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ADRENOMEDULLARY RESPONSES TO ACUTE AND CHRONIC ETHANOL ADMINISTRATION TO RATS

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Abstract—The variations in levels of adrenal dopamine (DA), noradrenaline (NA) and adrenaline (A) after acute and chronic ethanol administration have been studied in rats. A relatively moderate dose of ethanol (2 g/kg) induced significant increases in DA levels, while NA and A concentrations did not change, or decreased depending on the interval of time elapsed after ethanol injection. These findings, together with those obtained in rats pretreated with α-methyl-*p*-tyrosine methyl ester (AMT), indicate an increased turnover of adrenal catecholamines (CA) after acute ethanol treatment. Chronic ethanol intake leads to significant increases in DA levels in the adrenal glands of rats subjected to ethanol feeding for 12 and 16 days; no changes were observed in NA or A concentrations in these groups of animals. After 30 days of ethanol ingestion, the levels of the three CA are within the control range, a fact that could suggest some adaptation of the sympatho-adrenal system to ethanol. After 16 days of treatment, tolerance to acute effects of ethanol on adrenomedullary system was not clear.

Early studies indicated that ethanol stimulates the release of catecholamines (CA) from sympathetic neurons and/or the adrenal medulla. Studies on alcoholics indicate a derangement in noradrenergic function [1], and increases in CA excretion have been reported [2–5]. Some authors have indicated that the effect of ethanol on the secretory activity of the adrenal medulla appears to be indirect [6], while others demonstrated that ethanol may exert a direct effect on tissue CA turnover [7]. More recently, Cohen *et al.* [8] postulated that changes in adrenal CA synthesis and release after acute ethanol do not require adrenergic innervation, but that chronic ethanol administration affects adrenal CA primarily via a trans-synaptic process. Likewise, slight increases in adrenal CA content have been found after chronic oral ethanol administration [9, 10]; but, also, a reduction has been reported [11]. In addition, as regards tolerance development from repeated exposure to ethanol, few studies have examined this fact in peripheral noradrenergic tissues [12, 13].

In most studies of the peripheral nervous system the term CA is used synonymously with noradrenaline (NA) and adrenaline (A); although dopamine (DA) is present in small amounts in the periphery [14, 15], DA receptors have been identified in a variety of peripheral organs [16, 17]. Also, it has been postulated that increased adrenergic outflow not only stimulates peripheral CA turnover, but increases the proportional release of DA from adrenergic nerves and the adrenal [18, 19].

To our knowledge, no work has studied simultaneous variations of the three adrenal CAs after ethanol administration. Therefore, it was considered of interest to evaluate the adrenal DA, NA and A alterations induced by acute and chronic ethanol administration and also to know whether chronic ethanol intake results in tolerance to the response of adrenal CA to acute ethanol treatment.

MATERIALS AND METHODS

Female Wistar rats, initially weighing 200–260 g, were used. The rats were housed four to a cage for the acute experiments and two to a cage for the chronic experiments, in a ventilated room at a temperature of about 24°C and with a 12-hr light–dark schedule.

The animals in the acute study were injected intraperitoneally (i.p.) with 2 g/kg of ethanol. The animals in the chronic study were placed on an all-liquid diet consisting of chocolate-flavored Meritene (Wander, Spain), with 35% or 38% of the total calories represented either by added ethanol or by an isocaloric volume of sucrose solution. In addition, liquid diets were supplemented with vitamins (Vitamin Diet Fortification Mixture, INC Pharmaceuticals Inc., Cleveland, U.S.A.). In order to adapt the animals to liquid diet ingestion, they were placed on a preliminary 4-day liquid diet of sucrose; after this time elapsed, the sucrose was substituted with the appropriate amounts of ethanol to provide a diet with 35% of the total calories represented by ethanol. After 3 days on this diet, the proportion of ethanol was increased to 38% of the total caloric value. Each pair of rats per cage was provided with 160 ml/day of the liquid diet. Equal volumes of the diet were given to both the ethanol and the sucrose animals. The mean daily ethanol intake was about 15 g/kg. In the chronic study some groups of control animals had free access to lab. chow and water. Animals were weighed twice a week; the procedure followed did not induce a loss of body weight in either the ethanol-liquid diet or in the sucrose-liquid diet animals [20].

In the time–response acute experiments, different groups of rats were injected with equal doses of alcohol and then killed 0.5, 1, 2, 4 or 6 hr after the injection. Control animals received saline. The response to a test dose of ethanol in chronic ethanol-treated rats was studied by giving an i.p.

dose of 2 g/kg of ethanol to groups of rats that had been maintained on the ethanol diet for 16 days. The animals were killed 1, 2 or 4 hr after the injection.

Changes in the turnover of adrenal CA, after acute ethanol administration, were estimated by observing the depletion of DA, NA and A after inhibition of their synthesis with α-methyl-*p*-tyrosine methyl ester, AMT (H 44/68, Sigma, U.S.A.), a blocker of tyrosine hydroxylation; in each case, 250 mg/kg AMT was given (i.p.) 4 hr before death.

In all experiments, the times of injection were staggered to ensure that rats were always killed between the hours of 10:30 and 11:30 a.m. The rats were killed by decapitation with the aid of a guillotine; the adrenal glands were quickly removed, weighed and kept on ice until homogenization. Adrenal glands were homogenized in 0.4 N perchloric acid prior to purification and concentration of the amines by absorption in activated alumina according to the method described by Shellenberger and Gordon [21]. For the simultaneous fluorescence assay of DA, NA and A we used, slightly modified, the hydroxyindole method as described by Nagatsu [22]. Briefly, NA and A were differentiated by using the oxidation reaction at different pH (pH 3.5 for A and pH 7.0 for A plus NA). DA was differentiated from A and NA by oxidation at pH 7 and making use of the differences in the activation and fluorescence spectra of the trihydroxyindol fluorophores. Through the method were carried out internal standards (in the range of samples to be measured) of DA, NA and A which allow evaluation of the cross interference, in particular between A and NA since by the method used, the cross interference between DA and A or NA was unappreciable. The activation and fluorescence wavelengths for A oxidated at pH 3.5 were 410 μm, 520 μm respectively. The fluorescence intensity of A plus NA oxidated at pH 7.0 was read at the 390 μm activation peak and at the 490 μm fluorescence peak. The fluorescence intensity corresponding to DA was read with the activation and emission wavelengths set at 335 μm and 375 μm respectively. When possible, all samples belonging to a particular set of experiments were analyzed within a single assay. As this was not always possible in all assays, an additional control of standardization was provided by assaying adrenal gland pools obtained from intact rats. An Aminco–Bowman spectrophotofluorometer was used. The data were evaluated statistically by the Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

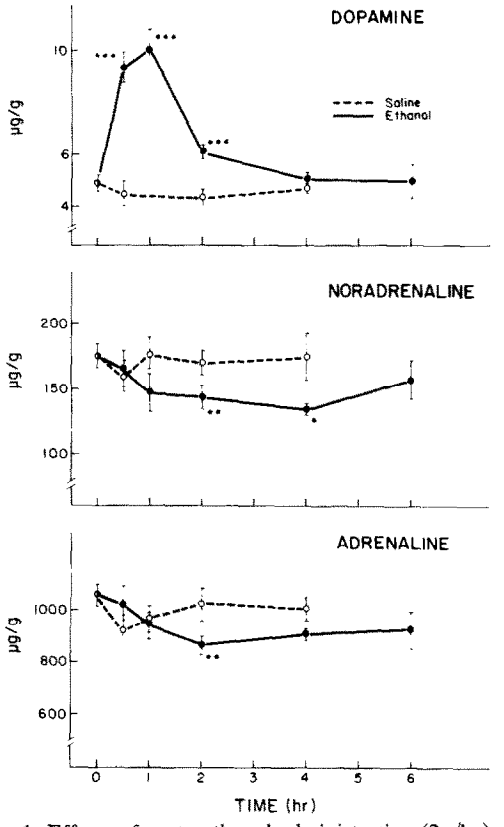

Fig. 1. Effects of acute ethanol administration (2 g/kg) on adrenal levels of catecholamines. Values are the mean ± S.E.M. of 10–24 rats: ●——●, ethanol; ○ - - - - ○, saline. Significant differences: ethanol vs corresponding saline group; ***$P < 0.001$; **$P < 0.05$; *$P < 0.02$.

RESULTS

The effects of a single dose (2 g/kg) of ethanol on adrenal CA content in animals killed at different time intervals are given in Fig. 1. None of the levels of the three catecholamines varied, with regard to non-treated animals, in any of the control groups, i.e. after the injection of saline. In rats killed 0.5,

Table 1. Effects of acute administration of ethanol (2 g/kg) on adrenal catecholamine levels after tyrosine hydroxylase inhibition by AMT in the rat

Treatment	Time (hr)	Dopamine (μg/g)	Noradrenaline (μg/g)	Adrenaline (μg/g)
Non-treated	—	4.90 ± 0.21	176 ± 10	1064 ± 28
AMT	—	2.37 ± 0.20*	143 ± 12†	1031 ± 88
AMT plus ethanol	1	2.31 ± 0.22	145 ± 14	1169 ± 76
AMT plus ethanol	2	1.79 ± 0.08‡	110 ± 7§	975 ± 34
AMT plus ethanol	4	2.13 ± 0.14	128 ± 10	1019 ± 47

Means ± S.E.M. of 8–10 rats per group.
Significant differences vs non-treated: *$P < 0.001$; †$P < 0.05$
Significant differences vs AMT: ‡$P < 0.02$; §$P < 0.05$
Animals were killed always 4 hr after AMT injection.
Time between ethanol injection and decapitation is noted for each group.

Table 2. Adrenal catecholamine variations after chronic ethanol intake

Treatment	Dopamine (μg/g)	Noradrenaline (μg/g)	Adrenaline (μg/g)
Non-treated	4.9 ± 0.21	176 ± 10	1064 ± 28
Sucrose 16 days	5.1 ± 0.26	173 ± 9	1048 ± 42
	(100 ± 5.1)	(100 ± 5.2)	(100 ± 4.0)
Ethanol 12 days	6.9 ± 0.08*	171 ± 6	1003 ± 39
	(135 ± 1.6)	(99 ± 3.5)	(96 ± 3.7)
Ethanol 16 days	7.4 ± 0.51*	164 ± 13	857 ± 68
	(145 ± 10.0)	(95 ± 7.5)	(83 ± 6.4)
Ethanol 30 days	5.8 ± 0.53	166 ± 18	903 ± 57
	(114 ± 10.3)	(96 ± 10.4)	(86 ± 5.4)

Values are the mean ± S.E.M. of 10–12 rats. Percentage of control in brackets.
Significant difference vs sucrose 16 days: *$P < 0.001$

1 or 2 hr after the injection of ethanol, adrenal DA was greatly increased ($P < 0.001$), being two times higher than the control after 1 hr; DA levels returned to normal values 4 hr after ethanol injection. The contents of NA and A were decreased ($P < 0.05$), with regard to the saline-injected animals, at the 2 hr time interval. Normal levels of A were observed afterwards, but NA was still decreased ($P < 0.05$) after 4 hr, the normal adrenal content of this hormone being recovered after 6 hr. After acute ethanol administration, there was observed a clear degree of ataxia and behavioral depression.

The results obtained in the AMT-treated groups of rats subjected to acute ethanol administration are given in Table 1. The DA and NA levels after the inhibition of tyrosine hydroxylase, by means of the above-mentioned inhibitor, had declined significantly ($P < 0.001$ and $P < 0.05$, respectively) at the study time (4 hr) whereas A content showed no significant changes with regard to non-treated rats. After a single administration of ethanol (2 g/kg) to rats pretreated with AMT, significant decreases in adrenal DA ($P < 0.02$) and NA ($P < 0.05$) levels were found in the animals killed 2 hr after ethanol treatment, in comparison to rats treated only with the inhibitor.

The variations of CA in the adrenal glands of rats subjected to chronic ethanol intake for 12, 16 and 30 days are given in Table 2. Between the groups of rats on lab. chow and those on sucrose-liquid diet, no significant differences were observed in the content of any of the three adrenal bioamines. Highly significant ($P < 0.001$) increases in DA were observed in the groups of animals subjected to ingestion of the ethanol-liquid diet for 12 and 16 days. NA and A did not vary significantly, with regard to controls, in any of the studied groups of animals. The chronic ethanol-treated rats appeared calm and, in general, showed less locomotive activity than the sucrose-treated animals.

In order to see if some degree of tolerance had been developed after chronic ethanol treatment, rats on the ethanol-liquid diet for 16 days received (i.p.) a test dose (2 g/kg) of ethanol on the last day and were killed 1, 2 or 4 hr afterwards. Results (Fig. 2) revealed a significant increase of the three CA ($P < 0.01$. $P < 0.02$ and $P < 0.05$ for DA, NA and A, respectively) in animals killed 1 hr after the injection of ethanol. At 2 and 4 hr intervals of time, levels of DA were significantly ($P < 0.01$) decreased, while the content of the other two bioamines at these two times were within the same range with regard to the animals on the ethanol-liquid diet only. Rats receiving an injection of ethanol after chronic treatment showed more long-lasting behavioral symptoms than those observed in acute ethanol-treated rats.

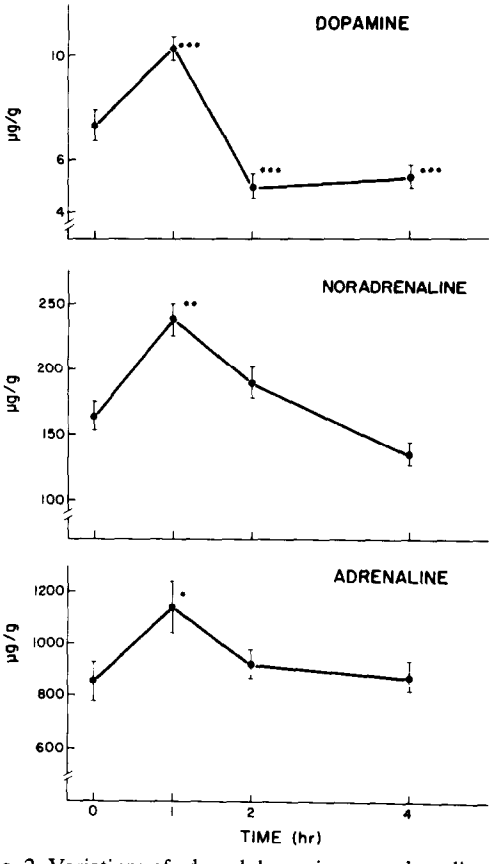

Fig. 2. Variations of adrenal dopamine, noradrenaline and adrenaline levels after the administration of a test dose of ethanol (2 g/kg) to chronic ethanol-treated rats. Values are the mean ± S.E.M. of 8–16 rats. Significant differences: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ vs animals on the ethanol-liquid diet only.

DISCUSSION

Different studies have shown that ethanol affects noradrenergic neurons in both brain and peripheral organs [9] and the rise in CA excretion in the urine after acute ethanol administration was suggested to be due to stimulation of the adrenal medulla [2, 4]. Although the ethanol action can be time-dependent [23], in most studies the effects of acute ethanol administration have been examined at one single interval of time. In our study, the responses at five different time periods were studied. With a single relatively moderate dose of ethanol, a highly significant twofold increment of DA was observed at 0.5 hr and 1 hr of elapsed time after ethanol administration; significant decreases in the levels of NA and A, were found 2 hr after ethanol injection. Values for the three CA returned to normal levels after 6 hr. The DA-raising effect of ethanol could be considered neurogenic, since it is well known that the synthesis of adrenomedullary hormones is stimulated by increased impulse flow in the secretory nerves of the adrenal medulla [24]. However, Cohen *et al.* [8] showed that a single high dose of ethanol caused significant increases of activity in adrenal tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine-*N*-methyltransferase (PMNT) in both intact and denervated rats. Studies *in vitro* [7] demonstrated that ethanol increased spontaneous CA release. These criteria would be compatible with our data, since adrenal A and NA are decreased, probably due to a releaser action of ethanol, while the increase in adrenal levels of DA may be explained by a compensatory mechanism.

In the animals sacrificed 2 hr after injection the ethanol injection to rats pretreated with AMT leads to a significant decrease in DA and, to a lesser extent, in NA levels. Since TH is considered rate-limiting in the biosynthesis of CA [25], our results indicated that 2 hr after treatment ethanol increases essentially the turnover of adrenal CA. Our data support the usefulness of adrenal DA evaluation as an indicator of adrenomedullary hormone biosynthesis [14].

In some studies related to chronic ethanol administration, alcohol was given by rather forced and stressful procedures. Oral ingestion of ethanol is considered a semivoluntary procedure [26] that more closely parallels the human model. With ethanol-liquid diets, a nearly even distribution of fluid intake between day and night hours can be achieved [27], although a nocturnal peak in liquid diet consumption has been reported [28].

Some authors [9, 10] showed a slight increase (14%) in total adrenal CA content after chronic ethanol administration; the rise being due mainly to an increment in A content, concomitant with an increase in PNMT activity. More recently, Cohen *et al.* [8], studying chronic ethanol effects in both intact and denervated rats, reported that total CA and the A/NA ratio were significantly greater in the intact group of animals. Under our experimental conditions, after 12 or 16 days of daily intake of ethanol, rises of adrenal DA were observed, although adrenal NA and A levels did not vary; the DA rises may be indicative of an increase in CA synthesis. After 30 days of treatment, the levels of DA as well as NA and A were within the control range, a fact that could be interpreted as some kind of adaptation to ethanol. Adams *et al.* [11] reported that, in rats subjected to chronic ethanol intoxication, the levels of A were markedly decreased, suggesting that the severe depletion of adrenomedullary A is due to a neural stimulation of A secretion. Concerning this study, it must be pointed out that ethanol was administered by intragastric intubation every 6 hr during a 96 hr period; this procedure induced a severe intoxication and, therefore, ought to be considered as highly stressful.

We had previously observed that chronic ethanol produced tolerance to the stimulatory effect of alcohol on the adrenocortical activity [20]. In the present study, although the adrenomedullary response to acute ethanol was modified as a result of chronic consumption of alcohol, the development of tolerance was not clear. In our opinion, the high increase of the three CA after the administration of a test dose of ethanol to chronic treated rats may indicate an increased biosynthesis in response to ethanol at the interval time of 1 hr; afterwards the DA, NA and A levels decreased; thus, it seems that ethanol induced an increase of adrenomedullary activity. To our knowledge none of the previously published studies about the effect of ethanol on adrenal CA have studied tolerance development to the acute effects. Our results indicated on the time course, an alteration of the adrenomedullary response to acute ethanol administration in chronic treated animals, but further studies on the subject are necessary.

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