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Electrical Stimulation and Lesions of the Medial Forebrain Bundle of the Rat: Changes in Voluntary Ethanol Consumption and Brain Aldehyde Dehydrogenase Activity

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Abstract. Repeated sessions of electrical stimulation or lesions in the ventral aspect of the medial forebrain bundle (VMFB) region of the brain in rats resulted in a significant increase and a decrease in voluntary ethanol (10% v/v) intake, respectively. Whole brain and midbrain-diencephalon (MB-DE) aldehyde dehydrogenase (ALDH) were measured in different groups of experimental and control animals before, immediately after, and 30 days after the termination of the stimulation regimen, or 8 days and 30 days after the induction of the lesions. By the end of the stimulation regimen, the levels of MB-DE ALDH of the experimental (ethanol-drinking: stimulated) animals were significantly higher than those of control animals (ethanol-drinking: nonstimulated, water-drinking: stimulated, and water-drinking: nonstimulated). A marked decrease in MB-DE ALDH activity was noted in lesioned animals but not in cyanamide-treated or in implanted control animals. Neither the stimulation nor the lesions had any demonstrable effect on whole brain ALDH activity. Cyanamide administration caused a pronounced decrease in ethanol intake and in levels of liver ALDH activity. The increase in MB-DE ALDH activity in the ethanol-drinking, stimulated animals was attributed to the interaction between the VMFB activation and the ethanol drinking, while the reduction in ALDH activity was attributed to the degeneration of biogenic-amine-containing nerve fibers.

Key words: MFB stimulation – MFB lesion – Ethanol intake – Brain aldehyde dehydrogenase – Liver aldehyde dehydrogenase

There is accumulating evidence that acetaldehyde, the highly reactive oxidation product of ethanol, interacts with biogenic amine metabolism in the brain in addition to producing its better-known toxic effects in the periphery (Rahwan, 1975; Cohen, 1976). Since biogenic amines play a principal role in neural systems mediating motivation and reward in mammals (e.g., German and Bowden, 1974; Mogenson and Phillips, 1976), such interactions could underlie some of the behavioral consequences of ethanol ingestion. However, the fact that acetaldehyde does not readily accumulate in brain tissue of ethanol-treated animals bearing pharmacologically compatible concentrations of acetaldehyde in their circulation (e.g., Sippel, 1974; Tabakoff et al., 1976) gives rise to confusion concerning the physiologic and biochemical mechanisms that mediate this effect (Deitrich and Erwin, 1975; Deitrich, 1976).

A possible clue to the nature of the principal mechanism that controls the acetaldehyde-mediated effects of ethanol may be deduced from demonstrations of a systematic relationship between the cerebral-aldehyde oxidizing multienzyme complex aldehyde dehydrogenase (EC 1.2.1.3, ALDH) and voluntary ethanol consumption in rats (Amir, 1977, 1978). ALDH occurs in all regions of the brain (Erwin and Deitrich, 1966) and is normally catalizing the oxidation of various endogenous aldehydes such as those that arise from the oxidative deamination of biogenic amines (Von Wartburg et al., 1975). But the fact that acetaldehyde has a higher affinity to ALDH relative to that of the natural substrates of the enzyme (Lahti and Majchrowicz, 1969) suggests that brain ALDH may also function as a 'metabolic barrier' to control the amount of acetaldehyde present in tissue during ethanol ingestion (Sippel, 1974; Tabakoff et al., 1976; Pettersson and Kiessling, 1977). It is thus conceivable that the interrelationships among brain ALDH, its natural substrates, and acetaldehyde may

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be a principal biochemical counterpart of some of the behavioral consequences of ethanol ingestion; accordingly, ALDH deficiency in the brain may predispose abstention from ethanol in animals, while high levels of the enzyme may be associated with strong preference for the drug.

A further possibility is that spontaneous or imposed changes in ethanol preference by animals may involve alterations in the properties or function of the aldehyde-oxidizing system of the brain. Previous studies have shown that ethanol preference in rats can be modified by prolonged electrical stimulation or lesions of neurons in the ventral aspects of the medial forebrain bundle (MFB) region of the brain (Amit et al., 1970, 1976; Amit and Stern, 1971; Wayner et al., 1971). The particular mechanisms mediating these effects are not yet fully understood (e.g., Wayner et al., 1972), but the fact that ALDH is part of the metabolic apparatus of central aminergic neurons (Duncan and Sourkes, 1974) suggests that such manipulations may influence ethanol consumption by modifying the aldehyde-oxidizing capacity of neurons that course through the VMFB. The present investigation examined the effect of VMFB stimulation and lesions on brain ALDH activity and voluntary ethanol consumption in rats.

EXPERIMENT I

This experiment examined the effect of intermittent stimulation of the VMFB on voluntary ethanol consumption and brain ALDH activity. The stimulation parameters used were modified from Amit et al. (1971).

Materials and Methods

Animals. A total of 80 male Wistar rats were used (325–375 g at the start of the experiment). Rats were housed singly in a temperature-regulated room (22 ± 1°C) under normal light-dark conditions (light on from 9 a.m. to 9 p.m.). Purina laboratory chow and water were available ad lib. Forty-four animals were implanted under sodium pentobarbital (50 mg/kg) with a single 250-μ bipolar electrode (Plastic Products) aimed at the ventral MFB in the area of the lateral hypothalamus [coordinates: 0.8 mm posterior to bregma, 1.7 mm lateral to the midline, and 8.6 mm below the surface of the skull (Pellegrino and Cushman, 1967)]. These animals were randomly assigned to an ethanol-drinking, stimulated group (ETOH-ST, $N = 22$) and to a water-drinking, stimulated group (H_2O-ST, $N = 22$). The unimplanted animals were randomly assigned to an ethanol-drinking, nonstimulated group (ETOH-NST, $N = 18$) and to a water-drinking, nonstimulated group (H_2O-NST, $N = 18$).

Procedure

Phase I. All ethanol-drinking animals were given continuous access to ethanol (10% v/v) and tap water for 30 days. A 10% ethanol solution was found previously to be well below the final acceptance concentration of Wistar rats used in our laboratory (Russell and Stern, 1973), thus providing an adequate concentration for evaluating ethanol preference in these animals (e.g., Eriksson, 1968). The position of the tubes containing the ethanol solutions was alternated daily from side to side of each cage to control for the development of position preference. Ethanol intake was recorded daily (9–10 a.m.) and was expressed in g of absolute ethanol consumed/kg body weight (g/kg). At the end of this phase (Day 31), 4 animals from each of the four groups (i.e., ETOH-ST, ETOH-NST, H_2O-ST, H_2O-NST) were randomly selected and sacrificed for brain ALDH determination.

Phase II. Starting at Day 31 all ETOH-ST and H_2O-ST animals received thirty daily 30-min sessions of intermittent electrical stimulation consisting of 5-s trains of square pulses (30 pps, 1.0 ms in duration), alternating with 5-s nonstimulation periods. The current amplitude ranged from 3–4 Vs and was reduced if animals exhibited any disruptive (running, jumping, or circling) locomotor activity. The stimulation technique used in the present study differed from that of Amit et al. (1970), who delivered 20-s trains of sine-wave stimulation alternating with 20-s of nonstimulation periods. The duration of the stimulation sessions and the intensities of stimulation used here were the same as those used by Amit et al. All nonstimulated animals were placed in the stimulation boxes for 30 min every day to control for handling. Ethanol and water were never offered in the stimulation boxes but were always available in the home cage. Ethanol intake was monitored daily as described. At the termination of this phase (Day 61) 7–8 animals were randomly selected from each of the four groups and were sacrificed for brain ALDH determination.

Phase III. The remaining ETOH-ST and ETOH-NST animals were maintained for an additional 30 days following the termination of the stimulation regimen and poststimulation ethanol intake was monitored as described. These animals and the remaining control animals (7–9 animals/group) were sacrificed at the termination of this phase (Day 91) for brain ALDH determination.

Determination of Brain ALDH Activity

Tissue Preparation. Rats were decapitated and their brains rapidly removed, washed with ice-cold distilled water, and disected on ice into midbrain-diencephalon and cortex-cerebellum. Each part was homogenized in sufficient 0.25 M sucrose containing 1% Triton X-100, to make 20% homogenates. Homogenates were centrifuged for 90 min at 100000 × g at 0°C and the clear supernatant was used as the enzyme source.

Assay of ALDH. Total ALDH activity was determined by measurement of the rate of enzyme-catalyzed NAD-dependent production of indole-3-acetic acid from indole-3-acetaldehyde (modified from Deitrich, 1966). Incubations were carried in duplicates for 10 min at 30°C using 0.2 ml of 0.02-M indole-3-acetaldehyde bisulfite (final concentration 0.004 M) and 0.1 ml of 0.03-M NAD (final concentration 0.003 M) as substrates (Sigma Chemical Co.) in 0.5 ml of 0.08-M potassium pyrophosphate buffer (final concentration 0.04 M), pH 9.3. The reactions were started by the addition of 0.2 ml of the 100000 × g enzyme source bringing the total volume to 1.0 ml. Unreacted indole acetaldehyde was extracted into dichlorethane and the activity was estimated from the fluorescence of the indole acetic acid formed (Deitrich, 1966) and was expressed in micromoles per h × g tissue wet wt.$^{-1}$. Recovery of known amounts of indole acetic acid was quantitative from solutions where no NAD and enzyme were added and over 90% NAD and enzyme. At the level of substrates used there was no detectable nonenzymatic reaction between NAD and indole acetaldehyde (Duncan and Tipton, 1971), and the enzyme-catalized production of indole acetic acid was linear for at least 15 min, indicating complete saturation

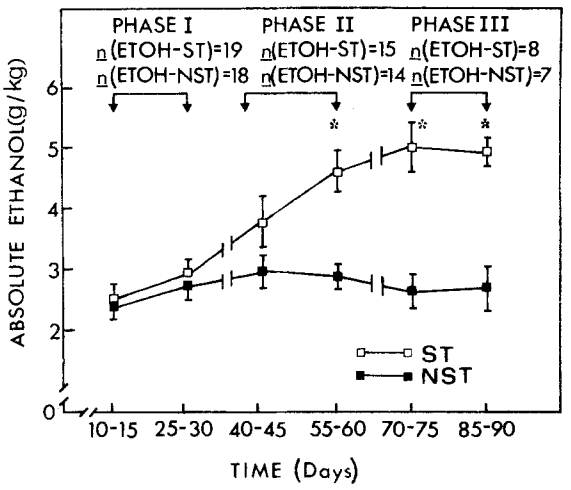

Fig. 1. Mean (± SEM) intake of absolute ethanol (g/kg body wt./day) of stimulated and nonstimulated animals in blocks of 5 days, in 10-day intervals. *Phase I:* before stimulation; *Phase II:* during stimulation; *Phase III:* after stimulation. * Significant difference (*t*-test, $P < 0.001$) from nonstimulated animals

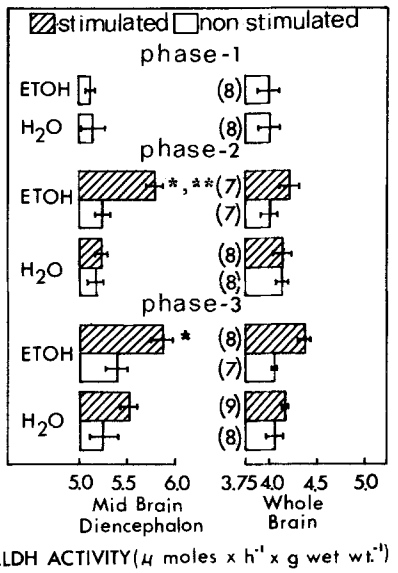

Fig. 2. Midbrain-diencephalon and whole brain (combined MB-DE and cortex-cerebellum) ALDH activity in the ETOH-ST, ETOH-NST, H_2O-ST, and H_2O-NST groups in the 3 phases of experiment. *Bars and horizontal lines:* means ± SEM (*N*) of ALDH activity. Data in Phase I represent combined values of ALDH activity of all ETOH-drinking and H_2O-drinking animals sacrificed at that point. * Significant difference from ETOH-NST; ** significant difference from H_2O-ST

of the enzyme. Protein concentrations were measured by the method of Lowry et al. (1951) with bovine serum albumin (Sigma Chemical Co.) as the standard.

Histology. To confirm electrode placements in the stimulated animals, four rats were sacrificed with an overdose of chloral hydrate and perfused with saline and 10% formal saline solution. Frozen sections of their brains were sliced and stained with thionin. A reconstruction of electrode placements in these animals revealed a distribution within the ventral MFB in the area of the lateral hypothalamus.

Results

A considerable increase in ethanol consumption, but not in total fluid intake, was noted in most of the ETOH-ST animals following the stimulation regimen. Control animals exhibited steady patterns of ethanol intake with very slight day-to-day variation. Since a number of animals from each of the two ethanol-drinking groups were sacrificed at Days 31 and 61 for enzyme determination, thus resulting in an unequal number of subjects across the different phases of the experiment, the data on ethanol consumption were assessed by comparing the means of the amount of ethanol consumed by each group in 5-day blocks in 10-day intervals using *t*-tests (Fig. 1). By the end of the stimulation regimen (Days 55–60), ethanol intake of the ETOH-ST group was significantly higher than that of the ETOH-NST group ($t = 4.50$, $df = 27$, $P < 0.001$). The ETOH-ST animals continued to consume high levels of ethanol for the remainder of the experiment (Phase III), and the differences between means of ethanol intake in the ETOH-ST and ETOH-NST groups were highly significant (i.e., Days 70–75: $t = 4.32$, $df = 13$, $P < 0.001$; Days 85–90: $t = 9.72$, $df = 13$, $P < 0.001$). Since the nature of the data required the application of several discontinuous statistical tests, thus increasing the possibility of obtaining spuriously significant results, the Bonferoni test was used to compensate for multiple discontinuous *t*-testing (Miller, 1966). The results remained significant.

Data on midbrain-diencephalon (MB-DE) and whole brain (combined values of MB-DE and cortex-cerebellum) ALDH activity of the different groups measured at the three phases of the experiment are shown in Figure 2. These data were assessed by an analysis of variance followed by a comparison between individual pairs of groups (Tukey HSD test), using an overall error term in the analyses. Activity of MB-DE ALDH was significantly higher in the ethanol-drinking than in the water-drinking animals [$F(1,67) = 10.01$, $P < 0.01$] and varied significantly within each category as a function of treatment (i.e., ST, NST) and time (i.e., Phase I, II, III) [$F(4,67) = 8.36$, $P < 0.001$]. The comparison between group means revealed that poststimulation (Phase II and III)

MB-DE ALDH activity of the ETOH-ST group was significantly higher ($P < 0.01$) than that measured in the ETOH-NST animals. Further, at the end of the stimulation period (Phase II) MB-DE ALDH activity of the ETOH-ST group was significantly higher ($P < 0.01$) than that of the H_2O-ST animals. No significant differences in MB-DE ALDH activity were recorded between the H_2O-ST and H_2O-NST groups. No significant differences in whole brain ALDH activity were noted between any pairs of groups in any of the three phases of the experiment.

Discussion

Ethanol intake by the ETOH-ST animals increased significantly during the course of the stimulation regimen and stabilized at about twice the level of ethanol consumed by the ETOH-NST animals. The possibility that the increase in levels of MB-DE ALDH activity measured in these animals resulted from enhanced ethanol intake and increases in brain levels of acetaldehyde is not likely; despite the fact that normal ALDH levels in cortex-cerebellum are considerably lower than that of MB-DE (Amir, unpublished), no changes in aldehyde-oxidizing capacity were noted in these brain regions in the ETOH-ST animals. Furthermore, acetaldehyde is not likely to accumulate in brain tissue of ethanol-ingesting animals under normal circumstances (Sippel, 1974; Tabakoff et al., 1976; Pettersson and Kiessling, 1977), thus ruling out the possibility of an acetaldehyde-dependent induction of ALDH activity. An alternative possibility is that the increase in MB-DE ALDH levels in the ETOH-ST animals resulted from a physico-chemical interaction between the activation of aminergic neurons and the metabolism of acetaldehyde in brain tissue.

Electrical stimulation of amine-containing neurons facilitates the turnover rate of biogenic amines and increases the activity of enzymes associated with their metabolic apparatus (Alousi and Weiner, 1966; Murrin and Roth, 1973; Walters and Eccleston, 1973; Kopin, 1974). It is conceivable that the amount of ALDH that is actually functional in situ depends to some extent on the steady-state level of endogenous aldehydes that arise from amine metabolism, which in turn depends on the pattern of activity of aminergic neurons. Since the total cerebral-aldehyde oxidizing capacity normally exceeds that which is required for the oxidation of endogenous aldehydes (Erwin and Deitrich, 1966), stimulation-dependent increases in the turnover rate of biogenic amines and their aldehyde products is not likely to significantly affect the level of ALDH activity (i.e., the H_2O-ST group). On the other hand, since acetaldehyde blocks the oxidation of endogenous aldehydes by competing for ALDH (Lahti and Majchrowicz, 1969), the presence of even subtle amounts of ethanol-derived acetaldehyde in the cellular milieu in close temporal contiguity with electrical stimulation of neurons (i.e., the ETOH-ST animals) could ultimately result in a physiologically demonstrable substrate-dependent changes in enzyme activity. Further, if ALDH is a physiologic mechanism in the control of ethanol-related behaviors, then such effect might lead to changes in ethanol preference by the stimulated animals.

EXPERIMENT II

Ventral MFB lesions were found to block ethanol consumption in rats (Amit et al., 1976) and were also shown to decrease midbrain ALDH levels (Duncan et al., 1972; Agid et al., 1973). The present experiment examined the possible relation between these effects. Further, a comparison was made between the effects of the lesion and the effects produced by cyanamide (Temposil), a potent ALDH inhibitor, which is often used to block ethanol consumption in humans (Consbruch and Derwart, 1968).

Materials and Methods

Subjects. A total of 45 male Wistar rats were used (400–450 g at the start of the experiment). Animals were housed and fed as described in Experiment I. Thirty-two rats were implanted with a single monopolar stainless steel electrode (250 μ in diameter) insulated to within 0.5 mm of the tip, aimed at the ventral MFB in the area of the lateral hypothalamus (coordinates were the same as in Experiment I).

Procedure. The implanted animals were divided into lesioned ($N = 16$) and nonlesioned ($N = 16$) groups. The unimplanted animals ($N = 13$) were used as the cyanamide-treated group. All animals were given free choice between ethanol (10% v/v) and tap water for 30 days as previously described. The ethanol was removed from all cages on Day 31 and all animals received their scheduled treatments. Medial forebrain bundle lesions were produced under ether anaesthesia by passing a constant anodal current (2 mA for 20 s) through the implanted electrode. A large referential electrode located on the body of the rats served as a current return. Histological verification of the location and size of the lesions were carried out in four animals 30 days after the induction of the lesions. A considerable destruction of tissue and almost complete loss of cellularity around the tip of the electrode was noted in these animals. The size of the lesions, however, was small, approximating 1–1.5 mm in diameter. The cyanamide-treated group received a series of 10 daily injectons (i.p., starting at Day 31) of 20 mg/kg cyanamide (citrated calcium carbamide in saline; Cyanamide of Canada Ltd.). The control animals (implanted, not lesioned) received daily injections of saline. Ethanol was again offered to all animals on Day 35 and continued for the remainder of the experiment. Ethanol solutions were removed from all cages during the treatment period (Days 31–35) to prevent the occurrence of a sickness-dependent abstention from ethanol or the development of conditioned aversion-type behavior in the lesioned and the cyanamide-treated animals. The

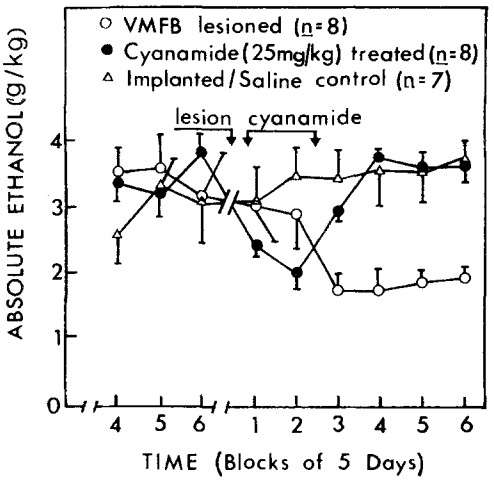

Fig. 3. Mean (± SEM) intake of absolute ethanol (g/kg body wt./day) of VMFB-lesioned, cyanamide-treated, and control animals in blocks of 5 days. Blocks 4–6 are baseline. *Arrows:* time of induction of lesions and start and termination of cyanamide treatment

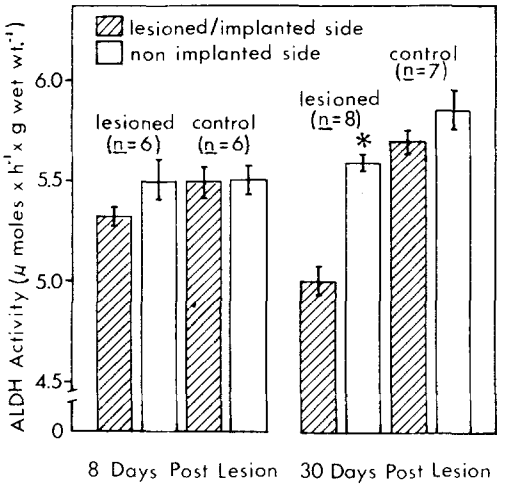

Fig. 4. The effect of VMFB lesions on midbrain-diencephalon ALDH activity. Assays were carried out at 8 days and 30 days postlesion. *Bars and vertical lines:* means ± SEM of ALDH activity of lesioned/implanted and nonlesioned side of midbrain-diencephalon. * Significant difference from lesioned side ($P < 0.05$, Tukey HSD test)

control animals exhibited steady pattern of ethanol intake with only small (5–10%) day-to-day variations.

ALDH Determination. Determinations of brain and liver ALDH activity were conducted on different experimental and control animals at Day 36 and at the termination of the experiment (Day 60). The preparation of brain tissue and the assay conditions were the same as those described in Experiment I. The effects of the lesions on midbrain-diencephalon ALDH activity were estimated by comparing the activity of ALDH on the lesioned side to that of the nonlesioned side of the brain. Similar comparisons were made on the implanted, nonlesioned animals. Total liver ALDH activity was measured from the 100000 × g supernatant of 10% detergent-treated (1% Triton X-100), liver homogenates. The assay conditions were the same as those for brain ALDH.

Results

Food intake, water intake, and body weights were not affected by the unilateral VMFB lesions or the cyanamide administration. By contrast, there was a marked decrease in ethanol intake in both VMFB-lesioned and cyanamide-treated animals (Fig. 3). The data on ethanol consumption by the three groups were assessed by comparing means of ethanol intake calculated in 5-day blocks. Analysis of the posttreatment ethanol intake (Days 35–60) revealed significant difference among the three groups [$F(2,20) = 4.91$, $P < 0.05$]. Further, there was a significant interaction between the type of treatment and time [$F(2,100) = 12.29$, $P < 0.001$]: the rate of decrease in ethanol consumption of the VMFB-lesioned animals was slower than that of cyanamide-treated animals. However, ethanol intake by the cyanamide-treated animals returned to baseline level following the termination of the cyanamide regimen, while ethanol intake by the lesioned animals remained very low for the remainder of the experiment.

The effect of MFB lesions on MB-DE ALDH is illustrated in Figure 4. The activity of ALDH in the lesioned side of the MB-DE was significantly lower than that measured in the contralateral side [$F(1,24) = 23.18$, $P < 0.001$], but the effect depended on the time of sampling [$F(1,24) = 9.50$, $P < 0.01$]. A significant decrease in ALDH activity in the lesioned side was noted 30 days after the induction of the lesion ($P < 0.01$), but not in the first determination (5 days after the induction of the lesion).

Data on whole brain and whole MB-DE ALDH activity are presented in Figure 5. No significant differences in whole brain ALDH activity were observed between the groups. By contrast, MB-DE ALDH levels varied significantly as a function of treatment [$F(2,35) = 9.08$, $P < 0.001$] and the time of sampling [$F(1,35) = 4.06$, $P < 0.05$]. Analysis of the interaction between treatment and time of sampling [$F(2,35) = 5.09$, $P < 0.01$] revealed that MB-DE ALDH activity of the lesioned animals was significantly lower than that of the cyanamide-treated and control animals at the termination of the experiment (30 days postlesion). No difference in ALDH activity was observed between the cyanamide-treated and the control group at that time.

There was a significant effect of treatment on liver ALDH activity [$F(2,35) = 9.70$, $P < 0.001$; see Fig. 6]. However, the interaction between treatment and time of sampling of liver ALDH [$F(2,35) = 3.92$, $P < 0.05$]

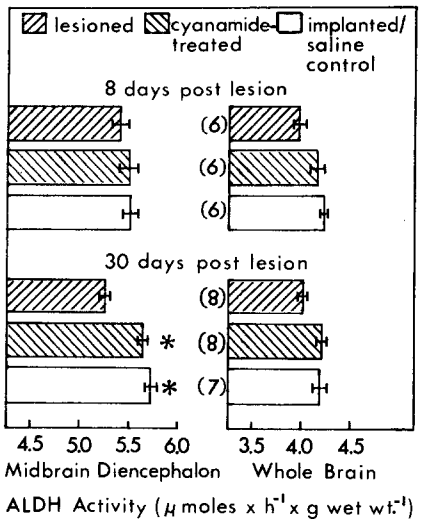

Fig. 5. Midbrain-diencephalon and whole brain (combined values of MB-DE and cortex-cerebellum) ALDH of the VMFB-lesioned, cyanamide-treated, and control animals at 8 days and 30 days postlesion. *Bars and horizontal lines:* means ± SEM of enzyme activity. *Numbers beside bars (right):* number of animals utilized in each assay. * Significant difference from lesioned group ($P < 0.05$, Tukey HSD test)

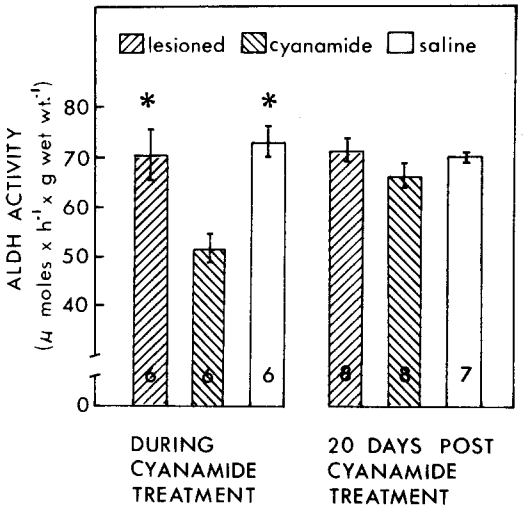

Fig. 6. Liver ALDH activity of VMFB-lesioned, cyanamide-treated, and control animals during and 20 days postcyanamide administration. *Bars and vertical lines:* means ± SEM of enzyme activity. *Numbers inside bars:* number of animals utilized in each assay. * Significant difference from cyanamide-treated group ($P < 0.05$, Tukey HSD test)

revealed that liver ALDH of the cyanamide-treated animals was significantly lower than that of the lesioned or control animals only during the cyanamide treatment. There were no significant differences between the groups at the end of the experiment.

Discussion

Ventral MFB lesions resulted in a significant decrease in both ethanol consumption and levels of MB-DE ALDH activity, suggesting that the presence of normal MB-DE ALDH levels may be a necessary condition for the maintenance of undisrupted ethanol directed behavior in rats.

A decrease in MB-DE ALDH activity could probably result from a destruction of any one of a number of different biogenic amine-containing nerve pathways including the ascending noradrenaline and serotonin fibers as well as those of the nigrostriatal dopamine pathway (e.g., Duncan et al., 1972; Agid et al., 1973). The results of the histological verification of the location and size of the lesion in four experimental animals suggest that the loss in ALDH observed here could result from the degeneration of noradrenaline- and dopamine-containing neurons (Ungerstedt, 1971). The finding that only a small reduction in ALDH was noted agrees with the fact that noradrenaline neurons do not contain high ALDH activity (Deitrich and Erwin, 1975).

The physiological mechanism that mediated the decrease in ethanol consumption in the lesioned animals is ambiguous. The reduction in ethanol intake in the cyanamide-treated animals probably resulted from a temporary inhibition of ALDH activity in the liver and in other peripheral tissue (Deitrich et al., 1975), and was probably due to an increase in the level of circulating acetaldehyde in these animals. The reduction in MB-DE ALDH activity could have resulted in a significant decrease in the aldehyde oxidizing capacity of the midbrain, leading to a localized change in the level of brain acetaldehyde. This possibility agrees with the hypothesis that ALDH serves as a ubiquitous brain barrier against circulating acetaldehyde, preventing its accumulation in tissue (Sippel, 1974). An alternative possibility is that reduction in ALDH activity in the midbrain could result in a localized increase in the concentrations of endogenous aldehydes, a process that could be facilitated when ethanol-derived acetaldehyde is present to inhibit the residual ALDH activity in tissue (Lahti and Majchrowicz, 1969). An increase in the level of endogenous aldehydes could probably change the pharmacological effects of ethanol, which in turn could result in changes in ethanol-related behavior.

GENERAL DISCUSSION

Recent experiments suggested that the level of brain ALDH may be a principal biochemical predisposition for ethanol preference in rats (Amir, 1977 and unpublished observations). The present data provide further

evidence that ALDH directly influences the regulation of voluntary ethanol consumption by rats.

It was previously postulated that alterations in ethanol consumption following VMFB manipulations resulted from neurochemical changes that involve the interaction of acetaldehyde with biogenic amines in midbrain structures (Amit and Stern, 1972). The results reported here implicate the involvement of MB-DE ALDH in this process. Accordingly, experimentally induced changes in ALDH activity possibly result in considerable changes in the metabolic disposition of various endogenously-occurring and exogenously derived aldehyde in the midbrain, affecting their biochemical interactions with biogenic-amine metabolism (Deitrich and Erwin, 1975; Deitrich, 1976). Changes in the interrelationships among MB-DE ALDH, its neural substrates, and acetaldehyde may underlie alterations in the behavioral response to ethanol.

An important implication of the data involves the particular role of acetaldehyde in the regulation of ethanol preference. Previous studies have demonstrated that repeated injections of various aldehydes into the brain enhanced ethanol preference in rats (Myers and Veale, 1969; Myers et al., 1972). Similar results have been obtained with repeated intracerebral injections of various acetaldehyde-biogenic amine condensation products (Melchior and Myers, 1977; Myers and Melchior, 1977). The present results suggest that these compounds do not act under normal physiological conditions to enhance ethanol preference in rats. Conversely, since ethanol ingestion is correlated with brain ALDH levels (e.g., Amir, 1977 and unpublished observations) and since experimentally induced changes in ALDH result in changes in ethanol consumption, a central inhibitory effect is implicated. Acetaldehyde and its condensation products have been suggested to play a principal role in the development of physical dependence on ethanol (Rahwan, 1975; Cohen, 1976). Nonetheless, under normal conditions rats will not voluntarily consume sufficient amounts of ethanol to allow significant accumulation of acetaldehyde in brain tissue (Sippel, 1974; Tabakoff et al., 1976), or to render them physically dependent on the drug (Wallgren and Barry, 1970; Israel and Mardones, 1971).

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