INTRACEREBRAL ADMINISTRATION OF ACETALDEHYDE: ITS RELATIONSHIP TO THE CENTRAL CATECHOLAMINES

Brian R. Smith

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ABSTRACT

INTRACEREBRAL ADMINISTRATION OF ACETALDEHYDE: ITS RELATIONSHIP TO THE CENTRAL CATECHOLAMINES

Brian R. Smith

The neuropharmacological and behavioral effects of possible interactions between intraventricular infusions of acetaldehyde, the first oxidation product of ethanol, and the catecholamines, norepinephrine and dopamine, were examined in laboratory rats. In the first two experiments, it was observed that acute and chronic intraventricular infusions of acetaldehyde failed to produce alterations in catecholamine release. As previous reports have indicated that its peripheral administration results in changes in catecholamine metabolism, the present findings suggest that acetaldehyde may have different neurochemical effects when administered centrally. In the last experiment it was observed that the propensity to self-administer acetaldehyde intraventricularly was correlated with the rate of norepinephrine metabolism. Even though the presence of acetaldehyde in the brain may not cause changes in monoamine release possible that its neuropharmacological effects may be

dependent upon the rate of catecholamine metabolism. These findings were discussed with regards to their involvement in subserving voluntary ethanol consumption.

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The behavioral effects of alcohol have been known to man throughout the ages. Because of its social acceptance and its availability, its use has become widespread. Alcohol abuse has become a major problem for society as it has been shown to be a contributing factor in such difficulties as family breakdown, automobile accidents and crime (Le Dain, 1973). The exact physiological mechanisms involved in mediating the effects of alcohol, however, remain a mystery.

In attempts to understand the psychopharmacological effects of alcohol, a vast amount of research has been undertaken over the past 50 years. A variety of suitable models of alcoholism have been developed using laboratory animals. Despite its aversive taste laboratory animals have been shown to consume large amounts of alcohol (Kahn & Stellar, 1960; Richter & Campbell, 1940; Wilson, 1972). Animals also learn to self-administer intravenous (Deneau, Yanagita & Seevers, 1969; Winger & Woods, 1973) and intragastric routes of administration (Amit & Stern, 1969; Yanagita and Takahashi, 1973).

Although these demonstrations indicate that alcohol has positive reinforcing effects that initiate and maintain alcohol oriented behavior the underlying neurochemical mechanisms were, until recently, very unclear. 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 & Stern, 1971; Amir & Stern, 1978).

It had been demonstrated that electrodes implanted in the lateral hypothalamus would support electrical self-stimulation (Olds, Travis & Schwing, 1960; Stein, 1968). Furthermore, the lateral hypothalamus had been shown to be traversed by some of the major catecholamine (CA) pathways (Lindvall & Björklund, 1974; Ungerstedt, 1971). These findings led to the hypothesis that the CA, norepinephrine (NE) and dopamine (DA), may subserve reward and may mediate motivated behavior (Fibiger, 1978; German & Bowden, 1974). Because stimulation of the lateral hypothalamus appeared to increase ethanol self-administration (Amit & Stern, 1971), it was suggested that those mechanisms which were thought to support electrical self-stimulation may also be involved in voluntary ethanol consumption.

This served as a basis for further studies examining the effects of central CA manipulations on ethanol self-administration. It has been observed that electrolytic lesions of the ventral lateral hypothalamus

attenuated ethanol preference (Amit, Meade, Levitan & Singer, 1976). Similarly, when the neurotoxin, 6-hydroxydopamine, was infused into the lateral ventricle of ethanol preferring rats, it resulted in an attenuation of ethanol consumption (Brown & Amit, 1977; Myers & Melchior, 1975). Furthermore, it was observed that pretreatment with alpha-methyltyrosine (an inhibitor of tyrosine hydroxylase, the rate limiting enzyme in CA synthesis) inhibited the ethanol-induced locomotor excitation in mice as measured in an open-field (Carlsson, Engel & Svensson, 1972). Similar effects have been demonstrated in human subjects, where pretreatment with alpha-methyltyrosine suppressed ethanol-induced stimulation and euphoria following programmed ingestion of alcohol (Ahlenius, Carlsson, Engel, Svensson & Sodersten, 1973). Also Myers and Veale (1968) have shown that the administration of alpha-methyltyrosine produced a transitory attenuation of ethanol consumption in rats. These results suggest that the central CA may play a functional role in mediating the pharmacological effects of ethanol.

A number of studies have investigated the possible effects of ethanol on central CA. It has been shown that CA neurons located in the lateral hypothalamus were highly affected by electrophoretically

administered ethanol (Wayner, Ono & Nolley, 1975). Furthermore, injections of ethanol in rats have resulted in an elevation in CA turnover as measured by increased accumulation of metabolites (Karoum, Wyatt & Majchrowicz, 1976). It has also been demonstrated that acute administrations of ethanol produced alterations in CA release and synthesis (Corrodi, Fuxe & Hokfelt, 1966; Pohorecky, 1974; Pohorecky & Jaffe, 1975). Since ethanol appears to alter CA metabolism and CA manipulation reduced ethanol intake and induction of stimulation and euphoria, this suggests that there may be an interaction ethanol and the central CA which may subserve the reinforcing properties of ethanol.

In recent years there has been some evidence 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 enzyme alcohol dehydrogenase (Hawkins & Kalant, 1972), which occurs mainly in the liver (Jacobsen, 1952; Hawkins & Kalant, 1972), although, it has been shown to take place in other parts of the body (Lundquist, 1971; Raskin & Sokoloff, 1972) including the brain (Raskin & Sokoloff, 1968, 1970; Tyce, Flock & Owen, 1968). Furthermore, because of its high lipid solubility, acetaldehyde is capable of

diffusing into the brain (Akabane, 1970).

Attempts to demonstrate the presence of acetaldehyde in the brain, however, have produced equivocal results. Earlier studies suggested that acetaldehyde was detectable in the brains of ethanol-treated rats at levels parallel to those in cerebral blood (Duritz & Truitt, 1966; Kiessling, 1962a, b; Majchrowicz, 1973; Majchrowicz & Ridge, 1973; Ridge, 1963). These findings were discounted on the grounds that acetaldehyde is formed non-enzymatically during sample preparation (Sippel, 1973). In fact, when this non-enzymatic formation was prevented by treatment with thiourea (Sippel, 1972) no acetaldehyde was detected in brain tissue until blood levels exceeded 200 μM (Sippel, 1974). These results suggest that elevated blood levels of acetaldehyde do not produce a concomitant increase in brain acetaldehyde. However, it has been demonstrated that blood acetaldehyde levels do correspond to those found in the cerebro-spinal fluid of rats injected with ethanol (Kiianmaa & Virtanen, 1977; Petterson & Kiessling, 1977).

Although, alcohol dehydrogenase is present in the brain (Raskin & Sokoloff, 1968, 1970) it is relatively inactive, and very little acetaldehyde is actually formed in the brain (Mukherji, Kashiki, Ohyanagi & Soviter, 1975; Tabakoff & von Wartburg, 1975). However, further

studies examining brain enzyme activity have suggested the possible presence of acetaldehyde. Amir (1977; 1978a, b; Amir & Stern, 1978) has shown that aldehyde dehydrogenase activity is correlated with ethanol preference in rats. It was suggested that the capacity of the brain to metabolize aldehydes could be related to the possible involvement of acetaldehyde in ethanol consumption. Furthermore, chronic administration of ethanol resulted in increased aldehyde dehydrogenase activity (Amir, 1978b).

In an attempt to investigate the central effects of acetaldehyde on behavior Brown and his colleagues (1978a) observed that while intraperitoneal injections of acetaldehyde resulted in a pronounced conditioned taste aversion, intraventricular infusions failed to induce any such effect (Brown, Amit, Smith & Rockman, 1978a). In a subsequent experiment it was observed that naivé rats would learn to self-administer acetaldehyde directly into the cerebral ventricles, whereas, ethanol was not self-administered via this route (Amit, Brown & Rockman, 1977; Brown, Amit & Rockman, 1979a). Furthermore, it was observed that the propensity to self-administer acetaldehyde by rats was positively correlated with subsequent ethanol preference (Brown, Amit & Smith, 1979b). These studies suggest that acetaldehyde may mediate the pharmacologically reinforcing properties of ingested

ethanol.

To examine the hypothesis further, human subjects were administered either Antabuse [R], Temposil [R] or a placebo prior to the ingestion of ethanol. Antabuse [R] (tetraethylthiuran disulfide; disulfiram) is clinically used in the treatment of alcoholism. Its primary action is to inhibit aldehyde dehydrogenase (Hald & Jacobsen, 1948) with the result that there is an elevation of blood acetaldehyde levels. The use of this agent in combination with ethanol produces a set of symptoms including vasodilation, changes in heart rate, decrease in blood pressure, dizziness, nausea, vomiting and respiratory depression (Hald & Jacobsen, 1948; Jacobsen, 1952; Raby, 1953; Walsh, 1971). Temposil [R] (citrated calcium carbamide) also interferes with acetaldehyde metabolism and results in similar effects when combined with ethanol (Consbruch & Derwart, 1968; Ferguson, 1956). These drugs have been used in the treatment of alcoholics over the past 25 years. At low doses of ethanol, there were no observable behavioral effects in the placebo treated subjects, however, there was enhanced euphoria and stimulation in the Antabuse [R] and Temposil [R] treated subjects. It was suggested that slight changes in acetaldehyde levels may have produced the central effects observed (Amit, Brown,

Amir, Smith & Sutherland, 1979).

Acetaldehyde's effects on the central CA have also been investigated. It was shown that inhalation of acetaldehyde by mice resulted in an increase in the concentration of brain CA (Ortiz, Griffiths & Littleton, 1974). Similarly, injections of acetaldehyde produced alterations in the CA content of rat brains (Duritz & Truitt, 1966) and it was shown to be more effective than ethanol in causing changes in the metabolism of the CA (Thadani & Truitt, 1977). The available evidence suggests that acetaldehyde may be present in the cerebro-spinal fluid and possibly in brain tissue following the administration of ethanol (Kiianmaa & Virtanen, 1977; Petterson & Kiessling, 1977; Sippel, 1974) and given its effects on the central CA, it is conceivable that acetaldehyde may mediate some of the central effects of ethanol.

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). Condensation products called tetrahydroisoquinolines (TIQ) have been observed in rat brain homogenates (Davis & Walsh, 1970) and in acetaldehyde perfused cow adrenals (Cohen & Collins, 1970). A number of investigators have

demonstrated that these TIQ alkaloids possess many neurochemical properties (Cohen, 1978). It was observed that they are taken up and stored by CA neurons (Cohen, Mytilineou & Barrett, 1972; Locke, Cohen & Dembiec, 1973) and as a result, the reuptake of naturally occurring CA is inhibited (Heikkila, Cohen & Dembiec, 1971; Tuomisto & Tuomisto, 1973). Furthermore, TIQ have been shown to be stored in CA synaptic vesicles (Tennyson, Cohen, Mytilineou & Heikkila, 1973), released upon electrical or chemical stimulation (Greenberg & Cohen, 1973; Rahwan, O'Neill & Miller, 1974) and can activate receptors (Mytilineou, Cohen & Barrett, 1974). Given the neurochemical properties of TIQ alkaloids, it is possible that they may act as "false transmitters" and they may contribute to some of the neurophysiological effects of ethanol consumption.

It has been reported that 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 measured in brain tissue (Collins & Bigdeli, 1975). Also, tetrahydropapaveroline (a dopamine-dopaldehyde condensate) was observed following L-DOPA treatment in conjunction

with ethanol administration (Sandler, Carter, Hunter & Stern, 1973; Turner, Baker, Algeri, Frigenio & Garattini, 1974). Recently, it was reported that 6-methoxysalsolinol was detected in striatal tissue of mice following chronic ethanol exposure (Hamilton, Blum & Hirst, 1978).

As TIQ alkaloids are neurochemically active compounds, it is conceivable that they may be formed during ethanol ingestion and they may play a functional role in mediating the pharmacological actions of ethanol. In a recent series of experiments, Myers and his co-workers found that during and following intraventricular infusions of tetrahydro-papaveroline, salsolinol, and a variety of other alkaloid derivatives, ethanol intake increased markedly compared to control rats infused with artifical cerebro-spinal fluid (Melchior & Myers, 1977; Myers & Melchior, 1977a, b; Myers & Oblinger, 1977). Using the same compounds in a similar paradigm, Brown and his colleagues failed to induce any enhancement in voluntary ethanol drinking (Brown, Amit & Smith, 1978). It was suggested that the differences in the findings were due to procedural inconsistencies.

It has been shown in a number of studies that NE, rather than DA or serotonin appears to be the neurotransmitter that is most functionally involved in ethanol self-administration (e.g., Amit, Brown, Levitan

& Ogren, 1977; Brown & Amit, 1977; Myers & Melchior, 1975). Therefore, it is conceivable that the TIQ which would be most effective in altering ethanol consumption is the acetaldehyde-NE derivative, 1-methyl-4,6, 7-trihydroxy TIQ, which has been found in acetaldehyde--perfused cow adrenals (Cohen, 1971; Cohen & Collins, 1970). However, attempts to synthesize this compound biochemically have not been successful (Collins & Kernozeck, 1972).

From the foregoing, it appears that acetaldehyde and the CA play a functional role in the mediation of ethanol consumption. The nature of the interaction of these two contributing components remains to be elucidated. It is possible that acetaldehyde may be present in the brain following the administration of ethanol and it is capable of interacting with the central CA. The results of this interaction may subserve some of the central effects that underlie the consumption of ethanol.

EXPERIMENT 1

There have been numerous studies examining the effects of ethanol administration on the central catecholamines, norepinephrine and dopamine. It has been reported that injections of ethanol resulted in an elevation of CA release as measured by increased accumulation of metabolites (Karoum, Wyatt & Majchrowicz, 1976). It has also been demonstrated that acute administration of ethanol produced an increase in CA release and synthesis (Corrodi, Fuxe & Hokfelt, 1966; Pohorecky, 1974; Pohorecky & Jaffe, 1975).

It has been shown that acetladehyde, the primary metabolite of ethanol, by itself, can alter the release rate of the CA. Inhalation of acetaldehyde vapor reuslted in an increase in the concentration of brain CA (Ortiz, Griffiths & Littleton, 1974), while, injections of acetaldehyde, produced alterions in their content within rat brains (Duritz & Truitt, 1966; Thadani & Truitt, 1977). The available evidence suggests that those mechanisms mediating the effects of acetaldehyde may also subserve the pharmacological effects of ethanol.

However, the above studies did not administer acetaldehyde directly into the brain. A number of recent studies have shown that centrally administered acetaldehyde results in behavioral and

physiological effects which are contrary to those produced when it is given peripherally (Brown, Amit, Smith & Rockman, 1978a; Amit, Brown & Rockman, 1977). Using the method of measuring the decline in brain CA following inhibition of synthesis (Brodie, Costa, Dlabac, Neff & Smookler, 1966; Corrodi, Fuxe & Hokfelt, 1966), the effects of an intraventricular infusion of acetaldehyde on CA release were investigated.

Method

Subjects. Eighteen male Wistar rats (Canadian Breeding Farms Ltd.) weighing 275-300g at the beginning of the experiment were used. The animals were individually housed in stainless steel cages (24 cm x 19 cm x 17 cm) covered with plexiglass tops, in a room regulated for constant temperature and humidity and a 12-hour day-night cycle. Food (Purina Lab Chow) and water were available ad libitum.

Procedure. Following several days of acclimitization, the animals were anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/kg). A 22-gauge stainless steel cannula guide (Plastic Products Inc.) was stereotaxically aimed at the left lateral ventricle of each of the animal. With the incisor bar set at 0.0, the stereotaxic co-ordinates were 1.0 mm posterior to Bregma, 1.5 mm lateral to the mid-sagittal line, and 3.6 mm

ventral to the dura. The cannula guide was secured in position by cranioplast cement anchored to the skull by 5 stainless steel screws. A stainless steel stilette was inserted into the guide to keep it free from obstruction.

After four days recovery, the animals were randomly assigned to four groups. Group 1 received an intraperitoneal (I.P.) injection of alpha-methyl-para--tyrosine methyl ester HCl (250 mg/kg; AMPT; Sigma Chemical Co.) followed two hours later by an intraventricular infusion of acetaldehyde at a dose of 320 µg (20 µl of 2% v/v acetaldehyde; Aldrich Chemicals Co.). The fluid was infused with a micrometer syringe via polyethylene tubing, attached to a 28-gauge cannula which was inserted into the chronically implanted guide. The infusion was delivered at a constant rate over 30 seconds. Group 2 received an infusion of acetaldehyde (320 µg) one minute prior to an I.P. injection of AMPT (250 mg/kg). Group 3 received an I.P. injection of AMPT followed 2 hours later by an intraventricular infusion of Ringer's solution (20 µl; Abbott Laboratories). Animals in Group 4 were handled but did not receive any pharmacological manipulation. The amount of acetaldehyde used was based on pilot work and on those used in previous experiments which demonstrated a behavioral effect (Brown et al., 1978a; Myers & Veale, 1979).

Three hours after the injection of AMPT, all the

animals were sacrificed by decapitation and the brains were rapidly extracted and cut in half. The left side was fixed in 10% formal saline and saved for histological confirmation of the cannula placement while the right side was immediately frozen on dry ice. Flourometric assay procedures were used on these samples to determine brain NE and DA levels (Chang, 1964; Shellenberger & Gordon, 1971).

Results

Figure 1 shows the mean whole brain levels of the CA, NE and DA, for each of the treatment groups. A one-way analysis of variance yielded significant differences among NE levels in the four groups $(F(3,14)=29.88, p<.001)$. Also, there were significant differences between the brain DA levels of the treatment groups $(F(3,14)=40.219, p<.001)$. Subsequent post hoc analysis (Tukey tests for multiple comparisons; $\alpha = .05$; Kirk, 1968) of the differences between the means under each of the conditions, showed that there were significant depletions of NE and DA as a result of the AMPT injections. However, there were no significant differences between the two acetaldehyde infused groups and the Ringer's infused group. Histological examination of the left side of the brain confirmed that all the cannula guides were properly implanted into the ventricles.

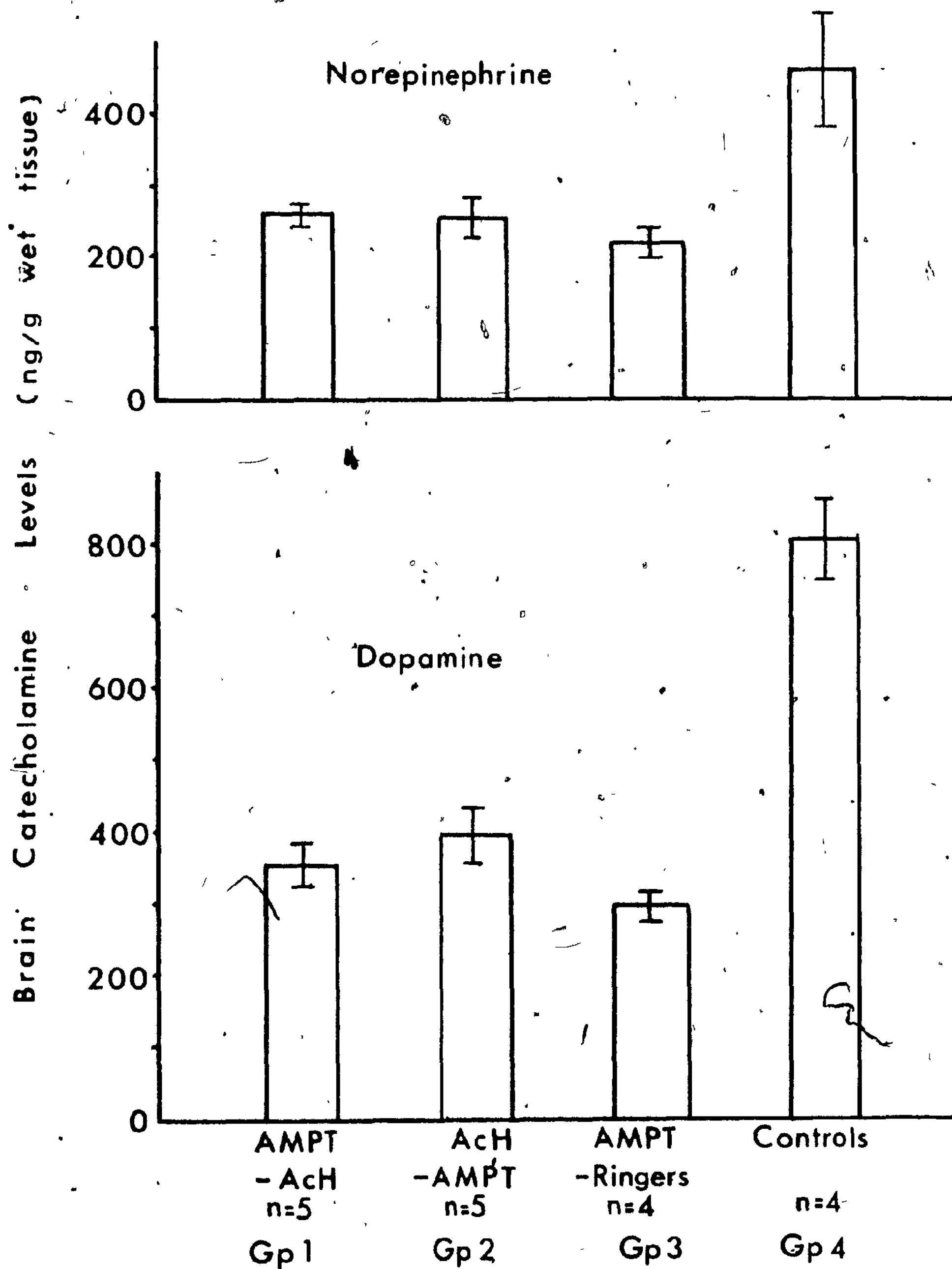

Figure 1. Mean whole brain catecholamine levels of rats treated with alpha-methyl-para-tyrosine (AMPT) and acetaldehyde (AcH). Verticle lines represent S.E.M.

Discussion

In the present experiment the effects of intraventricular administration of acetladehyde on CA release was examined. The rate of release of NE and DA was estimated by the technique of monitoring the decline of brain CA following synthesis inhibition with alpha-methyltyrosine (Brodie et al., 1966; Corrodi et al., 1966).

It was observed that there were no differences in CA release between the Ringer's infused animals and those receiving acetaldehyde just prior to the administration of alpha-methyl-para-tyrosine (AMPT).

As acetaldehyde has been reported to have a relatively short duration of action and is highly lipid soluble (Akabane, 1970), these two factors may limit the duration of its central pharmacological actions. It is possible that any effect by acetaldehyde on the CA may have terminated prior to the onset of the synthesis inhibitor. However, when the acetaldehyde was infused two hours after the AMPT administration there were no alterations observed in NE and DA release rate.

The present failure to observe alterations in CA metabolism following an acute intraventricular infusion of acetaldehyde may be the result of the limited extent of the pharmacological actions of a single infusion. Furthermore, it is conceivable that the pharmacological

actions of acetaldehyde may differ depending upon the route of administration.

EXPERIMENT 2

In the previous experiment it was observed that an acute infusion of acetaldehyde had little effect on the turnover of norepinephrine and dopamine. It has been shown that chronic treatment can produce alterations in central mechanisms. For example, Amir (1978b) has demonstrated that prolonged ethanol administration resulted in alterations in brain aldehyde dehydrogenase activity. Also, chronic inhalation of ethanol and acetaldehyde produced changes in brain catecholamine metabolism in mice (Ortiz, Griffiths & Littleton, 1974).

In the present experiment, the effect of chronic intraventricular infusions of acetaldehyde on catecholamine turnover, as estimated by the procedure used in Experiment 1, was investigated.

Method

Subjects. Nineteen male Wistar rats (Canadian Breeding Farms Ltd.) weighing 275-300g served as subjects for this experiment. The animals were individually housed in stainless steel cages covered with plexiglass tops, in a room regulated for constant temperature and humidity and a 12-hour day-night cycle. Food (Purina Lab Chow) and water were available ad libitum.

Procedure. A 22-gauge cannula guide was implanted into the left lateral ventricle of each animal according

to the surgical procedures described in Experiment 1. Following 3 to 4 days of recovery from surgery, the animals were connected to an infusion apparatus which consisted of a multichannel infusion pump (Harvard Apparatus). The fluid was delivered, via polyethylene tubing, to a flow-thru swivel (Brown, Amit & Weeks, 1976) connected to a shielded plastic tube which terminated in a 28-gauge internal cannula. The cannula was then inserted and secured into the chronically implanted guide. This system allowed the animals freedom of movement in the home cage at all times. The animals were randomly assigned to the treatment groups. The experimental group received 64 μg of acetaldehyde (4 μl of 2% v/v acetaldehyde) per infusion. A control group received 4 μl of Ringer's solution for every infusion, while a third group did not receive any infusions but was chronically connected to the infusion apparatus for the entire experiment. Infusions were delivered every 30 minutes for three consecutive days (72 hours). The cannulae were removed and flushed daily and fresh solutions were prepared every 24 hours. The animals' weight were recorded daily during the infusion period. The acetaldehyde dose selected was based on previous experiments in which this substance was infused intraventricularly (Myers & Veale, 1969; Amit et al., 1977) and also on the calculated capacity of the rat brain to

oxidize acetaldehyde (Sippel, 1974).

At the conclusion of this three day period the treatment and Ringer's control groups received intraperitoneal injections of alpha-methyl-para-tyrosine (250 mg/kg). Three hours following these injections all the animals were sacrificed by decapitation. The extraction procedure was similar to that employed in Experiment 1, with the brains cut in half, and a flourometric assay was performed on the samples in order to determine brain NE and DA levels.

Results

Figure 2 shows the mean brain levels of the CA, for the treatment groups. A one-way analysis of variance yielded significant differences among the three groups for NE levels ($F(2,16)=28.662$, $p<.001$) and for DA ($F(2,16)=74.731$, $p<.001$). Post hoc analysis (Tukey tests for multiple comparisons, $\alpha= .05$; Kirk, 1968) showed that there were significant depletions of both NE and DA as a result of the AMPT injections, however, there was no difference between the chronic acetaldehyde infused animals and the Ringer's infused group. Histological examination of the left side of the brains confirmed that all the cannula guides were properly implanted into the ventricles of each animal.

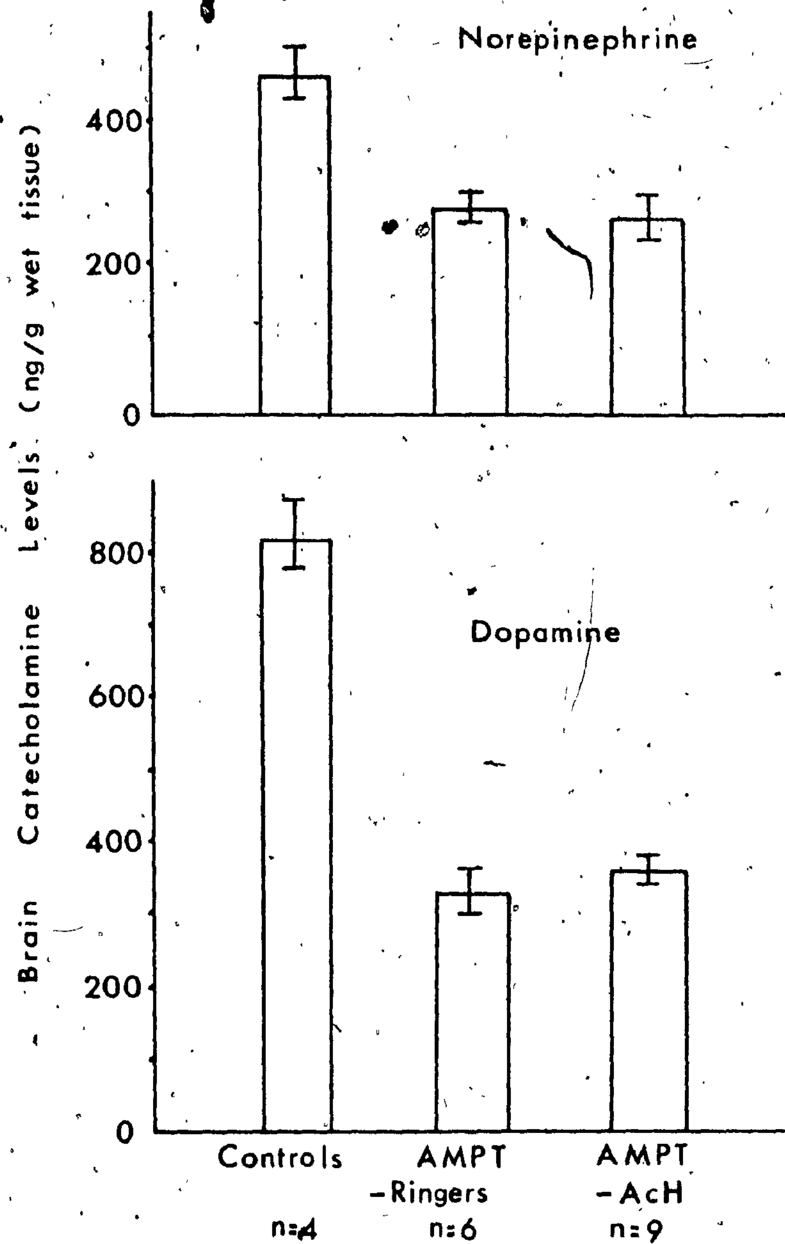

Figure 2. The mean brain levels of NE and DA following chronic acetaldehyde (AcH) infusion and alpha-methyl-para-tyrosine (AMPT) administration. Verticle lines represent S.E.M.

Discussion

In this experiment, it was found that intraventicular infusions of acetaldehyde failed to alter CA release, even when infused around-the-clock for 72 hours. The present findings are inconsistent with the previous reports indicating that acetaldehyde administration produces alterations in the metabolism of NE and DA (Duritz & Truitt, 1966; Ortiz, Griffiths & Littleton, 1974; Thadani & Truitt, 1977).

The lack of agreement between the results of the present experiment and past reports may be due to the differences in the route of administration of acetaldehyde. In those studies showing alterations in metabolism acetaldehyde was administered peripherally through injection (Duritz & Truitt, 1966), by inhalation (Ortiz et al., 1974) and by intracisternal infusion (Thadani & Truitt, 1977).

Another potentially important factor that may contribute to the inconsistencies may be the various methods used of determining CA metabolism. Voluntary ethanol consumption normally results in only negligible amounts of acetaldehyde to be present in the brain (Sippel, 1974; Tabakoff, Anderson & Ritzmann, 1976), however, these minute quantities may produce central pharmacological effects (Amit et al., 1977). It is conceivable that

alterations may occur in metabolism following acetaldehyde central administration, but the magnitude of the changes may be very slight so as to be undetectable using the method employed in the previous two experiments. Perhaps using a more sophisticated approach would yield differing results.

It has been demonstrated that acetaldehyde when given peripherally, possesses profound toxic effects (Asmussen, Hald & Larsen, 1948; Hald & Jacobsen, 1948). However, when administered centrally, via intraventricular infusions, acetaldehyde failed to produce any observable aversive effects (Brown et al., 1978a) and, in fact, was found to be self-administered indicating its ability to act as a reinforcer (Amit et al., 1977). It is conceivable that acetaldehyde, when present in the brain, possesses different pharmacological properties then those in the periphery.

EXPERIMENT 3

It has been demonstrated that acetaldehyde, the primary metabolite of ethanol, was self-administered into the cerebral ventricles of rats (Amit, Brown & Rockman, 1977). It was also observed that ethanol was not self-administered intraventricularly suggesting that acetaldehyde may be responsible for the positive reinforcing effects of ingested ethanol.

Amir (1977, 1978a) has also shown that the levels of activity of aldehyde dehydrogenase, an enzyme capable of oxidizing acetaldehyde, were positvely correlated with voluntary ethanol consumption in rats. Furthermore, it has been shown that the propensity to self-administer acetaldehyde was positively correlated with subsequent ethanol preference in rats (Brown, Amit & Smith, 1979b). These findings suggest that common mechanisms may mediate the self-administration of acetaldehyde and ethanol.

Previous reports have demonstrated that the central CA, NE and DA, may be involved in mediating the effects of ethanol (e.g., Amit, Stern & Wise, 1970; Corrodi, Fuxe & Hokfelt, 1966; Pohorecky, 1974) and also of acetaldehyde (Duritz & Truitt, 1966; Ortiz, Griffiths & Littleton, 1974; Thadani & Truitt, 1977). The present investigation attempted to determine whether an animal's propensity to self-administer acetaldehyde intraventricularly is

related to possible changes in CA release.

Method

Subjects. Sixty-one male Wistar rats (Canadian Breeding Farms Ltd.) weighing 275-300g at the start of the experiment were used. Prior to surgery and during the recovery period, the animals were individually housed in stainless steel cages in a room regulated for constant temperature and humidity and a 12-hour day-night cycle. Food (Purina Lab Chow) was available ad libitum.

Procedure. A 22-gauge stainless steel cannula guide was stereotaxically implanted into the left lateral ventricle according to the surgical procedures outlined in Experiment 1.

After four days recovery, the animals were individually placed in an operant chamber (Ralph Gerbrands Co., Model C) with the food hopper blocked off. The testing room was regulated for constant temperature and humidity and had a 12-hour day-night cycle. For eleven consecutive days the animals were maintained in the operant chambers and had free access to food, water and an operant lever. Each depression of the lever activated a pump (Razel, Inc.) for 10 seconds, during which a 4 μl infusion was delivered via polyethylene tubing to a flow-thru swivel (Brown, Amit & Weeks, 1976) suspended above the chamber. A shielded plastic tube, connected to the outlet

of the swivel, terminated in a 28-gauge cannula which was inserted and secured into the chronically implanted guide. During the 10 second infusion period, additional lever presses did not reactivate the pump and were not recorded. A multi-channel event recorder was used to monitor all infusions.

The experimental group received infusions of 2% acetaldehyde (v/v; 64 μg/infusion) while the control group was infused with Ringer's solution (pH adjusted to that of acetaldehyde; pH=4.5-4.8). Additional animals were yoked to the experimental animals so that each lever press effected by an experimental animal also resulted in an infusion of 2% acetaldehyde into its yoked partner. Lever presses made by the yoked animals did not activate the pump and result in an infusion, however, these presses were recorded. Once daily the animals were weighed and the cannulae removed and flushed so as to minimize blockage.

On the eleventh day the animals were injected intraperitoneally with alpha-methyl-para-tyrosine methyl ester HCl (250 mg/kg; Sigma Chemicals Co.). The animals were sacrificed by decapitation three hours later and the brains were rapidly extracted and cut in half. The left side was fixed in 10% formal saline and saved for histological confirmation of the cannula placement while the right side was immediately frozen on dry ice.

Flourometric assay procedures were used on these samples to determine brain norepinephrine and dopamine levels (Chang, 1964; Shellenberger & Gordon, 1971). The levels of these catecholamines were then correlated (Pearson Product-Moment) with the lever presses over the 11 day period for each animal.

Results

Figure 3 shows the mean number of lever presses recorded each day for the three groups. A two-way analysis of variance with repeated mesaures over the days factor yielded significant differences in the total daily infusions among the groups ($F(2,28)=3.96$, $p<.05$). There was also a significant days effect ($F(10,580)=4.69$, $p<.05$), however, there was no interaction ($F(20,580)=1.01$, $p>.05$). Post-hoc analysis of the group differences (Scheffé tests; $\alpha= .05$) indicated that the experimental and yoked groups lever pressed at higher rates than the control group, but there were no differences between the experimental and yoked animals' pressing rates. A correlation between the number of acetaldehyde infusions received by each yoked animal and its own lever pressing resulted in an insignificant relationship ($r= -0.17$, $n=21$, $p>.05$).

Table 1 shows the correlation coefficients computed between the NE and DA levels of each animal and the

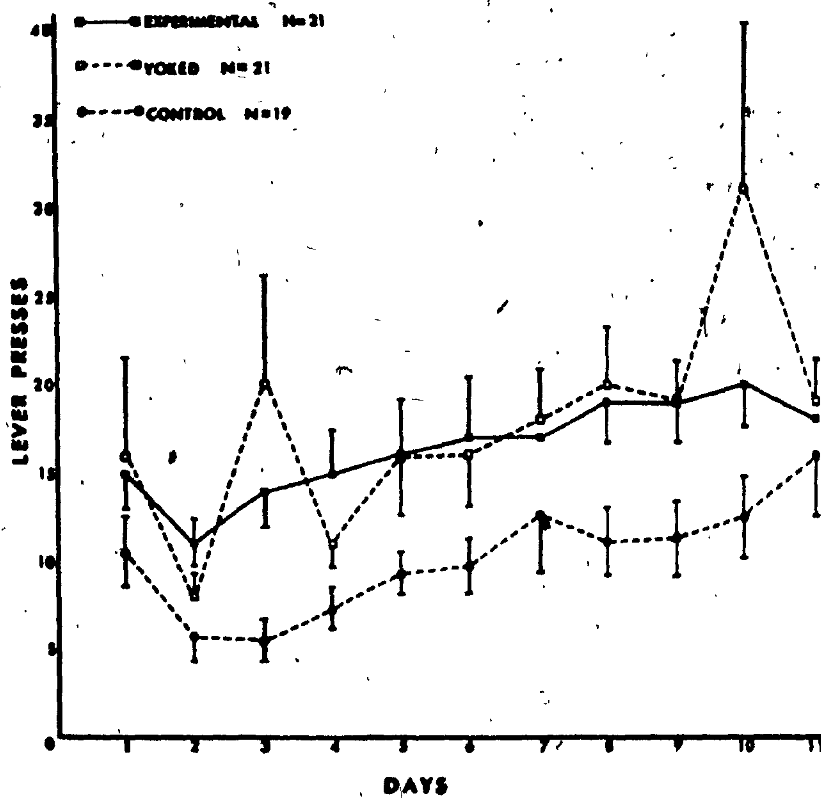

Figure 3. The mean number of lever presses per day for the three treatment groups. Verticle lines represent S.E.M.

CORRELATION COEFFICIENTS (r)

GROUPS	NOREPINEPHRINE	DOPAMINE
EXPERIMENTAL N=21	-0.41 *	-0.50 **
YOKED N=21 — INFUSIONS	-0.09	0.04
YOKED N=21 — LEVER PRESSES	-0.37 *	-0.40 *
CONTROL N=19	0.14	-0.09

* $p < 0.05$ ** $p < 0.01$

Table 1. The correlation coefficients computed between the NE and DA levels of each animal and its lever or infusion rate.

number of infusions or lever presses of that animal. It was observed that there was a significant negative relationship between the experimental group's lever pressing (infusions) and its CA levels (NE: $r = -.41$, $n=21$, $p<.05$; DA: $r = -.50$, $n=21$, $p<.01$). This was also seen with the yoked group's lever pressing (NE: $r = -.37$, $n=21$, $p<.05$; DA: $r = -.40$, $n=21$, $p<.05$), however, there was no significant correlation between the yoked group's infusion rate and their CA levels. There was also no correlation between the control animals' pressing rate for Ringer's solution and their NE and DA levels. Histological examination of the fixed brain halves confirmed that all the cannula guides were properly implanted into the left lateral ventricles.

Discussion

Consistent with previous findings (Amit, Brown & Rockman, 1977; Brown, Amit & Smith, 1979b) it was shown that naive rats receiving acetaldehyde will lever press at higher rates than controls. As there was no difference in the experimental and yoked groups' lever pressing rates, it is possible that the elevated pressing may have been the result of locomotor stimulation induced by the presence of acetaldehyde. However, there was no relationship between the number of lever presses by a yoked animal and the amount of acetaldehyde that it

received suggesting that the increased pressing may not be simply due to acetaldehyde-induced motor excitation. Also, Brown et al. (1979a) reported that programmed infusions to rats did not increase their activity more than those of Ringer's control animals.

It was also shown that the experimental and yoked groups' pressing was correlated to their CA levels and hence to their release rate. This did not appear to be due to induction by acetaldehyde as no such relationship was observed between the infusion rate in the yoked animals and their NE and DA metabolism.

The present findings are similar to those observed in Experiments 1 and 2 where the mere physical presence of acetaldehyde failed to produce alterations in CA release, however, it would appear that metabolism is related to the rate of acetaldehyde self-administration and possibly to its ability to act as a reinforcer. It has been suggested that acetaldehyde may alter neurochemical functioning by its ability to condense with biogenic amines to produce alkaloids that are capable of acting as pseudo-transmitters (e.g., Cohen, 1976, 1978; Rahwan, 1975). It is possible that these alkaloids may mediate the excitation observed, as reflected by increased lever pressing.

GENERAL DISCUSSION

In the present series of experiemnts, it was observed that intraventricular infusions of acetaldehyde failed to produce alterations in norepinephrine and dopamine release. The present findings are inconsistent with previous reports indicating that when acetaldehyde is present in the brain, there are changes in catecholamine metabolism and content (Duritz & Truitt, 1966; Ortiz, Griffiths & Littleton, 1974; Thadani & Truitt, 1977).

It has been shown that peripheral administration of acetaldehyde results in severe toxic effects (Asmussen, Hald & Larsen, 1948; Hald & Jacobsen, 1948), however, its central administration failed to produce aversive effects (Brown, Amit, Smith & Rockman, 1978a). It was also demonstrated that intraventricular infusions of acetaldehyde possessed reinforcing properties (Amit, Brown & Rockman, 1977). Therefore, in addition to the behavioral and physiological differences, it is possible that acetaldehyde may produce different neurochemical effects when administered centrally. However, it is conceivable that the magnitude of the alterations may be so minute as to be undetectable with the method of estimating CA metabolism that was employed. Perhaps a more sophisticated technique, such as measuring the reuptake of labelled neurotransmitter (Thadani & Truitt,

1977), may yield differing results.

The present findings indicate that, even though acetaldehyde administration may not induce changes in NE and DA metabolism, there appears to be a relationship between its psychopharmacological properties and CA release. In Experiment 3, it was observed that the propensity to self-administer acetaldehyde intraventricularly was correlated with CA metabolism. Even though the presence of acetaldehyde in the brain may not produce alterations in CA release, the occurrance of its neurochemical effects may be related to the rate of NE and DA metabolism.

It has been reported that acetaldehyde may condense with biogenic amines to form alkaloids (Cohen, 1976; Rahwan, 1975) and that they may act as false transmitters (Cohen, 1978). These alkaloids have been suggested to contribute to some of the neurophysiological effects of ethanol consumption (Cohen, 1976, 1978; Rahwan, 1975).

The present studies suggest that the rate of catecholamine metabolism may mediate the reinforcing properties of acetaldehyde and a possible interaction may subserve the pharmacological effects of ingested ethanol. Further research is necessary to elucidate the mechanisms by which an interaction between acetaldehyde and the catecholamines may be responsible for the positive

reinforcing effects of ingested ethanol.

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