Pharmacology Biochemistry & Behavior, Vol. 14, pp. 377–383. Printed in the U.S.A.

Suppression of Alcohol Drinking with Brain Aldehyde Dehydrogenase Inhibition

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Received 26 October 1980

SINCLAIR, J. D. AND K. O. LINDROS. *Suppression of alcohol drinking with brain aldehyde dehydrogenase inhibition.* PHARMAC. BIOCHEM. BEHAV. **14**(3) 377–383, 1981.—Calcium cyanamide, an aldehyde dehydrogenase (ALDH) inhibitor used in the treatment of alcoholism, strongly suppressed voluntary ethanol drinking by rats. Such inhibitors have generally been believed to act primarily by limiting drinking through acetaldehyde accumulation after ethanol consumption. Administration of a low dose of 4-methylpyrazole (4-MP) that abolished acetaldehyde accumulation did not, however, remove the suppression produced by cyanamide. 4-MP alone did not affect the unsuppressed alcohol intake by Long Evans rats or the drinking by rats of the ANA strain developed for low levels of ethanol consumption. When given from the start with cyanamide, 4-MP did affect the development of the suppression, but probably by its effect in lessening the degree of brain ALDH inhibition: a high correlation ($r = +0.825$, $p < 0.001$) was found between brain ALDH activity and ethanol consumption. The results suggest that cyanamide suppresses alcohol drinking also in the absence of acetaldehyde accumulation probably by some action related to its direct inhibition of brain ALDH.

Cyanamide Calcium carbimide Acetaldehyde Voluntary ethanol drinking Aldehyde dehydrogenase 4-Methylpyrazole

A NUMBER of studies have demonstrated a relation between increased acetaldehyde levels and decreased voluntary alcohol intake [7,17]. This has led to a widely accepted view that at least in some situations drinking is limited by the accumulation of acetaldehyde when ethanol is metabolized. Thus disulfiram (Antabuse®), calcium cyanamide (calcium carbimide, Temposil®, Dipsan®), and other inhibitors of aldehyde dehydrogenase (ALDH) are thought to suppress the alcohol drinking of humans and of laboratory animals [10, 14, 16, 25] by inhibiting the metabolism of acetaldehyde which then causes aversive levels of acetaldehyde to accumulate if more than a very low dose of ethanol is consumed. It has also been suggested that the higher blood acetaldehyde levels seen in Orientals [11, 23, 24] may be partially responsible for their lower alcohol consumption. Further support for the hypothesis that acetaldehyde limits ethanol intake has been seen in the findings that rat and mice strains that consume less ethanol voluntarily also show higher acetaldehyde levels than do high drinking strains after a fixed dose of ethanol [6, 12, 15, 26]. Similarly, high negative correlations have been found within strains between the individual ethanol intakes and the acetaldehyde levels produced after a test dose of ethanol [7].

The evidence relating acetaldehyde increases and voluntary alcohol intake decreases has, however, been only correlational, and the hypothesis suggesting a causal relationship between them is open to a number of questions. (a) How is the aversive effect of acetaldehyde signalled to the brain, since normally brain tissue does not contain appreciable concentrations of acetaldehyde even when the blood levels are relatively high [28, 29, 30]? (b) During normal voluntary alcohol intake in rats the blood acetaldehyde levels are extremely low. Is acetaldehyde really aversive at these low concentrations? (c) Previous studies [1, 2, 3] have shown higher correlations between brain ALDH activity and drinking than between liver ALDH activity and drinking. Why is this so, since blood acetaldehyde levels are regulated primarily by liver ALDH [17,19]?

The questons suggested that the hypothesis of acetaldehyde limiting alcohol drinking needed to be re-examined and tested independently of changes in ALDH activity. It was known that low doses of 4-methylpyrazole (4-MP) that partially inhibited alcohol metabolism would allow nearly all the acetaldehyde produced from ethanol to be metabolized within the liver, thus virtually eliminating acetaldehyde accumulation in the blood [18]. Our initial purpose was, therefore, to see if administration of 4-MP would increase the voluntary ethanol drinking by rats. When this was not found, we turned to other paradigms in which it had been thought that acetaldehyde accumulation was even more important for restricting alcohol consumption: in the ANA strain of rats developed for low levels of ethanol consumption [9] and known to develop high acetaldehyde levels after ethanol administration [6,12], and in rats given cyanamide to suppress their alcohol drinking. Lowering acetaldehyde levels with 4-MP still did not increase ethanol consumption. These

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results indicated to us that acetaldehyde accumulation probably is not a limiting factor in normal alcohol drinking and is not responsible for the suppression from cyanamide.

GENERAL PROCEDURES

Mature (4–6 month old) male rats were individually housed in suspended galvanized wire cages in a room with a 12 hr light/dark cycle, constant 55% humidity and 22–24°C temperature. The animals had continual access to food and tap water. In order for the daily ethanol intake to stabilize, the rats were always given preliminary access to 10% (v/v) ethanol, prepared from 96% ethanol and tap water, for one month just prior to each of the experiments. Water and ethanol solution were presented in 100 ml graduated Richter bottles with glass spouts, attached to the fronts of the cages.

When 4-MP was given, it was added (1 mM) to both drinking fluids. With this concentration the daily intake of 4-MP ranged from 7–9 mg/kg body weight for the Long Evans rats and from 8–11 mg/kg for the lighter AA and ANA rats. Calcium cyanamide (Dipsan®) was administered by thoroughly mixing 200 mg of it with each kg of powdered food. Except in Experiment 2, the new Astra rat diet (Astra-Ewos AB, Södertälje, Sweden) was used.

EXPERIMENT 1

Method

4-MP was given for 10 days to 9 Long Evans rats on continual access to 10% (v/v) ethanol and water. The alcohol consumption during this time was compared to that during the preceding 10 days and during the subsequent 10 days when 4-MP was not present.

Results

Alcohol intake was not significantly affected by 4-MP. The rats drank a mean (±SD) of 1.84 ± 2.12 g of ethanol/kg body weight during the 10 days before 4-MP, 1.91 ± 1.85 g/kg/day while 4-MP was present, and 1.94 ± 1.82 g/kg/day during the following 10 days. Water and food intakes, and the ratios of ethanol consumption to these measures also did not differ significantly between periods.

EXPERIMENT 2

Method

Fourteen ANA rats were raised and maintained on the old Astra diet shown to contain cyanamide [22]. They were divided into two equal groups matched for their ethanol intake during the last week of the preliminary ethanol access period. One group thereafter always had 4-MP in its drinking fluids. Both groups had one more week of continual access to ethanol solution and water, and then for the next 3 weeks they were forced to drink ethanol (or ethanol+4-MP) by giving the alternative fluid, water or water+4-MP, only every third day.

Results

As expected, the voluntary ethanol intake was very low: 0.36 g/kg/day during the last week of the preliminary period. 4-MP did not significantly ($p>0.05$) increase the alcohol intake above this level or above the level shown by the control group during the week of continual access to alcohol and water. It also did not significantly increase either the forced ethanol consumption (5.10 ± 0.81 vs 4.39 ± 0.90 g/kg/day for the 4-MP group and controls, respectively, $p>0.05$) or the voluntary ethanol consumption on the intervening days when water was available (0.21 ± 0.18 vs 0.30 ± 0.15 g/kg/day for the 4-MP and control groups, respectively, $p>0.05$).

EXPERIMENT 3

Method

Twenty-two rats of the heavy drinking AA strain [9] were divided into 3 groups matched for ethanol intake during the last week of preliminary access. A control group continued with no additional treatments, but cyanamide was given thereafter to both of the other groups. Beginning 6 days later, one group was given 4-MP in its drinking fluids. After an additional 6 days, access to alcohol was removed for all groups, 16 hr prior to a test of acetaldehyde accumulation.

For this test, each animal was intubated with 1.5 g/kg of ethanol, using the same type of fluid that had been given previously for drinking, i.e., 10% (v/v) ethanol solution for the control group and cyanamide group and 10% ethanol+1 mM 4-MP for the cyanamide+4-MP group. Tail blood samples (0.1 ml) were taken 85 min later directly into 0.9 ml ice-cold distilled water. Liver samples were then taken by the freeze clamp technique after cervical dislocation, powdered under liquid nitrogen, and extracted in perchloric acid containing thiourea [27]. Other portions of the liver samples and the total removed brains were stored under liquid nitrogen until homogenization in 4 volumes 0.25 M sucrose containing 1% Triton X-100 and 1 mM mercaptoethanol. ALDH activity was determined from the rate of removal of 80 μM acetaldehyde in pyrophosphate buffer (3.0 ml), pH 7.4, supplemented with 0.2 mM 4-MP, 5 mM NAD, and 1 mM $MgCl_2$. The reaction was started by adding 50 μl liver homogenate or 400 μl brain homogenate. Acetaldehyde concentrations were determined by head-space gas chromatography [8].

Results

Cyanamide rapidly suppressed the alcohol consumption of both groups to which it was given (Fig. 1). Subsequent addition of 4-MP to one group did not remove the suppression; the alcohol consumption of the cyanamide+4-MP group remained essentially the same as that by the group given only cyanamide. Food and water intakes also were unaffected by 4-MP. 4-MP did, nevertheless, completely prevent the accumulation of acetaldehyde during ethanol metabolism (Table 1). The ALDH activities were virtually the same in the cyanamide and the cyanamide+4-MP groups, but both had significantly lower activities than the control group.

EXPERIMENT 4

Method

In Experiment 3, the effect of 4-MP on cyanamide-suppressed drinking was observed for only 6 days, in order that the acetaldehyde accumulation and ALDH tests could be made. In the present experiment, 10 AA rats were treated identically to the cyanamide+4-MP group in Experiment 3, except that they continued on joint administration of cyanamide and 4-MP for 2 weeks.

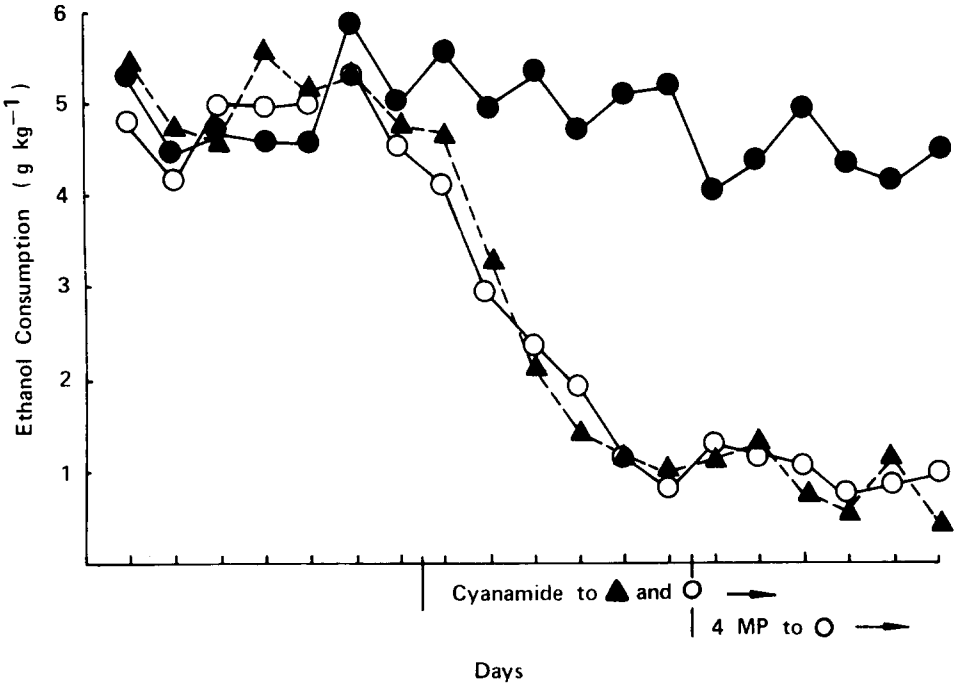

FIG. 1. The effects of cyanamide and subsequent 4-MP on voluntary consumption of ethanol. Cyanamide significantly suppressed the consumption by both groups to which it was given (▲ and ○) relative to the intake by the controls (●) and relative to their own precyanamide levels. The subsequent administration of 4-MP to one group (○) had no effect on alcohol consumption, although it eliminated the acetaldehyde accumulation caused by cyanamide (see Table 1). Similar results are obtained if alcohol consumption is expressed as the ratio of ethanol solution intake to total fluid intake.

Results

The longer period of 4-MP administration also failed to remove the suppression induced by cyanamide. The daily intake of ethanol remained below 1 g/kg/day throughout the 2 weeks and showed no tendency for increasing.

EXPERIMENT 5

Method

The purpose of this control experiment was to see if the failure of 4-MP to reverse the suppression from cyanamide was due to some inflexibility of the drinking behavior, for instance, as a result of conditioned taste aversions which prevented ethanol consumption from returning to the higher, precyanamide baseline even though acetaldehyde accumulation had been stopped. To test this possibility, 8 Long Evans rats, with continuous access to ethanol, had cyanamide added to their food for 6 days and then removed. If inflexibility were responsible for the previous results, ethanol drinking would be expected to remain suppressed regardless of whether acetaldehyde accumulation were prevented by 4-MP or by removal of cyanamice.

Results

No evidence for inflexibility was found. Cyanamide reduced ethanol consumption to 21% of the pretreatment baseline (from 2.67 to 0.55 g/kg/day), but alcohol intake returned to the baseline within 3 days after the termination of the cyanamide treatment.

EXPERIMENT 6

Method

Fifteen AA rats were divided into three equal matched groups, with similar treatments to those in Experiment 3, except that the cyanamide+4-MP group began receiving 4-MP at the same time that the cyanamide was first introduced.

Results

Cyanamide alone significantly suppressed ethanol drinking from 6.92 ± 1.32 g/kg/day during the week before treatment to 1.61 ± 2.36 g/kg/day during the treatment week. Cyanamide+4-MP also significantly ($p<0.001$) suppressed alcohol drinking, but not as much as cyanamide alone: from 6.99 ± 1.09 g/kg/day before treatment to 3.23 ± 0.86 g/kg/day during treatment. The difference between these groups reached significance ($p<0.05$) on 3 of the 7 days, although the means for the entire week failed to be significantly different.

EXPERIMENT 7

Method

This was basically a replication of Experiment 6, but

TABLE 1

EFFECTS OF CYANAMIDE AND 4MP ON ACETALDEHYDE CONCENTRATIONS IN TAIL BLOOD AND FREEZE-STOPPED LIVER 85 MIN AFTER INTUBATION WITH 1.5 g/kg OF ETHANOL AND ON LIVER AND BRAIN ALDH ACTIVITIES (MEAN ± SD)

Chronic treatment Acute treatment	Cyanamide Ethanol n=8	Cyanamide + 4MP Ethanol + 4MP n=8	Control Ethanol n=6
Acetaldehyde concentration			
Blood (μmol/l)	317.4 ± 102.7†	2.2 ± 2.6‡	3.6 ± 6.1
Liver (nmol/g wet wt/min)	331.3 ± 65.7†	17.1 ± 11.8*‡	42.2 ± 25.3
ALDH activity (μmol/g wet wt/min)			
Brain	0.09 ± 0.02†	0.09 ± 0.02†	0.18 ± 0.02
Liver	1.36 ± 0.32†	1.48 ± 0.36†	4.59 ± 0.61

*Significantly different from controls, $p<0.05$.
†Significantly different from controls, $p<0.00001$.
‡Cyanamide + 4MP group significantly different from cyanamide group, $p<0.00001$.

using 28 Long Evans rats (10 in the cyanamide+4-MP group, 9 in each of the other two groups), instead of AA rats. In addition, after the 6 day treatment period, the animals were sacrificed and brain ALDH activity was measured with the methods used in Experiment 3.

Results

The drinking patterns (Fig. 2) were essentially the same as in Experiment 6. Cyanamide+4-MP significantly ($p<0.001$) suppressed alcohol drinking relative to the pretreatment level and relative to the controls, but cyanamide alone produced a somewhat greater suppression. Although the difference between these two groups for the entire 6 days was not significant, it was significant on 2 of the individual days.

The brain ALDH activities paralleled these consumption results. Cyanamide+4-MP reduced the activity to 0.084 ± 0.015 μmol/g/min in contrast to 0.125 ± 0.008 for the controls, but not as much as cyanamide alone, which reduced the enzyme activity to 0.072 ± 0.014 μmol/g/min. Approximately these same results were obtained in another study in which cyanamide and 4-MP were administered but without giving the rats access to ethanol, thus showing that this effect of 4-MP on the development of cyanamide inhibition of brain ALDH was not dependent upon the presence of ethanol or acetaldehyde derived from it.

The relationship between brain ALDH activities and the mean ethanol during the 6 days of treatment is shown in Fig. 3. Over all three groups there was a very high positive correlation: r=+0.825 ($p<0.001$). A very high correlation was also found within the cyanamide+4-MP group: r=+0.845

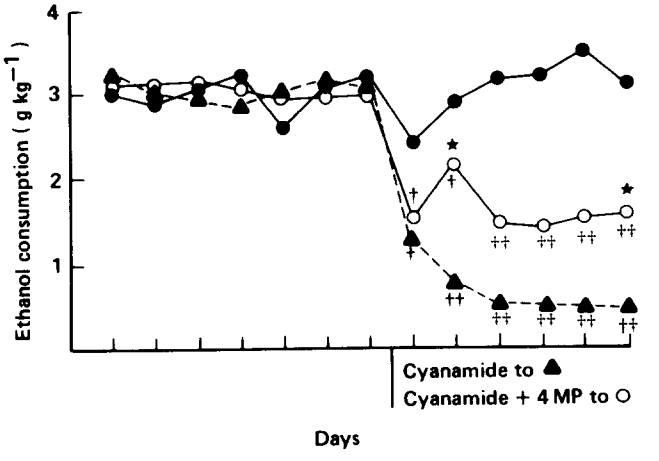

FIG. 2. The effects of cyanamide alone (▲) and concurrent cyanamide plus 4-MP (○) on voluntary ethanol consumption. Both treatments significantly (†$p<0.05$, ††$p<0.01$) reduced alcohol intake relative to that by the controls (●), but the suppression tended to be less in the cyanamide+4-MP group with the difference between them and the cyanamide alone group reaching significance on two days (*$p<0.05$).

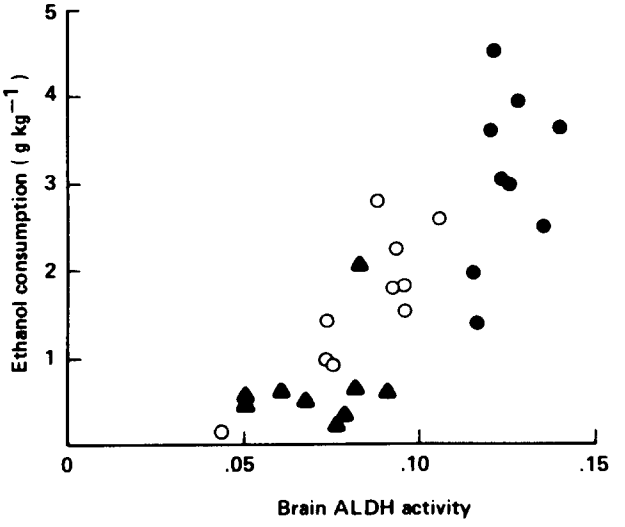

FIG. 3. The relationship between the mean alcohol intakes of the individual animals from the groups shown in Fig. 2 (using the same symbols as in that figure) during the final six days and their brain ALDH activities.

($p<0.01$). The correlation in the control group was much lower (+0.356) apparently because of the low variability in their enzyme activities; the correlation in the cyanamide alone group was still lower (+0.266) probably because of the small variability in their ethanol intakes.

DISCUSSION

The previous findings that had suggested that acetaldehyde accumulation was a limiting factor in alcohol consumption had confounded changes in acetaldehyde levels with changes in ALDH activity. With our procedures, which allow the two factors to be altered independently, the acetaldehyde levels were no longer related to alcohol drinking behavior.

4-MP clearly prevents acetaldehyde accumulation during ethanol metabolism as shown previously [18] and here in Experiment 3. Nevertheless, removal of acetaldehyde does not free normal Long Evans rats to drink larger quantities of ethanol (Experiment 1). Prevention of acetaldehyde accumulation also did not increase the very low voluntary alcohol intake of ANA rats (Experiment 2) nor did it significantly increase their forced ethanol consumption when no water was available.

Cyanamide was found to be a very potent and reliable means for suppressing alcohol drinking; the suppression was seen in every rat tested with cyanamide. Alcohol consumption after a week on cyanamide normally fell to about one-fifth of the precyanamide level, with the heavy drinking AA strain still consuming about twice as much alcohol as Long Evans rats when both were on cyanamide.

The suppression of ethanol intake with ALDH inhibitors such as cyanamide has previously been seen as clear evidence for the role of acetaldehyde in limiting alcohol drinking. Prevention of acetaldehyde accumulation with 4-MP did not, however, remove the suppression. Instead it had no discernible effect on drinking. In contrast, when cyanamide itself was removed, the suppression rapidly disappeared and ethanol consumption increased back to the presuppression level.

Acetaldehyde accumulation is also not needed for the development of the cyanamide-induced suppression: a significant suppression occurred even when 4-MP administration was begun simultaneously with cyanamide treatment. The finding that the suppression that developed with cyanamide+4-MP may have been somewhat less than that with cyanamide alone could be seen as suggesting that acetaldehyde might nevertheless intensify the suppression. However, the high correlations between brain ALDH and ethanol intake show that it is more likely that the cyanamide+4-MP group had less suppression of their drinking because their brain ALDH was less inhibited.

Despite the observed effects of intubated 4-MP on acetaldehyde (Table 1), it might be argued that having 4-MP in the drinking fluids had no effect on metabolism and thus did not alter drinking. Although this possibility was not tested directly in the present experiments, it has been shown previously that an even lower concentration (0.4 mM) of 4-MP in a liquid diet containing ethanol significantly reduced acetaldehyde levels resulting from ingestion of the diet [21]. Furthermore, the changes seen in Experiment 7 show that having 4-MP in the drinking fluids can alter metabolism.

Our finding that 4-MP does not increase alcohol drinking is in agreement with recent results by Carr *et al.* [5]. They also found that unlike pyrazole, 4-MP does not cause weight losses, changes in food or total fluid intake, or conditioned taste aversions, nor does 4-MP affect the consumption of an equi-aversive tasting quinine-sucrose solution. Their experiments, however, raise the possibility of a possible confounding factor for the interpretation of our results, since they found that 4-MP itself can suppress alcohol drinking. In rats having alternate day access to ethanol and thus drinking large amounts (about 6 g/kg) of alcohol on the days when it was available, injection of 30 mg/kg of 4-MP IP about an hour before ethanol was returned greatly reduced the alcohol consumption.

We also have seen a suppression from 4-MP. In a pilot study, 4-MP (1 mM) was added to the drinking fluids of an unusual Long Evans rat that normally drank about 8 g/kg of ethanol daily, and 4-MP did consistently reduce its alcohol intake by about 20%.

The most likely explanation of these results is that 4-MP reduced the metabolic capacity to eliminate ethanol so that it was impossible for the animals to maintain their high levels of ethanol drinking. Intubation with 47 mg/kg of 4-MP has been shown to produce an 80% maximal inhibition of alcohol metabolism [20]. Generalizing this result to a free drinking situation is difficult, but nevertheless, it would be expected that animals receiving such large doses of 4-MP would be unable to consume 6 g/kg/day of ethanol without becoming severely intoxicated and that the lower dose of 4-MP (about 7 mg/kg) obtained by the rat in our pilot study would prevent consumption of 8 g/kg of ethanol per day.

It could be argued that such suppressive effects from 4-MP could counteract the influence from prevention of acetaldehyde accumulation and thus prevent alcohol consumption from increasing. There are, however, several reasons why this is very unlikely. (a) Before 4-MP was given the rats in our experiments were drinking very low levels of ethanol: about 2 g/kg/day in Experiment 1 and less than 1 g/kg/day in Experiments 2–4. Consequently, they were far below their metabolic capacity and would be less likely to be affected by a reduction in that capacity. (b) The doses of 4-MP obtained by our rats are about one-third that given by Carr *et al.*. Intubation with such lower doses produced a maximal inhibition of alcohol metabolism of about 50% [20], and obtaining such doses of 4-MP gradually from the drinking fluids probably produces even less inhibition. Thus the total capacity for metabolizing ethanol in rats with 4-MP in their drinking fluids should have remained above 4 g/kg/day, i.e., several times higher than the consumption levels. (c) The alcohol drinking in our experiments neither increased nor decreased significantly when 4-MP was given. To explain this one would have to assume that the suppressive influence from 4-MP happened to be of the same magnitude as the suppressive effect of acetaldehyde accumulation that it replaced in each of the first four experiments. (d) If suppressive effects from 4-MP were responsible for regulating the level of alcohol drinking, the high correlation between brain ALDH activity and alcohol drinking is very difficult to explain.

Therefore, it seems more likely that with low levels of alcohol drinking and the dose used here 4-MP does not have a suppressive effect on ethanol consumption and that its failure to increase drinking was because acetaldehyde accumulation had not been limiting alcohol intake. The suppression of alcohol consumption by cyanamide thus appears to be a result of its direct inhibition of brain ALDH. This proposal is also supported by the findings that alcohol consumption is more highly correlated with ALDH activity in the brain than

with liver ALDH activity [1, 2, 3]. The means by which brain ALDH affects alcohol consumption may be related to the enzyme's actions in the metabolism of catecholamines and serotonin and the possible role of these substances in the selection of ethanol [1].

Brain ALDH activity is clearly only one of several factors affecting ethanol consumption. This can be seen in the data from the control animals in Fig. 3. There was little variation in their brain ALDH activity, but large differences in their voluntary alcohol intakes, and only a slight tendency for a correlation between the brain ALDH activity and the alcohol consumption. Similarly, brain ALDH activity has been found not to be related to the different ethanol intake levels of the AA and ANA rat strains [13]. Other factors can apparently supersede or obscure any effect related to small endogenous variations in brain ALDH activity. Perhaps high correlations with ethanol consumption can be found only when there is a wide range of brain ALDH activities, such as were produced through the administration of cyanamide and 4-MP in Experiment 7.

Our results argue against not only direct aversive actions of acetaldehyde but also against indirect actions as causes for the cyanamide-induced suppression of ethanol drinking. For instance, the tetrahydroisoquinoline condensation products between acetaldehyde and biogenic amines [4] could not be responsible for the suppressions, because acetaldehyde accumulation was not needed to produce the suppression. A role for the ring-substitution condensation products, such as tetrahydropapaveroline, between biogenic amines and their endogenous aldehydes cannot be excluded because inhibition of brain ALDH could favor their production.

ALDH inhibitors constitute one of the strongest and most reliable means for suppressing ethanol consumption. The present results do not prove that acetaldehyde and the alcohol-sensitizing reaction never restrict drinking, but they do show that the suppression of alcohol consumption observed here was caused by some other action of the inhibitor. Hopefully, this will remove the complacency generated by the feeling that we knew the mechanism by which these inhibitors affected alcohol drinking and will stimulate investigations searching for the real means for their effects.

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