LITERATURE CITED

1. R. U. Ostrovskaya, G. M. Molodavkin, S. S. Trofimov, et al., Byull, Éksp. Biol. Med., No. 12, 62 (1982).
2. R. U. Ostrovskaya, V. V. Parin, and N. M. Tsybina, Byull. Éksp. Biol. Med., No. 1, 51 (1972).
3. R. U. Ostrovskaya and S. S. Trofimov, Byull. Éksp. Biol. Med., No. 2, 170 (1984).
4. N. M. Tsybina, R. U. Ostrovskaya, T. V. Protopopova, et al., Khim.-Farm. Zh., No. 9, 10 (1974).
5. B. W. Agranoff, R. E. Davis, L. Casols, et al., Science, 158, 1600 (1967).
6. J. Bureš and O. Burešova, J. Comp. Physiol. Psychol., 56, 268 (1963).
7. A. Cherkin and A. Van Harreveld, Brain Res., 156, 265 (1978).
8. A. Delina-Stula and A. Vassout, Neuropharmacology, 17, 1063 (1978).
9. W. B. Essman, Biology of Memory, Budapest (1971), p. 213.
10. H. Frey, G. Popp, and W. Löscher, Neuropharmacology, 18, 581 (1979).
11. C. Guirgea, D. Lefevre, C. Lescrenier, et al., Psychopharmacologia, 20, 160 (1971).
12. E. Keller, J. L. Davis, K. H. Tachiki, et al., J. Neurochem., 37, 1335 (1981).
13. E. Roberts, Adv. Biochem. Psychopharmacol., 27, 91 (1981).
14. W. Sieghart, J. Neurochem., 37, 1116 (1981).
15. P. Zarzecki, P. S. Blum, G. E. Cordingley, et al., Brain Res., 89, 187 (1975).

BINDING OF ^{3}H-SPIPERONE IN THE MOUSE BRAIN AFTER INTRAPERITONEAL INJECTION

A. M. Zharkovskii, T. A. Zharkovskaya and K. S. Chereshka

UDC 615.214.2.033.81.073

KEY WORDS: ^{3}H-spiperone; dopamine receptors; neuroleptics; tolerance.

Methods of radioligand binding with neuronal membranes *in vitro* have become widely used in the study of the action of psychotropic drugs on various brain receptors [4]. In the last few years methods of binding *in vivo* also have been successfully developed, and they possess a number of important advantages [2, 3, 5]. In particular, by this method it is possible to assess the character of binding of a radioactive ligand in the living organism, while maintaining the ionic composition, the presence of mediators, and pH [2]. To study the action of neuroleptics on dopamine and serotonin receptors, ^{3}H-spiperone, which has high affinity for these receptors [1-3, 6], is used as the radioligand.

In experiments *in vivo* minimal doses of ^{3}H-spiperone are injected into the caudal vein of mice or rats, and a short time (30 min-4 h) later, the level of radioactivity in the brain tissues is measured. Intravenous injection into mice is technically quite difficult, for it is not possible to guarantee that all the ligand enters the blood stream, and this results in loss of both animals and substance. Intravenous injection in rats is not difficult, but in this case a large quantity of ligand is required, and this makes the experiments too expensive. In the investigation described below binding of ^{3}H-spiperone was studied after intraperitoneal injection into mice.

EXPERIMENTAL METHOD

Experiments were carried out on male mice weighing 20 ± 1 g. Different quantities of ^{3}H-spiperone (16.7 Ci/mmole, from Amersham Corporation, England) were injected intraperitoneally into the animals. For comparison, ^{3}H-spiperone was injected into the caudal vein of animals of another group. The mice were decapitated 1.5 h after injection of the radioligand and the brain was removed in the cold and divided into parts: frontal cortex, basal

Department of Pharmacology and Laboratory of Psychopharmacology, Research Institute of General and Molecular Pathology, Tartu University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 314-315, March, 1985. Original article submitted June 15, 1984.

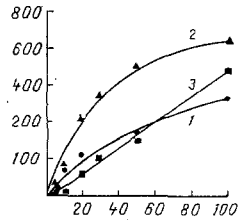

Fig. 1

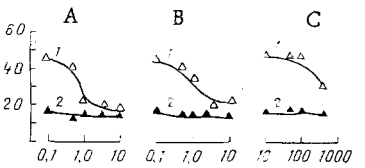

Fig. 2

Fig. 1. Binding of ^{3}H-spiperone in cortex, basal ganglia, and cerebellum of mice 1.5 h after intraperitoneal injection. Abscissa, quantity of ^{3}H-spiperone (in μCi) injected; ordinate, radioactivity (in cpm/mg tissue). 1) Specific binding (total binding in cerebellum) in cortex, 2) specific binding in basal ganglia, 3) nonspecific binding in cerebellum.

Fig. 2. Effect of haloperidol (A), chlorpromazine (B), and sulpiride (C) on binding of ^{3}H-spiperone (5 μCi) in basal ganglia of mice after intraperitoneal injection. Abscissa, dose of neuroleptics (logarithmic scale, in mg/kg); ordinate, radioactivity (in cpm/mg tissue). 1) Binding in basal ganglia, 2) binding in cerebellum.

TABLE 1. Binding of ^{3}H-spiperone (5 μCi) in Brain Structures of Mice after Its Intravenous and Intraperitoneal Injection (M ± m)

Mode of injection	No. of animals	Total binding, cpm/mg			Cortex	Basal ganglia
		cortex	basal ganglia	cerebellum	cerebellum	cerebellum
Intraperitoneally	5	40,4±7,2	49,9±6,4	17,0±1,7	2,38	2,9
Intravenously	6	56,9±8,7	62,1±2,4	16,5±1,9	3,45	3,76

ganglia (corpus striatum, olfactory tubercle, nucleus accumbens) and cerebellum. The tissue was frozen, weighed, and transferred into polypropylene test tubes, to which 0.5 ml of BTS tissue solubilizer (from Beckman, USA) was added. The tissue was dissolved in the solubilizer for 4 h at 40°C, after which 20 μl of glacial acetic acid was added to produce decolorization, and the contents of the tubes were transferred to flasks for scintillation counting, after addition of 10 ml of toluene scintillator, containing 13% of BBS-3 solubilizer (from Beckman). The flasks were allowed to stand for 2 h in darkness at 4°C to extinguish chemiluminescence, after which radioactivity was counted in an LS-7500 counter with an efficiency of 34-37%. The difference in the quantity of radioactivity in brain structures and cerebellum, in which only nonspecific binding was determined [2], was taken as specific binding.

To evaluate the action of the substances on ^{3}H-spiperone binding, 2 h before decapitation the mice were given an intraperitoneal injection of various doses of the following neuroleptics: haloperidol, chlorpromazine, sulpiride. The identity of the radioactivity in the tissue to unlabeled spiperone was determined by the method in [2]. Unlabeled spiperone was generously provided by Janssen Pharmaceutica (Belgium).

EXPERIMENTAL RESULTS

Data on specific binding of ^{3}H-spiperone in the basal ganglia and cortex after intraperitoneal injection are given in Fig. 1. Binding in the cortex and basal ganglia was saturating in character and reached a plateau at 100 μCi ^{3}H-spiperone. In the cerebellum, in which there are no dopamine receptors [3], the quantity of radioactivity rose in a straight line. Table 1 gives data on binding of ^{3}H-spiperone in brain structures of the mice after its intravenous and intraperitoneal injection. With intravenous injection the quantity of radioactivity in the basal ganglia and cortex was a little higher than after intraperitoneal injection, and the structure/cerebellum ratio was correspondingly higher. The somewhat smaller quantity of radioactivity in the brain after intraperitoneal injection was evidently connected with the greater metabolism of ^{3}H-spiperone at the periphery. To estimate whether radioactivity determined was identical with labeled ^{3}H-spiperone, brain homogenate from mice

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

A. I. Varkov and L. A. Malikova

UDC 616.89-008.441.13-092.9-07:616.831-008.944.52:577.175.52

KEY WORDS: alcoholism; brain; catecholamines.

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,

Department of Neuropharmacology, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 316-317, March, 1985. Original article submitted June 15, 1984.

0007-4888/85/9903-0311$09.50