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ACETALDEHYDE: - A POSITIVE REINFORCER MEDIATING ETHANOL CONSUMPTION IN LABORATORY RATS

Zavie W. Brown

A Thesis

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ABSTRACT

ACETALDEHYDE:- A POSITIVE REINFORCER MEDIATING ETHANOL CONSUMPTION IN LABORATORY RATS.

Zavie W. Brown, Ph.D.
Concordia University, 1978

The presence of acetaldehyde, the first oxidation product of ethanol, in the brains of laboratory rats was examined with regard to its possible involvement in the self-administration of ethanol. In contrast to the ability of acetaldehyde to induce a conditioned taste aversion when administered intraperitoneally, attempts to induce a conditioned aversion to a saccharin solution by intraventricular infusions of acetaldehyde were not successful. Subsequently, it was found that naive rats would learn to self-administer acetaldehyde but not ethanol directly into the cerebral ventricles. Furthermore, it was shown that the rate of intraventricular self-administration of acetaldehyde was positively correlated with the amount of ethanol voluntarily consumed in subsequent tests. These results suggest that acetaldehyde has positive central reinforcing effects that may mediate the consumption

of ethanol. In the final experiment, chronic infusions of various tetrahydroisoquinoline alkaloids, products of the condensation of aldehydes with biogenic amines, were found to be ineffective in altering ethanol intake. A model for the regulation of ethanol consumption is proposed.

PREFACE

The present thesis reports on four experiments designed to examine the relation between the presence of acetaldehyde in the brain and the voluntary consumption of ethanol in laboratory rats. Following a general introduction, the experiments are presented as separate reports based on manuscripts prepared, submitted, and in the case of Experiment "A" accepted for publication. This format of presentation unavoidably involves some degree of repetition.

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Introduction

The suggestion that acetaldehyde, the primary metabolite of ethanol, may be responsible for some of the physiological, pharmacological and behavioral effects of ethanol has generated much interest in recent years (Raskin, 1975; Truitt and Walsh, 1971). Attention was first drawn to acetaldehyde as a possible intervenant in the effects alcohol by the observations that high blood concentrations of acetaldehyde result in characteristic aversive reactions that reduce the appetite for alcohol (Asmussen, Hald and Larsen, 1948; Hald and Jacobsen, 1948; Jacobsen, 1952). In addition to demonstrating the presence of acetaldehyde in the brain following ethanol administration (Kiianmaa and Virtanen, 1977; Petterson and Kiessling, 1977; Sippel, 1974; Tabakoff, Anderson and Ritzmann, 1976), it has been shown that acetaldehyde is capable of altering the metabolism of central catecholamines (CA) (Duritz and Truitt, 1966; Ortiz, Griffiths and Littleton, 1974; Walsh, 1971) that have been implicated in the mediation of motivated behavior (e.g., Fibiger, 1978; German and Bowden,

1974; Stein, 1968; Zigmond and Stricker, 1972). Furthermore, it has been postulated that pharmacologically active alkaloids (see Cohen, 1976), that can be formed in the presence of acetaldehyde, may be involved in the development of alcohol dependence (see Rahwan, 1975; Myers and Melchior, 1977a,b). Little is known, however, about the behavioral changes brought about by the accumulation of acetaldehyde in the brain. In the experiments to be reported in the present thesis, the psychopharmacological effects of centrally administered acetaldehyde and their relationship to voluntary consumption of ethanol in laboratory rats were examined.

Psychopharmacological Factors in Ethanol Self-administration

Lester and Freed (1973) proposed that an adequate animal model of alcoholism requires that an animal be willing to work for ethanol and to voluntarily consume sufficient amounts of ethanol in order to sustain intoxication and ultimately induce a state of physical dependence. Although ethanol can function as a positive

reinforcer that may be involved in the development and maintenance of ethanol self-administration, there is little evidence implicating physical dependence in the mediation of ethanol consumption in laboratory animals.

A. Ethanol as a reinforcer

Animals will learn to perform an operant in order to receive intragastric (Amit and Stern, 1969; Davis, Smith and Werner, 1977; Yanagita and Takahashi, 1973) or intravenous (Deneau, Yanagita and Seevers, 1969; Winger and Woods, 1973) infusions of ethanol indicating that ethanol has positive reinforcing properties. Because of its aversive taste at higher concentrations (Kahn and Stellar, 1960; Richter and Campbell, 1940; Wilson, 1972) it has been more difficult to establish orally administered ethanol as a reinforcer in laboratory animals. Although more ethanol is consumed when its taste is masked by sucrose or saccharin, the increased intake does not persist when unadulterated ethanol is presented (Eriksson, 1969; Myers, Stoltman and Martin, 1972). Despite its aversive taste, however, naive rats learned to press a lever for

drops of a 10% (v/v) ethanol solution suggesting that the pharmacological effects of ingested ethanol are positively reinforcing (Meisch and Beardsley, 1975; Sinclair, 1974).

Various techniques have been used to induce preferential consumption of ethanol in laboratory animals. For example, it has been found that temporary removal or intermittent presentation of ethanol results in an enhanced voluntary intake (Amit, Stern and Wise, 1970; Sinclair and Senter, 1968). Substituting ethanol for water in the schedule-induced polydipsia paradigm resulted in the drinking of intoxicating amounts of ethanol (Falk, Samson and Winger, 1972; Senter and Sinclair, 1967) and in some cases a subsequent preference for ethanol persisted (Meisch and Thompson, 1974). It has been reported by some investigators that following a period in which ethanol was the sole available fluid, animals showed enhancement in voluntary consumption of ethanol (Myers, 1961; Wallgren and Forsander, 1963) while others have failed to induce a preference in similarly treated animals (Richter, 1926; Rodgers, Ward, Thiessan and

Whitworth, 1967). Attempts to encourage ethanol drinking by providing electric shock (Senter, Smith and Lewin, 1967), rewarding brain stimulation (Martin and Myers, 1972), or food (Black and Martin, 1972), as extrinsic reinforcement contingent upon the ingestion of ethanol have been successful. Upon termination of such contingencies, however, the enhanced ethanol intake does not persist. Daily sessions of non-contingent electrical stimulation of the lateral hypothalamus in rats has been shown to be effective in increasing the free-choice consumption of a previously rejected ethanol solution (Amir and Stern, 1978; Amit and Stern, 1971; Amit et al., 1970). Since animals will voluntarily consume ethanol despite its aversive taste, it suggests that ethanol ingestion does produce pharmacological effects that are positively reinforcing.

B. <u>The role of physical dependence in ethanol self-administration</u>

Despite the traditional view that physical dependence (operationally defined by the manifestation of withdrawal symptoms when ethanol

is withheld) is a necessary criterion for alcoholism (Lester and Freed, 1973), there is evidence from several sources that does not support this notion. In order to induce a state of physical dependence in animals, it is generally necessary to maintain high blood ethanol levels without interruption for several days (Goldstein, 1975; Hunter, Riley, Walker and Freund, 1975). Although a variety of procedures have been developed to induce physical dependence (see Friedman and Lester, 1977; Mello, 1973), the effects of these manipulations on voluntary ethanol consumption have been equivocal. For example, chronic gastric intubation of intoxicating doses of ethanol in animals was shown in some studies to increase subsequent oral self-administration of ethanol (Deutch and Koopmans, 1973; Sinclair, Walker and Jordan, 1973), while other investigators found no effect (Begleiter, 1975; Myers et al., 1972). Similarly, when ethanol was presented as the only source of fluid (Cicero and Smithloff, 1973; Ratcliffe, 1972) or as a partial source of calories (Freund, 1973; Walker, Hunter and Riley, 1975), animals

eventually became physically dependent as evidenced by the withdrawal symptoms upon removal of ethanol. These animals did not, however, show any subsequent preference for ethanol. Induction of physical dependence by ethanol vapor inhalation (Goldstein, 1974) has not been shown to alter voluntary ethanol intake.

Noteworthy are the patterns of ethanol self-administration in animals that are physically dependent. It has been shown that despite free access to ethanol, animals will periodically abstain voluntarily from self-administration and undergo the aversiveness of the withdrawal syndrome (Deneau et al., 1969; Winger and Woods, 1973; Woods, Ikomi and Winger, 1971). Similar patterns have been reported in human alcoholics who have been observed under some circumstances to go through periods of withdrawal even though alcohol was available (Mello and Mendelson, 1972). From these reports, it appears that physical dependence is neither a necessary nor a sufficient condition for the self-administration of ethanol in laboratory animals. Alternatively, ethanol appears to have inherent positive

reinforcing effects that may be crucial in the development and maintenance of ethanol-oriented behaviors.

C. Biochemical factors

Serotonin and ethanol self-administration. In 1968, Myers and Veale proposed that 5-HT may be involved in the mediation of ethanol consumption in rats. Unfortuntely, the data relevant to this hypothesis have not been consistent. For example, in behavioral genetics studies, Ahtee and Eriksson (1972) showed that rats genetically bred for ethanol preference (AA) had higher brain content of 5-HT than water preferring animals (ANA), whereas Berger and Weiner (1977) found no difference in 5-HT metabolism between high and low ethanol drinking rats. Reductions in brain content of 5-HT by p-chlorophenylalanine (pCPA) injections inhibited the consumption of ethanol in rats (Myers and Veale, 1968; Veale and Myers, 1970); opposite effects were found when 5-HT neurons were destroyed by central infusions of the neurotoxin 5,6-dihydroxytryptamine (Ho, Tsai, Chen, Begleiter and Kissin, 1974; Melchior and Myers, 1976; Myers and Melchior,

1975a). Using similar procedures, Kiianmaa (1975) found that destruction of 5-HT neurons produced no significant change in ethanol intake. These divergent results have been partly attributed to the increase in circulating acetaldehyde levels following pCPA treatment and the concomitant development of a conditioned aversion to the taste of ethanol (Nachman, Lester and LeMagnen, 1970). Studies using an alternative approach of elevating the levels of 5-HT in the brain have also led to conflicting results. When 5-HT levels were raised by administering the precursor tryptophan, it was found that ethanol intake increased (Myers and Melchior, 1975b; Sprince, Parker, Smith and Gonzoles, 1973). On the other hand, both peripheral and central injections of 5-hydroxytryptophan, the immediate precursor of 5-HT, reduced the preference for ethanol (Myers and Martin, 1973; Geller, Purdy and Merritt, 1973). Suppression of ethanol consumption in rats was also observed following intraventricular infusions of 5-HT itself (Hill, 1974).

Because of the numerous discrepancies that

have been reported, it is difficult to determine the extent to which central 5-HT mechanisms may be involved in the mediation of ethanol self-administration.

Catecholamines and ethanol self-administration

Ahtee and Eriksson (1975) have reported that the concentrations of dopamine (DA) in the brains of the ethanol-preferring AA rats were significantly higher than in the ANA strain although norepinephrine (NE) levels were not different. It has also been shown that electrical stimulation of the lateral hypothalamus, an area traversed by the major CA pathways (Lindvall and Bjorklund, 1974; Ungerstedt, 1971), resulted in a pronounced long-lasting preference for ethanol over water in laboratory rats (Amir and Stern, 1978; Amit and Stern, 1971; Amit et al., 1970). There have been a number of studies in which it has been shown that the disruption of CA systems results in an attenuation of ethanol consumption. Myers and Veale (1968) reported that treatment with alpha-methyl-para-tyrosine (AMPT), an inhibitor of the CA synthesizing enzyme tyrosine hydroxylase, produced a transitory reduction in

the preference for ethanol in rats. AMPT has also been shown to antagonize ethanol-induced stimulation and euphoria in humans (Ahlenius, Carlsson, Engel, Svensson and Sodersten, 1973) as well as in laboratory rats (Carlsson, Engel and Svensson, 1972). Electrolytic lesions in the area of the lateral hypothalamus of rats resulted in a marked reduction of ethanol intake (Amit, Meade, Levitan and Singer, 1976b). Similarly, the destruction of CA neurons by 6-hydroxydopamine (6-OHDA) attenuated ethanol consumption (Brown and Amit, 1977; Myers and Melchior, 1975a). There is a report, however, that after long-term exposure to ethanol following 6-HDA treatment, the preference for ethanol temporarily exceeded that of control animals (Kiianmaa, Fuxe, Jonsson and Ahteé, 1975).

The findings of Brown and Amit (1977) and Kiianmaa et al. (1975) suggest that central systems containing NE rather than DA or 5-HT subserve voluntary consumption of ethanol in rats. This notion has been supported by recent studies in which it was shown that central NE depletions produced by FLA-57, a non-toxic dopamine-beta-

hydroxylase inhibitor (Elorvall and Corrodi, 1970), suppressed the intake of ethanol in rats (Amit, Brown, Levitan and Ogren, 1977a). Furthermore, ethanol-preferring rats given ethanol as the sole fluid during treatment with FLA-57, subsequently rejected ethanol when presented in a free-choice with water; this implies that the reinforcing effects of ethanol were blocked by FLA-57 and ethanol-drinking behavior was extinguished (Brown, Amit, Levitan, Ogren and Sutherland, 1977). Davis et al. (1977) have provided additional support for the proposed noradrenergic involvement in ethanol reinforcement. They found that rats treated with AMPT or the DBH inhibitor U14,624 failed to re-acquire a response contingency for intragastric infusions of ethanol whereas haloperidol, a DA receptor blocker, had no effect. These findings suggest that central CA and NE in particular may be integrally involved in the reinforcing effects of ethanol that support its self-administration.

The Metabolism of Acetaldehyde

Acetaldehyde formation

When ethanol is consumed, it is oxidized to acetaldehyde by alcohol dehydrogenase (ADH. alcohol: NAD oxidoreductase; E.C. 1.1.1.1) primarily in the liver (Jacobsen, 1952; Hawkins and Kalant, 1972) and to a minor extent in other organs of the body (Lundquist, 1971; Raskin and Sokoloff, 1972) including the brain (Raskin and Sokoloff, 1968, 1970; Tyce, Flock and Owen, 1968). After chronic ethanol intake or when blood levels are elevated above 20mM, ethanol may also be metabolized by a microsomal ethanol oxidizing system (MEOS. Lieber and DeCarli, 1968) or by the enzyme catalase (E.C. 1.11.1.6. Keilin and Hartree, 1945), although these enzymes are relatively insignificant compared to ADH (Hawkins and Kalant, 1972). Normally, the hepatic oxidation of ethanol follows zero-order kinetics; when blood ethanol concentrations exceed 4 mM, the formation of acetaldehyde proceeds at a steady rate (Loomis, 1950; Lundquist, 1971). It has been reported that prolonged consumption of ethanol

results in an induction of ADH activity in the liver (Hawkins, Kalant and Khanna, 1966; Videla, Bernstein and Israel, 1973). There are, however, conflicting reports that indicate no increase in the capacity to oxidize ethanol following chronic ethanol consumption (Bartlett and Barnet, 1949; von Wartburg, 1971).

Elimination of acetaldehyde

The acetladehyde that is formed by the oxidation of ethanol is rapidly metabolized further to acetate by the mitochondrial enzyme aldehyde dehydrogenase (ALDH. aldehyde:NAD oxidoreductase; E.C. 1.2.1.3.). This enzyme is found in large quantities in the liver and to a lesser extent in the kidneys, adrenals, lungs, heart, intestines and in the brain (Deitrich, 1966). The widespread presence of ALDH is due to its function in the metabolism of amine-derived aldehydes and of small amounts of endogenously formed acetaldehyde (Deitrich, 1966). Since ethanol oxidation in the liver by ADH proceeds at a slower rate than acetaldehyde elimination by ALDH, there should be no accumulation of circulating acetaldehyde

in the body regardless of the concentration of ethanol (Jacobsen, 1952; Lundquist, 1971). Furthermore, Hawkins and Kalant (1972) have pointed out that since ethanol produces dose-dependent effects at concentrations above that which results in maximal acetaldehyde formation, it is unlikely that acetaldehyde could be responsible for all of the pharmacological effects of ethanol.

Two other enzymes, xanthine oxidase (E.C. 1.2.3.1) and aldehyde oxidase (E.C. 1.2.3.2), are also capable of oxidizing acetaldehyde, but because of their low affinity for acetaldehyde, their contribution in vivo is minimal (Mackler, Mahler and Green, 1954). It has also been shown that acetaldehyde can react enzymatically with a number of other compounds in the body (e.g., glyceraldehyde-3-phosphate, glycine, α-ketoacid pyruvate, and α-ketoglutarate) to form condensation products (Westerfeld and Bloom, 1966; Gilbert, 1957). However, the involvement of these condensation pathways in the elimination of ethanol-derived acetaldehyde is considered to be insignificant (Lundquist, 1971).

Finally, it should be mentioned that when ALDH is saturated, acetaldehyde can be reduced back to ethanol by the enzyme aldehyde reductase (alcohol:NADP oxidoreductase; E.C. 1.1.1.2; Gershman, 1975; von Wartburg, Berger, Ris and Tabakoff, 1975).

The Occurrence of Acetaldehyde

Peripheral accumulation of acetaldehyde

Following ingestion of moderate quantities of ethanol, only small amounts of acetaldehyde can be detected in the blood (Eriksson, 1977; Eriksson and Sippel, 1977; Lubin and Westerfeld, 1945; Lundquist and Wolthers, 1958) presumably because of the high rate of acetaldehyde oxidation (Feldman and Weiner, 1972; Sheppard, Albersheim and McClearn, 1970). When large doses of ethanol (>2 g/kg) are acutely administered, all the available ethanol oxidizing pathways may be activated so that the formation of acetaldehyde may exceed the capacity of liver ALDH to metabolize it completely (Lindros, Vihma and Forsander, 1972; Raskin and Sokoloff, 1972). Chronic exposure to ethanol produces mitochondrial damage (Cederbaum, Lieber and

Rubin, 1974), resulting in a reduced availability of nicotinamide adeninedinucleotide (NAD), the coenzyme necessary for ALDH functioning (Grunnet, 1973). Because of the consequent decrease in liver mitochondrial ALDH activity (Amir, 1978b; Hasumura, Teshke and Lieber, 1975; Koivula and Lindros, 1975; Korsten, Matsuzaki, Feinman and Lieber, 1975), the elimination of acetaldehyde is retarded and the destruction of hepatic mitochondria is further exacerbated (Cederbaum et al., 1974; Hasumura et al., 1975).

Blood concentration of acetaldehyde can also be enhanced by a number of agents that are capable of inhibiting hepatic ALDH. One such agent, tetraethylthiuran disulfide (disulfiram; Antabuse®), is used clinically in the treatment of alcoholism. When administered in combination with ethanol, this drug produces a set of symptoms including vasodilatation, changes in heart rate, decrease in blood pressure, dizziness, nausea, vomiting, and respiratory depression that in some extreme cases may result in death (Hald and Jacobsen, 1948; Jacobsen, 1952; Raby, 1953; Walsh, 1971). Intravenous

infusion of acetaldehyde in humans has been shown to evoke a similar ethanol-disulfiram reaction (Asmussen et al., 1948). Similar sensitization effects to ethanol can be produced by a number of other compounds which interfere with the metabolism of acetaldehyde, including citrated calcium carbamide (Temposil® (Consbruch and Derwort, 1968; Ferguson, 1956), carbon disulfide (Williams, 1937), Coprine (derived from the fungus Coprinus atramentarius) (Caldwell, Genest and Hughes, 1969), ethylene dinitrate and related compounds (Komura, 1974), and n-butyraldoxime (Koe and Tenen, 1970). It is interesting to note that other agents that are normally used for other pharmacological purposes also are capable of elevating blood acetaldehyde levels. For example, the 4-halopyrazoles, which when administered acutely, inhibit ADH and almost completely block the formation of acetaldehyde from ethanol (Lester, Keokosky and Felzenberg, 1968; Theorell, Yonetani and Sjoberg, 1969), tend to raise acetaldehyde concentrations by inhibiting ALDH when administered chronically (Koe and Tenen,

1975). The monoamine oxidase (MAO) inhibitors, pargyline and Lilly 51641 (Sanders, Collins, Peterson and Fish, 1977) and the catechol-O-methyl transferease (COMT) inhibitor, pyrogallol (Collins, Custod, Rubenstein and Tabakoff, 1976) also interfere with the metabolism of acetaldehyde and increase its concentration in the circulation.

Presence of acetaldehyde in the brain

Because of the high lipid solubility of acetaldehyde, it can readily diffuse into most body tissues (Akabane, 1970). However, attempts to determine whether or not acetaldehyde is present in the brain following ethanol administration have yielded equivocal results. In some of the earlier reports which claimed to have detected acetaldehyde in the brains of ethanol-treated animals (Duritz and Truitt, 1966; Kiessling, 1962a,b; Majchrowicz, 1973; Ridge, 1963) the alterations in brain acetaldehyde paralleled those seen in the cerebral blood (Majchrowicz, 1973; Ridge, 1963). The relatively high levels of brain acetaldehyde that were measured in these studies were attributed to its

non-enzymatic formation during the preparation of the samples (Sippel, 1973). In later studies, when the non-enzymatic formation of acetaldehyde was prevented by thiourea (Sippel, 1972), no measurable amounts could be found in the brain tissue of rats unless sufficient ethanol was administered to elevate blood levels of acetaldehyde above 200 uM (Sippel, 1974; Sippel and Eriksson, 1975; Tabakoff et al., 1976). Unlike the earlier reports of Ridge (1963) and Majchrowicz (1973), these results indicated that the increases in brain concentrations of acetaldehyde are not concomitant with increases in blood levels. However, it has been shown that acetaldehyde accumulation in the cerebrospinal fluid does correspond to that in the blood (Kiianmaa and Virtanen, 1977; Petterson and Kiessling, 1977). Because of the differences between the concentration of acetaldehyde in the brain tissue and that in the blood or cerebrospinal fluid, it has been proposed that there may be some form of enzymatic barrier preventing the penetration of acetaldehyde through the capillary and ventricular walls

(Petterson and Kiessling, 1977; Sippel, 1974; Tabakoff et al., 1976).

Additional information concerning the presence of acetaldehyde in the brain has been provided by a number of studies in which enzymatic activity in the brain has been examined. Although ADH is present in the brain, since it is relatively inactive, only negligible amounts of acetaldehyde are formed by this route (Mukherji, Kashiki, Ohyanagi and Slovitar, 1975; Raskin and Sokoloff, 1968, 1974; Tabakoff and von Wartburg, 1975; Veloso, Passonneau and Veech, 1972). It has also been shown that ALDH which is present in the brain in excess of the requirement for amine metabolism, is increasingly active following prolonged administration of ethanol to rats (Amir, 1978b). It has been suggested that ALDH may function as a metabolic barrier preventing the accumulation of acetaldehyde in the brain and thereby possibly modulating the central effects of ingested ethanol. Additional support for the notion that acetaldehyde may be present in the brain has been provided by Ammon, Estler and

Heim (1969) who showed that acetaldehyde rather than ethanol itself was responsible for the decrease in transacetylation of Coenzyme A in the brains of mice.

It should be noted that a necessary condition for the detection of acetaldehyde in the brain using present available methods is that ethanol must be administered in doses greatly exceeding those that would normally be self-administered. For the same methodological reasons, it has not been possible to determine the threshold levels of acetaldehyde in the blood and in the brain that are necessary to exert some pharmacological effect (Walsh, 1971).

Interaction of Acetaldehyde with Biogenic Amines

Effects on biogenic amine metabolism

The sympathomimetic effects evoked in the presence of high conentrations of circulating acetaldehyde (e.g., Walsh, 1971) have been related to the concomitant release of neuroamines in the periphery (Eade, 1959; Perman, 1958; Schneider, 1971; Truitt and Duritz, 1967). Although there are few studies on the effects of acetaldehyde on the metabolism of the biogenic

amines in the brain, the effects of its precursor, ethanol, have been more thoroughly examined. Ethanol treatment in animals has most often been found to have no effect on brain CA levels (Corrodi, Fuxe and Hokfelt, 1966; Duritz and Truitt, 1966; Efron and Gessa, 1963; Haggendal and Lindquist, 1961; Hunt and Majchrowicz, 1974; Pohorecky, 1974) although there have been some reports of altered content of CA (Carlsson, Magnusson, Svensson and Waldeck, 1973; Erikson and Matchett, 1975; Gursey and Olsen, 1960; Griffiths, Littleton and Ortiz, 1974). The turnover of brain norepinephrine (NE) following acute ethanol injection has been reported both to decrease (Pohorecky, 1974; Thadani and Truitt, 1977) and to increase (Carlsson and Lindquist, 1973; Carlsson et al., 1973; Corrodi et al., 1966; Hunt and Majchrowicz, 1974). Similarly, dopamine (DA) turnover has been shown to increase (Carlsson et al., 1973; Karoum, Wyatt and Majchrowicz, 1976) and to decrease (Hunt and Majchrowicz, 1974). Bacopoulos, Bhatnagar and van Orden (1978) showed that the turnover of NE and DA in various brain regions was differentially

affected by acute injections of ethanol in laboratory rats. The effects of ethanol on brain 5-HT are equally confusing. Different analyses have shown that 5-HT levels decreased (Gursey and Olsen, 1960), increased (Bonnycastle, Bonnycastle and Anderson, 1962) or remained unchanged (Efron and Gessa, 1963; Haggendal and Lindquist, 1961; Truitt and Duritz, 1967). Examination of 5-HT turnover in the brain has not yielded any more consistent results (Frankel, Khanna, Kalant and LeBlanc, 1976; Hunt and Majchrowicz, 1974; Kuriyama, Rauscher and Sze, 1971; Palaic, Desaty, Albert and Panisset, 1971). In a critical review of the relation between ethanol and the biogenic amines, Lahti (1975) concluded that at physiologically compatible concentrations of acutely administered ethanol, there were no appreciable changes in the levels of NE, DA and 5-HT, although the turnover of NE was increased. He also reported that chronic exposure to ethanol was usually found to decrease the content and increase the turnover of brain NE.

The effects of acetaldehyde itself on the

metabolism of centrally occurring neuroamines has also been examined. Ortiz et al. (1974) showed that acetaldehyde inhalation in mice for as few as two days, markedly increased the brain concentrations of NE, DA and 5-HT. By comparison, ethanol inhalation was necessary for a minimum of 8 days in order to produce comparable alterations. Similarly, Duritz and Truitt (1966) found that rats and rabbits pretreated with disulfiram (an ALDH inhibitor) and then injected with ethanol or acetaldehyde, showed changes in the content of catecholamines but not 5-HT. Thadani and Truitt (1977) also reported that acetaldehyde was more effective than ethanol in altering the metabolism of NE in the rat brain.

The alterations in amine metabolism in the brain following ethanol or acetaldehyde metabolism may be accounted for by a combination of factors. One possibility is that ethanol consumption shifts the metabolism of biogenic amines from the oxidative to a reductive pathway presumably due to competitive inhibition of ALDH by acetaldehyde, and to the reduced availability

of the NAD cofactor during ethanol oxidation (Davis, Brown, Huff and Cashaw, 1967a,b; Lahti and Majchrowicz, 1969; Walsh, Truitt and Davis, 1970).

Condensation reactions with biogenic amines

The biogenic amines are capable of condensing with aldehydes by a Pictet-Spengler reaction to form a variety of alkaloids (see: Cohen, 1976; Rahwan, 1975). McIsaac (1961) showed that serotonin (5-HT) could react with acetaldehyde to form tetrahydro-B-carbolines. Dajani and Saheb (1973) have confirmed these findings and have suggested that the B-carbolines may be involved in the mediation of the pharmacological effects of ethanol and in the development of physical dependence on ethanol. It has also been demonstrated that tetrahydroisoquinoline (TIQ) alkaloids, derived from CA and various aldehydes including acetaldehyde, can be formed in rat brain homogenates (Davis and Walsh, 1970; Walsh, Davis and Yamanaka, 1970) and in perfused animal organs (Cohen and Collins, 1970). These alkaloids have been shown to possess a number, of transmitter-like properties. For example,

TIQs are taken up and stored by CA neurons (Cohen, Mytilineau and Barrett, 1972; Locke, Cohen and Dembiec, 1973) and as a consequence of the affinity of these alkaloids for the amine transport pumps, the normal reuptake of CA may be inhibited (Heikkila, Cohen and Dembiec, 1971; Tuomisto and Toumisto, 1973). Electron microscopy has confirmed that the TIQs are accumulated in the storage vesicles of CA nerves (Tennyson, Cohen, Mytilineau and Heikkila, 1973). Furthermore, TIQs competitively inhibit the enzymatic breakdown of CA by MAO and COMT, thus altering the metabolic disposition of the neural system (Collins, Cashaw and Davis, 1973; Giovine, Renis and Bertolino, 1976; Cohen and Katz; 1975). Not only are TIQs taken up and stored by CA neurons but they can be released into the synapse upon electrical or chemical stimulation (Greenberg and Cohen, 1973; Rahwan, O'Neill and Miller, 1974), and can activate receptors (Mytilineau, Cohen and Barrett, 1974). In view of the possible neurochemical actions of the TIQs, Cohen (1973, 1976) has proposed that these alkaloids may function as "false" transmitters

thereby contributing to the neurophysiological and behavioral changes that accompany alcohol ingestion.

Simpson (1975) has argued against the probability of the involvement of TIQs in the sympathomimetic effects of ethanol or acetaldehyde on the basis that their accumulation is probably limited to small amounts, insufficient to exert any pharmacological effects. Furthermore, TIQs do not readily cross the blood brain barrier (see: Rahwan, 1975) making it unlikely that their peripheral formation will be relevant to the mediation of the central effects of ingested ethanol.

Although peripherally formed TIQs may not penetrate the brain, there have been reports that under certain conditions some of these alkaloids have been found in the brain. For example, Collins and Bigdeli (1975) reported that when blood acetaldehyde levels in rats were increased following the administration of ethanol together with pyrogallol and pargyline, small amounts of salsolinol (a DA-acetaldehyde condensate) were detectable in brain tissue.

However, O'Neill and Rahwan (1977) were unable to detect any salsolinol in the brains of mice that had been chronically exposed to ethanol vapor. L-DOPA treatment, with or without ethanol, resulted in the formation of measurable levels of tetrahydropapaveroline (THP; a DA-dopaldehyde condensate) in the brains of rats (Turner, Baker, Algeri, Frigenio and Garattini, 1974). Collins and Bigdeli (1975), however, were unable to detect THP although they admitted that their procedures may not have been sufficiently sensitive. Recently, it was reported that chronic exposure to ethanol led to the formation of methoxy-salsolinol in the rat brain (Hamilton, Blum and Hirst, 1978). It has also been demonstrated that B-carbolines may be formed centrally in vivo (Hsu and Mandell, 1975; Wyatt, Erdelyi, DoAmoral, Elliot, Renson and Barchas, 1975).

Since TIQ alkaloids are neurochemically active compounds that may be present in the brain following ethanol treatment, it is conceivable that they may be functionally related to the pharmacological actions of ethanol.

In summary, acetaldehyde may be present in the brain following ethanol administration. Furthermore, it is capable of altering the metabolism of the biogenic amines either via competitive enzyme inhibition or by the condensation with the neurotransmitters to form neurally active TIQ alkaloids. Therefore, based on the available evidance, it is not unreasonable to suggest that acetaldehyde may be responsible for some of the central effects of ethanol.

The Involvement of Acetaldehyde in Ethanol Consumption

A. Peripheral mechanisms

When an organism consumes alcohol, acetaldehyde normally does not accumulate in the body because of the high metabolic capacity of the liver (Eriksson and Sippel, 1977; Hawkins and Kalant, 1972). Artificially induced high circulating levels of acetaldehyde have been shown to be incompatible with the maintenance of ethanol consumption. For example, disulfiram-induced increases in blood acetaldehyde in human subjects results in aversive physiological

symptoms that discourage further intake of alcohol (Hald and Jacobsen, 1948). Similar attenuating effects of disulfiram on ethanol consumption have been reported in ethanol preferring mice (Schlesinger, Kakihana and Bennett, 1966). A variety of other compounds that inhibit the metabolism of acetaldehyde have been shown to reduce ethanol intake in laboratory animals. The ADH inhibitors, 4-halopyrazole and butyraldoxime, and the MAO inhibitors, pargyline and Lilly 51641, which reportedly also inhibit ALDH and elevate acetaldehyde concentrations, attenuate the consumption of ethanol (Koe and Tenen, 1970, 1975; Sanders et al., 1977). The ALDH inhibitor Temposil® (citrated calcium carbamide) also is capable of reducing the appetite for ethanol (Amir and Stern, 1978; Ferguson, 1956). On the other hand, little success has been achieved in attempts to demonstrate an enhancement in ethanol preference by increasing, via ALDH induction, the capacity to metabolize acetaldehyde. It was shown that although liver ALDH activity was increased by treatment with

phenobarbitol (Deitrich, 1966) or other common inducers of drug metabolism (Marselos and Hanninen, 1974), there was no systematic effect on forced or voluntary consumption of ethanol in rats (Marselos, Eriksson and Hanninen, 1975; Marselos and Pietikainene, 1975).

Some evidence supporting the view that the level of circulating acetaldehyde is inversely related to voluntary ethanol consumption has been provided by behavioral genetics studies. In addition to the findings that ethanol-preferring animals have a higher capacity to oxidize ethanol to acetaldehyde in the liver than non-preferring strains (Eriksson, 1973; Eriksson and Pikkarainen, 1968; McClearn, Bennett, Hebert, Kakihana and Schlesinger, 1964; Sheppard, Albersheim and McClearn, 1968), a direct correlation has also been found between ethanol preference and the capacity to eliminate acetaldehyde. For example, C57BL mice, which prefer a 10% (v/v) ethanol solution to water, were shown to have greater hepatic ALDH activity (Sheppard et al., 1968) and lower blood acetaldehyde levels following ethanol

administration (Schlesinger et al., 1966; Sheppard, Albersheim and McClearn, 1970) than the non-preferring DBA strain. Similarly, it has been reported that ANA rats, compared to the ethanol-drinking AA strain, have lower levels of ALDH activity (Koivula, Koivusala and Lindros, 1975) and consequently greater concentrations of blood acetaldehyde after acute ethanol injection (Eriksson, 1973). Inconsistent with these findings, however, is the report that C57BL mice showed higher blood levels of ethanol and acetaldehyde than DBA mice following acute injections of ethanol (Lin, 1975).

Studies examining sex differences in ethanol preference have also supported the proposal that consumption of ethanol is related to the capacity of the organism to eliminate acetaldehyde. It has been reported that females of the C57BL strain of mice drink more ethanol than their male counterparts (Eriksson and Malmstrom, 1967; Eriksson and Pikkarainen, 1968, 1970). Although these authors attributed the observed differences to the greater capacity

of the females to oxidize ethanol, it has been shown more recently that the concentration of exhaled acetaldehyde, which correlates well with blood levels (Eriksson and Sippel, 1977), is substantially greater in the males (Redmond and Cohen, 1972). Similarly, the differences in the drinking patterns and tolerance to alcohol between different human races has been attributed to the efficiency of acetaldehyde metabolism (Deitrich, 1976).

Although ethanol drinking is attenuated when blood acetaldehyde concentrations become elevated, it is unlikely that such a mechanism could regulate the voluntary consumption of ethanol. The aversive effects of the accumulation of acetaldehyde may however, temporarily terminate the intake of ethanol in order to prevent extensive toxicosis and morphological damage. On the other hand, the interaction of acetaldehyde with central systems may be part of a mechanism that mediates the self-administration of ethanol.

B. Central mechanisms

Based on the evidence that acetaldehyde may be found in the brain following ethanol

administration (e.g., Kiianmaa and Virtanen, 1977; Petterson and Kiessling, 1977; Sippel, 1974; Tabakoff et al., 1976) and that it is capable of altering neurochemical functioning (Duritz and Truitt, 1966; Ortiz et al., 1974) there has been some speculation about its role in the mediation of the reinforcing effects of ethanol.

There has been some controversy as to whether or not acetaldehyde may be responsible for the development of physical dependence on ethanol. Ortiz et al. (1974) showed that chronic inhalation of acetaldehyde produced withdrawal symptoms similar to those observed in ethanol-treated mice. On the other hand, Thurman and Pathman (1975) have provided contrary evidence. They showed that disulfiram treatments alone elevated endogenous levels of acetaldehyde, but did not produce any withdrawal symptoms when the treatments were terminated. Furthermore, they failed to produce withdrawal symptoms in C67BL mice which had been exposed to high levels of circulating acetaldehyde by chronic

treatment with ethanol and disulfiram. Finally, they demonstrated that administration of t-butanol, a substance that is not oxidized to acetaldehyde, resulted in behavioral symptoms similar to those seen following withdrawal of ethanol in physically dependent animals. The latter results with t-butanol were also reported by other investigators (Bellin and Edmonds, 1976; Wallgren, 1973). Whether or not acetaldehyde is involved in the induction of physical dependence may bear little relevance to its putative role in ethanol consumption since physical dependence does not appear to be a sufficient or necessary criterion for ethanol self-administration.

The involvement of TIQ alkaloids derived from acetaldehyde and the biogenic amines in the dependence upon ethanol has also been suggested (see: Blum, Hamilton, Hirst and Wallace, 1978; Cohen, 1976; Rahwan, 1975). It has been shown that intracerebral infusions of TIQs alters the severity of ethanol withdrawal symptoms in mice in a dose-related fashion (Blum, Eubanks, Wallace, Schwertner and Morgan, 1976). In 1970,

Davis and Walsh proposed that the underlying basis for alcoholism may be the acetaldehyde-induced formation of THP which is an intermediate in the biosynthesis of morphine in the poppy plant (Kirby, 1967; Shamma, 1972). This hypothesis was met with criticism based on the inability to demonstrate in vivo THP formation (Halushka and Hoffman, 1970), the lack of similarity between ethanol and morphine withdrawal symptoms (Seevers, 1970) and the failure of naloxone, an opiate antagonist, to precipitate withdrawal in ethanol-dependent mice (Goldstein and Judson, 1971). More recently, there has been some support for the notion that there may be some common neurochemical and behavioral mechanisms mediating the effects of morphine and ethanol. For example, the self-administration of ethanol and morphine was suppressed in laboratory rats following inhibition of CA synthesis by AMPT (Glick, Zimmerberg and Charap, 1973; Pozuelo and Kerr, 1972; Myers and Veale, 1968) or by destruction of CA neurons with electrolytic (Amit et al., 1976b; Glick, Cox and Crane,

1975) or neurochemical lesions (Brown and Amit, 1977; Meade and Amit, 1974; Myers and Melchior, 1975a). Depletions of brain content of NE by the DBH inhibitor FLA-57, resulted in an attenuation of the oral consumption of both ethanol (Amit et al., 1977a) and morphine (Brown, Amit, Sinyor, Rockman and Ogren, 1978). These findings have been substantiated by the reports that the acquisition of an operant task for intragastric infusions of ethanol (Davis et al., 1977) or intravenous injections of morphine (Davis, Smith and Khalsa, 1975) could be disrupted by treatment with the DBH inhibitor U14,624.

Furthermore, there have been a few studies that have demonstrated a cross dependence between ethanol and morphine. For example, animals that prefer ethanol to water also show a greater tendency to ingest morphine solutions (Nichols and Hsiao, 1967). Morphine injections alleviated convulsions in ethanol-withdrawn mice (Blum, Wallace, Schwerter and Eubanks, 1976) and also attenuated the voluntary consumption of ethanol in rats and mice

(Ho, Chen and Morrison, 1976, 1977; Sinclair, Atkins and Walker, 1973) although ethanol injections did not affect morphine intake (Gelfand and Amit, 1976). In contrast to previous failures to demonstrate any effects of opiate antagonists on ethanol drinking animals (Goldstein and Judson, 1971; Ho et al., 1976, 1977) it has recently been reported that naloxone can attenuate withdrawal symptoms in ethanol dependent mice and inhibit ethanol narcosis (Blum, Hamilton and Wallace, 1977) and that naltrexone increases voluntary ethanol intake in hamsters (Ross, Hartmann and Geller, 1976). It has also been reported that ethanol, morphine and salsolinol produce naloxone-reversible reductions of brain calcium in rats (Ross, Medina and Cardenas, 1974). Despite the numerous demonstrations of commonalities between ethanol and morphine, the possibility that opiate-like alkaloids mediate the reinforcing effects of ethanol is still tenuous.

There have been a few studies which have provided more direct evidence for the involvement of acetaldehyde and its alkaloid

derivatives in the central mediation of the pharmacological effects of ethanol. Myers and his co-workers have shown that chronic intraventricular infusions of acetaldehyde, as well as a number of other aldehydes and alcohols, increased the preference for ethanol in rats (Myers, 1963; Myers and Veale, 1969) and in monkeys (Myers, Veale and Yaksh, 1972). More recently, this research group has demonstrated the enhancement of ethanol consumption in rats during and following central infusions of a number of TIQs and B-carbolines (Melchior and Myers, 1977; Myers and Melchior, 1977a,b; Myers and Oblinger, 1977). In many cases, the increased ethanol intake was reported to have produced intoxication and withdrawal symptoms. Although these findings raise interesting possibilities for the involvement of acetaldehyde and its alkaloid derivatives in the effects of ethanol, the procedures utilized have been questioned. Using similar paradigms, other investigators (Cicero and Smithloff, 1973; Friedman and Lester, 1975; Jones, Essig and Creager, 1970; Koz and Mendelson,

1967) have been unable to substantiate the findings of Myers (Myers, 1963; Myer et al., 1972). Because voluntary ethanol consumption normally results in only negligible accumulation of acetaldehyde in the brain (Sippel, 1974; Tabakoff et al., 1976) and since peripherally formed TIQs probably do not cross the blood brain barrier (Rahwan, 1975), it is therefore questionable whether sufficient alkaloids are ever present in the brain to exert any pharmacological effect.

Additional related research has shown that ALDH activity in the brain is positively correlated with ethanol preference in rats (Amir, 1977, 1978a,b; Amir and Stern, 1978). It was suggested that the ability of the brain to eliminate acetaldehyde will determine the amounts of ethanol that the organism will voluntarily consume. However, since brain acetaldehyde levels were not measured in these studies, it was not possible to determine whether the increases in ALDH activity in the brain were attributable directly to the presence of acetaldehyde or to the changes

in the metabolism of the biogenic amines following ethanol consumption.

From the limited evidence that is available, it is conceivable that acetaldehyde may play a role in the mediation of the reinforcing effect of ethanol. However, the nature of the central pharmacological effects of acetaldehyde as they relate to voluntary ethanol consumption are not as yet clearly understood.

The Present Investigation

A series of experiments was designed in an attempt to elucidate some of the behavioral consequences of the presence of acetaldehyde in the brain. In the first study, injections of acetaldehyde administered peripherally and centrally were compared with respect to the development of aversive reactions which could interfere with consummatory behaviors. In the next experiment, laboratory rats were given the opportunity to self-administer ethanol or acetaldehyde directly into the cerebral ventricles in order to determine whether these substances have central reinforcing effects.

Subsequently, the relationship between lever pressing for intraventricular infusions of acetaldehyde and voluntary ethanol consumption was examined. The final study looked at the ethanol drinking patterns in rats in which various TIQ alkaloids were chronically infused into the ventricles of the brain.

Experiment "A"

Induction of a Conditioned Taste Aversion by Peripherally but Not Centrally Administered Acetaldehyde

Summary

Acetaldehyde, the primary metabolite of ethanol, produces a variety of aversive symptoms when present in the body in high concentrations. Laboratory rats learned to avoid a saccharin flavored solution which had previously been consumed just prior to a single intraperitoneal injection of acetaldehyde. However, when acetaldehyde was administered directly into the cerebral ventricles, there was no manifestation of a conditioned taste aversion. It was suggested that the aversive effects of acetaldehyde are mediated by peripheral toxic reactions rather than by its pharmacological actions in the brain.

A number of investigators have proposed that acetaldehyde, the primary metabolite of ethanol, may be directly involved in some of the pharmacological consequences of ethanol administration (Hawkins and Kalant, 1972; Jacobsen, 1952; Lieber, 1968; Lundquist, 1971). Normally, the ethanol-derived acetaldehyde is rapidly metabolized so that only small amounts remain to circulate in the body (Akabane, 1970; Eriksson and Sippel, 1977; Truitt and Walsh, 1971). However, when blood acetaldehyde levels become elevated either by inhibition of ALDH (Hald and Jacobsen, 1948), the enzyme responsible for the metabolism of acetaldehyde, or by intravenous injection of acetaldehyde (Asmussen, Hald and Larsen, 1948), human subjects manifest a variety of unpleasant physiological symptoms including vasodilatation, respiratory difficulties, dizziness, nausea and vomiting. In fact, high concentrations of acetaldehyde in the blood have been shown to be incompatible with ethanol consumption in animals (Schlesinger et al., 1966) and man (Consbruch and Derwart, 1968; Hald and Jacobsen, 1948).

There have been reports that following acute

injections of ethanol in laboratory animals, acetaldehyde could be detected in the cerebrospinal fluid (Kiianmaa and Virtanen, 1977; Petterson and Kiessling, 1977) as well as in brain tissue when blood acetaldehyde concentrations were elevated (Eriksson and Sippel, 1977; Sippel, 1974). Some evidence has also been provided suggesting that acetaldehyde may be pharmacologically active in the brain. For example, the metabolism of monoamines may be altered following ethanol administration, presumably through the competitive inhibition of ALDH by acetaldehyde (Deitrich and Erwin, 1975; Duritz and Truitt, 1966; Ortiz, Griffiths and Littleton, 1974). Furthermore, Amir (1977) found that ethanol intake in rats was positively correlated with ALDH enzyme activity in the brain. Myers and his co-worker have reported that intraventricular infusions of acetaldehyde, including a number of other aldehydes, alcohols and alkaloid derivatives, increased the voluntary consumption of ethanol in laboratory animals (Myers, 1963; Myers and Melchior, 1977a,b; Myers and Veale, 1969; Myers, Veale and Yaksh, 1972) although attempts to replicate some of these experiments have been unsuccessful (e.g., Friedman and Lester, 1975).

The conditioned taste aversion (CTA) paradigm has become a useful procedure for the examination of the interoceptive aversive effects of various agents. Laboratory animals will readily learn to avoid drinking a flavored solution which had previously been consumed prior to treatment with substances that produce noxious reactions (e.g., Garcia and Koelling, 1966; Nachman, 1970). CTA learning has also been demonstrated with self-administered drugs including ethanol (Berman and Cannon, 1974; Cappell, LeBlanc and Endrenyi, 1973; Lester, Nachman and LeMagnen, 1970; Sklar and Amit, 1977). It has been suggested that the paradoxical aversion to self-administered drugs in naive subjects may be a function of their positively reinforcing pharmacological effects (Amit, Levitan, Brown and Rogan, 1977b; Berman and Cannon, 1974; Riley, Jacobs and Lolordo, 1978; Sklar and Amit, 1977; White, Sklar and Amit, 1977).

It has recently been reported that an injection of ethanol paired with the consumption of saccharin resulted in a CTA in DBA but not in C57BL mice (Horowitz and Whitney, 1975). Since the DBA strain have low ALDH activity (Sheppard, Albersheim and McClearn, 1968), the induction of

the CTA was presumably due to the consequent accumulation of acetaldehyde in the body (Schlesinger et al., 1966; Sheppard et al., 1970). The purpose of the present experiment was to determine whether acetaldehyde itself, which has known aversive effects in the periphery, could induce a CTA when administered intraperitoneally or intraventricularly.

Experiment 1

Systemic injections of ethanol have been shown to produce a CTA in laboratory animals (e.g., Lester et al., 1970; Sklar and Amit, 1977). The present experiment attempted to determine whether rats would learn to associate a novel taste with the effects of a subsequent peripheral injection of acetaldehyde.

Method

Subjects. Subjects were male Wistar rats (Canadian Breeding Farms Ltd.) weighing 200 to 250 g. The animals were housed individually in stainless steel cages in a room regulated for constant temperature (22°C ± 1°) and humidity and a 12-hour day-night cycle. Purina rat chow and water were available ad libitum for 5 days until the start of the experimental manipulations.

Procedure. For 6 consecutive days all animals were allowed only limited access (20 minutes daily) to a single bottle of tap water. On day 7 (conditioning day), following random assignment to the treatment groups, the animals were allowed to drink a .1% (w/v) sodium saccharin solution for 10 minutes followed 1 minute later by an intraperitoneal injection (5 ml/kg) of either 5% (v/v) acetaldehyde (.2 g/kg), 7.5% (v/v) acetaldehyde (.3 g/kg) or the vehicle (Ringer's solution with the pH = 4.0; adjusted to that of the acetaldehyde solutions with hydrochloric acid). These doses were selected on the basis of reports that the LD_{50} for acetaldehyde in rats is approximately .5 g/kg i.p. (Akabane, 1970). The volume of saccharin solution consumed by each animal was recorded. For the next 5 days, the animals were again exposed to water for 20 minutes daily. On the sixth day (test day) after conditioning day, all animals were given a single-bottle choice of .1% saccharin for 10 minutes and the volumes consumed were recorded. At the termination of the experiment, 3 of the animals from each treatment group were sacrificed and examined for inflammation of the peritoneum.

Results

Figure A.1 shows the mean saccharin consumption on the conditioning and test days for each of the treatment groups. A two-way analysis of variance with repeated measures on the days factor, yielded significant main effects of groups ($F(2,37)=11.3160$, $p < .001$) and of days ($F(1,37)=17.1134$, $p < .001$) as well as a significant interaction effect ($F(2,37)=28.8768$, $p < .001$). Post hoc analysis (Newman-Keuls tests for multiple comparisons; $\alpha = .05$) of the differences between the means under each of the conditions, showed that the mean saccharin consumption on conditioning day did not differ significantly among the groups. However, on test day, the acetaldehyde-treated animals consumed significantly less than the control animals although there was no significant difference between the two drug groups. The Newman-Keuls tests further revealed that the acetaldehyde-injected groups significantly decreased their intake of saccharin from conditioning day to test day while the vehicle group drank significantly more.

It was observed that within 2-3 minutes following the injections of acetaldehyde, some but not all of the animals became ataxic and were

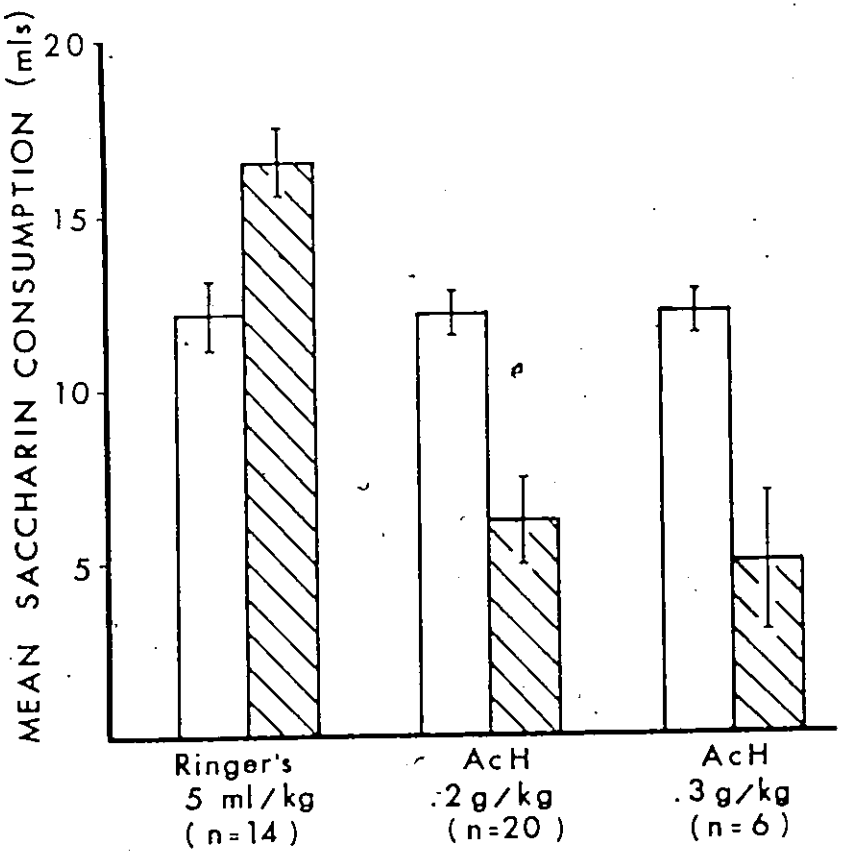

Figure A.1

The mean saccharin consumption on conditioning day (open bars) and test day (striped bars) in animals injected intraperitoneally with acetaldehyde (.2 or .3 g/kg) or Ringer's vehicle. Vertical lines represent the S.E.M.

sedated for periods up to 15 minutes. However, the magnitude of the CTA, as measured by the reduction in saccharin intake, did not appear to be related to the duration of the sedative effects. The autopsies did not reveal any sign of gross morphological changes in the peritoneal cavity.

Discussion

High blood acetaldehyde levels are known to produce a number of unpleasant physiological effects (Asmussen et al., 1948; Hald and Jacobsen, 1948). Aside from the brief period of sedation, there were no observable aversive reactions to the peripheral injections of acetaldehyde. Nevertheless, these animals did learn to associate the novel tasting saccharin solution with the pharmacological effects of acetaldehyde. It is possible therefore, that the demonstrated CTAs induced by ethanol (Berman and Cannon, 1974; Cappell et al., 1973; Lester et al., 1970; Sklar and Amit, 1977) may in fact be attributable to the accumulation of circulating acetaldehyde following injection of high doses of ethanol.

Experiment 2

In the preceeding experiment, it was shown that the presence of acetaldehyde in the body following systemic injections results in a conditioned taste aversion. However, the nature of the CTA to acetaldehyde is not clear. On one hand, it is well established that illness-producing agents can evoke CTAs (e.g., Garcia and Koelling, 1966; Nachman, 1970); conceivably the acetaldehyde-induced CTA observed in the previous experiment may simply have been a consequence of post-injection toxicosis. Alternatively, it has been suggested that CTAs to self-administered drugs may be due to their positive reinforcing central effects (Amit et al., 1977b; Berman and Cannon, 1974; Cappell et al., 1973; Riley et al., 1978; Sklar and Amit, 1977; White et al., 1977).

Since acetaldehyde can be found in the brain when blood concentrations are elevated (Eriksson and Sippel, 1973; Kiianmaa and Virtanen, 1977; Petterson and Kiessling, 1977; Sippel, 1974) it is possible that the CTA produced by the systemic injections of acetaldehyde may be due to its central pharmacological effects. Support for this notion has been provided by similar studies with

morphine. Morphine, which is self-administered by laboratory animals both peripherally (Deneau et al., 1969; Khavari, Peters, Baity and Wilson, 1975; Weeks and Collins, 1968) and centrally (Amit, Brown and Sklar, 1976a; Belluzzi and Stein, 1977; Stein and Olds, 1977) also induces a CTA when injected systemically (Cappell et al., 1973; Sklar and Amit, 1977) or intraventricularly (Switzman, Amit and Sinyor, 1978). This experiment tested the ability of acetaldehyde to induce a CTA when infused directly into the cerebral ventricles.

Method

Subjects. Male Wistar rats (Canadian Breeding Farms Ltd.) weighing 225-250 g were housed and maintained for 2 to 3 days under conditions described in Experiment 1. Under general anaesthesia (sodium pentobarbitol, 60 mg/kg i.p.), a 22-guage stainless steel cannula guide (Plastic Products Inc.) was surgically implanted into the left lateral of each of the animals. With the incisor bar set at 0.0 the stereotaxic co-ordinates were 1.0 mm posterior to Bregma, 1.5 mm lateral to the mid-sagittal-line, and 3.7 mm ventral to the dura. The cannula guide was secured

to the skull with stainless steel screws and cranioplast cement. A stainless steel stilette was inserted into the cannula guide to keep it free from obstruction.

Procedure. After a 7 day recovery period, the animals were placed on a water deprivation schedule as described in Experiment 1. On conditioning day, 1 minute following a 10 minute exposure to a .1% saccharin solution, the animals were infused with the test solutions using a micrometer syringe connected with polyethylene tubing to a 28-gauge cannula that was inserted into the chronically implanted guide. Acetaldehyde was administered intraventricularly in doses of 64 µg (4 µl of 2% v/v acetaldehyde), 320 µg (20 µl of 2% acetaldehyde) or 800 µg (20 µl of 5% acetaldehyde). The amounts of acetaldehyde infused were based on pilot work done to determine the sedative effects of different doses. Also Myers and Veale (1969) showed that infusions of 2 µl of a .5% (v/v) acetaldehyde solution produced some behavioral effect. The control animals received 4 or 20 µl of the vehicle (Ringer's solution) which was buffered with hydrochloric acid to a pH of 4.0 equivalent to

that of the acetaldehyde solutions. All infusions were delivered over a 15-20 second interval. After 5 intervening daily presentations of water, the animals were again given the opportunity to drink the saccharin solution on the test day.

At the termination of the experimental procedures, all animals were sacrificed with an overdose of sodium pentobarbitol and were perfused intracardially with saline followed by 10% formalin. The brains were extracted, stored in formalin and then sliced into 40 μ coronal sections.

Results

Figure A.2 summarizes the conditioning and test day mean saccharin intake of the groups of animals infused intraventricularly with the different doses of acetaldehyde or the vehicle solution. A two-way analysis of variance with repeated measures yielded a significant main effect of days ($F(1,24)=8.3438$, $p < .01$) but revealed that there was no significant main effect of treatment ($F(3,24)=.2493$, $p > .05$) nor a significant interaction effect ($F(3,24)=1.2187$, $p > .05$).

Following infusions of the 64 and 320 μg doses of acetaldehyde, there were no observable

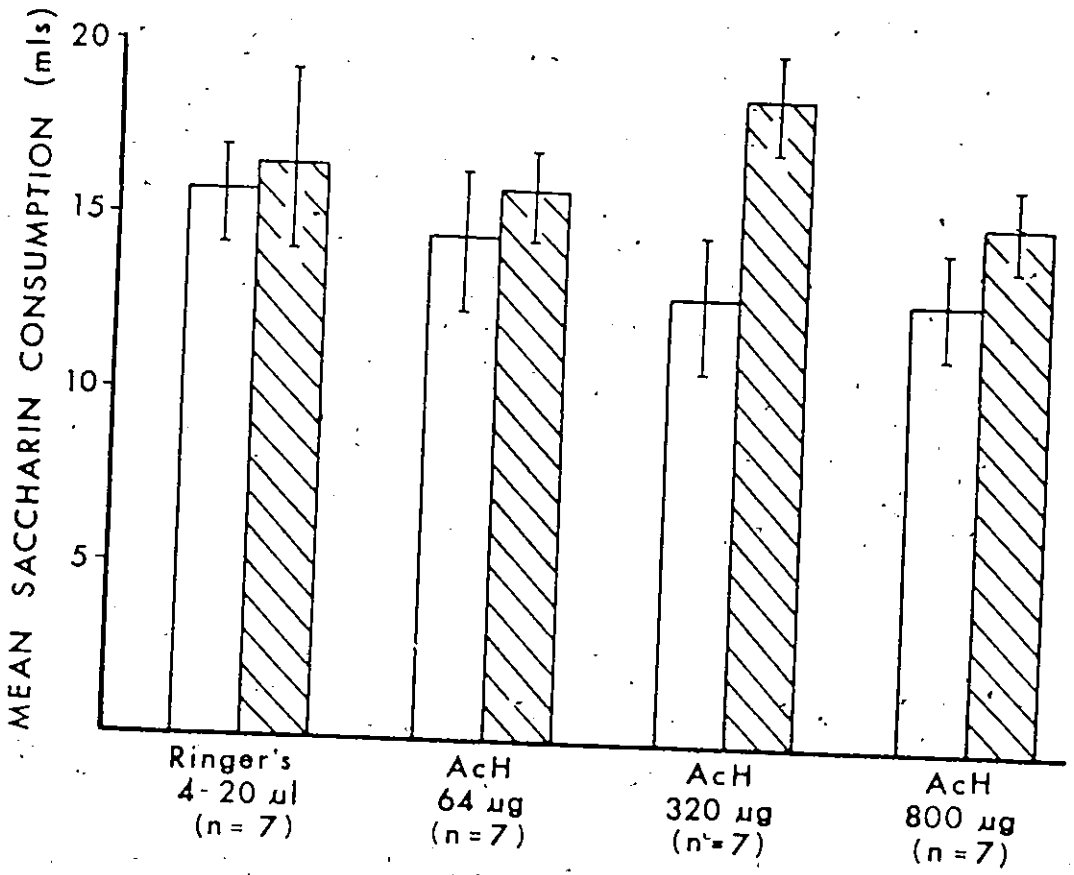

Figure A.2

The mean saccharin consumption on conditioning day (open bars) and test day (striped bars) in groups of rats infused intraventricularly with acetaldehyde (64, 320, or 800 µg) or Ringer's vehicle. Vertical lines represent the S.E.M.

effects on the animals' behavior. However, some of the animals treated with the 800 µg dose became ataxic and were sedated within 1 minute of the infusion. After 3 to 5 minutes the animals recovered from the sedative effects and appeared normal. Histological examination of the brains confirmed that all cannula guides were properly implanted into the ventricles. Furthermore, no damage to brain tissue was evident in any of the sections. Some ventricular enlargement was found but it was not systematically related to the differences in volumes or doses of acetaldehyde infused.

Discussion

In contrast to its effects when injected intraperitoneally, acetaldehyde failed to induce a CTA when infused into the cerebral ventricles. In fact, it was shown that saccharin intake increased slightly following its association with intraventricular acetaldehyde. Based on the observed sedative effects, the doses of acetaldehyde that were administered either intraventricularly or intraperitoneally were more or less comparable. These results therefore suggest that the aversive effects of acetaldehyde

are mediated by its peripheral toxicity rather than by its central pharmacological actions.

The failure to demonstrate a CTA with intraventricular infusions of acetaldehyde may be a function of the relatively short duration of its pharmacological effects (Akabane, 1970). It has been shown that cocaine, which has a short duration of action, does not induce a CTA unless multiple sequential injections are administered (Cappell and LeBlanc, 1975; Goudie, Dickins and Thornton, 1978; Switzman, Cytryniak and Amit, 1978). However, in the present case, based on Sippel's (1974) estimation that the rat brain is capable of oxidizing approximately 16 ug of acetaldehyde per minute, the largest dose administered (800 ug) would have required a minimum of 50 minutes to be eliminated thus providing sufficient time for an association to be formed between the taste of saccharin and the pharmacological effects of acetaldehyde in the brain. Similarly, it may be argued that because of its high lipid solubility (Akabane, 1970), acetaldehyde may diffuse out of the brain relatively quickly thereby limiting the duration of its central pharmacological actions. By

administering a series of intraventricular infusions it may be possible to extend the temporal effects of acetaldehyde in the brain and consequently induce a CTA. Since acetaldehyde, which is a toxic agent (e.g., Akabane, 1970), did not produce any brain tissue damage, it also supported the notion that the infused acetaldehyde may have been rapidly eliminated. Alternatively, it is possible that acetaldehyde may have been transformed to less toxic substances such as the tetrahydroisoquinoline alkaloids (Shizgal and Amit, 1973; Experiment "D") which have been shown to be formed in the brain (Davis and Walsh, 1970; Collins and Bigdeli, 1975). Although the nature of the central pharmacological actions of acetaldehyde is not clear, the present results at least indicate that they do not appear to have any observable aversive effects.

Experiment "B"

Intraventricular Self-Administration of Acetaldehyde but not Ethanol in Naive Laboratory Rats

Summary

For 11 consecutive days, naive rats were maintained in operant chambers where they were given the opportunity to self-administer acetaldehyde (1%, 2%, or 5% v/v), ethanol (2% or 10% v/v), or pH control solutions directly into the cerebral ventricles. Only the animals that had access to the 2% and 5% acetaldehyde solutions showed rates of lever pressing significantly higher than controls. It was suggested that acetaldehyde rather than ethanol itself may mediate the positive reinforcing effects of ethanol in the brain.

When ethanol is consumed, it is oxidized to acetaldehyde primarily in the liver where it is rapidly metabolized further to acetate leaving only small quantities of acetaldehyde to circulate in the blood (Eriksson and Sippel, 1977; Larsen, 1959; Majchrowicz, 1975). A number of investigators have examined the physiological and toxicological consequences of ethanol consumption and have questioned the extent to which the accumulation of acetaldehyde may be responsible for these effects (Hawkins and Kalant, 1972; Jacobsen, 1952; Lieber, 1968; Lundquist, 1971; Truitt and Walsh, 1971).

The possibility that acetaldehyde may be pharmacologically active in the brain has generated much interest and research. It has been demonstrated that the brain has the enzymatic capacity to synthesize (Raskin and Sokoloff, 1968, 1974) and to oxidize acetaldehyde (Erwin and Deitrich, 1966; Mukherji et al., 1975; Tabakoff and Gelpke, 1975) suggesting that acetaldehyde could be present in the brain following ethanol administration. Early attempts to measure the content of acetaldehyde in the brain following ethanol

treatment in laboratory animals yielded variable and conflicting results (Duritz and Truitt, 1966; Kiessling, 1962a,b; Majchrowicz, 1973; Ridge, 1963). In more recent studies, where the non-enzymatic formation of acetaldehyde was prevented (Sippel, 1972), the presence of acetaldehyde in brain tissue could be detected only when its concentration in cerebral blood was greatly elevated (Sippel, 1974; Tabakoff et al., 1976). However, in the cerebrospinal fluid the levels of acetaldehyde were similar to those measured in the blood following acute injections of ethanol in rats (Kiianmaa and Virtanen, 1977; Petterson and Kiessling, 1977). Because of the difference in content of acetaldehyde in the brain tissue as compared to the blood or the cerebrospinal fluid, it has been suggested that there may exist a metabolic barrier preventing the accumulation of acetaldehyde in brain tissue (Petterson and Kiessling, 1977; Sippel, 1974). Amir (1977, 1978a,b) showed that brain ALDH activity was directly correlated with voluntary ethanol consumption in rats and proposed that this enzyme may act as a

barrier to acetaldehyde reaching the brain.

The central pharmacological effects of acetaldehyde have also been examined in several studies. There have been numerous experiments showing that ethanol produces variable alterations in the metabolism of the central biogenic amines (e.g., Carlsson et al., 1973; Corrodi et al., 1966; Duritz and Truitt, 1966; Hunt and Majchrowicz, 1974; Pohorecky, 1974). It has been proposed that these neurochemical effects may be attributable to the competitive inhibition of brain ALDH by acetaldehyde (Lahti and Majchrowicz, 1969; Walsh, Truitt and Davis, 1970). In fact, there have been a few reports showing that treatment with acetaldehyde itself can alter the brain content of biogenic amines (Duritz and Truitt, 1966; Ortiz et al., 1974). Since central CA, and NE in particular, have been implicated in the mediation of the pharmacological and reinforcing properties of ethanol (Amit, 1977a; Amit and Stern, 1971; Brown and Amit, 1977; Davis et al., 1977; Myers and Melchior, 1975a), it is possible that the acetaldehyde-induced neurochemical changes may subserve the central

effects of ethanol. Another hypothesis that has received much attention in recent years is that acetaldehyde may condense with CA to form TIQ alkaloids, neuropharmacologically active compounds that may mediate the central actions of ethanol (Cohen, 1976; Myers and Melchior, 1977a,b; Rahwan, 1975).

Finally, a number of studies have attempted to relate the central effects of acetaldehyde to ethanol self-administration. In contrast to the toxic effects of peripheral accumulation of acetaldehyde which results in a loss of appetite for ethanol (Hald and Jacobsen, 1948; Schlesinger et al., 1966), the presence of acetaldehyde in the brain does not appear to produce a conditioned taste aversion (Experiment "A"). In fact, it has been demonstrated that intraventricular infusions of acetaldehyde, as well as a variety of other aldehydes, alcohols and alkaloids, markedly increase ethanol consumption in laboratory animals (Melchior and Myers, 1977; Myers, 1963; Myers and Melchior, 1977a,b; Myers and Oblinger, 1977; Myers and Veale, 1969; Myers, Veale and Yaksh, 1972), although the procedures used in these experiments have been questioned (Friedman and Lester, 1975).

Recently, it has been demonstrated that laboratory rats will self-administer amphetamine (Gustafson and Pickens, 1975) and morphine (Amit, Brown and Sklar, 1976a; Belluzzi and Stein, 1977; Stein and Olds, 1977) directly into the cerebral ventricles. In the present experiment, naive rats were given the opportunity to lever press for intraventricular infusions of acetaldehyde or ethanol solutions in order to determine whether or not they possess central reinforcing effect.

Method

Subjects. Male Wistar rats (Canadian Breeding Farms Ltd.) weighing 300-325 g served as subjects. A 22-guage cannula guide was implanted into the left lateral ventricle of each animal according to the surgical procedures described in Experiment "A".

Procedure. After 5 to 7 days of recovery from surgery, the animals were placed individually in operant chambers (Ralph Gerbrands Co. - Model C) with the food hopper blocked off. The infusion apparatus consisted of a pump (Razel Inc.) connected via polyethylene tubing to a flow-thru swivel (Brown, Amit and Weeks, 1976) suspended above the operant chamber. A shielded plastic tube connected to the outlet of the

swivel terminated in a 28-guage internal cannula, which was inserted into and secured to the permanently mounted cannula guide. For 11 consecutive days the animals were maintained in the operant chambers where they had free access to food, water, and the operant lever. The testing room was regulated for constant temperature (22° C ±1°) and a 12-hour light-dark cycle. The animals were not pre-trained to press the lever. When an animal pressed the operant lever, the pump was activated for 10 seconds and an infusion of 4 µl of fluid was delivered into the ventricle. During the 10-second infusion interval, additional presses did not reactivate the pump and were not recorded. All infusions were automatically monitored on a multi-channel event recorder. Animals received infusions of either acetaldehyde (1%, 2%, or 5% v/v: 32, 64, or 160 µg/infusion respectively), Ringer's solution with the pH adjusted to that of the acetaldehyde solutions (pH=4.5-4.8), ethanol (2% or 10% v/v: 64 or 320 µg/infusion respectively), or Ringer's solution with the pH adjusted to that of the ethanol solutions

(pH=6.0-6.2). Ringer's solution served as the vehicle for both acetaldehyde and ethanol. The selection of doses of acetaldehyde and ethanol was based partly on previous experiments in which these substances were administered intraventricularly (Myers, 1963; Myers and Veale, 1969) and partly on the calculated capacity of the rat brain to oxidize acetaldehyde (Sippel, 1974). Fresh solutions were prepared every 48 hours from stock solutions of ethanol (95% v/v) and acetaldehyde (Aldrich Chemical Co.). The acetaldehyde stock was periodically distilled and was stored at cold temperatures. Once daily the animals were weighed and the cannulae were removed and flushed in order to minimize blockage. Due to unavoidable cannula occlusion or damage in a number of animals, the experiment had to be terminated after the eleventh day.

Histology. At the end of the 11-day test period the animals were killed and were perfused intracardially with saline followed by 10% formalin. The brains were removed, stored in formalin, and were subsequently sliced into 40 μ coronal sections.

Activity Controls. In order to rule out the possibility that the results of this experiment could have been attributable to a drug-induced motor activation, a simple test was devised. Additional animals that had undergone surgery, as described earlier, were placed individually in the operant chambers and were connected to the infusion apparatus. Although the animals had access to the operant levers, presses were recorded but did not activate the pumps. Instead, the pumps were programmed to automatically deliver a 4 µl infusion every 5 minutes for a one-hour period. The substances infused included Ringer's solution (pH=4.7), acetaldehyde (2% v/v), ethanol (10% v/v), and lithium chloride (80 µg) a compound known to produce hypermotility when infused into the brains of rats (Blair, Sinyor, Cytryniak, Switzman and Amit, 1978). Following the programmed infusion period, spontaneous lever pressing was recorded for an additional 3 hours during which time the pumps remained disconnected.

Results

The patterns of lever pressing for acetaldehyde or its Ringer's pH control solutions during the daytime (8 A.M. - 8 P.M.) and night-time (8 P.M. - 8 A.M.) hours over the 11-day period are shown in Figure B.1. As can be seen from these graphs, there was no difference in the self-administration rates during the daytime hours when general activity was low. A two-way analysis of variance with repeated measures over the days factor yielded significant differences in the total daily infusions among the groups ($F(3,31)=4.3670$, $p < .02$). There was also a significant days effect ($F(10,310)=18.1197$, $p < .001$) and interaction effect ($F(30,310)=1.8685$, $p < .01$). Post-hoc analysis of the group differences (Scheffé tests; $\alpha = .05$) indicated that whereas the 1% acetaldehyde group did not differ from the control group, the animals receiving 2% or 5% acetaldehyde were significantly different from the 1% acetaldehyde and control groups. Furthermore, there was no significant dose effect between the 2% and 5% acetaldehyde groups. A similar analysis of the data for the ethanol and its Ringer's pH control

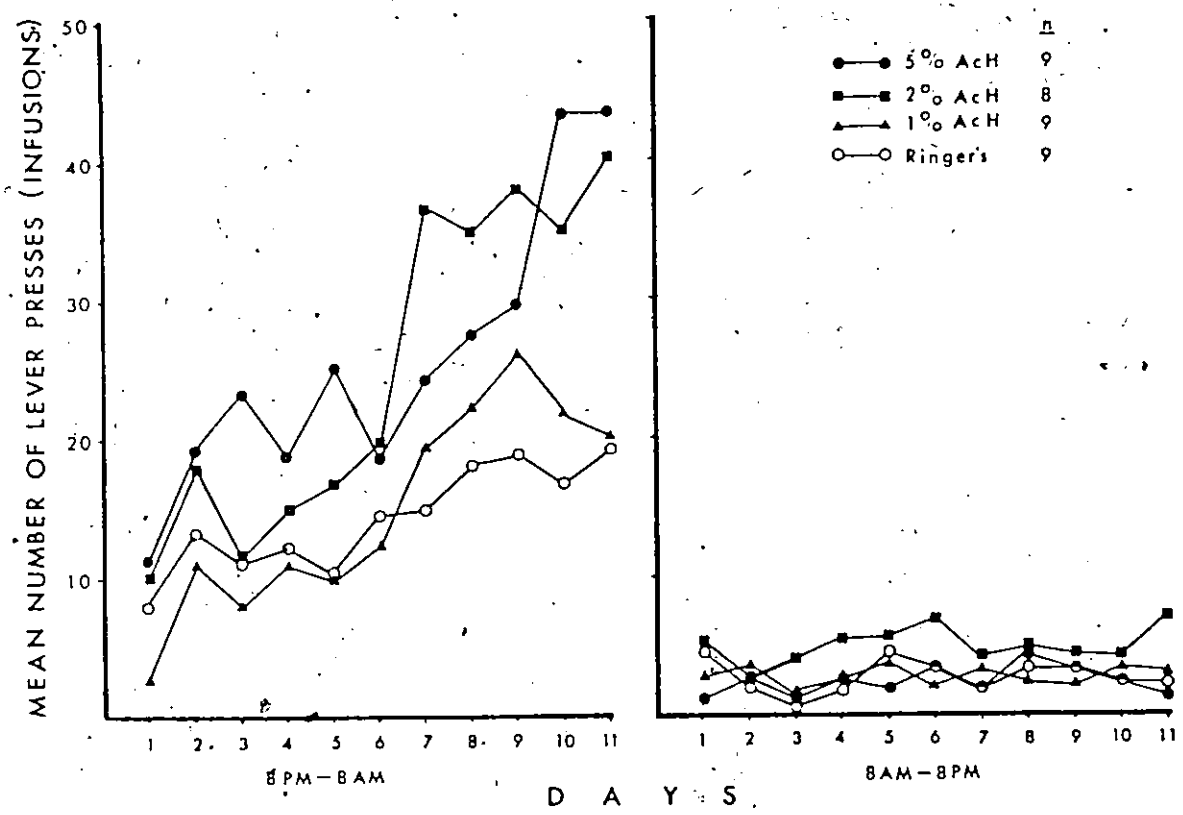

Figure B.1

The mean number of lever presses (infusions) during the night-time (left panel) and the daytime (right panel) over 11 consecutive days for rats receiving acetaldehyde solutions (1%, 2%, or 5% v/v) or the vehicle.

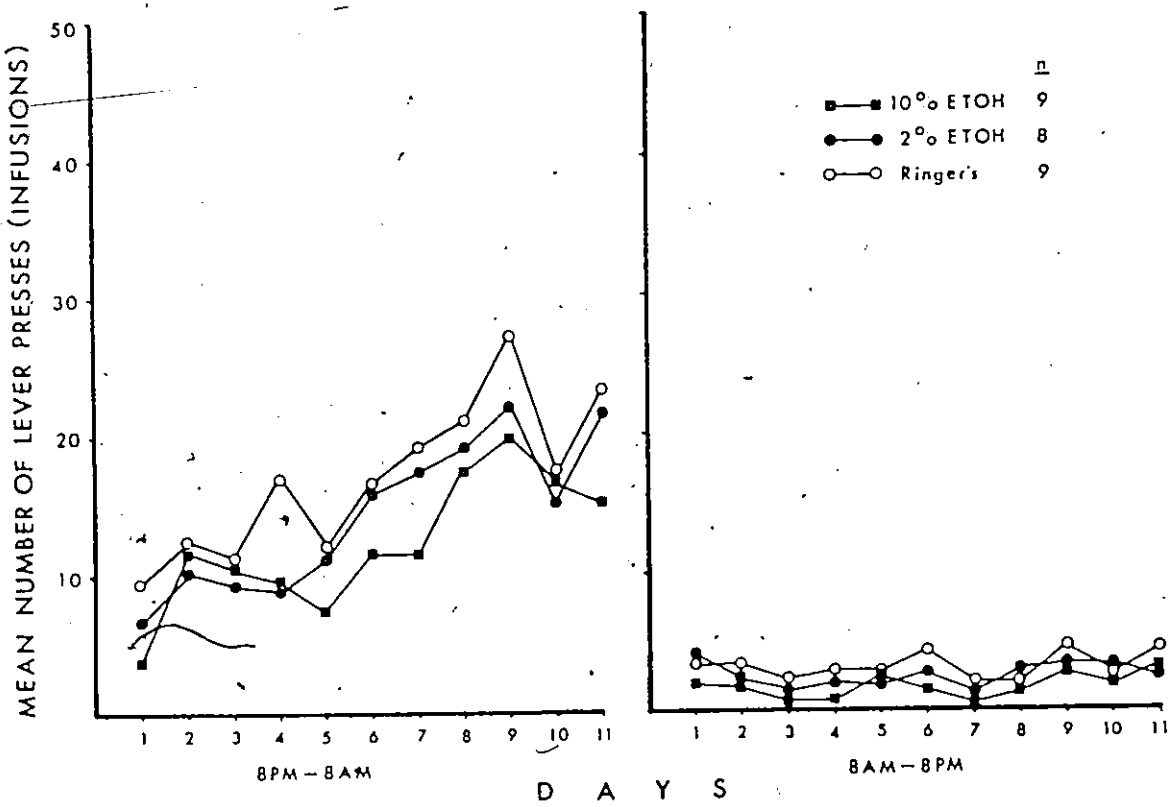

Figure B.2

The mean number of lever presses (infusions) during the night-time (left panel) and the daytime (right panel) over 11 consecutive days for rats receiving ethanol solutions (2% or 10% v/v) or the vehicle.

groups showed that while there was a significant effect of days ($F(10,230)=9.1524$, $p < .001$) there was no difference among the groups ($F(2,23)=.9752$, $p > .05$) nor was there any significant interaction effect ($F(20,230)=.4588$, $p > .05$). The daytime and night-time patterns of operant responding for these groups are represented in Figure B.2.

Histology. Histological examination of the brains confirmed that all cannula guides had properly penetrated the lateral ventricles. Despite the relatively large volumes of fluid self-infused by some of the animals, there was only slight tissue damage and ventricular enlargement that were not systemmatically related to the nature or the number of infusions. It has been reported that infusions of volumes up to 100 µl within a 30 minute period did not result in any increase in cerebrospinal fluid pressure in rats (Ringle and Herndon, 1969). Figure B.3 compares coronal sections of the brains of representative animals from some of the groups.

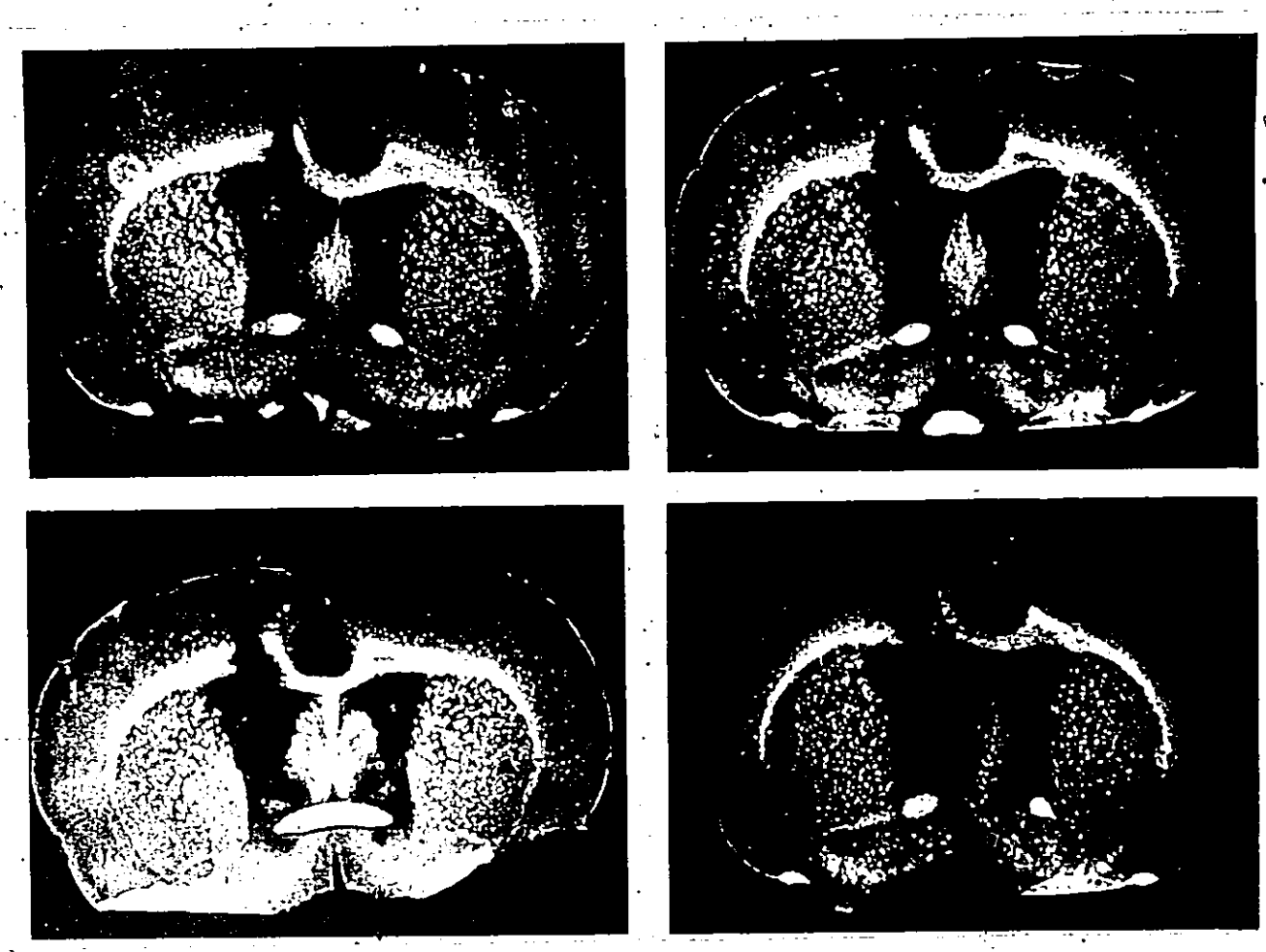

Figure B.3

Photomicrographs of coronal sections from brains of rats that received infusions of Ringer's solution (upper left), 10% ethanol (upper right), 2% acetaldehyde (lower left), and 5% acetaldehyde (lower right).

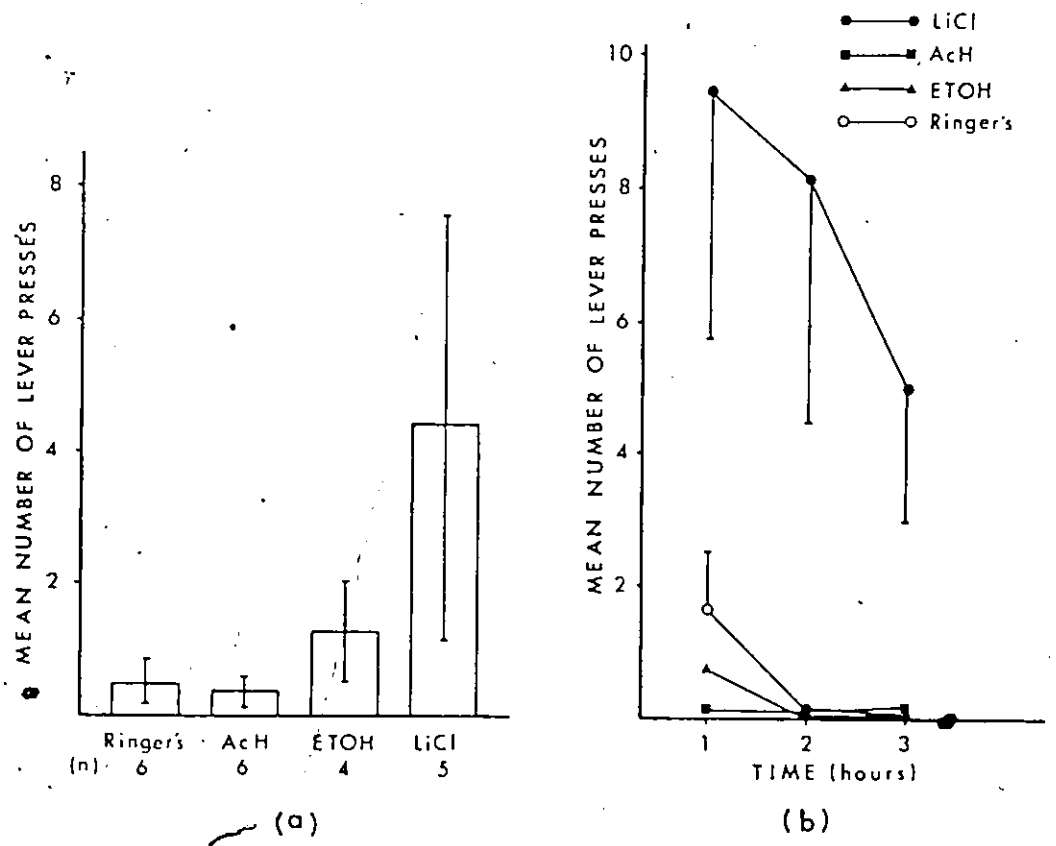

Figure B.4

a) Mean number of spontaneous lever presses in a one-hour period during which groups of rats were infused with acetaldehyde, ethanol, lithium chloride, or Ringer's solutions every 5 minutes. Vertical lines represent S.E.M.

b) Mean number of spontaneous lever presses over a 3-hour period after the one-hour infusion sequence for the same animals. Vertical lines represent S.E.M.

Activity Controls. As can be seen from Figure B.4(a), during the one-hour programmed infusion period the animals receiving acetaldehyde, ethanol, or Ringer's solutions showed minimal activity as measured by the number of spontaneous lever presses. By contrast, lithium chloride produced an observable increase in activity which was reflected in the number of presses of the lever. Similarly, during the 3-hour post-infusion period, the mean number of lever presses performed by the lithium-treated animals was significantly greater than for the remaining groups ($F(3,17)=7.4737$, $p < .01$; Scheffé test $p < .01$) while the acetaldehyde group was not significantly different from the ethanol or Ringer's animals ($p > .05$) (see Figure B.4(b)).

Discussion

The present results indicate that within an 11-day period naive laboratory rats will learn to perform an operant response in order to receive microinfusions of acetaldehyde into the cerebral ventricles, suggesting that acetaldehyde has direct central reinforcing effects.

The rates and patterns of intraventricular self-administration of acetaldehyde are comparable to those reported for intravenous self-infusions of ethanol in monkeys (Deneau et al., 1969; Winger and Woods, 1973). Similar rates have also been reported for intravenous (Weeks and Collins, 1968) and intraventricular (Amit et al., 1976a) self-administration of morphine in rats. Most of the self-administration of acetaldehyde occurred during the night-time hours when rats are normally active. All groups showed a significant increase in lever pressing over days, possibly due to habituation to the operant chamber. However, the relative increases for the 2% and 5% acetaldehyde groups were significantly greater.

On the other hand, ethanol, which is readily self-administered by peripheral routes (Amit and Stern, 1969, 1971; Davis et al, 1977; Deneau et al., 1969; Woods and Winger, 1973) was not self-administered intraventricularly at the doses presented. These findings suggest that the reinforcing effects of ethanol in the brain may be mediated by its metabolite,

acetaldehyde. When infused intraventricularly, ethanol did not appear to have positive reinforcing effects possibly because of the brain's limited capacity to oxidize ethanol to acetaldehyde (Raskin and Sokoloff, 1968, 1974; Tabakoff and von Wartburg, 1975).

Since acetaldehyde in high concentrations may act as a neurotoxin (Shizgal and Amit, 1973), the proposed enzymatic barrier that may exist in the cerebral capillary and ventricular walls (Petterson and Kiessling, 1977; Sippel, 1974) could serve as a protective mechanism. According to Sippel (1974) the rat brain is capable of oxidizing a maximum of 16 ug of acetaldehyde per minute. Based on this estimate, the brain should have been able to eliminate the acetaldehyde from each infusion of the 2% and 5% solutions within 4 or 10 minutes respectively, thereby avoiding an accumulation of acetaldehyde in the brain. The 1% acetaldehyde infusion may have been metabolized too quickly to allow any appreciable pharmacological effect. Although the transitory presence of acetaldehyde in the brain appears to be

positively reinforcing, the mechanism of action involved are not well understood.

It has been hypothesized that acetaldehyde may mediate the central pharmacological actions of ethanol by altering CA metabolism (Duritz and Truitt, 1966; Ortiz et al., 1974; Thadani and Truitt, 1977) or by condensing with CA to form neurochemically active TIQ alkaloids (Cohen, 1976; Cohen and Collins, 1970; Davis and Walsh, 1970; Rahwan, 1975). It is possible that these biochemical changes are induced by minute amounts of acetaldehyde which escape oxidation, penetrate the ventricular wall and affect proximal CA containing structures such as the hypothalamus (Lindvall and Bjorklund, 1974; Ungerstedt, 1971). Further research is necessary to clarify the role of central acetaldehyde in the regulation of ethanol consumption and in the mediation of its pharmacological effects.

Experiment "C"

Intraventricular Self-Administration of Acetaldehyde and Voluntary Consumption of Ethanol in Rats

Summary

For 11 consecutive days, naive rats were given the opportunity to acquire an operant response for infusions of acetaldehyde or Ringer's vehicle into the cerebral ventricles. The experimental group showed significantly higher rates of lever pressing than the control animals. Following the intraventricular self-administration period, all the animals in the acetaldehyde group were exposed to a free-choice between increasing concentrations of ethanol and water. The consumption of ethanol in the range of concentrations of 9% to 21% (v/v) was directly correlated to the previously established rates of self-administration of acetaldehyde. The results suggest that the central mechanisms mediating the reinforcing effects of acetaldehyde also subserve the pharmacologically reinforcing properties of ingested ethanol.

Ethanol is self-administered by laboratory animals by the oral (e.g., Amit and Stern, 1971; Sinclair, 1974), intragastric (Amit and Stern, 1969; Davis et al., 1977), and intravenous routes (Deneau et al., 1969; Winger and Woods, 1973). However, the mechanisms by which it exerts its positive reinforcing effects have not yet been clearly understood. Numerous studies have shown that ethanol produces alterations in the metabolism of central CA (e.g., Carlsson et al., 1973; Corrodi et al., 1966; Hunt and Majchrowicz, 1974; Pohorecky, 1974) which have been implicated in the mediation of motivated behavior (e.g., Fibiger, 1978; German and Bowden, 1974; Stein, 1968; Zigmond and Stricker, 1972). Conversely, there has also been accumulating evidence that manipulations of central CA, and NE in particular, alter the voluntary consumption of ethanol in laboratory animals (Amit et al., 1977a; Amit and Stern, 1971; Brown and Amit, 1977; Davis et al., 1977; Myers and Melchior, 1975a), suggesting that these neurotransmitters subserve the reinforcing effects of ethanol.

In the previous experiment ("B") it was found that ethanol, which readily diffuses into the brain

(Raskin and Sokoloff, 1974), was not self-administered by rats into the cerebral ventricles. On the other hand, acetaldehyde, the primary metabolite of ethanol, was self-administered intraventricularly, suggesting that acetaldehyde may be responsible for the positive reinforcing effects of ingested ethanol. It has been shown that when acetaldehyde is present in the brain, it alters the metabolism of CA (Duritz and Truitt, 1966; Ortiz et al., 1974; Thadani and Truitt, 1977) or condenses with the CA to form TIQ alkaloids (Collins and Bigdeli, 1975). These changes in CA metabolism have been related to the mechanisms that may be involved in the regulation of ethanol intake. Myers and his co-workers have reported that intraventricular infusions of acetaldehyde, ethanol, and several alkaloids resulted in an enhanced preference for ethanol in laboratory animals (Melchior and Myers, 1977; Myers, 1963; Myers and Melchior, 1977a,b; Myers and Veale, 1969). Amir (1977,1978a) has also found a positive correlation between voluntary ethanol consumption in rats and levels of activity of brain ALDH, an enzyme capable of oxidizing acetaldehyde.

The accumulation of acetaldehyde in the blood produces aversive physiological reactions (e.g. vasodilatation, decreased blood pressure, respiratory difficulties, nausea, etc.) that result in an attenuation of ethanol intake in man (Hald and Jacobsen, 1948) and laboratory animals (Schlesinger et al., 1966). On the other hand, the presence of acetaldehyde in the brain was shown to be reinforcing (Experiment "B") rather than aversive (Experiment "A"). The present investigation attempted to determine whether an animal's propensity to self-administer acetaldehyde into the cerebral ventricles is related to its predisposition to voluntarily consume ethanol.

Method

Subjects. Twenty-eight male Wistar rats (Canadian Breeding Farms Ltd.), weighing 300-325 g at the beginning of the experiment served as subjects. A 22-gauge stainless steel cannula guide was implanted into the left lateral cerebral ventricle of each animal in manner previously described (Experiment "A"). During the 5-day recovery period, the animals were indiyidually housed in stainless steel cages with food and water available ad libitum.

Procedure. The procedures for the intraventricular self-administration phase of this experiment were identical to those used in Experiment "B", except that only 2% acetaldehyde (64 ug/infusion) or Ringer's vehicle (4 ul/infusion) were available. Following the 11-day self-administration period, all the animals that had acetaldehyde available (n=20) were returned to the home cage where for the next 4 days, they were presented with water in 2 Richter tubes (Kimax) mounted on the front of the cage. Food was freely available. Subsequently, each animal was exposed to a free-choice between water and ethanol presented on alternate days in a regimen of increasing concentrations as follows: 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 17, 19, 21, and 25% (v/v). On the intervening days both drinking tubes were filled with water. Body weight and fluid intake were recorded daily.

At the termination of the experiment, the animals were given an overdose of sodium pentobarbitol and were perfused intracardially with saline followed by 10% formalin. The brains were extracted, stored in formalin, and later were sliced into 40 μ coronal sections.

Results

Figure C.1 shows the mean number of lever presses performed by animals receiving either 2% acetaldehyde or the Ringer's vehicle solution. A two-way analysis of variance with repeated measures over the days factor, revealed that the animals that had access to the acetaldehyde solution, pressed the lever significantly more than did the control animals ($F(1,26)=7.3545$, $p < .02$). There was also a significant main effect of days ($F(10,260)=4.0153$, $p < .001$) but no significant interaction effect ($F(10,260)=.6486$, $p > .05$).

The total number of acetaldehyde infusions self-administered by each animal over the 11-day period was found to be directly correlated with the total amount of ethanol (g/kg) consumed over the range of concentrations between 9 and 21% ($r=.4274$, $n=20$, $p < .025$). Table C.1 lists the correlation coefficients between the mean number of acetaldehyde infusions over 11 days and either the ethanol intake (g/kg) or ethanol preference (ethanol volume/total fluid volume) for each of the concentrations presented. Significant correlations were found for the concentrations ranging between 9 and 21% but not for the lower or higher concentrations.

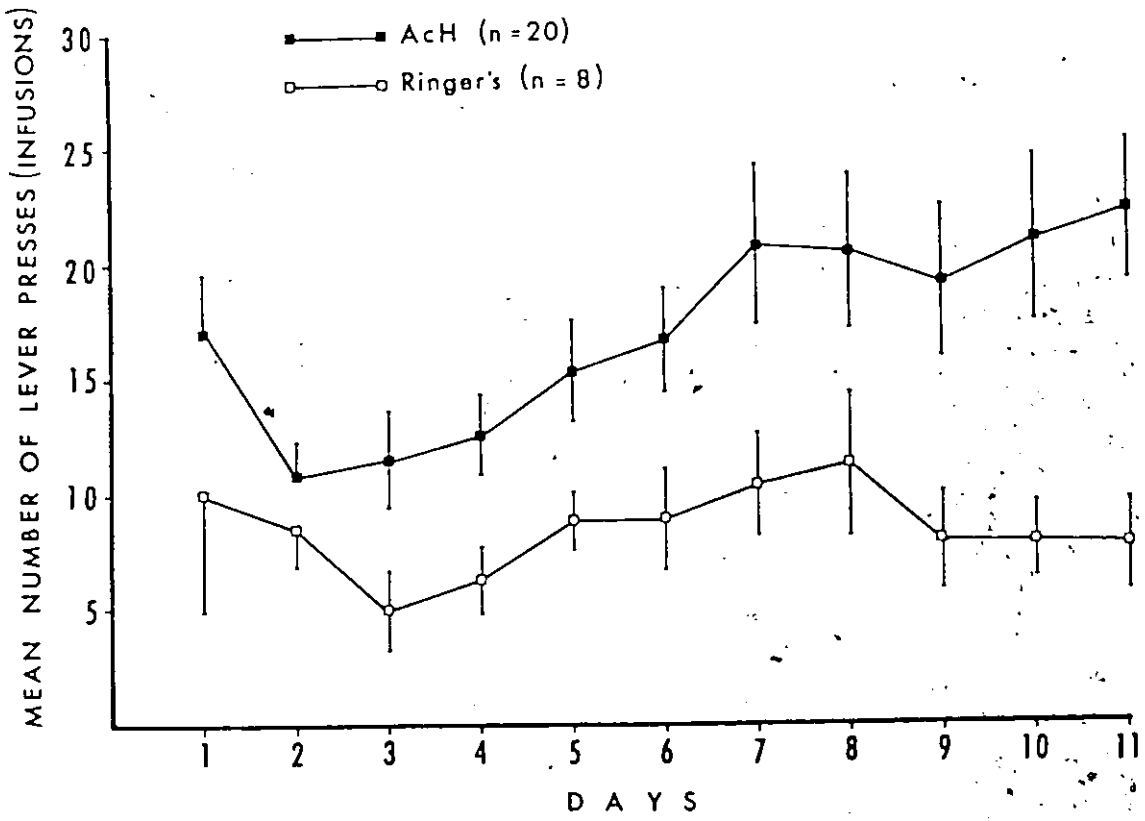

Figure C.1

Mean number of intraventricular self-infusions of acetaldehyde (2% v/v) or Ringer's pH control solutions in naive rats over an 11-day period. Vertical lines represent S.E.M.

Table C.1 Correlation coefficients between the total number of acetaldehyde infusions over 11 days and subsequent ethanol intake (g/kg) or ethanol preference (ethanol volume/total fluid volume: E/T) for each of the concentrations presented in a free-choice with water.

Ethanol % (v/v)	r Ethanol intake (g/kg) and Acetaldehyde infusions	r Ethanol preference (E/T) and Acetaldehyde infusions
3	.3133	.1827
3	.2067	.3453
5	.4027	.3465
5	.3307	.3754
7	.3627	.3543
7	.3431	.2997
9	.3581	.3948*
9	.4030*	.3584
11	.3869*	.4003*
11	.3800*	.2957
13	.4930*	.4317*
13	.4030*	.4466*
15	.4348*	.4526*
17	.4608*	.4859*
19	.4499*	.4931*
21	.3644	.4252*
25	.3559	.3354

* $p < .05$

Histological examination confirmed that the cannula guides had penetrated the ventricles in all subjects. No gross morphological damage was seen in the brains of any of these animals.

Discussion

Consistent with the findings of the previous experiment, it was shown that naive rats will self-administer acetaldehyde into the cerebral ventricles. This propensity to self-administer acetaldehyde was found to be directly correlated with voluntary ethanol consumption, suggesting that the mechanism that subserves the reinforcing effects of acetaldehyde may also be involved in the mediation of the pharmacologically reinforcing consequences of ingested ethanol.

It was not surprising to find a lack of correlation between acetaldehyde self-administration and consumption of the more dilute ethanol solutions, since rats may normally prefer ethanol for its taste at concentrations up to 7% (v/v) (Myers and Carey, 1961; Richter and Campbell, 1940; Wilson, 1972). Conversely, at the higher concentrations (21% v/v), the taste of ethanol may have been sufficiently aversive so as to disrupt the self-

selection of ethanol for its reinforcing effects.

Although peripheral accumulation of acetaldehyde results in an aversive reaction and a loss of appetite for ethanol (Hald and Jacobsen, 1948; Schlesinger et al., 1966), its presence in the brain has been shown to be positively reinforcing (Experiment "B") and may conceivably be the basis for the central mediation of ethanol consumption. A number of hypotheses have been proposed to explain the mechanism of action of acetaldehyde in the brain as it may relate to ethanol self-administration. Amir (1977, 1978a) has shown that the activity of brain ALDH is correlated with voluntary ethanol consumption and is increased following prolonged exposure to ethanol (Amir, 1978b). In the present study, it is conceivable that the animals that self-administered more acetaldehyde may have had a relatively greater induction of brain ALDH activity and could subsequently metabolize larger amounts of acetaldehyde reaching the brain following the consumption of ethanol.

An alternate hypothesis is that acetaldehyde may mediate the pharmacological effects of ethanol by its ability to alter the metabolism of central

biogenic amines (Duritz and Truitt, 1966; Ortiz et al., 1974; Thadani and Truitt, 1977), that have been implicated in the mediation of ethanol self-administration (Amit et al., 1977; Amit and Stern, 1971; Brown and Amit, 1977; Davis et al., 1977; Myers and Melchior, 1975a). Therefore, in the present case, it is possible that the animals' experience with intraventricular acetaldehyde may have differentially produced long-lasting increases in the turnover of CA which may have predisposed the animals to subsequently drink greater amounts of ethanol. It is also possible that during the acetaldehyde self-administration period there may have been some neuronal adaptation to acetaldehyde. Although no withdrawal symptoms were observed during the 4-day intervening period following acetaldehyde self-administration, it is conceivable that the animals subsequently drank ethanol as a source of acetaldehyde in order to satisfy an "artificial need state".

Although the present findings cannot determine which of the proposed mechanisms of actions of acetaldehyde is correct, they do suggest that common mechanisms mediate the self-administration

of both acetaldehyde and ethanol. Alternatively, since ethanol-derived acetaldehyde appears to function as a central reinforcer (Experiment "B"), it may be responsible for the maintenance of voluntary ethanol consumption.

Experiment "D"

Examination of the Role of Tetrahydroisoquinoline Alkaloids in the Mediation of Ethanol Consumption in Rats

Summary

Ethanol drinking laboratory rats were infused intraventricularly with salsolinol (100 µg/infusion) once per day for 20 days. Other animals were infused every 30 minutes 24 hours per day for 5 consecutive days with salsolinol (2 µg/infusion), tetrahydropapaveroline (.25 µg/infusion), 1-methoxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (2 µg/infusion), 4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline (2 µg/infusion), or 1-methoxy-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (2 µg/infusion). During the infusion and post-infusion periods there were no significant differences in ethanol intake or preference compared to animals infused with the Ringer's vehicle solution. The possible involvement of tetrahydroisoquinoline alkaloids in the mediation of ethanol consumption is discussed.

Although the central mechanisms mediating the consumption of ethanol have not as yet been determined, a number of hypotheses have been proposed based on the available evidence. The biogenic amines in the brain that have been implicated in the mediation of reward (e.g. Fibiger, 1978; German and Bowden, 1974; Stein, 1968) have also been shown to play a functional role in the pharmacological actions of ethanol. This notion has been supported by the numerous demonstrations that ethanol administration produces alterations in the metabolism of the central biogenic amines (e.g. Bonnycastle et al., 1962; Carlsson et al., 1973; Corrodi et al., 1966; Hunt and Majchrowicz, 1974). While the effects of serotonin (5-HT) manipulations on ethanol consumption have been equivocal (e.g. Hill, 1974; Myers and Veale, 1968; Myers and Melchior, 1975a,b), there has been greater consistency in the reports that CA, and NE in particular, may mediate the reinforcing properties of ethanol (e.g. Amit et al., 1977; Amit and Stern, 1971; Brown and Amit, 1977; Davis et al., 1977; Myers and Melchior, 1975a).

Several investigators have reported that acetaldehyde, the primary metabolite of ethanol, was detected in the brains of laboratory animals following ethanol administration (e.g., Majchrowicz, 1973; Petterson and Kiessling, 1977; Sippel, 1974; Tabakoff, Anderson and Ritzmann, 1976). Although the accumulation of acetaldehyde in the periphery results in an aversive physiological reaction (Asmussen et al., 1948; Hald and Jacobsen, 1948), its presence in the brain has been shown to have positive reinforcing effects (Experiment "B") that may subserve the voluntary consumption of ethanol in rats (Experiment "C"). Since acetaldehyde itself also increases brain levels and turnover of biogenic amines (Duritz and Truitt, 1966; Ortiz et al., 1974; Thadani and Truitt, 1977), it is conceivable that ethanol may exert its central pharmacological effects via its metabolite.

In 1970, two independent research groups simultaneously reported that CA could condense with aldehydes via a Pictet-Spengler reaction to form TIQ alkaloids in perfused cow adrenal glands (Cohen and Collins, 1970) and in rat brain

homogenates (Davis and Walsh, 1970). The formation of TIQs has also been demonstrated in vivo. Parkinsonian patients being treated with L-DOPA, showed measurable quantities of the dopamine derived alkaloids, salsolinol and THP, in the urine following ethanol consumption (Sandler, Carter, Hunter and Stern, 1973). In laboratory rats, treatment with L-DOPA either alone or with ethanol resulted in detectable levels of THP in the brain (Turner et al., 1974). In a later study, where blood acetaldehyde levels were markedly increased by pretreatment with the MAO inhibitor, pargyline and the COMT inhibitor, pyrogallol, small amount of salsolinol were found in the brain although no measurable amounts of THP were reported (Collins and Bigdeli, 1975). On the other hand, O'Neill and Rahwan (1977) were not able to find any salsolinol in the brains of mice chronically exposed to ethanol vapor. However, using a similar vapor inhalation technique, 6-methoxysalsolinol (isosalsoline) was detected in striatal tissue in mice (Hamilton et al., 1978). Tetrahydro-B-carbolines have also been formed in vitro and in vivo from condensation reactions

between 5-HT and aldehydes (Dajani and Saheb, 1973; McIsaac, 1961).

If TIQs are at all involved in the mediation of voluntary ethanol consumption, then because they presumably accumulate in the brain following exposure to ethanol, it would appear to follow that an excess of TIQs would suppress intake. Consequently, if TIQs are exogenously introduced into the brains of laboratory animals, their voluntary consumption of ethanol should be attenuated. However, in a recent series of experiments, Myers and his co-workers found that during and following intraventricular infusions of THP, salsolinol, B-carbolines, and a variety of other alkaloid derivatives, ethanol intake increased markedly compared to control rats infused with artificial cerebrospinal fluid (Melchior and Myers, 1977; Myers and Melchior, 1977a,b; Myers and Oblinger, 1977). It was claimed that some of the rats in these experiments consumed amounts of ethanol sufficient to produce intoxication and withdrawal symptoms when ethanol was removed. Moreover, withdrawal-like symptoms were sometimes observed

following TIQ infusions even before exposure to ethanol. Since the rats in these experiments had only limited access to ethanol prior to the alkaloid-infusion period, it may have precluded the observation of a TIQ-induced decrease in ethanol intake. In the present study, ethanol-experienced rats, with stable patterns of intake, were infused with a variety of alkaloids to examine their effects on voluntary ethanol consumption.

Experiment 1

Method

Subjects. According to the procedures outlined in Experiment "A", a cannula guide was implanted into the left lateral cerebral ventricle of each of 11 male Wistar rats (Canadian Breeding Farms Ltd.) weighing 225 to 250 g. Throughout the experiment, the animals were individually housed in stainless steel cages in a room regulated for constant temperature (22° C ±1°) and a 12-hour light-dark cycle. Purina rat chow was available ad libitum and fluids were presented in 2 Richter tubes (Kimax) mounted on the front of the cage. Fluid intake

and body weight were recorded throughout the experiment.

Procedure. Following a 5 to 6 day period for recovery from surgery, each animal was given a free choice between water and increasing concentrations of ethanol presented on alternate days. Only water was available on the intervening days. Ethanol solutions were prepared from a 95% (v/v) stock solution and tap water. The drinking tubes were rotated regularly in order to prevent the development of a position bias. The initial concentration of ethanol was 3% (v/v) and this was increased by 2% on each successive ethanol presentation if an animal previously drank more than half of its total daily fluid intake from the ethanol tube. This procedure was continued until each animal stabilized its intake of a particular concentration where 40 to 60% of its total fluid consumption was in the form of ethanol. The concentration self-selected in this manner, remained the test solution for the animal for the remainder of the experiment.

After recording 3 days of stable baseline intake, the animals were intraventricularly

infused once each day (between 15 and 17 h) for 20 consecutive days with either 1-methyl-6,7-dihydroxy TIQ (salsolinol; Aldrich Chemical Co.: 100 µg/2 µl: n=6) or Ringer's solution (2 µl: n=5). All infusions were administered with a micrometer syringe, connected with polyethylene tubing to a cannula that was inserted into the chronically implanted guide. The volumes of ethanol and water consumed were recorded during the infusion period and for an additional 7 ethanol presentations thereafter.

At the termination of the experiment, all the animals were given a lethal dose of pentobarbitol and were perfused intracardially with saline and 10% formalin. The brains were extracted and sliced into 40 µ coronal sections.

Results

Figure D.1 shows the ethanol intake during the baseline, infusion and post-infusion periods for animals receiving salsolinol or Ringer's solutions. In terms of absolute ethanol intake (g/kg), a two-way analysis of variance with repeated measures over periods, indicated that although there was a significant main effect of

periods ($F(2,18)=8.0852$, $p < .001$), there was no significant main effects of groups ($F(1,9)=.1300$, $p > .05$) or interaction effect ($F(2,18)=.7660$, $p > .05$). A similar analysis of the data expressed as a preference ratio (ethanol volume/total fluid volume) also showed that the groups were not significantly different ($F(1,9)=1.0134$, $p > .05$). However, there was a significant periods effect ($F(2,18)=21.1300$, $p < .001$) and interaction effect ($F(2,18)=3.7428$, $p < .05$). Post hoc tests (Scheffé;$\alpha=.05$) revealed that only in the post-infusion period did the salsolinol-treated animals show an increased ethanol preference compared to the control group. Histology confirmed that the cannulae had all been properly implanted into the ventricles.

Discussion

These results do not support the present proposal that intraventricular infusions of TIQs would suppress ethanol consumption. The data are also inconsistent with the findings of Myers and Oblinger (1977) who showed that single daily infusions of THP alone or in combination with salsolinol were sufficient to enhance ethanol

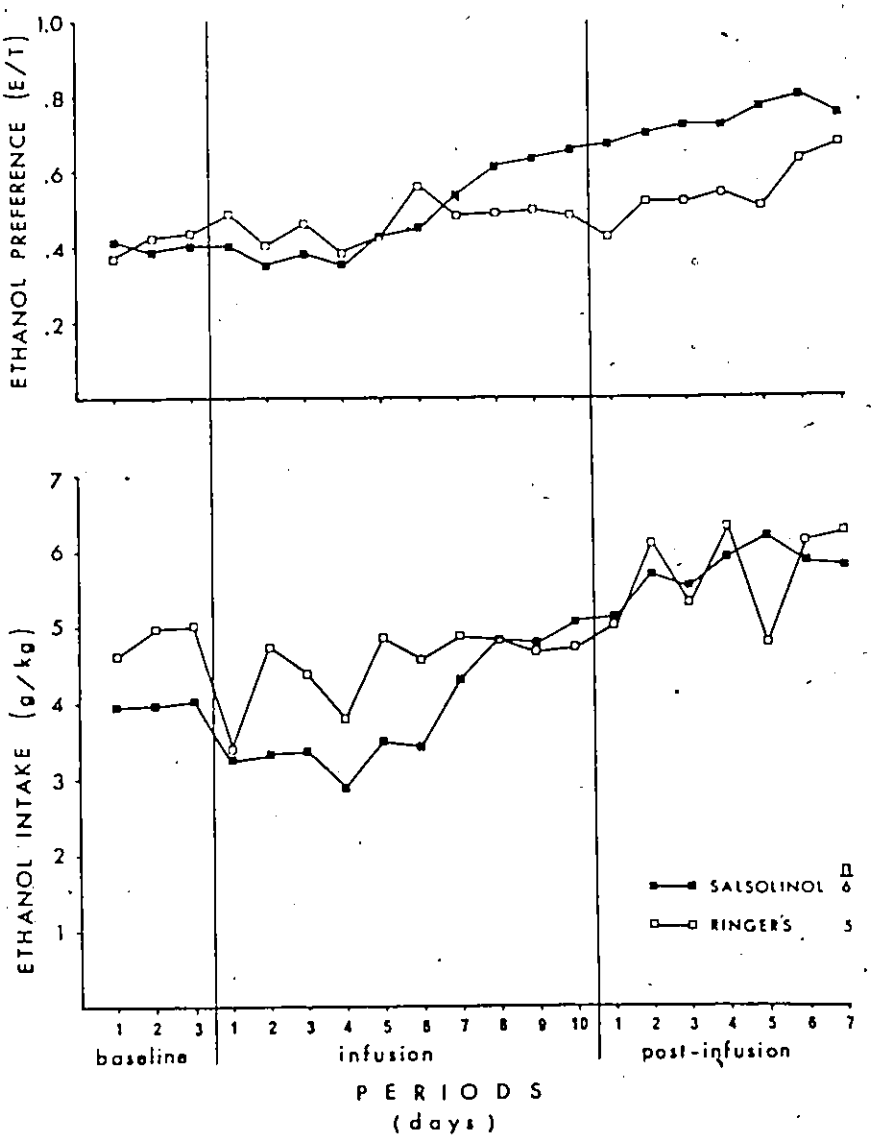

Figure D.1

Ethanol consumption (g/kg and preference ratio) over baseline, infusion and post-infusion periods in rats infused with salsolinol (100 µg/infusion) or Ringer's solution once per day for 20 days.

drinking. The present failure to see any suppression in ethanol intake in ethanol-experienced rats following infusions of salsolinol may be a result of the limited extent of the pharmacological actions of single daily treatments. Furthermore, it is possible that salsolinol is not the specific alkaloid which may be related to the central reinforcing effects of ethanol.

Experiment 2

Since single daily infusions of salsolinol had little effect on ethanol intake, in this experiment, the effects of multiple daily infusions of a variety of TIQ alkaloids in ethanol-drinking rats were examined.

Method

Subjects. Male Wistar rats (225-250 g) were maintained individually in stainless steel cages in a controlled environment. Food was available ad libitum and fluids were presented in a pair of Richter tubes mounted on the front of the cage.

Procedure. Ethanol solutions were presented in a free-choice with water on alternate days. Only water was available on the intervening days.

Commencing with a 3% (v/v) ethanol solution, the concentrations were increased by 2% every third ethanol presentation until the 15% level was reached. Animals failing to drink a minimum of 8 ml of the 15% ethanol solution were eliminated from the experiment. A cannula guide was surgically implanted into the left lateral ventricle of each of the remaining animals in accordance with the procedures previously described. Following recovery from surgery (2-4 days), the animals were transferred to a room where the infusions were to be delivered and were maintained in stainless steel cages (24 cm x 19 cm x 17 cm) covered with plexiglass tops. A 13% ethanol solution was presented daily in a free-choice with water in Richter tubes which were rotated as usual to avoid development of a position habit. After establishing a stable pattern of ethanol intake, the animals were connected to the infusion apparatus which consisted of a multichannel infusion pump (Harvard Apparatus) fitted with syringes connected by polyethylene tubing to the cannulae. Every 30 minutes, an automatic timer activated the pump

for 10 seconds during which 2 µl of fluid was delivered to each cannula. After the 2-day baseline period during which Ringer's solution was infused, the animals were randomly assigned to the treatment groups which were infused every 30 minutes for 5 consecutive days with one of the following compounds: tetrahydropapaveroline (THP; Burroughs Wellcome Laboratories; .25 µg; n=6), 1-methyl-6,7-dihydroxy TIQ (salsolinol; Aldrich Chemical Co.; 2 µg; n=5), 4,6,7-trihydroxy TIQ (4,6,7-THTIQ; Dr. M. Collins; 2 µg; n=5), 6-methoxy-4,7-dihydroxy TIQ (6M-4,7-DHTIQ, Dr. M. Collins; 2 µg; n=6), 1-methoxy-3-carboxy-6,7-dihydroxy TIQ (1M-3C-6, 7-DHTIQ; Dr. M. Collins; 2 µg; n=6), or Ringer's vehicle (pH adjusted to 3.6-4.6 with hydrochloric acid; n=6). The cannulae were removed and flushed daily and fresh solutions were prepared every 48 hours. Ethanol and water intake were recorded for an additional 5 days following the infusion period.

The animals were subsequently overdosed with sodium pentobarbitol and were perfused with saline followed by 10% formalin. The extracted brains were sliced into 40 µ coronal sections.

Results

Figures D.2 to D.6 show the mean absolute ethanol intake and preference ratios for each of the TIQ-infused groups compared to the Ringer's control group. An overall two-way analysis of variance with repeated measures on the periods factor, showed that in terms of absolute ethanol intake (g/kg) there was no significant main effect of groups ($F(5,28)=1.0115$, $p > .05$) or periods ($F(2,56)=1.9519$, $p > .05$) nor a significant interaction effect ($F(10,56)=1.4642$, $p > .05$). Likewise, analysis of the data expressed as preference ratios (ethanol volume/total fluid volume) did not produce any significant results: groups - $F(5,28)=.2431$, $p > .05$; periods - $F(2,56)=.1374$, $p > .05$; interaction - $F(10,56)=.9383$, $p > .05$.

Histological examination verified that all the cannula had penetrated the lateral ventricles. Except for some ventricular distension and the tract of the cannula guide itself, there was little sign of any tissue damage.

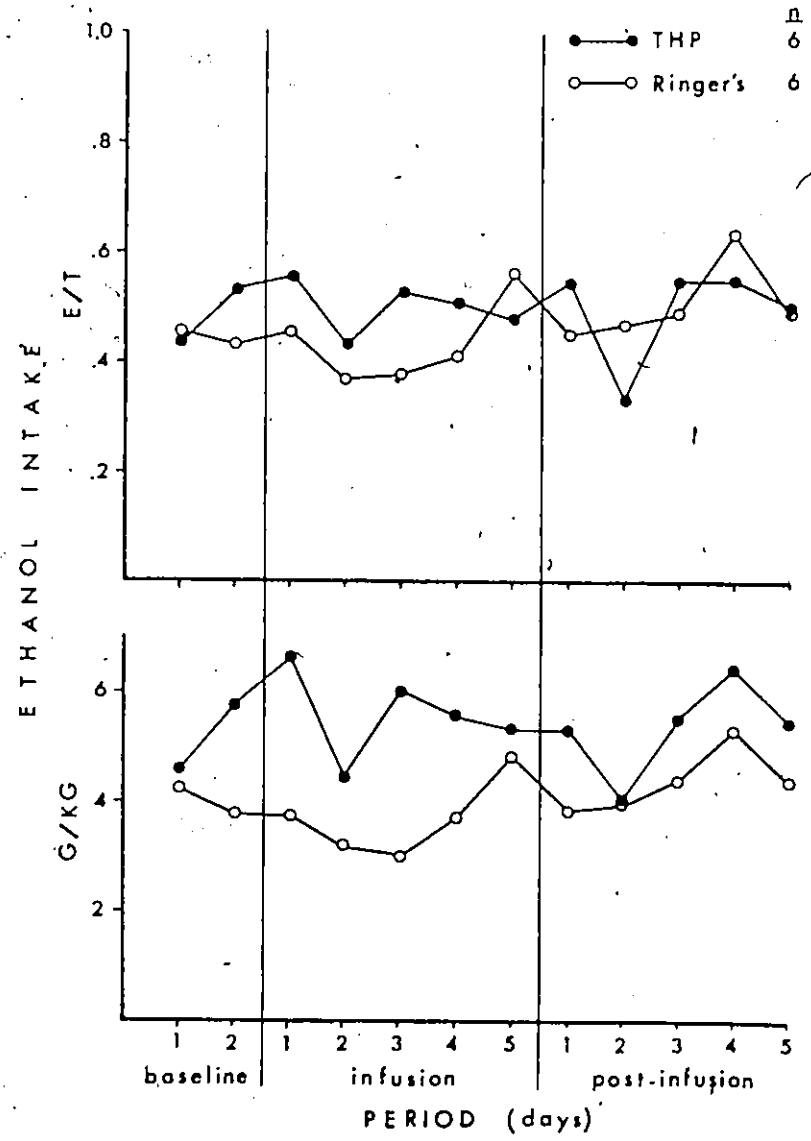

Figure D.2

Mean ethanol intake (g/kg) and preference ratio (ethanol volume/total fluid volume; E/T) for rats infused with THP (.25 ug; n=6; closed circles) every 30 minutes for 5 days compared to animals receiving Ringer's solution (n=6; open circles).

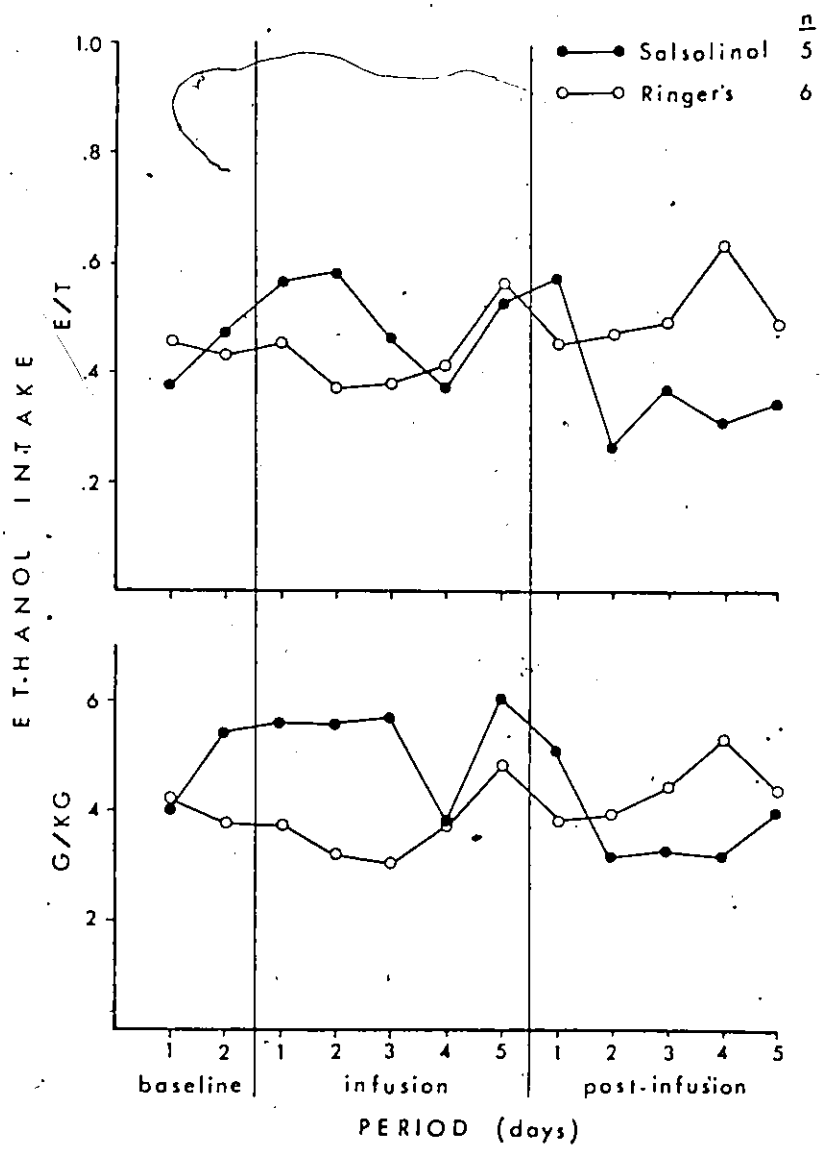

Figure D.3

Mean ethanol intake (g/kg) and preference ratio (E/T) for rats infused with salsolinol (2 ug; n=5; closed circles) compared to the control animals (n=6; open circles).

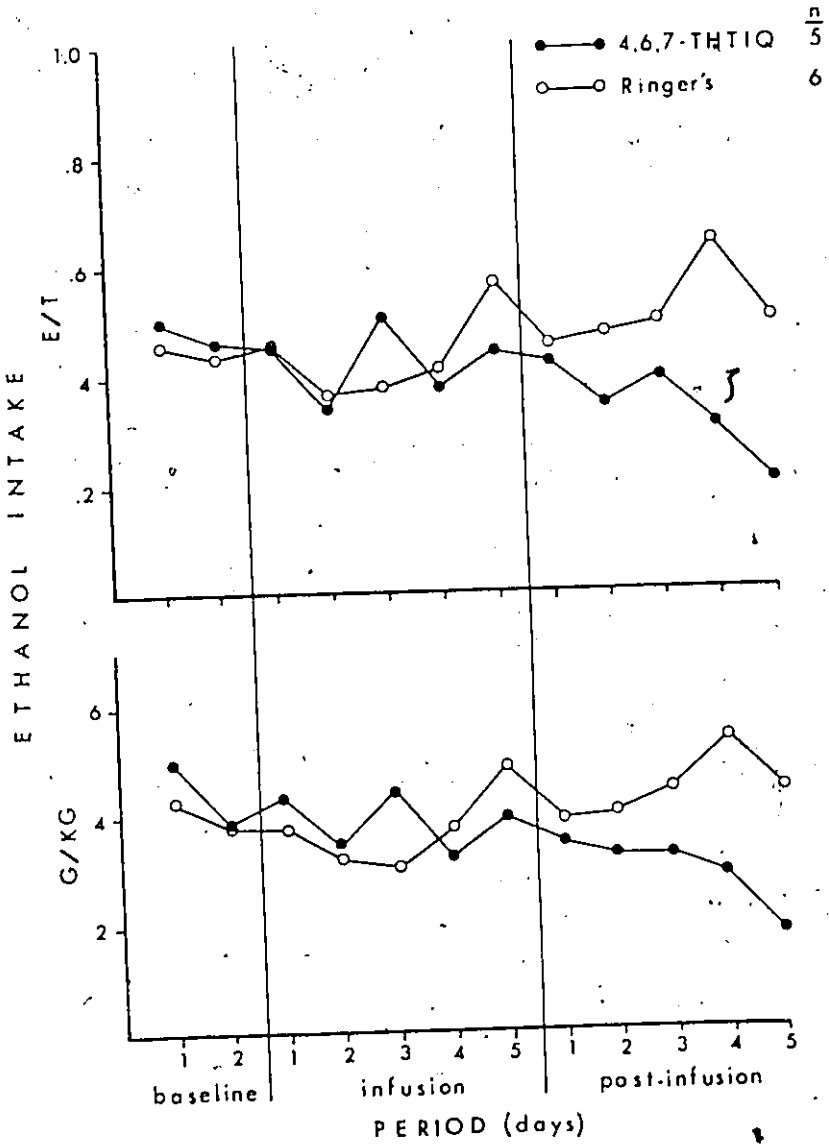

Figure D.4

Mean ethanol intake (g/kg) and preference ratio (E/T) for rats infused with 4,6,7-THTIQ (2 μg; n=5; closed circles) compared to the control animals (n=6; open circles).

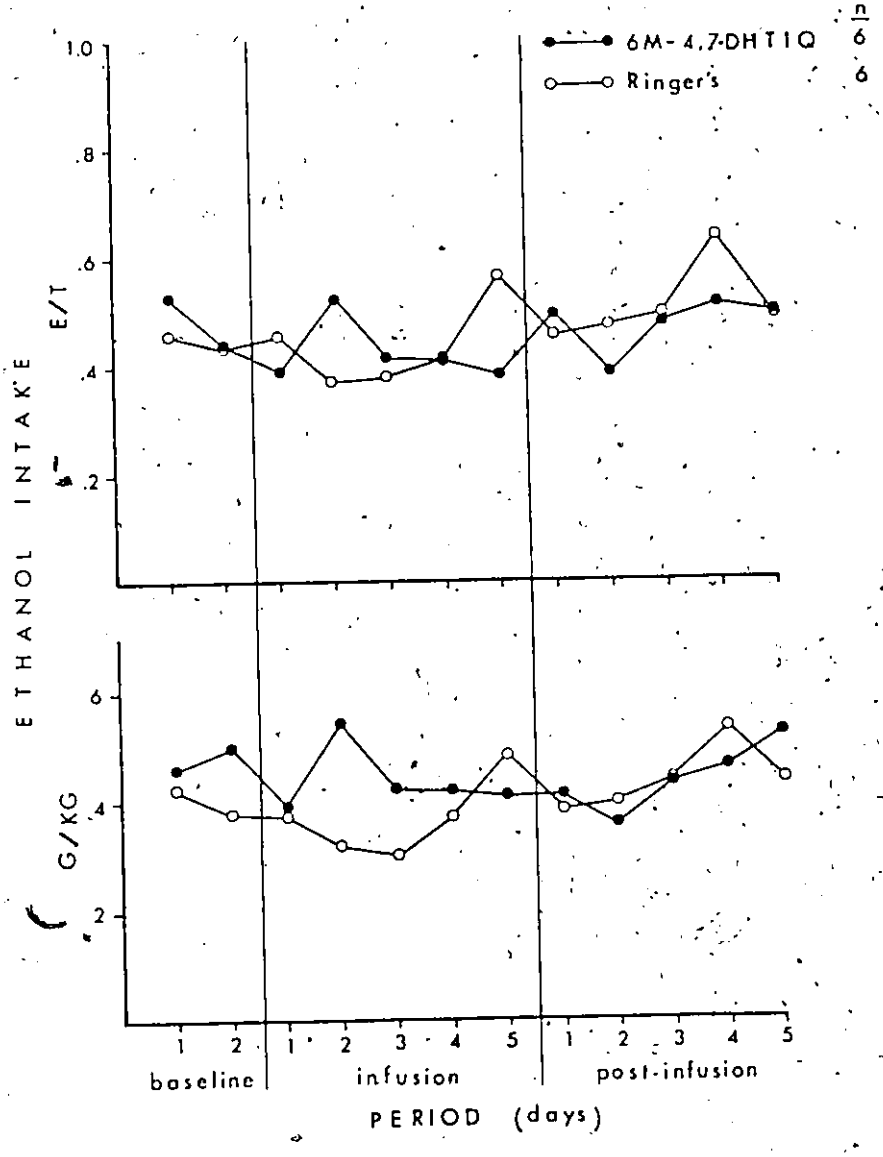

Figure D.5

Mean ethanol intake (g/kg) and preference ratio (E/T) for rats infused with 6M-4,7-DHTIQ (2 ug; n=6; closed circles) compared to the control animals (n=6; open circles).

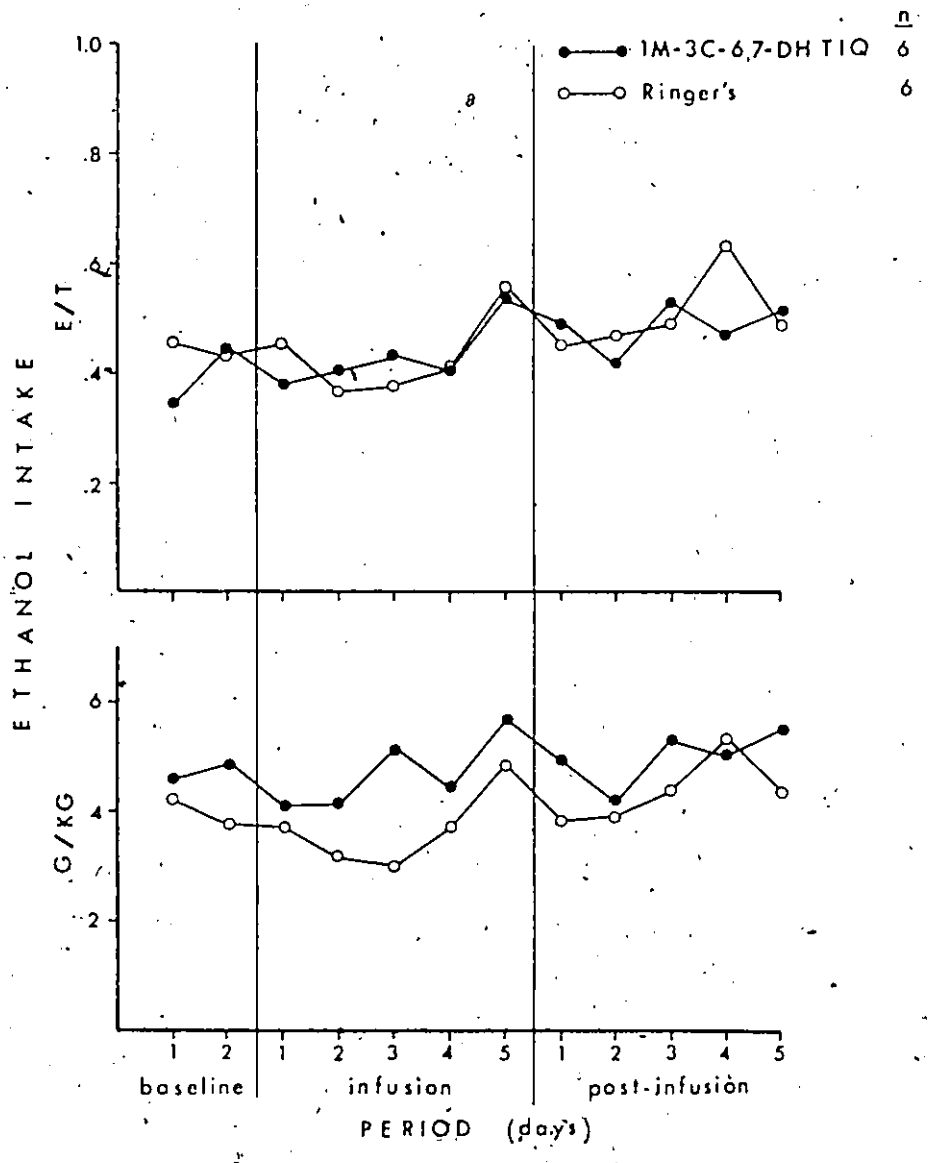

Figure D.6

Mean ethanol intake (g/kg) and preference ratio (E/T) for rats infused with 1M-3C-6,7-DHTIQ (2 µg; n=6; closed circles) compared to the control animals (n=6; open circles).

Discussion

In this experiment, it was found that a variety of TIQ alkaloids, even when infused every 30 minutes for 5 consecutive days, failed to alter voluntary ethanol consumption in rats. These results also do not support the hypothesis that intracranial infusions of TIQs should produce an attenuation in ethanol intake. However, it has been shown in a number of studies that NE, rather than dopamine or 5-HT, appears to be the neurotransmitter that is most functionally involved in ethanol self-administration (e.g., Amit et al., 1977; Brown and Amit, 1977; Davis, Smith and Werner, 1977; Myers and Melchior, 1975a). Therefore, it is conceivable that the TIQ which would be most effective in altering ethanol consumption is the acetaldehyde-NE derivative, 1-methyl-4,6,7-trihydroxyTIQ, which has been found in acetaldehyde-perfused cow adrenals (Cohen, 1971; Cohen and Collins, 1970). Unfortunately, attempts to synthesize this compound biochemically have not been successful (Collins and Kernozek, 1972). It is anticipated that intraventricular

infusions of this alkaloid may induce a reduction in ethanol self-administration.

The present findings are also inconsistent with the reports by Myers and Melchior who showed that intraventricular infusions of the same compounds, in most cases produced marked increases in voluntary ethanol consumption (Melchior and Myers, 1977; Myers and Melchior, 1977a,b). Furthermore, in contrast to the signs of intoxication and withdrawal observed in their studies, the animals in the present experiment never displayed any unusual symptoms.

The lack of agreement between the results of the present experiment and those reported by Myers and Melchior may be attributable to procedural inconsistencies. Since differences in the pharmacological and behavioral effects of ethanol have been related to differences in animals strain (e.g., Amir, 1978a; Eriksson, 1973; Rodgers, 1966), it is possible that Myers' Sprague-Dawley rats were more susceptible to the TIQ manipulations than the Wistar rats used in this experiment. Another crucial factor that may account for the differential results is the

manner in which ethanol was presented. As a test of ethanol preference, Myers used a 12-day regimen of increasing concentrations of ethanol (3-30% v/v) prior to and during the TIQ infusions. In the present experiment, the rats had prior experience with ethanol and were consistently drinking relatively large quantities of a 13% ethanol solution (4-6 g/kg/day) in the baseline period (refer to Figures D.2 to D.6). It is noteworthy that the increases in ethanol intake following TIQ infusions as reported by Myers and Melchior, in most cases did not exceed these baseline levels. It has been argued whether or not the presentation of increasing ethanol concentrations provides a more valid measurement of ethanol preference than the use of a single solution (Eriksson, 1968; Myers, 1968). In Myers' test sequence, which ranges from 3% to 30% (v/v), the voluntary intake of ethanol may to some extent have been determined by taste factors (Myers and Carey, 1961; Richter and Campbell, 1940; Wilson, 1972) or by the limitation of total fluid requirement. On the other hand, when an animal has an established pattern of consumption

of a fixed concentration of ethanol, the effects of any pharmacological manipulation would be less likely to be confounded by taste and volume factors. Nevertheless, because of the apparent robustness of the phenomenon reported by Myers and Melchior, one would have expected it to be manifested regardless of variations in animal strain or the method of ethanol presentation.

Another potentially important factor that may contribute to the inconsistencies, may be the fact that Myers and Melchior added ascorbic acid to lower the pH of the alkaloid solutions, while hydrochloric acid was used in the present experiment. Cohen (1977) has pointed out that ascorbic acid can induce the activity of catalase which is capable of oxidizing ethanol to acetaldehyde. It is therefore possible that the reported effects of the infusions of TIQs on ethanol consumption may be attributable to ascorbate-induced synthesis of acetaldehyde in the brain.

It is also difficult to relate the various proposed mechanisms of action of TIQs to the data obtained by Myers and Melchior. One popular

hypothesis is that TIQs may somehow be involved in the induction of physical dependence on ethanol (see Rahwan, 1975). Presumably, the TIQ alkaloids, which have potent neurochemical effects in the brain (see Cohen, 1976), may create an "artificial need state" which can be satisfied by TIQ formation from ingested ethanol. Based on this opiate-type model, the introduction of TIQs directly into the brain should alleviate the "need" and consequently result in an attenuation rather than an enhancement of ethanol consumption.

An alternative hypothesis is that the TIQs may be the substrate of reinforcement which supports ethanol self-administration. According to this model, an animal consumes ethanol to procure the precursor for TIQs. Consequently, if TIQs are exogenously introduced, it should preclude the necessity to consume ethanol rather than induce an increased ethanol intake. However, in a recent pilot study, it was found that rats will not self-administer either salsolinol or THP into the cerebral ventricles, suggesting that these TIQs do not function as

positive reinforcers.

Although TIQs have been detected in the brains of animals subjected to radical treatments with ethanol and enzyme inhibitors (Collins and Bigdeli, 1975), it is possible that under conditions of voluntary ethanol consumption, insufficient amounts of TIQs would be found in the brain to exert any pharmacological effects. Therefore, another consideration is that TIQs may simply be an artefactual by-product of ethanol administration which may play no mediational role in ethanol consumption.

General Discussion

The present series of experiments examined the involvement of acetaldehyde, the oxidative product of ethanol, in the mediation of ethanol consumption in laboratory rats. In the first experiment ("A"), it was shown that peripheral injections of acetaldehyde are capable of inducing a conditioned taste aversion to a novel flavor which had originally been consumed just prior to the acetaldehyde treatment in rats. These findings are consistent with the traditional view that acetaldehyde is a toxic substance (Akabane, 1970) which when present in the body in high concentration produces aversive physiological reactions that may discourage the consumption of alcohol (Hald and Jacobsen, 1948; Schlesinger et al., 1966). In contrast to its peripheral noxious effects, when infused into the cerebral ventricles of laboratory rats, acetaldehyde did not induce a CTA suggesting that its pharmacological effects in the brain were not perceived as being aversive.

Based on the reports that acetaldehyde is present in the brain following ethanol

administration (Petterson and Kiessling, 1977; Sippel, 1974; Tabakoff et al., 1976) and that it is capable of altering CA metabolism (Duritz and Truitt, 1966; Ortiz et al., 1974), it was hypothesized that the central pharmacological actions of acetaldehyde may in fact be positively reinforcing. In Experiment "B", it was found that rats would learn to press a lever for intraventricular infusions of acetaldehyde but not ethanol, suggesting that the positive reinforcing effects of ethanol may be mediated centrally by its metabolite. In fact, in Experiment "C", it was found that the rate of intraventricular self-administration of acetaldehyde was directly correlated with the subsequent voluntary consumption of ethanol solutions.

Additional evidence from other sources has suggested that acetaldehyde in the brain may be functionally related to ethanol consumption. Myers and his co-workers showed that chronic infusions of acetaldehyde into the cerebral ventricles, induced an increase in ethanol intake in laboratory animals (Myers and Veale, 1969;

Myers et al., 1972). Amir (1977, 1978a) has recently found that the activity of brain ALDH, which is capable of oxidizing acetaldehyde, was directly correlated with ethanol preference in rats. It is possible that the alterations in brain ALDH activity that are related to ethanol consumption (Amir, 1978b), may be functionally involved in the effects of ethanol. These changes in ALDH activity may alter CA metabolism and/or modulate the amount of acetaldehyde present in the brain following ethanol ingestion. Taken together, these data suggest that acetaldehyde may act as a positive reinforcer and may be responsible for the central pharmacological actions that maintain voluntary ethanol consumption in rats.

Despite all the available evidence implicating acetaldehyde in the effects of ethanol, it must be acknowledged that in most of these studies relatively large amounts of ethanol or acetaldehyde were necessary to demonstrate any toxicological, biochemical, or behavioral effects. During voluntary consumption of ethanol, because of the relative kinetics for the metabolism of ethanol

and acetaldehyde, very little acetaldehyde escapes into the circulation (e.g., Eriksson, 1977; Eriksson and Sippel, 1977). Consequently, the problem arises as to how sufficient acetaldehyde can reach and penetrate the brain, if the circulating levels necessary to achieve this are toxic and produce aversive reactions in the periphery. Whereas these peripheral mechanisms may be instrumental in preventing excessive consumption of ethanol in order to avoid extensive toxicity, it also precludes the availability of sufficient quantities of acetaldehyde to the brain. However, the possibility remains that trace amounts of acetaldehyde may be capable of eliciting central pharmacological effects. It remains to be determined what levels of acetaldehyde are necessary in the periphery and in the brain to evoke threshold effects.

Because acetaldehyde may be present in the brain during ethanol intoxication (e.g., Petterson and Kiessling, 1977; Sippel, 1974; Tabakoff et al., 1976), it is possible that it could condense with the CA or 5-HT to form alkaloids which have been hypothesized to be involved in some of the central

effects of ethanol (Cohen, 1976; Rahwan, 1975). In contrast to the reports that intraventricular infusions of TIQs enhanced the voluntary consumption of ethanol in rats (Melchior and Myer, 1977; Myers and Melchior, 1977a,b; Myers and Oblinger, 1977), in Experiment "D" of the present study, no effects were seen with any of the alkaloid compounds tested. Nevertheless, the possibility that TIQs are functionally involved in ethanol self-administration cannot be ruled out.

Various hypotheses attempt to explain the mechanisms by which TIQs may operate to regulate the consumption of ethanol. One possibility is that TIQs which are capable of functioning as pseudo-transmitters (Cohen, 1976), may displace CA and assume their role in mediating the effects of ethanol. Extending this hypothesis further, it is conceivable that the brain may adapt to the TIQ-induced neurochemical changes thereby creating an "artificial need state". An analogous model has previously been proposed describing an acquired homeostatic mechanism in which ethanol ingestion is perpetuated in order to maintain the availability of TIQs for neural functioning in the adapted

system (Amit, 1970; Amit and Stern, 1972). Another related proposal made by Davis and Walsh (1970), focussed on the fact that THP, one of the alkaloids that may be formed following ethanol administration, is a precursor to morphine (Kirby, 1967; Shamma, 1972). It was implied by these authors that the etiology of alcoholism may be the same as for opiate addiction. Although this hypothesis was severely criticized (Goldstein and Judson, 1971; Halushka and Hoffman, 1970; Seevers, 1970), there have been some demonstrations of commonalities between the pharmacological and behavioral effects of morphine and ethanol (see Blum et al., 1977). Based on the opiate model, one would predict that if an animal has developed a "need" for TIQs, direct infusion of the alkaloids would satisfy the "need" and preclude the necessity of ingesting ethanol as a precursor for TIQs. However, the results of the studies of Myers and Melchior as well as those of Experiment "D" do not conform to this model.

Another hypothesis that may be entertained is that TIQs, which may be spontaneously formed

in the brain after the ingestion of ethanol, may provide a signal for satiety which would terminate drinking. However, no suppression of ethanol drinking was reported following intraventricular TIQ infusions in either the Myers and Melchior studies or in Experiment "D". It is also possible that TIQs are simply formed as a consequence of ethanol intoxication but play no role in the mediating of the effects of ethanol. Since NE has been implicated in the pharmacological actions of ethanol, it is conceivable that the condensation product of NE and acetaldehyde may prove to be the most functionally relevant of the alkaloids.

Based on the current literature and the evidence provided in the present experiments, it is possible to postulate a model that will most parsimoniously describe the central mechanisms putatively involved in the regulation of voluntary ethanol consumption. Figure GD.1 outlines the components of the proposed regulatory system. When an organism ingests alcohol, it is metabolized primarily by the liver to acetaldehyde, some of which may reach the brain via

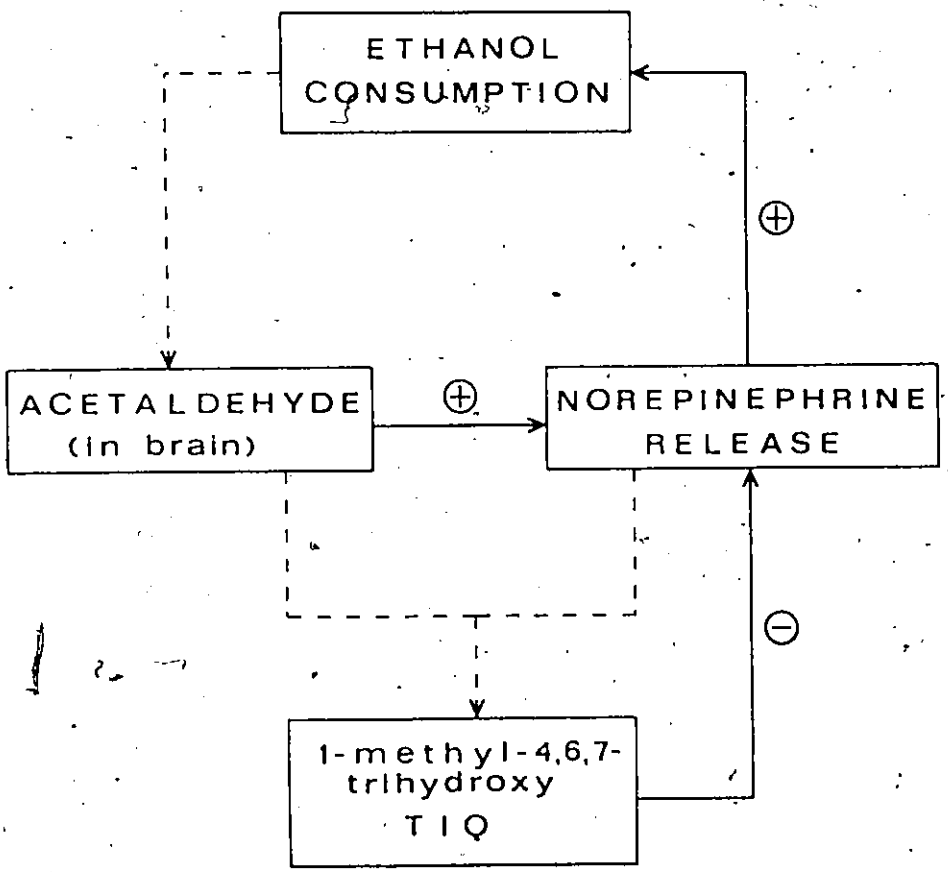

Figure GD.1

Proposed model for the regulation of ethanol self-administration.

⊕ ——→ stimulatory effects

⊖ ——→ inhibitory effects

- - - - - - - - → metabolic effects

the circulation. The presence of acetaldehyde in the brain may stimulate the release of CA the effect of which is presumed to be rewarding (e.g., Arbuthnott, Fuxe and Ungerstedt, 1971). In the case of ethanol, NE is the neurotransmitter which has been implicated most consistently in the reinforcing effects of ethanol. Therefore, in the present model, NE is specified as the relevant transmitter substance. It is proposed that the acetaldehyde-induced release of NE reinforces the ethanol-drinking behavior which further increases the supply of acetaldehyde to the brain and perpetuates the release of NE. Whereas this relatively simple system may account for the initiation and maintenance of ethanol self-administration, it does not provide for the termination of drinking. It is possible that satiation may result from the acute tolerance of the noradrenergic neurons to the continued presence of acetaldehyde. Alternatively, ethanol satiation may be mediated by TIQ alkaloids. As the consumption of ethanol, supported by the release of NE, is perpetuated, the increased availability of acetaldehyde in the presence of

the released NE favors the spontaneous formation of 1-methyl-4,6,7-trihydroxy-TIQ. The accumulating TIQ may be preferentially taken up by the noradrenergic neurons and function as pseudo-transmitters. As more NE is replaced by the TIQ, the acetaldehyde stimulation of the noradrenergic neurons will result in the release of the TIQ instead of NE. As a consequence, the ingestion of ethanol, which is no longer being reinforced by NE release, terminates temporarily. With the consequent reduced synthesis of acetaldehyde and the relative unavailability of NE, the formation of the alkaloid attenuates. Ultimately, as the TIQ is eliminated and NE replenishes the neurons, the system once again becomes capable of reinforcing ethanol consumption.

The experiments described in this thesis, provide the first direct demonstrations of the positive reinforcing effects of acetaldehyde in the brain that appear to be related to the voluntary consumption of ethanol. Evidence was also provided suggesting that TIQs, which are likely to be formed following the administration of high doses of ethanol, may play no central

mediational role in ethanol self-administration in rats. Further research is necessary to elucidate the mechanisms by which acetaldehyde may be responsible for the positive reinforcing effects of ingested ethanol.

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