjects

The subjects were 21 naive, male Long-Evans rats weighing 200-240g at the start of the experiment. They were housed individually in stainless steel cages in a humidity and temperature controlled animal colony maintained on a 12 hour light/dark cycle (lights on at 0800). Rat chow was available ad lib, and fluids were presented in two glass Richter tubes mounted on the front of the cage.

Drugs

3-amino-1,2,4-triazole (Sigma Chemical Co.) was prepared fresh daily in a concentration of 1g/2 ml of vehicle by heating and mixing with 0.9% saline.

Procedure

Following one week of acclimatization to the colony, the animals were presented with an ethanol acquisition schedule in which ethanol was given in a free choice with water on alternate days. Only water was available on intervening days. The first presentation was of a 2% (v/v) ethanol solution prepared by mixing a 95% stock solution with tap water. On each subsequent presentation, the concentration was increased by 1% to a final concentration of 10%. Once the 10% concentration was reached, one further water day was given, followed by a switch to 10 days of everyday presentation of 10% ethanol in a free choice with water. Following this maintenance period, only water was presented for a further five days, following which a second acquisition period was instituted.

Prior to the first acquisition day, the rats were randomly assigned to either drug or vehicle groups. One group (n=11) received 0.5 g/kg AT (ip),

and the other group (n=10) received equivalent volumes of saline (ip). Injections were given on ethanol presentation days five hours before the onset of the dark cycle, and were given only during the first acquisition period. No injections were given during the maintenance period or the second acquisition period.

Catalase Activity

Following the completion of the experiment (24 hours after the last ethanol presentation) the animals were anesthetized with metofane and perfused intracardially with heparinized saline. The brains were removed and 10% homogenates were prepared with 0.1% Triton X-100 in 10mM potassium phosphate buffer, pH 7.0. Catalase activity was measured using a Yellow Springs oxygen monitor equipped with a Clark style oxygen electrode (DeMaster, Redfern, Shirota, & Nagasawa, 1986). Protein was determined using the Lowry method (Lowry, Rosebrough, Far & Randall, 1951) and bovine serum albumin as the standard.

RESULTS AND DISCUSSION

First Acquisition Period

Data for mean ethanol consumption (g/kg) during the acquisition and maintenance periods are presented in Figure 1 (top panel). A two-way analysis of variance with repeated measures (drug x days) on the acquisition period yielded a significant effect of drug treatment ($F(1,19)=16.01, p<0.001$), reflecting the finding that the AT-treated rats drank significantly less ethanol than the control group. There was also a significant effect of ethanol concentration ($F(8,152)=3.68, p<0.001$), reflecting an increase in ethanol intake as the concentration increased. However, there was no interaction of drug group and ethanol concentration ($F(8,152)=1.986, p>0.05$).

There was also a significant effect of drug treatment ($F(1,19)=14.458$, $p<0.01$) and of ethanol concentration ($F(8,152)=7.183$, $p<0.001$) on ethanol preference (ethanol consumption in ml divided by total fluid consumption) (Figure 1, bottom panel).

There was no effect of drug treatment on total fluid consumption ($F(1,19)=0.639$, $p>0.05$), suggesting that the AT-treated rats compensated for their decreased ethanol intake by increasing their water intake. There was also no effect of AT on body weight ($F(1,19)=1.77$, $p>0.05$) during the acquisition period, which suggested that the effect of AT on ethanol consumption was not part of a larger general suppressive effect of AT on food and fluid intake.

Maintenance Period

A significant difference between the groups in ethanol intake (g/kg) remained during the 10 day maintenance period during which no injections were given ($F(1,19)=5.46$, $p<0.05$) (see Fig. 1, top panel). This effect was unlikely to have been due to continued catalase depletion because catalase levels have previously been reported to recover to approximately 80% of control levels within 48 hours following a dose of 0.5 g/kg AT (Aragon et al., 1991). Therefore, this finding suggested that the acquisition period was a critical time in the development of ethanol consumption without which the animals would not spontaneously drink significant amounts of 10% ethanol. This result is consistent with previous reports on the importance of either an acquisition period, or a period of prolonged ethanol exposure in the development of high levels of voluntary ethanol intake (Wise, 1973).

Ethanol intake (g/kg) changed significantly across days during the maintenance period ($F(9,171)=2.079$, $p<0.05$), but there was no significant interaction between days and drug group ($F(9,171)=2.324$, $p>0.05$) during this

time. The significant effect of days reflected the decrease in ethanol intake that occurred when the animals were switched from the alternate day ethanol presentations of the acquisition period to the everyday presentations of the maintenance period. This finding is consistent with many reports in the literature that animals consume more ethanol on an alternate day schedule than on an every day presentation schedule (Pinel, Mucha & Rovner, 1976; Wise, 1973).

A significant difference in ethanol preference ratios between the groups ($F(1,19)=4.92$, $p<0.05$) was also noted during the maintenance period (Figure 1, bottom panel). This finding was consistent with the g/kg intake results, which showed that drinking did not spontaneously recover in the AT-treated group following the termination of injections.

There were no differences between the groups in terms of total fluid intake ($F(1,19)=0.067$, $p>0.05$), or body weight ($F(1,19)=1.24$, $p>0.05$) during the maintenance period.

Second Acquisition Period

Ethanol intake (g/kg) during the second acquisition is presented in Figure 2 (top panel). Two-way ANOVA revealed no significant effect of former drug treatment on this measure ($F(1,19)=3.154$, $p>0.05$); however, there was a significant effect of ethanol concentration ($F(8,152)=20.739$, $p<0.001$), and a significant interaction between previous drug group and ethanol concentration ($F(8,152)=2.195$, $p<0.05$). A test of simple main effects revealed that the group previously administered AT consumed less ethanol than controls at concentrations of 6% ($F(1,30)=4.32$, $p<0.05$), 8% ($F(1,30)=7.93$, $p<0.01$), and 10% ($F(1,30)=4.17$, $p<0.05$). This result can be explained by observing that ethanol drinking in the control group increased slightly in the second acquisition period relative to the first acquisition period

levels, suggesting that previous alcohol consumption increased the second acquisition period drinking. Thus, since the AT-treated animals did not drink ethanol during the first acquisition period, it should be expected that their drinking would be lower than that of the saline-treated animals during the second acquisition period. However, since drinking in the AT group in the second acquisition was comparable to typical levels observed in untreated rats during their first acquisition, drinking can be said to have recovered in the AT-treated group.

There were no differences between the groups in terms of preference ratios ($F(1,19)=1.997, p>0.05$) during the second acquisition, which further supports the contention that drinking fully recovered when a second acquisition period was introduced.

Neither total fluid consumption ($F(1,19)=1.167, p>0.05$) nor body weight ($F(1,19)=1.331, p>0.05$) differed between the groups during the second acquisition.

Catalase Activity

There was no significant difference between the groups in mean catalase activity at the end of the experiment (mean catalase activity for saline group 0.582 (+/- 0.071) nM O₂/min/ug protein, AT group 0.581 (+/- 0.044), $t(19)=0.048, p>0.05$) demonstrating that there was no long term effect of AT treatment on catalase activity, and that the groups consisted of animals in the same range of catalase activity.

Overall, this experiment showed that animals treated with AT during the acquisition period failed to acquire levels of ethanol consumption as high as those of saline treated animals during the drug treatment period. The AT treated animals did not recover their drinking spontaneously following the termination of drug treatment, and required a second acquisition period to do

so. This result suggested that the acquisition period may be a critical time in the development of voluntary ethanol consumption, when the animal may learn about the association of taste factors with the post-absorptive, pharmacological effects of ethanol. However, the possibility remained that the decrease in ethanol consumption was due to an aversive association between the effects of drug treatment and the novel flavor of ethanol. Therefore, the next experiment was designed to address this possibility.

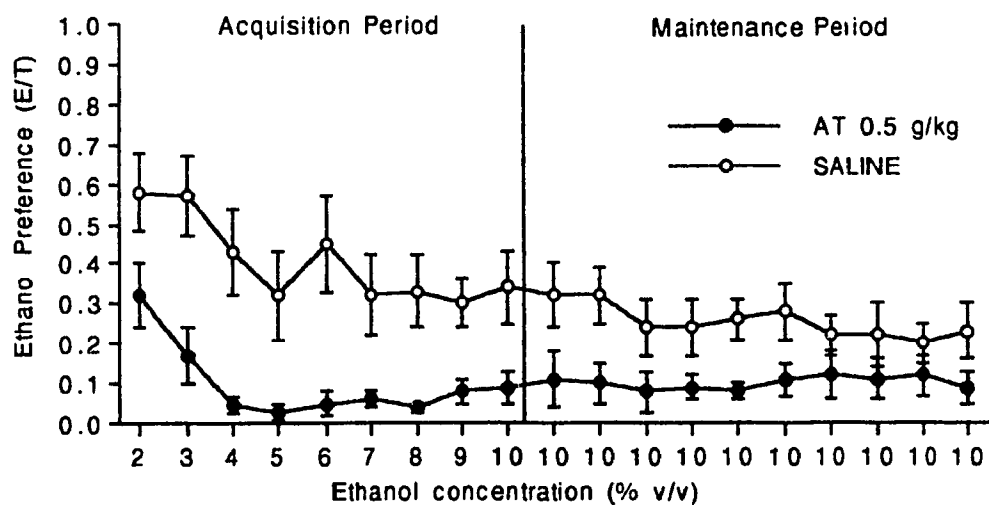
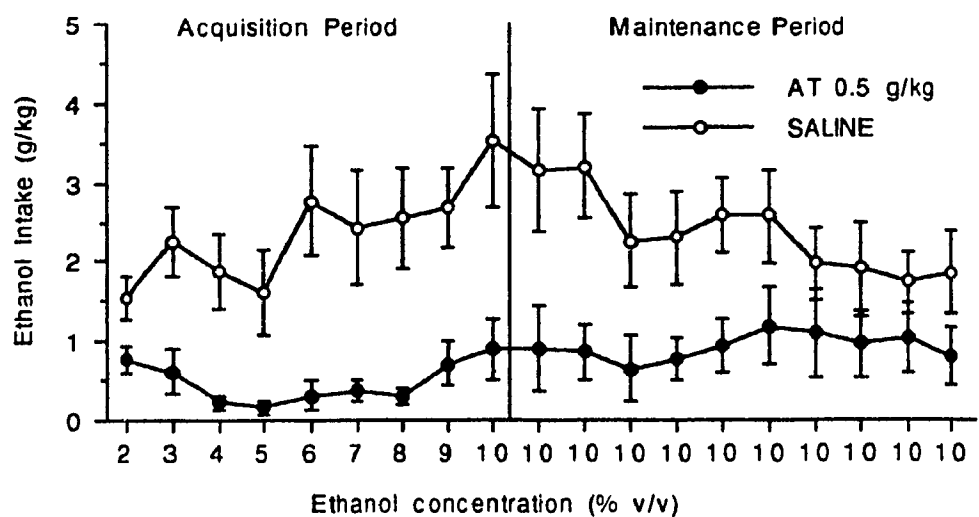


Figure 1. Mean (\pm SEM) ethanol intake in g/kg (top panel), and mean (\pm SEM) ethanol preference in the saline (open circles) and AT (0.5 g/kg) (filled circles) treated groups during the acquisition and maintenance periods.

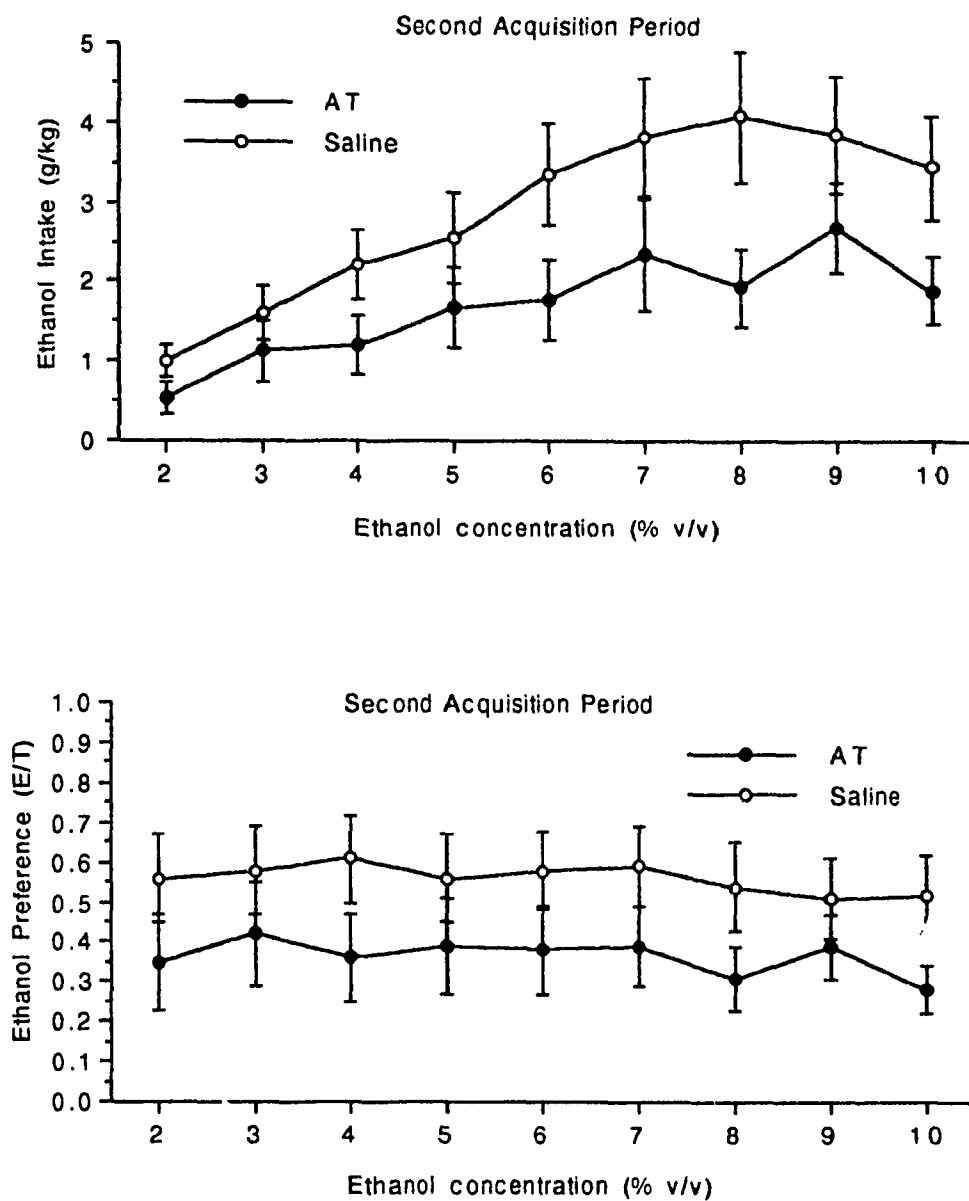


Figure 2. Mean (\pm SEM) ethanol intake in g/kg (top panel), and mean (\pm SEM) ethanol preference in the group previously treated with saline (open circles) and the group previously treated with AT (filled circles) during the second acquisition period.

Experiment 1B

Experiment 1A showed that AT administration attenuated ethanol consumption when administered during the acquisition period. However, it was important to determine whether this effect was specific to ethanol consumption. If the effect was specific, then it could be attributed to the AT-induced decrease in catalase activity, and the presumed subsequent decrease in acetaldehyde production. However, if the effect was non-specific, another explanation of the data would be required. Therefore, the purpose of the present experiment was to assess the specificity of the effects of AT on ethanol consumption by looking at its effects on the consumption of another novel flavored fluid. The same procedure was used as in Experiment 1A, except that a saccharin-quinine solution was used instead of ethanol. Saccharin-quinine solutions were prepared with increasing concentrations of quinine in an attempt to obtain similar preference ratios to those obtained with the increasing ethanol concentrations used in Experiment 1A.

METHOD

Subjects

The subjects were 17 naive, male Long-Evans rats, housed under the same conditions as in Experiment 1A.

Drugs

AT was used and prepared as in Experiment 1A.

Procedure

On day one of acquisition, the rats received 0.4% sodium saccharin solution in free choice with water. On subsequent presentations of the saccharin, quinine sulfate (Fisher Scientific) was added in increasing concentrations to bring consumption to levels comparable to that seen with ethanol consumption during acquisition. The concentrations of quinine sulfate used were 0.001% (acquisition day 2), 0.002% (day 3), 0.003% (day 4), 0.004% (day 5), 0.006% (day 6), 0.009% (day 7), 0.011% (day 8), 0.015% (day 8), 0.03% (day 9), 0.04% (day 10). Saccharin concentrations were held constant at 0.4% throughout. Saccharin-quinine (SQ) solutions were presented on alternate days, with only water available on the intervening days, as in the ethanol acquisition procedure. Injections of either 0.5 g/kg AT (n=8) or saline (n=9) were given five hours before the dark cycle as in Experiment 1A. Following the injection period, a ten day maintenance period was instituted during which no injections were given and the animals were maintained on a solution of 0.4% saccharin and 0.04% quinine presented every day in free choice with water.

RESULTS

Acquisition Period

Two-way ANOVA on saccharin consumption (ml) revealed a significant difference between the drug groups ($F(1,15)=45.146$, $p<0.001$), with the AT treated group consuming less SQ than the control group (Figure 3, top panel). There was a significant effect of days ($F(10,150)=19.062$, $p<0.001$), resulting from the decrease in consumption across days as the quinine concentration was increased. There was also a significant interaction of drug group and days ($F(10,150)=8.07$, $p<0.001$) as a combined result of the AT group consuming less SQ from the start of the acquisition period, and the control group decreasing their consumption over days.

Two-way ANOVA on preference ratios yielded the same pattern of results as seen with the intake data. There was a significant effect of drug group ($F(1,15)=64.513$, $p<0.001$), a significant days effect ($F(10,150)=20.087$, $p<0.001$), and a significant interaction ($F(10,150)=5.277$, $p<0.001$) (Figure 3, bottom).

There was no significant difference between the drug groups in terms of body weight ($F(1,15)=3.022$, $p>0.05$), which suggested that AT did not affect food intake. There was a significant difference in total fluid consumption between the groups ($F(1,15)=19.715$, $p<0.001$). This effect was due to the enhanced SQ consumption in the saline-treated animals. The AT treated animals drank significantly more water than controls ($F(1,15)=58.863$, $p<0.001$), suggesting that the effect of AT within the context of this experiment was specific to saccharin consumption, and was not due to a general suppression of fluid intake.

Maintenance Period

Saccharin-quinine consumption (ml) remained significantly different between the groups during the maintenance period ($F(1,15)=16.743, p<0.001$), with the AT group consuming less SQ than the controls (Figure 3, top). There was a significant days effect ($F(9, 135)=2.325, p<0.05$), and a significant interaction between groups and days ($F(9,135)=2.003, p<0.05$) reflecting slight variations in consumption in the control group during the maintenance period.

Two-way ANOVA on preference ratios yielded a significant effect of previous drug group during the maintenance period ($F(1,15)=14.696, p<0.01$), a significant days effect ($F(9,135)=2.958, p<0.01$), but no significant interaction ($F(9,135)=1.519, p>0.05$) (see Fig. 3, bottom). These results suggested that AT had a relatively long-lasting effect on SQ consumption, as was the case with ethanol intake.

Total fluid consumption was not significantly different between the groups during the maintenance period ($F(1,15)=4.277, p>0.05$), and there was no effect of days ($F(9,135)=1.734, p>0.05$), although there was a significant interaction of group and days ($F(9,135)=2.853, p<0.01$) due to slight fluctuations in total fluid consumption in the saline group during the maintenance period.

Body weight during the maintenance period was significantly greater for the saline-treated animals than for the AT-treated animals ($F(1,15)=4.751, p<0.05$). This finding was surprising since there was no difference in body weight between the groups during the acquisition period. The differences in body weight may have been due to some latent effect of AT treatment; however, this does not seem to be likely in the absence of a similar

effect in Experiment 1A under the same drug treatment regimen. There was an effect of days on body weight ($F(9,135)=38.212, p<0.001$), reflecting an increase in body weight over time for both groups, but no significant interaction ($F(9,135)=0.963, p>0.05$), which suggested that both groups grew at the same rate, and that the AT group did not lose weight during the maintenance period.

To summarize, this experiment was designed to assess the specificity of the effect of catalase inhibition by AT on ethanol consumption through the administration of AT during a SQ acquisition period. AT-treated animals consumed significantly less of the SQ solution than did control animals, paralleling the results of Experiment 1A. Furthermore, there was no recovery of consumption during the maintenance period when no drug was administered, as was found in the ethanol acquisition experiment, suggesting a carry-over effect of AT administration for both ethanol and SQ consumption.

Taken together, experiments 1A and 1B revealed that administration of the catalase inhibitor AT resulted in a decrease in consumption when the drug was administered concurrently with the presentation of novel-flavored ethanol or SQ solutions. Because the consumption of novel-flavors is more easily disrupted by pairing with a drug treatment than is the consumption of familiar flavors (Fenwick, Mikulka & Klein, 1975), and because previous work has shown that AT treatment disrupts the consumption of a familiar flavored ethanol solution (Aragon & Amit, 1992), it was necessary to assess the effects of AT on the consumption of familiar ethanol and SQ solutions.

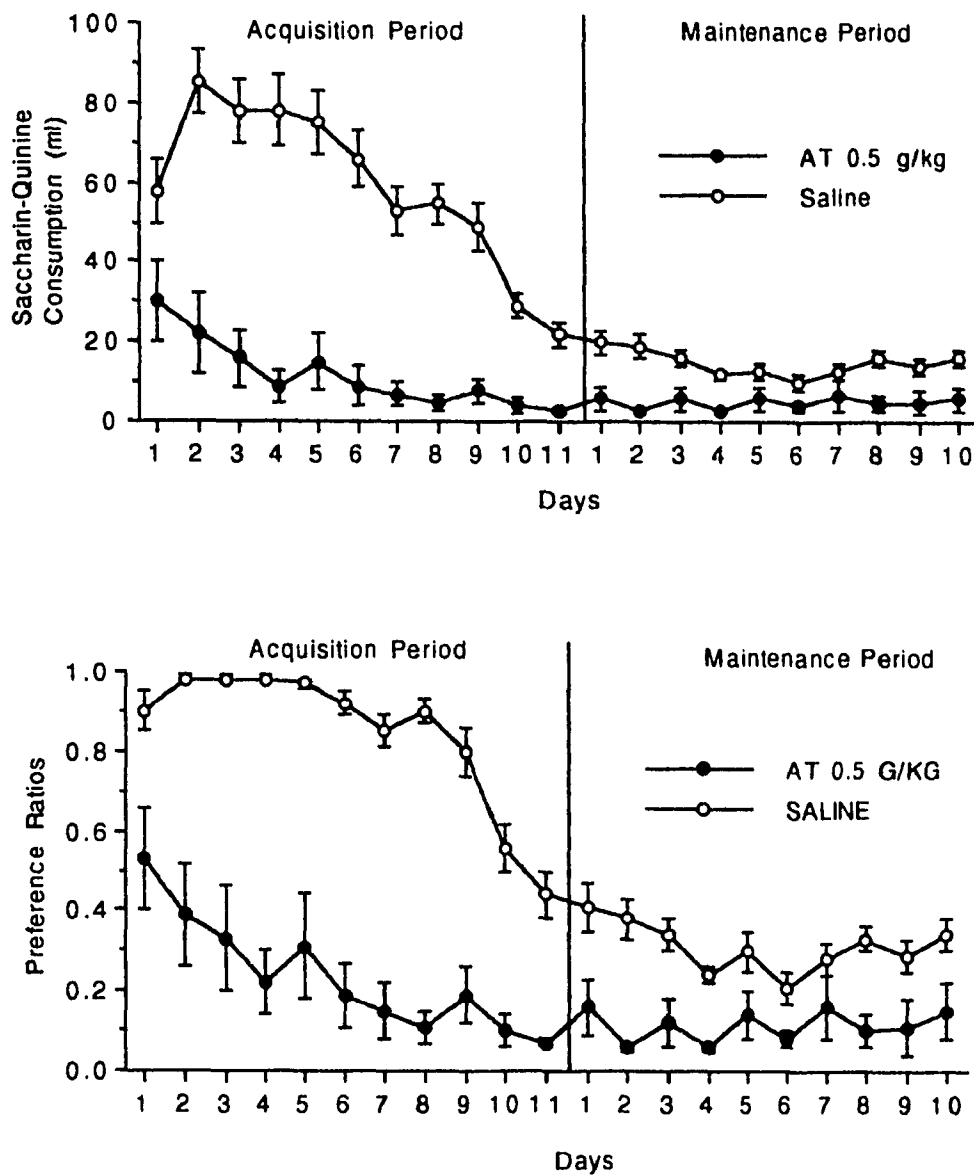


Figure 3. Mean (+/- SEM) saccharin-quinine intake in milliliters (top panel), and saccharin-quinine preference ratios (bottom panel) during the acquisition and maintenance period in saline (open circles) and AT treated animals (filled circles).

Experiment 2

The results of Experiments 1A and 1B, which showed that 0.5 g/kg AT decreased the consumption of novel ethanol or SQ solutions, prompted the need for the second set of experiments in which the effects of AT on the consumption of familiar ethanol or SQ solutions was examined. Although AT has previously been shown to reduce ethanol consumption in a dose-dependent fashion (Aragon & Amit, 1992), it was necessary to determine whether this effect was specific to ethanol. Several other drugs which at first were shown to reduce ethanol intake were later found to also reduce the consumption of SQ solutions (Amit et al., 1991; Gill et al., 1985). Therefore, the present series of experiments was designed to replicate previous findings with AT on maintained ethanol consumption, and to extend these findings by examining the effects of AT on maintained SQ consumption. In contrast to Experiment 1, in which the ethanol and SQ solutions were introduced on the same day as the first drug administration, the present set of experiments examined the effects of AT on familiar ethanol and SQ solutions that the animals were consuming at a stable level. In Experiments 2A and 2B, the effects of a moderate dose of AT (0.5 g/kg) on ethanol and SQ intake were studied. A higher dose of AT (1.0 g/kg) was used in experiments 2C and 2D to again assess the effects of AT on maintained ethanol and SQ consumption, respectively.

Experiment 2A

METHOD

Subjects

The subjects were 20 male Long-Evans rats (Charles River, Canada) weighing 215-240g at the start of the experiment. They were housed individually in stainless steel cages in a humidity and temperature controlled animal colony maintained on a 12 hour light/dark cycle (lights on at 0800). Rat chow was available ad lib, and fluids were presented in two glass Richter tubes mounted on the front of the cage.

Drugs

3-amino-1,2,4-triazole (Sigma Chemical Co.) was prepared fresh daily in a concentration of 1g/2 ml of vehicle by heating and mixing with 0.9% saline.

Procedure

Following one week of acclimatization to the colony, the animals were presented with an ethanol acquisition regimen in which ethanol was presented in free choice with water on alternate days. Only water was available on intervening days. Fluid consumption was measured daily. The first ethanol presentation was of a 2% (v/v) solution prepared by mixing a 95% stock solution with tap water. On each subsequent presentation, the concentration was increased by 1% to a final concentration of 10%. Once the 10% concentration was reached, one further water day was given, followed by a switch to 10 days of everyday presentation of 10% ethanol in free choice with water. The last 5 days of this 10% maintenance period constituted the baseline ethanol intake. Following the baseline period, a five day treatment period was initiated. During this time, 10% ethanol was available in free choice with water as it was during the maintenance period. However, during

the treatment period, the animals were given intraperitoneal injections of either saline (n=10) or AT (0.5 g/kg) (n=10) five hours before the onset of the dark cycle. This time period was chosen to replicate previous work (Aragon & Amit, 1992). This time period was also chosen because catalase activity has been shown to be maximally decreased between 3 and 6 hours post injection, and to remain decreased for at least 24 hours (Aragon, Rogan & Amit, 1991). Following the treatment period, there were five more days of everyday ethanol presentation in free choice with water which constituted the post-treatment period.

RESULTS

Because of the large within group variation inherent to ethanol drinking studies, all measures for this set of experiments are expressed in terms of percent change from baseline for each rat. The five baseline days were averaged for each rat, and a percent change from baseline score was obtained for each rat for each treatment and post-treatment day by subtracting the baseline score from the daily score, and dividing the result by the baseline score. Two-way analysis of variance with repeated measures on days as the within factor, and drug group as the between factor were then performed on all percent change from baseline measures.

Two-way analysis of variance (group x days) revealed a significant effect of drug ($F(1,18)=4.606$, $p<0.05$) on percent change in ethanol intake (g/kg) across the treatment and post-treatment days (Figure 4, top), suggesting that AT treatment decreased maintained ethanol consumption, consistent with previous reports (Aragon & Amit, 1992). There was no main effect of days ($F(9, 162)=0.895$, $p>0.05$), and no interaction of drug group by

days ($F(9, 162)=1.843, p>0.05$), suggesting that AT decreased ethanol consumption throughout the treatment and post-treatment periods.

A similar pattern of results was obtained when percent change in ethanol preference ratios were analyzed. Ethanol preference was defined as ethanol intake divided by total fluid (ethanol + water) intake. There was a significant effect of group ($F(1,18)=4.602, p<0.05$), but no effect of days ($F(9,162)=0.901, p>0.05$), and no interaction ($F(9,162)=1.343, p>0.05$) (Figure 4, bottom). Again, this result reflected the decrease in ethanol consumption in the AT treated group during the treatment and post-treatment periods.

There was no effect of AT treatment on percent change in total fluid consumption ($F(1,18)=1.185, p>0.05$), suggesting that AT did not have a general effect on fluid intake, and that the rats treated with AT compensated for their decrease in ethanol intake by increasing their water intake.

There was a significant effect of group on percent change in body weight ($F(1,18)=6.359, p<0.05$), a significant effect of days ($F(9,162)=108.33, p<0.001$), and a significant interaction of group and days ($F(9,162)=2.507, p<0.01$). The group difference may have been due to AT having some general suppressive effect on food consumption, or it may have been due to the saline group increasing their weight from the extra calories they received from their extra ethanol intake.

Overall, this experiment confirmed previous findings that AT decreased ethanol intake, and suggested that catalase activity was important in the regulation of stable voluntary ethanol intake. However, given the results of Experiment 1B, in which the effects of AT were found to be non-specific to ethanol consumption, Experiment 2B was designed to assess the specificity of AT's effect on maintained ethanol consumption.

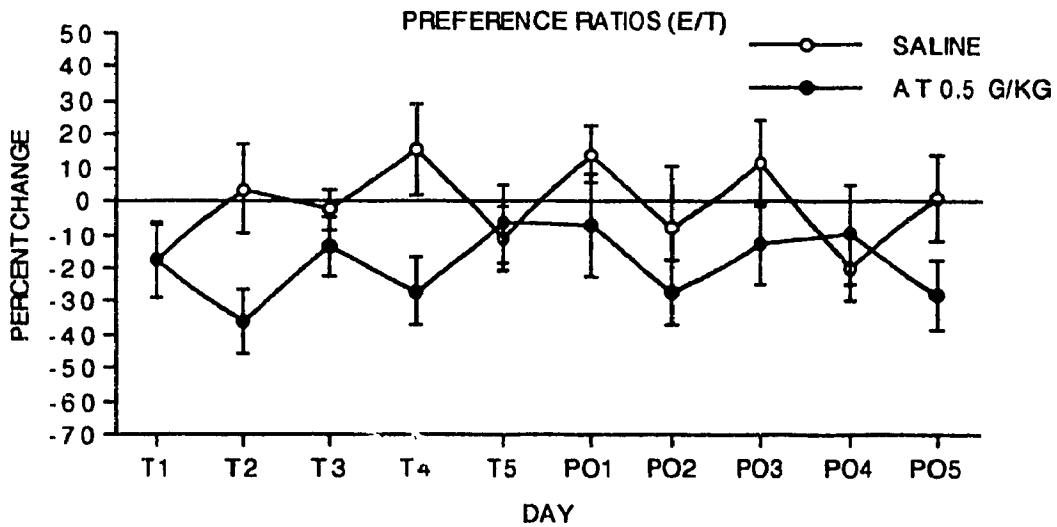
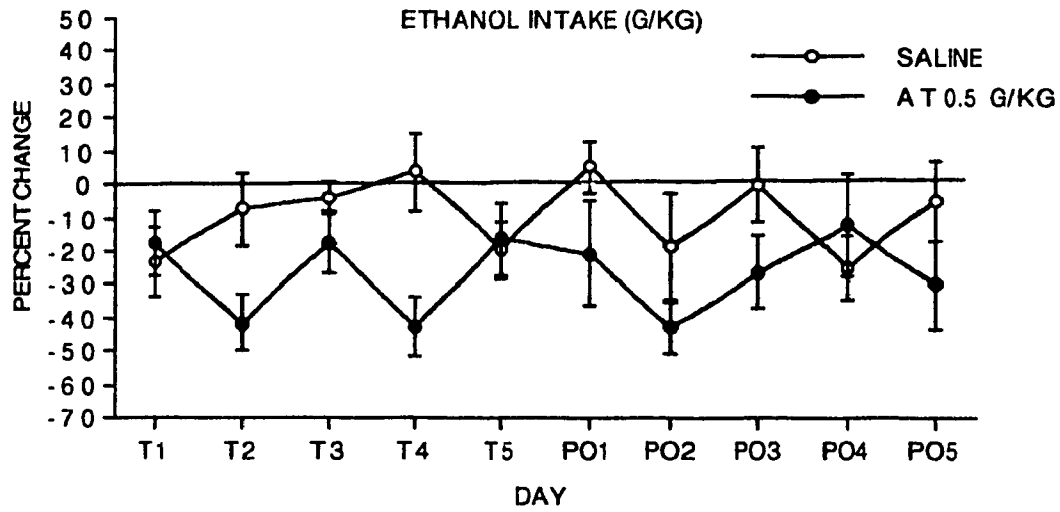


Figure 4. Percent change from baseline in ethanol intake in g/kg (top panel), and percent change from baseline in ethanol preference ratios (bottom panel) in saline (open circles) and AT treated (filled circles) animals.

Experiment 2B

Subjects

The subjects were 20 male Long-Evans rats (Charles River, Canada) weighing 200-240g at the start of the experiment. They were housed under the same conditions as in Experiment 2A.

Drugs

3-amino-1,2,4-triazole (Sigma Chemical Co.) was prepared fresh daily in a concentration of 1g/2 ml of vehicle by heating and mixing with 0.9% saline.

Procedure

The animals were gradually introduced to saccharin with increasing concentrations of quinine using the acquisition procedure of Experiment 1B. On Day 1 of acquisition, the rats received a 0.4% sodium saccharin solution in free choice with water. On subsequent presentation days, quinine sulfate (Fisher Scientific) was added to the saccharin solution in increasing concentrations. The concentrations used were 0.001% (acquisition day 2), 0.002% (day 3), 0.003% (day 4), 0.004% (day 5), 0.006% (day 6), 0.009% (day 7), 0.011% (day 8), 0.015% (day 8), 0.03% (day 9), 0.04% (day 10). Saccharin-quinine solutions were presented on days alternating with only water presentation. The position of the SQ bottle was alternated daily to prevent the development of a position bias. Following acquisition, the rats were switched to ten days of everyday presentation of saccharin-quinine (0.4% saccharin and 0.04% quinine) in free choice with water, the last five days of which constituted the baseline intake period. Following baseline, a five day treatment period was initiated, during which injections of either 0.5 g/kg AT (n=10) or saline (n=10) were administered (ip) five hours before the dark

cycle. Following the injection period, a five day post-treatment period was instituted during which no injections were given.

RESULTS AND DISCUSSION

Two-way analysis of variance on percent change from baseline scores of SQ intake (ml) revealed no significant effect of group ($F(1,18)=2.86$, $p>0.05$), no effect of days ($F(9,162)=1.003$, $p>0.05$), and no significant interaction of group and days ($F(9,162)=0.43$, $p>0.05$) (Figure 5, top). This finding suggested that AT did not have an effect on SQ intake, and that the effect of AT in Experiment 2A was therefore specific to ethanol intake.

Similarly, there was no significant effect of group ($F(1,18)=2.502$, $p>0.05$), no effect of days ($F(9, 162)=0.930$, $p>0.05$), and no group by days interaction ($F(9,162)=0.503$, $p>0.05$) on percent change in SQ preference ratios (Figure 5, bottom), again suggesting that AT had no effect on SQ intake.

Percent change in total fluid intake was also not different between the drug groups ($F(1,18)=2.032$, $p>0.05$), and there was no effect of days ($F(9,162)=1.517$, $p>0.05$), and no group by days interaction ($F(9,162)=0.849$), $p>0.05$). This finding again supported the notion that the effect of AT treatment was specific to ethanol intake, and did not have a general suppressive effect on consumption.

Body weight did not differ between the drug treatment groups ($F(1,18)=0.053$, $p>0.05$), although there was an effect of days ($F(9,162)=96.346$, $p<0.001$), reflecting the animals' growth over time. There was no interaction of group by days ($F(9, 162)=1.044$, $p>0.05$), again suggesting that AT treatment did not have an effect on food intake. This finding also argued against the possibility that the differences in body weight seen in Experiment 2A were due to a suppressive effect of AT on feeding, and

suggested that the differences were due to differences in calories obtained from ethanol consumption.

Together, the results of Experiments 2A and 2B suggested that the effect of AT was specific to ethanol intake when the flavored fluid was familiar, in contrast to the findings of Experiment 1 in which 0.5 g/kg of AT resulted in a decreased intake of both ethanol and SQ solutions. These results supported the hypothesis that catalase activity was important in the control of ethanol intake. However, the reduction in ethanol intake as a result of AT treatment in Experiment 2A was not as large as had previously been reported (Aragon & Amit, 1992). Furthermore, there appeared to be a trend toward a decrease in SQ intake in Experiment 2B (Figure 5). Therefore, a higher dose of AT (1.0 g/kg) was used in a Experiments 2C and 2D to again assess the effects of AT on the intake of familiar ethanol and SQ solutions.

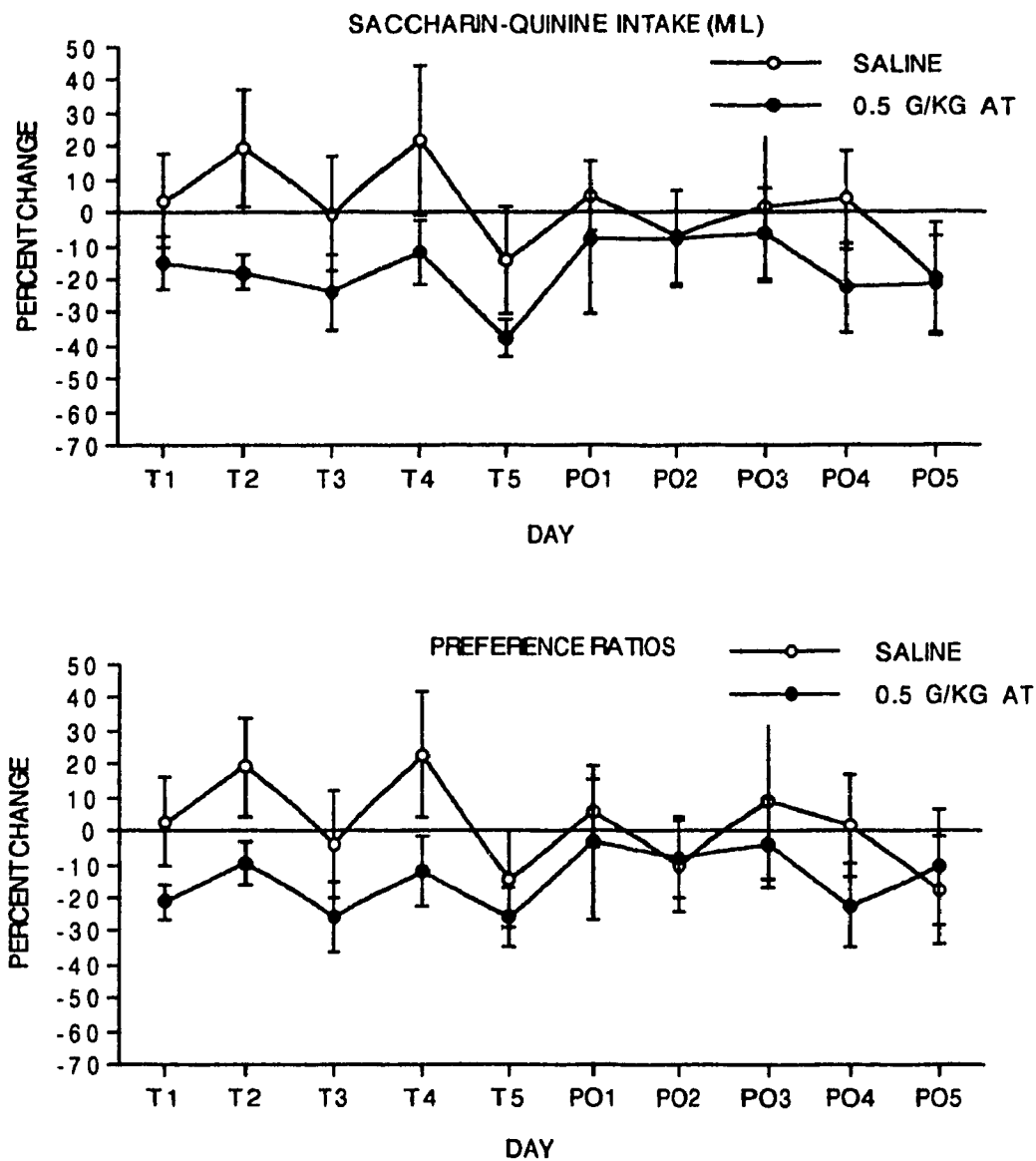


Figure 5. Percent change from baseline in saccharin-quinine intake in milliliters (top panel), and percent change from baseline in saccharin-quinine preference ratios (bottom panel) in saline (open circles) and AT treated (filled circles) animals.

Experiment 2C

METHOD

Subjects

The subjects were 14 male Long-Evans rats (Charles River, Canada), housed and maintained under the same conditions as in Experiments 2A and 2B.

Drugs

3-amino-1,2,4-triazole (Sigma Chemical Co.) was prepared fresh daily in a concentration of 1g/2 ml of vehicle by heating and mixing with 0.9% saline.

Procedure

The procedure used was identical to that in Experiment 2A except that 1.0 g/kg AT was used.

RESULTS AND DISCUSSION

Two-way analysis of variance on percent change from baseline scores revealed a significant effect of drug treatment on ethanol intake (g/kg) ($F(1,12)=6.031, p<0.05$), but no effect of days ($F(9,108)=0.626, p>0.05$), and no interaction of drug group and days ($F(9,108)=0.944, p>0.05$) (Figure 6, top). These results indicated that AT treatment decreased ethanol intake during the treatment period, and that this effect carried over to the post-treatment period, as was found in Experiment 2A using the 0.5 g/kg dose of AT. The decrease in consumption during the treatment period suggested that catalase may play an important role in mediating voluntary ethanol consumption. However, the continued reduction in ethanol intake seen during the post-treatment period suggested that some factors other than catalase inhibition may have been involved in the observed reduction in ethanol intake.

In contrast to the difference seen in g/kg ethanol intake, there were no differences between the groups in terms of percent change in preference ratios ($F(1,12)=2.018$, $p>0.05$), no effect of days ($F(9,108)=0.532$, $p>0.05$), and no interaction ($F(9,108)=0.439$, $p>0.05$) (Figure 6, bottom). However, these results can be explained by the finding that percent change in total fluid intake was also decreased significantly in the AT group relative to controls ($F(1,12)=7.882$, $p<0.05$). Thus, since the preference ratio represented the amount of ethanol intake relative to total fluid intake, and since both ethanol intake and total fluid intake were decreased in the AT group, the ratio remained constant. Therefore, the percent change in preference ratios for the AT group was not significantly different from controls. The decrease in total fluid intake in the AT group can be attributed entirely to their decreased ethanol intake, since there was no difference between the groups in percent change in water intake ($F(1,12)=0.082$, $p>0.05$).

Percent change in body weight did not differ between the groups ($F(1,12)=1.621$, $p>0.05$), again arguing against AT having a non-specific effect on food consumption. There was an effect of days ($F(9,108)=15.794$, $p<0.001$), reflecting growth, and there was an interaction of groups and days ($F(9,108)=2.247$, $p<0.05$), which suggested that the two groups gained weight at different rates; however, in the absence of an overall group difference this finding does not support a non-specific action of AT on food intake.

This experiment confirmed previous findings which showed that AT administration decreased ethanol intake in rats (Aragon & Amit, 1992). However, in light of the discrepancy between Experiments 1 and 2 in terms of the specificity of AT's effect, it was necessary to assess the specificity of the effect of 1.0 g/kg AT by examining a SQ control group. This was the purpose of the following experiment.

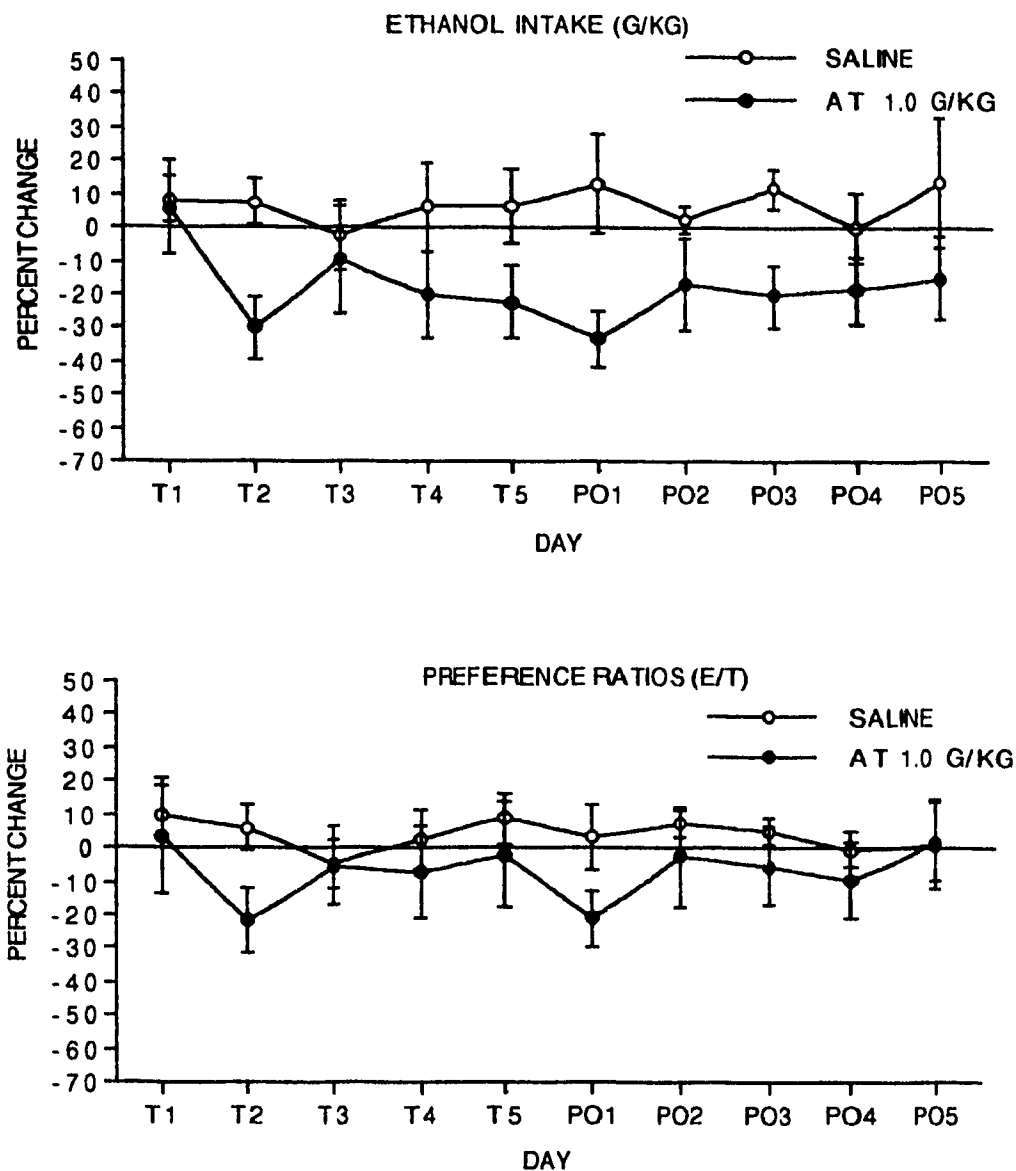


Figure 6. Percent change from baseline in ethanol intake in g/kg (top panel), and percent change from baseline in ethanol preference ratios (bottom panel), in saline (open circles) and AT treated (filled circles) animals during treatment with 1.0 g/kg AT, and during 5 post-treatment days.

Experiment 2D

METHOD

Subjects

The subjects were 18 male Long-Evans rats (Charles River, Canada) housed and maintained under the same conditions as in the previous experiments.

Drugs

3-amino-1,2,4-triazole (Sigma Chemical Co.) was prepared fresh daily in a concentration of 1g/2 ml of vehicle by heating and mixing with 0.9% saline.

Procedure

The procedure used was identical to that in Experiment 2B except that 1.0 g/kg AT was used.

RESULTS AND DISCUSSION

Percent change from baseline data was again used, and two-way analysis of variance on SQ intake (ml) revealed a significant effect of drug treatment ($F(1,16)=22.498$, $p<0.001$), but no effect of days ($F(9,144)=0.508$, $p>0.05$), and no interaction between groups and days ($F(9,144)=0.812$, $p>0.05$) (Figure 7, top). These results parallel what was seen with ethanol consumption following both 0.5 and 1.0 g/kg AT in that the consumption of the flavored fluid decreased during the treatment period, and remained attenuated during the post-treatment period.

Preference ratios were also significantly lower in the AT group as compared to the saline group ($F(1,16)=16.367$, $p<0.001$), reflecting the decrease in SQ preference in the AT group (Figure 7, bottom). There was no effect of days ($F(9,144)=0.481$, $p>0.05$), and no interaction of days and group

($F(9,144)=0.874$), $p>0.05$) on the measure of percent change in preference ratios. This finding corresponded to the findings with ethanol intake, which suggested that intake was decreased across both the treatment and post-treatment periods.

Percent change in body weight was significantly different between the groups ($F(1,16)=96.217$, $p<0.001$), with the AT treated group showing less of an increase in weight than the control group. There was also a significant effect of days ($F(9,144)=43.406$, $p<0.001$), reflecting the animals' growth over time, and a significant interaction ($F(9,144)=10.409$, $p<0.001$), reflecting the slower rate of growth in the AT treated animals. These results suggested that AT animals may have been consuming less food than the controls.

Overall, Experiment 2D showed that AT disrupted the consumption of a familiar SQ solution. The reason for the decrease in ethanol and SQ consumption in this experiment, and in Experiment 1, needed to be further explored. This was the purpose of the next experiment.

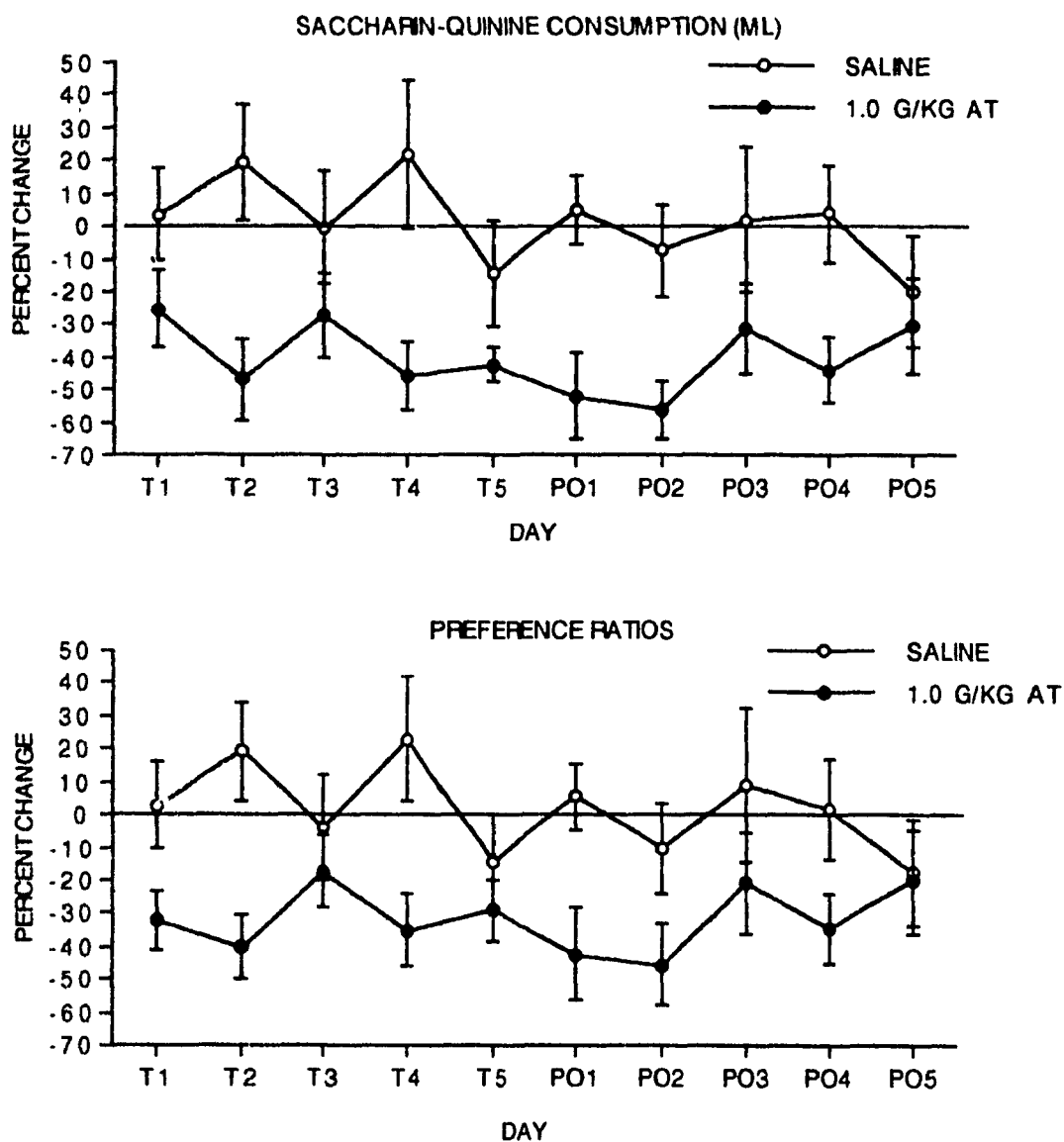


Figure 7. Percent change from baseline in saccharin-quinine intake in milliliters (top panel), and percent change from baseline in saccharin-quinine preference ratios (bottom panel) in saline (open circles) and AT treated (filled circles) animals during a five day treatment with 1.0 g/kg AT, and during five post-treatment days.

Experiment 3

The previous experiments demonstrated that 0.5 g/kg of AT decreased the consumption of novel and familiar ethanol solutions, but not familiar SQ solutions. However, 1.0 g/kg of AT decreased the consumption of both novel and familiar ethanol and SQ solutions. Furthermore, the decreases in consumption were relatively long lasting. The reason for the decrease was not immediately clear, since it was hypothesized that AT would decrease only ethanol consumption by inhibiting the central production of acetaldehyde and its presumed reinforcing effects. One possible explanation of the results was that AT induced a conditioned taste aversion (CTA), which resulted in the decreased fluid consumption. This possibility was tested in the present experiment.

In the traditional CTA paradigm (forward CTA), a drug or other physiological agent (conditioning agent) is administered immediately following the consumption of a novel or distinctively flavored substance (Hunt & Amit, 1987). It is presumed that the aversive interoceptive cues produced by the drug become associated with the flavor of the ingested substance, which results in an avoidance of that flavor on subsequent presentations. Many psychoactive and emetic agents are capable of inducing a CTA, which is thought to result from the novelty of the drug state or from a drug-induced illness (Hunt & Amit, 1987; Sklar & Amit, 1977).

The forward CTA paradigm was used in the present study to determine whether AT was capable of inducing a CTA. However, in the drinking studies of Experiments 1 and 2, the drug AT was administered five hours before the dark cycle, and thus, five hours before the majority of the drinking took place. Therefore, to invoke the CTA explanation in these experiments, it must be demonstrated that AT is capable of inducing a CTA

when administered before fluid presentation (backward CTA). Backward conditioning typically does not result in as strong or as stable CTAs as does forward conditioning, and the strength of the aversion usually decreases as the interval between drug administration and the ingestion of the food or fluid increases (Barker, Smith, Suarez, 1977). For example, lithium chloride (LiCl), an agent which is very effective in the forward conditioning of a CTA (Kulkosky et al., 1980), did not produce a CTA when the interval between LiCl administration and saccharin ingestion exceeded 30 minutes (Barker et al., 1977). However, backward conditioning has been demonstrated with the dopamine-B-hydroxylase inhibitor FLA-57 when this drug was administered 3-4 hours before fluid presentation (Sirota & Boland, 1987). Therefore, it was thought necessary to test for the possibility of backward conditioning in the present experiment.

It has previously been shown that 1.0 g/kg of AT did not induce a CTA when administered 4 hours before the consumption of a novel saccharin solution (Aragon, Spivak & Amit, 1985). However, the paradigm involved a one bottle test in which saccharin was the only fluid available to the fluid deprived rats. Since the drinking studies of Experiments 1 and 2 involved a two bottle choice between water and either ethanol or SQ, the CTA experiments of the present study involved a two bottle test to more closely duplicate the testing conditions of Experiments 1 and 2. Furthermore, two bottle tests are known to be more sensitive tests of CTA than are one bottle tests (Hunt & Amit, 1987), therefore, it was thought prudent to employ the two bottle test. The ability of AT (0.5 g/kg and 1.0 g/kg) to induce both backward and forward CTA's was investigated in the present experiment.

METHOD

Subjects

The subjects were 49 male Long-Evans rats weighing 290-340g at the start of the experiment. The animals were housed and maintained under the same conditions as in the previous experiments.

Drugs

3-amino-1,2,4-triazole (Sigma Chemical Co.) was prepared fresh daily in a concentration of 1g/2 ml of vehicle by heating and mixing with 0.9% saline.

Procedure

Baseline. The animals were allowed to adapt to the housing conditions for two weeks, with food and water available ad lib. Following this adaptation period, the animals were switched to a 23 hour 40 minute water deprivation schedule. Water was available for only 20 minutes each day, and was presented in plastic drinking tubes fitted with ball-bearing spouts and mounted on the front of the cages. Fluid consumption was measured daily. Water was presented for nine consecutive days until daily intake was relatively stable for each animal.

Backward CTA. On the tenth deprivation day, a 0.1% saccharin solution was presented for 20 minutes in the drinking tube instead of water (Pairing Day 1). Five hours prior to the presentation of the drinking tube, the animals were given one of three injections (ip.). One group received saline (n=9), the second group received 0.5 g/kg AT (n=8), and the third group received 1.0 g/kg AT (n=8). Following Pairing Day 1, there were five days of water presentation, followed by Pairing Day 2, in which the procedure of Pairing Day 1 was repeated. Pairing Day 2 was followed by five more water days. Test Day 1 followed this period, and consisted of the saccharin solution

being presented in free choice with water for the 20 minute drinking period. A two bottle test was used because the drinking studies of Experiments 1 and 2 employed two bottle tests, and because the two bottle test has been shown to be a more sensitive test of CTA (Hunt & Amit, 1987). Test Day 1 was followed by five more water days, and then by Test Day 2, which was identical to Test Day 1. No injections were given on either of the Test Days.

Forward CTA. The procedure was identical to that of the backward CTA, except that injections (0.0 g/kg, n=7; 0.5 g/kg, n=8; 1.0 g/kg, n=8) were administered immediately following the 20 minute saccharin presentation on Pairing Days 1 and 2.

RESULTS AND DISCUSSION

Backward CTA

A two-way analysis of variance with drug groups as the between factor and days Pairing 1 (P1), Pairing 2 (P2), Test 1 (T1), and Test 2 (T2) as the within factor, was performed on saccharin intake (ml). There was a significant effect of days ($F(3,66)=5.209$, $p<0.01$), reflecting a slight decrease in saccharin intake in all groups on Test Day 1 (Figure 8, top). However, there was no effect of drug ($F(2,22)=3.296$, $p>0.05$), and no drug by days interaction ($F(6,66)=1.047$, $p>0.05$), which showed that 0.0, 0.5, and 1.0 g/kg of AT administered five hours before fluid consumption did not have a differential effect on saccharin intake. This finding indicated that AT did not induce a backward CTA, consistent with previous reports (Aragon et al., 1985).

Because the test day involved a two bottle choice between water and saccharin, test day preference ratios were also examined. A preference ratio was obtained for each animal by dividing saccharin consumption by total

fluid intake. A two-way analysis of variance of drug by days for T1 and T2 showed again that there was no effect of AT dose on saccharin preference ($F(2,20)=1.208$, $p>0.05$), no effect of days ($F(1,20)=0.315$, $p>0.05$), and no interaction ($F(2,20)=0.554$, $p>0.05$) (Figure 8, bottom). This finding provided further support that AT did not induce a backward CTA.

Forward CTA

Two-way analysis of variance on saccharin intake across days P1, P2, T1, and T2 for the animals receiving 0.0, 0.5, or 1.0 g/kg of AT following saccharin consumption revealed a significant effect of drug ($F(2,20)=22.498$, $p<0.001$), days ($F(3,60)=18.768$, $p<0.001$), and a significant interaction of drug and days ($F(6,60)=3.917$, $p<0.01$) (Figure 9, top).

Post hoc test of simple main effects revealed that there was no significant difference between the groups on day P1 ($F(2,20)=0.382$, $p>0.05$), indicating that the groups were evenly matched at baseline. There was a significant effect of groups on days P2 ($F(2,20)=8.517$, $p<0.01$), T1 ($F(2,20)=14.664$, $p<0.001$), and T2 ($F(2,20)=16.839$, $p<0.001$). Further analysis with Tukey post hoc tests revealed that on day P2 the group given 1.0 g/kg AT drank significantly less saccharin than the control group ($p<0.05$). On both Test Days 1 and 2, both drug groups (0.5 and 1.0 g/kg) drank significantly less than the control group ($p<0.05$). These results indicated that AT did induce a CTA when it was administered following saccharin consumption.

Preference data on Test Days 1 and 2 were also analyzed by two way analysis of variance with drug as the between factor and days as the within factor. There was a significant effect of drug ($F(2,21)=23.225$, $p<0.001$), but no effect of days ($F(1,21)=1.003$, $p>0.05$), and no interaction of drug and days ($F(2,21)=1.062$, $p>0.05$) (Figure 9, bottom). The drug effects were further

analyzed with Tukey post hoc tests, which revealed that both the 0.5 and 1.0 g/kg groups had significantly lower saccharin preference ratios than the control group on Test Day 1 ($p < 0.05$). This finding supported the saccharin intake results which showed that AT can produce a CTA when administered immediately following saccharin intake. However, on Test Day 2 only the 1.0 g/kg group differed significantly from the control group ($p < 0.05$). This finding suggested that the 0.5 g/kg group may have been starting to recover from the effects of drug treatment on Pairing Days 1 and 2.

Overall, the results of this experiment showed that AT did not produce a backward CTA, but did induce a forward CTA. In the context of the present thesis, this finding suggested that the decreases in consumption that were observed in Experiments 1, 2, and 3 were not likely to have been entirely due to a CTA mechanism.

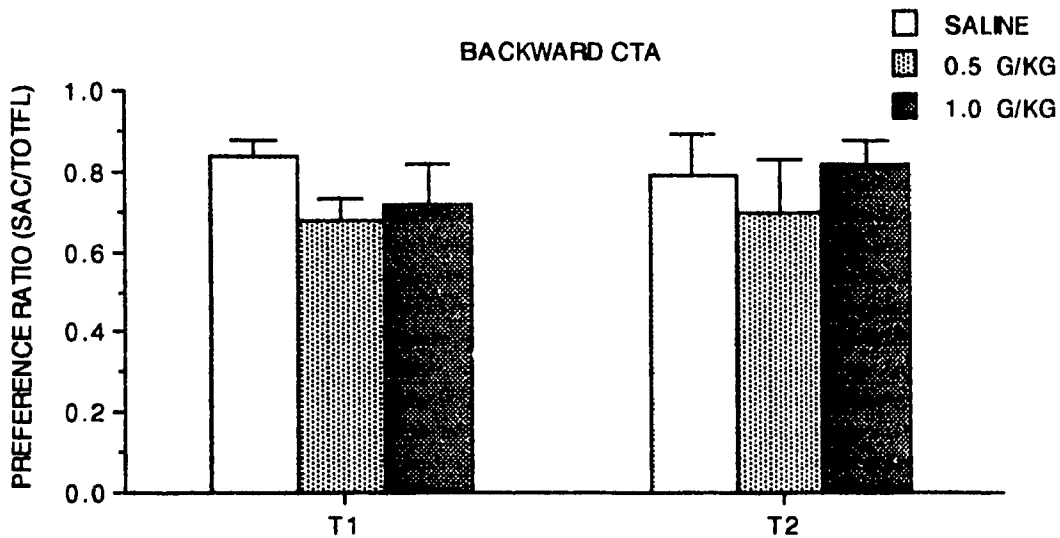
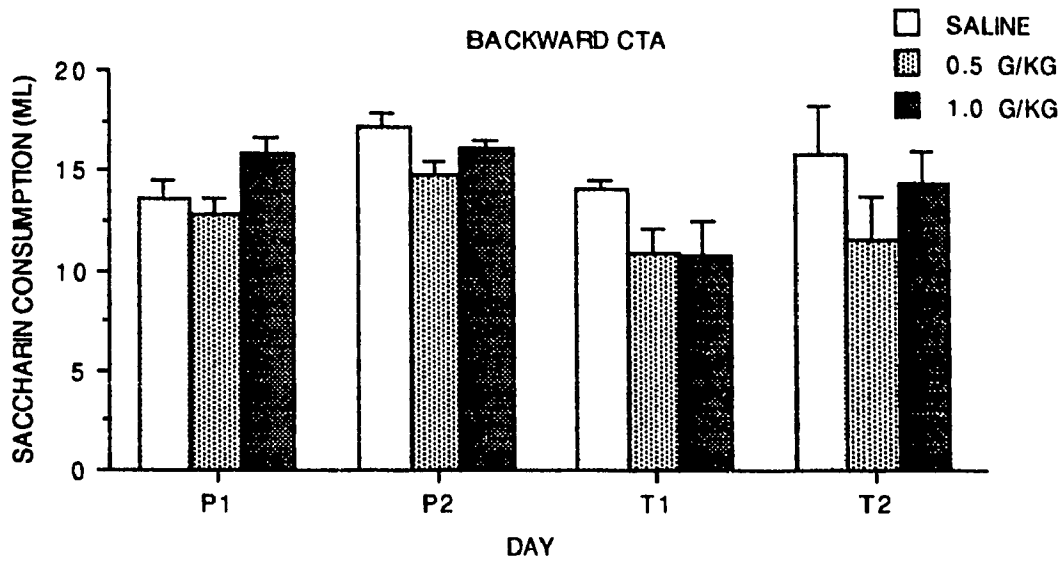


Figure 8. Top panel: Mean saccharin consumption (ml) on pairing days 1 and 2 (P1, P2), and test days 1 and 2 (T1, T2) in rats treated with 0.0, 0.5, or 1.0 g/kg AT. Bottom panel: Mean saccharin preference ratios on test days 1 and 2 (T1, T2) in rats treated with 0.0, 0.5, or 1.0 g/kg AT.

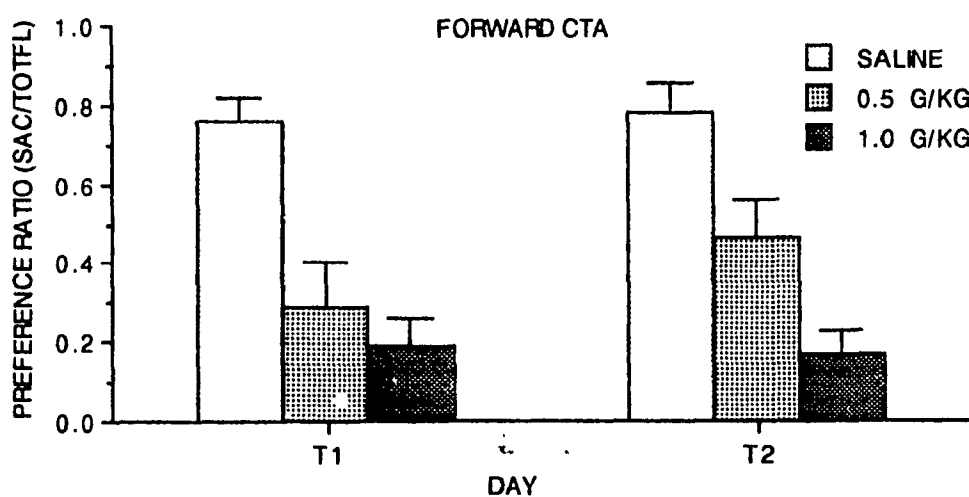
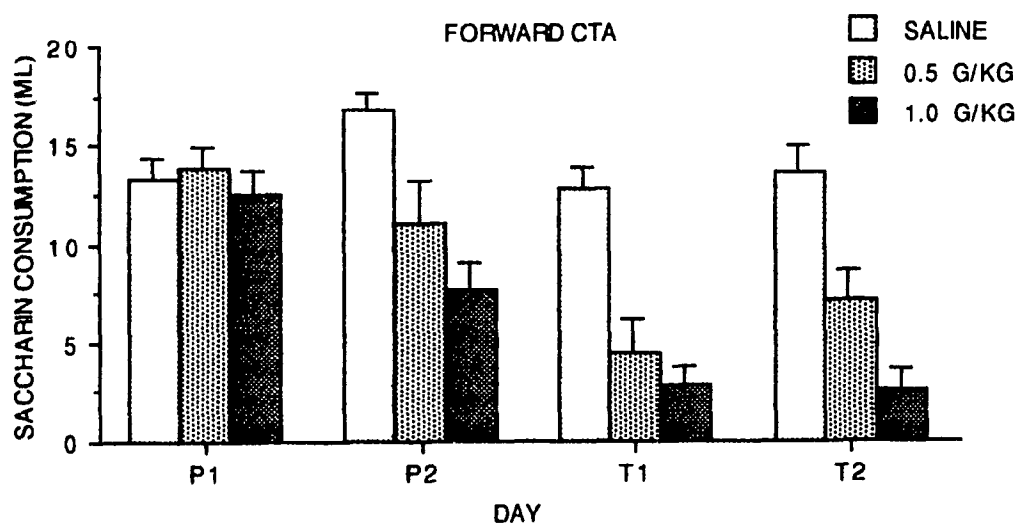


Figure 9. Top panel: Mean saccharin consumption (ml) on pairing days 1 and 2 (P1, P2), and test days 1 and 2 (T1, T2) in rats treated with 0.0, 0.5, or 1.0 g/kg AT. Bottom panel: Mean saccharin preference ratios on test days 1 and 2 (T1, T2) in rats treated with 0.0, 0.5, or 1.0 g/kg AT.

GENERAL DISCUSSION

The purpose of this thesis was to demonstrate the importance of the role of catalase activity in the acquisition of voluntary ethanol intake in rats. The reason for this goal was linked to the fact that considerable evidence has implicated central acetaldehyde as mediating many of the behavioral and reinforcing effects of ethanol (e.g., Amit & Smith, 1985). Furthermore, a large body of biochemical and behavioral data has shown that the enzyme catalase oxidizes ethanol to acetaldehyde in the brain (Aragon, Rogan & Amit, 1992; Cohen et al., 1980; Gill et al., 1992), and that this oxidation has behavioral significance for many ethanol induced behaviors in rats and mice (Aragon & Amit, 1993; Aragon, Pesold & Amit, 1992; Aragon, Spivak & Amit, 1985; 1989;1991).

In the attempt to further characterize the importance of catalase activity in mediating ethanol consumption, the effect of catalase manipulations on voluntary ethanol intake in rats was studied in the present thesis using the catalase inhibitor AT. Preliminary work showed that catalase inhibition induced by AT administration dose-dependently attenuated voluntary intake of a familiar ethanol solution in rats (Aragon & Amit, 1992). Therefore, the present work was undertaken to extend these results to the acquisition of ethanol intake, and to assess the specificity of the effect of AT on ethanol consumption by studying the effects of AT on SQ intake.

The present series of experiments revealed that while AT did decrease the consumption of ethanol during acquisition and during maintained ethanol consumption, it also decreased the consumption of a novel SQ solution at 0.5 g/kg of AT, and of a familiar SQ solution at 1.0 g/kg of AT. In Experiment 1A, 0.5 g/kg AT was administered to rats during an ethanol acquisition period.

AT treated animals drank significantly less ethanol than controls during the acquisition period, and consumption remained lower even after drug treatment was terminated. Experiment 1B showed that the same pattern of results occurred when the novel fluid was SQ instead of ethanol. This finding made it impossible to interpret the results of the ethanol acquisition in terms of the role of catalase-mediated acetaldehyde production in the development of voluntary ethanol intake. Since catalase is not presently known to be involved in SQ metabolism, these results suggested a more general effect of AT administration on the consumption of novel fluids.

Previous work had shown that AT decreased the consumption of a familiar ethanol solution (Aragon & Amit, 1992). Since the consumption of fluids with novel flavors is more easily disrupted than is the consumption of familiar flavored fluids (Fenwick et al., 1975; Kalat, 1974; Kalat & Rozin, 1970), it was necessary to test the effect of AT on the consumption of a familiar SQ solution, to see if consumption would again be decreased. Experiment 2 examined the effects of two doses of AT on maintained ethanol and SQ intake. Experiment 2A showed that 0.5 g/kg AT decreased ethanol consumption during the treatment and post-treatment periods, whereas Experiment 2B revealed a non-significant decrease in SQ consumption. This finding suggested that catalase played a role in the consumption of ethanol, and that blocking catalase activity with AT resulted in the decrease in ethanol intake. However, Experiments 2C and 2D showed that 1.0 g/kg AT decreased both ethanol and SQ intake throughout the treatment and post-treatment periods. Initially, this finding, together with the results of Experiments 2A and 2B, suggested that although some of the decrease in ethanol intake may have been due to a decrease in the reinforcing effects of

ethanol, some of the reduction must also have been due to another more generalized mechanism.

These results suggested that perhaps AT was inducing a conditioned taste aversion (CTA), which resulted in the decreased intake of the flavored fluids. Therefore, Experiment 3 was designed to test this hypothesis using both backward and forward CTA paradigms. Although AT did produce a forward CTA at both doses tested, it did not induce a backward CTA at either dose. Since the drinking studies of Experiments 1 and 2 employed what could be viewed as an essentially backward CTA paradigm, it was unlikely that the decreases in drinking observed in the present studies were entirely due to a CTA mechanism. This and other alternative interpretations of the data will be examined in the following sections.

ALTERNATIVE INTERPRETATIONS

Conditioned Taste Aversion

The main finding that argues against a CTA explanation of the data is that the drinking studies of Experiments 1 and 2 involved the administration of AT five hours before the dark cycle, and thus five hours before the majority of the drinking took place. Thus the procedure was essentially a backward conditioning procedure in that the unconditioned stimulus of AT was experienced by the animals five hours before the majority of their experience with the conditioned stimulus flavored solutions. However, Experiment 3 clearly showed that AT did not induce a backward CTA, in that there was no significant decrease in saccharin intake on any of the test days in the groups given AT five hours before saccharin presentation.

Despite the findings of the backward CTA study, the possibility that the reduction in drinking seen in Experiments 1 and 2 was due to a CTA mechanism still remained, since AT did induce a strong forward CTA. The

fluids in Experiments 1 and 2 were available for 24 hours each day, except for when the bottles were removed to administer injections and change the fluids. Therefore, it was possible that if small amounts of fluid were ingested closely following the AT injections, the flavor could have become associated with the effects of the AT injection. With shorter UCS-CS intervals, the backward procedure is essentially a forward procedure, since the illness and the flavor may be experienced contiguously (Barker et al., 1977). Thus, this might have been the mechanism for the decreased SQ and ethanol intake. This possibility could be addressed in a future study by having the flavored fluids available only during the dark cycle, and not available in the hours immediately preceding or following drug treatment.

Another notable finding was that body weight was decreased in some of the AT groups in some experiments. Though body weight was not reduced by AT in all experiments, the fact that it was reduced in some, and when seen, was always reduced in the AT group, suggested that AT may have had an effect on food intake. In Experiment 1, there were no differences in body weight between saline and AT treated animals in either the SQ acquisition or the ethanol acquisition period, which suggested that AT did not affect food intake. However, in Experiment 1B, body weight was lower in the A^m group as compared to controls during the maintenance period, when no AT injections were given. Thus, this finding suggested that AT may have had a latent effect on food intake. A reduction in body weight in the AT group was also found in Experiment 2A with 0.5 g/kg AT. However, under the same drug treatment conditions there was no difference in body weight found in Experiment 2B, when SQ was the novel fluid. Thus, the difference in weight gain in Experiment 2A could have been due to the extra calories the saline group received from their higher ethanol intake. A strong effect of AT on

body weight seems unlikely since there was no effect of AT on body weight in the acquisition period of Experiment 1 during which 11 injections of 0.5 g/kg AT were administered, as opposed to the 5 injections administered in Experiment 2A. Using 1.0 g/kg AT, a significant effect of drug treatment on body weight was found in Experiment 2D, but not in 2C. Thus, while it is still possible that AT had an effect on food consumption as a result of some aversive or toxic effects, the inconsistency of the effect suggests that this was not a likely reason for any observed differences in fluid intake.

Another finding which warrants consideration with respect to the CTA interpretation of the data is the lack of recovery of drinking following the termination of drug treatment. In Experiment 1, AT-treated animals' drinking did not recover during a 10 day drug free maintenance period in either the ethanol or SQ conditions. In Experiment 2, ethanol consumption remained decreased throughout the five day post-treatment period following 0.5 g/kg AT, as did both ethanol and SQ consumption following 1.0 g/kg AT. These decreases in consumption could not be due to continued catalase depletion, since catalase activity is known to recover to normal levels within 48 hours of administration of 0.5 and 1.0 g/kg AT (Aragon, Rogan, & Amit, 1991). Thus, the continued decreases in consumption may not be inconsistent with a CTA interpretation of the data. For example, one pairing of lithium chloride following ethanol consumption has been shown to result in an avoidance of the ethanol solution throughout an 18 day post-treatment period (Boland & Stern, 1980). However, the aldehyde dehydrogenase inhibitor 4-methylpyrazole has been shown to decrease ethanol consumption in rats throughout a five day treatment and post-treatment period, but did not decrease SQ intake, and did not result in a CTA (Carr et al., 1980). Thus, the absence of immediate recovery of pre-treatment drinking levels following the

drug treatment period does not necessarily imply that a CTA was involved. In this respect, it is important to try to differentiate between the effects of drug treatments on the intake of ethanol and other fluids.

Several drugs which at first were thought to decrease ethanol intake by interfering with the reinforcing effects of ethanol were later found to be capable of also inducing prolonged decreases in saccharin consumption. For example, parachlorophenylalanine (p-CPA), a tryptophan hydroxylase inhibitor, decreased ethanol consumption for up to 33 days following 11 pairings of ethanol with p-CPA injections (Veale & Myers, 1970). Consumption of a saccharin solution was also decreased by p-CPA treatment, but took only six days to recover following 11 pairings of saccharin and p-CPA injections (Parker & Radow, 1976). The serotonin reuptake inhibitor zimeldine was shown to decrease the free choice consumption of both ethanol and SQ solutions throughout a five day drug treatment and five day post-treatment period (Gill et al., 1985), as was found with the dopamine β -hydroxylase inhibitor FLA-57 (Sirota & Boland, 1987). Like AT, zimeldine was subsequently found to induce a forward but not a backward CTA (Gill et al., 1986). However, FLA-57 was shown to induce both forward and backward CTAs, to both novel and familiar solutions (Amit et al., 1991). Thus, these examples illustrate that although a drug treatment may decrease ethanol and saccharin intake, the magnitude of the effect may not be the same for both fluids, and may be specific to certain fluid presentation and drug treatment conditions. These findings suggest that a simple CTA interpretation of the results may be too simplistic, and illustrate the need to explore some alternative interpretations of the data.

Taste Reactivity Hypothesis

Another possible explanation of the present results comes from many recent reports that ethanol intake and saccharin intake are correlated in many strains of rats (Gosnell & Krahn, 1992; Kampov-Polevoy, Kasheffskaya, & Sinclair, 1990; Overstreet et al., 1993; Sinclair, Kampov-Polevoy, Stewart & Li, 1992). The relationship between saccharin and ethanol consumption appears to be reciprocal in that saccharin intake is a good predictor of subsequent ethanol intake (Gosnell & Krahn, 1992; Overstreet et al., 1993). Conversely, rats initially selected for high ethanol intake will subsequently drink more saccharin than rats initially selected for low ethanol intake (Sinclair et al., 1992). This also appears to be true of mice in that the high ethanol drinking C57BL/6J mice (Whitney & Horowitz, 1978) also drink more saccharin than do the low alcohol drinking DBA mice (Forgie, Byerstein & Alexander, 1988). These findings suggested some commonalities in either the behavioral or neurochemical responses to ethanol and saccharin ingestion.

Evidence of behavioral similarities between the ingestion of ethanol and saccharin solutions in rats also comes from studies that manipulated the schedules of fluid presentation. Periodic presentation and withholding of ethanol, or periods of prolonged ethanol withdrawal, will increase the subsequent consumption of ethanol in rats (Sinclair & Bender, 1979; Wise, 1973). This effect has also been noted for quinine, saccharin (Wayner, et al., 1972), saline and citric acid solutions (Wayner & Fraley, 1972). When directly compared, similar patterns of consumption are seen with both ethanol and sodium saccharin solutions under a variety of schedule manipulations, which suggested that there may be similar taste factors which control the intake of these solutions (Pinel & Huang, 1976).

Rats trained to avoid ethanol solutions have been shown to display similar orofacial responses to oral infusions of both ethanol and sucrose-quinine solutions (Keifer, Bice, Orr & Dopp, 1990). This finding suggested that these two solutions may have similar taste properties. It is possible that some pharmacological manipulations might render certain tastes more or less palatable, thus affecting the subsequent intake of those flavors. While it is purely speculative at this point, it is possible that the decrease in fluid consumption in the present experiments was due to some effect of AT on taste reactivity to these solutions.

DIRECTIONS FOR FUTURE RESEARCH

In future work it would be both interesting and useful to attempt to further differentiate between the effects of AT on the intake of ethanol and other flavored fluids. This is important since AT did not have the same effect on ethanol and SQ intake in Experiments 2A and 2B. If consistent differences in the the effect of AT on ethanol and SQ intake do exist, it would provide a better indication of the amount of variability in ethanol consumption that can be accounted for by catalase activity. This goal could be achieved by extending the post-treatment period to examine the time course of recovery between the two fluid conditions in order to see whether there are any qualitative differences in this respect. Furthermore, since a different effect was found with 0.5 and 1.0 g/kg of AT on maintained ethanol consumption, it would be useful to examine the effects of several doses of AT on ethanol and SQ consumption. It would also be important to study the effects of AT on a more extensive set of concentrations of both ethanol and SQ solutions. Some drugs have been shown to have inverse effects on saccharin intake as a function of saccharin concentration (Touzani, Akarid, & Velley,

1991), therefore, it is important to pursue this possibility in future studies. In addition, it would be useful to study the effects of AT on the consumption of other flavors, such as salty and bitter, since the consumption of these flavors has also been shown to covary with ethanol intake in some strains of rats (Sinclair et al., 1992).

Considering the weight of the data which suggests that catalase activity plays an important role in the reinforcing and behavioral properties of ethanol, it is important to continue this line of research. This would be possible if another pharmacological agent could be identified which would block catalase activity, without any possible illness-inducing effects. Preliminary work from this laboratory with the nitric oxide synthase blocker L-nitro-arginine-methylester (L-NAME) has been promising in this respect.

The role of catalase in modulating voluntary ethanol intake could also be further explored by studying the effects of AT on ethanol self-administration in a paradigm in which taste factors are bypassed. For example, the effects of AT on intracerebral, intragastric, or intraventricular ethanol or acetaldehyde self-administration could be assessed without the possible confounding factors of oral self-administration.

Conclusion

It was hoped that the importance of the role of catalase in mediating learning about the pharmacological effects of ethanol during acquisition could be elucidated in the present experiments. Instead, the results pointed to the possibility that AT had a more general effect on the consumption of flavored fluids, which may have been due to an effect on taste perception, or to an illness-inducing effect of the drug. This work illustrates the need for careful controls of possible confounding factors in studies of voluntary oral ethanol

intake in animal models. Since the effect of AT on consumption was similar for both ethanol and SQ solutions, it is not reasonable to draw any firm conclusions about the role of acetaldehyde on the reinforcing properties of ethanol from this work. However, these findings do not preclude the possibility that catalase-mediated acetaldehyde formation may still be important in the mediation of voluntary ethanol intake.

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