CHANGES IN SENSITIVITY OF BRAIN DOPAMINE AND SEROTONIN RECEPTORS DURING LONG-TERM TREATMENT WITH CARBIDINE

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Carbidine*, a derivative of α-carboline, combines the properties of neuroleptic and antidepressant [1, 3]. In experiments on animals carbidine differs in certain tests from the known neuroleptics. For instance, unlike the latter, it potentiates amphetamine stereotypy [3]. The writers showed previously [2, 5] that carbidine, in behavioral experiments and radioligand binding experiments, exhibits mainly the properties of a blocker of serotonin (S_2) receptors and can block, but to a much lesser degree, dopamine receptors of the striatum, which is a property of most known neuroleptics. It has been shown in recent years [9, 12] that after discontinuing chronic administration of neuroleptics, hypersensitivity of dopamine receptors in the striatum and limbic system develops as a compensatory reaction.

It was accordingly decided to study the state of the dopamine and serotonin receptors of the brain during chronic administration of carbidine to animals, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 220-250 g. In the experiments of series I, 0.8-M NaCl solution, carbidine in a dose of 10 mg/kg/day (provided by Professor K. S. Raevskii), and haloperidol in a dose of 1 mg/kg/day were injected intraperitoneally for 23 days. The daily dose of the neuroleptics was divided into two parts and injected twice a day. On the 4th day after stopping the neuroleptics, apomorphine hydrochloride (0.5 mg/kg) was injected subcutaneously into the animals and the intensity of stereotyped sniffing and chewing was determined according to a conventional scale. The intensity of the sniffing and chewing movements was estimated separately on a 4-point scale: 0) animal's behavior does not differ from the control, 1 point) single stereotyped movements, 2 points) stereotyped movements last not less than 30 sec during 1 min of observation, 3 points) stereotyped movements continuous during period of observation (1 min) and end in response to tapping on the cage, and 4 points) stereotyped movements continuous and do not cease after tapping of the cage.

In the experiments of series II the animals received an injection of 5-hydroxytryptophan (5-HTP) in a dose of 150 mg/kg combined with the monoamine oxidase inhibitor pargyline (50 mg/kg). 5-HTP was injected 20 min, and pargyline 1.5 h before the experiment. The number of heat twitches during 1 min was counted. The remaining animals of the control and experimental groups were decapitated. The frontal cortex and striatum were taken from them at 0-4°C. Parts of the brain from two rats were pooled and binding of ^{3}H-spiperone and ^{3}H-LSD was determined. Binding of ^{3}H-spiperpone (specific radioactivity 20 Ci/mmole, from Amersham Corporation, England) was determined by the method in [8], according to which the membrane suspension was used without preliminary washing. Specific binding was determined as the difference between binding in the absence and in the presence of 1 μM (+)-butaclamol (from Ayerst, Canada). Binding of ^{3}H-LSD (specific activity 11.8 Ci/mmole, from Amersham Corporation) in the cortex was carried out by the method in [7] with slight modification. Since ^{3}H-LSD has been shown to bind with different receptors, in order to detect the serotonin component of binding, the determination was made in the presence of 100 nM (-)-sulpiride (from Ravizza, Italy). In this concentration (-)-sulpiride selectively eliminated the dopamine component of binding. Specific binding of ^{3}H-LSD was determined as the difference between binding in the absence and presence of 1 μM pipamperone (Jansen Pharmaceutica, Belgium). In this con-

*3,6-dimethyl-1,2,3,4,4a,9a-hexahydro-γ-carboline dihydrochloride.

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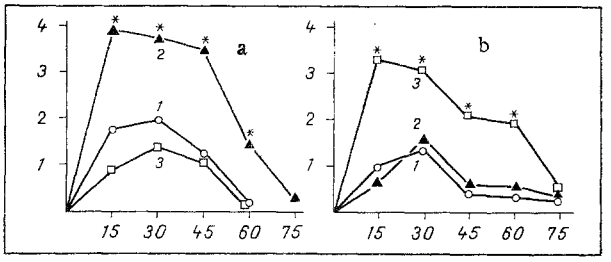
TABLE 1. Binding (in fmoles/mg protein) of ^{3}H-Spiperone in Striatum and Cortex, and of ^{3}H-LSD in Cortex of Rats on 5th Day After Discontinuation of Carbidine and Haloperidol (M ± m; n = 5-6)

Substance	mg/kg day	Binding of ^{3}H-spiperone		Binding of ^{3}H-LSD (cortex)
		Striatum	Cortex	
Control		105,2±24,6	201,0±19,2	129,0±7,5
Carbidine	10,0	154,1±39,2	140,7±18,2*	96,2±8,3*
Haloperidol	1,0	256,6±36,5*	202,5±18,2	108,2±12,0

Legend. *P < 0.05. Concentration of ^{3}H-spiperone in striatum 0.5 mM, in cortex 1.0 nM; concentration of ^{3}H-LSD 4 nM.



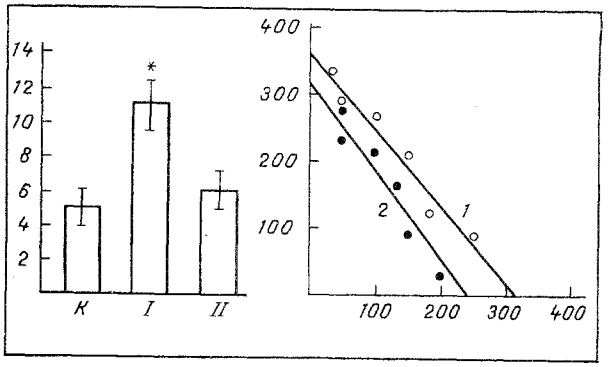


Fig. 1 Fig. 2 Fig. 3

Fig. 1. Intensity of stereotyped movements: sniffing (a) and gnawing (b) induced by subcutaneous injection of apomorphine (0.5 mg/kg) in rats on 4th day after discontinuation of physiological saline (1), carbidine (2), and haloperidol (3). Abscissa, time after injection of apomorphine (in min); ordinate, intensity of stereotyped movements (in points). *P < 0.05.

Fig. 2. Number of head twitchings induced by injection of 5-HTP (150 mg/kg) in combination with pargyline (40 mg/kg) on 6th day after discontinuation of carbidine (1) and haloperidol (II). C) Control. Remainder of legend the same as to Fig. 1

Fig. 3. Kinetic of ^{3}H-spiperone binding (0.1-2.0 nM) in cortex of rats after discontinuation of physiological saline (1) and carbidine (2). Abscissa, amount of ligand bound (in fmoles/mg protein); ordinate, ratio of bound to free ligand. 1) Maximal binding of 3.5 fmoles/mg protein; K_d = 0.88 nM; 2) 245.6 fmoles/mg and 0.77 nM respectively. Mean results of two experiments given on Scatchard plot.

centration pipamperone, as preliminary experiments showed, displaced ^{3}H-LSD only from serotonin (S_2) receptors.

Statistical analysis of the data for apomorphine stereotypy was carried out by the Mann–Whitney U test, and for analysis of the remaining data Student's t test was used. The kinetic parameters of binding, K_d, and maximal binding were determined by regression analysis.

EXPERIMENTAL RESULTS

On the 5th day after discontinuation of the neuroleptics, and increase in the intensity of apomorphine stereotypy was observed in the animals; after discontinuation of carbidine this took place on account of increased stereotyped sniffing, whereas stereotyped gnawing and licking were vitrually absent (Fig. 1). Conversely, after long-term administration of haloperidol and increase in stereotyped gnawing was observed (Fig. 1). After discontinuation of car-

bidine the number of head twitchings induced by 5-HTP increased (Fig. 2). After discontinuation of haloperidol an increase in the number of twitchings also was observed, but the differences were not statistically significant. On the 5th day after discontinuation of carbidine specific binding of ^{3}H-spiperone in the striatum was unchanged, but in the frontal cortex it was reduced. After injection of haloperidol, on the other hand, an increase in binding was observed in the striatum. A decrease in ^{3}H-LSD binding also was observed in the cortex of rats receiving carbidine (Table 1). A study of the kinetics of ^{3}H-spiperone binding in the cortex showed that the decrease in binding was due to a decrease in density of S_2 receptors (Fig. 3).

Changes observed on discontinuation of long-term carbidine treatment thus differed from changes caused by long-term haloperidol treatment. According to data of other workers [9, 12], enchanced apomorphine stereotypy is observed after discontinuation of neuroleptics, and this is interpreted as increased sensitivity of dopamine receptors in response to their prolonged blockade. After discontnuation of carbidine we also observed enhancement of stereotyped behavior of the animals, but the enhancement of stereotypy took place on account of only one component, namely sniffing, whereas after discontinuation of haloperidol the intensity of gnawing also was increased. The syndrome of stereotyped gnawing has been shown to be linked primarily with stimulation of the dopamine receptors of the striatum [12], whereas other components of stereotyped behavior may be determined by the effect of neuroleptics on other parts of the brain. It can be tentatively suggested that carbidine, unlike typical neuroleptics, does not increase the sensitivity of the dopamine receptors of the striatum, but the increase in the intensity of sniffing was connected with its effect on other parts of the brain or on other neurotransmitter systems. Evidence in support of this view is given by the binding experiments in which, after discontinuation of carbidine, no significant increase in ^{3}H-spiperone binding was observed in the striatum. After discontinuation of carbidine the intensity of hyperkinesia induced by 5-HTP increased, indicating a change in sensitivity of the serotonin receptors.

After discontinuation of carbidine binding of ^{3}H-spiperone and ^{3}H-LSD in the cortex was reduced. It has been shown that these radioligands label predominantly S_2 receptors in the cerebral cortex [7, 10, 11]. It was recently established that certain other neuroleptics, namely phenothiazine derivatives and clozapine [6], can reduce the density of S_2 receptors, but by contrast with the latter, after discontinuation of carbidine no increase in density of dopamine D_2 receptors was observed in the striatum. As was shown previously [4, 10], chronic administration of cyclic antidepressants also reduces the density of S_2 receptors. It can therefore be postulated that the antidepressive properties of carbidine are due to its effect on brain S_2 receptors.

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