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Abstract

Concentrations of norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were determined in eleven brain regions of rats following acute and repeated ethanol administration: (a) an intraperitoneal (i.p.) injection of 1, 2, 3 or 4g ethanol/kg body weight and (b) i.p. injection of 1 or 2g ethanol/kg body weight for seven consecutive days. After acute administration, the concentrations of monoamines and their metabolites appeared to be altered in all brain regions examined except substantia nigra and dorsal amygdala, with maximal variation 2 or 3h after 3g ethanol administration. After repeated administration, the alterations following injections of 2.0g/kg were more marked than the injections of 1.0g/kg. Generally, the levels of NE, DA and 5-HT were decreased while the levels of HVA, DOPAC and 5-HIAA were increased with a few exception. The most prominent findings were seen in the striatum, nucleus accumbens and locus coeruleus. These data indicate that concentrations of monoamines and their metabolites can be determined simultaneously in discrete brain regions and that monoaminergic systems in the brain respond region-specifically to ethanol treatment.

KEYWORDS: ethanol, dopamine, norepinephrine, serotonin, striatum

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The Effect of Acute and Repeated Ethanol Administration on Monoamines and Their Metabolites in Brain Regions of Rats

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Concentrations of norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were determined in eleven brain regions of rats following acute and repeated ethanol administration: (a) an intraperitoneal (i.p.) injection of 1, 2, 3 or 4g ethanol/kg body weight and (b) i. p. injection of 1 or 2g ethanol/kg body weight for seven consecutive days. After acute administration, the concentrations of monoamines and their metabolites appeared to be altered in all brain regions examined except substantia nigra and dorsal amygdala, with maximal variation 2 or 3h after 3g ethanol administration. After repeated administration, the alterations following injections of 2.0g/kg were more marked than the injections of 1.0g/kg. Generally, the levels of NE, DA and 5-HT were decreased while the levels of HVA, DOPAC and 5-HIAA were increased with a few exception. The most prominent findings were seen in the striatum, nucleus accumbens and locus coeruleus. These data indicate that concentrations of monoamines and their metabolites can be determined simultaneously in discrete brain regions and that monoaminergic systems in the brain respond region-specifically to ethanol treatment.

Key words : ethanol, dopamine, norepinephrine, serotonin, striatum

It has been reported that various neurotransmitter systems are affected by ethanol. The concentrations of NE, DA, 5-HT and their metabolites in the central nervous system have been suggested to be involved in ethanol sensitivity, tolerance and dependence (1-6). However, reports have been contradictory, with data suggesting increases, decreases, or no changes in several transmitter systems (7-17). This lack of

agreement may have derived from differences in the species or strains of animals used, the dose of ethanol given, the route of drug administration, time after treatment, length of treatment in chronic studies, the areas of the brain examined, and inconsistent ethanol level in brain and blood.

Development of the technique of high performance liquid chromatography with electrochemical detection (HPLC-ECD) has permitted simultaneous quantification of the biogenic amines NE, DA and 5-HT and their principal metabolites

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in the same brain. The purpose of the present study was to assess the effects of ethanol exposure on brain monoamine neurotransmitter systems in rats. The concentrations of NE, DA, 5-HT and their metabolites, DOPAC, HVA and 5-HIAA were determined by a HPLC-ECD in eleven discrete brain regions in rats following acute and repeated exposure to ethanol. A comparison was made of the neurotransmitter concentrations and regional distribution between the results obtained in this study with rats and those of other studies.

Materials and Methods

Male Wistar rats weighing about 300g were used throughout the study. All animals were housed individually and were maintained at constant temperature and humidity with a 12h darklight cycle, and were allowed free access to food and water.

Acute ethanol treatment was performed by intraperitoneal (i.p.) injection of a dose of 3.0g/kg body weight as

Table 1 Serum ethanol levels after administration of ethanol (a)

Ethanol (3g/kg b.w.) Times after injection(h)	Serum ethanol (g/l) Mean \pm S.D.
0.5 (n=3)	2.80 \pm 1.02
1 (n=3)	2.63 \pm 0.69
2 (n=3)	2.97 \pm 0.08
3 (n=3)	2.80 \pm 0.10

(b)

Ethanol (g/kg b.w.)	Serum ethanol (g/l) 2h after injection Mean \pm S.D.
1 (n=3)	0.97 \pm 0.79
2 (n=3)	1.79 \pm 0.00
3 (n=3)	2.90 \pm 0.08
4 (n=3)	3.50 \pm 0.19

(a) Rats were injected intraperitoneally with ethanol 3.0g/kg body weight. (b.w.) Rats were killed at 0.5, 1.0, 2.0 and 3.0 h after the injection of ethanol. (b) Ethanol was administered intraperitoneally to rats in the amounts of 1.0, 2.0, 3.0, or 4.0g/kg body weight. Rats were killed 2 h after each dose of ethanol.

an 18g% solution in saline. Rats were killed at 0.5, 1.0, 2.0 and 3.0h after the injection of ethanol. Control animals were given an equal volume of saline and killed 2 h later. In another acute study, ethanol (1.0, 2.0, 3.0, and 4.0g/kg body weight) was administered i.p. to rats as a diluted solution in saline, while only saline was given to control animals in an equal volume. The rats were killed 2 h after these treatments. In repeated studies, rats were injected i. p. with ethanol in the amounts of 1.0 and 2.0g/kg body weight as a diluted solution in saline for seven consecutive days, while control animals were treated with an equal volume of saline repeatedly. The rats were killed 2 h after the last injection of ethanol or saline.

At the completion of each experiment, the rats were killed by the near-freezing technique (18). Whole brains were rapidly removed and frozen on dry ice, and stored at -70°C until dissected into discrete brain regions. Trunk blood from each animal was also collected immediately after decapitation.

The stored whole brains were sliced into one millimeter thick coronal sections on a cryostat at -20°C . Slices were dissected at -20°C according to the atlas of Pellegrino *et al.* (19) into eleven brain regions including: ventro-medial hypothalamus (VMH), lateral hypothalamus (LH), nucleus paraventricularis (PTN), striatum, nucleus accumbens (N. accumbens), substantia nigra (S. nigra), ventral tegmental area (VTA), ventral amygdala (V. amygdala), dorsal amygdala (D. amygdala), raphe dorsalis and locus coeruleus. All procedures were carried out with brain tissue in a frozen state. These brain samples were again stored at -70°C until assayed for the content of monoamines and their acid metabolites. Tissue was homogenized with 0.05N HCl and the homogenate was centrifuged at $20,000\times g$ for 20min. An aliquot of supernatant was used for assay of neurotransmitters. NE, DA, 5-HT, DOPAC, HVA and 5-HIAA were determined by HPLC-ECD.

Ethanol concentration in trunk blood was determined using a commercial kit (Boehringer Mannheim GmbH, W. Germany). The protein content of the homogenate was determined by the procedure of Lowry *et al.* (21).

Data were expressed as means \pm S.D. Williams-Wilcoxon test and Student's *t*-test were used for comparisons to the control.

Results

Effects of Acute Ethanol Administration

Time-course study. The effect of acute ethanol

administration (3.0g/kg body weight, i. p.) on plasma ethanol concentrations are shown in Table 1-a. We observed an average plasma concentration of 2.80 ± 1.02 g/l 0.5h after administration. No further changes were detected in the plasma ethanol level 1h to 3h after administration, as compared with the value at 0.5h.

The concentrations of monoamines and their metabolites appeared to be altered in all brain regions examined except the S. nigra and D. amygdala, with maximal variation 2h or 3h after treatment after 3 g ethanol administration. Gener-

ally, NE, DA and 5-HT concentrations were decreased while DOPAC, HVA and 5-HIAA concentrations were increased with a few exception. The most prominent findings were seen in the striatum, N. accumbens, raphe dorsalis and locus coeruleus (Fig. 1).

Dose study. The effects of acute administration with four different doses (1.0, 2.0, 3.0, and 4.0g/kg body weight) on plasma ethanol concentrations are shown in Table 1-b. The changes in plasma ethanol concentrations were dose-dependent.

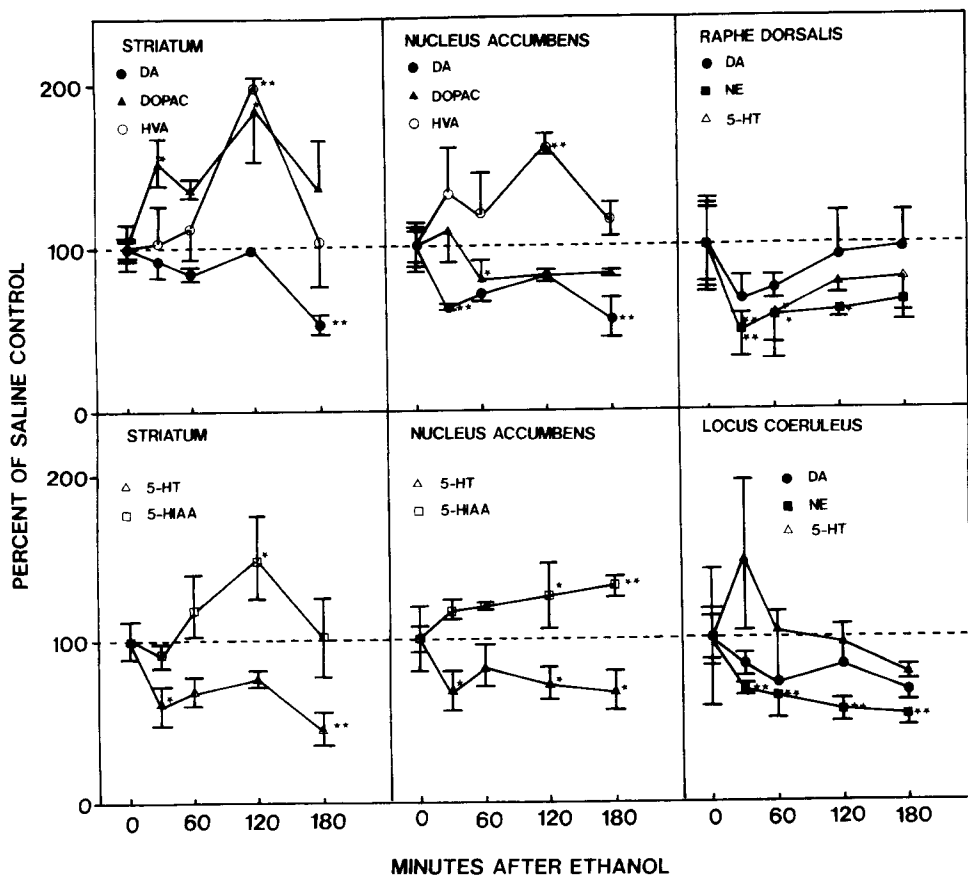


Fig. 1 Time-course of the NE, DA, DOPAC, HVA, 5-HT and 5-HIAA concentrations following intraperitoneally ethanol injection. Rats were injected intraperitoneally with ethanol 3.0g/kg body weight. Rats were killed at 0.5, 1.0, 2.0 and 3.0h after the injection of ethanol. Monoamine and their metabolite concentrations are expressed as percentages of mean levels in the control group. Each value is the mean of at least 3 rats. *,**Significantly different from the control group (Williams-Wilcoxon test: *p<0.05, **p<0.01). Abbreviation: NE, norepinephrine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

Like the acute time-course study, the levels of NE, DA and 5-HT were significantly reduced after administration, while their metabolite concentrations were significantly elevated with a few exception. The most marked findings were seen in the striatum and N. accumbens (Fig. 2).

In the raphe dorsalis, the NE concentration was reduced after injections of 3.0 ($p < 0.05$) and 4.0g/kg ($p < 0.05$). Also, the NE concentrations in the V. amygdala decreased after injections of 1.0 ($p < 0.05$) and 2.0 ($p < 0.01$)g/kg.

In the VTA, concentrations of NE ($p < 0.01$), DA ($p < 0.01$) and 5-HT ($p < 0.01$) significantly decreased without any variation in the concentrations of their metabolites after injections of 1.0g/kg.

In the locus coeruleus, the NE concentration ($p < 0.01$) decreased after administration with

maximal reduction following the injection of 4.0g/kg. The 5-HIAA concentration ($p < 0.01$) in the V. amygdala increased after injection of 4.0g/kg without any alteration in the 5-HT concentration.

Effects of Repeated Ethanol Administration

Trunk blood samples collected from the 1.0g/kg ethanol-injected rats at the time of sacrifice showed an average serum ethanol concentration (SAC) of 0.24 ± 0.09 g/l. In the 2.0g/kg ethanol-injected rats, the average SAC was over six times that of the 1.0g/kg treatment (1.53 ± 0.11 g/l).

The concentrations of monoamines and their metabolites in various brain regions after repeated injections of two different doses of ethanol are shown in Table 2.

The alterations following injections of 2.0g/kg were more marked than those following injections of 1.0g/kg, and about half of the alterations after

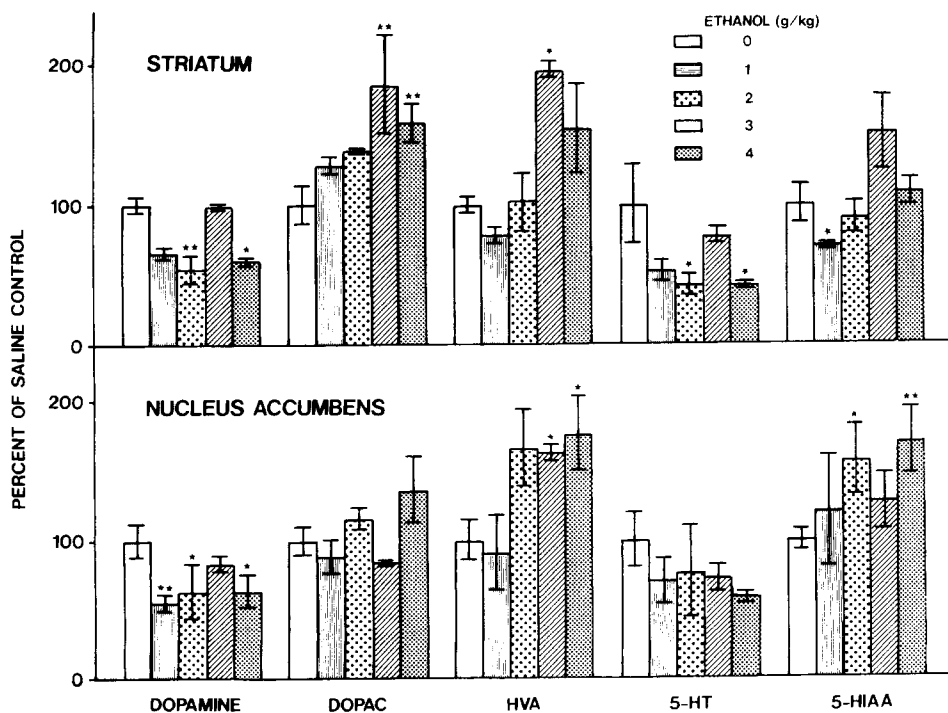


Fig. 2 Effect of acutely administered ethanol on the DA, DOPAC, HVA, 5-HT and 5-HIAA concentrations in rat brain regions. Ethanol was administered intraperitoneally to rats in the amounts of 1, 2, 3 and 4g/kg body weight as a diluted solution in saline. Rats were killed 2h after each dose of ethanol or saline. Monoamine and their metabolite concentrations are expressed as percentages of mean levels in the control group. Each value is the mean of at least 3 rats. Significantly different from the control group (Williams-Wilcoxon test: * < 0.05 , ** < 0.01). Abbreviations used are the same as those of the legend in Fig. 1.

1.0g/kg were opposite to those observed in the acute study.

Following daily injections of 1.0g/kg, NE concentrations were reduced in the VTA and

Table 2 Effects of chronically administered ethanol on the NE, DA, DOPAC, HVA, 5-HT and 5-HIAA levels in rat brain regions.

	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Striatum						
Saline	—	298.2 ± 99.8	160.9 ± 21.6	43.3 ± 7.0	6.3 ± 2.0	16.1 ± 2.5
Ethanol 1g	—	234.4 ± 32.3	190.2 ± 50.0	44.5 ± 9.7	5.5 ± 1.0	17.3 ± 2.2
Ethanol 2g	—	168.3 ± 66.7**	237.0 ± 56.3	57.8 ± 5.9**	3.9 ± 1.2**	19.3 ± 3.3*
Substantia nigra						
Saline	16.3 ± 2.1	27.7 ± 5.1	20.6 ± 4.5	4.2 ± 1.2	23.3 ± 4.0	36.9 ± 5.2
Ethanol 1g	16.9 ± 2.1	34.4 ± 3.1*	18.2 ± 6.0	4.5 ± 1.6	28.1 ± 5.0	35.0 ± 6.6
Ethanol 2g	13.1 ± 2.1*	17.7 ± 4.3*	16.7 ± 5.3	4.9 ± 1.1	17.1 ± 3.2*	35.0 ± 9.0
Nucleus accumbens						
Saline	—	172.6 ± 46.5	150.3 ± 44.5	29.6 ± 6.8	10.8 ± 2.8	21.7 ± 3.1
Ethanol 1g	—	255.0 ± 106.3	108.3 ± 27.8*	34.8 ± 8.2	13.5 ± 4.5	26.0 ± 6.1
Ethanol 2g	—	106.5 ± 30.8*	229.0 ± 69.4	51.5 ± 7.8**	9.2 ± 3.2	35.1 ± 5.8**
Ventral tegmental area						
Saline	34.8 ± 6.6	20.8 ± 18.5	18.6 ± 14.5	6.2 ± 3.8	18.1 ± 5.9	24.1 ± 13.4
Ethanol 1g	24.7 ± 10.0*	10.1 ± 7.3	9.9 ± 7.1	3.3 ± 1.7	11.4 ± 8.1*	18.2 ± 18.2
Ethanol 2g	21.6 ± 6.9**	3.3 ± 1.4	5.0 ± 2.5*	1.8 ± 0.7**	7.0 ± 4.6**	19.4 ± 16.1
Ventro-medial hypothalamus						
Saline	103.2 ± 35.8	58.6 ± 26.5	28.4 ± 14.3	—	13.8 ± 5.6	26.1 ± 10.2
Ethanol 1g	116.4 ± 35.9	52.7 ± 34.8	28.6 ± 12.0	—	14.9 ± 5.6	33.0 ± 11.5
Ethanol 2g	82.8 ± 30.7	45.6 ± 21.4	33.6 ± 14.9	—	8.8 ± 2.5	30.0 ± 10.9
Lateral hypothalamus						
Saline	72.5 ± 20.2	8.7 ± 2.8	11.8 ± 3.7	—	19.3 ± 2.9	38.4 ± 7.6
Ethanol 1g	75.1 ± 19.4	13.2 ± 3.2*	9.2 ± 1.2	—	23.8 ± 5.1	32.7 ± 7.6
Ethanol 2g	55.9 ± 22.1	6.8 ± 2.6	11.0 ± 5.4	—	15.4 ± 4.8	39.5 ± 11.9
Nucleus paraventricularis						
Saline	129.5 ± 36.2	8.0 ± 2.5	13.2 ± 2.6	—	22.1 ± 4.8	47.6 ± 4.3
Ethanol 1g	139.9 ± 36.3	9.4 ± 2.5	12.7 ± 3.4	—	15.6 ± 3.7*	40.9 ± 3.2**
Ethanol 2g	77.7 ± 11.8**	7.0 ± 1.3	12.4 ± 2.2	—	10.8 ± 1.9**	40.6 ± 4.4**
Ventral amygdala						
Saline	14.7 ± 1.5	2.4 ± 0.6	1.9 ± 0.6	—	14.2 ± 2.2	18.0 ± 1.8
Ethanol 1g	16.2 ± 2.3	2.5 ± 0.8	1.7 ± 0.9	—	14.8 ± 2.8	20.9 ± 5.0
Ethanol 2g	12.7 ± 1.7	1.8 ± 0.4	1.2 ± 0.5	—	10.2 ± 1.1**	20.4 ± 2.1
Dorsal amygdala						
Saline	22.3 ± 1.6	8.1 ± 2.1	4.9 ± 1.8	—	25.1 ± 2.7	31.9 ± 4.5
Ethanol 1g	25.2 ± 5.9	11.3 ± 3.0*	4.0 ± 1.1	—	26.3 ± 4.1	27.4 ± 5.0
Ethanol 2g	13.7 ± 3.0*	7.3 ± 2.2	5.2 ± 2.0	—	17.2 ± 2.6**	29.5 ± 5.4
Raphe dorsalis						
Saline	92.8 ± 18.5	7.5 ± 3.0	5.9 ± 1.7	—	54.7 ± 12.0	110.4 ± 33.9
Ethanol 1g	74.6 ± 12.7*	5.0 ± 1.0	4.1 ± 1.4	—	39.1 ± 9.7*	120.5 ± 11.9
Ethanol 2g	61.7 ± 24.4**	5.3 ± 2.1	5.3 ± 2.4	—	30.8 ± 11.2**	141.9 ± 46.2
Locus coeruleus						
Saline	57.2 ± 11.2	6.5 ± 2.3	8.8 ± 3.5	2.8 ± 0.6	26.4 ± 9.6	37.8 ± 10.5
Ethanol 1g	45.0 ± 17.3	5.0 ± 2.6	5.6 ± 4.3	1.9 ± 0.8	17.4 ± 4.4*	38.9 ± 7.7
Ethanol 2g	43.9 ± 13.6	6.8 ± 2.2	6.4 ± 1.5	2.6 ± 0.5	19.9 ± 4.6	60.1 ± 16.0**

Rats were injected intraperitoneally with ethanol in the amounts of 1.0 or 2.0g/kg body weight for seven consecutive days. The rats were killed 2h after the last injection of ethanol or saline. Results are expressed as a mean ± S.D., of 7-9 rats, of pmoles/mg protein. Significantly different from the control group (Williams-Wilcoxon test: *p < 0.05, **p < 0.01). Abbreviations used are the same as those of the legend in Fig. 1.

raphe dorsalis. DA concentrations were elevated in the S. nigra, LH and D. amygdala, while the HVA concentration was unaltered and the DOPAC concentration decreased in the N. accumbens. 5-HT concentrations were reduced in the VTA, raphe dorsalis, PTN and locus coeruleus. The 5-HIAA concentration was also decreased in the PTN (Table 2).

Following daily injections of 2.0g/kg, NE concentrations were lowered in the S. nigra, VTA, raphe dorsalis, D. amygdala and PTN. DA concentrations were lowered in the striatum, N. accumbens and S. nigra. HVA concentrations were elevated in the striatum and N. accumbens while they were lowered in the VTA. The DOPAC concentration was also altered in the VTA alone. The 5-HT concentration was lowered in seven brain regions; striatum, S. nigra, VTA, V. amygdala, raphe dorsalis, D. amygdala and PTN. The 5-HIAA concentrations were elevated in the striatum, N. accumbens and locus coeruleus. However, the 5-HIAA concentration was decreased in the PTN (Table 2).

Discussion

The results of the present study clearly show that ethanol influences the dopaminergic system in the striatum and the N. accumbens. For example, the acute and repeated administration of ethanol decreased the concentration of DA and increased the concentration of its metabolite, HVA, in these brain regions studied which contain dopaminergic terminals.

In our result, the increased concentration of HVA by acute ethanol (3g/kg) treatment is in general agreement with reports which described a similar increase in DA metabolite concentration in the striatum of alcohol-preferring rats (22) and of mice (23). In this study, a comparable rise in metabolite concentration was also observed in the N. accumbens, another major dopaminergic nucleus, which was not investigated in the two papers cited above. The decreased concentrations

of DA in the striatum and N. accumbens by acute ethanol treatment in the present study are not generally in agreement with the report by Dar and Wooles (23), who showed that the higher dose of ethanol increased the striatal DA levels, whereas a low dose of ethanol had no effect. Further investigation is needed to explain the depletion of DA by acute ethanol treatment in this study.

In the repeated ethanol-treated rats (2g/kg), HVA concentration increased and DA concentration decreased in the striatum and N. accumbens almost similarly to acutely treated rats. However, HVA ratio (HVA concentration/DA concentration) in those two regions of rats in repeated study was higher than that in acute study, possibly indicating a much more increased DA turnover in repeated study. Contrary to the repeated administration of 2g/kg ethanol, DA concentration increased and metabolites decreased in some brain regions following repeated administration of lower doses (1g/kg). This inconsistent response to lower doses might be interpreted in terms of nonspecific reaction to insufficient agent.

The present data also show that ethanol affects the serotonergic system in the striatum, N. accumbens, S. nigra, VTA, VMH, LH, PTN, V. amygdala, raphe dorsalis and locus coeruleus. The acute and repeated administration of ethanol decreased the concentration of 5-HT and increased the concentration of its metabolite, 5-HIAA, in the striatum and N. accumbens. The finding of decreased 5-HT and increased 5-HIAA levels by acute ethanol are generally in agreement with the report of Suthanthirarajan (16) who described that the 5-HT concentrations in the hypothalamus, striatum, midbrain, pons medulla, cerebellum and cerebral cortex showed a reduction at 30 and 60 min after ethanol ingestion, with a corresponding rise in the level of 5-HIAA indicating increased turnover of the amine.

In our study, the 5-HT concentrations in the S. nigra, PTN, V. amygdala, D. amygdala and raphe dorsalis of the rats repeatedly treated with ethanol (2g/kg) were lowered, whereas 5-HT concentrations in those brain regions of the acute

ethanol rats were not changed. An increased concentration of 5-HIAA without any change in the 5-HT level by acute or chronic ethanol treatment was shown in a report by Pohorecky *et al.* (12). The increased levels of both 5-HT and 5-HIAA in the frontal cortex, anterior striatum and N. accumbens were observed one hour after the i.p. injection of ethanol in a report by Murphy *et al.* (6), who suggested enhanced intraneuronal metabolism. Our findings of increased 5-HIAA and decreased 5-HT levels in the striatum and N. accumbens by repeated ethanol administration generally agree with the report of Gothóni and Ahtee (14) who showed that chronic ethanol administration decreased 5-HT and increased 5-HIAA concentrations in the rat brain.

Additionally, our results show that ethanol influences the noradrenergic system in some brain regions. The acute administration of ethanol decreased the concentrations of NE in the raphe dorsalis and locus coeruleus. This is not in agreement with the report of Suthanthirarajan (16), who described that the concentration of NE showed a significant rise in the hypothalamus and striatum after 30 and 60 min of ethanol ingestion. Hellevuo and Kiianmaa (24) reported that acute ethanol did not affect the levels of NE in any region of the brain. The present data show that the repeated administration of ethanol induced a less marked but significant decrease in NE levels than acute ethanol. The finding of decreased NE levels by repeated ethanol administration is not in agreement with reports of increased NE concentrations (15, 17) and of no change in NE levels (22). These discrepant findings may have arisen from methodological or procedural differences such as the species and strain of animals employed, the processing of tissues for analysis. Moreover, if the concentration of MHPG (3-methoxy-4-hydroxyphenylglycol), an NE metabolite, in brain regions is determined together with the concentration of NE, some more detailed information could be obtained about the effect of ethanol on NE metabolism.

In conclusion, the present study has shown

that concentrations of monoamines and their metabolites can be determined simultaneously in discrete brain regions. Although there are contradictory differences between the present study and some of other reports, our findings suggest that ethanol significantly interferes with not only dopamine, but also serotonin and norepinephrine metabolism, and support the proposition that changes in dopaminergic, serotonergic and noradrenergic transmission mechanisms may be involved in the central nervous system effects of acute and repeated ethanol administration.

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