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Dopamine levels in the striatum and the effect of alcohol and reserpine

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In the search for elucidation of the biochemical mechanism of the action of addictive drugs, the effects of ethanol on the neural transmission in the striatum are of particular interest. This part of the brain is very rich in dopamine (DA), and could be involved to a greater extent than other brain regions in alcohol-induced formation of endogenous, morphine-like alkaloids [1, 2]. Possible interaction of ethanol with biogenic amines has been studied, and changes in the turnover of DA in the rat brain [3], as well as an increase in catecholamine levels in the mouse brain, after acute and chronic administration of ethanol [4] have been reported.

In the studies presented in this paper, our main interest was focused on changes occurring in the neural transmission processes in the corpus striatum of rats subjected to acute and prolonged administration of alcohol; the rats were not given a choice of drinking fluid. They were started on a solution with a low content of ethanol at a very early age. The experiments lasted approximately 2 months, during which time the concentration of alcohol in the drinking water was increased gradually from 1.6 to 16.2%. Control rats, often litter-mates of experimental animals, were kept in identical experimental conditions on ordinary water. This experimental procedure was an attempt to simulate the usual drinking patterns of man, and to produce tolerance to alcohol rather than dependence. The effects of acute and chronic administration of alcohol on dopaminergic and cholinergic striatal systems were examined. We assayed the activities of choline acetyltransferase and tyrosine hydroxylase, and determined the levels of dopamine in corpus striatum.

Sprague–Dawley rats of both sexes were used. In chronic experiments, immature rats (80–100 g body weight) were kept on solutions of ethanol instead of drinking water. Animals were housed in pairs in cages with wire floors, and the consumption of fluid was measured daily. The record of their growth was also kept daily for a period of over 2 months. Ethanol was mixed with water at an initial concentration of 1.6% for the first 2–3 days, and the concentration was increased gradually to 16.2%. Control rats were kept under identical experimental conditions. In acute experiments, intraperitoneal injections of 4 g/kg of ethanol were given, and the rats were killed at predetermined intervals of time. After quick decapitation, the corpus striatum was dissected, while the material was kept on ice. The striatum was reached by opening the lateral ventricles; during the dissection of the nucleus from the lateral walls of the ventricles care was taken not to include any white matter or non-striated cortical areas. Dopamine was estimated in the striatum of rats according to the method described by Shellenberger and Gordon [5] and choline acetyltransferase using the procedure described by us previously [6]. Tyrosine hydroxylase activity was assayed in homogenates of corpus striatum in 9 vol. of 0.32 M sucrose using Dounce homogenizer (clearances of either 0.002 or 0.004 inch). The enzyme was assayed by measuring the liberation of $^{14}CO_2$ from L-[1-^{14}C]tyrosine [7], using a slightly modified medium. The final volume of 0.455 ml contained: 19.4 μM L-[1-^{14}C]tyrosine (0.5 μCi), 121 mM NaCl, 4.8 mM KCl, 2.0 mM sodium EDTA, 2.1 mM sucrose, 31.3 mM sodium phosphate (pH 6.6) and 5.4 mg tissue. The evolved $^{14}CO_2$ (trapped in 0.16 to 0.12 ml of NCS tissue solubilizer) was counted in 15 ml of toluene scintillation fluid containing 6 g/liter of PPO and 75 mg/liter of POPOP.*

The results obtained on two groups of 16 rats each were taken as an example of changes in body weight between the alcohol-treated and control rats. While the mean body weight was initially 72–73 g, the group receiving alcohol after 2 months of treatment weighed 251 $\pm$ 35 g, whereas the control rats weighed 276 $\pm$ 30 g ($0.02 < P < 0.05$).

Fluid consumption varied considerably; on day 1 the rats drank very little, but on the next day there was an increase to 20–25 ml/rat/day, an amount which was maintained throughout the whole experiment. However, each time the increase in the concentration of alcohol was introduced, there was a slight decrease in the amount of fluid consumed that day. Water consumption was higher in control groups (we do not have exact values), and this probably explains the higher body weight.

On the basis of these results, we are inclined to assume that there was very little difference in general growth and development of immature rats subjected to chronic alcohol uptake.

The greatest concentration of ethanol (16.2%) was maintained for a period of 7–10 days before killing the animals. This was equivalent to about 3–4 g alcohol/day/rat. The rats maintained on this high dose of alcohol were visibly affected, but were not unconscious at any time. When 4 g/kg was administered to rats in acute experiments intraperitoneally, they lost consciousness within 10 min and did not regain the righting reflex until 1 hr later.

Alcohol administered acutely (4 g/kg, i.p.) produced a strong intoxication in rats. The righting reflex was lost within a few minutes after administration of ethanol and did not return until 1 hr later, at which time a significant increase in the content of DA was observed (Table 1). Another group was killed 15 min after ethanol injection, and in this group the DA content was at the normal levels. When the rats were maintained on ethanol drink for 2 months, an increase (60 per cent) was still present in animals withdrawn from alcohol for 48 hr.

Since higher levels of dopamine could influence tyrosine hydroxylase (TH), the enzyme involved in the synthesis of DA, we examined the activity of TH. Interestingly, the high rises in DA content had no influence on tyrosine hydroxylase.

To include the cholinergic neural mechanism in the striatum, we measured the activity of choline acetyltransferase, the enzyme involved in the synthesis of acetylcholine. A possible interrelation between the two systems in the striatal area has been previously postulated [8, 9]. Similar to TH activity, choline acetyl-transferase levels were unchanged (Table 2).

* PPO = 2,5-diphenyloxazole; and POPOP = 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene.

Table 1. Effect of administration of ethanol on striatal dopamine levels*

Experimental procedure	Duration of treatment	No. of experiments	Dopamine levels in μg/g of wet weight	
			Saline	Ethanol
Acute	1 hr	6	7.90 ± 1.53	10.65 ± 1.14†
Acute	4 hr	6	6.81 ± 0.12	7.18 ± 2.1
Acute	15 min	4	8.11 ± 1.29	7.89 ± 1.05
Chronic	2 months	6	8.02 ± 1.61	13.09 ± 2.53†
Chronic withdrawal	2 months 48 hr	4	6.87 ± 1.00	10.97 ± 1.05†

* In acute experiments, rats received 4 g/kg of 20% ethanol solution (i.p.). In chronic treatment, ethanol was administered to immature rats (70–100 g) with drinking water, starting with 1.6% and increasing it to 16.2% gradually during a period of 2 months. Dopamine levels were assayed as described in Methods. Values are expressed as means ± S. D.

† $0.001 < P < 0.01$.

Since it seemed that the synthesis of dopamine was not affected by ethanol, we looked for a further explanation by examining the double action of reserpine and alcohol on DA levels. Mardones *et al.* [10], in their quantitative electroencephalographic studies of the effects of ethanol, established the interaction between ethanol and reserpine. According to their results the pretreatment of rabbits with reserpine reduced markedly the initial synchronization, and abolished the delayed stimulant effect of alcohol. Although our experiments were conducted on rats, we decided to examine the influence of reserpine-induced depletion of catecholamines on the alcohol-induced increase in DA levels. Reserpine is a very long acting drug usually administered in small doses 24–48 hr in advance, but we established that 5 mg/kg of reserpine completely depleted striatal DA within 1 hr. It was important to find a dose of reserpine with strong action, but below the maximal effect. About one-third of the normal DA levels (35 ± 10 per cent) remained in the striatum 2 hr after 1.5 mg/kg of reserpine.

When alcohol (4 g/kg) was administered at this time, again as in the absence of reserpine, an increase in dopamine levels was found (62 ± 17 per cent). However, because of their wide scatter and the small number of animals tested, this difference was not statistically significant. Thus, further work is required to show whether the effects of alcohol and reserpine are independent or related by the action at a common locus.

There are several methods for inducing alcohol dependence in rodents [11–13]. All of these methods assure strong intoxication, physical dependence, and subsequent severe withdrawal symptoms after only a short period of continuous administration.

These methods can be classified as forced administration. Voluntary drinking, is not only difficult to induce in rodents, but also it is usually devoid of signs assuring obvious physical dependence.

In the methods presented here, the rats were not given a choice of drinking fluid; therefore, the method should be classified as forced administration method.

Brain monoamines have been implicated in the neurochemical changes related to the central action of alcohol [14]. Corrodi *et al.* [15] found that the turnover of norepinephrine (NE), but not that of dopamine, was increased after acute administration of ethanol. This was a delayed effect (4 hr), and it was confirmed by the findings of Davis *et al.* [16], who reported on the increase in urinary excretion of catecholamine metabolites. Thadani and Truitt [17], on the other hand, found a decrease in the turnover of norepinephrine. One explanation for these discrepancies was provided by the results of Hunt and Majchrowicz [3], who suggested that the effect of a single dose of alcohol is biphasic and also different for NE and DA. During the first few hours after administration of alcohol, NE turnover is increased, whereas that of DA is unaffected. Later on, the NE turnover is slightly reduced, while that of DA is definitely increased.

In experiments described here, ethanol was found to increase the steady-state levels of DA within 1 hr. Four hr after a dose which, although intoxicating, did not maintain the loss of righting reflex, the levels of dopamine were at normal levels. We also noticed that a lower dose of alcohol (1 g/kg) slightly reduced the levels of striatal DA.

Much higher levels of DA were found in the striatum of rats treated chronically with alcohol. Such treatment did not interfere with the well-being of the animals and did not change their rate of growth. Our method of chronic treatment did not cause any dramatic display of withdrawal symptoms, but the tolerance to alcohol was increased. Higher levels of DA were still present even after

Table 2. Choline acetyltransferase activity in the striatum of ethanol-treated rats*

Experimental procedure	Duration of treatment	No. of experiments	ChAc activity in nmoles/hr/g of wet weight		Significance
			Saline	Ethanol	
Acute	1 hr	4	9.99 ± 0.49	9.90 ± 0.77	NS
Chronic	2 months	6	7.38 ± 0.77	7.59 ± 0.89	NS
Chronic withdrawal	2 months 60 hr	2	7.48	6.25	
Chronic withdrawal	2 months 16 hr	4	7.20 ± 1.36	6.39 ± 1.32	NS

* Treatment with ethanol was the same as that described in Table 1. Choline acetyltransferase activity was assayed as described in Methods.

NS = not significant.

48 hr of abstinence. This last observation differs from that of Griffiths *et al.* [18], who also reported an increase of 50 per cent in catecholamines during the chronic administration of alcohol, but a return to normal within 10 hr upon withdrawal from ethanol.

Neither after acute, nor after prolonged, treatment with ethanol did we observe any changes in the activity of tyrosine hydroxylase, the enzyme involved in the biosynthesis of DA. Changes in the levels of DA in the striatum of alcohol-treated rats could be produced by the influence of ethanol on the adrenergic storage site. To test this possibility, we studied the depleting action of reserpine on monoamines in alcohol-treated rats, but the results were equivocal; DA levels after reserpine and alcohol were not significantly higher than those after reserpine alone.

We also found that alcohol did not influence the activity of choline acetyltransferase. Graham and Erickson [19] reported that the reduction in free acetylcholine found during intoxication with alcohol may merely reflect decreased neuronal activity rather than represent an important causative factor in ethanol-induced central nervous system depression. Our results suggest that the reduction in ACh levels does not result from a reduction in the synthetic ability of the cholinergic system, confirming Graham's suggestion.

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