receiving 10 μCi ^{3}H-spiperone intraperitoneally was centrifuged and the supernatant was chromatographed on Silufol plates in a chloroform–methanol (9:1) system [2].

As the experiments showed, 75 + 5% of the radioactivity migrated along with unlabeled spiperone, rather less than after intravenous injection (90%) [2]. The effect of various neuroleptics on binding of ^{3}H-spiperone also was investigated after its intraperitoneal injection (Fig. 2). Haloperidol and chlorpromazine actively displaced ^{3}H-spiperone from its binding sites in the basal ganglia. Sulpiride was less active, but in a dose of 200 mg/kg it also displaced the ^{3}H-spiperone, in agreement with observations by other workers [3]. The neuroleptics, however, did not affect binding of ^{3}H-spiperone in the cerebellum. The level of nonspecific binding, determined after injection of maximal doses of neuroleptics, was higher, incidentally, and never reached the level determined in the cerebellum (Fig. 2).

The results of these experiments thus show that after intraperitoneal injection ^{3}H-spiperone binds specifically with receptors of the cortex and basal ganglia of the mouse brain. Neuroleptics with varied chemical structure displace ^{3}H-spiperone from its binding sites. Although after intraperitoneal injection the level of specific binding is rather lower than after intravenous injection, the relative simplicity, reproducibility, and economy of the method make it more acceptable.

LITERATURE CITED

1. L. N. Allikmets, A. M. Zharkovskii (A. M. Zarkovsky), and A. M. Nurk, Europ. J. Pharmacol., 75, 145 (1981).
2. V. Höllt, A. Czlonkowski, and A. Herz, Brain Res., 130, 176 (1977).
3. P. M. Laduron, P. F. M. Janssen, and J. E. Leysen, Life Sci., 23, 581 (1978).
4. S. H. Snyder and J. R. Bennett, Jr., Ann. Rev. Physiol., 38, 153 (1976).
5. I. F. Tallman, J. W. Thomas, and D. W. Gallager, Life Sci., 24, 873 (1979).

CATECHOLAMINE LEVELS IN THE RAT BRAIN AT DIFFERENT STAGES OF EXPERIMENTAL ALCOHOLISM

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The role of positive reinforcement structures in the development of a craving for alcohol and dependence on it is nowadays accepted [5, 15]. If the catecholaminergic nature of these structures [11] and data on changes in the catecholaminergic system under the influence of alcohol in man and animals [1, 2, 12] are taken into account, there are good grounds for linking disturbances in the physiological function of this system with the development of a craving for alcohol and dependence on it. However, the contradictory nature of the data on this problem, due to the use of different techniques of alcoholism on animals of different species and strains, does not allow any definite conclusion to be drawn on participation of the dopaminergic and noradrenergic system in the development of a craving for alcohol and dependence on it [4].

The aim of this investigation was to determine noradrenalin (NA), dopamine (DA), and homovanillic acid (HVA) levels in the rat brain at different stages of experimental alcoholism.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats. In the experiments of series I on animals divided on the basis of the duration of ethanol anesthesia test (4.5 g/kg,

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TABLE 1. Concentrations (in ng/g) of NA, DA, and HVA in Brain of Rats Predisposed (1) and Not Predisposed (2) to Alcohol Consumption (M ± m)

Experimental conditions	NA (hypothalamus)		DA (striatum)		HVA (striatum)	
	1	2	1	2	1	2
Intact rats	1416±93	1256±218	7015±1186	7227±972	612±51,7	616±57,9
Ethanol, 2.5 g/kg intraperitoneally, single dose	1015±35	1145±80	6385±260	6285±472	555±49,0	576±62,4
Consump. of 15% ethanol during 10 days	1392±102	1550±89	9205±865	9026±865	540±88,2	643±69,5

TABLE 2. Concentrations (in ng/g) of NA, DA, and HVA in Rat Brain after 4 and 8 Months of Alcoholization and after Withholding of Alcohol (M ± m)

Experimental conditions	NA (hypothalamus)		DA (corpus striatum)		HVA (corpus striatum)	
	control	expt.	control	expt.	control	expt.
Alcholization						
4 months	1107±254	1341±201	8208±1007	8765±1337	555±77,5	631±20,5
8 months	1203±240	1156±235	7773±1694	6667±1016	604±49,9	713±69,5
Withholding of alcohol	—	1168±107	—	7126±909	—	627±67,7

intraperitoneally) into animals predisposed and not predisposed to alcohol consumption, the duration of ethanol anesthesia was 80 and 180 min respectively [6].

The initial catecholamine concentration in the animals' brain was determined 30 min after a single intraperitoneal injection of 25% ethanol solution in a dose of 2.5 g/kg, and also after consumption of 15% ethanol solution by the animals for 10 days under conditions of free choice between it and water. The level of absolute alcohol consumed per diem was 2.8 g/kg in predisposed and 0.3 g/kg in unpredisposed animals. In series III catecholamines were determined in rats consuming 15% alcohol solution for 4 and 8 months, and in animals of the latter group, 24 h after alcohol was withheld. Periods of alcoholization of 10 days and 4 and 8 months correspond to stages of formation of a craving for alcohol, of fully formed craving, and of physical dependence [4]. Withholding alcohol for 24 h from rats alcoholized for 8 months leads to the development of abstinence [7].

The NA concentration was determined in the hypothalamus, and DA and HVA in the corpus striatum of the rats and pooled brain structures from two animals. The substances for testing were isolated on columns with Sephadex G-10 [14]. A 0.02 M solution of iodine was used as oxidizing agent for determination of DA and NA, and a 0.01% solution of $K_3Fe(CN)_6$ for determination of HVA [8]. The determinations were done on a "Hitachi" spectrofluorometer (Japan).

The results were subjected to statistical analysis by comparison of means [3].

EXPERIMENTAL RESULTS

The catecholamine levels were identical in predisposed and unpredisposed animals. Ethanol, given in a single dose to predisposed animals, induced a sharp fall (by 30%) in the NA concentration ($P < 0.05$) compared with that in unpredisposed rats. After alcoholization for 10 days the NA level in the predisposed animals was the same as in the control, whereas in unpredisposed animals it was increased by 19% ($P < 0.05$). The DA level (Table 1) rose during this period in rats of both groups ($P < 0.05$).

Alcoholization for 4 months did not cause statistically significant changes in the levels of the above-mentioned neurotransmitters compared with those found after alcoholization for 10 days. After 8 months of alcoholization a fall was observed in the NA (17%, $P < 0.05$) and DA (31%, $P < 0.01$) concentrations and a rise in the HVA concentration (25%, $P < 0.01$) in the animals.

A fall in the HVA concentration (13%, $P < 0.05$) but no change in the NA and DA levels were observed in these rats 24 h after withholding of alcohol. In the control animals (rats of the same age, but not consuming alcohol) no changes were found (Table 2).

These results showing a fall in the NA concentration under the influence of a single dose of alcohol in rats predisposed to its consumption are probably evidence of increased

liberation and utilization of NA in these animals, i.e., of activation of the noradrenergic system, and this is confirmed by data in the literature [9, 13]. Considering the evidence of activation of the positive reinforcement system under the influence of alcohol in rats predisposed to its consumption [5], it can be concluded that the euphoric action of alcohol is realized through the noradrenergic system. After 10 days of alcoholization the NA level becomes stabilized, probably on account of increased NA synthesis accompanied by a high level of its utilization. The identical direction of the changes in DA and HVA concentrations in rats of different groups and differences in the NA concentration observed at this stage are probably evidence of the leading role of the noradrenergic system in the formation and maintenance of a craving for alcohol.

In the stage of physical dependence a raised HVA concentration was found in the rats, indicating increased activity of the dopaminergic system. Changes in the activity of this system also take place in the period of abstinence. These data, and also the absence of differences in the NA level at the physical dependence stage relative to the early stages, are evidence of a qualitative change in the mechanisms of alcohol motivation and of the predominant role of the dopaminergic system in the development of alcohol dependence and in the manifestation of the abstinence syndrome.

LITERATURE CITED

1. I. P. Anokhina, in: The Pathogenesis, Clinical Aspects, and Treatment of Alcoholism [in Russian], Moscow (1976), pp. 15-19.
2. I. P. Anokhina, N. N. Ivanets, B. M. Kogan, et al., in: Problems in the Pathogenesis of Mental Diseases [in Russian], Moscow (1979), pp. 92-96.
3. M. L. Belen'kii, Elements of Quantitative Evaluation of a Pharmacologic Effect [in Russian], Leningrad (1963).
4. Yu. V. Burov, Vest. Akad. Med. Nauk SSSR, No. 5, 72 (1982).
5. Yu. V. Burov and S. A. Borisenko, Farmakol. Toksikol., No. 3, 291 (1979).
6. Yu. V. Burov, G. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).
7. I. V. Viglinskaya, in: Experimental and Clinical Psychopharmacology [in Russian], Moscow (1980). pp. 34-42.
8. N. E. Anden, B. E. Roos, and B. Werginius, Life Sci., 2, 448 (1963).
9. N. G. Bacopoulos, R. K. Bhatnagar, and L. S. Van Orden, J. Pharmacol. Exp. Ther., 204, 1 (1978).
10. C. C. Chag, J. Neuropharmacol., 3, 643 (1964).
11. M. S. Olds and A. Inweler, Brain Res., 36, 385 (1972).
12. B. Tabakoff and P. L. Hoffman, J. Neurochem., 25, 43 (1978).
13. P. V. Thadani and E. B. Truit, Biochem. Pharmacol., 26, 1147 (1977).
14. B. Westerink and J. Korf, Europ. J. Pharmacol., 38, 281 (1976).
15. R. A. Wise, in: Drug Alcohol Depend., 6, 82 (1980).