CHANGE IN NUMBER OF BENZODIAZEPINE RECEPTORS IN DIFFERENT PARTS OF THE RAT BRAIN AFTER NEUROLEPTIC WITHDRAWAL

A. M. Zharkovskii and T. A. Zharkovskaya

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Neuroleptics not only have an antipsychotic action, but they also give rise to various changes in animal behavior. These effects are linked with the effect of these drugs on dopaminergic, serotoninergic, GABA-ergic, and chlolinergic brain systems [1, 2, 4, 12]. It was shown previously that neuroleptics also have an antiaggressive and a tranquilizing action [1]. Benzodiazepines, which are the most effective tranquilizers at the present time, exert their action through benzodiazepine (BD) receptors in brain tissue [11].

It was therefore decided to study the effect of neuroleptics on BD receptors in the rat brain, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 230-250 g. The following drugs were injected into the animals (10-12 rats in each group): haloperidol (1.0 mg/kg), sulpiride (50 mg/kg), and chlozapine (10.0 mg/kg) daily for 16 days. The daily dose of the neuroleptics was divided into two parts and injected twice a day intraperitoneally. Control animals received physiological saline. On the 5th day after discontinuation of the neuroleptics the animals were killed and, in the cold, the striatum, olfactory tubercle, and frontal cortex were isolated. Pieces of brain were frozen at −20°C. Rats of a separate group underwent unilateral destruction of dopaminergic neurons with the aid of 6-hydroxydopamine (6-HDA). Under ether anesthesia 8 μg of 6-HDA in 4 μl of 0.2% ascorbic acid was injected into the animals by means of a stereotaxic apparatus, taking coordinates form an atlas of the rat brain [9], into the right side of the substantia nigra (pars compacta). A subcutaneous injection of 0.5 mg/kg apomorphine was given to the rats 14 days after the operation, and animals in which apomorphine induced at least six contralateral turns in 2 min were used in the subsequent experiments. These rats were killed 2 days later, the striatum was excised on the side of destruction, and binding of [^{3}H]flunitrazepam was determined. The striatum on the intact side served as the control. Membranes were isolated and binding carried out by the method in [11] with slight modification. Binding was carried out in 1 ml of medium containing 0.8 ml of membrane suspension, 100 μl of [^{3}H]flunitrazepam (specific activity 80 Ci/mmole, Amersham Corporation, England) in a final concentration of 0.625 nM and 100 μl of displacing substances in different concentrations. The tubes were incubated for 60 min at 0°C and the [^{3}H]flunitrazepam bound with the membranes was separated by filtration through GF/B filters (Whatman, England). Radioactivity was determined on an LS-6800 scintillation counter (Beckman, USA). Nonspecific binding was determined in the presence of 10^{-6} M cold flunitrazepam. The labeled dilution method was used to determine the binding parameters [6]. According to this method the quantity of radioactivity was constant and the concentration of unlabeled flunitrazepam increased (0.5-20 nM). Protein was determined by Lowry's method [10]. In experiments *in vitro* optical isomers of butaclamol, generously provided by the firm of "Ayerst," and spiperone, provided by the firm of Janssen Pharmaceutica, also were used.

EXPERIMENTAL RESULTS

[^{3}H]Flunitrazepam bound with high affinity to all brain structures studied. The method of labeled dilution used for analysis of binding gave similar results to those obtained by

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TABLE 1. Effect of Neuroleptics (dose in μM inhibiting binding by 50%) on Binding of [^{3}H]Flunitrazepam (0.5 nM) with Rat Brain Membranes (M ± m; n = 3)

Substance	Striatum	Cortex
Spiperone	1,4±0,4	—
Haloperidol	9,2±2,4	10,5±4,5
Sulpiride	82,6±12,8	170,0±37,2
Clozapine	20,2±4,0	8,0±3,2
(+)-Butaclamol	2,5±0,4	11,4±3,8
(-)-Butaclamol	12,0±3,1	150,2±25,6

TABLE 2. Binding of [^{3}H]Flunitrazepam (in fmoles/mg) with Membranes from Different Parts of Brain of Rats Previously Treated with Neuroleptics for 16 Days (M ± m; n = 3)

Substance	Striatum			Limbic system		Cortex	
	daily dose, mg/kg	maximal binding	K_d, nM	maximal binding	K_d, nM	maximal binding	K_d, nM
Control	—	600±35	1,6±0,3	650±48	2,4±0,4	1250±120	2,4±0,4
Haloperidol	1,0	425±19*	1,9±0,3	567±21*	1,8±0,5	770±75*	2,9±0,2
Sulpiride	50,0	325±45*	1,7±0,4	375±24*	1,6±0,5	730±64*	2,9±0,3
Clozapine	10,0	210±24*	1,3±0,4	420±32*	2.0—0,4	—	—

Legend. *P < 0.05 compared with control. Experiments carried out on 5th day after withdrawal.

the classical method, according to which the concentration of labeled ligand in the incubation medium increased [5, 9]. For instance, extrapolation of the straight line on a Scatchard plot revealed one binding site with high affinity. The density of the receptors was highest in the cortex: 1250 ± 120 fmoles/mg protein, whereas in the striatum and limbic system it was 600 ± 35 and 650 ± 48 fmoles/mg respectively.

In experiments *in vitro* the neuroleptics were found to be weak inhibitors of binding of [^{3}H] flunitrazepam. The substances displaced the radioligand only in micromolar concentrations (Table 1). By strength of displacement of this ligand the neuroleptics were arranged in the following order: spiperone > (+)-butaclamol > haloperidol > (—)-butaclamol > clozapine > sulpiride. Some degree of stereospecificity of displacement also was observed. For instance, (+)-butaclamol was 5-10 times more active than (—)-butaclamol, whereas in binding experiments in which labeled thioperidol was used, the dextrorotatory isomer was 150 times more active than the levorotatory isomer [13].

Despite the fact that neuroleptics exhibited low affinity for BD receptors in experiments *in vitro*, chronic administration of these drugs for 16 days caused a significant decrease in [^{3}H]flunitrazepam binding on the 5th day after withdrawal (Table 2). This decrease was independent of the nature of the neuroleptic and was equally marked after injection both on the typical neuroleptic haloperidol, and of the atypical neuroleptics chlozapine and sulpiride.

The greatest decrease in binding (by about 40%) was determined in the cortex, rather less in other structures. The decrease in binding took place on account of a decrease in the number of receptors, whereas the discociation constants (K_d) showed no significant change (Table 2).

In rats with unilateral injury to the dopaminergic pathways due to 6-HDA, a decrease in the number of BD-receptors also was found in the striatum on the side of injury (Fig. 1).

The results of this investigation show that after withdrawal of the neuroleptics there was a considerable decline in the number of BD-receptors in the striatum. This effect was not connected with any direct action of neuroleptics on receptors, since the substances tested in experiments *in vitro* inhibit [^{3}H]flunitrazepam binding only in micromolar concentrations, and this is evidently achieved trans-synaptically through dopaminergic and GABA-ergic systems. A compensatory increase in activity of the dopaminergic system in response

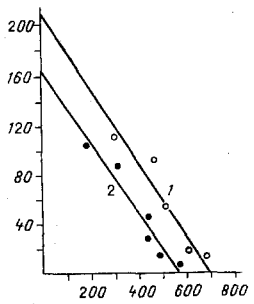

Fig. 1. Scatchard plot of [^{3}H]flunitrazepam binding in rats receiving preliminary injection of 6-HDA (8 μg/4 μl) into right substantia nigra. Abscissa, quantity of bound ligand (in fmoles/mg); ordinate, ratio of bound and free forms (in fmoles/mg protein relative to nanomoles of free ligand). 1) Left striatum (control), 2) right striatum. Binding experiments carried out on 16th day after injection of 6-HDA.

to prolonged blockage in turn causes changes in activity of the GABA-ergic system and, as a result of this, a decrease in the number of BD receptors. This hypothesis is confirmed by data showing that the BD-receptor is part of a more intricate GABA-benzodiazepine complex [8]. The experiments with 6-HDA also suggest that a certain proportion of BD-receptors are located on dopaminergic terminals of the nigrostriatal system.

Bearing in mind the hypothesis that after long-term administration of an antagonist, as a compensatory reaction its withdrawal induces an increase in the number of corresponding receptors [3, 7, 12] and, conversely, it can be postulated that long-term administration of neuroleptics has an agonistic action on BD-receptors.

The results suggest that the changes found in the density of BD-receptors lie at the basis of the antiaggressive, tranquilizing, and other effects of neuroleptics.

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