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Brain Aldehyde Dehydrogenase and Central Acetaldehyde in the Mediation of Ethanol Consumption

Karen J. Spivak

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
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ABSTRACT

Brain Aldehyde Dehydrogenase and Central Acetaldehyde in the Mediation of Ethanol Consumption

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The rôles of brain aldehyde dehydrogenase (ALDH) and centrally-acting acetaldehyde were examined with regard to their involvement in mediating an ethanol drinking bout. In Experiment 1, it was observed that there was a relationship between the amount of ethanol consumed in a 10 min. restricted access paradigm ("simulated initial" drinking bout) and total amount consumed in a 24 hr access paradigm. These results suggested that a drinking bout was a pharmacologically meaningful event.

In Experiments 2 and 3, it was demonstrated that animals pretreated with cyanamide (an ALDH inhibitor) or 4-methylpyrazole (4MP- an alcohol dehydrogenase inhibitor) + cyanamide increased ethanol intake. However, in Experiment 4 it was shown that animals trained to ingest a saccharin-quinine solution or water during a 10 min. restricted access period, also increased fluid intake following pretreatment with cyanamide. These results suggested that cyanamide may produce a general increase in fluid intake. The magnitude of increase, however, was appreciably lower

for ethanol drinking subjects compared to water and saccharin-quinine drinking animals. In Experiment 5, the effects of cyanamide on ethanol and saccharin-quinine consumption were examined using a 24 hr access paradigm. The results suggested that cyanamide specifically suppressed ethanol intake and preference. The results of Experiment 6 suggested that those specific effects of cyanamide on ethanol may be attributed to the inhibition of brain ALDH, since pretreatment with the ALDH inhibitor coprine produced similar results. Furthermore, pretreatment with 4MP+cyanamide and 4MP+coprine resulted in a bidirectional effect on ethanol intake which was related to individual subjects' baseline levels of consumption in a 24 hr access paradigm. Together, the restricted access and 24 hr access paradigms provided evidence suggesting that the pharmacological consequences of a drinking bout may, in part, determine the pattern and intake of ethanol when given continuous ethanol availability. In addition, the results of the present series of experiments suggested that brain ALDH and by implication, centrally-acting acetaldehyde may play a role in regulating ethanol drinking behavior.

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In recent years, a great deal of evidence has emerged suggesting a role for acetaldehyde, the primary metabolite of ethanol, in some of the behavioral, pharmacological and positive reinforcing properties of ethanol (for review, see Lindros, 1978; 1985; Amir, Brown & Amit, 1980; Smith, Amit, Aragon & Socaransky, 1985). Numerous studies, however, have demonstrated that acetaldehyde, when present in peripheral circulation at high concentrations following ethanol administration, can evoke various aversive symptoms in both humans and experimental animals (see Lindros, 1978).

In addition to these well-established aversive effects, it has also been demonstrated that naive laboratory rats will learn to perform an operant maintained by response-contingent intracerebroventricular (Amit, Brown & Rockman, 1977; Amit & Smith, 1985; Brown, Amit & Rockman, 1979) or intravenous (Myers, Ng & Singer, 1984; Takayama & Uyeno, 1985) infusions of acetaldehyde. These results suggest that acetaldehyde may possess positive reinforcing properties (Amir et al, 1980; Brown et al, 1979).

It would appear, therefore, that acetaldehyde may act both as an aversive or positive reinforcing agent. Given that acetaldehyde is the primary metabolite derived from ethanol and given acetaldehyde's psychopharmacological properties, it has been postulated that the enzymes responsible for the formation and degrada-

tion of acetaldehyde may play a role in a number of the psychopharmacological effects of ethanol, including voluntary ethanol consumption (Amir, 1977; Aragon & Amit, 1985; Lindros, Kiovula & Eriksson, 1975), locomotor activity (Spivak, Aragon & Amit, 1987) and toxicosis (Mizoi et al, 1983). Accordingly, the possible contribution of these enzyme systems in modulating the pharmacological and behavioral effects of ethanol may provide invaluable information concerning acetaldehyde's role in ethanol's actions. More importantly, it has been suggested that genetic or predispositional factors towards alcoholism may be potentially related to differences in the metabolism of ethanol (Amit, Smith & Aragon, 1986; Bosron & Li, 1986). Indeed, some investigators have gone so far as to suggest that "alcoholism may be in fact acetaldehydism" (Raskin, 1975; von Wartburg & Buhler, 1984). Consequently, a better understanding of the interaction between the enzyme systems, the metabolic fate of acetaldehyde and its behavioral consequences may well provide critical insights into the motivational properties of ethanol and alcohol abuse.

The following sections will provide evidence supporting the notion that centrally-acting acetaldehyde may mediate some of the psychopharmacological effects of ethanol. A review of the psychopharmacological actions of acetaldehyde as well as evidence

suggesting that ethanol metabolizing enzymes in brain may play a role in regulating central levels of acetaldehyde will also be presented. However, before discussing these areas, the first few sections of the introduction provide an overview of the peripheral mechanisms that mediate the formation and metabolism of acetaldehyde and the evidence for the occurrence of acetaldehyde both in the periphery and brain.

Metabolism of Ethanol; Acetaldehyde Formation

Ethanol is highly soluble in water and after oral ingestion both in humans and animals, is readily absorbed from the gastrointestinal tract where it diffuses rapidly and uniformally throughout the body (Erickson, 1979). The elimination of absorbed ethanol is extremely efficient with over 90% being metabolized in the body (Erickson, 1979).

There is general agreement that the principal enzyme responsible for the initial metabolism of ethanol to acetaldehyde is the NAD+ - dependent alcohol dehydrogenase (see Hawkins & Kalant, 1972; Lundquist, Tygstrup, Winkler, Mellemgaard & Munck-Petersen, 1962). Alcohol dehydrogenase is located primarily in the cytosolic region of the cell (Havre, Margolis, Abrams & Landau, 1976) and is abundant in the liver where over 90% of ethanol ingested is oxidized to acetaldehyde (Hawkins & Kalant, 1972). Smaller amounts of alcohol

dehydrogenase have been detected in extrahepatic tissue including the kidneys, gastric and intestinal mucosa (Lundquist, 1971; Raskin & Sokoloff, 1972) and trace amounts have also been found in brain (Buhler, Pestalozzi, Hess & von Wartburg, 1983; Raskin & Sokoloff, 1968; 1970).

Although alcohol dehydrogenase is the principal ethanol-oxidizing enzyme in the liver, ethanol may also be metabolized by the NADPH- and O_2- dependent microsomal ethanol-oxidizing system (MEOS: Lieber & DeCarli, 1968; Lieber, 1977,) and by the peroxidatic H_2O_2- dependent catalase system (Keilin & Hartree, 1945; Wendell & Thurman, 1979). The participation of these non-alcohol dehydrogenase pathways in ethanol metabolism appears to be minimal under normal conditions. However, some investigators argue that at high blood ethanol concentrations (> 20 mM) and after chronic ethanol ingestion, these ethanol metabolizing systems may have a more active role in ethanol metabolism and subsequently enhance acetaldehyde formation (e.g. Hawkins & Kalant, 1972; Leiber, 1977). Nevertheless, other investigators have found very little evidence to support any relevant contribution to the synthesis of acetaldehyde from non-alcohol dehydrogenase pathways in vivo at high blood ethanol levels or after chronic administration (Khanna, Lindros, Israel & Orrego, 1977; Lindros, Salaspuro & Pikkarainen, 1977).

Metabolism of Ethanol: Elimination of Acetaldehyde

Acetaldehyde that is formed through the oxidation of ethanol is rapidly metabolized to acetate by the NAD-dependent enzyme aldehyde dehydrogenase (Hawkins & Kalant, 1972; Lundquist, 1971; Weiner, 1979). Because aldehyde dehydrogenase (ALDH) is the primary enzyme responsible for oxidizing biogenic aldehydes to acids, it is widely distributed in mammalian tissues (Deitrich, 1966) and can be found in the mitochondrial, cytosolic and endoplasmic reticular regions of the cell (Pettersson & Tottmar, 1982; Tottmar, Pettersson & Kiessling, 1973). Although a large quantity of ALDH is found in the liver, considerable levels are found in kidney, small intestine and brain (Deitrich, 1966; Koivula, Turner, Huttunen & Koisvusalo, 1981; Pettersson & Tottmar, 1982; Pietruszko, Reed, Vallari, Major, Saini & Hawley, 1981). Two major classes of ALDH have been found in liver of both humans and rats (Deitrich, 1966; Goedde & Agarwal, 1987; Harada, Agarwal & Goedde, 1978). A low Km isoenzyme of ALDH with a high affinity for acetaldehyde is located in the mitochondria of cells (Pettersson & Tottmar, 1982; Weiner, 1986) and a high Km isoenzyme of ALDH with a low affinity for acetaldehyde is located in the cytosolic fraction of cells (Pettersson & Tottmar, 1982). With improved methods for cellular fractionation and enzyme assays, hepatic mitochondrial ALDH which has a

high affinity to acetaldehyde, has been implicated as the principal enzyme responsible for acetaldehyde oxidation (Lindros, Vihma & Forsander, 1972; Socaransky, Aragon, Amit & Blander, 1984; Svanas & Weiner, 1985).

As with ethanol metabolism, the liver is the primary site of acetaldehyde elimination and is capable of metabolizing 90-95% of acetaldehyde produced during ethanol oxidation (see Lindros, 1978). Acetaldehyde metabolism can occur in extrahepatic tissue including kidney, muscle (Deitrich, 1966) and brain (Deitrich, 1966; Mukherji, Kashiki, Ohyanagi & Sloviter, 1975, Pettersson & Tottmar, 1982a). Hepatic ALDH, however, is so efficient in eliminating acetaldehyde that only part of the extrahepatic capacity is normally used (Lindros, 1978).

It has been postulated that acetaldehyde may play a role in the mediation of some of the actions of ethanol in the central nervous system (Smith et al, 1985; Walsh, 1971). To this end, the source of acetaldehyde responsible for these effects may arise from circulating blood acetaldehyde levels derived from the hepatic oxidation of ethanol or from direct ethanol oxidation in brain. The pharmacological effects of acetaldehyde in the central nervous system will therefore depend on the amount of acetaldehyde that leaves the liver and reaches brain (via peripheral

circulation) or that is formed directly in brain. In the next two sections, a brief overview of the occurrence of acetaldehyde in the periphery and brain is presented.

Occurrence of Acetaldehyde in the Periphery

Minute amounts of acetaldehyde have been detected in the peripheral circulation of rats following the administration of moderate doses of ethanol (Eriksson, 1977; Eriksson & Sippel, 1977). When larger doses of ethanol (> 2gm/kg) are administered, the rate of ethanol oxidation may exceed the rate of hepatic acetaldehyde elimination capacity, resulting in elevated peripheral acetaldehyde concentrations (Lindros et al, 1972; Raskin & Sokoloff, 1972; Weiner, 1979). However, a recent study using mice demonstrated that no acetaldehyde could be detected in blood even after a challenge with a high dose of ethanol (3 gm/kg), when proper control samples were included in the assay procedure (Eriksson, Atkinson, Petersen & Deitrich, 1984). It is possible, nevertheless, that the discrepency in blood acetaldehyde levels, cited above, may be partly attributed to the use of different rodent strains (Eriksson & Sippel, 1977).

Chronic ingestion of ethanol may also lead to elevated blood acetaldehyde levels (Lindros, Stowell, Pikkarainen & Salaspuro, 1980, Palmer & Jenkins, 1980).

It is known that prolonged ethanol exposure may disrupt liver function (e.g. Hasumura, Teschke & Lieber, 1975). One such consequence of this disruptive influence may be a decrease in hepatic mitochondrial ALDH activity, thereby reducing the capacity of the liver to oxidize acetaldehyde and subsequently increasing circulating levels of acetaldehyde (Hasumura et al, 1975; Jenkins & Peter, 1980; Palmer & Jenkins, 1982).

Peripheral blood concentrations of acetaldehyde can also be elevated by pharmacological agents that are capable of inhibiting hepatic ALDH. Two such agents widely used in the treatment of alcoholism are disulfiram (Antabuse) and the cyanamide derivative, calcium carbimide (Temposil) (Ritchie, 1970; Sellers, Naranjo & Peachey, 1981). In the presence of ethanol, these agents induce a reaction referred to as the Disulfiram-alcohol-reaction (DAR: Truitt & Walsh, 1971; Kitson, 1977). Symptoms of this reaction include vasodilation, tachycardia, decrease in blood pressure, dizziness, nausea and vomiting (Kitson, 1977). In more severe cases, respiratory depression, cardiovascular collapse and death may occur (Jacobsen, 1952).

The effects of these compounds on both man and animal have been attributed to their ability to inhibit ALDH activity, thereby causing an accumulation of acetaldehyde in blood and tissue after ethanol administration (Kitson, 1977; Marchner & Tottmar, 1978). It has

been demonstrated both in humans as well as laboratory animals that the administration of disulfiram or cyanamide reduces voluntary consumption of ethanol (Amit, Brown, Amir, Smith & Sutherland, 1980; Eriksson, 1980; Mottin, 1973; Sinclair & Lindros, 1981). However, there have also been other reports indicating no change in voluntary ethanol intake in laboratory rodents following treatment with cyanamide (e.g. Amit, Levitan & Lindros, 1976). The discrepancy in findings may be due to procedural differences in cyanamide treatment as well as the use of different drinking schedules. For example, in the study by Sinclair and Lindros (1981) cyanamide was presented in the food and animals had continuous access to ethanol. Subjects in the study by Amit et al (1976) were presented with an ethanol solution every second day and cyanamide was administered intraperitoneally only on days when animals had access to ethanol.

The widely accepted explanation for the demonstrated reductions in voluntary ethanol consumption is that acetaldehyde, at high levels in the blood (as a consequence of ALDH inhibition), is toxic and produces aversive effects (Lindros et al, 1975; Schlesinger, Kakihana & Bennet, 1966; Sellers et al, 1981). This view has recently been challenged by Sinclair and Lindros (1981), who demonstrated that prevention of the accumulation of blood acetaldehyde by a concurrent

treatment with cyanamide and the alcohol dehydrogenase inhibitor 4-methylpyrazole still resulted in the suppression of ethanol drinking in rats. These authors concluded that acetaldehyde accumulation in the periphery was not responsible for the suppression of ethanol drinking following cyanamide pretreatment.

However, the contention that high circulating levels of acetaldehyde may limit subsequent ethanol drinking has been supported by investigations of the innate ethanol-sensitivity observed in some Orientals (Goedde, Harada & Agarwal, 1979; Mizoi et al, 1983; Wolff, 1972). These studies revealed that at least 50% of Japanese lack the hepatic mitochondrial low Km enzyme ALDH (Harada, Misawa, Agarwal & Goedde, 1980; Mizoi et al, 1983). Following the consumption of low to moderate doses of ethanol, these individuals have much higher blood acetaldehyde levels than that found in Caucasians and Japanese without this deficiency after ingestion of similar amounts of ethanol. Because of the inability to metabolize acetaldehyde quickly and efficiently, these Orientals may have a heightened sensitivity to ethanol and experience dysphoric reactions (Goedde et al, 1979; Mizoi et al, 1979). This reaction is similar to that observed in alcoholics who consume ethanol while receiving disulfiram or calcium carbimide (see, Brien & Loomis, 1986; Kitson, 1977). On the basis of these findings, it has been

postulated that the low Km ALDH deficiency in Orientals may, in fact, be a genetic factor protecting them from developing alcoholism and may also help explain the lower prevalence of alcoholism among Orientals (Goedde et al, 1979; Goedde & Agarwal, 1987). Taken together, the findings suggest that elevated blood acetaldehyde levels may be aversive and deter subsequent drinking. However, recently it has been reported that at a low dose of ethanol, non- alcoholic subjects pretreated with disulfiram or calcium carbimide experienced "euphoria" which was associated with slightly elevated blood acetaldehyde levels (Brown, Amit, Smith, Sutherland & Selvaggi, 1983).

It should be noted that in most cases, elevated blood acetaldehyde levels are detectable only when acetaldehyde levels are abnormally (e.g. ALDH deficiency) or artificially (e.g. with ALDH inhibitors) increased. However, under conditions in which animals voluntarily consume ethanol (i.e. free choice paradigm) and after moderate consumption in Caucasians and some Orientals, acetaldehyde cannot readily be detected in blood (for review, see Eriksson, 1980; 1983; Lindros, 1983).

The inability to detect such low levels of acetaldehyde may be partly attributed to the fact that most studies have used assay procedures that determine unbound acetaldehyde in plasma extracts (e.g. Eriksson,

1983). Recently, it has been questioned whether the low levels of acetaldehyde detected in blood plasma actually reflect the true concentration of acetaldehyde in blood (DiPadova, Alderman & Lieber, 1986). Using an improved method for acetaldehyde measurement in red blood cells, DiPadova et al (1986) and Baraona, DiPadova, Tabasco and Lieber (1987) reported significantly higher concentrations of acetaldehyde in red cells and total blood than in plasma extract. Moreover, it was demonstrated that acetaldehyde could bind reversibly to red blood cells (erythrocytes) and be transported to and taken up by extrahepatic tissues (Baraona et al, 1987).

These findings suggested that acetaldehyde can be detected in total blood following the administration of ethanol and can be transported to various tissues, including brain. Therefore, it is conceivable that some quantities of acetaldehyde, albeit low, formed through the hepatic oxidation of ethanol, can be transported in blood to the brain.

Presence of Acetaldehyde in the Brain

It has been frequently reported that ethanol diffuses through body tissue and can be readily detected in brain tissue (e.g. Ritchie, 1970). Acetaldehyde, because of its high lipid affinity, can also easily diffuse through various organs including

the brain (Akabane, 1971; Lindros, 1978). However, attempts to measure or detect the presence of acetaldehyde in brain following exposure to ethanol has yielded ambiguous results. Earlier studies reported the presence of acetaldehyde in the brain of ethanol-treated animals at levels equal to or greater than acetaldehyde levels measured in cerebral blood (Duritz & Truitt, 1966; Kiessling, 1962; Majchrowitz, 1973). The relatively high levels of acetaldehyde observed in these earlier studies have been disputed because of technical and methodological difficulties (Eriksson, 1980; Eriksson et al, 1984; Lindros, 1983; Sippel, 1972). The problem with all these procedures was the spontaneous non-enzymatic formation of acetaldehyde by ethanol oxidation during sample preparation (Sippel, 1972; Truitt, 1970). Sippel (1972) demonstrated that the addition of thiourea to the deproteinized brain homogenate prevented the non-enzymatic release of acetaldehyde. When thiourea was used in the assay procedure of subsequent studies, acetaldehyde levels were extremely low or undetectable in brain tissue of rats after an injection of 3 gm/kg of ethanol (Eriksson & Sippel, 1977; Sippel, 1974). Acetaldehyde was detected in brain only if cerebral blood levels exceeded 200 nM following pretreatment with cyanamide (Eriksson and Sippel, 1977). In contrast, Tabakoff, Anderson and Ritzman (1976) injected mice with 3 gm/kg

of ethanol and detected very low brain acetaldehyde levels (about 6 nM) when the concentration of acetaldehyde in the blood was approximately 70 nM.

Since acetaldehyde levels in the central nervous system would reflect brain tissue concentration more accurately than peripheral blood levels (Eriksson & Sippel, 1977; Nuutinen, Salaspuro, Valle & Lindros, 1984), some studies have examined acetaldehyde concentrations in cerebrospinal fluid. In one study, cerebrospinal fluid of rats was shown to contain acetaldehyde at concentrations lower than that found in blood but at levels appreciably higher than those found in brain following ethanol administration (3 gm/kg) (Kiianmaa & Virtanen, 1978). Pettersson and Kiessling (1977) also demonstrated the presence of acetaldehyde in cerebrospinal fluid of rats. However, they reported a direct relationship between concentrations of acetaldehyde in blood and cerebrospinal fluid. The discrepency between these two studies may be attributed to the large number of methodological differences. However, what was apparent from these studies was that acetaldehyde levels in cerebrospinal fluid after acute ethanol administration did not reflect acetaldehyde concentrations found in brain. Despite the differences in acetaldehyde concentrations in cerebrospinal fluid and brain, the presence of acetaldehyde in cerebrospinal fluid suggests that acetaldehyde may have a

functional role in mediating some of the actions of ethanol in the central nervous system.

Various theories have been proposed to account for the lack of a direct relationship between levels of acetaldehyde in blood or cerebrospinal fluid and those found in brain. It has been suggested that the cerebral capillary walls may act as a special enzymatic blood brain barrier, limiting the entry of circulating acetaldehyde to the brain (Eriksson & Sippel, 1977; Kiianmaa & Virtanen, 1978; Sippel, 1974; Tabakoff et al, 1976). However, the presence of acetaldehyde in cerebrospinal fluid indicates that acetaldehyde may cross the blood brain barrier. It has also been proposed that the presence of high affinity brain ALDH may result in lower levels of acetaldehyde in brain than in cerebrospinal fluid (Pettersson & Kiessling, 1976). A study by Westcott, Weiner, Shultz and Myers (1980) supports this notion. Using a push-pull perfusion technique, these authors detected acetaldehyde in the interstitial fluid of rat brain (5-20 nM) after intragastric administration of ethanol (4.5 gm/kg). At the termination of this procedure, the rats were sacrificed and the brains were extracted for acetaldehyde determination. Similar to the results of previous studies (Eriksson & Sippel, 1977; Kiianmaa & Virtanen, 1978), Westcott et al (1980) did not detect acetaldehyde in whole brain unless blood levels of

acetaldehyde were artificially elevated using the ALDH inhibitor disulfiram. Westcott et al (1980) concluded that the presence of acetaldehyde in the extracellular fluid of the brain indicated that acetaldehyde could cross the blood brain barrier. Furthermore, the absence (or undetectable levels) of acetaldehyde in whole brain tissue may have resulted from the rapid oxidation of acetaldehyde by brain ALDH. In addition, these authors suggested that acetaldehyde present in the interstitial fluid of the brain could impinge upon the environment of the neuron, thereby exerting some pharmacological effect.

Additional studies examining brain enzyme activity have suggested the possible presence of acetaldehyde in cerebral tissue. The brain does possess the necessary oxidative machinery for ethanol metabolism. Ethanol can be metabolized via brain alcohol dehydrogenase. Alcohol dehydrogenase has been detected in the brain (Buhler et al, 1983; Raskin & Sokoloff, 1970; 1972) and its activity has been reported to increase following chronic ethanol administration in mice (Raskin & Sokoloff, 1974). However, only minute amounts of alcohol dehydrogenase is present in the brain and very little acetaldehyde may actually be formed through this route (Lindros, 1978; 1985). It is therefore unlikely that alcohol dehydrogenase plays a role in ethanol metabolism in brain.

Ethanol metabolism may also occur via the formation of reactive hydroxyl radicals during the oxidation of ascorbate (Cohen, 1977). Another potential oxidative pathway may be via cytochrome P-450, whose presence in brain has been established (Paul, Axelrod & Diliberts, 1977). There is, however, no direct evidence to support the role of these two systems in ethanol metabolism in brain.

Ethanol oxidation may occur through the peroxidatic activity of brain catalase. Studies using various biochemical and histochemical methods have verified that catalase is present in brain (Brannan, Maker & Raes, 1981; Gaunt & DeDuve, 1976; McKenna, Arnold & Holtzman, 1976). In addition, Cohen, Sinet and Heikkila (1980) presented biochemical evidence of ethanol oxidation in rat brain in vivo via the peroxidatic activity of brain catalase. In this study, treatment with ethanol prior to the administration of the catalase inhibitor 3-amino-1,2,4-triazole prevented the inhibition of catalase in rat brain suggesting that ethanol competed successfully with the inhibitor. These authors concluded that their results constituted indirect evidence for ethanol metabolism in brain. In addition, in vitro studies using neuroglia and neuroblastoma cell lines have shown that neuroglia cells were extremely efficient in metabolizing ethanol to acetate (Wickramsinghe, 1987). Moreover, neuroblastoma

cells significantly reduced ethanol metabolism in the presence of 3-amino-1,2,4-triazole, suggesting that catalase may play a role in ethanol metabolism in neural tissue.

The brain also possesses the necessary oxidative machinery for the removal of acetaldehyde. Mukherji et al (1975) demonstrated that the addition of radioactively-labelled acetaldehyde to an isolated rat brain preparation resulted in a significant incorporation of radioactivity into the free amino acids of the brain sample. The authors therefore suggested that the rat brain was capable of metabolizing acetaldehyde. Since ALDH is present in considerable quantities in brain (Deitrich, 1966; Koivula et al, 1981), ALDH has been proposed to be the most likely candidate responsible for the elimination of acetaldehyde as it has a high affinity towards this substrate (Duncan & Tipton, 1971; Erwin & Deitrich, 1966; Mukherji et al, 1975).

In summary, the presence of acetaldehyde in brain may arise from circulating blood acetaldehyde produced by the hepatic oxidation of ethanol. However, acetaldehyde appears to be detected in brain only following a very high dose of ethanol or when acetaldehyde metabolism has been blocked with ALDH inhibitors (e.g. Eriksson & Sippel, 1977; Westcott et al, 1980). Alternatively, the brain does possess the oxidative machinery for the production and degradation of acet-

aldehyde, suggesting that acetaldehyde metabolism can occur directly in brain. To date, the presence of appreciable amounts of acetaldehyde in brain during ethanol administration has not been conclusively demonstrated. However, the evidence provided by numerous behavioral studies presented in the following sections supports the contention that centrally-acting acetaldehyde may play a role in the mediation of some of the central actions of ethanol.

Psychopharmacological Effects of Acetaldehyde

Aside from the well-known aversive effects (Eriksson, 1980a), acetaldehyde has also been shown to possess primary positive reinforcing properties, implicating this compound also in voluntary ethanol consumption (see Amir et al, 1980; Amit et al, 1986; Lindros, 1985).

In earlier studies, Myers and his coworkers demonstrated that intraventricular infusions of acetaldehyde, as well as a variety of alcohols, aldehydes and alkaloids, increased ethanol drinking in rats and Rhesus monkeys (Myers & Veale, 1969; Myers, Veale & Yaksh, 1972; Myers & Melchoir, 1977). However, using similar paradigms other investigators have been unable to replicate some of those findings (Friedman & Lester, 1975; Amit, Smith, Brown & Williams, 1982). Brown et al (1979) demonstrated that naive rats would self-

administer acetaldehyde into the cerebral ventricles but would not perform the operant task when ethanol infusions were used as the reinforcer. These same authors also reported that the propensity to self-administer acetaldehyde intraventricularly was positively correlated with ethanol preference (Brown, Amit & Smith, 1980). It was therefore suggested that the central reinforcing effects of acetaldehyde may mediate, at least in part, voluntary ethanol consumption in rats (Brown et al, 1980).

Consistent with these findings, it has been shown that animals will learn to self-administer acetaldehyde intravenously, suggesting that low peripheral concentrations of acetaldehyde may also be reinforcing (Myers, Ng & Singer, 1982; 1984; Myers, Ng, Marsuki, Myers & Singer, 1984; Takayama & Uyeno, 1985). Moreover, it was also shown that animals with a prior history of acetaldehyde self-administration subsequently consumed more ethanol when they were later presented with a free choice of increasing concentrations of ethanol and water (Myers et al, 1984; Myers, Ng, Marsuki et al, 1984). These results suggested that acetaldehyde may possess positive reinforcing properties and prior exposure to acetaldehyde may facilitate the subsequent acquisition of ethanol intake. Furthermore, it has been reported that naive rats self-administered acetaldehyde intravenously at much higher rates compared to

animals lever pressing for intravenous infusions of ethanol (Takayama & Uyeno, 1985). These findings suggested that acetaldehyde may be a more potent reinforcer than ethanol.

Using another paradigm to examine the reinforcing properties of acetaldehyde, Smith, Amit and Splawinsky (1984) investigated the effects of multiple intracerebroventricular infusions of acetaldehyde on conditioned place preference. Earlier studies examining the phenomenon of place conditioning used aversive agents such as irradiation as the unconditioned stimulus (Garcia, Kimeldorf & Hunt, 1957). In a study by Garcia et al (1957), rats showed a clear aversion to environmental cues that had been paired with irradiation. Recently, conditioned place preference has been shown to occur following the administration of self-administered drugs such as morphine (Blander, Hunt, Blair & Amit, 1984), heroin (Schenk, Hunt, Colle & Amit, 1983) and cocaine (Spyraki, Fibiger & Phillips, 1982). Consistent with these findings, Smith et al (1984) demonstrated that multiple intracerebroventricular infusions of acetaldehyde induced conditioned placed preference in rats.

The implication of these studies is that acetaldehyde may be active at low doses which nevertheless appear to support intravenous and intraventricular self-administration of acetaldehyde. Moreover, these

findings suggest that acetaldehyde may possess positive reinforcing properties. Further support for this notion can be found in several studies examining ethanol's effects on human subjects. As mentioned earlier, the ALDH inhibitors disulfiram and calcium carbimide are commonly used as adjuncts in the treatment of alcoholism. It is presumed that the aversive symptoms associated with ethanol consumption while on this medication will deter alcoholics from further drinking. In an earlier study, it was reported that patients consuming low to moderate doses of ethanol following the administration of calcium carbimide (Temposil) expressed feelings of well-being with an increasing desire to drink (Minto & Roberts, 1960). In a more recent study, subjects pretreated with the ALDH inhibitors disulfiram or calcium carbimide reported enhanced euphoria and stimulation which was associated with elevated blood acetaldehyde levels following the consumption of low doses of ethanol (Brown et al, 1983). No such effects were reported in placebo-treated subjects consuming the same amounts of ethanol. The authors concluded that the potentiation of the "euphoric" effects of low doses of ethanol with the ALDH inhibitors appeared to be attributed to the increased availability of acetaldehyde to the brain. In another study where blood acetaldehyde levels were not manipulated, it was reported that ethanol-induced

increases in mood (i.e. talking easy and feeling good) following the ingestion of ethanol was positively correlated with blood acetaldehyde levels (Behar et al, 1983). The results of these behavioral studies suggest that acetaldehyde at low doses may be positively reinforcing and may mediate ethanol consumption. On the basis of this notion, it has been suggested that acetaldehyde has central pharmacological effects which appear to be involved in the actions of ethanol (e.g. Amit et al, 1986; Smith et al, 1985). The following section provides evidence suggesting that the source of centrally-acting acetaldehyde responsible for these effects may be derived directly in brain.

The Role of Ethanol-Metabolizing Enzymes in Mediating the Psychopharmacological Effects of Ethanol

It is possible that the presence of centrally-acting acetaldehyde in the brain may conceptually arise from circulating blood acetaldehyde levels produced by hepatic oxidation of ethanol. This route, however, has been questioned since the levels of acetaldehyde resulting from voluntary ethanol consumption in animals are very low or even below the level of detection in peripheral blood and consequently in brain (Deitrich, 1987; Eriksson & Sippel, 1977; Sippel, 1974). In view of more recent findings, however, it is possible that acetaldehyde, reversibly bound to erythrocytes, may be

transported from hepatocytes to the brain (Baraona et al, 1987; DiPavoda et al, 1986). A second possible route contributing to the central pharmacological actions of acetaldehyde may be direct ethanol oxidation in brain via the enzyme catalase. Ethanol readily passes into brain tissue (Ritchie, 1970) and catalase has been suggested to play a putative role in the oxidation of ethanol in rat brain (Cohen et al, 1980).

Over the last few years, there has been increasing evidence to suggest that catalase-derived acetaldehyde may mediate some of the psychopharmacological effects of ethanol. Acetaldehyde has been shown to possess both aversive and reinforcing properties (see Lindros, 1978; 1985). A common property shared by a variety of self-administered drugs including ethanol and acetaldehyde is the ability to induce a conditioned taste aversion (CTA) (for review, see Goudie, 1979; Hunt & Amit, 1987). Earlier studies demonstrated that rodents exposed to a novel flavored solution and then injected with an aversive or emetic agent (e.g. lithium chloride) reduced their intake of that flavored substance on subsequent presentations (e.g. Nachman, 1963; Nachman & Ashe, 1973). This reduction in intake of the test substance was assumed to be due to an association between the taste of the substance and some aversive action of the drug. It has been suggested that the nature of CTAs induced by self-administered

drugs including ethanol are qualitatively different than CTAs induced by emetic agents such as lithium chloride (Hunt & Amit, 1987). While the evidence would in fact suggest that lithium chloride-induced CTAs are mediated by conditioned illness or toxicity, other studies suggest that CTAs induced by self-administered drugs may be functionally related to the positive reinforcing properties of these agents (Sklar & Amit, 1977; Switzman, Amit, White & Fishman, 1978). Thus, CTAs induced by self-administered drugs including ethanol may not be based on a single pharmacological variable such as gastrointestinal malaise (as in the case of lithium chloride) but are related to a constellation of stimulus properties of these drugs, reflecting the "euphoric/dysphoric" interaction of the drugs' effects (Colpaert, 1978; Hunt & Amit, 1987).

There are numerous studies demonstrating CTAs induced by ethanol (e.g. Cappell, LeBlanc & Endrenyi, 1973; Lester, Nachman & LeMagnen, 1970). The role of acetaldehyde, if any, in mediating this effect is at present unclear. Because of its toxic properties, it is conceivable that circulating blood acetaldehyde levels produced by ethanol metabolism may play some role in ethanol-induced CTAs. However, acetaldehyde is self-administered by rats indicating that this metabolite may mediate the positive reinforcing effects of ethanol (Brown et al, 1978). It is possible then, that

both ethanol self-administration and ethanol-induced CTA may be mediated by acetaldehyde and are functionally related to the "euphoric/dysphoric" properties of acetaldehyde. Work by Aragon, Spivak and Amit (1985) has implicated catalase-derived acetaldehyde in brain in mediating CTAs induced by ethanol. Rats, pretreated with the catalase inhibitor 3-amino-1,2,4-triazole (AT), did not demonstrate a CTA normally induced by ethanol. It was therefore suggested that the blockade of ethanol-induced CTA by AT was due to its interference in the production of centrally-formed acetaldehyde via brain catalase. More directly, this effect seemed specific to ethanol, as pretreatment with AT failed to attenuate or block CTAs induced by morphine or lithium chloride (an emetic agent). The authors concluded that brain catalase may participate specifically in ethanol metabolism in brain and that centrally-formed acetaldehyde may mediate CTAs induced by ethanol.

To determine the contribution of catalase-derived acetaldehyde in mediating other psychopharmacological effects of ethanol, the same authors investigated the role of centrally-formed acetaldehyde in the depressant effects of ethanol using the open field paradigm (Aragon, Spivak & Amit, 1985). The results indicated that rats pretreated with AT did not demonstrate depression of locomotor activity following ethanol

administration (2 gm/kg). In yet another study, these authors reported that pretreatment with AT attenuated ethanol-induced narcosis in rats (Aragon, Spivak & Amit, 1987). Together, these findings suggest that acetaldehyde formed centrally via the peroxidatic activity of brain catalase may also be important in mediating the psychopharmacological effects of ethanol-induced locomotor depression and narcosis.

As a consequence of these findings, Aragon and Amit (1987) compared a variety of ethanol-related behaviors in rats pretreated with AT or vehicle to the behavioral responses of the two mouse strains C57BL/6 and DBA/2. These mouse strains were chosen because it was shown that C57BL/6 mice had approximately 35% less brain catalase activity than DBA/2 (Aragon & Amit, 1987). The authors reported, for example, that following a challenge with ethanol, C57BL/6 mice did not demonstrate an ethanol-induced CTA (Horowitz & Whitney, 1975) and slept for a shorter period of time (Damjanovich & MacIunes, 1973) than DBA/2 mice. The behavioral responding of C57BL/6 mice following ethanol administration was similar to that reported earlier for rats pretreated with AT. Pretreatment with AT resulted in a blockade of ethanol-induced CTA (Aragon et al, 1986) and attenuated sleep time in rats (Aragon et al, 1987). The authors suggested that this similarity in ethanol-related behaviors provided further support for

the notion that centrally-acting acetaldehyde produced through the activity of brain catalase may mediate many of the psychopharmacological actions of ethanol.

It appears that catalase-derived acetaldehyde may play a role in mediating many of the central actions of ethanol. It is conceivable then, that centrally-produced and centrally-acting acetaldehyde may also mediate ethanol-motivated behaviors under conditions of normal voluntary ethanol intake (Amir et al, 1980; Amit et al, 1986; Aragon & Amit, 1985; Lindros, 1985). Recently, Aragon, Sternklar and Amit (1985) demonstrated a direct relationship between brain catalase activity and voluntary ethanol consumption in rats. Animals that consumed more ethanol were also found to have higher levels of brain catalase activity. The authors also reported that there was no induction of brain catalase in animals following 25 days of forced-choice exposure to ethanol. It was therefore postulated that the central formation of acetaldehyde via brain catalase may play a regulatory role in ethanol intake. In an attempt to demonstrate that catalase activity plays a role in regulating ethanol consumption, Amit and Aragon (1987) measured catalase activity in tail blood of naive rats collected prior to exposure to ethanol. Following a stable period of ethanol consumption, animals were sacrificed and brain

homogenates were assayed for catalase activity. The authors reported a relationship between blood and brain catalase activity and levels of ethanol consumption in rats. These results suggested that brain catalase and its potential ability to synthesize acetaldehyde directly in brain may play a role in regulating ethanol intake.

It has also been reported that a direct relationship exists between brain ALDH activity and ethanol consumption under a variety of manipulations and conditions (Amir, 1977; Amir, 1978a; Amir & Stern, 1978; Sinclair & Lindros, 1981; Socaransky et al, 1985; Socaransky, et al, 1984). Two recent studies investigated the role of brain ALDH in mediating some of the psychopharmacological effects of ethanol (Spivak, Aragon & Amit 1987; 1987a). These authors used various enzyme inhibitors in an attempt to assess the putative roles of, primarily, liver ALDH (as reflected by blood acetaldehyde levels) and brain ALDH in mediating ethanol-induced CTA (Spivak et al, 1987) and locomotion (Spivak et al, 1987a). In the CTA study, animals pretreated with cyanamide or 4-methylpyrazole (4MP) and cyanamide (a treatment manipulation which prevents the accumulation of blood acetaldehyde) both demonstrated a potentiation of ethanol-induced CTA at the lowest dose (0.4 gm/kg) and an attenuation of CTA at the highest dose (1.2 gm/kg) in comparison to the control groups.

This effect could not be attributed to elevated blood acetaldehyde levels since pretreatment with 4MP and cyanamide prevents the peripheral accumulation of acetaldehyde (Hillbom, Sarviharju & Lindros, 1983; Sinclair & Lindros, 1981). Similar results were obtained from a study examining the effects of cyanamide on ethanol-induced locomotor activity (Spivak et al, 1987a). Animals pretreated with cyanamide or 4MP and cyanamide demonstrated more locomotor depression at the lowest dose of ethanol and an attenuation of locomotor depression at the highest dose compared to the control group. The results of the two studies suggest that brain ALDH may also play a role in mediating some of the psychopharmacological effects of ethanol, perhaps by regulating acetaldehyde levels in brain.

These results support the findings from an earlier study examining the role of brain ALDH in ethanol consumption. Sinclair and Lindros (1981) concluded that the suppression of ethanol consumption by cyanamide was due to its direct inhibition of brain ALDH and not to elevated blood acetaldehyde levels. They suggested a role for brain ALDH in the central regulating mechanisms of ethanol drinking. This notion is consistent with other reports suggesting a systematic relationship between ethanol intake and brain ALDH activity (Amir, 1977; 1978b; Socaransky et al, 1985).

Taken together, these studies suggest a role for centrally-acting acetaldehyde in the mediation of many of the psychopharmacolgical effects of ethanol and in the mediation of ethanol consumption. Furthermore, the enzymes which regulate the production and degradation of acet- aldehyde in brain may be a critical link in mediating the psychopharmacological effects of acetaldehyde. On the basis of these findings, it has been postulated that the modulation of ethanol intake may be dependent, in part, on the brain's capacity to form and eliminate acetaldehyde centrally (Amit et al, 1986; Aragon and Amit, 1985; Socaransky et al, 1984).

On the surface, an "apparent contradiction" appears to exist between the roles of brain catalase and brain ALDH in ethanol consumption. This contradiction refers to the fact that both enzymes are correlated with ethanol consumption, yet each enzyme appears to have a different function. Brain catalase may play a role in the production of acetaldehyde (through the metabolism of ethanol) and ALDH may play a role in the degradation of acetaldehyde in brain. To address this "apparent contradiction", Aragon and Amit (1985) proposed a two dimensional model of ethanol consumption which emphasized a dynamic relationship between these two enzyme systems in brain. The authors developed a mathematical function relating the synthesis and turnover (or degradation) of acetaldehyde

based on the kinetic properties of catalase and ALDH, respectively. They reported a highly significant correlation between ethanol consumption and the levels of acetaldehyde per unit of time, derived from the model and determined by the synthesis and degradation of acetaldehyde. Moreover, the correlation coefficent derived from the mathematical model was higher than either of the individual correlations between ethanol intake and brain catalase or brain ALDH. These findings suggested that the regulation of ethanol intake may be dependent on the regulation of levels of acetaldehyde in brain per unit of time, mediated by a potential dynamic interaction between brain catalase and ALDH. Aragon and Amit (1985) further suggested that the central enzymatic control of ethanol consumption may be based on the resultant central effects of acetaldehyde.

The Present Investigation

In the traditional voluntary ethanol consumption paradigm, animals are presented with continuous access to a free choice of ethanol and water. Typically, ethanol intake is measured once daily at the end of a 24 hr period. However, it is well established that animals consume ethanol in a series of discrete bouts over the 24 hr access period (Gentry, Rappaport & Dole, 1983; Gill, France & Amit, 1986; Iso, 1987; Marcucello, Munro & MacDonall, 1984) and, in

particular, during the active or night cycle (Gill et al, 1986; Iso, 1987). As a result of employing the traditional oral consumption paradigm, series of discrete drinking behaviors are collapsed to reflect a single behavioral event (i.e. total ethanol intake in 24 hr). In order to understand the behavioral determinants that underlie ethanol-motivated behavior, it is most crucial to understand the components that comprise the behavior. Gill et al (1986) reported that ethanol intake in a 24 hr paradigm was characterized by short rapid bouts occurring predominantly during the night cycle. Total ethanol intake was reflected by the size and frequency of bouts over the 24 hr period.

On the basis of the ethanol drinking patterns established in the 24 hr access paradigm, Gill et al (1986) attempted to determine the pharmacological efficacy of these discrete drinking bouts. They devised a "simulated drinking bout" whereby animals were trained to consume ethanol within a restricted 10 min. drinking period. The results indicated that animals drank sufficient quantities of ethanol (0.6 gm/kg) during the "simulated bout" to produce pharmacologically meaningful blood and brain ethanol levels as well as to alter performance in a passive avoidance task and exploratory/locomotor activity. The authors concluded that animals were motivated to ingest pharmacologically meaningful levels of ethanol both in

the limited access and 24 hr access paradigms. These results are supported by recent findings demonstrating that animals consumed sufficient quantities of ethanol to produce detectable and pharmacologically relevent blood ethanol levels during a 30 min. (e.g. Grant & Samson, 1985; Samson, 1986) and 60 min. (Linseman, 1987; Stewart & Grupp, 1984) restricted access period.

Together, these studies suggest that the rapid consumption of ethanol over a short period of time (i.e. drinking bouts) may be pharmacologically meaningful to animals. It is therefore possible that given continuous ethanol availability, ethanol drinking behavior may be determined by the pharmacological consequences of these individual bouts over time. Given that ethanol consumption is a behavioral phenomenon by definition, ethanol self-administration may be viewed as an acquired motivated behavior. In line with conventional learning theory then, the probability and frequency of a behavioral response should increase when the behavior produces favorable changes in the environment. This process is commonly known as reinforcement. Since ethanol is endowed with positive reinforcing properties (e.g. Amit, Sutherland & White, 1975), exposure to the drug may trigger a learning process whereby the animal learns to discriminate the positive reinforcing properties of ethanol and thus acquires the motivation to ingest

ethanol. Within the context of learning theory, an ethanol drinking bout can be considered as a behavioral response, and the consequences derived from the response may determine subsequent drinking behavior. Consequently, the reinforcing efficacy of ethanol for each rat may be determined by the size and frequency of bouts over a 24 hr drinking period.

Recently, several studies have reported a direct relationship between brain catalase and brain ALDH activity and ethanol consumption in a 24 hr access paradigm (Aragon et al, 1985; Sinclair & Lindros, 1981; Socaransky et al, 1985). The findings suggest that these enzymes may play a role in regulating ethanol intake by modulating the levels of acetaldehyde in brain. The reinforcing properties of acetaldehyde may therefore be related to its rate of formation and degradation in brain by catalase and ALDH respectively. This is supported by the findings that a faster rate of production (i.e. higher levels of brain catalase activity) and a faster rate of degradation (i.e. higher levels of brain ALDH activity) of acetaldehyde correlated with higher levels of ethanol intake (Aragon et al, 1985; Socaransky et al, 1984). Interestingly, it has also been suggested that a rapid rate of onset and a brief duration of action are characteristic of a drug's potential as a reinforcer (Falk, 1983; Busto & Sellers, 1986). It follows then, that if acetaldehyde

plays a role in mediating the reinforcing properties of ethanol and brain catalase and ALDH regulate the levels of acetaldehyde in brain, these central enzymes may play a role in regulating the size and or frequency of bouts in a 24 hr access period. Moreover, since the animal must emit a drinking response before reinforcement can occur, the initial drinking bout may serve to influence in some fashion the pattern of drinking for rats during their active cycle.

The focus of the present series of experiments was to investigate the role of centrally-acting acetaldehyde and brain ALDH in mediating the initial drinking bout. In an attempt to simulate a drinking bout, a 10 min. restricted access paradigm was employed. Gill et al (1986) reported that the amount of ethanol consumed during the restricted access period was comparable to the quantities consumed in a single bout by animals given free access to ethanol. It was hypothesized that if levels of acetaldehyde in brain are a physiological parameter that control ethanol intake, then variations in brain ALDH activity may alter the reinforcing efficacy of the initial drinking bout and subsequently give rise to differences in ethanol consummatory behavior. The present series of experiments were designed to:

1) demonstrate that a relationship exists between a "simulated" 10 min. drinking bout and total ethanol consumption in a 24 hr access paradigm;

2) further demonstrate that a "drinking bout" is a pharmacologically meaningful event;

3) demonstrate that ethanol consumption can be altered by manipulating the enzymes responsible for acetaldehyde metabolism in brain;

4) attempt to differentiate the relative contribution of central and or peripheral actions of acetaldehyde in mediating ethanol consumption.

The central focus of the present dissertation is to investigate the role of brain ALDH and by implication, centrally-acting acetaldehyde in the mediation of ethanol consumption. By achieving a better understanding of this phenomenon, it is hoped that new light will be shed on the involvement of acetaldehyde in mediating ethanol-motivated behavior.

EXPERIMENT 1

Laboratory rats in a 24 hr free choice paradigm consume ethanol in a series of discrete drinking bouts (Gill et al, 1986; Marcucella et al, 1984). Gill et al (1986) devised a "simulated" drinking bout whereby animals were trained to drink ethanol within a 10 min. drinking session. The results indicated that animals drank sufficient quantities of ethanol during the restricted access to produce pharmacologically meaningful blood and brain ethanol levels as well as to alter performance in a passive avoidance task and exploratory/locomotor behavior.

It would be important to determine whether the "simulated" drinking bout has any meaningful relationship to drinking behavior in a 24 hr access paradigm. In an attempt to address this issue, naive animals trained to drink ethanol in a variety of experiments were pooled to determine whether the 10 min. restricted access to ethanol (simulated drinking bout) was related to total ethanol consumption in 24 hr continuous access.

METHODS

Subjects

Subjects were 273 male Long Evans rats (Charles River Breeding Farms) initially weighing 150-225 grams. The animals were housed individually in stainless steel cages with free access to laboratory chow and water

throughout the experiment. Fluids were presented in two glass Richter tubes mounted on the front of the cages. The animal colony room was illuminated on a 12 hr day/night schedule.

Procedure

The ethanol training procedure comprised of two phases. Phase I consisted of the ethanol screening procedure. After 7 days adaptation to the laboratory housing conditions, animals were screened for ethanol consumption by presenting ethanol solutions (prepared from 95% v/v stock solution with tap water) in an ascending order from 2% to 10% on an alternate-day schedule. On each subsequent ethanol presentation, the concentration was increased by 1%. Animals were exposed only once to ethanol concentrations of up to 5%. Concentrations from 6% to 10%, however, were presented twice with intervening water days between each exposure. On intervening days, both Richter tubes were filled with water. The position of the ethanol and water tubes was alternated daily in order to eliminate position bias. Following the screening period, animals were switched to a schedule of everyday ethanol presentation in a free choice with water. After 7 consecutive days of exposure to 10% ethanol solution, animals were placed in new cages with free access to food and water (presented in a single water bottle) and deprived of ethanol for a two week period.

Phase II consisted of training animals to drink ethanol within a restricted time period. The restricted access procedure included several steps. Following the two week deprivation period, animals received a 24 hr free choice between water (presented in the water bottle) and a 10% ethanol solution presented in rubber stoppered plastic tubes fitted with steel ball-bearing spouts. For the next 7 days, ethanol was presented each day for a 45 min. period. Water bottles were removed just prior to ethanol presentation and were immediately returned following the drinking session. For the following 10 days, (Days 1 to 10), animals were presented with ethanol for a 10 min. period each day. Treatment manipulations began on Day 11.

RESULTS

A baseline measure of ethanol intake in the 24 hr access paradigm (24 HR) was determined by calculating mean ethanol intake (gm/kg) on the last three days of 10% maintenance (Phase I) for each animal. Baseline measures for ethanol intake in the 10 min. restricted access period (BOUT SIZE) were determined by calculating mean ethanol intake (gm/kg) over the last five training days (Days 6 to 10). Ethanol intake was measured to the nearest 0.5 ml. Subjects whose mean bout size was less than 0.25gm/kg were eliminated from the present analysis and all future experiments.

Informal observations of the subjects' drinking behavior led to the conclusion that such low quantities of ethanol (less than 1.5 ml) were more related to spurious factors such as leakage or animals pushing the tube than actual drinking behavior. Consequently, a total of 35 animals were eliminated from the experimentsand the total number of subjects used in the present analysis was 238. A Pearson product-moment correlation revealed that the amount of ethanol consumed by each animal in the 10 min. restricted access was correlated with the total amount of ethanol consumed over a 24 hr period ($r=.361$; $N=238$; $p<.001$). To facilitate visual inspection of the data, Figure 1 represents mean bout size for animals grouped on the basis of their ethanol intake in the 24 hr paradigm in one gm/kg increments. As shown in Figure 1, there was a gradual increase in bout size as animals consume greater quantities of ethanol in a 24 hr access paradigm.

DISCUSSION

The present findings indicated that there was a relationship between the amount of ethanol consumed in the restricted access paradigm and the total amount consumed in a 24 hr access paradigm. Animals, who ingested larger amounts in the restricted access, also drank more ethanol over a 24 hr period. These findings

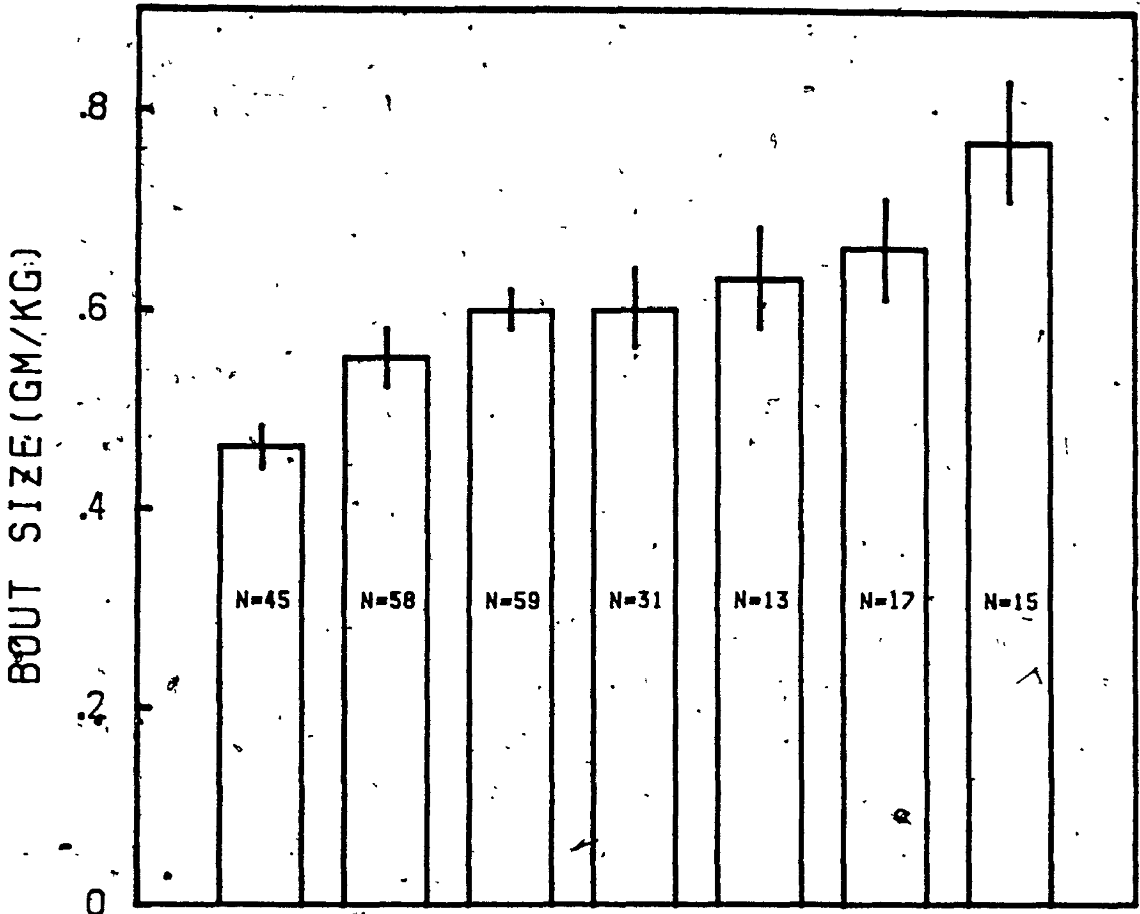

Figure 1. Mean ethanol intake during the 10 min. restricted access (mean bout size) as a function of baseline intake during continuous (24 hr) access, expressed in one gm/kg increments.

suggested that the "simulated" drinking bout may in fact reflect the initial drinking bout and that it may serve to influence in some fashion, the pattern of drinking of rats during their active cycle. Indeed, drinking behavior in the restricted access paradigm was very similar to a drinking bout in 24 hr access as described by Gill et al (1986). Upon presentation, animals immediately drank the ethanol solution and drank continuously for a 2-4 min. period.

It is also possible that the amount of ethanol consumed in the limited access paradigm reflected a compensatory response to the restricted availability of ethanol. These animals were allowed unlimited access to varying concentrations of ethanol for a period of four weeks and have learned how to regulate their ethanol intake. Ethanol exposure was then subsequently restricted and this may have influenced their drinking behavior in the restricted access paradigm. MacDonall and Marcucella (1979) demonstrated that animals consumed more ethanol per hour when ethanol availability was limited to one hour compared to three hours. In the present studies, some animals may have subsequently increased their otherwise regulated drinking bout to compensate for the restricted access to ethanol. This may explain the more prominent increase in bout size for animals who consumed 5 gm/kg or more in the 24 hr access paradigm (see Figure 1). Despite

this potential influence of the restricted access per se, a relationship between the size of the drinking bout in a restricted access paradigm and total consumption in a 24 hr access paradigm was still apparent and significant. These findings suggested that the size of the bout and its subsequent pharmacological effects may be important variables in determining ethanol drinking behavior.

The results of the present study established a relationship between the amount of ethanol consumed in a "simulated" drinking bout and total consumption in a 24 hr access paradigm. Thus, the hypotheses to be tested in the following experiments were based on the assumption that alterations in the "initial" drinking bout would reflect alterations in ethanol drinking behavior in general. The following series of experiments were designed to investigate the role of brain ALDH and centrally-acting acetaldehyde in mediating a drinking bout.

EXPERIMENT 2

Several investigators have suggested that centrally-acting acetaldehyde may play a role in the regulation of ethanol intake (Amit et al, 1986; Lindros, 1985; Truitt and Walsh, 1971). This notion is supported by reports of a direct relationship between brain ALDH activity and voluntary ethanol consumption (Amir, 1977; Sinclair & Lindros, 1981; Socaransky et al, 1984). Furthermore, Sinclair and Lindros (1981) challenged the widely accepted view that reductions in voluntary ethanol consumption following cyanamide pretreatment (an ALDH inhibitor) was a consequence of the aversive effects produced by high circulating levels of acetaldehyde in the blood (e.g. Lindros, 1985; Schlesinger et al, 1965). They demonstrated that the prevention of the accumulation of blood acetaldehyde by concurrent treatment with cyanamide and the alcohol dehydrogenase inhibitor 4-methylpyrazole still resulted in the suppression of ethanol drinking in rats. These authors concluded that the suppression of ethanol consumption by cyanamide was due to its direct inhibition of brain ALDH and not due to elevated blood acetaldehyde levels.

The drinking data in the Sinclair and Lindros (1981) study was based on continuous access to ethanol. Consequently, the effects of cyanamide on ethanol consumption can only be determined by changes in total

ethanol intake. Since animals drink in discrete bouts with varying frequency, it can be assumed that cyanamide altered the size of the bout and or the frequency of bouts over the 24 hr access period. The advantage of a restricted access paradigm is that it allows one to examine a discrete component of the total drinking behavior, in this case, a drinking bout or bouts within a 10 min. drinking session. The amount of ethanol consumed in this "simulated" drinking bout was found to be related to 24 hr consumption (see Experiment 1) and has been shown to be pharmacologically meaningful (Gill et al, 1986; Stewart & Grupp, 1984). If such a drinking bout produces a pharmacologically relevant effect and brain ALDH has been implicated in mediating ethanol consumption in general, it would be of interest to determine whether brain ALDH and centrally-acting acetaldehyde may also play a role in regulating the size of a drinking bout. In an attempt to investigate this notion, it was hypothesized that brain ALDH activity, and by implication central levels of acetaldehyde, could be manipulated in the rat by the administration of agents which inhibit oxidative enzymes responsible for ethanol metabolism.

It has been reported that the administration of 4-methylpyrazole, an alcohol dehydrogenase inhibitor (Magnusson, Nyberg, Bodin & Hansson, 1972) decreases the rate of elimination of ethanol in the liver and

consequently limits the production of acetaldehyde in the periphery (Lindros, Stowell, Pikkarainen & Salaspuro, 1981; Sinclair & Lindros, 1981). This treatment is not expected to interfere with the central formation of acetaldehyde through the peroxidatic activity of catalase (Cohen et al, 1980; Aragon et al, 1985). Therefore, it was assumed that in brain ethanol could be metabolized by catalase to produce acetaldehyde. Treatment with cyanamide has been shown to interfere with the metabolism of acetaldehyde in the liver by inhibiting aldehyde dehydrogenase, thereby causing elevated levels of acetaldehyde in the periphery (e.g. Marchner & Tottmar, 1978). Brain ALDH activity was reported to be inhibited by cyanamide as well (Sinclair & Lindros, 1981).

It has been previously demonstrated that the simultaneous administration of the alcohol dehydrogenase and aldehyde dehydrogenase inhibitors 4-methylpyrazole and cyanamide respectively, prevented the accumulation of acetaldehyde in the periphery (Sinclair & Lindros, 1981; Sinclair, Lindros & Tehro, 1980). Moreover, brain ALDH activity was also inhibited (Sinclair & Lindros, 1981; Sinclair et al, 1980) and this manipulation may have served to increase central levels of acetaldehyde formed via brain catalase (Aragon & Amit, 1985). It was therefore hypothesized that the accumulation of acetaldehyde in

brain as a consequence of brain ALDH inhibition may alter ethanol consumption in animals pretreated with 4-methylpyrazole and cyanamide or cyanamide alone. If, however, peripherally-produced acetaldehyde is a major contributing factor in regulating ethanol intake, then differential drinking levels should be observed between these various treatment groups.

The focus of the present experiment was to investigate the potential relative roles of centrally- and or peripherally- acting acetaldehyde in mediating ethanol consumption when using a restricted access paradigm.

METHODS

Subjects

Subjects were 100 male Long Evans rats (Charles River Breeding Farms) initially weighing 175-225 grams. The housing conditions were identical to those outlined in Experiment 1.

Drugs

4-methylpyrazole (10mg/kg) and sodium cyanamide (25mg/kg) (Sigma Chemicals, St. Louis) were each dissolved in saline. All injections were administered intraperitoneally (i.p.), in a volume of 1.0 ml/kg.

Procedure

The ethanol screening procedure and restricted access procedure were described in Experiment 1. An outline of the procedure is presented in Table 1.

Table 1. Experimental Procedure

Days	Procedure
1-7	Habituation
8-35	Ethanol screening (2%-10%)
36-42	Ethanol maintenance (10%)
43-56	Ethanol deprivation
57	Acclimatization to ethanol in tube
58-64	Ethanol presented for 45 min.
65-74 (Training days)	Ethanol presented for 10 min.
66,69,72	Saline pretreatment (i.p.)
75-79 (Test days 1-5)	Drug pretreatment (i.p.)
80	Drug recovery day

Briefly, animals were initially screened for ethanol consumption by presenting ethanol solutions in an ascending order from 2% to 10% on an alternate-day schedule. After seven consecutive days of exposure to the 10% ethanol solution, animals were deprived of ethanol for a two week period.

Restricted access procedure: Following two weeks of ethanol deprivation, animals were presented with a choice of ethanol (10%) and water for one single 24 hr period. For the next seven days, ethanol was presented each day for a 45 min. period. Water bottles were removed just prior to ethanol presentation and were immediately returned following the drinking session. For the following 10 days (Days 65 to 74), animals were presented with ethanol for a 10 min. period each day. On Training days 66, 69 and 72 animals received two successive i.p. injections of saline (1.0 ml/kg) four hr prior to ethanol presentation. This was undertaken in an attempt to habituate the animals to the injection

procedure that followed. Based on mean ethanol intake (gm/kg) of the last five training days, animals were ranked and assigned to one of four treat- ment groups to ensure an equal distribution of drinkers in each group. For the next five days (Test days 1-5), four hr prior to ethanol presentation, animals received two successive i.p. injections. Half the animals received saline (S) and the other half received 4-methylpyrazole (4MP) as the first injection (Pretreatment 1). Subsequently, half the animals in each of these groups received saline (S) or cyanamide (CYAN) as the second injection (Pretreatment 2). This procedure resulted in four groups as outlined in the following table:

Table 2. Summary of treatment groups

	PRETREATMENT 2	
	Saline	Cyanamide
PRETREATMENT 1		
Saline	S+S (n=23)	S+CYAN (n=26)
4-methylpyrazole	4MP+S (n=25)	4MP+CYAN (n=26)

Following the injection period (Test days 1-5), ethanol was presented on one more day without drug treatment and drinking was measured.

RESULTS

A baseline measure of ethanol intake was determined by calculating mean ethanol intake (gm/kg) over the last five training days (Days 70-74) for each animal. A one-way analysis of variance (ANOVA) on baseline intake yielded no differences between groups

($F(3,96) = .41$; $p > .75$). All drinking data was therefore analyzed as percent change from baseline level of consumption for each animal. Mean ethanol intake (gm/kg) expressed as percent change from baseline intake is presented in Figure 2. A three-way ANOVA with repeated measures (Pretreatment 1 x Pretreatment 2 x Days) yielded a significant Pretreatment 1 x Pretreatment 2 interaction ($F(1,96 = 5.5$; $p < .02$) and a significant Pretreatment 2 x Days interaction ($F(5,480) = 6.49$; $p < .00001$). Post hoc Tukey tests revealed that groups treated with cyanamide (Pretreatment 2), independent of their Pretreatment 1 conditions (Saline or 4MP), drank significantly more ethanol than groups S+S and 4MP+S across all the test days ($q(2,430) = 28.4$; $p < .01$). There were no differences between groups on the day following the last injection day ($p > .05$). As shown in Figure 2, the increase in ethanol consumption for groups 4MP+CYAN and S+CYAN was evident on the first day of drug treatment and appeared to be stable across the treatment period. Although both cyanamide treated groups significantly increased drinking across the five Test days, Tukey tests for the Pretreatment 1 x Pretreatment 2 interaction revealed that overall, group 4MP+CYAN drank significantly more ethanol than groups S+CYAN, S+S and 4MP+S ($q(4,96) = 25.3$; $p < .05$). In particular, the mean

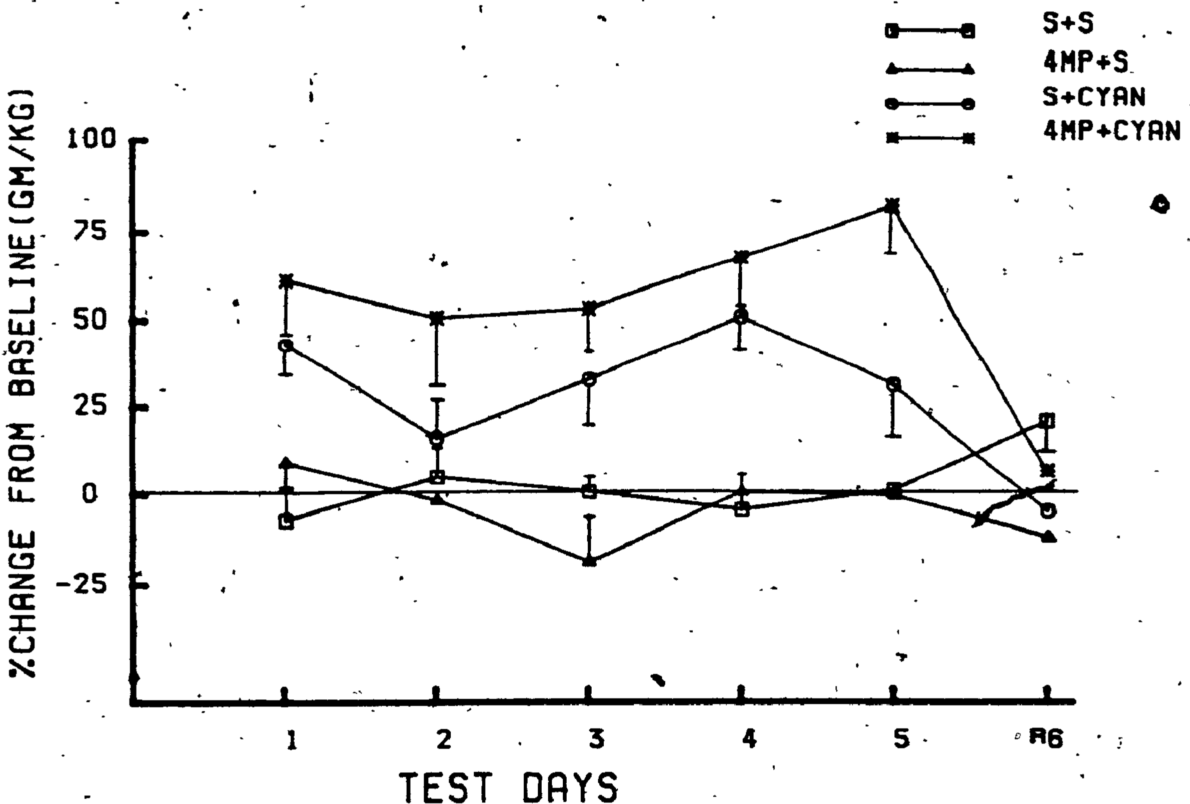

Figure 2. Mean ethanol intake (expressed as percent change from baseline intake) for all treatment groups. S= saline; 4MP= 4-methylpyrazole; CYAN= cyanamide; R6= drug recovery day.

increase in ethanol consumption over the five Test days for group 4MP+CYAN was almost twice as high as the increase demonstrated by group S+CYAN (62% increase vs 34% increase, respectively).

As previously mentioned in the procedure, animals were initially ranked on the basis of baseline levels of consumption and equally distributed to the four treatment groups. By employing this ranking method, each treatment group consisted of animals with varying baseline consumption levels. It was hypothesized that the treatment manipulations may have differentially affected animals who consumed larger or smaller quantities of ethanol. Consequently, it was decided to arbitrarily categorize animals on the basis of their baseline levels of consumption into LOW, MEDIUM and HIGH drinkers. 'LOW' drinkers were those animals consuming between 0.25-0.49 gm/kg in the 10 min. restricted access paradigm. 'MEDIUM' (MED) drinkers were subjects who drank between 0.50-0.79 gm/kg and 'HIGH' drinkers consumed 0.80 gm/kg or more in the restricted access paradigm. A closer analysis of the contribution of these drinking groups to the observed treatment effects was undertaken. Mean ethanol intake (expressed as percent change from baseline) as a function of treatment manipulation for each drinking group (HIGH, MED, LOW) is presented in Figure 3 (panels a,b,c, respectively). Individual three-way ANOVAs were

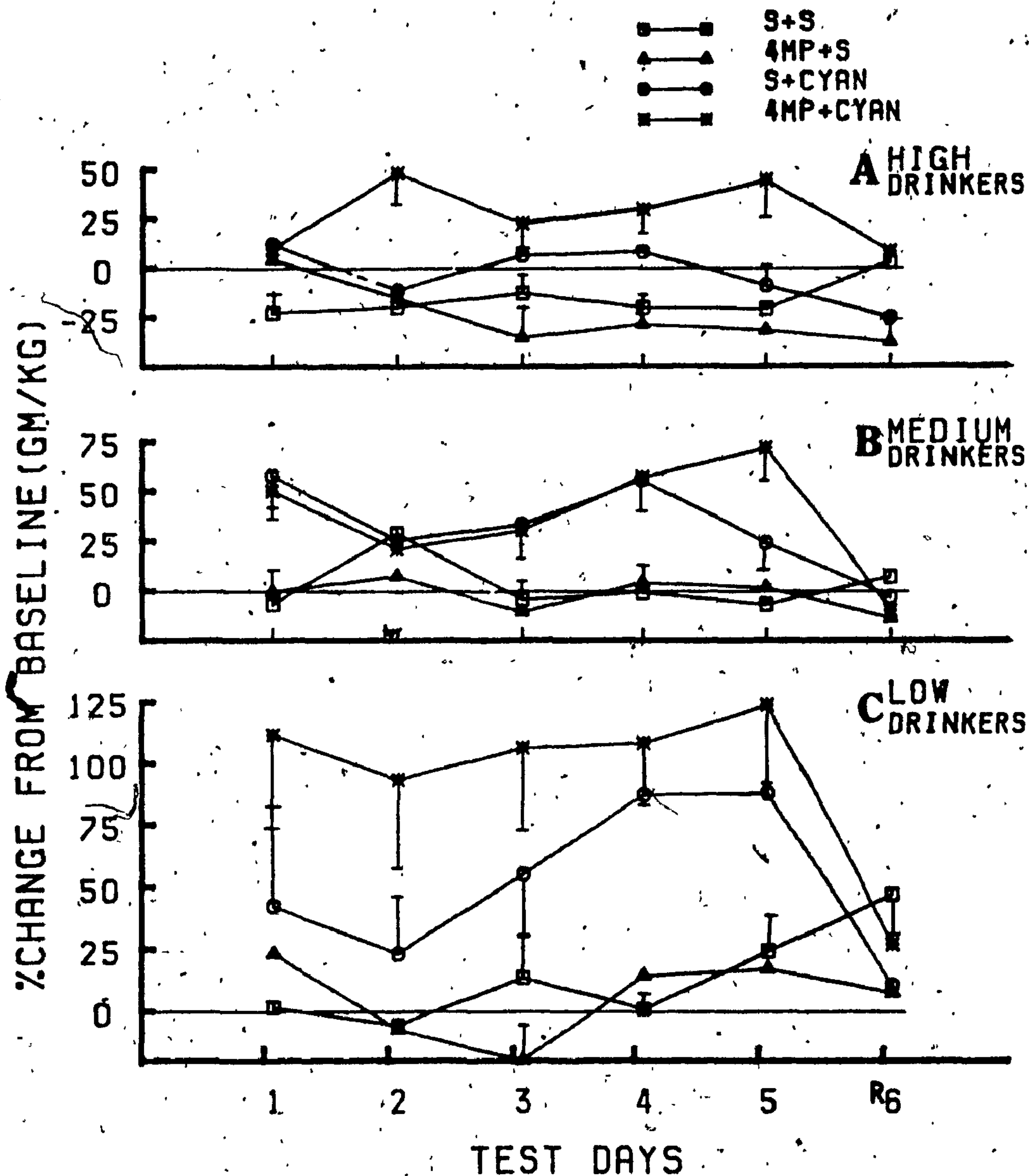

Figure 3. Mean ethanol intake (expressed as percent change from baseline intake) as a function of treatment manipulation (S+S, 4MP+S, S+CYAN and 4MP+CYAN) for each drinking group (HIGH, MEDIUM, LOW) (panels a,b,c respectively).

performed on each drinking group as a function of treatment manipulation. For HIGH drinkers, the analysis yielded a significant Pretreatment 1 x Pretreatment 2 x Days interaction ($F(5,105) = 2.57$; $p < .03$). Post hoc Tukey tests revealed that group 4MP+CYAN drank significantly more ethanol than groups S+S and 4MP+S on Test days 2 to 5 ($q(4,115) = 47.2$; $p < .05$) and significantly more ethanol than group S+CYAN on Test days 2 and 5 ($p < .05$). As shown in Figure 3, panel a, group 4MP+CYAN increased ethanol intake by about 30% across Test days. It is of interest to note that although groups S+S and 4MP+S both demonstrated a reduction in ethanol intake (about 20%), group S+CYAN did not fluctate from their baseline level during the injection period (see Figure 3a). However, on the day following the last injection day, groups pretreated with cyanamide decreased their ethanol intake compared to the previous day.

A three-way ANOVA for both MEDIUM and LOW drinkers revealed significant Pretreatment 2 x Days interactions ($F(5,205) = 3.69$; $p < .003$; $F(5,130) = 2.61$; $p < .03$, respectively). Tukey tests for MEDIUM drinkers revealed that animals pretreated with cyanamide (groups S+CYAN and 4MP+CYAN) drank significantly more ethanol than groups S+S and 4MP+S across all Test days except T2 ($q(2,212) = 35.9$; $p < .05$) (see Figure 3b). Post hoc Tukey tests for LOW drinkers revealed that animals pre-

treated with cyanamide independent of their Pretreatment 1 condition drank significantly more ethanol across all Test days than groups S+S and 4MP+S ($q(2,138) = 49.6$; $p < .05$). As shown in Figure 3c, however, the pattern of drinking was different for the two cyanamide treated groups. Group 4MP+CYAN demonstrated a 100% increase in ethanol consumption from Test days 1 to 5, whereas group S+CYAN demonstrated a gradual increase in ethanol intake over Test days to approximately 90% above baseline level. The mean increase in ethanol consumption over the five Test days for group 4MP+CYAN was almost double the increase observed for group S+CYAN (109% increase vs 59% increase, respectively).

Because of the individual variation in ethanol intake within each treatment group, a graphic representation of mean ethanol intake as a function of drinking level (HIGH, MED, LOW) for each treatment group is presented in Figure 4 (panels a,b,c,d, respectively). As shown in Figure 4a, animals pretreated with Saline (group S+S) did not appear to demonstrate variable drinking behavior as a consequence of the injection procedure. However, HIGH drinkers appeared to be sensitive to the injection procedure. In Figure 4b, HIGH drinkers pretreated with 4MP+S also demonstrated a reduction in ethanol consumption, whereas MED and LOW drinkers were not influenced by this

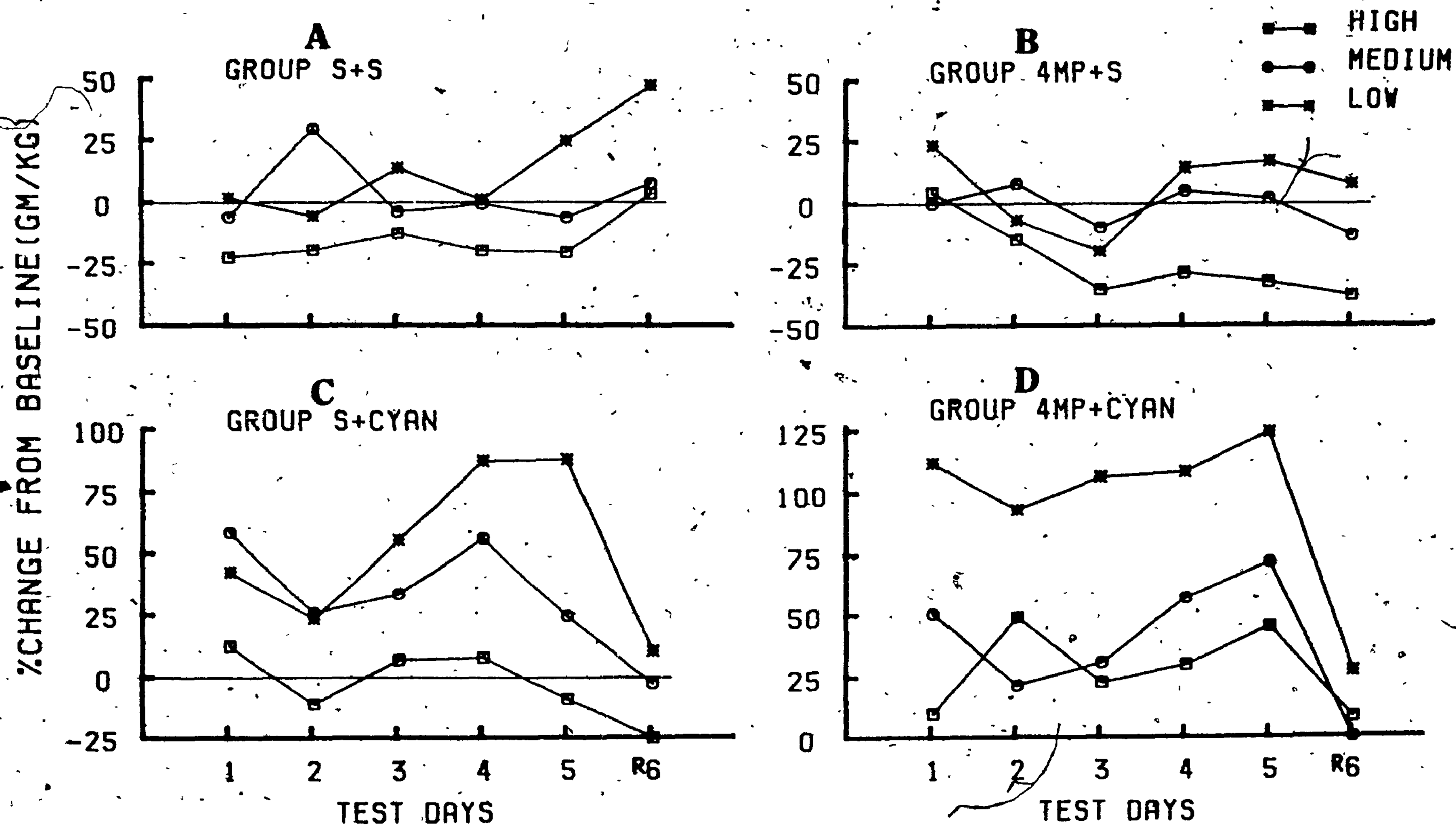

Figure 4. Mean ethanol intake (expressed as percent change from baseline intake) as a function of drinking level (HIGH, MEDIUM, LOW) for each treatment group (S+S, 4MP+S, S+CYAN and 4MP+CYAN) (panels a,b,c,d respectively).

treatment manipulation. The data of Group S+CYAN is presented in Figure 4c. The drinking behavior of this group appeared to be altered in a dose-dependent manner. HIGH drinkers demonstrated no change from baseline level, MED drinkers increased by approximately 40% and LOW drinkers increased ethanol intake to about 90% above their baseline levels. A similar dose-response relationship is shown in Figure 4d for group 4MP+CYAN. However, MED and HIGH drinkers appeared to demonstrate similar increases in ethanol intake, compared to the drinking response observed by group S+CYAN. Low drinkers showed the greatest increase in ethanol intake.

DISCUSSION

The results of the present experiment support the finding that a simulated drinking bout is a pharmacologically meaningful event (Gill et al, 1986) since the drinking levels in such a bout can be altered by manipulating acetaldehyde-metabolizing enzymes. It is evident that from Test days 1 to 5, animals pretreated with cyanamide independent of their Pretreatment 1 condition (groups 4MP+CYAN and S+CYAN) consumed significantly more ethanol than groups S+S and 4MP+S. The increase in ethanol intake was consistently demonstrated during the injection period. When cyanamide treatment was terminated, animals returned to baseline levels. The increase in ethanol consumption by cyana-

mide does not appear to be attributed to elevated blood acetaldehyde levels since animals pretreated with 4MP+CYAN (a condition which prevents accumulation of acetaldehyde in the periphery) also demonstrated increases in ethanol consumption. A common factor in both cyanamide treated groups was the inhibition of brain ALDH. These results therefore suggest that brain ALDH may play a role in regulating an ethanol "drinking bout(s)", perhaps by regulating central levels of acetaldehyde. It should be noted, however, that the increase in ethanol intake for group S+CYAN was much lower than that observed for group 4MP+CYAN. These results suggested that the peripheral accumulation of acetaldehyde appeared to play a role in limiting the increase in ethanol intake produced by cyanamide.

An interesting finding in the context of the present study was the immediate change in consumption on the first test day of cyanamide treatment. Moreover, the increase in ethanol intake persisted across test days and appeared to asymptote on the first test day (see Figure 2). Furthermore, as soon as drug treatment terminated, animals abruptly returned to baseline levels of consumption. These findings suggested that the effect of cyanamide on ethanol was immediate, occurring in the first few minutes during or following ethanol consumption and further suggested that animals were capable of detecting the termination

of cyanamide treatment. The next experiment was therefore designed to investigate the nature of the effect of cyanamide on ethanol using the restricted access procedure.

EXPERIMENT 3

In the previous experiment it was observed that animals pretreated with cyanamide independent of the Pretreatment 1 condition (groups 4MP+CYAN and S+CYAN) demonstrated an increase in ethanol intake across Test days. Of particular interest was the finding that this increase was evident on the first day of cyanamide treatment and abruptly ceased when drug treatment was terminated. If this phenomenon is related to a specific effect of cyanamide on ethanol during or quickly following ethanol consumption, then animals should be able to alter their drinking response in the presence and absence of cyanamide. The present experiment was designed to investigate the nature of cyanamide's effect on ethanol and its immediacy using the restricted access paradigm. Experiment 3 was divided into two parts. Part I consisted of the behavioral assay to test the hypothesis outlined above. In Part II, some of the subjects were used for assays to determine blood ethanol and acetaldehyde levels following restricted access to ethanol.

METHODS

Subjects

Ninety-nine subjects from Experiment 2 were given three additional weeks of restricted access to ethanol for a 10 min. period each day. One subject from group

S+CYAN was eliminated from this phase of the experiment due to sickness.

Drugs.

4-methylpyrazole (10mg/kg) and sodium cyanamide (25mg/kg) were prepared as outlined in Experiment 2.

Procedure

Following three additional weeks of restricted access to ethanol, the experiment began. On Day 22 (Drug day 1), four hr prior to ethanol presentation, animals were divided into their previous treatment groups and received i.p. injections of their respective drug treatments (e.g. S+S, 4MP+S, S+CYAN, 4MP+CYAN). These drugs were administered every other day (Drug days- odd days). On alternate days (even days), all animals received i.p. injections of saline (1.0 ml/kg) four hr prior to ethanol presentation (Saline days). This procedure was maintained for 10 consecutive days.

RESULTS

A baseline measure of ethanol intake was determined by calculating mean ethanol intake (gm/kg) over the last five days prior to Drug day 1 for each animal. A one-way ANOVA yielded no significant differences between groups on baseline levels of ethanol consumption ($F(3,95) = 1.9$; $p > .13$. Mean ethanol intake (expressed as percent change from baseline) for all treatment groups is presented in Figure 5. A three-way

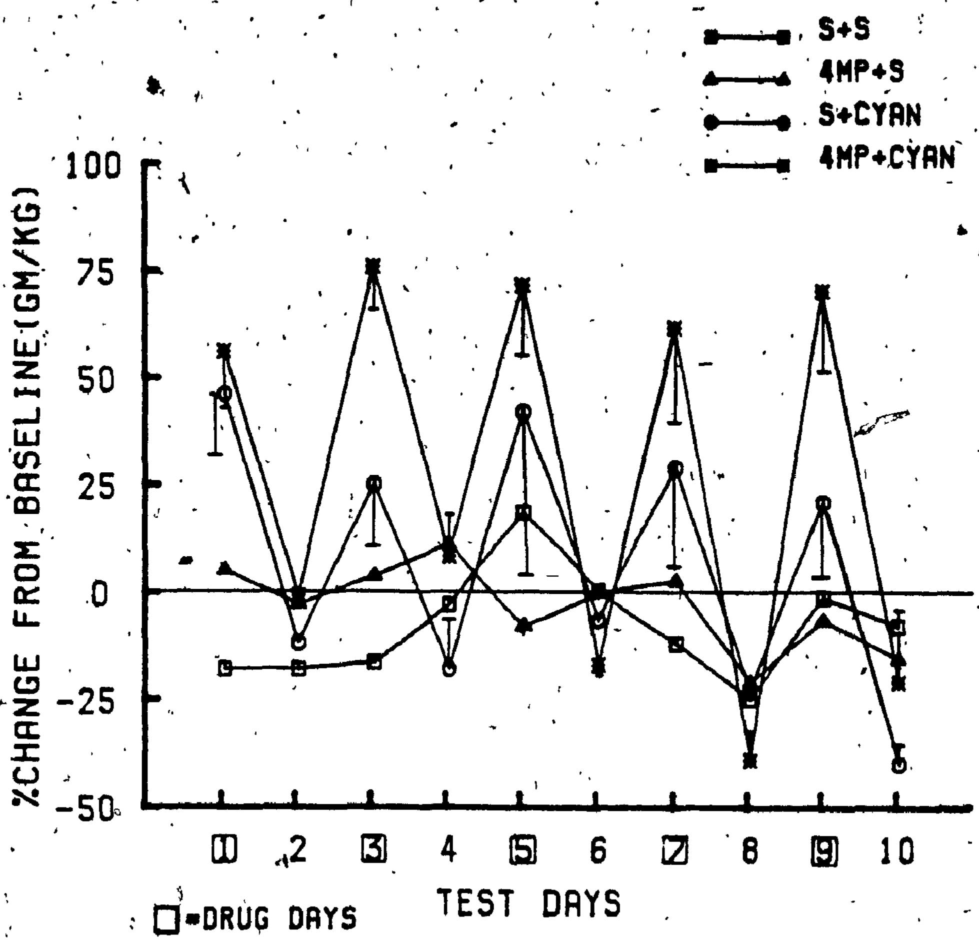

Figure 5. Mean ethanol intake (expressed as percent change from baseline intake) for all treatment groups.

ANOVA with repeated measures yielded a significant Pretreatment 2 x Days interaction ($F(9,855) = 9.3$; $p < .00001$). Post hoc Tukey tests revealed that groups pretreated with cyanamide independent of the Pretreatment 1 manipulation (groups 4MP+CYAN and S+CYAN) consumed significantly more ethanol on all Drug days than groups S+S and 4MP+S ($q(2,126) = 26.9$; $p < .05$). Groups did not differ from one another on Saline days ($p > .05$). It is evident from Figure 5 that group 4MP+CYAN consumed more ethanol on Drug days than group S+CYAN. However, an interesting pattern of ethanol consumption for groups 4MP+CYAN and S+CYAN was observed over the 10 testing days. Both groups demonstrated an increase in ethanol consumption on Drug days yet maintained baseline intake levels on Saline days. Tukey tests revealed that group S+CYAN consumed significantly more ethanol on Drug days than on corresponding Saline days ($q(2,855) = 30$; $p < .05$). This same pattern of intake was demonstrated for group 4MP+CYAN ($p < .05$).

PART II

A biochemical assay was carried out to confirm previous reports that a "simulated" drinking bout produced detectable blood ethanol levels. In addition, the assay would verify whether cyanamide did in fact produce detectable blood acetaldehyde levels following the drinking bout as well as inhibiting brain aldehyde dehydrogenase.

Subjects

Fifty-two subjects from Experiment 3 were used for the assays. An additional 10 naive male Long Evans rats weighing 480-550 grams were included for the ALDH assay.

Procedure

Following Experiment 3, the 52 subjects were deprived of ethanol for a 3 week period. Animals were then presented with ethanol for a 10 min. period each day. Subjects were given five additional training days prior to the start of ~~the~~ experiment. On testing day, four hr prior to ethanol presentation, animals were divided into their previous treatment groups and received i.p. injections of their respective drug treatments (e.g. S+S, 4MP+S, S+CYAN, 4MP+CYAN). Immediately following the 10 min. drinking session, animals were sacrificed by decapitation. Trunk blood of each animal was collected and later assayed for ethanol and acetaldehyde determinations by head-space gas chromatography.

To determine the effects of cyanamide on brain ALDH activity, the 10 naive subjects were sacrificed by decapitation following anaesthetization with ether four hr after i.p. administration of cyanamide (25 mg/kg; n=5) or saline (1.0 ml/kg; n=5). The brain of each subject was rapidly extracted, rinsed in ice cold saline and blotted lightly on dry filter paper.

Brain samples were first stored at -70° c and later assayed for ALDH activity levels.

Determination of Blood Ethanol and Acetaldehyde: The procedure used was based on that of Stowell (1979) with modifications by Iversen and Damagaard (1983). These modifications were used to ensure accurate ethanol and acetaldehyde levels by protecting the blood sample from acetaldehyde and ethanol degradation during sample preparation. A brief outline of the procedure is presented. Animal trunk blood was collected into test tubes which contained 150 I.U. of sodium heparin. Blood (1 ml) was mixed with 4 ml of ice cold semi-carbizide reagent, containing 25 mM of thiourea. This mixture was spun in a refrigerated centrifuge at 400 g to separate blood cells from serum. Serum (2 ml) was added to 0.5 ml of 3.0 M perchloric acid and then spun at 11,000 g to obtain a clear protein-free supernatant. At this time, 0.5 ml of the supernatant was pipetted into an 8 ml vial, stoppered and stored at -70° c until assayed for ethanol and acetaldehyde by head-space gas chromatography.

Preparation of Brain Tissue: Frozen brain samples were weighed and then placed into the homogenation medium. Whole brains were homogenized (Teflon on glass) in sufficient 0.25 M sucrose containing 1% Triton-X100 to make 10% brain homogenates. Homogenates were centrifuged for 60 min. at 100,000 g, at 0° c and then the

clear supernatant was used as the enzyme source. All samples were frozen at -70° c until assayed.

Assay of Aldehyde Dehydrogenase: Aldehyde dehydrogenase activity was assayed spectrophotometrically by measuring the rate of the enzyme-catalyzed NAD+-dependent production of NADH (modified from Deitrich, Troxell, Worth and Erwin, 1976). The reaction mixture consisted of 0.75 ml of 0.03 M pyrophosphate buffer (pH 9.6), 0.075 ml of 20.0 mM NAD, 0.275 ml of distilled water and 0.2 ml of the enzyme source, incubated for 10 min. at 23 c. The reaction was initiated by the addition of 0.2 ml of 36.3 µM acetaldehyde, bringing the total volume to 1.5 ml. Protein content was measured following the method of Lowry et al (1951) and bovine serum albumin was used as the standard. All assays were carried out in duplicate for both the enzyme activity and protein determinations.

RESULTS

Ethanol intake (gm/kg) during the 10 min. drinking session was calculated for each animal and subsequently compared to blood ethanol levels determined by head space gas chromatography. A significant positive correlation was revealed between the quantities of ethanol consumed and blood ethanol levels ($r(53) = 0.699$; $p < .001$). Mean bout size was 0.56 gm/kg and mean blood ethanol level was 40.4 mg/100 ml. Blood acet-

aldehyde levels were only detectable in animals pretreated with cyanamide alone (group S+CYAN; 294 ± 53 μM acetaldehyde).

The effect of cyanamide on brain ALDH activity is presented in Table 3.

Table 3. Brain ALDH activity S.E.M. (expressed as nM of NADH/min/mg protein) following pretreatment with cyanamide or saline.

TREATMENT	ALDH activity	% INHIBITION from saline control group
CYANAMIDE	1.16 ± .02 *	11
SALINE	1.31 ± .01	

* - significantly different from saline, p<.001

Independent T tests revealed that pretreatment with cyanamide produced a significant reduction in brain ALDH activity ($t(10) = 6.14$; $p < .001$).

DISCUSSION

The results from Part II confirm previous findings that the consumption of small quantities of ethanol result in detectable blood ethanol levels (Gill et al, 1986; Stewart & Grupp, 1984). Furthermore, animals ingested sufficient quantities of ethanol to produce concentrations of acetaldehyde that could be manipulated by the ALDH inhibitor cyanamide. Only animals pretreated with cyanamide alone (group S+CYAN) demonstrated elevated blood acetaldehyde levels. Pretreatment with 4-methylpyrazole together with cyanamide proved effective in preventing the accumulation of

acetaldehyde in blood for group 4MP+CYAN. The assay of brain ALDH activity suggested that cyanamide inhibited only 11% of brain ALDH activity compared to the saline control group. However, it has recently been reported that the same dose of cyanamide (25 mg/kg), administered under the same conditions, inhibited 30% of brain ALDH activity (Spivak et al, 1987). Furthermore, pilot work from our laboratory as well as findings from other studies (e.g. Sinclair & Lindros) have shown that cyanamide produced higher rates of brain ALDH inhibition than that reported in the present experiment. It is therefore possible that the relatively low rate of brain ALDH inhibition reported in the present study may be due to a methodological problem and that the actual inhibitory action of cyanamide on brain ALDH may be significantly higher.

The results of Experiment 3 (Part I) suggested that cyanamide had a specific effect on ethanol during or following ethanol consumption. Animals consistently demonstrated an increase in ethanol intake on days when they were pretreated with cyanamide, suggesting that cyanamide's effect on ethanol was discriminable to animals. The increase in ethanol intake cannot be attributed to elevated blood acetaldehyde levels, since animals pretreated with 4MP+CYAN (which prevents the accumulation of acetaldehyde in the periphery) also demonstrated consistent increases in ethanol consump-

tion on Drug days. These results therefore suggested that cyanamide's action on ethanol may occur at the central level, potentially mediated by brain ALDH.

A consistent finding in Experiments 2 and 3 was that pretreatment with cyanamide resulted in an increase in ethanol intake. It would be of interest to determine the nature of the increase as well as the specificity of this effect to ethanol. One possibility is that by inhibiting brain ALDH, cyanamide altered the psychopharmacological properties of ethanol and the increase in ethanol consumption reflected changes in the reinforcing efficacy of ethanol. An alternative explanation for the rapid change in consumption is that cyanamide may modify the taste of the ethanol solution. These hypotheses were tested in the following experiments.

EXPERIMENT 4

In the previous two experiments it was demonstrated that pretreatment with cyanamide increased ethanol consumption when a restricted access paradigm was used. It was particularly interesting that ethanol consumption increased on the very first day of cyanamide treatment. It has been postulated that this rapid change in consumption may reflect alterations in the psychopharmacological properties of ethanol. However, an alternative hypothesis is that this immediate increase in ethanol consumption may be related to cyanamide's ability to modify the taste of the ingested solutions.

The purpose of the present experiment was to further investigate the nature of the increase in ethanol intake by cyanamide. In order to test the "taste hypothesis", animals were trained to consume a saccharin-quinine solution or plain tap water using a 10 min. restricted access schedule. A saccharin-quinine solution was used in an attempt to create a distinct flavor comparable to ethanol on a preference-aversion gradient, yet possessing no pharmacological properties. The water group was included to determine the effects of cyanamide on fluid intake in general. It was hypothesized that if the increase in ethanol consumption was a consequence of cyanamide's ability to modify the taste of the solution, then it should also

modify consumption of a saccharin-quinine solution. If, however, the increase reflected a specific effect of cyanamide on ethanol, then cyanamide treatment should not alter intake levels of the saccharin-quinine solution. If water intake increased in the water drinking group, then the increase observed with ethanol may be attributable to a general effect on fluid induced by cyanamide. The present experiment was therefore designed to investigate the nature and specificity of the increase in ethanol intake produced by cyanamide when using the restricted access paradigm.

METHODS

Subjects

Subjects were 29 male Long Evans rats (Charles River Breeding Farms) initially weighing 125-150 grams. The housing conditions were identical to those in Experiment 1.

Procedure

An outline of the screening and restricted access procedures for the saccharin-quinine (SQ; n=15) and water (WAT; n=14) drinking groups is presented in Table 4. A brief outline of the procedure for each drinking group is presented below. For the saccharin-quinine (SQ) group, animals were first screened for SQ consumption using a 24 hr access paradigm. Animals were presented with a free choice between water and saccharin (0.1% w/v) adulterated with increasing

Table 4. Outline of Experimental Procedure

1. Group drinking saccharin-quinine (SQ)

Days	Procedure
1-7	Habituation
8-9	Saccharin (0.1%) and water for 24 hr continuous access
10-11	Quinine (0.0025%) added to saccharin
12-27	SQ screening (0.0025%-0.02% quinine)
28-34	SQ maintenance (with 0.02% quinine)
35-48	SQ deprivation
49	Acclimatization to SQ in tube
50-54	SQ presented for 45 min.
55-69	SQ presented for 10 min.
62,66	Saline pretreatment (i.p.)
70-74 (Test days 1-5)	Drug pretreatment (i.p.)
75	Drug recovery day
76-96	SQ presented for 10 min.
97,99,101,103,105 (Drug days)	Drug pretreatment (i.p.)
98,100,102,104,106 (Saline days)	Saline pretreatment (i.p.)

Group 2. Group drinking water (WAT)

Days	Procedure
1-52	Habituation
53-54	Saccharin (0.05%) presented for 45 min.
55-69	Tap water presented for 10 min.
62,66	Saline pretreatment (i.p.)
70-74 (Test days 1-5)	Drug pretreatment (i.p.)
75	Drug recovery day
76-96	Tap water presented for 10 min.
97,99,101,103,105 (Drug days)	Drug pretreatment (i.p.)
98,100,102,104,106 (Saline days)	Saline pretreatment (i.p.)

concentrations of quinine (0.0025%-0.02%). Subjects were initially presented with the saccharin solution for only two days. Animals were then exposed to each SQ concentration for two consecutive days. On each subsequent SQ presentation, the concentration of quinine was increased by 0.0025%. The position of the SQ and water tubes was alternated in order to eliminate position bias. A stable SQ concentration was chosen when SQ preference was equivalent to the preference ratios of animals with a free choice of water and ethanol (10% concentration). Once this criteria was met, animals were given seven additional days of exposure to the SQ solution and water (saccharin- 0.1%, quinine- 0.02%). On Day 35, animals were placed in new cages with free access to food and water (presented in a single water bottle) and deprived of saccharin-quinine for a two week period.

The restricted access training procedure began on Day 49. The procedure was identical to that used for ethanol-experienced subjects in Experiment 1 with some minor modifications. The 45 min. training period was reduced from seven days to five days and the 10 min. restricted access period was increased from 10 days to 15 days. These modifications were made in order to give subjects more training exposure to the 10 min. restricted access procedure. Water bottles were removed just prior to SQ presentation and returned

immediately after the drinking session. Animals received i.p. injections of saline (1.0 ml/kg) on training days 62 and 66. Based on mean SQ intake (ml) of the last five days (Days 65-69), animals were ranked and assigned to one of two treatment groups to ensure an equal distribution of drinkers in each group. For the next five days (Test days 1-5), four hr prior to SQ presentation, animals received i.p. injections of either cyanamide (25mg/kg- group CYANSQ; n= 7) or saline (1.0 ml/kg; group SALSQ; n= 8). Following the injection period, SQ was presented on one more occasion without drug treatment and drinking was measured.

Following three additional weeks of restricted access to SQ, the "alternate day" testing paradigm was employed. On Day 97 (Drug day 1), four hr prior to SQ presentation, animals were divided into their previous treatment groups and received i.p. injections of their respective drug treatments (e.g. cyanamide or saline). These drugs were administered every other day (Drug days- odd days). On alternate days (even days), all animals received i.p. injections of saline (1.0 ml/kg) four hr prior to SQ presentation (Saline days). This procedure was maintained for 10 consecutive days.

Subjects in the water drinking group were housed individually in stainless steel cages with free access to food and water (presented in a single water bottle) throughout the experiment. Although there was no

screening procedure for this group, subjects were handled as often as SO drinking subjects. Following an habituation period of 52 days, water drinking animals were presented with a saccharin solution (0.05% w/v) for a 45 min. period each day for two days. Water bottles were removed just prior to the saccharin presentation and immediately returned following the drinking session. During the next 15 training days of 10 min. restricted access, tap water was substituted for the saccharin solution. Animals received i.p. injections of saline on training days 62 and 66. The testing procedure was identical to that employed for group SO. One water group was pretreated with cyanamide (group CYANWAT; n= 7) and the other group was pretreated with saline (group SALWAT; n= 7) for five successive days. Following three additional weeks of restricted access to water, the "alternate day" testing paradigm, identical to that used for group SO was employed.

RESULTS

The present experiment was designed to investigate the effects of cyanamide on fluid intake other than ethanol. Since an ethanol drinking group was not used in the present experiment, ethanol consumption data for animals pretreated with cyanamide alone (CYANE; n= 26) and saline (SALE; n= 23) from Experiments 2 and 3 were presented for comparative purposes only. A baseline

measure of intake was determined by calculating mean SQ or water intake over the last five training days (Days 65-69) for each animal. Individual one-way ANOVAs performed on baseline intake revealed no differences between cyanamide or saline groups within each fluid group prior to the testing phase ($p > .05$). Mean fluid intake (expressed as percent change from baseline intake) for ethanol, SQ and water drinking groups is presented in Figure 6 (panels a,b,c, respectively). Individual two-way ANOVAs (Drug x Days) with repeated measures were performed on the SQ and water drinking groups. An ANOVA performed on SQ intake yielded a significant Drug x Day interaction ($F(5,65) = 3.3$; $p < .01$) and a significant main effect of Drug. Tukey tests indicated that animals pretreated with cyanamide significantly increased SQ intake on Test days 3,4 and 5 compared to the saline control group ($q(2,21) = 150.3$; $p < .05$). A significant Drug x Day interaction and main effect of Drug was also found for the water group ($F(5,75) = 5.4$; $p < .0003$). Tukey tests indicated that animals pretreated with cyanamide consumed more water during the testing phase (Test days 1-5) than controls ($q(2,18) = 126.6$; $p < .05$). As shown in Figure 6a, ethanol drinking animals pretreated with cyanamide consumed more ethanol across test days than the saline-treated group.

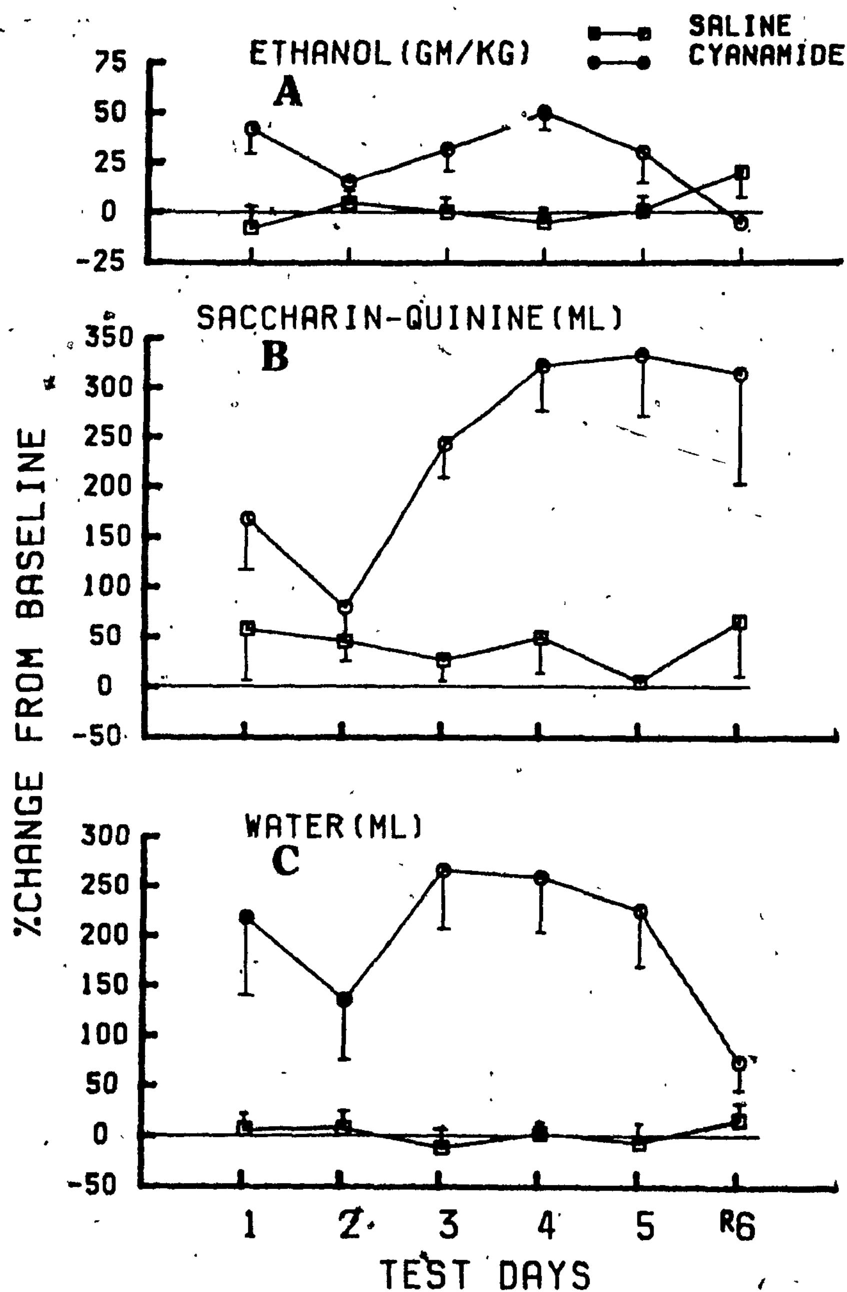

Figure 6. Mean fluid intake (expressed as percent change from baseline intake) for ethanol, saccharin-quinine and water drinking groups pretreated with saline or cyanamide (panels a,b,c respectively).

For the analysis of the "alternate day" test procedure, a baseline measure of intake was determined by calculating mean SQ or water intake over the last five days (days 92-96) prior to Drug day 1 for each animal. A one-way ANOVA on baseline intake for the water drinking group yielded no differences between saline or cyanamide treated groups prior to Drug day 1 ($p>05$). However, a one-way ANOVA revealed a significant group effect for SQ intake ($F(1,12)= 8.3$; $p<.01$). Baseline intake of animals previously pretreated with cyanamide was significantly higher than group SALSQ (CYANSQ- 6.5 ± 1.1 ml; SALSQ- 2.7 ± 0.7 ml). Consequently, the drinking data for the water drinking group was analyzed as percent change from baseline and the drinking data for the saccharin-quinine group was analyzed as raw scores. Ethanol and water intake (expressed as percent change from baseline) across the 10 Testing days is presented in Figure 7 (panels a and b, respectively). Saccharin-quinine intake is presented in Figure 8.

A two-way ANOVA on water intake yielded a significant Drug x Days interaction ($F(9,135)= 11.7$; $p<.00001$) and main effect of Drug. Tukey tests indicated that animals pre- ~~treated~~ with cyanamide (group CYANWAT) consumed more water across all Drug days compared to group SALWAT ($q(2,19)= 72.4$; $p<.05$). In addition, water intake was significantly higher on

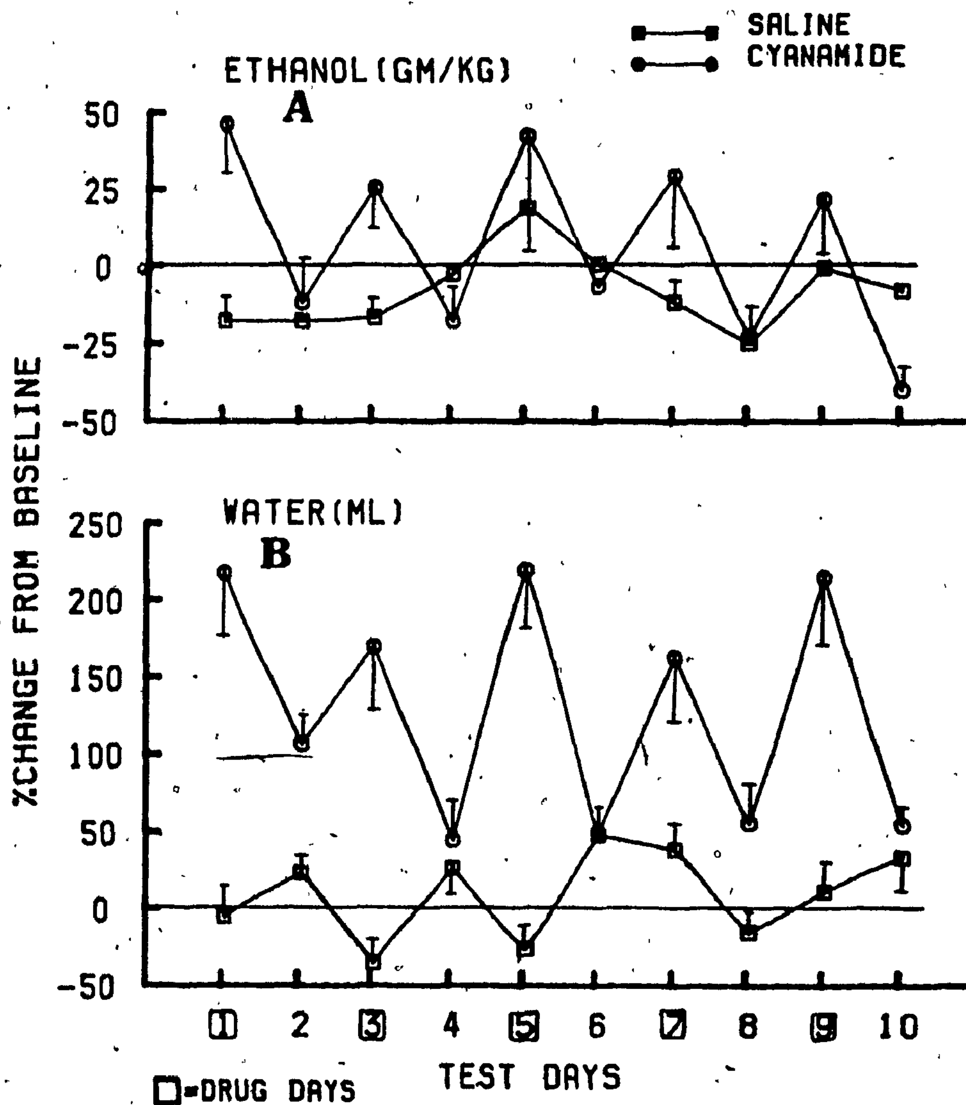

Figure 7. Mean fluid intake (expressed as percent change from baseline intake) for ethanol and water drinking groups pretreated with saline or cyanamide (panels a,b respectively).

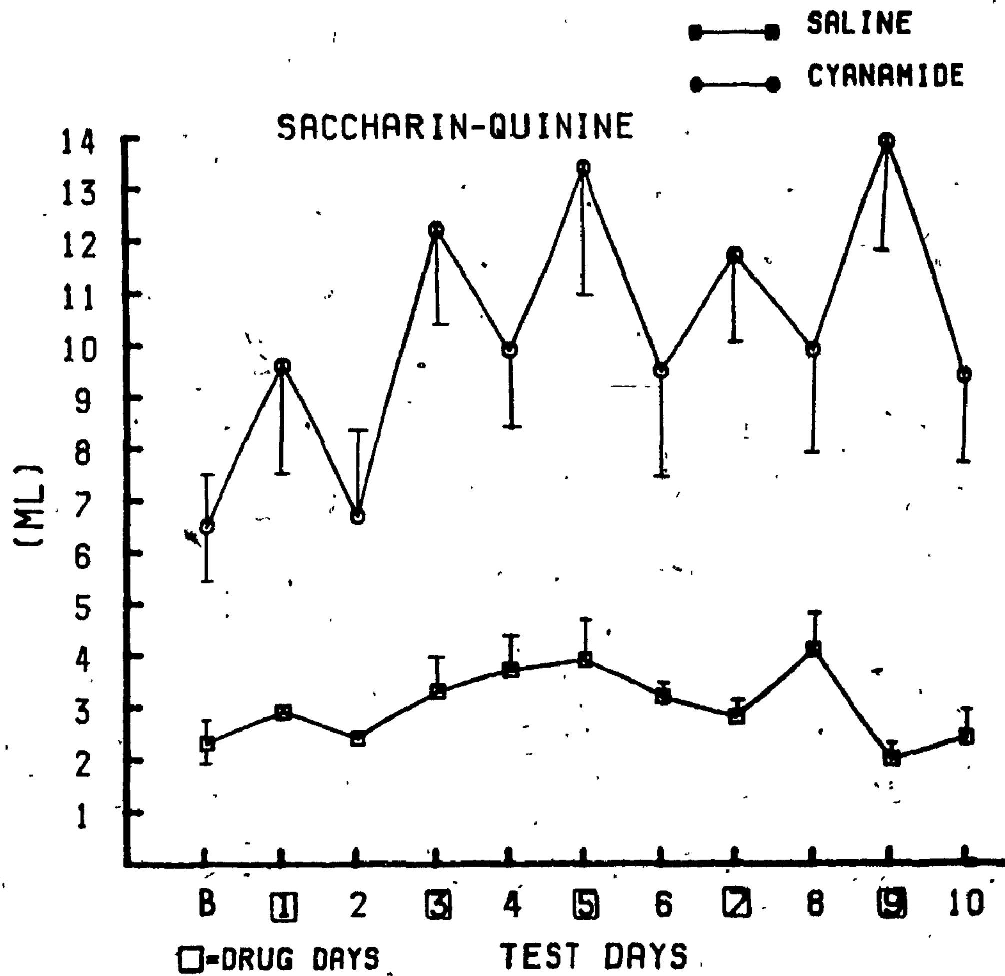

Figure 8. Mean saccharine-quinine intake (ml) for animals pretreated with saline or cyanamide.

Drug days than on corresponding Saline days for group CYANWAT ($q(2;135)= 52$; $p<.05$) (see Figure 7b). A two-way ANOVA performed on the raw drinking scores for the saccharin-quinine group yielded a significant Drug x Days interaction ($F(10,120)= 7.1$; $p<.00001$) and main effect of Drug. Tukey tests revealed that cyanamide treated animals (group CYANSQ) consumed more saccharin-quinine on all Drug days than on corresponding Saline days ($q(2,120)= 1.8$; $p<.05$). The mean increase in SQ intake on Drug days was approximately 100% above baseline levels. SQ intake for saline pretreated subjects (group SALSQ) did not significantly differ across all days ($p>.05$).

Although drinking data for the ethanol group was analyzed in Experiment 3, it is important to note that animals pretreated with cyanamide alone demonstrated a significant increase in ethanol consumption on Drug days yet maintained baseline intake levels on Saline days. As shown in Figures 7 and 8, the pattern of fluid intake for ethanol, water and SQ drinking groups pretreated with cyanamide is almost identical. However, the magnitude of increase on Drug days as well as the magnitude of decrease on Saline days is very different for the ethanol drinking group when the former is compared to saccharin-quinine and water drinking groups.

DISCUSSION

The results of the present experiment do not support the hypothesis that cyanamide merely modified factors relating to the taste of flavored substances. What is evident, however, is that cyanamide influenced the consumption of all the fluids tested including water. Although animals pretreated with cyanamide increased their intake of a SQ solution, animals trained to drink plain tap water also increased water intake following pretreatment with cyanamide.

A striking feature of the data is the similarity in pattern of consumption for ethanol, SQ and water consuming groups. As shown in Figure 6, the increase in fluid intake was evident from the first Test day throughout the five testing days. The "specificity" of cyanamide's effect was most markedly demonstrated in the "alternate day" test paradigm. The pattern of increased drinking on Drug days vs decreased drinking on the corresponding Saline day was a salient characteristic in all three drinking groups (see Figures 7 and 8). It is important to note, however, that the magnitude of increase was very different for the ethanol drinking group compared to the SQ and water drinking groups. In the first part of the experiment where animals were treated for five successive days, ethanol drinking animals pretreated with cyanamide demonstrated a mean increase of 40% in comparison to

increases of over 200% for the SQ and water drinking groups (see Figure 6). Moreover, in the "alternate day" test paradigm, ethanol drinking animals did not differ significantly from saline controls on Drug days; the cyanamide effect was mainly attributed to the reductions in consumption on Saline days relative to intake on the preceeding Drug day (see Results section in Experiment 3). On the other hand, the SQ and water drinking groups pretreated with cyanamide ingested significantly more fluid on Drug days than their respective saline control groups.

The present findings suggested that cyanamide treatment resulted in a general increase in fluid intake. However, the magnitude of increase was different for animals consuming ethanol. It was therefore suggested that cyanamide had a specific effect on ethanol which placed a limitation on the amount of ethanol that the animal could ingest. The following experiment addresses this notion.

EXPERIMENT 5

The series of experiments presented thus far were designed as a systematic investigation of the nature of cyanamide's effect on ethanol in an attempt to understand the role of brain ALDH and centrally-acting acetaldehyde in mediating a "drinking bout". The results suggested that cyanamide increased ethanol intake as well as saccharin-quinine and water consumption in a restricted access paradigm. It would appear then, that cyanamide produced a general increase in fluid intake. At present, it is not known whether this increase is related to a direct inhibition of ALDH by cyanamide or some other property of cyanamide itself. However, as reported in Experiment 4, the magnitude of increase in consumption seen in ethanol drinking animals was much lower than that observed in the saccharin-quinine and water consuming groups. Based on the above, it is possible that two properties of cyanamide may be influencing ethanol drinking behavior simultaneously. One may be related to the capacity of cyanamide to increase fluid intake and the other may be directly related to the inhibition of brain ALDH, which in turn, limited the amount of ethanol ingested. Thus, this concept assumes the presence of two overlapping but independent processes influencing ethanol drinking behavior. It is therefore conceivable that in the 10 min. restricted access paradigm, the general fluid

effect produced by cyanamide may initially be the more salient feature because of the limited availability to ethanol. It is possible that by extending the duration of exposure to ethanol, the potential "limiting" factor (involving brain ALDH) may become more prominent. This notion is supported by the finding that animals pretreated with cyanamide demonstrated a suppression of ethanol intake using a 24 hr access paradigm (Sinclair & Lindros, 1981; Eriksson, 1980). Moreover, if the "limiting" factor is specific to ethanol, then cyanamide treatment should not suppress saccharin-quinine intake using a 24 hr access paradigm.

To test these hypotheses, a 24 hr access paradigm was employed in the present experiment. Following a screening procedure for ethanol or saccharin-quinine consumption, animals were pretreated with cyanamide and total fluid intake over the 24 hr period was recorded.

METHODS

Subjects

Subjects were 28 male Long Evans rats initially weighing 125-150 grams. Housing conditions were identical to those in Experiment 1. Fluids were presented in two glass Richter tubes mounted on the front of the cage. The animal colony room was illuminated on a 12 hr day/night schedule. The light cycle changed at 8:00 PM/AM.

Procedure

Ethanol drinking group (n=14): The ethanol screening procedure was identical to that described in Experiment 1. Following the alternate day screening procedure (2%-10%), animals were switched to a schedule of every day ethanol presentation in a free choice with water for 12 consecutive days. Based on mean ethanol intake (gm/kg) of the last five days (Baseline intake-Days 8-12), animals were ranked and assigned to one of two treatment conditions to ensure an equal distribution of drinkers in each group. On Day 12, animals were injected i.p. with cyanamide (25 mg/kg- group CYANE) or saline (1.0 ml/kg- group SALE) at 5:00 PM (three hours prior to the onset of the night cycle). The Richter tubes were removed from the cages at 4 PM and returned at 8 PM. This was done in an attempt to maximize cyanamide's inhibitory action on ALDH activity since it has been reported that ethanol administered prior to cyanamide treatment interfered with cyanamide's ability to inhibit ALDH activity in the liver (Tottmar, Marchner & Lindberg, 1977). Animals were injected every afternoon at the same time for five consecutive days (Test days 1-5). Drinking was measured on one more occassion following the testing period.

Saccharin-quinine consuming group (n=14): The saccharin-quinine (SQ) screening procedure was

identical to that described in Experiment 4. Animals were initially exposed to a saccharin solution (0.1% w/v) and water for two days. Animals were then exposed to the saccharin solution adulterated with increasing concentrations of quinine (0.0025%-0.0175%). Animals were presented with each SQ concentration for two days. Using the criteria established in Experiment 4, the SQ concentration was set when SQ preference was equivalent to the preference ratios of animals with a free choice of water and ethanol (10% concentration). Subjects in the present experiment appeared to be more sensitive to the quinine concentrations used than subjects in Experiment 4. Consequently, two SQ concentrations were employed. Seven subjects were maintained at a quinine concentration of 0.0175% and the remaining animals were maintained on a quinine concentration of 0.0125%. Once the criteria was met, animals were given five additional days of exposure to the SQ solution and water. Based on mean SQ consumption (ml) over the last three days (Baseline intake- Days 3-5), animals were ranked, and assigned to one of two treatment conditions to ensure an equal distribution of drinkers in each group. On Day 5, animals were injected i.p. with cyanamide (25 mg/kg- group CYANSQ) or saline (1.0 ml/kg- group SALSQ) at 5:00 PM. The Richter tubes were removed from the cages at 4 PM and returned at 8 PM. Animals were injected every afternoon at the same time for five

consecutive days (Test days 1-5). Drinking was measured on one more occassion following the injection period.

RESULTS

Baseline values for mean absolute ethanol (gm/kg) or SQ (ml) intake, mean preference ratio (calculated as percent of ethanol or SQ in total daily fluid intake) and mean total daily fluid intake were calculated over the last three or five training days for SQ and ethanol drinking animals, respectively. Since ethanol is a pharmacological agent and drinking behavior may be specifically influenced by factors related to the pharmacological actions of ethanol, the ethanol and SQ drinking groups were analyzed separately.

Individual one-way ANOVAs were performed on baseline data for all the above measures. The analyses yielded no differences in baseline intake between groups within each fluid drinking group ($p>.05$). All data was therefore analyzed as percent change from baseline level. The data of all three measures (gm/kg, preference and total fluid intake) for the ethanol drinking group are presented in Figure 9. A two-way ANOVA on mean absolute ethanol intake (gm/kg) yielded a significant Days effect only ($p<.05$). As shown in Figure 9a, however, animals pretreated with cyanamide demonstrated a large decrease in ethanol consumption on

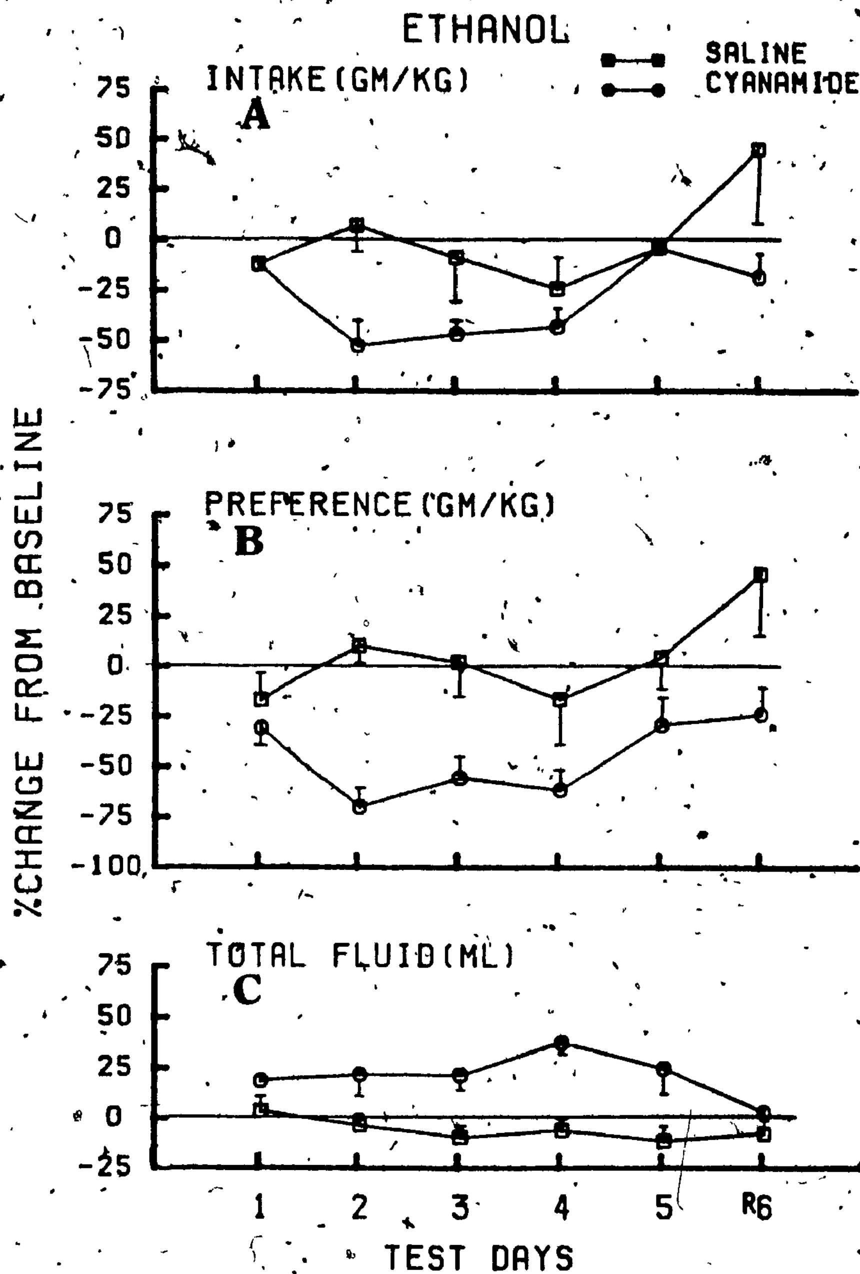

Figure 9. Mean absolute ethanol intake (gm/kg), preference and total fluid intake (expressed as percent change from baseline intake) for animals pretreated with saline or cyanamide (panels a,b,c respectively).

Test days 2-4 in comparison to group SALE. A two-way ANOVA on ethanol preference yielded a significant main effect of Drug ($F(1,12) = 13.3$; $p < .003$). The Drug x Days interaction was not significant ($F(5,60) = 2.1$; $p < .076$). Overall, group CYANE demonstrated a significant decrease in preference for ethanol compared to group SALE. Although the Drug x Days interaction was not significant, it is quite evident from Figure 9b that ethanol preference was strongly reduced for group CYANE on Test days 2-4. Consequently, post hoc Tukey tests were performed on the Drug x Days interaction. It was revealed that ethanol preference was significantly reduced for group CYANE on Test days 2-4 and recovery day 6 (R6) when compared to group SALE ($q(2,18) = 42.5$; $p < .05$). It is of interest to note that animals pre-treated with cyanamide decreased absolute ethanol intake on these corresponding Test days as well (see Figure 9a). A two-way ANOVA on total fluid intake for the ethanol drinking group yielded a significant main effect of Drug ($F(1,12) = 23.1$; $p < .0004$) and no significant Drug x Days interaction ($F(5,60) = 1.8$; $p > .13$). Overall, total fluid intake was significantly higher for animals pretreated with cyanamide (group CYANE) (see Figure 9c). Taken together, these analyses suggested that animals pretreated with cyanamide reduced their preference for ethanol as well as intake of absolute ethanol and increased their total fluid

intake. This would indicate that water intake increased over Test days.

Mean saccharin-quinine intake (ml), SQ preference and total fluid intake (expressed as percent change from baseline) are presented in Figure 10 (panels a,b,c respectively). A two-way ANOVA performed on mean SQ intake yielded no significant Drug x Days interaction ($F(5,60) = 1.8$; $p > .13$) nor main effect of Drug ($F(1,12) = 1.2$; $p > .30$) (see Figure 10a). A two-way ANOVA on SQ preference also yielded no significant interaction ($F(5,60) = 1.5$; $p > .20$) nor Drug effect ($F(1,12) = .02$; $p > .92$) (see Figure 10b). However, a two-way ANOVA on total fluid intake yielded a significant Drug x Days interaction ($F(5,60) = 4.5$; $p < .002$). Post hoc Tukey tests revealed that animals pretreated with cyanamide consumed more fluid than group SALSQ on all Test days ($q(2,15) = 27.6$; $p < .05$) (see Figure 10c). The increase in total fluid intake indicated that animals pretreated with cyanamide consumed more water over Test days than the saline pretreated group.

DISCUSSION

The results of the present experiment supported the notion that cyanamide may have a specific effect on ethanol. Subjects pretreated with cyanamide suppressed ethanol consumption and demonstrated a concomitant decrease in preference for ethanol. In contrast, SQ drinking animals pretreated with cyanamide demonstrated

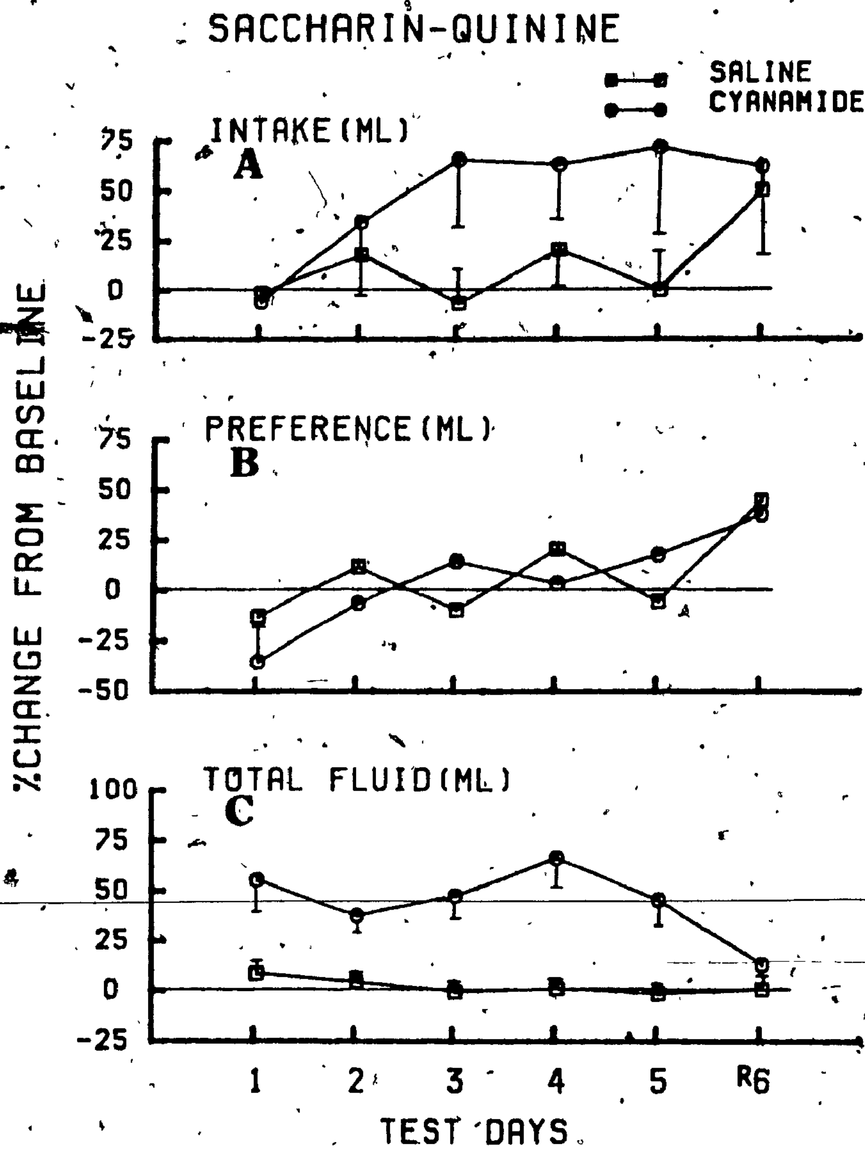

Figure 10. Mean saccharine-quinine intake (ml), preference, and total fluid intake (expressed as percent change from baseline intake) for animals pretreated with saline or cyanamide (panels a, b, c respectively).

no change in SO intake nor preference. A common feature to both cyanamide treated groups was a significant increase in total fluid intake. These results suggested that cyanamide may produce a general increase in fluid consumption (in this case, water intake) in a 24 hr access paradigm. However, the suppression of ethanol intake as well as the reduction in ethanol preference suggested that cyanamide may have a specific effect on ethanol.

These findings were consistent with other reports that cyanamide suppressed ethanol intake in a 24 hr access paradigm (Sinclair and Lindros, 1981; Sinclair et al, 1980; Lindros, Koivula & Eriksson, 1975). Unfortunately, these authors did not report whether or not water or total fluid intake was also altered by cyanamide treatment.

Given the present results and given the putative role of cyanamide, it is of interest to determine the contribution of brain ALDH in mediating the alterations in ethanol consumption by cyanamide. However, since cyanamide may also produce a general increase in fluid intake, it would be beneficial to use an ALDH inhibitor that does not possess this property. If cyanamide's effects on ethanol were attributed to brain ALDH inhibition, then similar effects should be observed using a different ALDH inhibitor. The next experiment was designed in an attempt to address this issue.

EXPERIMENT 6A

Cyanamide appears to possess at least two independent properties. It seems to enhance consumption of fluids in general and also specifically suppress ethanol intake. It has been suggested that the suppression of ethanol consumption may be attributed to the direct inhibition of brain ALDH by cyanamide (Sinclair & Lindros, 1981; Sinclair et al, 1980). However, recently it has been demonstrated that an active metabolite of cyanamide and not cyanamide itself is responsible for the inhibition of ALDH activity (Cederbaum & Dicker, 1985; DeMaster, Shirota & Nagasawa, 1984). Interestingly, the enzyme catalase seems to be involved in the conversion of cyanamide to its active metabolite (DeMasters et al, 1984; DeMaster, Shirota & Nagasawa, 1985; Svanas & Weiner, 1985). As a consequence of this conversion process, catalase activity was also reported to be inhibited. DeMaster, Redfern, Shirota and Nagasawa (1986) demonstrated that treatment with 0.68 mM of cyanamide inhibited approximately 50% of catalase activity in brain. It has also been shown that approximately 50% of brain catalase is inhibited with a dose of 25 mg/kg cyanamide (0.59 mM), used in the present series of experiments (C.M.G. Aragon, personal communication). Given brain catalase's potential role in regulating ethanol consumption (e.g. Aragon et al, 1985), it is possible

that the inhibition of brain catalase by cyanamide may also contribute to the observed alterations in ethanol consumption following cyanamide treatment.

In an attempt to further determine the contribution of brain ALDH in regulating a "drinking bout", the ALDH inhibitor coprine was used in the present experiment. Coprine, a constituent of the inky cap mushroom Coprinus atramentarius, has been reported to be a potent inhibitor of ALDH both in the liver and in brain (Tottmar & Lindberg, 1977; Pettersson & Tottmar, 1982a; Tottmar, Marchner & Lindberg, 1977), and has been reported to suppress ethanol intake in rats (Sinclair et al, 1980). Pilot work in preparation for the present study also indicated that coprine did not interfere with brain catalase activity.

Furthermore, one difference in the methodology underlying the 24 hr access and restricted access paradigms employed in the present series of experiments was that animals had a choice of two fluids (ethanol and water) in the former (24 hr) and only one fluid (ethanol) in the latter paradigm (restricted access). Since cyanamide may influence fluid consumption in general, animals pretreated with cyanamide may consume ethanol in the restricted access not specifically for ethanol but because it was the only fluid available to them in this paradigm. If animals were given a choice between water and ethanol during the restricted access,

the contribution of cyanamide's effects on fluid intake as well as it's direct action on ethanol may be clarified.

The goal of the present experiment was two fold. If brain ALDH plays a role in regulating a "drinking bout", then pretreatment with cyanamide or coprine should produce similar changes in ethanol consumption. Moreover, if either agent influences fluid intake in general, then differences in water and ethanol intake should be observed when animals are given a free choice during the 10 min. restricted access paradigm.

METHODS

Subjects

Subjects were 42 male Long Evans rats initially weighing 150-175 grams. Housing conditions were identical to those outlined in Experiment 1. Fluids were presented in two glass Richter tubes mounted on the front of the cage.

Drugs

4-methylpyrazole (10 mg/kg), sodium cyanamide (25 mg/kg) (Sigma Chemicals, St. Louis) and coprine (20 mg/kg) (courtesy of Dr. O. Tottmar) were each dissolved in saline and administered in a volume of 1.0 ml/kg. All injections were administered intraperitoneally (i.p.).

Procedure

The ethanol screening procedure and restricted access procedure were described in Experiment 2. A brief outline of the procedure is presented in Table 5.

Table 5. Outline of Experimental procedure

Days	Procedure
1-7	Habituation
8-35	Ethanol screening (2%-10%)
36-42	Ethanol maintenance (10%)
43-56	Ethanol deprivation
57	Acclimatization to ethanol in tube
58-62	Ethanol and water presented for 45 min.
63-77 (Training days)	Ethanol and water presented for 10 min.
70,74	Saline pretreatment (i.p.)
78-82 (Test days 1-5)	Drug pretreatment (i.p.)
83	Drug recovery day

Briefly, animals were initially screened for ethanol consumption by presenting ethanol solutions in an ascending order from 2% to 10% on an alternate day schedule. After seven consecutive days of exposure to the 10% ethanol solution, animals were deprived of ethanol for a two week period.

Restricted access procedure: Following two weeks of ethanol deprivation, animals were presented with a choice of water (presented in the water bottle) and a 10% ethanol solution presented in rubber stoppered plastic tubes fitted with steel ball-bearing spouts for one single 24 hr period. For the next five days a choice of ethanol and water (both presented in the plastic tubes) were presented each day for a 45 min. period. The water and ethanol tubes were alternated on each day to prevent position bias. The single water

bottle on the front of the cage was removed just prior to the free choice presentation and was immediately returned following the drinking session. For the following 15 days, animals were presented with a choice of ethanol and water for a 10 min. period each day. On Training days 70 and 74, animals received two successive i.p. injections of saline (1.0 ml/kg) four hr prior to ethanol and water presentation. Based on mean ethanol intake (gm/kg) of the last five training days, animals were ranked and assigned to one of five treatment groups to ensure an equal distribution of drinkers in each group. For the next five days, four hr prior to ethanol and water presentation, animals received two successive i.p. injections. A summary of the treatment groups is presented in Table 6.

Table 6. Summary of Treatment groups

Group		Treatment
S+S	(n= 8)	Saline + Saline
S+COP	(n= 9)	Saline + Coprine
S+CYAN	(n= 9)	Saline + Cyanamide
4MP+COP	(n= 8)	4-methylpyrazole + Coprine
4MP+CYAN	(n= 8)	4-methylpyrazole + Cyanamide

All drugs except coprine were administered every day for five days. Since Tottmar and Lindberg (1977) reported that liver ALDH activity was still significantly inhibited by coprine (27 mg/kg) twenty-four hours after administration and in order to control for accumulation effects, coprine was administered to

groups 4MP+COP and S+COP only on Test days 1,3 and 5. On Test days 2 and 4, saline was administered instead of coprine. Following the injection period (Test days 1-5), ethanol and water were presented on one more day without drug treatment and drinking was measured.

An additional 11 male Long Evans rats were used to verify that coprine inhibited brain ALDH activity at 4 hr and 24 hr after treatment. Six rats were sacrificed by decapitation 4hr (n=3) and 24 hr (n=3) following coprine treatment. The remaining five subjects were injected with saline and sacrificed 4 hr later. The brain of each subject was rapidly extracted, rinsed in ice cold saline and blotted lightly on dry filter paper. Brain samples were first stored at -70° c and later assayed for ALDH activity levels. The ALDH assay procedure was outlined in Experiment 3. In addition, nine male Long Evans rats were included to determine whether the dose of 4MP used was sufficient to prevent the peripheral accumulation of acetaldehyde by coprine. Five subjects were pretreated with saline + coprine (20 mg/kg) and four subjects were pretreated with 4MP (10 mg/kg) + coprine, four hr prior to i.p. administration of ethanol (1.2 gm/kg). Animals were sacrificed by decapitation 30 min. after ethanol administration. Trunk blood of each animal was collected and later assayed for acetaldehyde determinations by head-space

gas chromatography. The procedure for acetaldehyde determinations was outlined in Experiment 3.

RESULTS

The effects of coprine on brain ALDH activity at 4 hr and 24 hr following treatment are presented in Table 7. Independent T tests revealed that pretreatment with coprine produced a signficant reduction in brain ALDH activity at 4 hr ($t(4) = 7.88$; $p < .001$) and at 24 hr ($t(4) = 5.98$; $p < .001$) compared to saline controls. As mentioned earlier, pilot work indicated that coprine did not interfere with brain catalase activity (coprine (n=3): 0.103 ± .01 units of catalase/min/mg protein; saline (n=3): 0.111 ± .003 units of catalase /min/mg protein).

Table 7: Brain ALDH activity ± S.E.M. (expressed as nMoles of NADH/min/mg of protein) following pretreatment of coprine (4hr and 24 hr) or saline.

Treatment	ALDH activity	% INHIBITION from saline control group
Coprine (4 hr)	1.17 ± .009 *	11
Coprine (24	1.13 ± .03 *	14
Saline	1.31 ± .01	

*- significantly different from saline; $p < .001$

Peripheral blood acetaldehyde was only detected in animals pretreated with coprine alone (314 ± 19.4 µM acetaldehyde) following systemic administration of ethanol (1.2 gm/kg). The addition of 4MP to coprine

was efficient in preventing the accumulation of blood acetaldehyde levels by coprine.

A baseline measure of absolute ethanol intake was determined by calculating mean ethanol intake (gm/kg) over the last five training days (Days 73-77) for each animal. Baseline measures of mean preference ratio (calculated as percent of ethanol in total fluid intake) and mean total intake were also calculated. Individual one-way ANOVAs were performed on baseline data of all the above measures. The analyses yielded no differences in baseline intake between treatment groups ($p>.05$). All data was therefore analyzed as percent change from baseline level. The data of all three measures (absolute ethanol intake (gm/kg), preference and total fluid intake) for all treatment groups is presented in Figure 11 (panels a,b,c, respectively). A two-way ANOVA (Group x Days) on mean absolute ethanol intake yielded a significant Group x Days interaction ($F(5,185)= 3.64$; $p<.00001$) and a significant Group effect ($F(4,37)= 9.88$; $p<.00001$). Post hoc Tukey tests were performed on the interaction effect and are summarized in Table 8.

Table 8. Summary table of significant differences between groups across Test days using Tukey tests ($q(5,84) = 58.7$; $p < .05$) (R6= Drug recovery day). Each group was compared to all other groups across days, when appropriate.

			TEST DAYS			
GROUP	T1	T2	T3	T4	T5	R6
4MP+CYAN		S+COP S+CYAN	S+COP	S+COP	S+COP S+CYAN S+S	S+CYAN
4MP+COP			S+COP		S+COP	S+CYAN
S+S			S+COP S+CYAN		S+COP	S+CYAN

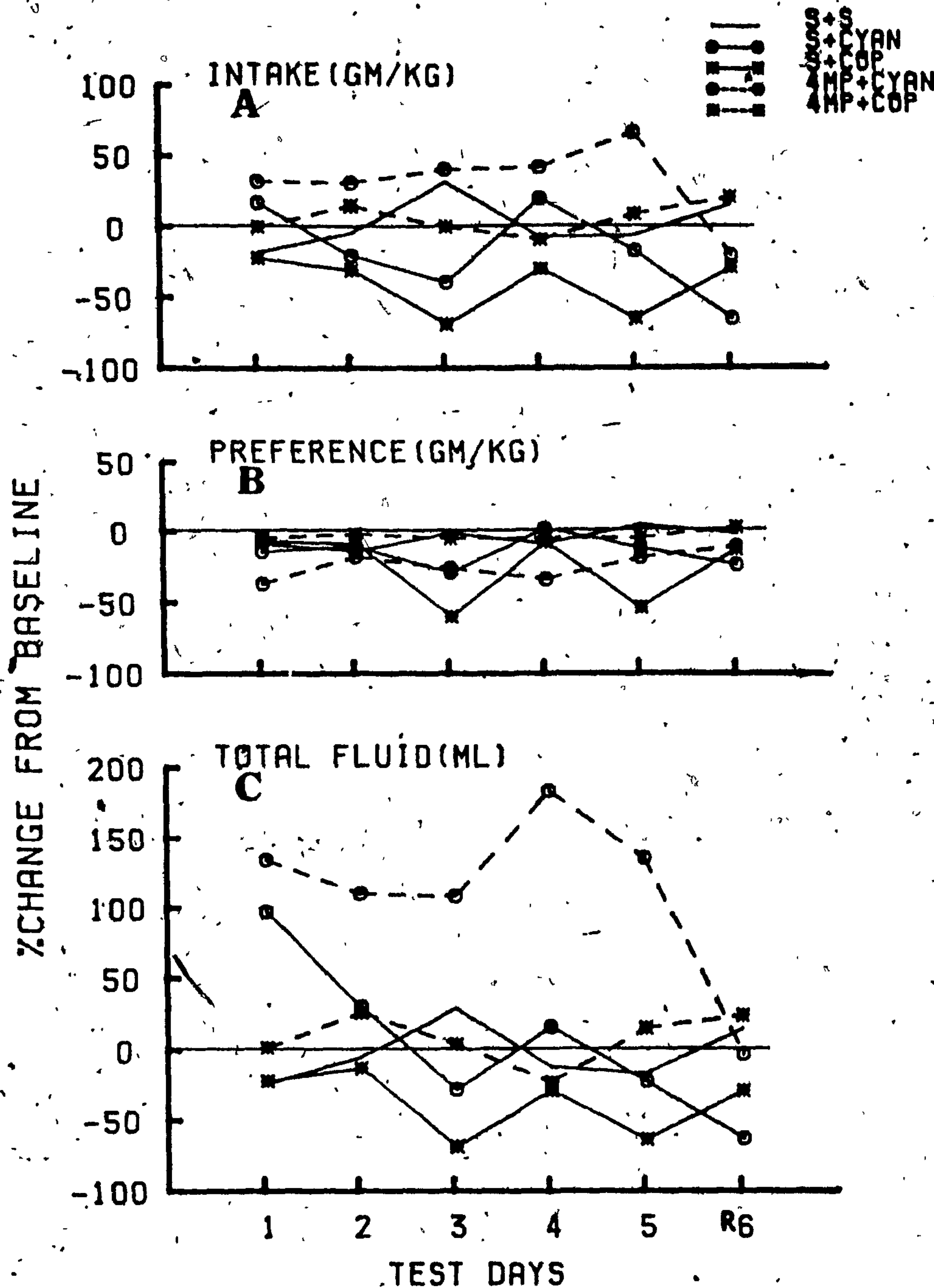

Figure 11. - Mean absolute ethanol intake (gm/kg), preference and total fluid intake (expressed as percent change from baseline intake) for all treatment groups (panels a,b,c respectively). S= saline; 4MP= 4-methylpyrazole; CYAN= cyanamide; COP= coprine.

Overall, Tukey tests revealed that animals pretreated with coprine alone (group S+COP) consumed significantly less ethanol than groups 4MP+CYAN, 4MP+COP and S+S across Test days ($q(5,37)= 35.6$; $p<.05$). In addition, group S+CYAN drank significantly less ethanol than group 4MP+CYAN ($p<.05$). There were no significant differences between groups S+CYAN and S+COP ($p>.05$). Moreover, no significant differences were revealed between groups 4MP+CYAN, 4MP+COP and S+S ($p>.05$) (see Figure 11a).

A two-way ANOVA of ethanol preference yielded a significant Group x Days interaction ($F(5,185)= 2.42$; $p<.001$) and a significant Group effect ($F(4,37)= 4.7$; $p<.004$). Tukey tests revealed that group S+COP demonstrated a significant decrease in preference compared to groups 4MP+COP and S+S on Test day 3, and to groups 4MP+COP, S+S and S+CYAN on Test day 5 ($q(5,86)= 36.8$; $p<.05$). As shown in Figure 11b, this effect is mainly attributed to the large reduction in preference on these particular Test days for group S+COP. Tukey tests performed on the Group effect revealed that overall, groups S+COP and 4MP+CYAN signficantly reduced preference for ethanol compared to groups 4MP+COP and S+S ($q(5,37)= 19.4$; $p<.05$).

A two-way ANOVA of total fluid intake yielded a significant Group x Days interaction ($F(5,185)= 3.8$; $p<.00001$). Tukey tests revealed that group 4MP+CYAN

consumed significantly more fluid than groups S+S, 4MP+COP and S+COP on all Test days and drank more fluid than group S+CYAN on Test days 3 to 5 ($q(5,62)= 96.9$; $p<.05$). Group S+CYAN consumed significantly more fluid than groups S+S, S+COP and 4MP+COP on Test day 1 ($p<.05$). It is of interest to note that although group 4MP+CYAN consumed more fluid in total and tended to consume more absolute ethanol, these animals demonstrated a significant reduction in preference (see Figure 11). These results suggested that total fluid intake for group 4MP+CYAN reflected not only an increase in ethanol intake, but an increase in water intake as well during the 10 min. restricted access. A two-way ANOVA was performed on the raw data for water intake. Raw data was used since baseline levels of water intake for all treatment groups were zero and consequently, percent change from baseline was an undefined value. The analysis yielded a significant Group x Days interaction ($F(6,222)= 2.94$; $p<.00001$). Water intake for all treatment groups is presented in Figure 12. Post hoc Tukey tests revealed that group 4MP+CYAN consumed significantly more water than groups S+S, 4MP+COP and S+COP across all Test days ($q(5,83)= 1.55$; $p<05$) and drank more water than group S+CYAN on Test days 3 to 5 ($p<.05$) (see Figure 12.)

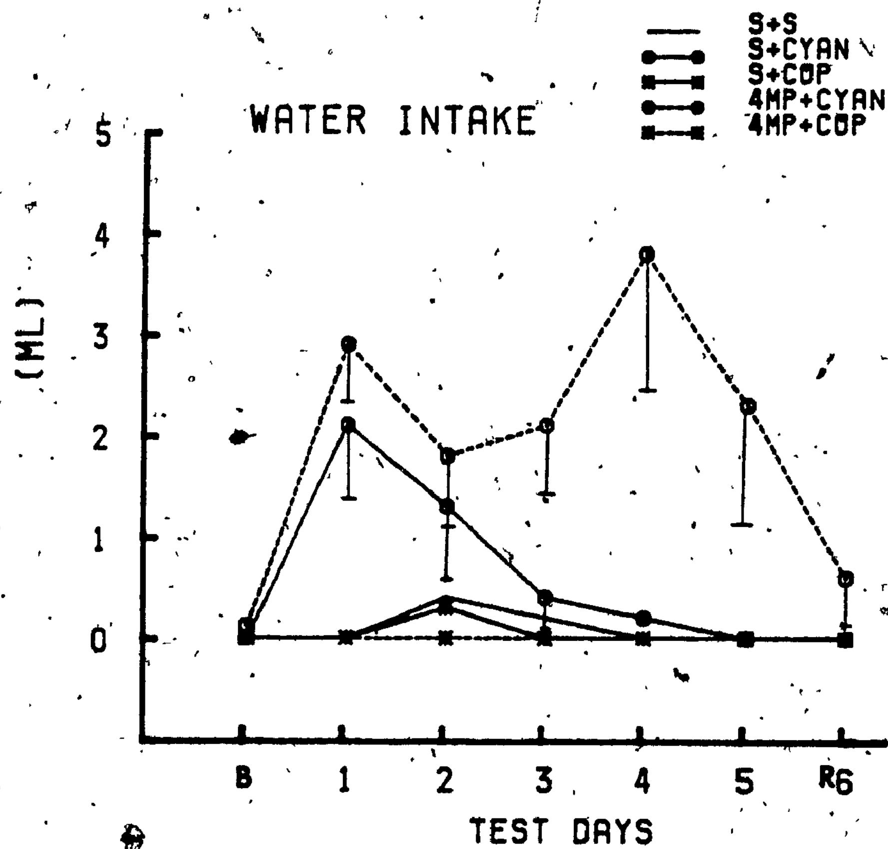

Figure 12. Mean water intake (ml) for all treatment groups.

DISCUSSION

The present findings revealed that animals pretreated with coprine alone (group S+COP) significantly reduced ethanol intake compared to animals pretreated with 4MP+COP. Moreover, animals pretreated with cyanamide alone (group S+CYAN) consumed significantly less ethanol than group 4MP+CYAN. These results suggested that the peripheral accumulation of acetaldehyde (due to the inhibition of liver ALDH activity) may play a role in the suppression of ethanol consumption in a 10 min. restricted access paradigm.

The results also suggested that coprine, unlike cyanamide, did not alter fluid intake in general. Animals pretreated with coprine (groups S+COP and 4MP+COP) did not increase water or ethanol intake during treatment (see Figure 11). Animals pretreated with cyanamide, however, demonstrated an increase in fluid intake. This effect was particularly evident in group 4MP+CYAN. In fact, group 4MP+CYAN demonstrated a significant reduction in ethanol preference presumably because these animals consumed significantly more water on Test days.

The assay of brain ALDH activity suggested that coprine (at 4 hr and 24 hr) inhibited approximatley 11% of brain ALDH activity compared to the saline control group. These results appeared to be appreciably lower that those reported by others using coprine (e.g.

Pettersson & Tottmar, 1982a). It is therefore possible, that the relatively low rate of brain ALDH inhibition reported in the present study may be due to a methodological problem (similar to that reported in Experiment 3 for the assay of brain ALDH activity with cyanamide). Consequently, the actual inhibitory action of coprine on brain ALDH activity may be appreciably higher than that reported in the present experiment.

If brain ALDH plays a role in mediating a "drinking bout", then animals pretreated with coprine and cyanamide alone or with the addition of 4MP should demonstrate similar changes in ethanol consumption. Sinclair and Lindros (1981) reported that pretreatment with 4MP+CYAN or cyanamide alone both resulted in the suppression of ethanol intake. They concluded that the suppression of ethanol consumption by cyanamide was attributed to its direct inhibition of brain ALDH. On the basis of the present findings, it would appear that brain ALDH does not play an immediate role in mediating the initial drinking bout(s) simulated in the 10 min. restricted access paradigm. Animals pretreated with coprine and cyanamide alone suppressed ethanol intake. In contrast, pretreatment with 4MP+COP and 4MP+CYAN produced no observable change in ethanol consumption. This latter finding is inconsistent with the results reported above by Sinclair and Lindros (1981), who reported that pretreatment with 4MP+CYAN suppressed

ethanol consumption. The discrepency in findings may be attributed to the different paradigms used in the two studies. Sinclair and Lindros (1981) used a 24 hr access paradigm whereas, in the present study, a 10 min. restricted access paradigm was employed. It was proposed earlier that the "simulated" drinking bout may reflect the initial drinking bout that may serve to influence in some fashion the pattern of drinking for rats in a continuous (24 hr) access paradigm. Following this notion, it is therefore conceivable that the inhibition of brain ALDH by cyanamide or coprine (without the concomitant increase in blood acetaldehyde levels) may alter the central actions of ethanol following a drinking bout. However, the consequence of this change may be only observed in subsequent drinking behavior and not during the initial drinking bout itself. On the other hand, animals pretreated with cyanamide or coprine alone (groups S+CYAN, S+COP), reduced ethanol intake in the 10 min. restricted access period and this suppression was consistent with that reported when using a 24 hr access paradigm (Sinclair & Lindros, 1981). It is possible that changes in the psychopharmacological effects of ethanol following a drinking bout may be more salient when acetaldehyde concentrations in blood are elevated by cyanamide.

The following experiment was designed to investigate whether pretreatment with cyanamide and coprine alone and with the addition of 4MP would all produce similar changes in ethanol consumption using a 24 hr access paradigm.

EXPERIMENT 6B

Subjects

Subjects were 17 male Long Evans rats weighing 490-600 grams at the start of the experiment. Animals were individually housed in stainless steel cages with free access to food and water throughout the experiment. Fifteen of the 17 animals employed in the present experiment were pretreated with 4MP+CYAN (n= 7) or 4MP+COP (n= 8) in Experiment 6A. The other two subjects were drug naive but had been trained to drink ethanol in the 10 min. restricted access.

Drugs

4-methylpyrazole (10 mg/kg), sodium cyanamide (25 mg/kg) and coprine (20 mg/kg) were each dissolved in saline. All drugs were administered i.p. in a volume of 1.0 ml/kg.

Procedure

Following the last injection day in Experiment 6A, subjects were given an additional seven days of 10 min. restricted access to ethanol and water. Animals were subsequently placed in new cages with two Richter tubes mounted in the front of the cage. Animals were

presented with a free choice of ethanol (10%) and water for 10 days (maintenance days) using a 24 hr access paradigm. On Day 11, subjects were divided into their two previous treatment groups (groups 4MP+CYAN and 4MP+COP). A group to be pretreated with coprine only (S+COP) was formed by taking two animals from groups 4MP+CYAN and 4MP+COP as well as one of the naive subjects. The second naive subject was added to group 4MP+CYAN to increase the sample size.

On Day 11 at 5 pm (three hr prior to the start of the night cycle), animals received i.p. injections of 4MP+CYAN (n=6), 4MP+COP (n=6) or S+COP (n=5) for six consecutive days. Richter tubes were removed from the cages at 4 pm and returned at 8 pm each day. This was done in an attempt to maximize cyanamide's inhibitory action on ALDH activity since it has been reported that ethanol administered prior to cyanamide treatment interfered with cyanamide's ability to inhibit ALDH activity in the liver (Tottmar et al, 1977). As in the previous experiment, coprine was administered every second day (Test days 1,3 and 5). On Test days 2, 4 and 6, saline was substituted for coprine.

RESULTS

The present experiment was designed to investigate and compare the effects of the various enzyme manipulations on ethanol consumption using a 24 hr access paradigm. Since a group pretreated with cyanamide

alone was not used in the present experiment, animals pretreated with cyanamide alone from Experiment 5 (group S+CYAN) were included in the present analyses for comparative purposes. A baseline measure of ethanol intake was determined by calculating mean ethanol intake (gm/kg) over the last four maintenance days for each animal. A one-way ANOVA yielded no significant differences between groups on baseline levels of ethanol consumption ($p>.05$). Mean ethanol intake, calculated in two day blocks, was therefore analyzed as percent change from baseline intake and the data is presented in Figure 13. A two-way ANOVA (Group x Block) yielded a significant Group x Blocks interaction only ($F(2,40)= 2.4$; $p<.045$). Post hoc Tukey tests revealed that the interaction effect was a function of a significant reduction in ethanol intake for group S+COP compared to group 4MP+CYAN in Block 3 ($q(4, 32)= 69.4$; $p<.05$). Although there were no other significant differences between any of the treatment groups, it is evident from Figure 13 that animals pretreated with cyanamide or coprine alone (groups S+CYAN and S+COP, respectively) demonstrated a reduction in ethanol intake across Test days. In contrast, groups 4MP+CYAN and 4MP+COP did not appear to alter ethanol intake over Test days. However, the drinking data for individual subjects in these groups revealed an interesting pattern of consumption. Some

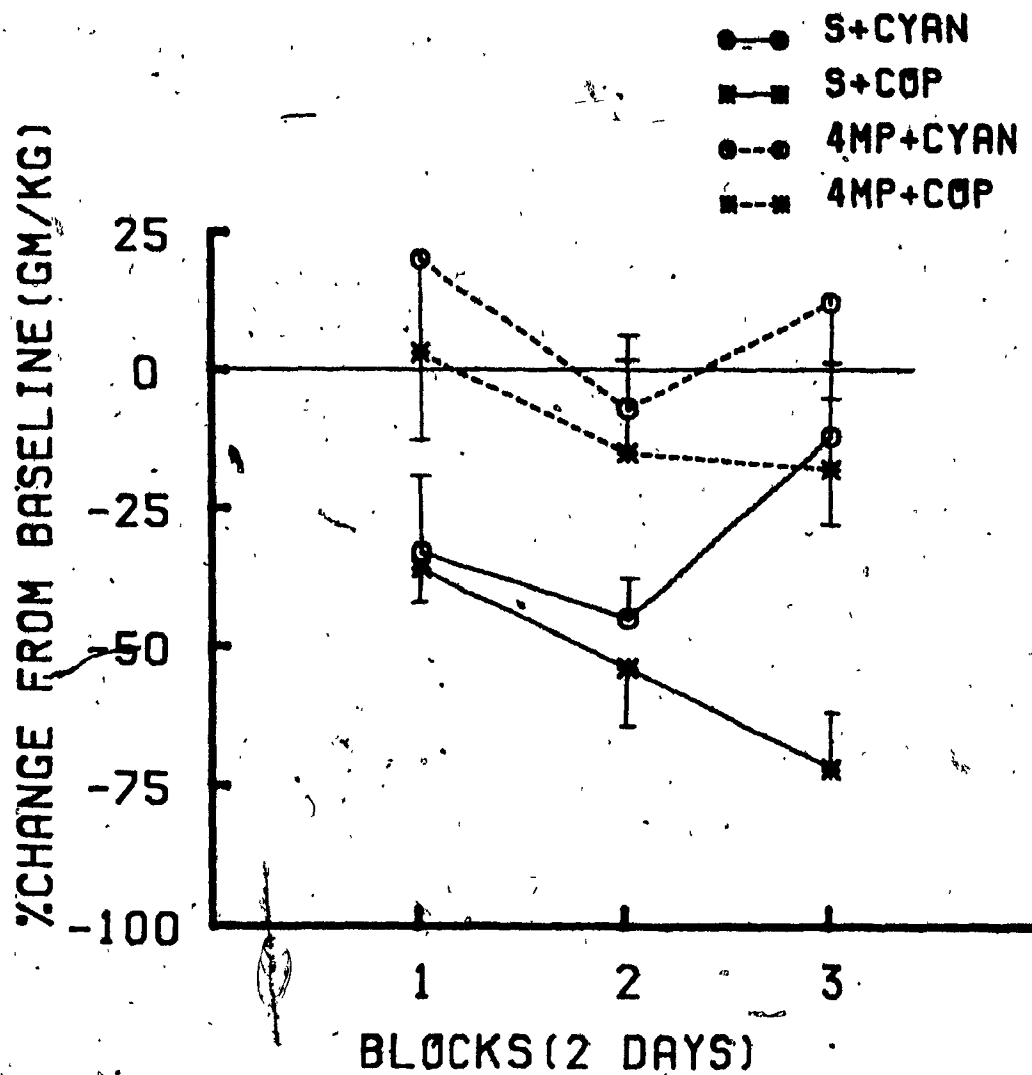

Figure 13. Mean ethanol intake (expressed as percent change from baseline intake) presented in two day blocks for all treatment groups.

animals appeared to increase intake and other subjects appeared to decrease ethanol intake. A comparison of individual baseline levels of intake to mean percent change from baseline over the six test days for these two groups combined yielded a significant correlation ($r(10) = -.60$; $p < .05$). Mean ethanol intake (expressed as mean percent change from baseline across Test days) as a function of baseline intake is presented in Figure 14 for all groups. Animals pretreated with 4MP+CYAN and 4MP+COP whose baseline levels were low appeared to drink more ethanol than animals with higher baseline levels of intake (see Figure 14a). No significant correlation was found for animals pretreated with coprine and cyanamide alone ($r(10) = -.36$; $p > .05$). As shown in Figure 14b, there was less variability in ethanol intake between animals in these groups.

Based on the range of baseline levels of ethanol intake for all animals (0.96-6.6 gm/kg), the midpoint value of 2.8 gm/kg was selected as the cutoff point. Animals whose baseline levels of consumption were below this cutoff were categorized as LOW drinkers and above this cutoff were categorized as HIGH drinkers. Ethanol intake (expressed as percent change from baseline level) for each animal as a function of baseline intake is presented in Figure 15 in two day blocks. As shown in Figure 15, animals were divided into LOW and HIGH drinkers within each two day block. In Block 1, LOW

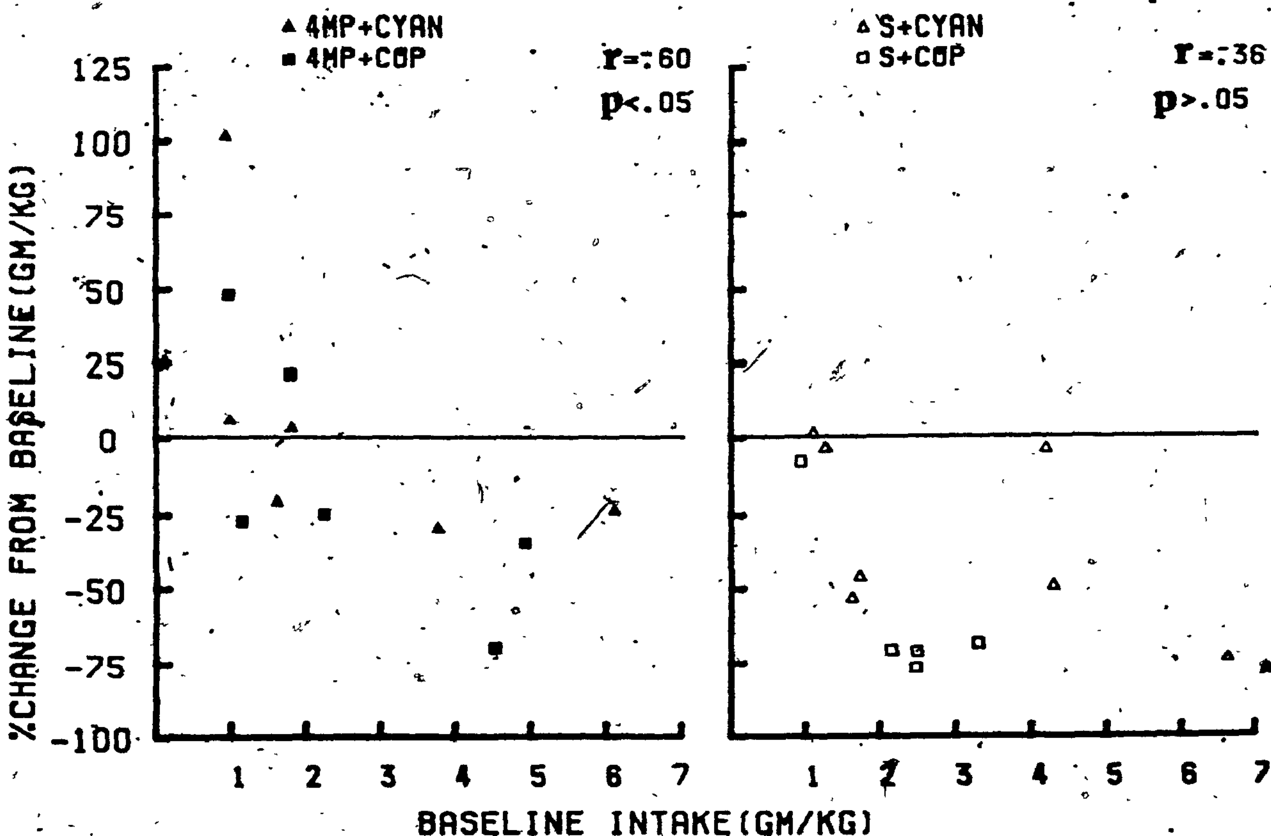

Figure 14. Mean ethanol intake (expressed as mean percent change from baseline intake across all Test days) for each animal as a function of baseline intake.

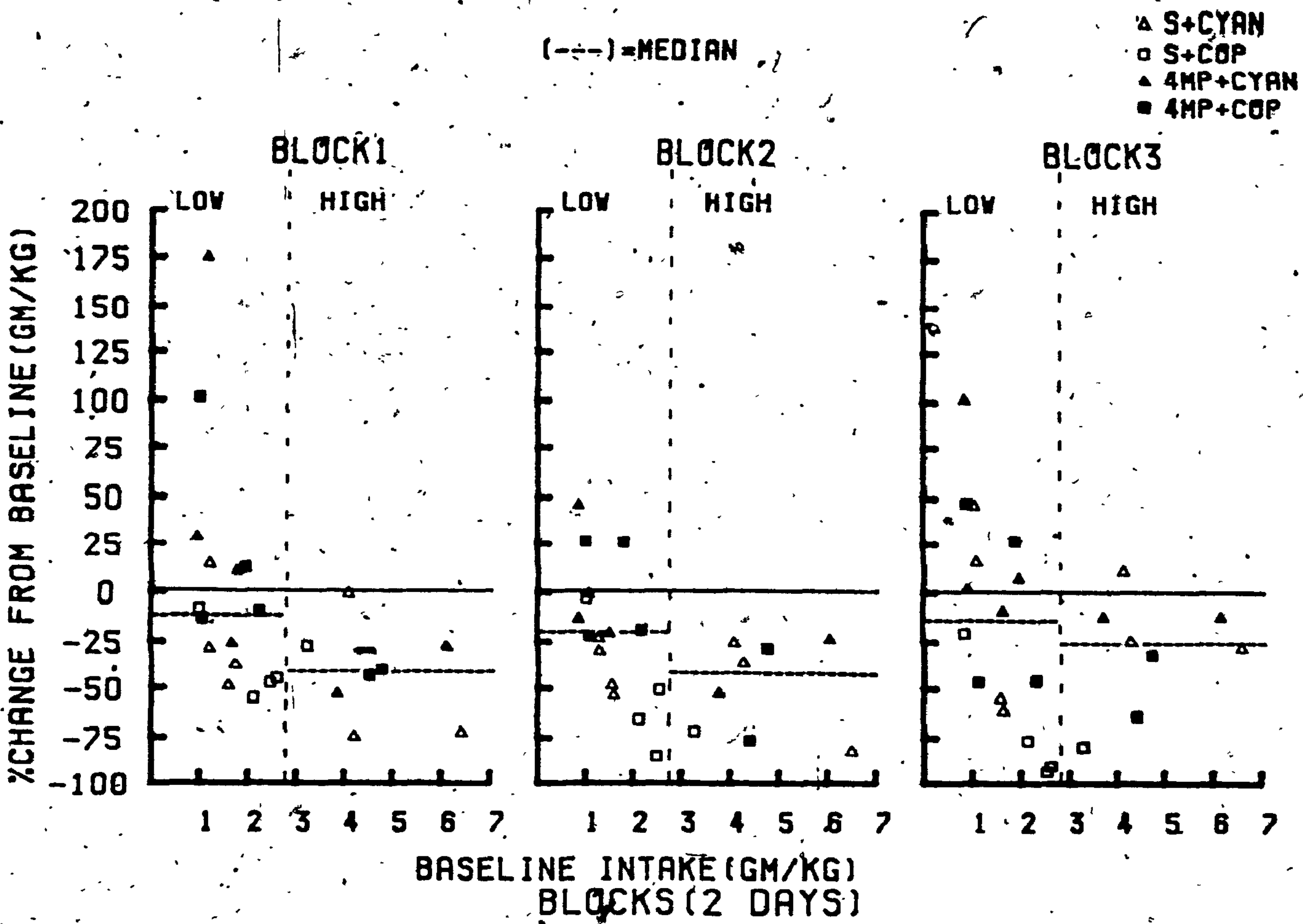

Figure 15. Ethanol intake (expressed as percent change from baseline intake) presented in two day blocks for each animal as a function of baseline intake. HIGH= baseline intake greater than 2.8 gm/kg (n= 8); LOW= baseline intake less than 2.8 gm/kg (n=16).

drinkers (less than 2.8 gm/kg) pretreated with 4MP+CYAN and 4MP+COP appeared to consume more ethanol than animals in group S+CYAN and S+COP. However, as shown in Block 1, HIGH drinkers in all treatment groups demonstrated a reduction in ethanol intake. Across all three blocks HIGH drinkers, regardless of pretreatment manipulation, demonstrated reductions in ethanol intake. Moreover, LOW drinkers pretreated with coprine or cyanamide alone consistently demonstrated reductions in ethanol intake in each two day block. In contrast, LOW drinkers in groups 4MP+CYAN and 4MP+COP consistently consumed more ethanol than LOW drinkers in group S+CYAN and S+COP in each two day block.

DISCUSSION

The results of Experiment 6B suggested that brain ALDH may play a role in regulating ethanol consumption. In general, pretreatment with 4MP+CYAN and 4MP+COP did not appear to alter ethanol consumption in a 24 hr access paradigm. The findings were consistent with those reported in Experiment 6A using a 10 min. restricted access. However, upon closer examination of individual drinking behavior in the 24 hr access paradigm, an interesting pattern was observed. Almost all subjects whose baseline level of consumption was greater than 2.8 gm/kg (HIGH drinkers) demonstrated reductions in ethanol intake regardless of pretreatment manipulation. This suppression cannot be attributed to

elevated blood acetaldehyde levels since animals pre-treated with 4MP+CYAN and 4MP+COP (a manipulation which prevents the peripheral accumulation of acetaldehyde) also demon- strated a reduction in ethanol intake. In the case of LOW baseline drinkers (less than 2.8 gm/kg), the opposite picture emerged. Animals pretreated with cyanamide or coprine alone (groups S+CYAN and S+COP) consistently reduced ethanol consumption in comparison to groups 4MP+CYAN and 4MP+COP. In contrast, these latter groups appeared to increase ethanol consumption. These results suggest that the peripheral accumulation of acetaldehyde may play a role in the suppression of ethanol intake for animals who initially drink smaller quantities of ethanol in a 24 hr access paradigm (i.e. groups S+CYAN and S+COP). Conversely, inhibition of brain ALDH activity without a conconmitant increase in blood acetaldehyde levels may enhance ethanol drinking behavior for LOW drinking animals. Although interpretations are cautiously drawn because of the small sample sizes, the pattern of intake for individual LOW and HIGH drinking animals in 24 hr access appeared to be differentially influenced by the enzyme manipulations employed in the present experiment. The results of Experiments 6A and 6B suggest that the inhibition of brain ALDH by cyanamide and coprine may alter the psychopharmacological effects of the initial drinking

bout. However, the pharmcological consequences of the initial bout may only be observable in when drinking behavior is repeated over time (e.g. 24 hr access).

GENERAL DISCUSSION

The present series of experiments examined the role of brain ALDH and possibly centrally-acting acetaldehyde in the mediation of ethanol drinking behavior. In Experiment 1, it was shown that the amount of ethanol consumed in a 10 min. "simulated" drinking bout was significantly correlated with ethanol intake in a 24 hr continuous access paradigm. On the basis of previous reports that animals consume ethanol in a series of discrete drinking bouts over a period of 24 hr (Gentry et al, 1983; Gill et al, 1986), the present findings suggested that the "simulated" drinking bout may reflect a drinking bout or more specifically, the initial drinking bout when given continuous ethanol availability. A series of experiments were subsequently designed and carried out in an attempt to determine the putative contribution of brain ALDH and centrally-acting acetaldehyde in regulating a drinking bout simulated in a 10 min. restricted access paradigm. It was hoped that the information gained by examining a drinking bout would elucidate how drinking behavior is regulated in general.

In Experiment 2, it was observed that pretreatment with cyanamide only or 4-methylpyrazole + cyanamide both produced increases in ethanol consumption. This effect could not be attributed to elevated blood acetaldehyde levels since animals pretreated with 4-methyl-

pyrazole + cyanamide (a condition which prevents the accumulation of peripheral blood acetaldehyde) demonstrated an increase in ethanol intake as well. Although both groups increased ethanol intake, animals pretreated with cyanamide alone consumed less ethanol than group 4-methylpyrazole + cyanamide, suggesting that the difference in ethanol intake may be attributed to elevated blood acetaldehyde levels produced by cyanamide. The magnitude of increase following cyanamide treatment appeared to be inversely related to baseline levels of consumption. Low drinkers demonstrated the largest increase, mid-range drinkers increased ethanol consumption by about 40% and high drinkers showed the smallest or no increase.

The specificity of cyanamide's effect on ethanol was examined in Experiment 3. Animals were pretreated with various enzyme inhibitors on alternate days while receiving ethanol. The results suggested that cyanamide may have a specific effect on ethanol since increases in ethanol intake were demonstrated only on days when subjects were pretreated with cyanamide. Together, results of Experiments 2 and 3 suggested that the effect of cyanamide on ethanol consumption was immediate and consistent, since it was observed on the first day of cyanamide treatment and persisted throughout the treatment period.

Experiment 4 was designed in an attempt to further understand the specificity and the nature of the increase in ethanol intake produced by cyanamide. Animals trained to drink a saccharin-quinine solution or plain tap water for a 10 min. drinking period were subsequently pretreated with cyanamide for five consecutive days and then on alternate days as outlined in Experiments 2 and 3. The results demonstrated that cyanamide treatment produced increases in both saccharin-quinine and water consumption. These findings suggested that the increase in ethanol consumption, reported earlier, reflected a general effect of cyanamide on fluid intake.

The present results were inconsistent with some previous reports that cyanamide suppressed ethanol consumption (Amit et al, 1980; Eriksson, 1980; Sinclair et al, 1980). However, those studies employed a 24 hr access paradigm whereas, in the present series of experiments, a 10 min. restricted access paradigm was used. It was conceivable that the general increase in fluid intake produced by cyanamide (i.e. an increase in ethanol intake) may be prominent only in the initial drinking bout but not when animals have continuous ethanol availability. It was possible then, that the pharmacological consequences of ethanol associated with this initial increase could subsequently change drink-

ing behavior over the course of the night cycle resulting in a suppression of ethanol intake.

To examine this issue further, a 24 hr access paradigm was employed in Experiment 5. Moreover, a saccharin-quinine group was included to determine if cyanamide would produce a general suppression of drinking over a 24 hr period or whether the effect was specfic to ethanol. The results suggested that cyanamide suppressed ethanol intake and preference and did not significantly alter saccharin-quinine consumption. However, both groups demonstrated a significant increase in water intake. These findings suggested that cyanamide may have two independent but overlapping properties; it enhanced the consumption of fluids in general and it appeared to specifically suppress ethanol intake.

In an attempt to tease apart these two independent properties of cyanamide, a free choice of ethanol and water was presented to animals in Experiment 6A, using the 10 min. drinking paradigm. In addition, if cyanamide's effects on ethanol were indeed attributed to the inhibition of brain ALDH, then alterations in drinking behavior should also be observed with the ALDH inhibitor coprine. The results demonstrated that animals pretreated with cyanamide or coprine alone, suppressed ethanol intake in the 10 min. drinking session. These findings suggested that the accumula-

tion of acetaldehyde in the periphery may have contributed to the observed suppression. It can be argued that brain ALDH may not play a role in regulating a drinking bout since animals pretreated with 4-methylpyrazole + cyanamide (4MP+CYAN) and 4-methlypyrazole + coprine (4MP+COP) did not demonstrate changes in ethanol intake. It was possible, however, that these manipulations did not produce an immediate effect on ethanol intake but the effect would become evident in subsequent drinking behavior.

A 24 hr access paradigm was employed in Experiment 6B to determine the effects of 4MP+CYAN and 4MP+COP on continuous ethanol drinking behavior. The results demonstrated that these manipulations altered ethanol intake in a 24 hr access paradigm. However, this effect was only evident when baseline levels of consumption were considered. It was apparent that animals whose baseline consumption levels were greater than 2.8 gm/kg (determined as the midpoint in ethanol drinking range) suppressed ethanol intake on Test days. These findings were comparable to animals pretreated with cyanamide or coprine only, and are consistent with previous reports that 4MP+CYAN suppresses ethanol intake in a 24 hr access paradigm (Sinclair & Lindros, 1981). In contrast, animals pretreated with 4MP+CYAN and 4MP+COP who consumed less than 2.8 gm/kg at baseline increased ethanol intake, suggesting that this

manipulation may enhance ethanol drinking behavior in low drinking animals. To summarize; the key findings in the present dissertation were:

1) A drinking bout is a pharmacologically meaningful event.

2) Cyanamide seems to possess at least 2 independent properties. It can enhance consumption of fluids in general and it may also have a specific effect on ethanol intake.

3) Those specific effects of cyanamide on ethanol appear to be attributed to the inhibition of brain ALDH, since pretreatment with coprine produced similar results.

4) The direction of the effect by cyanamide and coprine was related to individual subjects' baseline levels of consumption in a 24 hr paradigm.

5) The pharmacological consequences of a bout will in part determine the pattern and intake in 24 hr.

Together, the present findings suggest that brain ALDH and by implication, centrally-acting acetaldehyde, may play a role in regulating ethanol drinking behavior. The evidence supporting this notion will now be discussed in relation to the key findings stated above.

A Drinking Bout is a Pharmacologically Meaningful Event.

The present series of experiments demonstrated that the "simulated" drinking bout is a pharmacologically meaningful event. It was shown in Experiment 3 that the amount of ethanol consumed in a 10 min. drinking bout produced detectable blood ethanol levels. These findings were consistent with other studies demonstrating that animals consumed sufficient quantities of ethanol to produce detectable and pharmacologically relevant blood ethanol levels using various restricted access paradigms (Gill et al, 1986; Grant & Samson, 1985; Linseman, 1987). In addition, it was shown that blood ethanol levels significantly correlated with the amount of ethanol consumed in the 10 min. restricted access ($r = 0.70$). A similar correlation between ethanol consumption in a 10 min. restricted access paradigm and blood ethanol levels has been reported by Gill et al (1986). Furthermore, it was shown that pretreatment with cyanamide resulted in elevated blood acetaldehyde levels following a 10 min. drinking bout. These findings suggested that animals consumed sufficient quantities of ethanol in a 10 min. drinking session to produce manipulable levels of acetaldehyde.

Gill et al (1986) devised the 10 min. drinking bout to simulate one of several discrete drinking bouts

observed in a 24 hr access paradigm. They concluded that animals were motivated to consume "pharmaco-logically meaningful" or "intoxicating" levels of ethanol both in the restricted and 24 hr access paradigms. In the present thesis, it was demonstrated that a significant relationship existed between the amount of ethanol consumed in a "simulated" drinking bout and continuous ethanol availability. Since the size and frequency of bouts may be important determinants in ethanol drinking behavior, the "simulated" drinking bout may provide critical data on one of the behavioral components (i.e. drinking bout) that comprise ethanol motivated behavior.

Cyanamide May Possess Two Independent Properties

In the present series of experiments it was shown that cyanamide may possess at least two independent properties. It may enhance the consumption of fluids in general, and it may also have a specific effect on ethanol. It was evident that cyanamide produced an increase in fluid intake in both the restricted and 24 hr access paradigms. In the 10 min. access, the increase was observed in all fluids tested (i.e. ethanol, saccharin-quinine or water). In the 24 hr access paradigm, only water intake was significantly increased following cyanamide treatment. The mechanism by which cyanamide increased fluid intake is at present unclear. It is possible that the increase was related

to the direct inhibition of ALDH by cyanamide. However, animals pretreated with the ALDH inhibitor coprine, did not increase ethanol or water consumption in the two-bottle restricted access or 24 hr access paradigms. Therefore, it seems unlikely that the increase was related to the direct inhibition of ALDH activity. Another possibitity is that the increase in fluid intake was caused directly by some other property of cyanamide itself. Cyanamide is known to oxidize to urea (R.A. Deitrich, personal communication). Urea concentrations may become elevated in animals following the administration of cyanamide. To control the urea concentrations in blood, animals may excrete more urine and subsequently consume more fluid to compensate for water loss. It is of interest in this context to note that although animals in the 10 min. restricted access were not water deprived, animals pretreated with cyanamide still demonstrated an increase in fluid intake.

Although cyanamide produced a general increase in fluid intake, it also had a specific effect on ethanol consumption. For example, it was shown in Experiment 5 that animals pretreated with cyanamide demonstrated an increase in water intake but a suppression in ethanol consumption in a 24 hr access paradigm. In contrast, animals pretreated with cyanamide demonstrated an increase in ethanol consumption during the 10 min.

drinking session (Experiments 2 and 3). However, the magnitude of the increase was much lower for ethanol drinking animals than saccharin-quinine and water drinking subjects. These results suggested the presence of a factor which may limit the increase in ethanol consumption produced by cyanamide. One can speculate then, that if cyanamide did not produce this general increase in fluid intake which contributed to the increases in ethanol consumption, the smaller increases observed in ethanol drinking animals may have reflected an actual "suppression" of drinking. One factor contributing to this "suppression" effect may be elevated blood acetaldehyde by cyanamide. In fact, animals pretreated only with cyanamide (group S+CYAN) demonstrated a greater "suppression" of ethanol consumption than animals pretreated with 4MP+CYAN. A similar finding was demonstrated by Sinclair and Lindros (1981) using a 24 hr access paradigm. They reported that although both cyanamide and 4MP+CYAN treatment produced a suppression in ethanol intake, the effect was greater in animals pretreated with cyanamide alone. However, if elevated blood acetaldehyde levels were the only factor limiting ethanol intake by cyanamide, then pretreatment with 4MP+CYAN should have reversed the "suppression", since the addition of 4-methylpyrazole prevents the peripheral accumulation of acetaldehyde. The increase in ethanol intake

observed in animals pretreated with 4MP+CYAN was substantially lower than that observed in saccharin-quinine and water drinking groups. Thus, the addition of 4-methylpyrazole to cyanamide did not reverse the "suppression" of ethanol consumption. These findings suggested that acetaldehyde accumulation was not the only factor limiting ethanol intake since pretreatment with 4MP+CYAN prevents peripheral accumulation of acetaldehyde. A common factor to both cyanamide treated groups was the inhibition of brain ALDH activity by cyanamide. It is therefore possible that the inhibition of brain ALDH by cyanamide may be one of the factors responsible for limiting ethanol intake.

Furthermore, if cyanamide's effect on ethanol was mainly attributed to a general increase in fluid intake, then all ethanol drinking animals should have increased ethanol consumption by the same proportion. In fact, the increase in ethanol intake varied as a function of baseline intake (i.e. LOW, MEDIUM and HIGH drinkers). High drinkers demonstrated the lowest increase and low drinkers pretreated with 4MP+CYAN or cyanamide alone demonstrated the largest increase in ethanol intake. This similarity in effect may be attributed to the inhibition of brain ALDH by cyanamide. However, it is important to note that there were differences in the magnitude of increase for animals pretreated with cyanamide alone or 4MP+CYAN.

The magnitude of increase in ethanol intake for animals pretreated with only cyanamide was significantly smaller compared to animals pretreated with 4MP+CYAN. These findings suggested that elevated blood acetaldehyde levels may have contributed to the differences noted above.

An increase in ethanol consumption following cyanamide treatment has been reported in studies using a 24 hr access paradigm. Sinclair and Gribble (1985) demonstrated that five days of cyanamide administration during a period of ethanol deprivation produced a long-lasting increase in ethanol consumption when ethanol was subsequently presented to animals. Using an alternate day schedule of ethanol presentation, Amit et al (1976) reported that animals demonstrated a slight increase in ethanol intake following calcium carbimide (a derivative of cyanamide) treatment. In contrast, it has been demonstrated that pretreatment with cyanamide suppressed ethanol intake when using a continuous access paradigm (Sinclair et al, 1980; Sinclair & Lindros, 1981). These latter findings were confirmed in the present studies using a 24 hr continuous schedule of ethanol presentation. The discrepancy between studies using the 24 hr access paradigm may be attributed to the different procedures employed. The alternate day schedule used by Amit et al (1976) may be more similar to the deprivation schedule employed by

Sinclair and Gribble (1985), since animals in the former study only received ethanol every other day. It is possible that the deprivation factor in both studies played a role in the observed increases in ethanol consumption following cyanamide treatment. It can be argued that the 10 min. restricted access is also a deprivation-like procedure, since ethanol was presented to animals for a limited time period each day. Consequently, the mechanism underlying the increase produced by cyanamide may be similar in the studies stated above. However, in the present 10 min. restricted access paradigm, ethanol intake immediately returned to baseline levels following the termination of cyanamide treatment. Conversely, in the study by Sinclair and Gribble (1985), ethanol intake increased following the termination of cyanamide administration. The different temporal effects of cyanamide on ethanol intake suggests that different mechanisms may underlie the increase observed in the 10 min. restricted access and the ethanol deprivation schedules.

<u>The Specific Effect of Cyanamide may be Attributed to Brain ALDH Inhibition</u> .

The notion that brain ALDH may play a role in regulating a "drinking bout" was supported by the studies using the ALDH inhibitor coprine. The effects of 4MP+COP and coprine alone on ethanol intake parallelled that observed in animals pretreated with 4MP+CYAN

and cyanamide. However, coprine did not produce a general increase in fluid intake. If cyanamide's effects on ethanol were mainly attributed to the inhibition of brain ALDH and pretreatment with coprine produced similar effects on ethanol consumption, then it is proposed that coprine produced its effect via the same mechanism (i.e. brain ALDH inhibition). Thus, the similarity in alterations in ethanol intake produced by coprine and cyanamide strengthens the notion that these changes are specific to ethanol and more specifically, that brain ALDH may be involved in the observed changes.

This interpretation can be challenged since differences in ethanol intake were observed when a direct comparison was made between animals pretreated with coprine and cyanamide. Animals pretreated with 4MP+CYAN and 4MP+COP did not produce any observable changes in ethanol intake in the two-bottle choice 10 min. access paradigm. In contrast, animals pretreated with cyanamide and coprine alone, in the same paradigm, demonstrated a suppression of ethanol intake. These results tend to suggest that peripheral accumulation of acetaldehyde caused by these ALDH inhibitors may be a critical factor in altering the initial phases of ethanol consumption. Furthermore, it can be argued that the addition of 4-methylpyrazole to cyanamide and coprine served only to eliminate the peripheral accumu-

lation of acetaldehyde and allowed animals to resume their normal levels of ethanol consumption. Following this argument, one can conclude that peripheral acetaldehyde may be involved in regulating ethanol consumption. However, it has been argued that under unmanipulated conditions of voluntary ethanol consumption, acetaldehyde levels in the periphery may be too low to play an active role in regulating ethanol intake (Eriksson & Sippel, 1977; Lindros, 1985). Moreover, if the peripheral accumulation of acetaldehyde was the only motivational factor operating in the regulation of ethanol drinking behavior, then animals should have ceased drinking altogether following the first drinking bout because of the punishing, aversive effects associated with elevated levels of acetaldehyde. In the present series of experiments, even animals pretreated with coprine or cyanamide alone did not totally suppress ethanol intake. One must therefore assume the presence of another factor which motivated animals to consume ethanol despite the apparent aversive consequences of elevated blood acetaldehyde levels.

At first glance, it seemed that drinking behavior in the 24 hr access paradigm was quite similar to that observed in the 10 min. drinking session (see Experiment 6B). Animals pretreated with coprine and cyanamide alone both demonstrated a suppression of ethanol intake in the 24 hr access paradigm. In contrast,

pretreatment with 4MP+COP and 4MP+CYAN did not appear to alter ethanol intake from baseline levels. It could be argued, as above, that the prevention of the peripheral accumulation of acetaldehyde by 4-methylpyrazole merely allowed animals to consume their baseline levels of intake. However, if brain ALDH plays a role in regulating ethanol consumption, then, by the nature of the manipulation, either an increase or decrease in drinking behavior should have occurred in animals pretreated with 4MP+CYAN and 4MP+COP. A closer examination of ethanol intake in individual animals revealed that pretreatment with 4MP+COP and 4MP+CYAN resulted in bidirectional changes in drinking behavior. High drinking animals suppressed ethanol intake comparable to that observed in high and low drinking animals pretreated with coprine or cyanamide alone. In contrast, low drinking animals pretreated with 4MP+CYAN and 4MP+COP tended to increase ethanol intake. These results suggest that inhibition of brain ALDH activity may have altered ethanol drinking behavior in the 24 hr access paradigm, albeit in a complex fashion.

Taken together, it is proposed that peripheral acetaldehyde may not be the main factor involved in the process regulating ethanol drinking behavior when ethanol is freely available. Based on the findings that brain ALDH activity correlated with ethanol intake in a 24 hr access paradigm (e.g. Amir, 1977; Sinclair &

Lindros, 1981; Socoransky et al, 1984) and that pretreatment with 4MP+COP and 4MP+CYAN both suppressed and enhanced ethanol intake in a 24 hr access paradigm, it is suggested that cyanamide and coprine's primary effects on ethanol may be through their inhibitory action of brain ALDH.

The fact that there were no observable changes in ethanol consumption for groups 4MP+CYAN and 4MP+COP during the 10 min. drinking session but such changes were in fact observed in a 24 hr access paradigm, suggested that brain ALDH may not play an immediate role in mediating the initial drinking bout simulated in the restricted access paradigm. The discrete nature of a single 10 min. drinking session may be similar to the one trial learning described in the conditioned taste aversion (CTA) literature. In the 10 min. drinking paradigm, animals learn to make an association between the taste of the fluid (in this case, ethanol) and some pharmacological property of the drug. If one assumes that brain ALDH plays a role in the pharmacological effects of ethanol, then pretreatment with cyanamide and coprine should alter the pharmacological consequences of a drinking bout. The taste of ethanol would subsequently be the conditioned cue for these changes and alterations in ethanol intake would reflect this learned association between the taste and pharmacological properties of ethanol. In this

context, it has been postulated that CTAs induced by self-administered drugs including ethanol, may be functionally related to the positive reinforcing properties of these agents (for review, see Hunt & Amit, 1987). Moreover, CTAs induced by self-administered drugs may be related to a constellation of stimulus properties of these drugs, reflecting the "euphoric/dysphoric" interaction of the drugs' effects (Colpaert, 1978; Hunt & Amit, 1987). Indeed, this notion is supported by reports within the human drug abuse literature of the involvement of mixed "eurphoric/dysphoric" effects of psychoactive drugs in the maintenance of drug self-administration (Mello, 1983; Meyer & Mirin, 1979; see also, Hunt & Amit, 1987). For example, during a period of chronic opiate and alcohol intoxication, drug users reported increased dysphoria and anxiety (Mello, 1983). Moreover, at high doses of cocaine intake, there were reports of dysphoria and anxiety accompanied by a desire for more cocaine (Resnick, Kestenbaum & Schwartz, 1977). It would appear then, that CTAs induced by self-administered drugs may reflect a constellation of "euphoric/dysphoric" stimulus properties of the drugs' effects. Furthermore, there is evidence from both the human drug and animal CTA literature to suggest that the positive reinforcing properties of self-administered drugs may not be readily discriminable. For example, experienced

opiate users have reported that the strength of the initial nausea occurring following drug administration is often used as a positive correlated cue for predicting the intensity of a subsequent drug 'high' (Stolerman & Kumar, 1972). In the animal CTA literature it has been reported that it is difficult to demonstrate the formation of a CTA using centrally-administered drugs such as morphine (Hunt, Amit, Switzman & Sinyor, 1983) and acetaldehyde (Brown, Amit, Smith & Rockman, 1978), although systemic injections of these substances readily induces a CTA (e.g. Brown et al, 1978; Hunt, Spivak & Amit, 1985). Together, these findings suggested that the "dysphoric" effects of a self-administered drug may enhance the saliency of the more positive reinforcing properties of the drug (Hunt & Amit, 1987).

Within this context, the saliency of the central pharmacological cues in animals pretreated with cyanamide and coprine alone may be enhanced by the concomitant increase in blood acetaldehyde levels. Elevations in blood acetaldehyde concentrations produce various noxious physiological effects such as tachychardia, dizziness and nausea (see Kitson, 1977). Despite their seemingly aversive nature, these peripheral effects may facilitate learning an association between the taste stimulus (ethanol) and the centrally-mediated effects of ethanol (Hunt & Amit, 1987). The

addition of 4-methylpyrazole to coprine and cyanamide prevented the periperal accumulation of acetaldehyde following a drinking bout. Consequently, the central cues following ethanol consumption may be less salient and animals may not readily learn an association between the taste and the drug state.

The saliency of the central stimulus properties of ethanol following pretreatment with 4MP+CYAN and 4MP+COP may also be influenced by the duration of exposure to ethanol. It has been reported that the duration of exposure to the unconditioned stimulus (e.g. drug state) is an important factor in conditioning studies (e.g. Goudie & Dickens, 1978). For example, cocaine and heroin both have a rapid rate of onset and both act as relatively weak CTA inducing agents (Goudie, Dickens & Thornton, 1978; Switzman, Hunt & Amit, 1981). However, it has been demonstrated that the magnitude of CTA induced by cocaine can be potentiated by extending the duration of drug exposure by means of repeated injections (Foltin, Preston, Wagner & Shuster, 1981). Using a different conditioning paradigm, Smith (1983) reported that a single intracerebroventricular infusion of acetaldehyde failed to induce a conditioned place preference. However, multiple infusions of acetaldehyde over a period of five minutes reliably produced a conditioned place preference. These latter findings support the notion

that increased exposure to a drug may be an important factor in conditioning studies.

In Experiment 6, extending the presentation of ethanol from 10 min. to 24 hr produced an alteration in drinking behavior for animals pretreated with 4MP+CYAN and 4MP+COP. It appears then, that animals may learn to associate the taste and the pharmacological consequences of ethanol only with repeated exposure to the centrally-mediated event, which in this case was provided by continuous access to ethanol (i.e. 24 hr paradigm).

Cyanamide and Coprine: Bidirectional Effects in the 24 hr Access Paradigm.

The results from the studies contained in the present thesis demonstrated that ALDH inhibition did not result in a unidirectional effect on ethanol consumption. Although there was no effect on group means for animals pretreated with 4MP+CYAN and 4MP+COP in the 24 hr access (Experiment 6B), there was a significant effect across individual rats. It was shown that the change in ethanol intake was a function of the baseline level of intake. Generally, animals that consumed greater than 2.8 gm/kg of ethanol at baseline (i.e. HIGH drinkers) subsequently demonstrated a suppression of ethanol intake, whereas an increase in ethanol intake was observed in animals that consumed less than 2.8 gm/kg at baseline (LOW drinkers). In

contrast, pretreatment with coprine and cyanamide alone produced a suppression of ethanol intake in both high and low drinkers.

A similar "dose"-dependent change has been reported for C57 mice pretreated with 4-methylpyrazole alone (Gentry, 1985). Gentry (1985) demonstrated that the effect of 4-methylpyrazole on voluntary ethanol consumption in mice was directly related to their baseline level of intake; it suppressed ethanol intake in high drinking mice and increased intake in low drinking mice. Gentry (1985) suggested that 4-methylpyrazole exerted its effects through its manipulation of blood ethanol levels. In the present thesis, the "dose"-dependent effect was only observed in rats pretreated with 4-methylpyrazole in addition to the ALDH inhibitors. On the basis of Gentry's (1985) work, it is therefore possible that the change in ethanol intake in the present study was directly related to the pharmacological effects produced by 4-methylpyrazole independent of brain ALDH manipulations. In Experiment 2, animals pretreated with 4-methylpyrazole alone did not demonstrate any change in ethanol consumption during the 10 min. restricted access. When animals were subsequently divided into high, mid-range and low drinkers on the basis of baseline intake, it was shown that high drinkers reduced ethanol intake over days and no change in consumption was observed for the mid-range

and low drinkers. However, the pattern of intake in animals pretreated with saline was similar to that observed in 4-methylpyrazole-treated subjects. It would appear then, that the high drinkers in the saline and 4-methylpyrazole groups may have been more sensitive to the injection procedure than mid-range and low drinkers. Consistent with these finding, Gentry et al (1983) reported that daily injections of saline suppressed the high rate of ethanol consumption typically observed in C57 mice. The present findings therefore suggested that the pharmacological effects of 4-methylpyrazole alone may not be responsible for the "dose"-dependent effect observed in animals pretreated with 4MP+CYAN and 4MP+COP.

It is of interest to note that the "dose"-dependent effect was not only observed in the 24 hr access paradigm but in the 10 min. restricted access paradigm as well for animals pretreated with 4MP+CYAN. In Experiment 2, it was shown that increases in ethanol consumption for group 4MP+CYAN was a function of base-line intake. High drinkers demonstrated the smallest increase and low drinkers demonstrated the largest increase in ethanol intake. It was evident from the present series of experiments that the increase in ethanol consumption may be related to cyanamide's effect on general fluid intake. However, the magnitude of increase parallelled the direction of change

observed in high and low drinkers in the 24 hr paradigm; i.e. high drinkers demonstrated a decrease and low drinkers an increase in ethanol consumption. Therefore, the similarity of findings in the 10 min. restricted access and 24 hr access paradigms would tend to further strengthen the relationship between the behaviors demonstrated in a "simulated" drinking bout and those in the 24 hr access.

Pretreatment with cyanamide alone produced a "dose"-dependent change in ethanol intake during the 10 min. restricted access but this "dose-dependent" relationship was not observed in the 24 hr access paradigm. Given that animals consume ethanol in a series of discrete bouts in a 24 hr access paradigm (e.g. Gill et al, 1986), it is possible that each drinking bout would result in elevated blood acetaldehyde levels produced by cyanamide treatment. It is therefore conceivable that both high and low drinkers may become more sensitive to ethanol's effects as a consequence of the repeated exposure to elevated blood acetaldehyde levels (and its potential punishing effects) and subsequently suppress ethanol intake.

<u>The Pharmacological Consequences of a Bout Will in part Determine the Pattern and Intake of Ethanol.</u>

Various factors appear to play a role in regulating the size of individual bouts as well as the frequency of bouts both in the restricted access and 24

hr access paradigms. The amount of ethanol consumed by rats may depend on the reinforcing properties as well as the taste and peripheral consequences of ethanol. A combination of these and other factors may ultimately determine the maximal rate of responding for ethanol. For example, under "normal" conditions the amount of ethanol consumed by high drinkers may reflect a "ceiling effect", above which, it may trigger aversive consequences (i.e. motor incoordination, dizziness etc). Low drinking animals may be more sensitive to the taste or peripheral effects of ethanol and may not experience the reinforcing effects following the consumption of low quantities of ethanol. It is possible that as a consequence of consuming such low amounts of ethanol, insufficient levels of acetaldehyde which may play a role in mediating the reinforcing effects of ethanol, are produced in brain.

In the present experiments, cyanamide appeared to have a differential effect on ethanol intake in high and low drinkers. The mechanism underlying the "dose"-dependent effect is at present unclear. It is conceivable that the inhibition of brain ALDH may result in a concomitant increase in brain acetaldehyde concentrations following a drinking bout in animals pretreated with 4MP+CYAN and 4MP+COP. For high drinkers who may already be at their "ceiling", the

potentiation may be aversive. Animals may subsequently decrease ethanol consumption in an attempt to regulate the magnitude of this enhanced pharmacological state and return to their previous drug-state level. This notion is supported by the finding that animals are extremely accurate in regulating ethanol intake. Amit and Corcoran (1975) demonstrated that animals were very efficient in regulating the amount of absolute ethanol ingested when the concentration of solutions presented to them were systematically varied. It is possible then, that in the present study, animals were capable of discriminating the altered central effects of ethanol produced by cyanamide and coprine, and regulated their intake accordingly.

Support for the notion that brain ALDH inhibition may mediate the pharmacological effects of ethanol can be found in a human study examining the effects of calcium carbimide and disulfiram on ethanol consumption (Brown et al, 1983). Subjects pretreated with these ALDH inhibitors reported enhanced euphoria following the consumption of a low dose of ethanol whereas no discernible effects were reported in placebo treated subjects ingesting the same low dose of ethanol. Related to this finding was the observation that some alcoholic patients treated with the ALDH inhibitor disulfiram continued to consume low doses of ethanol

(Brown et al, 1983; Chevens, 1953). Chevens (1953) reported that some alcoholics preferred taking disulfiram when drinking ethanol because the combination evoked the same sensations they normally experienced from larger amounts of ethanol. Taken together, it is postulated that changes in the central metabolism of acetaldehyde as a consequence of brain ALDH inhibition may enhance the central pharmacological effects of ethanol. This enhancement of ethanol's effects may subsequently decrease the threshold for the amount of ethanol necessary to produce a central reinforcing effect. However, it is also possible that the ingestion of ethanol above the "ceiling" may be aversive, causing animals to reduce their intake.

A similar mechanism may also underly the increase in ethanol intake observed in low drinkers pretreated with 4MP+CYAN and 4MP+COP. As a consequence of the enzyme manipulations, the central pharmacological effects of ethanol may be enhanced even following the initial consumption of low quantities of ethanol. It is therefore possible that the animals were exposed to an enhanced reinforcing effect which may have subsequently overidden other factors previously limiting ethanol intake (e.g. taste, peripheral aversion). The increase in ethanol intake for low drinkers may reflect an increase in the frequency of responding for ethanol

as a consequence of its newly acquired reinforcing effects. Since low drinkers may initially be well below their "ceiling", they may be able to consume more ethanol than high drinkers. It is possible that similar underlying mechanisms may be functioning in high and low drinking animals pretreated with only coprine and cyanamide. However, the concomitant increase in blood acetaldehyde levels observed in high drinkers may mask the "positive" central effect of ethanol. In the case of low drinking animals who may initially be sensitive to the taste or peripheral effects of ethanol, elevated blood acetaldehyde may potentiate the noxious effects of ethanol resulting in a suppression of ethanol intake.

Summary and Conclusions

The results of the present series of experiments suggested that brain ALDH and by implication, centrally-acting acetaldehyde may play a role in the mediation of ethanol drinking behavior. It was demonstrated that the inhibition of ALDH activity by cyanamide and coprine produced changes in ethanol consumption. Animals pretreated with cyanamide or 4MP+CYAN both displayed similar changes in ethanol consumption in the 10 min. and 24 hr access paradigms. In the latter procedure, however, high drinkers in both treatment groups demonstrated a suppression in ethanol

intake. Nevertheless, the alterations in ethanol consumption cannot be attributed to elevated blood acetaldehyde levels by cyanamide, since pretreatment with 4MP+CYAN prevented the peripheral accumulation of acetaldehyde. The similarity in behavior between animals pretreated with 4MP+CYAN and cyanamide alone, may therefore be attributed to the inhibition of brain ALDH activity by cyanamide. This notion is supported by the finding that a parallel effect on ethanol consumption was observed with the ALDH inhibitor coprine. Thus, the similarity in alterations in ethanol intake produced by coprine and cyanamide strengthens the notion that these changes are specific to ethanol and more specifically, that brain ALDH may be involved in the observed changes.

On the basis of reports on a direct relationship between brain ALDH activity and ethanol consumption (e.g. Sindlair & Lindros, 1981; Socaransky et al, 1984) and on the basis of the present findings that manipulations of brain ALDH activity altered ethanol intake, it is postulated that brain ALDH plays a role in regulating ethanol drinking behavior. Furthermore, it is proposed that the regulation of ethanol drinking behavior may be mediated by the regulation of levels of acetaldehyde in brain by central ethanol metabolizing enzymes. The source of centrally-acting acetaldehyde

responsible for these effects may be derived directly in brain by catalase. It has been demonstrated that catalase can metabolize ethanol in brain in vivo (Cohen et al, 1980). In addition, it has been reported that a direct relationship exists between brain catalase activity and ethanol consumption in rats (Amit & Aragon, 1987; Aragon et al, 1985). It is therefore postulated that as a dynamic interactional system, the rate of formation and degradation of acetaldehyde by brain catalase and brain ALDH may be the specific factor within this system which actually regulates ethanol drinking behavior. The reinforcing properties of acetaldehyde may therefore be related to its rate of formation and degradation in brain by catalase and ALDH. Given that a rapid rate of onset and a brief duration of action characterizes a drug's potential as a reinforcer (Busto & Sellers, 1986; Falk, 1983) and given the findings that acetaldehyde possesses positive reinforcing properties (see Amit et al, 1986), it is suggested that ethanol self-administration may be based upon the central effects of acetaldehyde. This notion is supported by findings that the inhibition of brain catalase activity by 3-amino-1,2,4-triazole and the inhibition of brain ALDH activity by cyanamide disrupted ethanol-related behaviors considered to be centrally-mediated, such as conditioned taste aversion

(Aragon et al, 1985; Spivak et al, 1987), locomotion (Aragon et al, 1985a; Spivak et al, 1987) and narcosis (Aragon et al, 1987).

As previously stated, it has been reported that animals consume ethanol in a series of discrete drinking bouts (e.g. Marcucello et al, 1984; Gentry et al, 1983). Furthermore, consistent with previous findings (Gill et al, 1986), the present results demonstrated that an initial "drinking bout" was a pharmacologically meaningful event. Moreover, it was shown that a relationship existed between the amount of ethanol consumed in the "simulated" drinking bout and in continuous (24 hr) ethanol access. In the present series of experiments, the 10 min. restricted access and 24 hr access paradigm were employed in an attempt to elucidate the contribution of brain ALDH in mediating the initial drinking bout as well as regulating ethanol drinking behavior in general. Gill et al (1986) suggested that ethanol consumption could be considered as a "reinforcement-bound" behavior, where total intake may be a function of the frequency of bouts. Since the frequency of bouts will be influenced by the consequences of the previous response (i.e. drinking bout), it would follow that alterations in the central actions of ethanol following a drinking bout should influence subsequent drinking behavior. In this

context, the inhibition of brain ALDH activity by cyanamide and coprine did not produce an immediate change in ethanol consumption in the "simulated" initial drinking bout. However, in the 24 hr access paradigm, pretreatment with 4MP+CYAN and 4MP+COP did produce changes in ethanol consumption. These findings suggest that the inhibition of brain ALDH may not produce an immediate change in ethanol consumption during the initial drinking bout. However, it is postulated that the inhibition of brain ALDH, may nevertheless, alter the central actions of ethanol following the initial drinking bout and may influence subsequent drinking behavior.

Taken together, the results of the present series of experiments suggest that ethanol drinking behavior is not a single behavior but rather, is comprised of a series of discrete components. Thus, ethanol drinking behavior should be considered as a series of discrete drinking bouts and each drinking bout should be considered as a pharmacologically meaningful event. Consequently, the pattern and frequency of bouts will determine ethanol drinking behavior. Furthermore, the present findings suggest that central ethanol metabolizing enzymes may play a role in regulating ethanol drinking behavior by regulating the rate of formation and degradation of acetaldehyde in brain.

Despite a massive body of literature on this subject, the precise nature of the involvement of acetaldehyde in mediating ethanol consumption is still at present, unclear. Brain ALDH has been shown to be a major route of monoamine deamination (Duncan & Sourkes, 1974; Tabakoff & Gelpke, 1975). It has been postulated that acetaldehyde in brain may competitively inhibit brain ALDH, giving rise to increases in steady state levels of its endogenous substrates- biogenic aldehydes (for review see Deitrich & Erwin, 1975; 1980; Thandani & Truitt, 1977). The accumulation of these biogenic aldehydes may subsequently affect neuronal functioning and the behavioral response to ethanol (Deitrich & Erwin, 1975; Amir, 1977; 1978).

Recently, it was reported that cyanamide treatment (200 mg/kg in food) produced a 26% reduction in norepinephrine turnover in rat brain (Lindros, Sinclair, Ahtee & Attila, 1981). Since acetaldehyde may competitively inhibit brain ALDH, it is possible that acetaldehyde produces its effects by interfering with norepinephrine turnover as well. This hypothesis receives some support from studies demonstrating that acetaldehyde like ethanol, alters the turnover of norepinephrine (Duritz & Truitt, 1966; Oritz, Littleton & Griffiths, 1974; Thandani & Truitt, 1977; Walsh, 1971). Brain norepinephrine has been shown to play a

role in ethanol consumption (e.g. Amit, Brown, Levitan & Ogren, 1977; Corcoran, Lewis & Fibiger, 1983; Kiianmaa, 1980), locomotor activity (Mason, Corcoran & Fibiger, 1979) and CTA (Sklar & Amit, 1977). Therefore, a logical extension of this line of research in the future should focus on the possible interaction between central acetaldehyde and the norepinephrine system in the brain.

REFERENCES

Akabane, J. (1970). Aldehydes and related compounds. International Encyclopedia of Pharmacological Therapeutics, 2, 523-560.

Amir, S. (1977). Brain and liver aldehyde dehydrogenase: relations to ethanol consumption in Wistar rats. Neuropharmacology, 16, 781-784.

Amir, S. (1978). Brain and liver aldehyde dehydrogenase activity and voluntary ethanol consumption by rats: Relations to strain, sex and age. Psychopharmacology, 57, 97-102.

Amir, S., Brown, Z.W. & Amit, Z. (1980). The role of acetaldehyde in the psychopharmacological effects of ethanol. In H. Rigter & J.C. Crabbe (Eds.), Alcohol tolerance, dependence and addiction, (pp. 317-337. Amsterdam: Elsevier/North Holland.

Amir, S. & Stern, M.H. (1978). Electrical stimulation and lesions of the medial forebrain bundle of the rat: Changes in voluntary ethanol consumption and brain aldehyde dehydrogenase activity. Psychopharmacology, 57, 167-174.

Amit, Z. & Aragon, C.M.G. Catalase activity measured in rats naive to ethanol correlates with later voluntary ethanol consumption: Possible evidence for a biological marker system of ethanol intake. Submitted to Psychopharmacology, 1987.

Amit, Z., Brown, Z.W., Amir, S., Smith, B. &

Sutherland, E.A. (1980). Behavioral assessment of the role of acetaldehyde in the mediation of alcohol intake in animals and humans. In K. Eriksson, J.D. Sinclair & K. Kiianmaa (Eds.), Animal models in alcohol research, (pp. 159-165). New York: Academic Press.

Amit, Z., Brown, Z.W., Levitan, D.E. & Ogren, S.O. (1977). Noradrenergic mediation of the positive reinforcing properties of ethanol: I. Suppression of ethanol consumption in rats following dopamine-beta-hydroxylase inhibition. Archives Internationale de Pharmacodyanamie et Therapie, 230, 65-75.

Amit, Z., Brown, Z.W. & Rockman, G.E. (1977). Possible involvement of acetaldehyde, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol self-administration. Drug and Alcohol Dependence, 2, 495-500.

Amit, Z. & Corcoran, M.E. (1975). Regulation of ethanol intake by rats with an induced preference for ethanol. Neuropharmacology, 14, 1-7.

Amit, Z., Levitan, D.E. & Lindros, K.O. (1976). Suppression of ethanol intake following administration of dopamine-beta-hydroxylase inhibitors in rats. Archives Internationale de Pharmacodynamie et de Therapie, 223, 114-119.

Amit, Z. & Smith, B.R. (1985). A mult-dimensional examination of the positive reinforcing properties of acetaldehyde. Alcohol, 2, 367-370.

Amit, Z., Smith, B.R. & Aragon, C.M.G. (1986). Alcohol metabolizing enzymes as possible markers mediating voluntary alcohol consumption. Canadian Journal of Public Health, 77 (Suppl. 1), 15-20.

Amit, Z., Smith, B.R., Brown, Z.W. & Williams, R.L. (1982). An examination of the role of TIQ alkaloids in alcohol intake: Reinforcers, satiety agents or artifacts. In F. Bloom, J. Barchas, M. Sandler & E. Usdin (Eds.), Beta-carbolines and tetrahydroisoquinolines, (pp. 345-364). New York: Alan R. Liss Inc.

Amit, Z., Sutherland, E.A. & White, N. (1975/76). The role of physical dependence in animal models of human alcoholism. Drug and Alcohol Dependence, 1 435-440.

Aragon, C.M.G. & Amit, Z. (1985). A two-dimensional model of alcohol consumption: Possible interaction of brain catalase and aldehyde dehydrogenase. Alcohol, 2, 357-360.

Aragon, C.M.G. & Amit, Z. (1987). Genetic variation in ethanol sensitivity in C57BL/6 and DBA/2 mice: A further investigation of the differences in brain catalase activity. Annals of the New York Academy of Sciences, 492, 398-400.

Aragon, C.M.G., Spivak, K. & Amit, Z. (1985). Behavioral evidence for the role of brain catalase in the mediation of acetaldehyde related actions of ethanol. Alcoholism: Clinical and Experimental Research, 9, 209.

Aragon, C.M.G., Spivak, K. & Amit, Z. (1985a). Blockade of ethanol-induced conditioned taste aversion by 3-amino-1,2,4-triazole: Evidence for catalase-mediated synthesis of acetaldehyde in rat brain. Life Sciences, 37, 2077-2084.

Aragon, C.M.G., Spivak, K. & Amit, Z. Brain catalase inhibition attenuates ethanol but not pentobarbital induced narcosis in rats. Submitted to Pharmacology Biochemistry & Behavior, 1987.

Aragon, C.M.G., Sternklar, G. & Amit, Z. (1985). A correlation between voluntary ethanol consumption and brain catalase activity in the rat. Alcohol, 2, 353-356.

Baraona, E., DiPadova, C., Tabasco, J. & Lieber, C.S. (1987). Red blood cells: A new major modality for acetaldehyde transport from liver to other tissues. Life Sciences, 40, 253-258.

Behar, D., Berg, C.J., Rapoport, J.L., Nelson, W., Linnoila, M., Cohen, M., Bozevich, C. & Marshall, T. (1983). Behavioral and physiological effects of

ethanol in high risk and control children: A pilot study. Alcoholism: Clinical and Experimental Research, 7, 404-410.

Blander, A., Hunt, T., Blair, R. & Amit, Z. (1984). Conditioned place preference: An evaluation of morphine's positive reinforcing properties. Psychopharmacology, 84, 124-127.

Bosron, W.F. & Li, T-K (1986). Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases and their relationship to alcohol metabolism and alcoholism. Hepatology, 6, 502-510.

Brannan, T.S., Maker, H.S. & Raes, I.P. (1981). Regional distribution of catalase in rat brain. Journal of Neurochemistry, 86, 307-309.

Brien, J.F. & Loomis, C.W. (1985). Aldehyde dehydrogenase inhibitors as alcohol-sensitizing drugs: A pharmacological perspective. Trends in Pharmacological Sciences, December, 477-480.

Brown, Z.W., Amit, Z. & Rockman, G.E. (1979). Intraventricular self-administration of acetaldehyde but not ethanol in naive laboratory rats. Psychopharmacology, 64, 271-276.

Brown, Z.W., Amit, Z. & Smith, B.R. (1980). Intraventricular self-administration of acetaldehyde and voluntary consumption of ethanol in rats. Behavioral and Neural Biology, 28, 150-155.

Brown, Z.W., Amit, Z., Smith, B.R. & Rockman, G.E. (1978). Differential effects on conditioned taste aversion learning with peripherally and centrally administered acetaldehyde. Neuropharmacology, 17 931-953.

Brown, Z.W., Amit, Z., Smith, B.R., Sutherland, E.A. & Selvaggi, N. (1983). Alcohol-induced euphoria enhanced by disulfiram and calcium carbimide. Alcoholism: Clinical and Experimental Research, 7 276-278.

Buhler, R., Pestalozzi, D., Hess, M. & von Wartburg, J-P. (1983). Immunohistochemical localization of alcohol dehydrogenase in human kidney, endocrine organs and brain. Pharmacology Biochemistry & Behavior, 18, 55-60.

Busto, U. & Sellers, E.M. (1986). Pharmacokinetic determinants of drug abuse and dependence: A conceptual perspective. Clinical Pharmacokinetics 11, 144-153.

Cappell, H., LeBlanc, A.E. & Endrenyi, L. (1973). Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazepoxide. Psychopharmacologia, 29, 239-246.

Cederbaum, A.I. & Dicker, E. (1985). Inhibition of the peroxidatic activity of catalase towards alcohols by the aldehyde dehydrogenase inhibitor cyanamide. Toxicology Letters, 29, 107-114.

Chevens, L.C.F. (1953). Antabuse addiction. British Medical Journal, 1, 1450-1451.

Colpaert, F.C. (1978). Discriminative stimulus properties of narcotic analgesic drugs. Pharmacology Biochemistry & Behavior, 9, 863-887.

Cohen, G. (1977). An acetaldehyde artifact in studies of the interaction of ethanol with biogenic amine systems: The oxidation of ethanol by ascorbic acid. Journal of Neurochemistry, 29, 761-762.

Cohen, G., Sinet, P.M. & Heikkila, R. (1980). Ethanol oxidation by rat brain in vivo. Alcoholism: Clinical and Experimental Research, 4, 366-370.

Corcoran, M.E., Lewis, J. & Fibiger, H.C. (1983). Forebrain noradrenergic and oral self-administration of ethanol by rats. Behavioral & Brain Research, 8, 1-21.

Damjanovich, R.P. & MacIunes, J.W. (1973). Factors involved in ethanol narcosis: Analysis in mice of three inbred strains. Life Sciences, 13, 55-65.

Deitrich, R.A. (1966). Tissue and subcellular distribution of mammalian aldehyde-oxidizing capacity. Biochemical Pharmacology, 15, 1911-1922.

Deitrich, R.A. (1987). Specificity of the action of ethanol in the central nervous system: behavioral effects. In K.O. Lindros, R. Ylikahri & K.Kiianmaa (Eds.) Advances in biomedical alcohol research, (pp. 133-138). Oxford: Pergamon Press.

Deitrich, R.A. & Erwin, J.G. (1975). Involvement of biogenic amine metabolism in ethanol addiction. Federation Proceedings, 34, 1962-1968.

Deitrich, R.A. & Erwin, J.G. (1980). Biogenic amine-aldehyde condensation products: tetraisoquinolines and tryptolines (B-carbolines). Annual Review of Pharmacology, 20, 55-80.

Deitrich, R.A., Troxell, P.A., Worth, W.S. & Erwin, V.G. (1976). Inhibition of aldehyde dehydrogenase in brain and liver by cyanamide. Biochemical Pharmacology, 25, 2733-2737.

DeMaster, E.G., Redfern, B., Shirota, F.N. & Nagasawa, H.T. (1986). Differential inhibition of rat tissue catalase by cyanamide. Biochemical Pharmacology, 35, 2081-2085.

DeMaster, E.G., Shirota, F.N. & Nagasawa, H.T. (1984). The metabolic activation of cyanamide to an inhibitor of aldehyde dehydrogenase is catalyzed by catalase. Biochemical & Biophysical Research Communications, 122, 358-365.

DeMaster, E.G., Shirota, F.N. & Nagasawa, H.T. (1985). Catalase mediated conversion of cyanamide to an inhibitor of aldehyde dehydrogenase. Alcohol, 2, 117-121.

DiPadova, C., Alderman, J. & Lieber, C.S. (1986). Improved methods for the measurement of acetaldehyde concentrations in plasma and red blood cells.

Alcoholism: Clinical and Experimental Research, 10, 86-89.

Duncan, R.J. & Sourkes, T.L. (1974). Some enzymatic aspects of the production of oxidized metabolites of catecholamines and 5-hydroxytryptamine by brain tissue. Journal of Neurochemistry, 22, 663-669.

Duncan, R. & Tipton, K.F. (1971). The kinetics of brain aldehyde dehydrogenase. European Journal of Biochemistry, 22, 538-543.

Duritz, G. & Truitt, E.B. (1966). Importance of acetaldehyde in the action of ethanol on brain norepinephrine and 5-hydroxytryptamine. Biochemical Pharmacology, 15, 711-721.

Erickson, C.K. (1979). Factors affecting the distribution and measurement of ethanol in the body. In E. Majchrowicz & E.P. Noble (Eds.), Biochemistry and pharmacology of ethanol, vol. 1, (pp. 9-26). New York: Plenum Press.

Eriksson, C.J.P. (1977). The distribution and metabolism of acetaldehyde in rats during ethanol oxidation- II. Regulation of the hepatic acetaldehyde levels. Biochemical Pharmacology, 26, 249-252.

Eriksson, C.J.P. (1980). Problems and pitfalls in acetaldehyde determinations. Alcoholism: Clinical and Experimental Research, 4, 22-29.

Eriksson, C.J.P. (1980a). The aversive effect of acetaldehyde on alcohol drinking behavior in the rat. Alcoholism: Clinical and Experimental Research, 4 107-111.

Eriksson, C.J.P. (1983). Human blood acetaldehyde concentrations during ethanol oxidation (update 1982). Pharmacology Biochemistry & Behavior, 18, 141-150.

Eriksson, C.J.P., Atkinson, N., Petersen, D. & Deitrich, R.A. (1984). Blood and liver acetaldehyde concentrations during ethanol oxidation in C57 and DBA mice. Biochemical Pharmacology, 33, 2213-2216.

Eriksson, C.J.P. & Sippel, H.W. (1977). The distribution and metabolism of acetaldehyde in rats during ethanol oxidation- I. The distribution of acetaldehyde in liver, brain, blood and breath. Biochemical Pharmacology, 26, 241-247.

Erwin, V.G. & Deitrich, R.A. (1966). Brain aldehyde dehydrogenase localization, purification and properties. Journal of Biological Chemistry, 241 3533-3539.

Falk, J.L. (1983). Drug dependence: Myth or motive? Pharmacology Biochemistry & Behavior, 19, 385-391

Folton, R.W., Preston, K.L., Wagner, G.C. & Shuster, C.R. (1981). The aversive stimulus properties of

repeated infusions of cocaine. Pharmacology Biochemistry & Behavior, 15, 71-74.

Friedman, H.J. & Lester, D. (1975). Intraventricular ethanol and ethanol intake: A behavioral and radiographic study. Pharmacology Biochemistry & Behavior, 3, 393-401.

Garcia, J., Kimeldorf, D.J. & Hunt, E.L. (1957). Spatial avoidance in the rat as a result of exposure to ionizing radiation. British Journal of Radiation, 30, 318-321.

Gaunt, G.L. & DeDuve, C. (1976). Subcellular distribution of D-amino acid oxidase and catalase in rat brain. Journal of Neurochemistry, 26, 749-759.

Gentry, R.T. (1985). Voluntary ethanol consumption of ethanol and its consequences on C57 mice treated with 4-methylpyrazole. Alcohol, 2, 581-587.

Gentry, R.T., Rappaport, M.S. & Dole, V.P. (1983). Elevated concentrations of ethanol plasma do not suppress voluntary ethanol consumption in C57BL mice. Alcoholism: Clinical and Experimental Research, 7, 420-423.

Gill, K., France, C. & Amit, Z. (1986). Voluntary ethanol consumption in rats: An examination of blood/brain ethanol levels and behavior. Alcoholism: Clinical and Experimental Research, 10, 457-462.

Goedde, H.W. & Agarwal, D.P. (1987). Polymorphism of aldehyde dehydrogenase and alcohol sensitivity. Enzyme, 37, 29-44.

Goedde, H.W., Harada, S. & Agarwal, D.P. (1979). Racial differences in alcohol sensitivity: A new hypothesis. Human Genetics, 51, 331-334.

Goudie, A.J. (1979). Aversive stimulus properties of drugs. Neuropharmacology, 18, 971-979.

Goudie, A.J. & Dickens, D.W. (1978). Nitrous oxide-induced conditioned taste aversions in rats: The role of duration of drug exposure and its relation to the taste aversion self-administration paradox. Pharmacology Biochemistry & Behavior, 9, 587-592.

Goudie, A.J., Dickens, D.W. & Thornton, E.W. (1978). Cocaine-induced conditioned taste aversions in rats. Pharmacology Biochemistry & Behavior, 8, 757-761.

Grant, K.A. & Samson, H.H. (1985). Induction and maintenance of ethanol self-administration without food deprivation in the rat. Psychopharmacology, 86, 475-479.

Harada, S., Agarwal, D.P. & Goedde, H.W. (1978). Isozyme variations in aldehyde dehydrogenase (EC1.2.1.3) in human tissues. Human Genetics, 44 181-185.

Harada, S., Misawa, S., Agarwal, D.P. & Goedde, H.W. (1980). Liver alcohol dehydrogenase and aldehyde

dehydrogenase in the Japanese: Isozyme variation and its possible role in alcohol intoxication. American Journal of Human Genetics, 32, 8-15.

Hasumura, Y., Teschke, R. & Lieber, C.S. (1975). Acetaldehyde oxidation by hepatic mitochondria: Its decrease after chronic ethanol consumption. Science, 189, 727-728.

Havre, P., Margolis, J.M., Abrams, M. & Landau, B.R. (1976). Subcellular site of acetaldehyde oxidation in monkey liver. Biochemical Pharmacology, 25, 2757-2758.

Hawkins, R.D. & Kalant, H. (1972). The metabolism of ethanol and its metabolic effects. Pharmacological Reviews, 24, 67-157.

Hillbom, M.E., Sarviharju, M.S. & Lindros, K.O. (1983). Potentiation of ethanol toxicity by cyanamide in relation to acetaldehyde accumulation. Toxicology and Applied Pharmacology, 70, 133-139.

Horowitz, G.P. & Whitney, G. (1975). Alcohol-induced conditioned aversion: Genotypic specificity in mice (Mus musculus). Journal of Comparative and Physiological Psychology, 80, 340-346.

Hunt, T. & Amit, Z. (1987). Conditioned taste aversion induced by self-administered drugs: Paradox revisited. Neuroscience and Biobehavioral Reviews 11, 107-130.

Hunt, T., Amit, Z., Switzman, L. & Sinyor, D. (1983). An aversive naloxone-morphine interaction in rats. Neuroscience Letters, 35, 311-315.

Hunt, T., Spivak, K. & Amit, Z. (1985). Aversive stimulus properties of morphine: Evaluation using the drug preexposure conditioned taste aversion paradigm. Behavioral and Neural Biology, 44, 60-73.

Iso, H. (1986). Voluntary ethanol consumption in the absence of hunger and thirst drives. Psychopharmacology, 88, 101-104.

Iversen, H.L. & Damgaard, S.E. (1983). Determination of acetaldehyde in human blood using thiourea to inhibit ethanol interference. Clinica Chimica Acta, 135, 151-158.

Jacobsen, E. (1952). Deaths of alcoholic patients treated with disulfiram (tetraethylthiuram disulfide) in Denmark. Quarterly Journal of Studies on Alcohol, 13, 16-26.

Jenkins, W.J. & Peter, J.J. (1980). Selectively reduced hepatic acetaldehyde dehydrogenase in alcoholics. Lancet, 1(8169), 628-629.

Keilin, D. & Hartree, E.F. (1945). Properties of catalase: Catalysis of coupled oxidation of alcohols. Biochemistry Journal, 39, 293-301.

Khanna, J.M., Lindros, K.O., Israel, Y. & Orrego, H. (1977). In vivo metabolism of ethanol at high and

low concentrations. In R.G. Thurman, J.R. Williamson, H. Drott & B. Chance (Eds.), Alcohol and aldehyde metabolizing systems, vol. 3. New York: Academic Press.

Kiianmaa, K. (1980). Alcohol intake and ethanol intoxication in the rat: effect of a 6-OHDA-induced lesion of the ascending noradrenaline pathways. European Journal of Pharmacology, 6, 9-19.

Kiianmaa, K. & Virtanen, P. (1978). Ethanol and acetaldehyde levels in cerebrospinal fluid during ethanol oxidation in the rat. Neuroscience Letters, 10, 181-186.

Kiessling, K-H. (1962). The effect of acetaldehyde on rat brain mitochondria and its occurrence in brain after ethanol injection. Experimental Cell Research, 26, 432-434.

Kitson, T.M. (1977). The disulfiram-ethanol reaction. Journal of Studies on Alcohol, 38, 96-113.

Koivula, T., Turner, A., Huttunen, M. & Koisvusalo, M. (1981). Subcellular and perisynaptic distribution of rat brain aldehyde dehydrogenase activity. Journal of Neurochemistry, 36, 1893-1897.

Lester, D., Nachman, M. & LeMagnen, J. (1970). Aversive conditioning by ethanol in the rat. Quarterly Journal of Studies on Alcohol, 31, 578-586.

Lindros, K.O. (1983). Human blood acetaldehyde levels: with improved methods a clearer picture emerges. Alcoholism: Clinical and Experimental Research, 7, 70-75.

Lindros, K.O. (1985). Research on experimental and inborn alterations of acetaldehyde: Implications for treatment of alcoholism. The second Malmo symposium on alcohol, (pp. 115-125). Malmo: AB Ferrosan.

Lindros, K.O., Koivula, T. & Eriksson, C.J.P. (1975). Acetaldehyde levels during ethanol oxidation: A diet-induced change and its relation to liver aldehyde dehydrogenases and redox states. Life Sciences, 17, 1589-1598.

Lindros, K.O., Salaspuro, M. & Pikkarainen, P. (1977). Studies on the role of the ADH pathway in increased ethanol elimination after chronic alcohol intake in rat and man. In R.G. Thurman, J.R. Williamson, H. Drott & B. Chance (Eds.), Alcohol and aldehyde metabolizing systems, vol. 3, (pp. 343-354). New York: Academic Press.

Lindros, K.O., Sinclair, J.D., Ahtee, L. & Attila, L. (1981). Effect of cyanamide on brain norepinephrine and dopamine. Acta Pharmacologie et Toxicologie, 49(Suppl.4), Abstract 24.

Lindros, K.O., Stowell, A., Pikkarainen, P. & Salaspuro, M. (1980). Elevated blood acetaldehyde in alcoholics with accelerated ethanol elimination.

Pharmacology Biochemistry & Behavior, 13, 119-124

Lindros, K.O., Stowell, A., Pikkarainen, P. & Salaspuro, M. (1981). The disulfiram (Antabuse)-alcohol reaction in male alcoholics: Its efficient management by 4-methylpyrazole. Alcoholism: Clinical and Experimental Research, 5, 528-530.

Lindros, K.O., Vihma, R. & Forsander, O.A. (1972). Utilization and metabolic effects of acetaldehyde and ethanol in perfused rat liver. Biochemistry Journal, 126, 945-952.

Linseman, M.A. (1987). Alcohol consumption in free-feeding rats: Procedural, genetic and pharmacokinetic factors. Psychopharmacology, 92, 254-261

Lowry, O.H., Rosebrough, N., Farr, A., Randall, R., (1951). Protein measurement with the folin reagent. Journal of Biological Chemistry, 193, 265-275.

Lundquist, F. (1971). The metabolism of ethanol. In Y. Israel & J. Mardones (Eds.), Biological basis of alcoholism, (pp. 1-52). New York: John Wiley and Sons.

Lundquist, F., Tygstrup, N., Winkler, K., Mellemgaard, K. & Munck-Petersen, S. (1962). Ethanol metabolism and production of free acetate in the human liver. Journal of Clinical Investigation, 41, 955-961.

Magnusson, G., Nyberg, J.A., Bodin, N. & Hansson, E. (1972). Toxicity of pyrazole and 4-methylpyrazole in mice and rats. Experentia, 28, 1198-1200.

Majchrowicz, E. (1973). The concentration of ethanol and acetaldehyde in blood and brain of alcohol-dependent rats. Proceedings of the American Society of Neurochemistry, 4, 113.

Marchner, H. & Tottmar, O. (1978). A comparative study on the effects of disulfiram, cyanamide and 1-amino-cyclopropanol on the acetaldehyde metabolism in rats. Acta Pharmacologie et Toxicologie 43, 219-232.

Marcucella, H., Munro, I. & MacDonall, J.S. (1984). Patterns of ethanol consumption as a function of the schedule of ethanol access. Journal of Pharmacology and Experimental Therapeutics, 230, 658-664.

Mason, S.T., Corcoran, M.E. & Fibiger, H.C. (1979). Noradrenergic processes involved in the locomotor effects of ethanol. European Journal of Pharmacology, 54, 383-387.

McKenna, O., Arnold, G. & Holtzman, E. (1976). Microperoxisome distribution in the central nervous system. Brain Research, 117, 181-194.

Mello, N.K. (1983). A behavioral analysis of the reinforcing properties of alcohol and other drugs in man. In B. Kissin & H. Begleiter (Eds.), The pathogenesis of alcoholism, biological factors, vol. 7, (pp. 133-198). New York: Plenum Press.

Meyer, R.E. & Mirin, J.M. (1979). The heroin stimulus: Implications for a theory of addiction. New York: Plenum Medical Book Company.

Mizoi, Y., Ijiri, Y., Tatsuno, T., Kijima, T., Fujiwara, S., Adachi, J. & Hishida, S. (1979). Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. Pharmacology Biochemistry & Behavior, 10, 303-311

Mizoi, Y., Tatsuno, Y., Adachi, J., Kogame, M., Fukunaga, T., Fujiwara, S., Hishida, S. & Ijiri, I. (1983). Alcohol sensitivity related to polymorphism of alcohol-metabolizing enzymes in Japanese. Pharmacology Biochemistry & Behavior, 18, 127-133

Minto, A. & Roberts, F.J. (1960). "Temposil" a new drug in the treatment of alcoholism. Journal of Mental Science, 106, 288-295.

Mottin, J.L. (1973). Drug-induced attenuation of alcohol consumption. Quarterly Journal of Studies on Alcohol, 34, 444-472.

Mukherji, B., Kashiki, Y., Ohyanagi, H. & Sloviter, H. (1975). Metabolism of ethanol and acetaldehyde by the isolated perfused rat brain. Journal of Neurochemistry, 24, 841-843.

Myers, R.D. & Melchoir, C.L. (1977). Differential actions on voluntary alcohol intake of tetrahydroisoquinolines or a B-carboline infused chronically in the ventrical of the rat. Pharmacology

Biochemistry & Behavior, 7, 381-392.

Myers, R.D. & Veale, W.L. (1969). Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. Archives Internationale de Pharmacodynamie et de Therapie, 180, 100-113.

Myers, R.D., Veale, W.L. & Yaksh, T.L. (1972). Preference for ethanol in the Rhesus monkey following chronic infusion of ethanol into the cerebral ventricles. Physiology and Behavior, 8, 431-435.

Myers, W.D., Ng, K.T., Marzuki, S., Myers, R.D. & Singer, G. (1984). Alterations of alcohol drinking in the rat by peripherally self-administered acetaldehyde. Alcohol, 1, 229-236.

Myers, W.D., Ng, K.T. & Singer, G. (1984). Ethanol preference in rats with a prior history of acetaldehyde self-administration. Experentia, 40, 1008-1010.

Myers, W.D., Ng, K.T. & Singer, G. (1982). Intravenous self-administration of acetaldehyde in the rat as a function of schedule, food deprivation and photoperiod. Pharmacology Biochemistry & Behavior, 17 807-811.

Nachman, M. (1963). Learned aversion to the taste of lithium chloride and generalization of other salts. Journal of Comparative & Physiological Psychology, 56, 343-349.

Nachman, M. & Ashe, J.H. (1973). Learned taste aversions in rats as a function of dosage, concentration and route of administration of lithium chloride. Physiology and Behavior, 10, 73-78.

Nuutinen, H.U., Salaspuro, M.P., Valle, M. & Lindros, K.O. (1984). Blood acetaldehyde concentration gradient between hepatic and antecubital venous blood in ethanol-intoxicated alcoholics and controls. European Journal of Investigation, 14, 306-311.

Palmer, K.R. & Jenkins, W.J. (1982). Impaired acetaldehyde oxidation in alcoholics. Gut, 23, 729-733.

Oritz, A., Griffiths, P.J. & Littleton, J.M. (1974). A comparison of the effects of chronic administration of ethanol and acetaldehyde to mice: Evidence for a role of acetaldehyde in ethanol dependence. Journal of Pharmacy and Pharmacology, 26, 249-260.

Paul, S.M., Axelrod, J. & Diliberto, E.J. (1977). Catecholestrogen-forming enzymes of brain: Demonstration of cytochrome P-450 mono-oxygenase. Endocrinology, 101, 1604-1610.

Petersson H. & Kiessling, K-H. (1977). Acetaldehyde occurrence in CSF during ethanol oxidation in rats and its dependence on the blood level and on dietary factors. Biochemical Pharmacology, 26, 237-240.

Pettersson, H. & Tottmar, O. (1982). Aldehyde dehydrogenase in rat brain. Subcellular distribution and properties. Journal of Neurochemistry, 38, 477-487.

Pettersson, H. & Tottmar, O. (1982a). Inhibition of aldehyde dehydrogenases in rat brain and liver by disulfiram and coprine. Journal of Neurochemistry, 39, 628-634.

Pietruszko, R., Reed, D.M., Vallari, R., Major, L., Saini, N. & Hawley, R. (1981). Brain aldehyde dehydrogenase in human alcoholics and controls. Alcoholism: Clinical and Experimental Research, 5 78-84.

Raskin, N.H. (1975). Alcoholism or acetaldehydism? New England Journal of Medicine, 292, 422-423.

Raskin, N.H. & Sokoloff, L. (1968). Brain alcohol dehydrogenase. Science, 162, 131-132.

Raskin, N.H. & Sokoloff, L. (1970). Alcohol dehydrogenase activity in rat brain and liver. Journal of Neurochemistry, 17, 1677-1687.

Raskin, N.H. & Sokoloff, L. (1972). Enzymes catalyzing ethanol metabolism in neural and somatic tissues of the rat. Journal of Neurochemistry, 19, 273-282.

Resnick, R.B., Kestenbaum, R.S. & Schwartz, L.K. (1977). Acute systemic effects of cocaine in man: A controlled study by intranasal and intravenous routes. Science, 195, 696-698.

Ritchie, J.M. (1970). The aliphatic alcohols. In L.S. Goodman & A. Gilman (Eds) The pharmacological basis of therapeutics, (pp. 135-150). New York: The MacMillan Company.

Samson, H.H. (1986). Initiation of ethanol reinforcement using a sucrose-substitution procedure in food and water-sated rats. Alcoholism: Clinical and Experimental Research, 10, 436-442.

Schenk, S., Hunt, T., Colle, L. & Amit, Z. (1983). Isolation versus grouped housing in rats: differential effects of low doses of heroin in the place preference paradigm. Life Sciences, 32, 1129-1134.

Schlesinger, K., Kakihana, R. & Bennet, E.L. (1966). Effects of tetraethylthiuram disulfide (Antabuse) on the metabolism and consumption of ethanol in mice. Psychonomic Medicine, 28, 514-520.

Sellers, E.M., Naranjo, C.A. & Peachey, J.E. (1981). Drugs to decrease alcohol consumption. New England Journal of Medicine, 305, 1255-1262.

Sinclair, J.D. & Gribble, P.A. (1985). Cyanamide injections during alcohol deprivation increase alcohol drinking. Alcohol, 2, 627-630.

Sinclair, J.D. & Lindros, K.O. (1981). Suppression of alcohol drinking with brain aldehyde dehydrogenase inhibition. Pharmacology Biochemistry & Behavior, 14, 377-383.

Sinclair, J.D., Lindros, K.O. & Terho, K. (1980). Aldehyde dehydrogenase inhibitors and voluntary ethanol drinking by rats. In R.G. Thurman (Ed) Alcohol and aldehyde metabolizing systems-IV, (pp. 481-487). New York: Plenum Press.

Sippel, H.W. (1972). Thiourea, an effective inhibitor of the non-enzymatic ethanol oxidation in biological extracts. Acta Chemica Scandinavia, 24, 541-550.

Sippel, H.W. (1974). The acetaldehyde content in rat brain during ethanol metabolism. Journal of Neurochemistry, 23, 451-452.

Sklar, L.S. & Amit, Z. (1977). Manipulations of catecholamine systems block the conditioned taste aversion induced by self-administered drugs. Neuropharmacology, 16, 649-655.

Smith, B.R. (1983). Acetaldehyde and norepinephrine interactions in the mediation of some of the psychopharmacological properties of ethanol. Doctoral dissertation, Concordia University.

Smith, B.R., Amit, Z., Aragon, C.M.G. & Socaransky, S. (1985). Neurobiological correlates of ethanol self administration: The role of acetaldehyde. In C.A. Naranjo & E.M. Sellers (Eds.) Research advances in new psychopharmacological treatments for alcoholism, (pp. 45-63). New York: Elsevier

Smith, B.R., Amit, Z. & Splawinsky, J. (1984). Conditioned place preference induced by intraventricular infusions of acetaldehyde. Alcohol, 1, 193-195.

Socaransky, S.M., Aragon, C.M.G. & Amit, Z. (1985). Brain ALDH as a possible modulator of ethanol intake. Alcohol, 2, 361-365.

Socaransky, S.M., Aragon, C.M.G., Amit, Z. & Blander, A. (1984). Higher correlation of ethanol consumption with brain than liver aldehyde dehydrogenase in 3 strain of rats. Psychopharmacology, 84, 250-253.

Spivak, K., Aragon, C.M.G. & Amit, Z. (1987). Alterations in brain aldehyde dehydrogenase activity modify the locomotor effects produced by ethanol in rats. Alcohol and Drug Research, 7, 481-491.

Spivak, K., Aragon, C.M.G. & Amit, Z. (1987a). Alterations in brain aldehyde dehydrogenase activity modify ethanol-induced conditioned taste aversion. Alcoholism: Clinical and Experimental Research, in press.

Spyraki, C., Fibiger, H.C. & Phillips, A.G. (1982). Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. Brain Research, 253, 195-203.

Stewart, R.B. & Grupp, L.A. (1984). A simplified procedure for producing ethanol self-selection in rats. Pharmacology Biochemistry & Behavior, 21, 255-258.

Stolerman, I.P. & Kumar, R. (1972). Secondary reinforcment in opioid dependence. In J.M. Singh, L.H. Miller & H. Lal (Eds.) Drug addiction: Experimental psychology, vol. 1, (pp. 49-60). New York: Futura Publishing Co.

Stowell, A.R. (1979). An improved method for determination of acetaldehyde in human blood with minimal ethanol interference. Clinica Chemica Acta, 98, 201-205.

Svanas, G.W. & Weiner, H. (1985). Enzymatic requirement for cyanamide inactivation of rat liver aldehyde dehydrogenase. Biochemical Pharmacology, 34, 1197-1204.

Switzman, L., Amit, Z., White, N. & Fishman, B. (1978). Novel tasting food enhances morphine discriminability in rats. In F.C. Colpaert & J.A. Roscrans (Eds.) Stimulus properties of drugs: Ten years of progress, (pp. 199-207). Amsterdam: Elsevier/ North Holland.

Switzman, L., Hunt, T. & Amit, Z. (1981). Heroin and morphine: Aversive and analgesic effects in rats. Pharmacology Biochemistry & Behavior, 15, 755-759

Tabakoff, B., Anderson, R.A. & Ritzmann, R.F. (1976). Brain acetaldehyde after ethanol administration. Biochemical Pharmacology, 25, 1305-1309.

Tabakoff, B. & Gelpke, C.C. (1975). Alcohol and aldehyde metabolism in brain. In E. Majchrowicz (Ed.)

Biochemical pharmacology of ethanol, (pp. 141-164). New York: Plenum Press.

Takayama, S. & Uyeno, E.T. (1985). Intravenous self-administration of ethanol and acetaldehyde by rats. Japanese Journal of Psychopharmacology, 5, 329-334.

Thandani, P.V. & Truitt, E.B. (1977). Effect of acute ethanol or acetaldehyde administration on the uptake, release, metabolism and turnover rate of norepinephrine in rat brain. Biochemical Pharmacology, 26, 1147-1150.

Tottmar, O. & Lindberg, P. (1977). Effects on rat liver acetaldehyde dehydrogenase in vitro and in vivo by coprine, the disulfiram-like constituent of Coprinus atramentarius. Acta Pharmacologie et Toxicologie, 40, 476-481.

Tottmar, O., Marchner, H. & Lindberg, P. (1977). Inhibition of rat-liver aldehyde dehydrogenases in vitro and in vivo by disulfiram, cyanamide and the alcohol-sensitizing compound coprine. In R.G. Thurman & J.R. Williamson, H.R. Droft & B. Chance (Eds.) Alcohol and aldehyde metabolizing systems, vol. II, (pp. 203-212). New York: Academic Press.

Tottmar, S.O.C., Pettersson, H. & Kiessling, K.H. (1973). The subcellular distribution and properties of aldehyde dehydrogenase in rat liver. Biochemical Journal, 135, 577-586.

Truitt, E.B. (1970). Ethanol-induced release of acetaldehyde from blood and its effects on the determination of acetaldehyde. Quarterly Journal of Studies on Alcohol, 31, 1-12.

Truitt, E.B. & Walsh, M.J. (1971). The role of acetaldehyde in the actions of ethanol. In H. Kissin and H. Begleiter (Eds.) The biology of alcoholism, vol. I., (pp. 161-195). New York: Plenum Press.

von Wartburg, J.P. & Buhler, R. (1984). Biology of disease: Alcoholism and aldehydism: New biomedical concepts. Laboratory Investigation, 50, 5-15.

Walsh, M.J. (1971). The role of acetaldehyde in the interactions of ethanol with neuroamines. In M.K. Roach, W.M. Isaac & P.J. Creaven (Eds.) Biological aspects of alcohol, (pp. 233-266). Austin: University Texas Press.

Weiner, H. (1979). Aldehyde dehydrogenase. Mechanism of action and possible physiological role. In E. Majchrowicz & E.P. Noble (Eds.) Biochemistry and pharmacology of ethanol, vol. 1, (pp. 107-123). New York: Plenum Press.

Weiner, H. (1987). Subcellular localization of acetaldehyde oxidation in liver. Annals of the New York Academy of Sciences, 492, 25-33.

Wendell, G.D. & Thurman, R.G. (1979). Effect of ethanol concentration on rates of ethanol elimination in normal and alcohol treated rats in vivo.

Biochemical Pharmacology, 28, 273-279.

Westcott, J.Y., Weiner, H., Shultz, J. & Myers, R.D. (1980). In vivo acetaldehyde in the brain of the rat treated with ethanol. Biochemical Pharmacology, 29, 411-417.

Wickramasinghe, S.N. (1987). Neuroglial and neuroblastoma cell lines are capable of metabolizing ethanol via an alcohol-dehydrogenase-independent pathway. Alcoholism: Clinical and Experimental Research, 11, 234-237.

Wolff, P.H. (1972). Ethnic differences in alcohol sensitivity. Science, 175, 449-450.