ACETALDEHYDE AND NOREPINEPHRINE INTERACTIONS IN THE MEDIATION OF SOME OF THE PSYCHOPHARMACOLOGICAL PROPERTIES OF ETHANOL

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ABSTRACT

ACETALDEHYDE AND NOREPINEPHRINE INTERACTIONS IN THE MEDIATION OF SOME OF THE PSYCHOPHARMACOLOGICAL PROPERTIES OF ETHANOL

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The present investigation attempted to examine the roles of acetaldehyde, norepinephrine, and their possible interaction in the mediation of some of the psychopharmacological properties of ethanol. In section A, it was observed that centrally administered acetaldehyde could induce a conditioned place preference supporting previous reports demonstrating that acetaldehyde may have reinforcing properties. In section B, it was shown that ethanol had a time- and dose-dependent biphasic effect on noradrenergic activity and that the ethanol-induced stimulation was blocked by pretreatment with FLA-57, a dopamine-beta-hydroxylase inhibitor. It was suggested that the increased activity of the noradrenergic system may play an important role in the mediation of ethanol oriented behaviors

The final section examined the possible involvement of tetrahydroisoquinoline (TIQ) alkaloids, condensation products

between acetaldehyde and catecholamines, in some of the pharmacological effects of ethanol. It was observed that TIQ alkaloids were self-administered via the intracerebroventricular route, suggesting that they may have reinforcing properties; however, their central administration failed to result in alterations in voluntary ethanol intake. It was suggested that TIQ alkaloids may have psychopharmacological properties capable of influencing behavior but they do not appear to play a mediational role in ethanol consumption. These findings were discussed with respect to possible mechanisms of action of ethanol reinforcement.

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TABLE OF CONTENTS

LIST OF FIGURES

List of Tables

The use of alcohol throughout the centuries is well documented. Yet, despite a vast amount of research during the past 50 years, there remains a great deal of ambiguity regarding the biochemical mechanisms which underlie its effects (Lester, 1966; Littleton, 1975; Deitrich, 1976; Rahwan, 1974). A variety of models for alcohol use have been developed in an attempt to understand the psychopharmacological action of alcohol (see Meisch, 1977).

It has been shown that animals will consume large quantities of alcohol despite its apparent aversive taste (Kahn and Stellar, 1960; Richter and Campbell, 1940; Wilson, 1972). A number of techniques have been used in order to induce a preference for consumed alcohol in laboratory animals. One such technique is the schedule-induced polydipsia paradigm which results in the drinking of intoxicating quantities of ethanol (Falk, Samson and Winger, 1972; Senter and Sinclair, 1967). Elimination of the concurrent food reinforcement, however, does not result in a decrease in ethanol intake as a preference for ethanol over water remains (Meisch and Thompson, 1974). Intermittant presentation of alcohol also results in an increase in voluntary ethanol intake in rats (Amit, Stern and Wise, 1970;

Sinclair and Senter, 1968). Animals can also be demonstrated to learn to self-administer ethanol in a variety of operant paradigms. Rats have been shown to press a lever for drops of ethanol solution (Meisch and Beardsley, 1975; Sinclair, 1974) as well as to self-administer via the intravenous (Deneau, Yanagita and Seever, 1969; Winger and Woods, 1973; Woods, Ikomi and Winger, 1971) and intragastric routes of administration (Amit and Stern, 1969; Yanagita and Takahashi, 1973; Werner, Smith and Davis, 1977). These data demonstrating that animals will voluntarily consume and learn to self-administer ethanol, suggest that the psychopharmacological effects derived are positively reinforcing and that they may be important in the initiation and maintenance of alcohol-oriented behaviors.

The Involvement of the Central Catecholamines in Ethanol Self-Administration

In 1970, Amit, Stern and Wise demonstrated that electrical stimulation of the lateral hypothalamus resulted in an increase in voluntary ethanol consumption in rats and that this increased preference for ethanol over water persisted for some time following

the termination of the stimulation (Amit and Stern, 1971; Amir and Stern, 1978). The lateral hypothalamus has been shown to be traversed by some of the major catecholamine (CA) pathways (Lindvall and Bjorklund, 1974; Ungerstedt, 1971) suggesting an involvement of norepinephrine (NE) and dopamine (DA) in the mediation of voluntary ethanol consumption.

A number of studies have demonstrated that disruption of central CA can result in alterations in ethanol's effects. Myers and Veale (1968) have shown that administration of alpha-methyltyrosine (AMPT), an inhibitor of tyrosine hydroxylase, the rate limiting enzyme in CA synthesis (Moore and Dominic, 1971), produced a transitory attenuation in ethanol consumption in rats. It has also been demonstrated that AMPT administration interferes with the re-acquisition of intragastric self-administration of ethanol in rats (Davis, Werner and Smith, 1978). Furthermore, pretreatment with AMPT suppressed ethanol-induced euphoria following programmed ingestion of alcohol in man (Ahlenius, Carlsson, Engel, Svensson and Sodersten, 1973) and ethanol-induced locomotor stimulation in mice (Carlsson, Engel and Svensson, 1972; Engel, Strombom, Svensson and Waldeck, 1974). The administration of nialamide, a monoamine oxidase inhibitor, has been

shown to antagonize ethanol-induced locomotor stimulation in mice (Ahlenius, Brown, Engel, Svensson and Waldeck, 1974). It was suggested that this was the result of decreased CA synthesis caused by a feedback mechanism.

It has been observed that electrolytic lesions of the ventral hypothalamus reduced ethanol preference (Amit, Meade, Levitan and Singer, 1976). Similarly, when the neurotoxin 6-hydroxydopamine (6-OHDA) was infused into the lateral ventricles of ethanol prefering rats, it resulted in an attenuation of voluntary ethanol intake (Brown and Amit, 1977; Myers and Melchior, 1975). These results suggest that central CA play a fundamental role in mediating the psychopharmacological effects of ethanol. However, they do not provide data regarding the relative importance of NE or DA in ethanol's actions.

The findings of Brown and Amit (1977) suggest that those brain systems containing NE rather than DA subserve voluntary ethanol consumption in rats. Furthermore, 6-OHDA lesions of the ascending dopamine pathways failed to alter ethanol intake in rats (Kiianmaa, Anderson and Fuxe, 1979). These results are supported by others who have demonstrated that 6-OHDA lesions of NE pathways and not DA neurons produce

alterations in voluntary ethanol consumption (Kiianmaa, Fuxe, Jonsson and Ahtee, 1975; Kiianmaa, 1980; Mason, Corcoran and Fibiger, 1979). In addition, the administration of noradrenergic receptor blocking agents, phenoxybenzamine and yohimbine, which by themselves did not affect locomotion, significantly suppressed ethanol-induced locomotor stimulation (Liljequist, Berggren and Engel, 1981). These authors also observed that low doses of yohimbine, which stimulate NE activity, resulted in an enhancement of the ethanol-induced stimulation.

Central depletions of NE by the dopamine-beta-hydroxylase (DBH) inhibitors, FLA-63 (Svensson and Waldeck, 1969) and FLA-57 (4-methyl-1-homopiperazinedithiocarboxylic acid; Florvall and Corrodi, 1970) were observed to suppress locomotor stimulation by ethanol (Liljequist et al., 1981; Brown, Smith and Sinyor, 1978). The use of these DBH inhibitors has also been shown to attenuate voluntary ethanol intake in rats (Amit, Levitan and Lindros, 1976; Amit, Brown, Levitan and Ogren, 1977). Further experiments demonstrated that treatment with FLA-57 along with the presentation of ethanol as the sole fluid source resulted in a rejection of ethanol when later presented in a free-choice with water (Brown, Amit, Levitan, Ogren and

Sutherland, 1977). It appeared that FLA-57 blocked the reinforcing properties of ethanol and alcohol drinking behavior was subsequently extinguished. These findings suggest that the reinforcing properties of ethanol are mediated through an interaction with central noradrenergic systems.

Additional support for the hypothesis was provided by Davis et al. (1978) who showed that rats given the DBH inhibitor U14,624 (1-phenyl-3-(2-thiazolyl)-2-thiourea) failed to re-acquire a response contingency for intragastric infusions of ethanol, whereas, haloperidol, a DA receptor blocker had no effect. Subsequent experiments replicated the effect of FLA-57 on the reinforcing properties of ethanol in that treatment of the DBH inhibitor blocked intragastric self-administration of ethanol (Davis, Werner and Smith, 1979). From the foregoing, it would appear that the central CA, and in particular NE, subserve those reinforcing properties of ethanol that support its self-administration.

The Effects of Ethanol on Catecholamine Metabolism

A number of studies have investigated the possible effects of ethanol on central CA. It has been shown that the CA neurons in the lateral hypothalamus were highly affected by electrophoretically administered ethanol (Wayner, Ono and Nolley, 1975). In most cases, the treatment of ethanol has failed to induce alterations in brain CA levels (Corrodi, Fuxe and Hokfelt, 1966; Duritz and Truitt, 1966; Efron and Gessa, 1963; Haggendal and Linquist, 1961; Hunt and Majchrowicz, 1974; Pohorecky, 1974), however, there have been some reports of ethanol-induced changes in CA content in brain tissue (Carlsson, Magnusson, Svensson and Waldeck, 1973; Erickson and Matchett, 1975; Gursey and Olsen, 1960; Griffiths, Littleton and Ortiz, 1974).

Similarly, discrepancies in the effects of ethanol on the turnover of NE have occurred in the literature. Norepinephrine turnover has been reported to decrease (Pohorecky, 1974; Pohorecky and Jaffe, 1975; Thadani and Truitt, 1977) and to increase (Carlsson and Lindquist, 1973; Carlsson et al., 1973; Corrodi et al., 1966; Hunt and Majchrowicz, 1974; Borg, Kvande and Sedvall, 1981) following ethanol administration. Dopamine turnover as well has been shown to increase

(Carlsson et al., 1973; Karoum, Wyatt and Majchrowicz, 1976) and to decrease (Hunt and Majchrowicz, 1974). It has also been shown that acute ethanol administration may have differential effects on NE and DA turnover in various regions of rat brain (Bacopoulos, Bhatnager and van Orden, 1978). Hunt and Majchrowicz (1974) have observed a biphasic effect of ethanol, in that both increases and decreases in biogenic amine turnover can be seen over time. Since ethanol appears to alter NE metabolism and NE manipulation alters ethanol intake, as well as the induction of stimulation and euphoria, it suggests that there may be an interaction between ethanol and brain NE which may subserve the reinforcing properties of ethanol.

The Metabolism of Ethanol and Formation of Acetaldehyde

In recent years there has been evidence reported implicating acetaldehyde, the primary metabolite of ethanol, in the mediation of the pharmacological effects of alcohol. Acetaldehyde is generated through the oxidation of ethanol primarily by the action of the nicotinamide-adenine dinucleotide-dependent alcohol dehydrogenase (ADH: Hawkins and Kalant, 1972). This occurs mainly in the liver (Jacobsen, 1952; Hawkins and

Kalant, 1972), although it has been shown to take place in other parts of the body such as blood and vital organs (Lundquist, 1971; Raskin and Sokoloff, 1972). Ethanol may also be metabolized by a microsomal ethanol oxidizing system (MEOS: Lieber and DeCarli, 1968; Lieber, 1977) and by the enzyme catalase (Kieilin and Hartree, 1945; Lieber, 1977). Ethanol oxidation can also occur in the brain as ADH has been reported to be present in brain tissue (Raskin and Sokoloff, 1968, 1970; Tyce, Flock and Owen, 1968), however, ADH in the brain is relatively inactive and very little acetaldehyde is actually formed (Mukherji, Kashiki, Ohyanagi and Sloviter, 1975; Tabakoff and von Wartburg, 1975; Veloso, Passonneau and Veech, 1972). Other potential pathways for the oxidation of ethanol are via the formation of reactive hydroxyl radicals during the spontaneous oxidation of ascorbate (Cohen, 1977) and by the presence of cytochrome P-450 (Paul, Axelrod and Diliberto, 1977; Cohen, Sinet and Heikkila, 1980). However, direct evidence supporting these pathways does not, at present, exist (Cohen et al., 1980). A fourth pathway involving the peroxidatic activity of catalase has been proposed, as indirect evidence for the oxidation of ethanol in vivo has been demonstrated (Cohen et al., 1980).

The oxidation of acetaldehyde to acetate is the main route for its elimination. Several enzyme systems such as pyridine nucleotide-dependent aldehyde dehydrogenases and oxidases are responsible for aldehyde metabolism (ALDH: Hawkins and Kalant, 1972; Lundquist, 1971; von Wartburg, 1980). The primary pathway of metabolism is via the former system, ALDH, and it is found in large quantities in the liver, and to some extent, in the kidneys, adrenals, lungs, heart and brain (Deitrich, 1966). Its presence throughout the body appears to be due to its role in the metabolism of endogenously formed acetaldehyde and biogenic amine-derived aldehydes (Deitrich, 1966).

The Occurrence of Acetaldehyde in Brain

Acetaldehyde, because of its high lipid solubility, is capable of diffusing into many tissues, including brain (Akabane, 1970). Attempts to demonstrate the presence of acetaldehyde in the brain, however, have produced equivocal and controversial results. Earlier studies suggested that acetaldehyde was detectable in the brains of ethanol-treated rats (Duritz and Truitt, 1966; Kiessling, 1962a, 1962b; Majchrowicz, 1973; Ridge, 1963) and at levels which

paralleled those in cerebral blood (Majchrowicz, 1973; Ridge, 1963). However, the relatively high levels of acetaldehyde observed in these studies were later discounted on the grounds that acetaldehyde can be formed non-enzymatically during sample preparation (Sippel, 1973). In fact, when this non-enzymatic formation was prevented by treating the sample with thiourea (Sippel, 1972), no acetaldehyde was detected in brain tissue until blood levels exceeded 200 uM, far greater than those seen during normal ethanol oxidation (Sippel, 1974). These results suggest that elevated blood levels of acetaldehyde do not produce a concomitant increase in brain levels, however, it has been demonstrated that blood acetaldehyde levels do correspond to those found in the cerebrospinal fluid of rats injected with ethanol (Kiianmaa and Virtanen, 1978; Petterson and Kiessling, 1977).

Different theories have been presented to account for the differences between brain acetaldehyde levels and those found in blood or cerebrospinal fluid. Eriksson and Sippel (1977) for example, have suggested that all of the acetaldehyde is metabolized in the capillaries and therefore does not enter the brain. It has been proposed that this acts as an enzymatic barrier to the penetration of acetaldehyde into the

brain (Tabakoff, Anderson and Ritzmann, 1976; Sippel, 1974). Recently, using thiourea to prevent non-enzymatic formation, acetaldehyde was detected in brain fluid (Westcott, Weiner, Shultz and Myers, 1980). Levels between 5 and 20 uM were measured in the interstitial fluid following an intragastric administration of ethanol in rats (Westcott et al., 1980). Similar to the results of previous studies (Sippel, 1974), Westcott and co-workers did not detect acetaldehyde in brain tissue unless the blood levels of acetaldehyde were artificially elevated using the ALDH inhibitor, disulfiram (Hald and Jacobsen, 1948). It was suggested that the oxidation of acetaldehyde by ALDH in the cells is extremely rapid (Westcott et al., 1980). These results indicate that acetaldehyde is detectable in the extracellular fluid and that it could impinge upon the environment of the neuron and thereby exert some pharmacological effect.

Additional studies examining brain enzyme activity have suggested the possible presence of acetaldehyde. Amir (1977; 1978a; 1978b; Amir and Stern, 1978) has shown that aldehyde dehydrogenase activity is correlated with ethanol preference in rats and that chronic exposure to ethanol resulted in an induction of brain ALDH (Amir, 1978b). Amir has suggested (1977)

that the capacity of the brain to metabolize aldehydes could be related to the possible involvement of acetaldehyde in ethanol consumption. More direct evidence for the involvement of ALDH in voluntary ethanol consumption was provided by Sinclair and Lindros (1981). By manipulating blood acetaldehyde levels with the ALDH inhibitor calcium cyanamide (Consbruch and Derwart, 1968; Ferguson, 1956) and the ADH inhibitor 4-methylpyrazole (Lindros, 1975), these authors observed that only the inhibiton of brain ALDH resulted in a suppression of voluntary ethanol consumption. Furthermore, high correlations were found between brain ALDH activity and ethanol consumption similar to those observed by Amir (1977; 1978a;1978b). Lindros and Sinclair (1981) suggested that the suppression of ethanol consumption by these agents was unrelated to peripheral aversion but rather to a central regulating mechanism.

The Psychopharmacological Effects of Acetaldehyde

The peripheral accumulation of acetaldehyde has long been known to be extremely toxic (Hald and Jacobsen, 1948; Jacobsen, 1952) and in fact, this toxicity has become the basis of a treatment model for

alcoholism (Sellers, Naranjo and Peachey, 1981). Yet, at present little is known regarding the central actions of acetaldehyde. In an attempt to investigate the central effects of acetaldehyde on behavior, Brown and his colleagues (1978) observed that while intraperitoneal injections resulted in a pronounced conditioned taste aversion, intraventricular infusions failed to induce any such effect. In subsequent experiments by the same group, it was observed that naive rats would learn to self-administer acetaldehyde directly into the cerebral ventricles, whereas ethanol was not self-administered via this route (Amit, Brown and Rockman, 1977; Brown, Amit and Rockman, 1979). More recently, it has been reported that animals self-injected acetaldehyde intravenously (Myers, Ng and Singer, 1982). These results suggest that acetaldehyde may have both aversive and reinforcing properties (Eriksson, 1980). It was also proposed that acetaldehyde, rather than ethanol itself, may mediate the positive reinforcing properties of ethanol in the brain. Additional evidence in support of this notion was provided by the observation that the propensity to self-administer acetaldehyde by rats was positively correlated with subsequent ethanol preference (Brown, Amit and Smith, 1980a). These data suggest that the

central mechanisms mediating the reinforcing effects of acetaldehyde may also subserve the pharmacologically reinforcing properties of ingested ethanol.

To examine this hypothesis further, disulfiram (tetraethylthiuram disulfide), citrated calcium carbamide or a placebo was administered to human subjects prior to the ingestion of ethanol. The primary action of these two compounds is to inhibit ALDH causing an elevation in blood acetaldehyde levels (Hald and Jacobsen, 1948; Consbruch and Dewart, 1968; Ferguson, 1956). At low doses of ethanol, there was no observable behavioral effects in the placebo treated subjects, however, there was enhanced euphoria and stimulation in the disulfiram and calcium carbamide treated subjects. Blood ethanol levels were shown to be similar in all three groups, whereas, blood acetaldehyde levels were significantly higher in the two drug treated groups. It was suggested that the elevations in acetaldehyde levels may have produced the central effects observed (Amit, Brown, Amir, Smith and Sutherland, 1980; Brown, Amit, Smith and Sutherland, in press).

The Action of Acetaldehyde on the Metabolism of the Central Catecholamines

The effects of high acetaldehyde levels in the blood resulting in vasodilation, changes in heart rate, decreased blood pressure, dizziness, nausea, vomiting and respiratory depression (Hald and Jacobsen, 1948; Jacobsen, 1952; Raby, 1953; Walsh, 1971) have been related to a concomitant release of neuroamines in the periphery (Eade, 1959; Perman, 1958; Schneider, 1971; Truitt and Duritz, 1967). While there are many studies examining the effects of ethanol on the metabolism of biogenic amines in the brain, there are few reports regarding acetaldehyde's own actions. It was shown that inhalation of acetaldehyde for two days by mice resulted in an increase in the concentration of brain CA (Ortiz, Griffiths and Littleton, 1974). These results were similar to those observed for ethanol, however continuous inhalation of ethanol vapor for 8 days was necessary in order to have the same magnitude of effect (Ortiz et al., 1974). Similarly, injections of acetaldehyde produced alterations in the CA content of rat brains (Duritz and Truitt, 1966) and it was shown to be more effective than ethanol in causing changes in the metabolism of CA (Thadani and Truitt, 1977).

The alterations in the metabolism of central CA by

acetaldehyde may be the result of competitive inhibition of ALDH and to a reduced availability of the NAD cofactor during ethanol oxidation. This may cause a shift of biogenic amine metabolism from the oxidative to a reductive pathway (Davis, Brown, Huff and Cashaw, 1967a; 1967b; Lahti and Majchrowicz, 1969; Walsh, Truitt and Davis, 1970). Given these effects of acetaldehyde on the central CA and the available evidence suggesting that acetaldehyde may be present in the cerebrospinal fluid (Kiianmaa and Virtanen, 1977; Petterson and Kiessling, 1977) and in the interstitial fluid of the brain (Westcott et al., 1980) following ethanol administration, it is conceivable that acetaldehyde may mediate some of the central effects of ethanol.

Acetaldehyde and Catecholamine Interactions

It has been reported that aldehydes, including acetaldehyde, are capable of condensing with biogenic amines, via a Pictet-Spengler reaction, to form alkaloids (Cohen, 1976; Rahwan, 1975). These condensation products called tetrahydroisoquinoline alkaloids (TIQ) have been observed in rat brain homogenates (Davis and Walsh, 1970; Walsh, Davis and Yamanaka,

1970) and in acetaldehyde perfused cow adrenals (Cohen and Collins, 1970; Cohen, 1971). A number of investigators have demonstrated that these TIQ alkaloids possess many transmitter-like properties (Cohen, 1978). It was observed that they are taken up and stored by CA neurons (Cohen, Mytilineou and Barrett, 1972; Locke, Cohen and Dembiec, 1973) and as a result, the reuptake of naturally occurring CA is inhibited (Heikkila, Cohen and Dembiec, 1971; Tuomisto and Tuomisto, 1973). Furthermore, TIQ alkaloids have been shown, via electron microscopy, to be stored in CA synaptic vesicles (Tennyson, Cohen, Mytilineou and Heikkila, 1973). It has also been demonstrated that TIQ alkaloids competitively inhibit the enzymatic breakdown of CA by monoamine oxidase and catechol-O-methyl transferase (Collins, Cashaw and Davis, 1973; Giovine, Renis and Bertolino, 1976; Cohen and Katz, 1975). The importance of these findings is that the metabolic disposition of the neural system may be altered by the presence of TIQ alkaloids. As indicated above TIQs can be stored in the synaptic vesicles but they can also be released into the synapse by electrical or chemical stimulation (Greenberg and Cohen, 1973; Rahwan, O'Neill and Miller, 1974) and can activate receptors (Mytilineou, Cohen and Barrett, 1974). Given the

neurochemical properties of TIQ alkaloids, it is possible that they may act as "false transmitters" and that they may contribute to some of the neurophysiological effects of acetaldehyde and ethanol consumption.

Tetrahydroisoquinoline alkaloids can be formed in the periphery; however they do not readily cross the blood-brain barrier (Rahwan, 1975) and thus it seems unlikely that peripherally-formed TIQs would contribute to the central effects of ethanol. It has been reported that under certain conditions these alkaloids may occur in brain tissue. When blood levels of acetaldehyde were elevated following ethanol administration with pyragallol, a catechol-O-methyl transferase inhibitor, and pargyline, a monoamine oxidase inhibitor, small quantities of salsolinol (a dopamine-acetaldehyde condensate) were detected in brain tissue (Collins and Bigdeli, 1975). This is in contrast to O'Neill and Rahwan (1977) who failed to measure any salsolinol in the brains of mice that were ethanol-dependent following chronic exposure to ethanol vapor. Tetrahydropapaveroline (a dopamine-dopaldehyde condensate) was observed following L-DOPA treatment in conjunction with ethanol administration (Sandler, Carter, Hunter and Stern, 1973; Turner, Baker, Algeri, Frigenio and

Garattini, 1974), however this finding was not confirmed by others (Collins and Bigdeli, 1975). In addition, 6-methoxysalsolinol was detected in striatal tissue of mice following chronic ethanol exposure (Hamilton, Blum and Hirst, 1978) and more recently, using gas chromatographic/mass spectrometric methods, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (dopamine-formaldehyde condensate) was detected as a normal constituent of rat brain (Barker, Monti, Tolbert, Brown and Christian, 1981). Also, levels of salsolinol were measured in post mortem human brain of alcoholics and in control cases as well (Sjoquist, Eriksson and Winblad, 1982) indicating that endogenously formed salsolinol may be normally present. Since TIQ alkaloids are neurochemically active compounds, it is conceivable that they may play a functional role in the mediation of the pharmacological actions of ethanol.

It has been suggested that TIQ alkaloids derived from acetaldehyde and the biogenic amines may be involved in ethanol dependence (Blum, Hamilton, Hirst and Wallace, 1978; Cohen, 1976; Rahwan,1975). Intra-cerebral infusions of TIQs have been shown to alter the severity of ethanol withdrawal symptoms in mice in a dose-related fashion (Blum, Eubanks, Wallace, Schwertner and Morgan, 1976). Azevedo and Osswald

(1977) have shown neurotoxin-like degradation of adrenergic nerve terminals in rats and it has been suggested that this may contribute to ethanol withdrawal.

As with other areas of TIQ research, there is also conflicting evidence linking TIQ effects with opiate receptors. Davis and Walsh (1970) proposed that tetrahydropapaveroline (THP), which is the intermediate in the biosynthesis of morphine in the poppy plant (Kirby, 1967; Shamma, 1972) may be the underlying mechanism for alcoholism. This notion was severly critisized based on the inability, at that time, to detect THP in vivo (Halushka and Hoffman, 1970) and on the lack of similarity between ethanol and morphine withdrawal (Seevers, 1970). Salsolinol has been reported to possess morphine-like properties in the opiate-like guinea pig ileum preparation (Hamilton, Hirst and Blum, 1979), however, this action was not attributed to an interaction with opiate receptors (North, Collins, Milner, Karras and Koziol, 1981).

There have been a number of studies attempting to provide direct evidence for the involvement of acetaldehyde and biogenic amine condensation products in the central mediation of the psychopharmacological effects of ethanol. Myers and his co-workers found

that during and following intraventricular infusions of tetrahydropapaveroline, salsolinol and a variety of other alkaloid derivatives, ethanol intake increased markedly compared to control rats infused with artifical cerebrospinal fluid (Melchior and Myers, 1977; Myers and Melchior, 1977a; 1977b; Myers and Oblinger, 1977; Myers and Hoch, 1979). In many cases, a sevenfold increase was reported lasting several months following the termination of the TIQ infusions and withdrawal symptoms from the alkaloids alone were described. The results raise many questions regarding the possible role of acetaldehyde and isoquinoline alkaloids in the mediation of voluntary ethanol consumption, however the techniques employed in these studies have been called into question. Repeated attempts to replicate earlier findings of Myers (Myers, 1963; Myers, Veale and Yaksh, 1972), using the same techniques have failed (Cicero and Smithloff, 1973; Friedman and Lester, 1975; Jones, Essig and Creager, 1970; Koz and Mendelson, 1967).

In a series of experiments using the same compounds, Brown, Amit and Smith (1980b) failed to induce an enhancement in voluntary ethanol drinking. The differences in the findings were attributed to procedural inconsistences between the two sets of studies

(Deitrich and Erwin, 1980). Brown and co-workers (1980) utilized rats that were consuming a constant concentration of ethanol for an extended baseline period, whereas, Myers (Melchior and Myers, 1977; Myers and Melchior, 1977a; 1977b; Myers and Oblinger, 1977). presented a single series of increasing ethanol concentrations before and during the TIQ alkaloid administration. At present, only one study reports data similar to that of Myers, and even here, it is only a partial replication (Duncan and Deitrich, 1980). It was reported that small increases in ethanol intake can be observed following infusions of THP, but in contrast to Myers, this finding was only observed using very low doses. At higher levels of THP there is a tendency for rats to decrease their intake (Duncan and Deitrich, 1980).

In summary, the limited evidence available suggests that acetaldehyde may play a role in the mediation of the reinforcing effects of ethanol. The nature of its interaction with the catecholamines, either direct or via condensation products, remains to be elucidated. The present investigation attempts to explore the relative contributions of acetaldehyde, the biogenic amine, norepinephrine, and their possible

interactions in the mediation of some of the psychopharmacological effects of ethanol.

Given that acetaldehyde appears to be a self-administered drug, the purpose of section A was to determine whether central administration could induce a conditioned place preference. The goal of section B was to systematically examine the effects of ethanol on noradrenergic activity and in section C, a possible mode of interaction between brain acetaldehyde and norepinephrine was investigated.

Section A

Conditioned Place Preference Induced by Intracerebroventricular Administration of Acetaldehyde

Experiment 1

Acetaldehyde, the primary metabolic product of ethanol, has been implicated in several of the pharmacological properties of alcohol (Amir, Brown and Amit, 1980). It has been shown that peripheral accumulation of acetaldehyde is toxic (Hald and Jacobsen, 1948; Jacobsen, 1952), yet little is known of acetaldehyde's actions within the brain. Previous research demonstrated that animals can learn to press an operant lever in order to receive intracerebroventricular (ICV; Brown, Amit and Rockman, 1979; Smith, 1980) and intravenous injections of acetaldehyde (Myers, Ng and Singer, 1982). It was also shown that the rate of ICV self-administration was related to subsequent voluntary ethanol consumption (Brown, Amit and Smith, 1980). On the basis of these results it was suggested that acetaldehyde may have reinforcing properties and that it may act, at least in part, as a mediator of ethanol consumption (Amit, Brown, Rockman, Smith and Amir, 1980).

Despite these positive findings, however, attempts to demonstrate other central stimulus properties of acetaldehyde in different paradigms have failed. Peripheral injections of acetaldehyde were shown to

produce a strong conditioned taste aversion, whereas, central infusions were not effective in inducing taste aversions (Brown, Amit, Smith and Rockman, 1978). In addition, intraventricular administration of acetaldehyde failed to generalize to ethanol-like effects in a discriminative stimulus paradigm (Altshuler and Shippenberg, 1982).

An alternative procedure for the study of drug reinforcement is place conditioning (Mucha, Van Der Kooy, O'Shaughnessy and Bucenieks, 1982). Subjects are treated by explicitly pairing distinctive neutral environmental cues with the presentation of an unconditioned stimulus (UCS). The subjects are later given an opportunity to spend time in the presence of these environmental cues and the behavior of the subjects toward these cues provide information on the nature of the UCS.

Place conditioning has been demonstrated using irradiation as the UCS as rats show a clear aversion to environmental cues paired with exposure to irradiation (Garcia, Kimeldorf and Hunt, 1957). Conditioned place preferences have also been shown to occur following intravenous administrations of morphine and cocaine (Mucha et al., 1982), as well as following central infusions of morphine (Katz and Gormezano, 1979; Van

Der Kooy, Mucha, O'Shaughnessy and Bucenieks, 1982; Phillips and LePiane, 1980) and heroin (Bozarth and Wise, 1981).

These studies demonstrate that self-administered drugs are capable of producing a conditioned place preference suggesting that place conditioning may be an adequate technique for studying the reinforcing properties of psychoactive drugs. Given that acetaldehyde also appears to be a self-administered drug, the purpose of the present investigation was to determine whether central administration could induce a conditioned place preference.

Method

Subjects

Fifteen male Sprague-Dawley rats (Charles River Canada) weighing 250-275 gm at the start of the experiment were used. Animals were individually housed in stainless steel cages (24 cm x 19 cm x 17 cm) in a room controlled for constant temperature and humidity and a 12 hour light-dark cycle. Food and water were available ad libitum throughout the experiment in the home cage.

Surgery

Under anaesthesia (sodium pentobarbitol; 60 mg/kg

IP), a 22-guage stainless steel cannula guide (Plastic Products, Inc.) was chronically implanted into the left lateral ventricle of each animal using stereotaxic procedures. The following coordinates were used: incisor bar set at zero, with the tip of the cannula guide positioned 1 mm posterior to bregma, 1.5 mm lateral to the midsagittal suture and 3.6 mm ventral to the dura. The cannula guide was secured in position on the skull with cranioplast cement and five stainless steel screws. A stainless steel stilette was inserted into the guide to keep it free from obstruction.

Apparatus

The experimental chamber consisted of a narrow box (60 cm x 15 cm x 20 cm), which was divided in half. One side of the box had metal walls with a plain wooden floor while the opposite side had black stripe painted wooden walls with a wire mesh covered wooden floor. A partition constucted with walls similar to those of each side of the box could be placed in the middle of the chamber to divide it into two different boxes. Each side was equiped with an infusion apparatus consisting of a pump (Razel Inc.) connected via polyethylene tubing to a flow-through swivel (Brown, Amit and Weeks, 1976) which was suspended above the chamber. A shielded plastic tube connected to the outlet of the

swivel terminated in a 28-guage internal cannula.

Procedure

The following procedure was adapted from that used by Cunningham (1979) and Stewart and Grupp (1981). After 4 to 5 days recovery from surgery, the animals received ten conditioning trials of five minutes duration once per day. The animals were connected to the infusion apparatus and confined to one side of the chamber where they received a 20 ul infusion over the first thirty seconds. Five trials consisted of a single acetaldehyde infusion (20 ul of 2% v/v = 320 ug) paired with exposure to one side and five trials of a saline infusion paired with exposure to the other side. The choice of dose of acetaldehyde was based on previous work examining its effects following central administration (Brown et al., 1978). These drug and non-drug pairings were randomly alternated throughout the ten days with the restriction that no more than three consecutive trials of the same pairing occurred. In order to control for the influence of a possible side preference, irrespective of drug effects, half the animals received drug pairings on the wire mesh side while the rest had drug pairings on the wood floor side. At the conclusion of the conditioning period, the animals were given access to the full chamber for

five minute sessions, once daily, for three days and the time spent on each side was recorded.

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

Results

Figure 1 shows the amount of time spent on the drug and non-drug (saline) sides during the three test days. A two-way analysis of variance revealed that there were no differences in the time spent on each side ($F(1,70) = 1.856$, $p>.05$) and no interaction between possible drug paired preference and trials ($F(2,70) = .205$, $p>.05$).

Figure 2 shows the amount of time spent on each side of the chamber, irrespective of the drug infused. It can clearly be seen that the animals have a strong preference for the wire mesh side of the chamber throughout the three test sessions. A two-way analysis of variance yielded a significant main effect of side preference ($F(1,70) = 55.776$, $p<.0001$). No significant interaction observed ($p>.05$).

Histological examination confirmed the location of

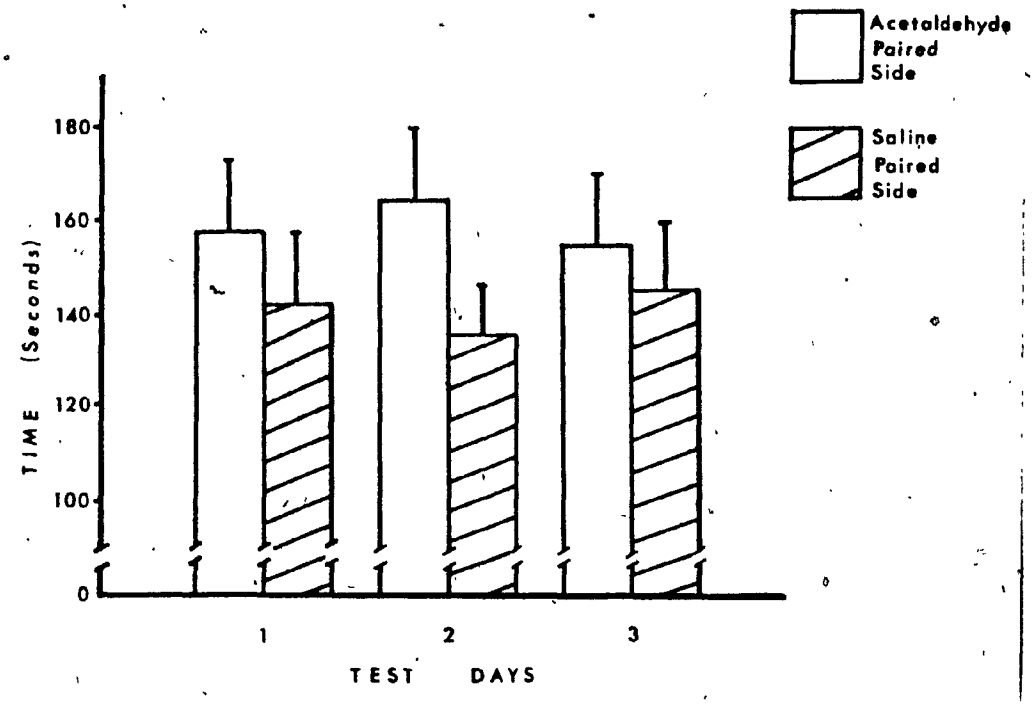


FIGURE 1. Mean time spent on drug and non-drug (saline) sides following conditioning sessions with acute drug infusions. Vertical lines represent the S.E.M.

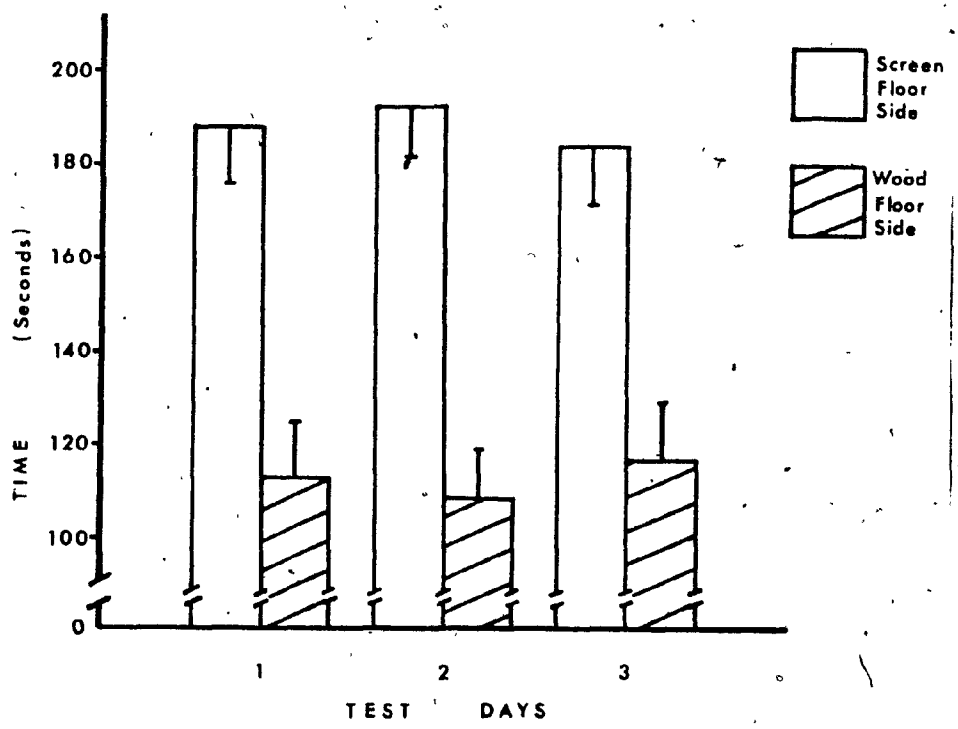


FIGURE 2 Mean time spent on each side of the chamber, irrespective of drug infused, following conditioning with acute drug infusions. Vertical lines represent the S.E.M.

the cannula guide in the lateral ventricle of each animal.

Discussion

The results of the present experiment indicate that a single intracerebroventricular (ICV) infusion of acetaldehyde failed to induce place conditioning as animals developed neither a preference nor an aversion to the acetaldehyde paired side. This failure to observe a conditioning effect with an acute central acetaldehyde infusion corresponds to previous research showing an absence of an acetaldehyde induced conditioned taste aversion (Brown et al., 1978). The present findings did indicate that the animals displayed a strong preference for the wire mesh floor side. This was true regardless of the drug paired with this side.

One possible explanation for the lack of place conditioning is the extremely rapid elimination rate of acetaldehyde from the brain (Akabane, 1970; Westcott, Weiner, Shultz and Myers, 1980). It is probable that a 30 second infusion may not have been of sufficient duration to produce conditioning effects. The duration of action of an unconditioned stimulus has been demonstrated to be an important factor in determining its

effectiveness as a conditioning agent (Goudie and Dickins, 1978). It is possible that place conditioning would have taken place if the duration of exposure to acetaldehyde would have been increased.

Experiment 2

In the previous experiment it was observed that acute intraventricular infusions of acetaldehyde failed to induce place conditioning. It was suggested that this lack of effect may be due to its short duration of action (Akabane, 1970; Westcott et al., 1980) as the length of exposure to the unconditioned stimulus has been demonstrated to be an important factor in conditioning studies (Goudie and Dickins, 1978). The purpose of the present experiment was to examine the effect of multiple intraventricular infusions of acetaldehyde on place conditioning.

Method

Subjects

Thirteen male Sprague-Dawley rats (Charles River Canada) weighing 250-275 gm were individually housed in stainless steel cages in a room regulated for constant temperature and humidity and a 12 hour light-dark cycle. Food and water were available ad libitum in the home cage.

Using the identical procedures outlined in the previous experiment, a stainless steel cannula guide was chronically implanted into the left lateral

ventricle of each animal.

Procedure

The apparatus and experimental procedure were identical to that employed in the previous study with the exception that a series of multiple infusions were delivered during each conditioning pairing trial. These pairings consisted of eight 20 second infusions occurring every 40 seconds during the five minute trial. Each individual infusion delivered 8 ul for a total volume of 64 ul over the five minutes. Acetaldehyde administration (1% v/v; 64 ug / 20 sec.) was paired with one side and saline infusions of similar volumes were paired with the opposite side. The concentration of acetaldehyde and the volume were based on data from research examining the reinforcing properties of acetaldehyde (Brown et al., 1979; Smith, 1980).

The group was split such that each half received acetaldehyde pairings on opposite sides of the chamber during the conditioning phase. The animals were given access to the entire chamber for three test sessions during which the time spent on each side was recorded.

At the termination of the experiment, the animals were sacrificed and perfused intracardially with saline and 10% formalin. The brains were extracted and sliced into 40 u coronal sections.

Results

Figure 3 shows the amount of time spent on the drug and non-drug (saline) paired sides during the three test days. It appeared that the animals tended to spend more time on the drug paired side during the second and third test days, however, this was not significant as there was no main effect of drug preference ($F(1,60) = .80$, $p>.05$). Great variability in the time spent on each side was observed (figure 3) and this was reflected in the lack of interaction between drug preference and trials ($F(2,60) = 2.24$, $p>.05$).

Figure 4 shows the amount of time spent on each side of the chamber regardless of the drug pairings and it can be seen that there is no consistent preference for either side. This was confirmed with a two-way analysis of variance indicating no main effects of side preference or interaction ($p>.05$).

Histology confirmed the placement of the cannula guide in each animal. It also showed that the infusion procedure did not result on any noticable ventricular expansion.

Discussion

The use of multiple infusions of acetaldehyde in

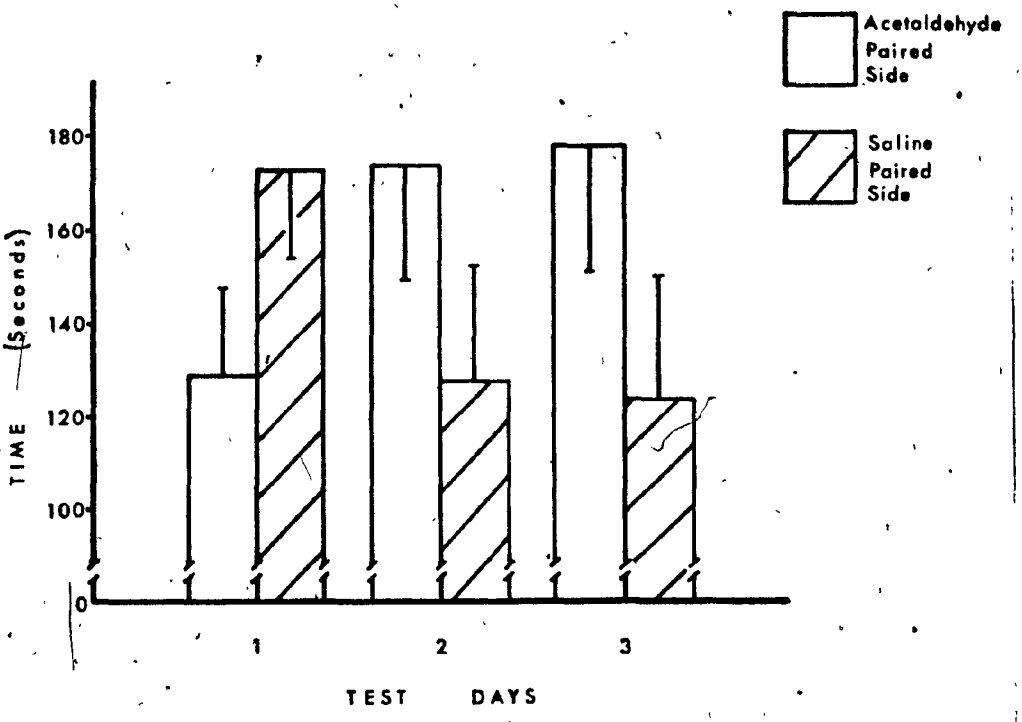


FIGURE 3 Mean time spent on drug and non-drug (saline) sides following conditioning with multiple drug infusions. Vertical lines represent the S.E.M.

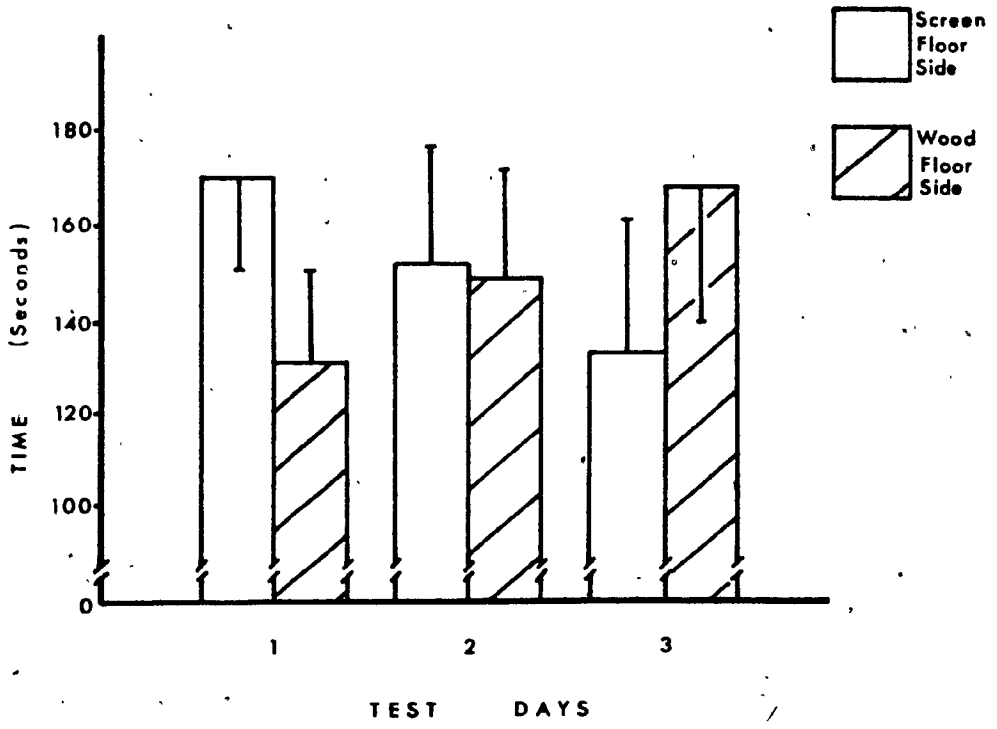


FIGURE 4 Mean time spent on each side of the chamber, irrespective of drug infused, following conditioning with multiple drug infusions. Vertical lines represent the S.E.M.

the present experiment failed to demonstrate a significant place conditioning effect. These results were similar to those observed in the first study using an acute administration. However, contrary to the general lack of variability observed in the previous experiment, the current results demonstrate a wide fluctuation in the behavior of rats receiving multiple infusions within and across test days. In addition, there was no consistent preference for any particular side of the chamber as was observed in the previous experiment. It would appear that the influence of a natural preference for one side of the chamber was inhibited by increasing the duration of exposure to acetaldehyde during the conditioning trials.

Central administration of acetaldehyde may be a weak conditioning agent and in the present paradigm, where there are only five drug trials over ten days, the ability of intraventricularly infused acetaldehyde to induce strong place conditioning may be decreased. This may be an important factor given the inherent variability of behavioral responses to acetaldehyde's central actions and indeed to ethanol in general (Brown, Amit and Smith, 1980a).

The present results suggest that increasing the duration of exposure to central infusions of acet-

aldehyde may produce alterations in the behavior of animals in a place conditioning paradigm. Although the findings do not demonstrate a place preference or aversion, the increased variability and the inhibition of natural side preference do indicate a change in the nature of the stimulus properties associated with the environmental cues. The present paradigm, however, does not appear to be sufficiently sensitive to determine the qualitative nature of these changes.

Experiment 3

The previous attempts to demonstrate place conditioning with intraventricular infusions of acetaldehyde have been unsuccessful in producing a preference or an aversion. In the last experiment, it was observed that multiple administrations of acetaldehyde resulted in an alteration of the behavioral response of rats to the paradigm, however, the nature of these changes remained undetermined. One factor considered was the increased variability in the behavioral response of the animals, which may have been due to the central actions of acetaldehyde or to the experimental procedure employed. In the present experiment an alternative method, previously used by several investigators (Katz and Gormezano, 1978; Phillips and LePiane, 1980), was employed in an attempt to decrease the influence of this variability.

Method

Subjects

Twenty-six male Sprague-Dawley rats (Charles River Canada) weighing 250-275 gm were individually housed under conditions described in the preceeding experiments. A stainless steel cannula guide was chronically

implanted into the left lateral ventricle using the procedures outlined in the earlier studies.

Procedure

The experimental chambers and the infusion apparatus were identical to that used in the initial experiments. After 4 to 5 days recovery from surgery, the animals were given access to the entire chamber for five minutes once per day for three days. The amount of time spent on each side was recorded.

Following this pre-exposure, the non-prefered side for each animal was identified and they were assigned to one of two groups such that the mean time spent for the last two days on the non-prefered side was approximately equal. Five conditioning trials followed (five minutes duration, once per day), during which one group received acetaldehyde infusions (1% v/v; 8 ul / 20 sec / 40 sec) while the other group received saline infusions. All drug administrations occurred in the non-prefered side for each individual animal. Animals were then given access to the entire chamber for five minutes. The time spent on the drug paired side (non-prefered) was recorded.

Animals were subsequently sacrificed and perfused with saline and 10% formalin. The brains were extracted and sliced into 40 u coronal sections.

Results

A t-test for independent samples indicated that there were no differences in the amount of time spent on the non-prefered side between the acetaldehyde and saline infused groups during the baseline period ($t(24) = .96$, $p > .05$). Figure 5 shows the mean difference from baseline for the two groups during the three test days. It can be seen that the animals receiving intraventricular acetaldehyde increased the time spent on the conditioning side more than the saline treated animals. A two-way analysis of variance revealed a significant main effect of drug treatment ($F(1,24) = 6.01$, $p < .05$) and insignificant effects of test trials ($F(2,24) = .10$, $p > .05$) and interaction ($F(2,48) = .002$, $p > .05$).

Histological examination confirmed the placement of the cannula guide in the left lateral ventricle of each animal, as well, no detectable differences could be seen between the brains of acetaldehyde and saline treated animals.

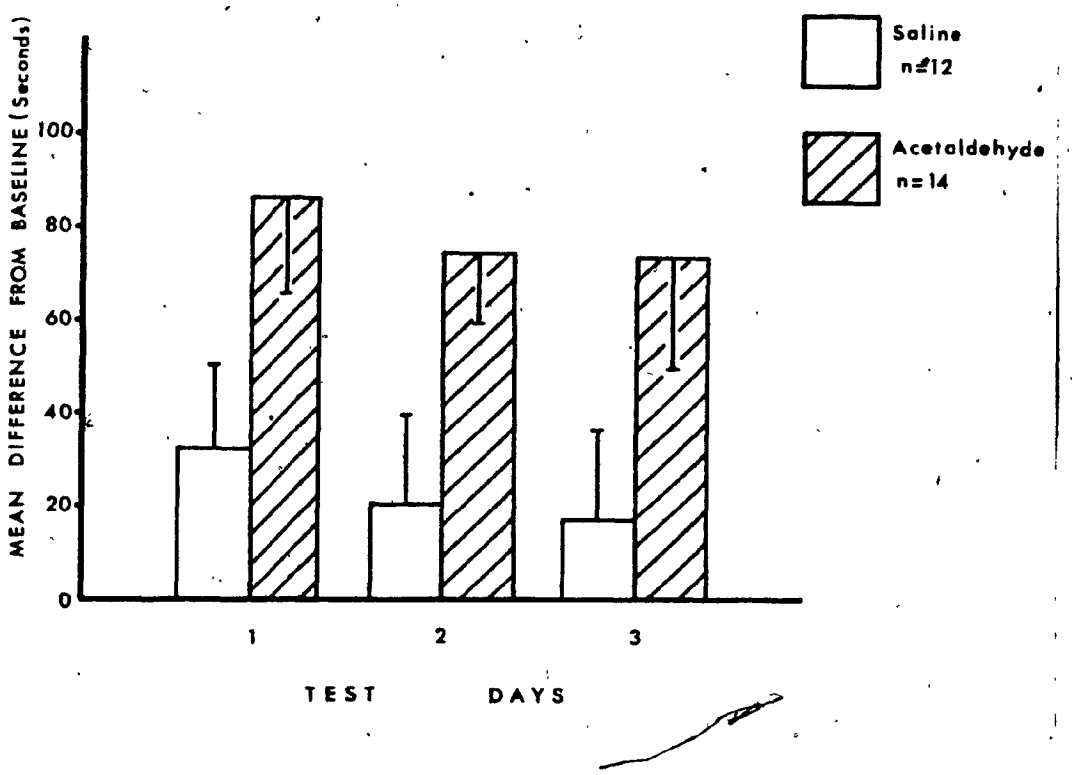


FIGURE 5 Mean difference from baseline for acetaldehyde and saline treated animals during three test sessions. Vertical lines represent the S.E.M.

Discussion

The present results indicate that intraventricular infusions of acetaldehyde are capable of inducing a conditioned place preference. Simple exposure to the conditioning procedure was seen to decrease the natural aversion to one side of the chamber which was observed in the saline group, however, the acetaldehyde treated animals demonstrated a significantly greater change in preference which lasted for the three test days. By using a change from baseline measure, the present study eliminated the influence of variability in responding of the animals in this procedure. The findings suggest that the previous method (experiment 2) was not of sufficient sensitivity to demonstrate the reinforcing properties of centrally administered acetaldehyde.

The results support the notion that acetaldehyde, when present in the brain, has positively reinforcing properties (Brown, Amit and Rockman, 1979). The dose level used in the present experiment is of particular importance in that the concentration of acetaldehyde was identical to that which previously had been shown to support self-administration (Smith, 1980). The eight infusions delivered during each five minute conditioning session represented the equivalent of 16 lever press infusions which was approximately half of

the mean daily rate of self-administering rats. (Brown, Amit and Smith, 1980a; Smith, 1980).

The multiple infusion technique may be of critical importance in any conditioning experiment with centrally administered acetaldehyde. The rapid elimination rate of acetaldehyde from the brain (Akabane, 1970; Westcott et al., 1980) indicates that any psychpharmacological effect will be of extremely short duration. The duration of the unconditioned stimulus has been shown to be an important factor in conditioning studies (Goudie and Dickins, 1978) and this may account for the failure to observe acetaldehyde induced conditioned taste aversion (Brown et al., 1978) or generalization in ethanol induced discriminative stimulus experiments (Altshuler and Shippenberg, 1982). Both of these studies utilized an acute administration of acetaldehyde, and a more continuous or repeated form of administration may have yielded positive results.

The ability of acetaldehyde to induce place conditioning is in concordance with other research demonstrating that self-administered drugs can produce a conditioned place preference (Katz and Gormezano, 1979; Phillips and LePiane, 1980; Bozarth and Wise, 1981; Mucha et al., 1982; Van Der Kooy et al., 1982). This suggests that this procedure may be an adequate tech-

nique for studying the motivational properties of self-administered substances.

An advantage of the place conditioning paradigm can be seen in the fact that the conventional self-administration paradigm is highly susceptible to the motor debilitating effects of various manipulations, however, the place conditioning procedure is relatively immune to these effects. The animals are tested drug-free, thereby enabling the use of a wide range of pharmacological tools to investigate the underlying mechanisms of drug reinforcement.

Section B

The Effect of Ethanol on Noradrenergic Activity in Rat Brain

Experiment 1

In addition to the research examining the possible involvement of acetaldehyde in the actions of ethanol, the central catecholamines have also been implicated in the mediation of some of the psychopharmacological effects of alcohol. In particular, emphasis has been placed on the involvement of norepinephrine (NE) in mediating those effects. Manipulations of central catecholamines, with the neurotoxin 6-hydroxydopamine, demonstrated the importance of NE in the mediation of voluntary ethanol consumption (Brown and Amit, 1977; Mason, Corcoran and Fibiger, 1979; Kiianmaa, 1980). As well, blockade of NE synthesis has been shown to attenuate ethanol self-administration (Davis, Smith and Werner, 1978; Davis, Werner and Smith, 1979) and voluntary ethanol consumption (Amit, Brown, Levitan and Ogren, 1977).

Studies examining the effects of ethanol on NE activity have yielded differential findings. Several reports have indicated that alcohol increased the activity (Corrodi, Fuxe and Hokfelt, 1966; Hunt and Majchrowicz, 1974) and synthesis of brain NE (Carlsson, Magnusson, Svensson and Waldeck, 1973), while others have reported a decrease in NE turnover following

ethanol administration (Pohorecky and Jaffe, 1975; Thadani and Truitt, 1973; Thadani, Kulig, Brown and Beard, 1976). Most of these studies have used various doses of ethanol with biochemical measurements taken at single time intervals, thus it is possible that these apparent opposite effects may be related to ethanol's dose-dependent biphasic actions (Pohorecky, 1977) as well as possible time-dependent effects (Hunt and Majchrowicz, 1974; Pohorecky, 1977).

The accumulation of brain 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO_4), a NE metabolite, was shown to be an accurate index of central noradrenergic activity (Korf, Aghajanian and Roth, 1973; Karasawa, Furukawa, Ochi and Shimizu, 1978). It has also been shown that MHPG-SO_4 concentration in lumbar cerebrospinal fluid was elevated in healthy intoxicated humans (Borg, Kvande and Sedvall, 1981) providing further evidence for ethanol's influence on brain noradrenergic mechanisms.

The purpose of the present study was to provide a systematic analysis of the time-dependent actions of ethanol on central noradrenergic activity through the measurement of brain MHPG-SO_4 and blood ethanol at various time intervals following acute ethanol administration.

Method

Subjects

Male Wistar rats (Charles River Canada) weighing 200-225 gm were used in the experiment. The animals were individually housed in stainless steel cages in a room regulated for constant temperature and humidity and a 12 hour light-dark cycle. Food and water were available ad libitum. The animals were given five days to acclimatize to the colony conditions and were handled daily prior to the start of the experiment.

Procedure

Each animal received an intraperitoneal (IP) injection of either 2 gm/kg of ethanol (8.5 ml/kg of 30% v/v) or Ringer's solution. The animals were sacrificed 15, 30, 60 or 120 minutes following the IP injection, by decapitation. Trunk blood was collected for gas chromatographic analysis of blood ethanol levels using the procedures outlined below. The brains were rapidly extracted, rinsed in ice cold saline and then frozen on dry ice. The brain samples were stored at -70°C then fluorometrically assayed for MHPG-SO_4 levels.

Determination of MHPG-SO_4

The procedure used was similar to the fluorometric

method described by Kohno, Matsuo, Tanaka, Furukawa and Nagasaki (1979). Briefly, whole brains were homogenized in 5 volumes of 0.2 N H_2SO_4, containing 0.1% sodium metabisulphite ($Na_2S_2O_5$) as a protective agent against oxidation. The homogenate was then centrifuged at 8000g for 5 minutes in the cold. One portion of the supernatant (approximately 1/6 to 1/8) was transfered into a polycarbonate tube. Tissue blanks and internal standards were prepared from tissue extracts at this time. Each sample was adjusted to pH= 6.0-6.5 with 0.3 N and 0.03 N $Ba(OH)_2$. Following a second centrifugation at 8000g for 10 minutes, the supernatant was decanted into a glass tube and passed through a 0.6 X 6 cm column of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals). The column was washed with 2 ml of distilled water, followed successively by 8 ml of 0.06 N HCl and 1 ml of 0.4 N perchloric acid (PCA). MHPG-SO_4 was eluted from the column with 2 ml of 0.4 N PCA containing 0.1% $Na_2S_2O_5$. Immediately after the separation of MHPG-SO_4, 0.1 ml of 1% cysteine and 0.1 ml of 70% PCA were added to the eluate. These reaction mixtures were heated in an oven at 100°C for 30 minutes. The same reagents were added to the tissue blanks, however, they were not heated. The samples were reheated again for 10 minutes following the

addition of 0.3 ml of freshly distilled ethylenediamine and then they were cooled in a water bath. The fluorescence was read at wavelengths of 325 mm, excitation, and 465 mm, emission, using an Aminco-Bowman spectrophotofluorometer.

Determination of blood ethanol

The procedure used was based on Stowell (1979) with modifications based on reports by Eriksson, Sippel and Forsander (1977). Animal trunk blood was collected into test tubes which contained 150 International Units (IU) of sodium heparin. One ml of blood was mixed with 4 ml of ice cold semicarbazide reagent. This mixture was spun in a refrigerated centrifuge at 400 g to separate blood cells from serum. Two ml of serum were added to 0.5 ml of 3.0 M perchloric acid and then spun at 11,000 g to obtain a clear protein-free supernatant. At this time, 0.5 ml of the supernatant was pipeted into an 8 ml vial, stoppered and stored at -70°C until assayed for ethanol by head-space gas chromatography (Eriksson et al., 1977).

Results

Figure 6 represents the effects of an intraperitoneal injection of ethanol on 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO_4) accumulation

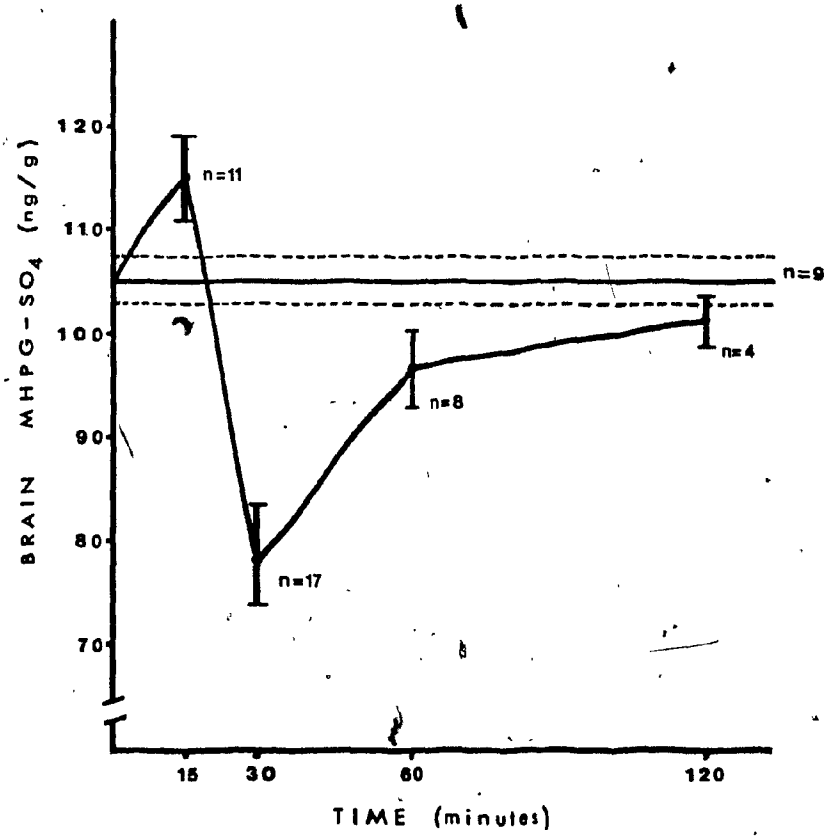


FIGURE 6 Effect of ethanol on MHPG-SO_4 levels in rat brain at various time intervals following IP injection. Horizontal line represents control levels. Dotted and vertical lines represent the S.E.M.

in rat brain. A one-way analysis of variance revealed a significant change in the levels of MHPG-SO_4 following ethanol administration ($F(4,44)= 15.65, p<.001$).

Dunnett's Test for comparisons involving a control mean (Dunnett, 1955) demonstrated that there was a significant decrease in MHPG-SO_4 levels 30 minutes following the ethanol injection. This effect was not seen at 60 or 120 minutes as the levels of MHPG-SO_4 had returned to control values at these times. As can be seen in Figure 6, there appeared to be an increase in MHPG-SO_4 levels at 15 minutes, however, it was not statistically significant.

The levels of ethanol in blood following a 2 gm/kg injection can be seen in Figure 7. A one-way analysis of variance indicated that there was a significant alteration in the levels of ethanol over the four time periods examined ($F(3,36)= 7.88, p<.001$). Post hoc Tukey tests revealed that peak levels of blood ethanol occured at 30 minutes followed by a slight decline at the 120 minute time sample.

Correlation coefficients were calculated between the levels of MHPG-SO_4 in brain tissue and the level of ethanol in peripheral blood at the various time intervals sampled. The 120 minute time interval was omitted as the number of animals sampled at this time was too

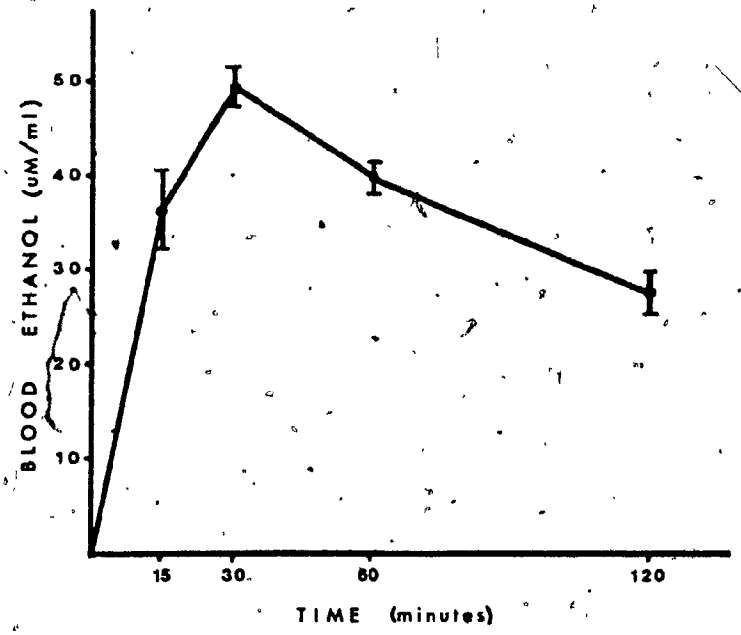


FIGURE 7 Blood ethanol concentrations at various time intervals. Vertical lines represent the S.E.M.

small. Figure 8 shows the scatter plots for the 15, 30 and 60 minute time intervals. The correlations obtained for the three sampling intervals were statistically significant ($p<.05$) suggesting a possible relationship between peripheral blood ethanol levels and central levels of MHPG-SO_4. These associations appear to change in a qualitative manner over the three time intervals sampled as the correlation at 15 minutes was positive while those at 30 and 60 minutes were negative.

Discussion

The present experiment demonstrated that acute ethanol administration can alter MHPG-SO_4 levels in rat brain and that the magnitude of these alterations is highly correlated with levels of circulating blood ethanol. Although the increase in MHPG-SO_4 levels observed at the 15 minute sampling interval was not statistically significant, the nature of the relationships between MHPG-SO_4 and ethanol indicate the presence of a biphasic effect. The significant correlation at 15 minutes was positive, while, those at 30 and 60 minutes were negative indicating a change in the direction of the association between ethanol and MHPG-SO_4. The inability to detect a statistically signi-

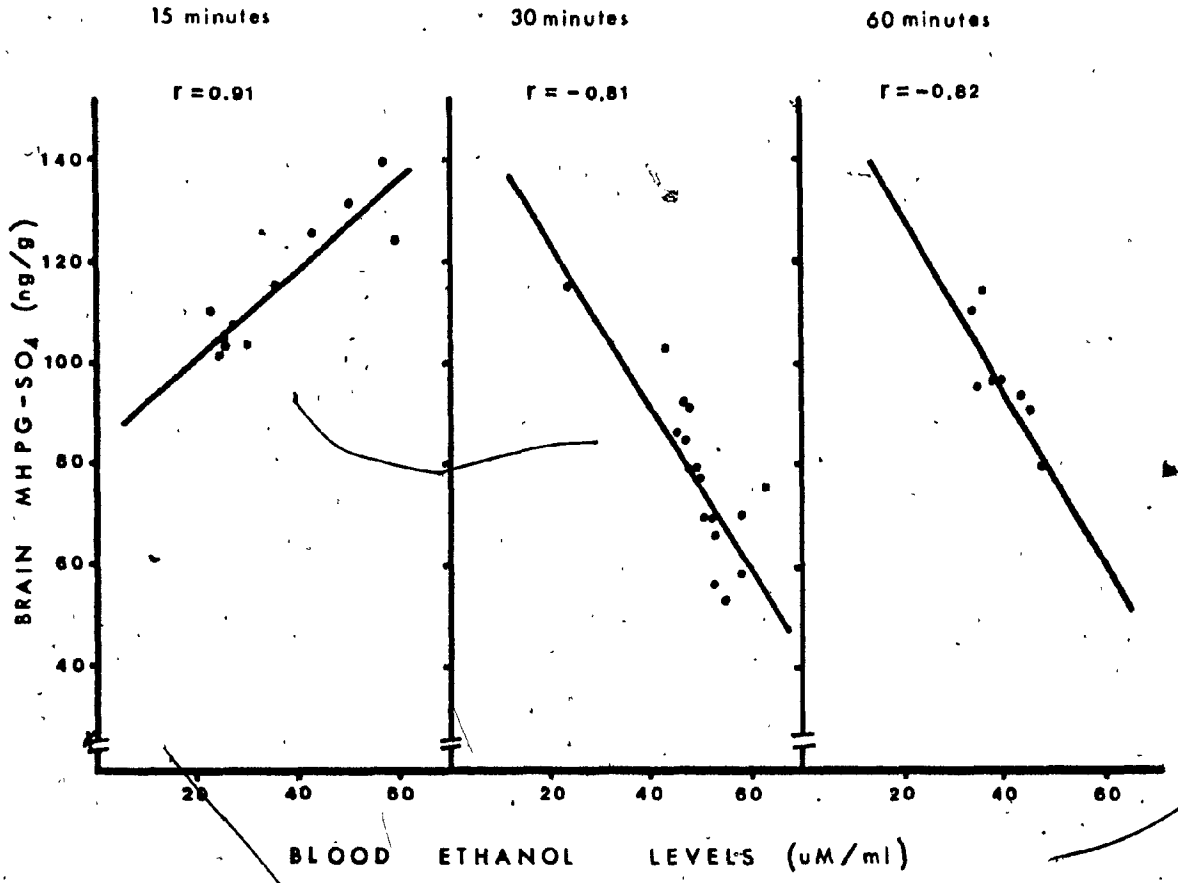


FIGURE 8 Scatter plots and correlation coefficients between brain MHPG-SO_4 levels and blood ethanol concentration at three time intervals.

ficant increase may be due to the time interval selected and perhaps the levels of MHPG-SO_4 may be higher at five or ten minutes folowing ethanol administration.

As indicated earlier, the accumulation of MHPG-SO_4 in brain has been shown to reflect central noradrenergic activity (Korf et al., 1973; Karasawa et al.,1978). The present findings confirm previous results indicating that ethanol seems to exert a biphasic effect on NE (Hunt and Majchrowicz, 1974). The magnitude of this effect appears to be strongly related to the levels of ethanol in the blood, therefore, the nature of the alterations produced by ethanol in the noradrenergic system seem to be not only time-dependent but also dose-dependent. This may account for the apparent divergent results observed by other investigators (Pohorecky and Jaffe, 1975; Thadani and Truitt, 1973; Thadani et al., 1976) as both the dose of ethanol and the time following its administration appear to be important in determining the nature of its effect on NE.

The results also reveal the presence of a highly significant relationship between peripheral blood ethanol levels and their central effect on noradrenergic activity as measured by the accumulation of MHPG-

SO. This was observed at 15, 30 and 60 minutes following ethanol administration. The notion that the observed changes in central NE activity are mediated by peripheral blood ethanol levels is supported by a previous report (Sunahara, Kalant, Schofield and Grupp, 1978) on observed equilibrium between blood and brain ethanol levels within 3 minutes following an IP injection of ethanol.

The mechanism by which ethanol may exert two directly opposite effects remains unclear. The decrease in noradrenergic activity may be a compensatory mechanism in response to the initial increase, perhaps, mediated by changes in receptor sensitivity (Skolnick and Daly, 1976; Starke and Endo, 1976) or due to possible direct effects of ethanol on cell membranes (Kalant, 1975). Recently it has been suggested that ethanol preferentially activates noradrenergic neurons and that this may account for the observed motor stimulation effects of low doses of ethanol. However, as ethanol levels increase there may be greater activation of GABA neurons resulting in a depressant action on motor activity (Liljequist, 1982).

An alternative explanation may conceivably involve acetaldehyde, ethanol's primary metabolite. Acetaldehyde has been implicated in the mediation of

several psychopharmacological actions of ethanol (Amir, Brown amd Amit, 1980). Since acetaldehyde was reported to trigger release of central NE (Thadani and Truitt, 1977) it is possible that some interaction between ethanol and acetaldehyde may account for these biphasic effects. Clearly, further research is necessary in order to elucidate the nature of ethanol's effect on norepinephrine and how these interactions may influence the behavioral response to ethanol.

Experiment 2

The blockade of norepinephrine (NE) synthesis by the dopamine-beta-hydroxylase (DBH) inhibitor, FLA-57, has been shown to attenuate voluntary ethanol consumption (Amit, Brown, Levitan and Ogren, 1977) and intragastric ethanol self-administration (Davis, Werner and Smith, 1979). In addition, depletions of NE by FLA-63 (DBH inhibitor) and FLA-57 were observed to suppress ethanol induced locomotor stimulation (Liljequist, Berggren and Engel, 1981; Brown, Smith and Sinyor, 1978). These findings have led some authors to suggest that NE may mediate the reinforcing properties of ethanol (Amit et al., 1977).

The results of the previous experiment demonstrated that ethanol administration produced a time- and dose-dependent biphasic effect on noradrenergic activity as measured by changes in the accumulation of the noradrenergic metabolite, 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO_4).

The purpose of the present experiment was to investigate the effects of FLA-57 on ethanol induced increase in MHPG-SO_4 accumulation in rat brain.

Method

Subjects

Eighteen male Wistar rats (Charles River Canada) weighing 175-200 gm were individually housed in stainless steel cages in a room regulated for constant temperature and humidity and with a 12 hour light-dark cycle. Food and water were available ad libitum.

Procedure

Following 4 to 5 days for acclimatization to colony conditions, the animals were injected intraperitoneally with either FLA-57 (30 mg/kg) or the vehicle solution. The FLA-57 was prepared by dissolving it in 1N NaOH and then adjusting the pH of the solution to 7.5 - 8.0 with 1N acetic acid. Ringer's solution (Abbott Laboratories) was added to bring the solution to a final concentration of 15 mg/ml. The vehicle consisted of 1N NaOH and 1N acetic acid mixed to pH = 7.5 - 8.0.

Animals received one injection per day for five consecutive days. Four hours following the last drug administration, each group was divided in two and ethanol (2 gm/kg; 30% v/v) or saline were administered. Ten minutes after this injection the animals were sacrificed by decapitation and the brains rapidly extracted. The brains were quickly rinsed in ice cold saline and immediately frozen on dry ice. Samples were

stored at -70°C then fluorometrically assayed for MHPG-SO levels using the same procedures outlined in the previous experiment.

At decapitation, trunk blood was also collected into test tubes containing 150 IU of sodium heparin. One ml of blood was mixed with 4 ml of ice cold semicarbazide reagent and this mixture was prepared using the method outlined in the previous experiment for gas chromatographic analysis of blood ethanol concentration.

Results

Figure 9 shows the levels of MHPG-SO_4 10 minutes after the administration of ethanol. As can be seen there appeared to be an elevation of MHPG-SO_4 in brain following ethanol alone, however, the combination of ethanol and FLA-57 seemed to result in a slight decrease. A one-way analysis of variance yielded a significant difference in the mean levels of MHPG-SO_4 for the four groups ($F(3,14) = 7.61$, $p<.01$). Post hoc Tukey tests indicated that there were significant differences between the ethanol alone group and the FLA-57 alone and FLA-57 - ethanol groups ($p<.05$). There was also a significant difference between the ethanol group and the vehicle - saline control group at

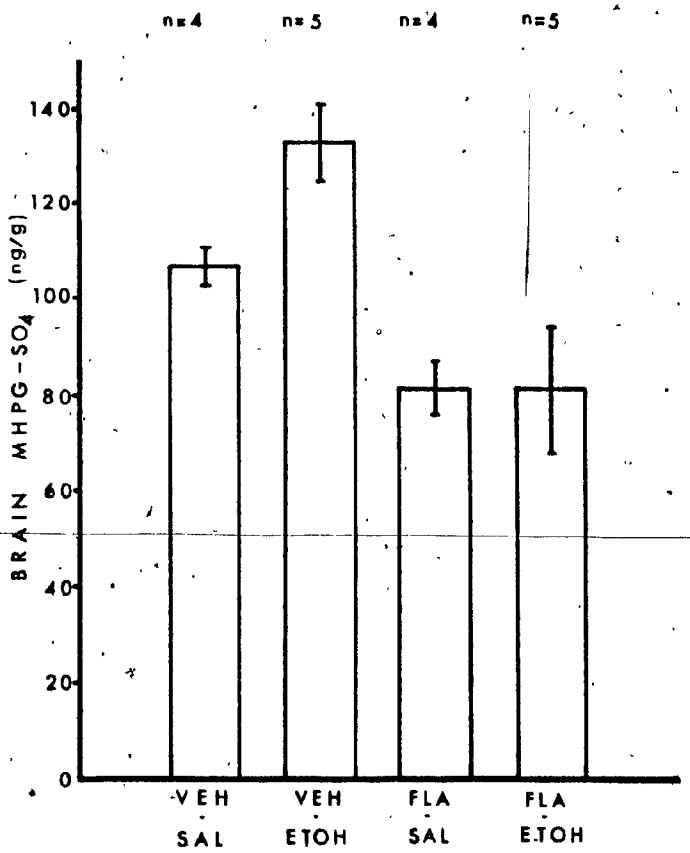


FIGURE 9 Effect of FLA-57 on ethanol induced increase in MHPG-SO_4 accumulation in rat brain. Vertical lines represent the S.E.M.

the α=.10 level of significance (p =.07).

Figure 10 shows the mean blood ethanol concentration for the two ethanol treated groups. No difference was observed in these levels (p>.05).

Discussion

The present findings showed that an acute ethanol administration produced an increase in the levels of MHPG-SO in rat brain. In the previous experiment a 10% increase in MHPG-SO_4 levels was observed 15 minutes following ethanol administration, however, it was not statistically significant. It was suggested that due to the biphasic nature of ethanol's effect an earlier time may have been more appropriate for observing a strong stimulatory action. The current results support this notion in that a 25% increase was observed 10 minutes after the ethanol injection. Possibly due to the relatively small sample size employed, post hoc analysis indicated that this increase only approached the traditional level of significance (p = .05), however, the concordance of the results with those of the previous experiment seems to suggest that ethanol does indeed produce an increase in MHPG-SO_4 levels.

It was also observed that FLA-57 blocked this ethanol induced increase. This effect does not appear

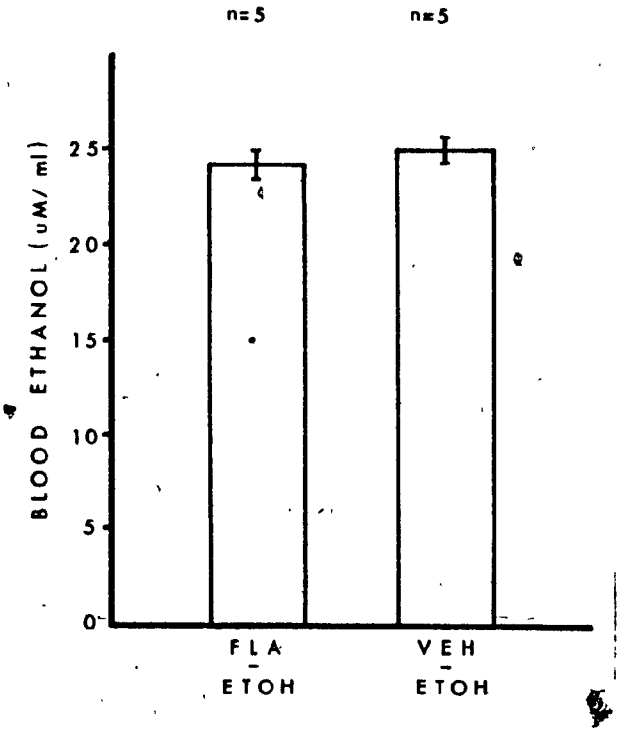


FIGURE 10 Effect of FLA-57 on blood ethanol concentration. Vertical lines represent the S.E.M.

to be due to changes in ethanol metabolism as blood ethanol concentrations of the two ethanol treated groups were not different. This finding was similar to that reported by Tottmar and his co-workers (Tottmar, Hellstrom, Holmberg and Lindros, 1982).

As the accumulation of MHPG-SO_4 in brain has been shown to reflect central noradrenergic activity (Korf et al., 1973; Karasawa et al., 1978), the present findings seem to suggest that ethanol produces a stimulatory effect on noradrenergic activity. In addition, the results also indicate that FLA-57 pre-treatment may inhibit the stimulatory action of ethanol.

Previous data has shown that FLA-57 pre-treatment attenuates ethanol consumption (Amit et al., 1977) and self-administration (Davis et al., 1979). While further research examining FLA-57's possible effect on the depressant action of ethanol on noradrenergic activity is neccessary, it is possible that ethanol induced stimulation of norepinephrine may mediate these ethanol oriented behaviors. The increased availability of norepinephrine combined with the presence of ethanol's metabolite, acetaldehyde, could create suitable conditions in the brain conducive to the formation of condensation products such as tetrahydroisoquinoline alkaloids (TIQ; Cohen and Collins, 1970).

These alkaloids have been suggested to mediate some of the psychopharmacological properties of ethanol (Cohen, 1978).

Section C

The Role of Tetrahydroisoquinoline Alkaloids in the Mediation of Voluntary Ethanol Intake in the Rat

Experiment 1

In 1970, two independent teams simultaneously demonstrated that catecholamines (CA) could condense with aldehydes to form tetrahydroisoquinoline (TIQ) alkaloids (Cohen and Collins, 1970; Davis and Walsh, 1970). Several investigators have also reported that following ethanol administration TIQ alkaloids could be detected in the brains of laboratory animals (Turner, Baker, Algeri, Trigenio and Garattini, 1974; Hamilton, Blum and Hirst, 1978), in the urine of Parkinsonian patients treated with L-Dopa (Sandler, Carter, Hunter and Stern, 1973) and in post mortem examinations of human brains of alcoholics (Sjoquist, Eriksson and Winblad, 1982). Since TIQs have been shown to have potent neurochemical effects (Cohen, 1976), these alkaloids, in their capacity to act as pseudo-transmitters, could conceivably mediate the consumption of ethanol.

In an attempt to provide direct evidence for the involvement of TIQ alkaloids in the central mediation of the psychopharmacological effects of ethanol, Myers and his co-workers conducted a series of experiments examining the effects of central infusions of a variety of TIQ compounds on voluntary ethanol intake (Melchior

and Myers, 1977; Myers and Melchior, 1977a; 1977b; Myers and Oblinger, 1977; Myers and Hoch, 1979). In every study, regardless of the alkaloid infused or the infusion regimen employed, Myers reported a marked increase in ethanol intake in rats following intraventricular infusions of TIQs compared to the intake of ethanol by control animals. In many cases a seven-fold increase was reported, lasting several months following the termination of the TIQ infusions and withdrawal symptoms from the alkaloids alone were described (Melchior and Myers, 1977; Myers and Melchior, 1977a; 1977b).

Contrary to these findings, another series of experiments using the same compounds, failed to induce any enhancement in voluntary ethanol drinking (Brown, Amit and Smith, 1980b). The differences in the findings were attributed to a number of procedural inconsistancies between the two sets of studies (Deitrich and Erwin, 1980).

Recently, in an attempted replication of this TIQ effect, Sinclair failed to observe any changes in ethanol consumption following single infusions of tetrahydropapaveroline (Sinclair and Myers, in press). This, despite the fact that the experiment was supervised by Myers in his own laboratory. However, the use

of a different strain of rat, a single infusion regimen plus various laboratory conditions were described as contributing to the negative findings (Sinclair and Myers, in press).

The purpose of the present experiment was to eliminate these procedural and technical differences and to determine whether TIQ infusions would then yield comparable results to those obtained by Myers.

Method 1A

Subjects

Male Wistar rats (Charles River Canada) weighing 250-275 gm were maintained individually in stainless steel cages covered with plexiglass tops. Food was available ad libitum and fluids were presented in a pair of Richter tubes which were rotated daily in a random fashion.

Procedure

After 3 to 4 days acclimatization to the housing conditions, the animals were exposed to the first test sequence in which ethanol solutions were presented in a free choice with water daily. Commencing with a 3% (v/v) ethanol solution, the concentrations were increased daily according to the following schedule: 3,4,5,6,7,9,11,13,15,20,25 and 30 percent. Following

this 12 day sequence, a cannula guide was surgically implanted into the left lateral ventricle of each animal in accordance with the procedures previously described (section A: experiment 1).

Following 2 days recovery, the animals were connected to an infusion apparatus consisting of a multichannel infusion pump (Harvard Apparatus) fitted with syringes connected through a swivel (Brown, Amit and Weeks, 1976) to polyethylene tubing to the cannulae. Every 30 minutes, an automatic timer activated the pump for 20 seconds during which 4 ul of fluid was delivered to each cannula. One group received tetrahydropapaveroline HBr (THP; Burroughs Wellcome Laboratories; 0.25 ug; n=5) while the other group received Ringer's vehicle (pH adjusted to 3.6 - 4.6 with hydrochloric acid; n=3). These infusions were maintained for two days prior to and throughout a second 12-day ethanol test sequence administered as before. The cannulae were removed and flushed daily and fresh solutions of THP and Ringer's were prepared every 48 hours.

At the termination of the experiment, cannula placement and patency were verified using a dye-infusion technique. The syringes were filled with a filtered solution of methylene blue and each animal received a 10 ul infusion over 30 seconds. The animals

were then given a lethal dose of sodium pentobarbital and the brains were extracted. The brains were cut either sagittally or coronally at the plane of the cannula implant.

Results 1A

Figure 11 shows the ethanol consumption of the groups receiving infusions of THP and Ringer's during both baseline and test periods. Ethanol intake was expressed as absolute ethanol intake (gm/kg) and as preference ratios (ethanol volume/total fluid volume). A three-way analysis of variance with repeated measures on the concentration factor was performed on the data and the results summarized in Table 1. As can be seen, the main effect of ethanol concentration was the only significant result. All other main effects and interactions were not significant. This was true for both absolute ethanol intake and preference ratio.

Examination on the brain sections revealed that the infused dye had diffused throughout the ventricular system confirming that the canulae were properly implanted and were not obstructed.

Method 1B

Subjects

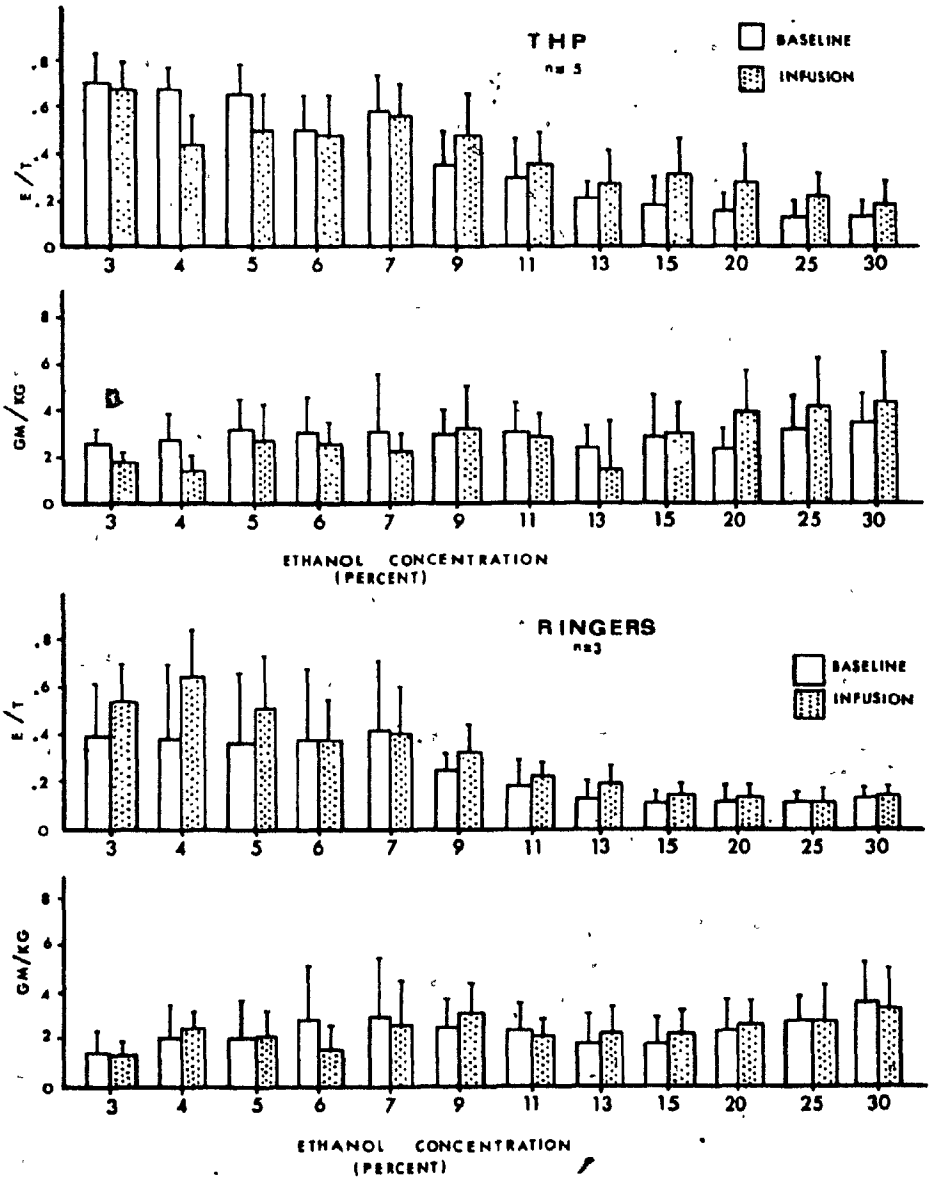


FIGURE 11 Ethanol consumption (gm/kg and ethanol preference) at various concentrations of the test sequences in the baseline phase and during infusions of THP or Ringer's solutions in Wistar rats.

TABLE 1

Summary of statistical analysis of the data on ethanol intake in Wistar rats infused with THP or Ringer's solutions.

Factor	Absolute Ethanol (gm/kg) df	F	Preference Ratio (E/T) df	F
Groups (G)	(1,6)	.307	(1,6)	.547
Periods (P)	(1,6)	.008	(1,6)	.269
Concentration (C)	(11,66)	1.873	(11,66)	8.753*
G X P	(1,6)	.026	(1,6)	.227
G X C	(11,66)	.216	(11,66)	.214
P X C	(11,66)	1.353	(11,66)	.455
G X P X C	(11,66)	.482	(11,66)	1.179

*$p < .05$

Male Sprague-Dawley rats (Charles River Canada) weighing 250-275 gm were maintained in conditions similar to those described in Experiment 1A.

Procedure

The method employed in the present experiment was identical in all respects to that used in Experiment 1A with the exception that 1-methyl-6,7-dihydroxy TIQ HBr (salsolinol; Aldrich Chemicals; 4 ug; n=5) and Ringer's vehicle (pH adjusted to 3.6 - 4.6 with hydrochloric acid; n=8) were infused into the left lateral ventricle of the animals.

Results 1B

Figure 12 shows the ethanol consumption of the groups receiving infusions of salsolinol and Ringer's during both baseline and test periods expressed as absolute ethanol intake (gm/kg) and preference ratio (ethanol volume/total fluid volume). A three-way analysis of variance with repeated measures on the concentration factor was performed on the data with the results summarized in Table 2. The only significant result observed was the main effect of ethanol concentration. All other main effects and interactions were not significant for both absolute ethanol intake and preference ratio.

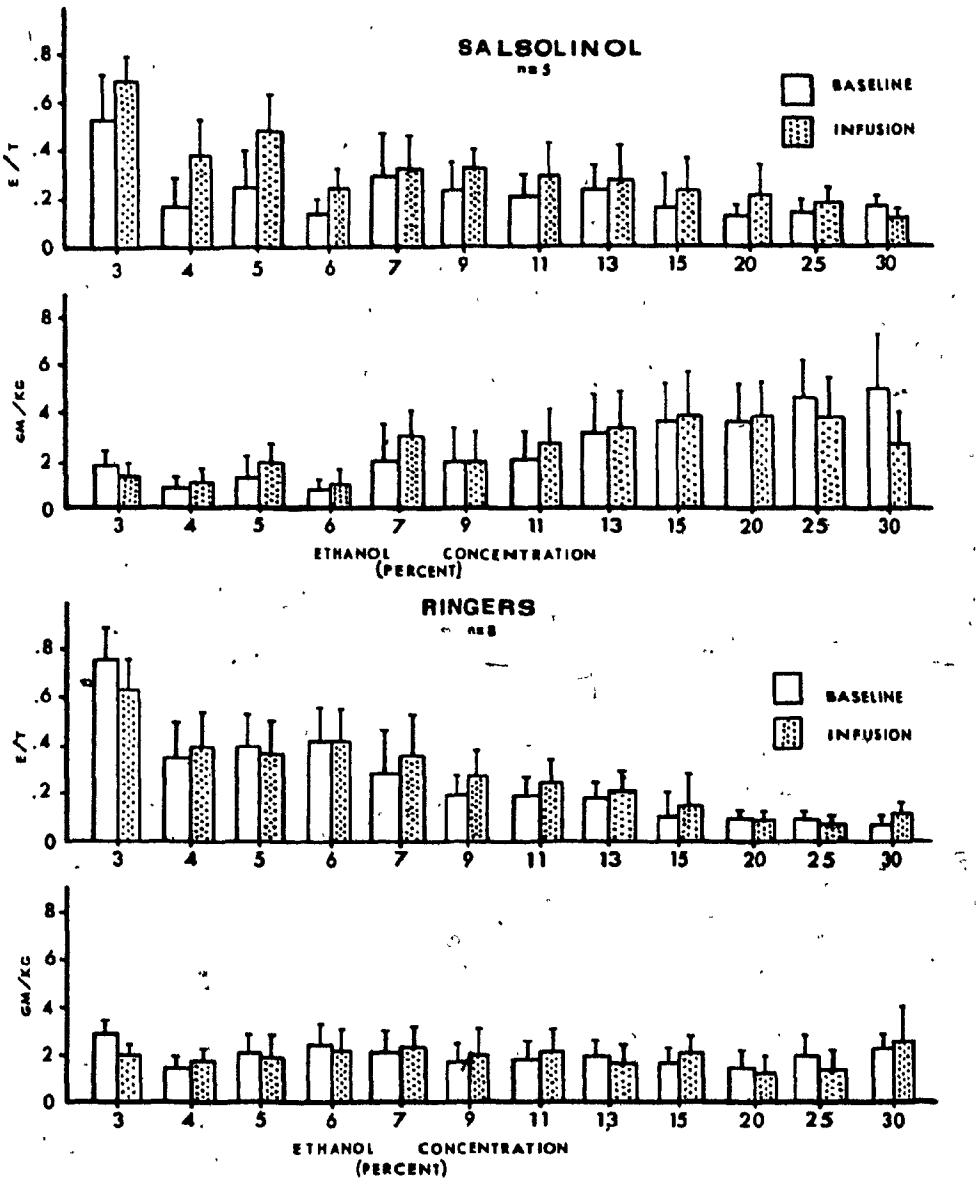


FIGURE 12 Ethanol consumption (gm/kg) and ethanol preference at various concentrations of the test sequences in the baseline phase and during infusions of salsolinol or Ringer's solutions in Sprague-Dawley rats.

TABLE 2

Summary of statistical analysis of the data on ethanol intake in Sprague-Dawley rats infused with salsolinol or Ringer's solutions.

Factor	Absolute Ethanol (gm/kg) df	F	Preference Ratio (E/T) df	F
Groups (G)	(1,11)	.215	(1,11)	.014
Periods (P)	(1,11)	.757	(1,11)	4.278
Concentration (C)	(11,121)	2.042*	(11,121)	15.128*
G X P	(1,11)	.419	(1,11)	2.288
G X C	(11,121)	1.182	(11,121)	1.696
P X C	(11,121)	1.608	(11,121)	.516
G X P X C	(11,121)	1.047	(11,121)	.934

*$p < .05$

Figure 13 represents the individual drinking patterns of two representative animals from the Ringer's infused groups. This figure demonstrates the wide variability between animals within the same group. In some cases, intake of ethanol in the range of 9% to 15% was increased during the Ringer's infusion period. By contrast, other animals failed to consume any substantial quantities of ethanol during either test sequence.

Examination of the sectioned brains following the dye infusion confirmed the placement and patency of all cannula guides.

Discussion

Consistent with the results of previous experiments (Brown et al., 1980b; Sinclair and Myers, in press), it was observed in the present experiment that intracerebroventricular infusions of THP or salsolinol failed to alter voluntary ethanol consumption in either Sprague-Dawley or Wistar rats. Despite implementation of the same procedures used in Myers' studies, the present data were inconsistent with their reported results (Melchior and Myers, 1977; Myers and Melchior, 1977a; 1977b). Myers and his associates have claimed that infusions of TIQ alkaloids produce increases in

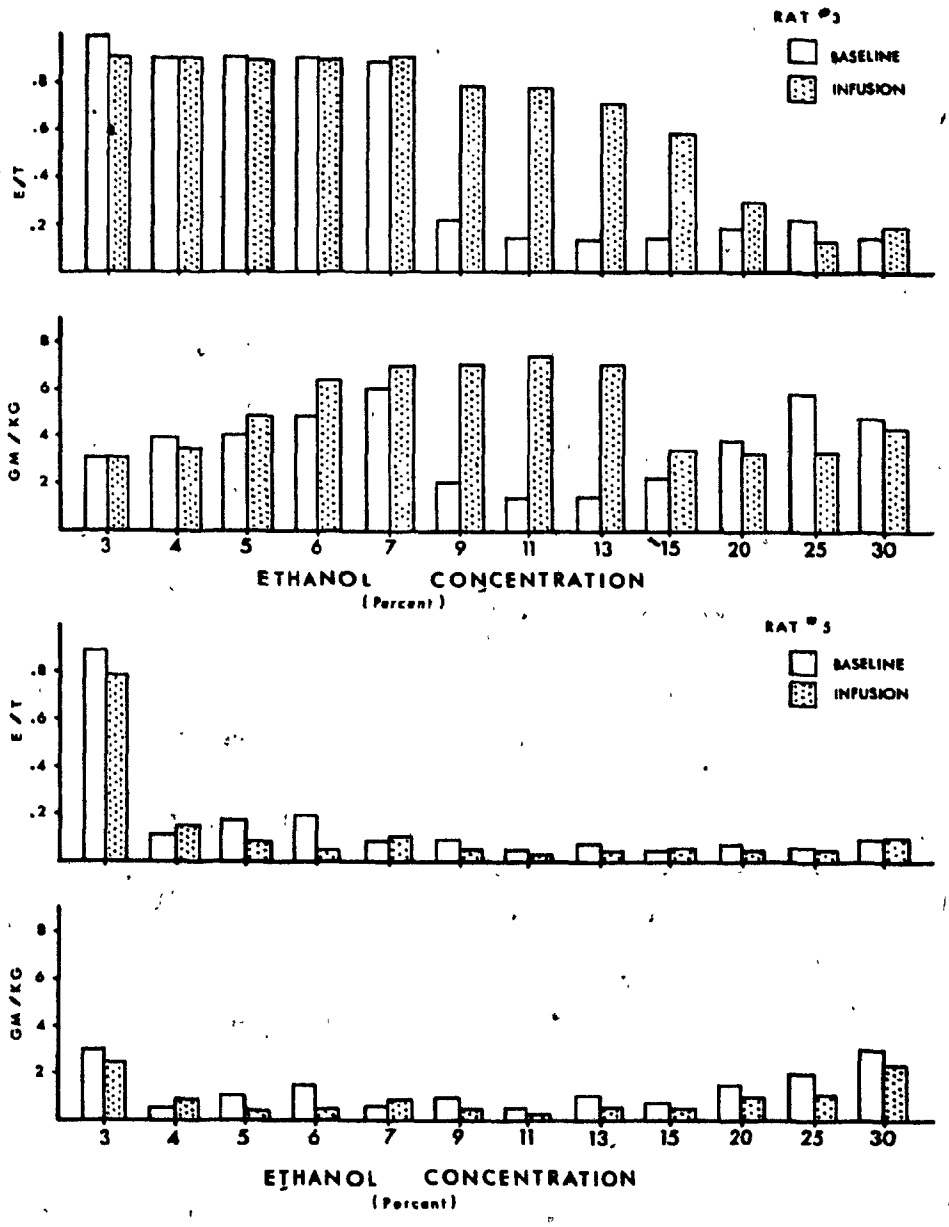


FIGURE 13 Ethanol consumption (gm/kg) and preference for two individual animals in the 12 day ethanol sequence during the baseline phase and during infusions with Ringer's solution.

ethanol consumption, yet, using the same compounds, paradigm and strain of animals, these findings were not confirmed in the present experiment.

It should be noted that in the current experiment the Sprague-Dawley rats consumed an average of almost 2 gm/kg per day throughout the baseline period. These drinking levels were in line with those seen in a systematic investigation of ethanol preference in various strains of animals (Wise, 1973). However, Myers reports that his Sprague-Dawley rats consumed less than 1 gm/kg of ethanol per day. It is possible that by using such low ethanol drinking animals, Myers' experiments may be biased towards observing increases in ethanol consumption, whereas the present study employed animals drinking in moderate ranges, thus allowing either decreases or increases in consumption.

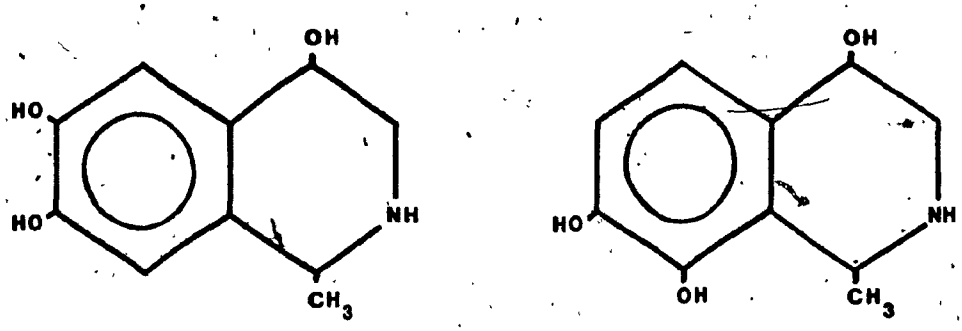
It can be seen from samples of individual drinking patterns (Figure 13) that Myers' ethanol presentation schedule resulted in great variability between animals within the same group. It is possible that the differences between Myers' observations and those reported in the present experiment may, in part, be due to variability in the drinking patterns which appears to be inherent in this experimental paradigm.

Another potentially important factor that may

contribute to the inconsistencies, is that Myers' used ascorbic acid as an oxidation redardant in his alkaloid solutions. It has been pointed out that ascorbic acid can induce the activity of catalase which is capable of oxidizing ethanol to acetaldehyde (Cohen, 1977). Markley and Deitrich (1980), in fact, have demonstrated that intraventricular infusions of acsorbate produced increases in ethanol intake similar to those reported in Myers' TIQ studies and in a study by Duncan and Deitrich (1980). It is therefore possible that the reported effects of infusions of TIQ alkaloids on ethanol consumption may be attributable to ascorbate induced synthesis of acetaldehyde in the brain.

Experiment 2

The failure to affect ethanol intake by intraventricular infusions of TIQ alkaloids suggests the possibility that TIQs may simply be an artifactual by-product of consumed ethanol without any mediational role whatsoever. However, it has been shown in the previous section of this dissertation that increased activation of noradrenergic neurons may be involved in the mediation of ethanol oriented behaviors. Thus it would follow that the critical alkaloid, if indeed this group of compounds plays any role in ethanol intake, would be the condensation product of acetaldehyde and norepinephrine. In fact, in the original paper by Cohen and Collins (1970) they indentified two isomers, products of this condensation (Figure 14). The purpose of the present experiment was to determine whether chronic intraventricular infusions of norepinephrine - acetaldehyde condensation products would produce alterations in voluntary ethanol consumption in laboratory rats.



1-M-4,6,7-THTIQ
PARA

1-M-4,7,8-THTIQ
ORTHO

FIGURE 14 Chemical structure of two isomers of norepinephrine-acetaldehyde condensation.

Method

Subjects

Male Wistar rats (Charles River Canada) weighing 175-200 gm were maintained individually in stainless steel cages in a room regulated for constant temperature and humidity and a 12 hour light-dark cycle. Food was available ad libitum. Fluids were presented in a pair of Richter tubes mounted on the front of the cage. Fluid intake and body weight were recorded daily and the tubes were rotated randomly to prevent the development of a position bias.

Procedure

Each animal was given a free choice between tap water and increasing concentrations of ethanol presented on alternate days. Only water was available on the intervening days. Ethanol solutions were prepared with 95% (v/v) stock solution and tap water. 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 it total daily fluid intake from the ethanol tube. This procedure was continued until each animal stabilized its intake of a particular concentration where 30-50% of its total fluid consumption was in the form of ethanol. Only those animals that met this criterion

by consuming at least a 9% (v/v) ethanol solution were used in the experiment. At this point, the ethanol solutions were presented in a free choice with water on a daily basis and the concentrations fixed for each animal became the test solution and were maintained for the duration of the experiment.

Under sodium pentobarbital anaesthesia, a 22-guage stainless steel guide cannula was chronically implanted into the left lateral ventricle of each animal using the procedures described in Section A. Following recovery from surgery and the re-establishment of a stable pattern of ethanol intake, the animals were connected to the infusion apparatus (see Section C:experiment 1). The automatic timer activated the pump every 15 minutes for 7 seconds during which a 2 ul infusion was delivered to each cannula. After a four day baseline period in which the animals received Ringer's infusions, they were assigned to treatment groups which were infused around the clock every 15 minutes for three consecutive days. Each animal received one of the following compounds; 1-methyl-4,6,7-trihydroxy TIQ (2 ug) (courtesy of Dr. R.L.Williams), a mixture cosisting of 60% 1-methyl-4,7,8-trihydroxy TIQ and 40% of the former isomer (2 ug) (courtesy of Dr. R.L.Williams) or Ringer's solu-

tion. The cannulae were removed, flushed and fresh solutions were prepared daily. The animals were weighed and fluid intake was recorded every day.

At the termination of the experiment the animals were infused with 25 ul of Trypan Blue stain and then sacrificed, followed by saline and 10% formalin perfusion. The extracted brains were then sliced into 40 u coronal or saggital sections.

Results

As can be seen from Figure 15 there was no change in the consumption of ethanol during the infusion of the two experimental compounds. A three-way analysis of variance revealed a significant group effect for both absolute ethanol intake (gm/kg; $F(2,12) = 6.59$, $p < .05$) and preference ratio (E/T; $F(2,12) = 4.38$, $p < .05$). This would appear to be due to the lower baseline and treatment intake of the Ringer's group and not due to any effect of the TIQ infusions themselves. All other main effects and interactions were not significant ($p > .05$).

Histological examination demonstrated the presence of blue stain throughout the ventricular system indicating that no occulsion of the cannulae had taken place and that the administered compounds were deli-

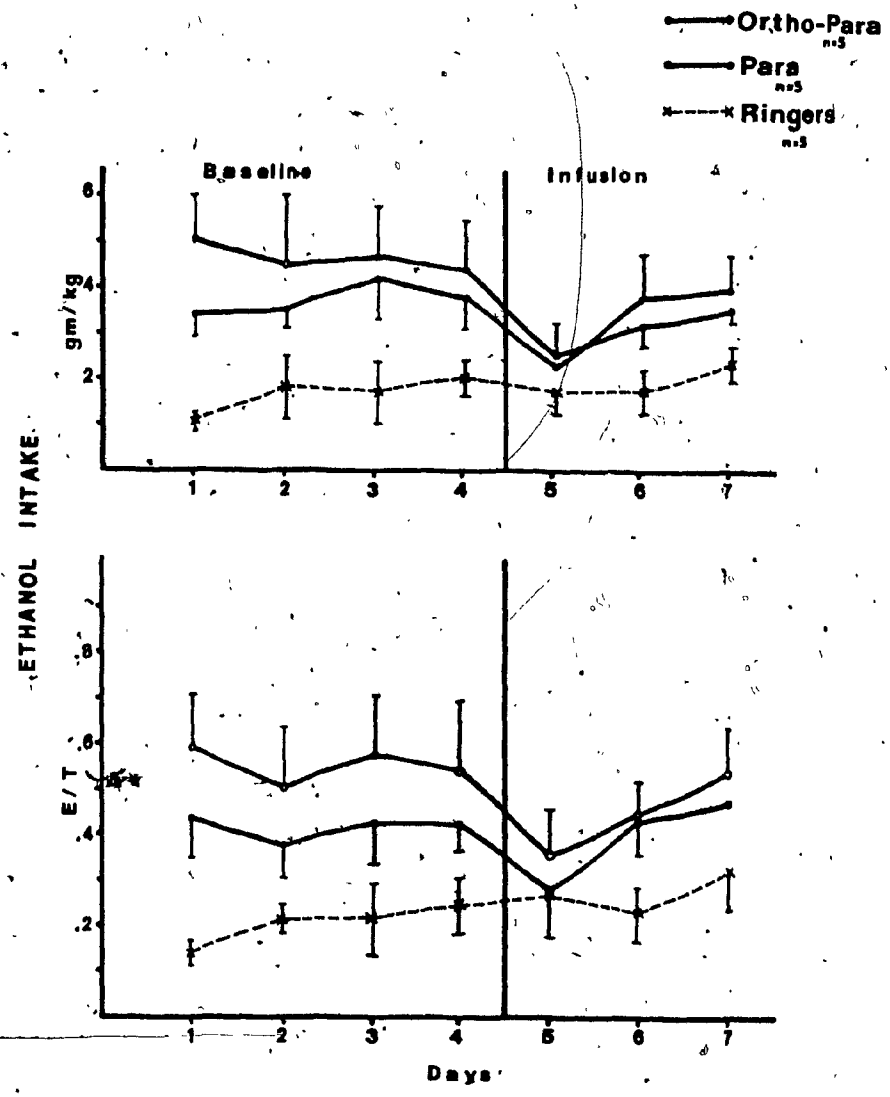


FIGURE 15 Effect of chronic infusions of norepinephrine-acetaldehyde condensation products on ethanol consumption (gm/kg and preference) in Wistar rats. Vertical lines represent the S.E.M.

vered to the lateral ventricle.

Discussion

The data obtained in this experiment lends further support to existing evidence (Brown et al., 1980b; Sinclair and Myers, in press) arguing against the involvement of TIQ alkaloids in the mediation or regulation of voluntary ethanol intake. These results are of particular importance in view on the fact that the TIQ alkaloids used were acetaldehyde - norepinephrine condensation products. It is important to note in this context that both acetaldehyde (Amir et al., 1980) and norepinephrine (Amit et al., 1977; Davis et al., 1978) were implicated in the mediation of ethanol reinforcement.

When the entire body of evidence, including the present results and the recent data obtained by Sinclair and Myers (in press) is carefully evaluated and the methodological difficulties inherent in the studies reporting positive data are critically examined, it must be concluded that, at present, no role for TIQ alkaloids has been demonstrated. While it is still possible that some link between these compounds and ethanol intake may be demonstrated in future, it would appear that at present they must be

considered irrelevant to the mechanisms underlying the regulation of voluntary ethanol consumption in rats.

Experiment 3

In the previous experiments it was observed that intraventricular infusions of a variety of TIQ alkaloids failed to produce alterations in voluntary ethanol intake. It was suggested that these compounds may not play a mediational role in ethanol consumption and may simply be artifactual by-products of exposure to ethanol.

Despite these negative findings, however, it is possible that TIQ alkaloids may have psychopharmacological properties capable of influencing behavior. It has been demonstrated that TIQs possess many transmitter-like properties (Cohen, 1978) and may act as pseudo-transmitters. In addition, it has been suggested that TIQ alkaloids may have opiate-like effects (Hamilton, Hirst and Blum, 1979; Altshuler, Phillips and Feinhandler, 1980), although it has also been suggested that these effects are not the result of opiate receptor interactions (North, Collins, Milner, Karras and Koziol, 1981). The purpose of the present experiment was to determine whether TIQ alkaloids were capable of acting as reinforcers when infused into the cerebral ventricles.

Method 3A

Subjects

Male Wistar rats (Charles River Canada) weighing 275-300 gm at the start of the experiment were used. These animals were implanted with a cannula guide aimed at the left lateral ventricle using the procedures previously described.

Procedures

Following a short recovery period of 4-5 days, the animals were placed individually into operant chambers (Ralph Gerbrands Co., Model C) with the food hopper blocked off. Each animal was connected to an infusion apparatus which consisted of a pump (Razel, Inc.) connected via polyethylene tubing to a flow-through swivel located above the operant chamber. A shielded plastic tube connected to the swivel outlet, terminated in a 28-guage internal cannula, which was inserted into and secured to the permanently mounted cannula guide. The animals were maintained in these chambers for 7 consecutive days, where they had access to food, water and the operant lever. The testing room was regulated for constant temperature and a 12 hour light-dark cycle. The animals were not pretrained to press the lever. When the lever was pressed, the pump was activated for 10 seconds during which a 4 ul infusion

was delivered into the ventricle. Additional presses during the 10 second infusion interval did not reactivate the pump and were not recorded. All infusions were recorded automatically on a multichannel event recorder.

The animals received infusions of 1-methyl-4,6,7-trihydroxy TIQ (2 ug; 1M-4,6,7-TH; para isomer) or a mixture compound consisting of approximately 60% 1-methyl-4,7,8-trihydroxy TIQ and 40% of the former isomer (2 ug; 1M-4,7,8-TH; ortho-para isomer). There was an additional group that received infusions of Ringer's solution after each lever press. Fresh solutions were prepared every 48 hours and once daily the animals were weighed and the cannulae were removed and flushed in order to minimize blockage.

At the termination of the experiment the animals were sacrificed and perfused intracardially with saline followed by 10% formalin. The brains were removed and subsequently sliced into 40 u coronal sections.

Results 3A

Figure 16 shows the mean number of lever presses per day for each of the three groups. As can be seen the two experimental groups bracketed the Ringer's control group. The 1M-4,6,7-TH group appeared to lever

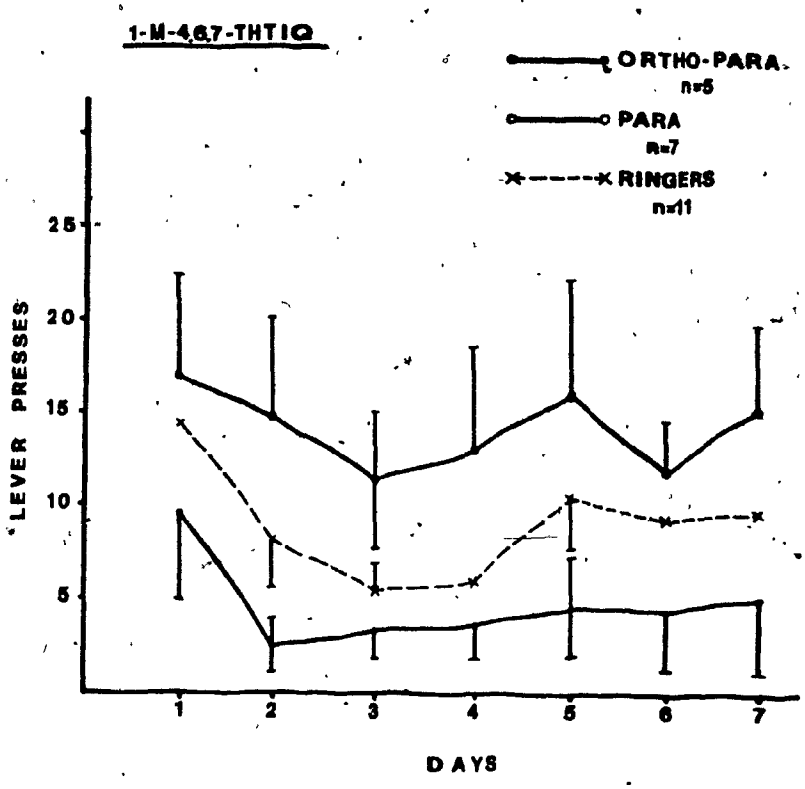


FIGURE 16 Intraventricular self-administration of two norepinephrine-acetaldehyde condensation products. Mean number of lever presses per day with vertical lines representing the S.E.M.

press at rates below those of the Ringer's group while the animals receiving the mixture compound (1M-4,7,8-TH) seemed to have an elevated rate of responding. A two-way analysis of variance revealed a significant main effect of groups ($F(2,20) = 8.89$, $p<.05$) but no effect of days ($F(6,120) = 1.74$, $p>.05$) and no interaction ($F(18,120) = 0.07$, $p>.05$). Post hoc tests (Sheffe) reveal that the two experimental groups do not differ from the Ringer's control group, however, the animals receiving the mixture compound (1M-4,7,8-TH) did lever press at significantly higher rates than the 1M-4,6,7-TH group.

As in the previous experiments, the cannula placement in each animal was verified by histological examination.

Method 3B

Subjects

Male Wistar rats (Charles River Canada) weighing 275-300 gm at the start of the experiment were implanted with a cannula guide aimed at the left lateral ventricle using the technique previously described.

Procedure

Using the exact procedure as outlined in the previous phase (experiment 3; method 3A), the animals

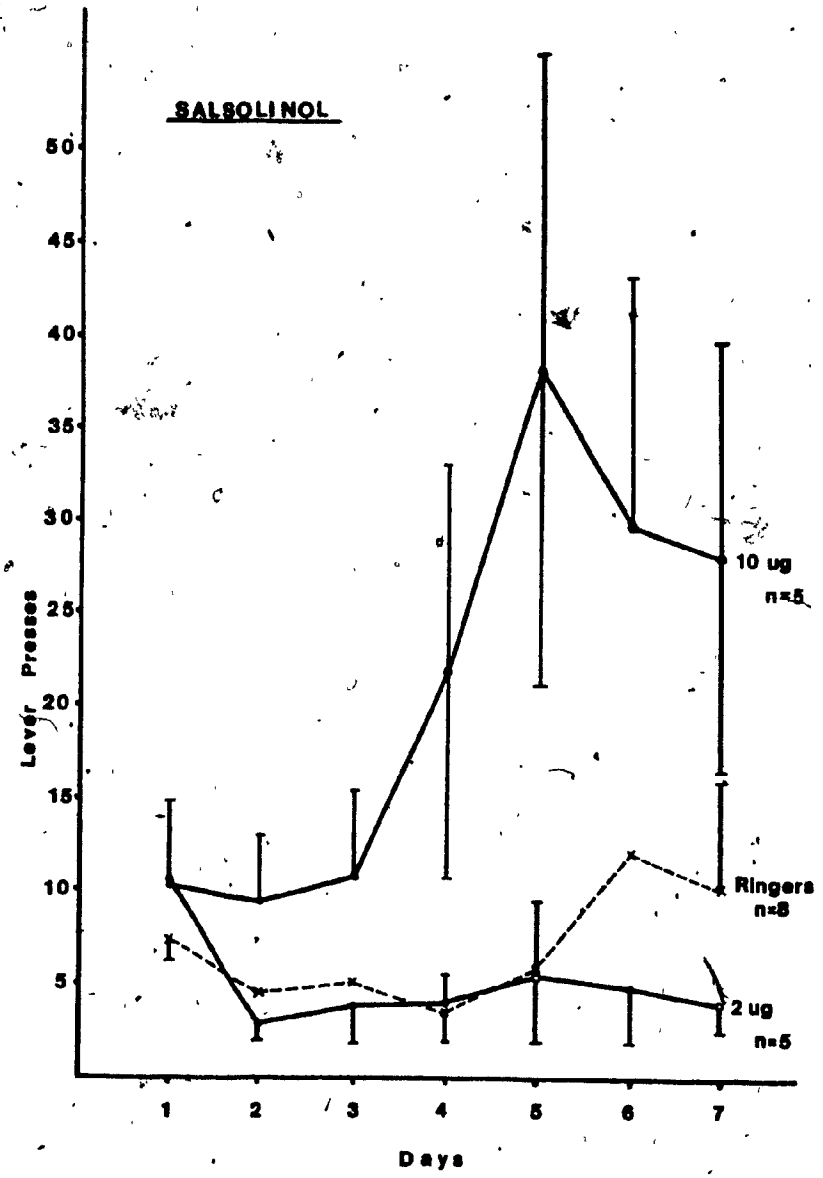


FIGURE 17 Intraventricular self-administration of salsolinol. Mean number of lever presses per day with vertical lines representing the S.E.M.

were placed into operant chambers for 7 consecutive days with free access to food, water and an operant lever. They were connected to the infusion apparatus which delivered a 4 ul infusion (10 seconds duration) following each lever press. The animals received infusions of salsolinol (courtesy of Dr. R.L.Williams) at doses of 10 ug or 2 ug per infusion. An additional group received Ringer's solution. Fresh solutions were prepared every 48 hours. Once daily, the animals were weighed and the cannulae were removed and flushed to minimize the possibility of blockage.

At the termination of the experiment, the animals were sacrificed and perfused intracardially with saline followed by 10% formalin. The brains were extracted and subsequently sliced into 40 u coronal sections.

Results 3B

The rate of lever pressing for the two doses of salsolinol and Ringer's solution is shown in Figure 17. It can be seen that the rate of pressing for the 10 ug dose of salsolinol was elevated above the 2 ug and Ringer's infused animals.

A two-way analysis of variance revealed no main effect of groups ($F(2,15) = 3.31$, $p>.05$). There was a significant effect of days ($F(6,90) = 2.34$, $p<.05$) and

a significant interaction ($F(12,120) = 1.94$, $p<.05$). Post hoc analysis (Sheffe) indicated that there were no differences between the rates of lever pressing of the 2 ug salsolinol animals and those of the Ringer's infused animals ($p>.05$), whereas, there was a significant elevation in the pressing rates of the 10 ug salsolinol animals which began on day 4 and continued until the termination of the experiment ($p<.05$).

Histological examination revealed that all the animals had cannulae which penetrated the lateral ventricle.

Discussion

The results of the present study suggest that TIQ alkaloids may have reinforcing properties. In the first phase it was observed that the norepinephrine - acetaldehyde condensation products may have isomer specific effects. It was seen that the rate of lever pressing for the ortho-para mixture (1M-4,7,8-TH) was higher than the rate of pressing for the para isomer (1M-4,6,7-TH). The inability to detect differences between the Ringer's infused group and the ortho-para TIQ infused group may be due to the mixed nature of the alkaloid. Infusions of the para isomer appeared to result in lower rates of pressing, possibly indicating

a depressant or aversive action. The use of a pure ortho isomer in future may result in significantly higher rates of pressing than those observed in the present study.

In the second phase, it was observed that animals readily self-administered salsolinol (dopamine - acetaldehyde condensation product) via the intraventricular route. Given the neurotransmitter-like properties of these alkaloids (Cohen, 1978), it is possible that the central administration of salsolinol may activate dopamine neurons which have been implicated in the mediation of brain reward (Wise, 1980).

In view of the failure of any of the TIQ alkaloids to affect ethanol consumption, it is suggested that the possible positive reinforcing properties of these compounds are inherent in their pharmacological actions and are independent of any role as mediator of voluntary ethanol intake in rats. Future research is required to understand the nature of the interactions between TIQ alkaloids, central neural systems and subsequent behavioral effects. If the efforts of several investigators to demonstrate the endogenous presence of these alkaloids in an ethanol free organism prove successful (Barker et al., 1981), then the present data may be of importance in the area of

motivation.

General Discussion

The purpose of the present dissertation was to investigate the nature of some of the brain mechanisms underlying many of the psychopharmacological properties of ethanol. Emphasis was placed on the role of central noradrenergic neurons and on a possible mode of interaction with acetaldehyde, the primary metabolite of ethanol.

In section A, it was observed that centrally administered acetaldehyde was able to induce a conditioned place preference. These results support and extend those of previous reports demonstrating that acetaldehyde may have reinforcing properties (Brown et al., 1979; Smith, 1980; Myers et al., 1982). It has been suggested that these properties may play an important role in the mediation of ethanol reinforcement (Amir et al., 1980; Brown et al., 1980).

In section B, it was observed that an acute ethanol injection resulted in a time- and dose-dependent biphasic effect on noradrenergic activity. Furthermore, pre-treatment with FLA-57, a dopamine-beta-hydroxylase inhibitor previously shown to attenuate ethanol reinforcement (Amit et al., 1977;

Davis et al., 1979), was seen to block the ethanol-induced increase in norepinephrine metabolism. These results suggest the possibility that ethanol-induced stimulation of norepinephrine neurons may play a role in the mediation of some ethanol oriented behaviors.

In an attempt to combine these two lines of research, investigators have suggested a possible role for aldehyde - catecholamine condensation products (Cohen and Collins, 1970; Davis and Walsh, 1970; Stern and Amit, 1972). The results of section B suggest that conditions in the brain following ethanol exposure may be conducive to the formation of tetrahydroisoqinoline (TIQ) alkaloids. It has been previously shown that TIQ alkaloids possess many transmitter-like properties (Cohen, 1978) and the results of section C indicate that their central administration may in fact influence behavior. However, as central administration of several TIQ alkaloids failed to alter voluntary ethanol consumption (section C), the behavioral effects observed appear to be inherent in their pharmacological properties and independent of any role as mediator of ethanol intake in rats.

The present findings demonstrate that acetaldehyde, when present in the brain, may possess reinforcing properties which have been suggested to mediate

voluntary ethanol consumption. In addition, the results suggest that activation of noradrenergic neurons may also be an important factor in the mediation of ethanol reinforcement. However, the involvement of TIQ alkaloids does not appear to be the mechanism of interaction between these two systems in the regulation of ethanol intake. An alternative hypothesis may involve a direct effect of acetaldehyde on norepinephrine neurons. It has been demonstrated that acetaldehyde could trigger the release of brain norepinephrine (Thadani and Truitt, 1977) and it may be possible that the reinforcing properties of ethanol are mediated by a direct activation of central noradrenergic neurons by acetaldehyde.

It is well known that organisms are capable of regulating their intake of ethanol and it is possible that the actions of acetaldehyde and its interactions with central noradrenergic and related enzyme systems may mediate this regulation. It has been observed that acetaldehyde may competitively inhibit aldehyde dehydrogenase (ALDH; Lahti and Majchrowicz, 1976). This enzyme was shown to play a major role in the matabolic pathway of monoamine deanimation (Duncan and Sourkes, 1974; Tabakoff and Gelpke, 1975) The acetaldehyde-induced release of norepinephrine and the inhibition of

ALDH could lead to an increased accumulation of biogenic aldehydes which has been shown to affect normal neural functioning (Tabakoff, 1974). While the presence of acetaldehyde may initially stimulate norepinephrine release, the subsequent rise in biogenic aldehydes could interfere with this stimulatory effect, possibly reducing acetaldehyde's reinforcing properties. This complex interaction between drug, neural system and enzyme metabolism may be a possible mechanism for satiety in the voluntary intake of ethanol. Initial exposure to acetaldehyde, through ethanol consumption, may result in reinforcement, however continued exposure may lead to an accumulation of biogenic aldehydes which could inhibit the mechanisms of reinforcement, thereby leading to satiety.

Previous research has indicated that this may be a viable hypothesis as it has been shown that the activity of brain ALDH may play a regulatory role in the mediation of voluntary ethanol consumption (Amir, 1977; 1978; Amir and Stern, 1978). High rates of enzyme activity have consistently been associated with elevated ethanol consumption (Schlesinger, Kakihana and Bennett, 1966; Sheppard, Albersheim and McLearn, 1968; Amir, 1977; 1978; Amir and Stern, 1978; Eriksson,

1980). These high rates of enzyme activity could reduce the rate of accumulation of biogenic aldehydes in the brain following catecholamine stimulation, thereby, increasing the brain's capacity to respond to central acetaldehyde and subsequently lead to increased ethanol consumption. This interaction not only describes a possible mechanism for satiety in alcohol consumption but could also account for the individual variation in behavioral responses so often observed following ethanol exposure.

Clearly, these biochemical interactions are hypothetical and additional research is required in order to elucidate the precise mechanisms whereby ethanol influences behavior. The present dissertation provides data regarding the nature of these mechanisms and the results indicate some of the appropriate avenues that future investigators may pursue in order to better understand the psychopharmacological actions of alcohol.

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