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ADAPTIVE CHANGES ON SIGMA- AND PHENCYCLIDINE RECEPTORS DURING LONG-TERM HALOPSRIDOL AND RACLOPRIDE TREATMENT IN RATS

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UDC 615.214.2.015.4:612.82.014.467].076.9

Key words: haloperidol; raclopride; sigma receptors; phencyclidine receptors.

Derivatives of arylcyclohexylamine and benzomorphan possess a psychomimetic action [7, 9, 10]. Phencyclidine and other arylcyclohexylamines interact with phencyclidine receptors, whereas N-allylnormetazocine (SKF 10,047) and benzomorphans have highest activity for sigma receptors [8, 11]. Among the antipsychotic drugs, powerful antagonists of sigma receptors have been discovered; haloperidol has the highest affinity for these receptors [8, 12]. The view is held that selective antagonists of sigma receptors may prove to be potential antipsychotic drugs [13]. Neuroleptics do not interact with phencyclidine receptors in experiments in vitro [8, 12], but it has recently been shown that long-term haloperidol administration leads to a marked increase in number and reduction in affinity of phencyclidine receptors [2]. These facts are evidence of a possible role of sigma and phencyclidine receptors in the mechanism of action of neuroleptics.

The aim of this investigation was to study adaptive changes on sigma and phencyclidine receptors during long-term treatment with neuroleptics. Two different neuroleptics were chosen for this purpose: haloperidol, a high-affinity antagonist of sigma and dopamine$_2$-receptors, and raclopride, a selective dopamine$_2$-receptor antagonist.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 200-250 g. Haloperidol (Gedeon Richter, Hungary) in a dose of 0.5 mg/kg daily, and raclopride (Astra Läkemedel AB, Sweden) in a dose of 1 mg/kg daily were injected intraperitoneally for 15 days. Experiments to study binding of ^{3}H-thienylphenylcyclidine (^{3}H:TPC, "Dupont-NEN," USA; specific activity 60 mCi/mmole) and ^{3}H-SKF 10,047 ("Dupont-NEN," USA; specific activity 40 mCi/mmole) were carried out 2 and 48 h after the last injection of the neuroleptics by the method in [8]. The forebrain of the rats (the brain stem and cerebellum were removed) was homogenized in 10 volumes of 50 mM Tris-HCl buffer (pH 7.7) at 20°C and centrifuged 3 times at 40,000*g* for 15 min. The membranes were incubated at 23°C for 30 min in the presence of 2 nM ^{3}H-TPC, to study phencyclidine receptors, and in the presence of 10 nM ^{3}H-SKF 10,047 to determine sigma receptors. Nonspecific binding of ^{3}H-TFC was determined with the aid of ketamine (0.1-100 μM) and ^{3}H-SKF 10,047, with the aid of haloperidol (2.5-10,000 mM). Binding was stopped by rapid filtration through Whatman 6F/B filters (treated with 0.1% polyethylamine solution), and the filters were then washed with 5 ml of cold incubation buffer (5 mM Tris-HCl, pH 8.1) at 20°C. The radioactivity of the samples was measured in Bray's scintillator by means of an LC-6800 beta counter ("Beckman," counting efficiency 50-53%).

In the behavioral experiments the action of ketamine (5 mg/kg), an agonist of phencyclidine receptors, and of apomorphine (0.15 mg/kg), an agonist of dopamine receptors, was investigated after long-term treatment with haloperidol and raclopride. The behavioral experiments were conducted 48 h after withdrawal of the neuroleptics. In the 15th minute after

Laboratory of Psychopharmacology, Tartu University. (Presented by Academician of the Academy of Medical Sciences of the USSR D. A. Eharkevich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 306-309, September, 1989. Original article submitted October 10, 1988.

 0007-4888/89/0009-1270$12.50

TABLE 1. Effect of 15-Day Course of Haloperidol (0.5 mg/kg daily) and Raclopride (1 mg/kg daily) on Binding of ^{3}H-TPC and ^{3}H-SKF 10,047 in Rat Brain ($M \pm m$)

Substance, dose	^{3}H-TPC				^{3}H-SKF 10,047			
	2 h after withdrawal		48 h after withdrawal		2 h after withdrawal		48 h after withdrawal	
	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}
Physiological saline	0,87±0,12	312±20	1,24±0,25	252±25	33±5	9,5±0,6	49±4	10,1±0,8
Haloperidol, 0.5 mg/kg	0,76±0,14	360±22	1,36±0,30	286±28	52±6*	12,3±0,6*	40±5	12,2±0,7
Raclopride, 1 mg/kg	0,81±0,15	344±18	1,42±0,20	280±23	38±5	12,7±0,7*	39±6	12,1±0,8

Legend. Mean values of two or three independent investigations are given. *$p < 0.05$ compared with physiological saline. K_d) Dissociation constant (in nM in the case of ^{3}H-SKF 10,047, in μM in the case of ^{3}H-TPC. B_{max}) Maximal number of binding sites (in pmoles/g tissue).

TABLE 2. Effect of 15-Day Course of Haloperidol (0.5 mg/kg daily) and Raclopride (1 mg/kg daily) on Behavioral Effects of Ketamine (5 mg/kg) in Rats ($M \pm m$)

Experimental conditions	Intensity, points		Orienting-investigative activity during 3 min		
	of stereotypy	of ataxia	number of impulses	number of rearings	number of hole sniffings
PS + PS	—	—	16±2,8	1,2±0,3	2,0±0,5
Haloperidol + PS	—	—	16±3,2	1,5±0,4	2,5±0,5
Raclopride + PS	—	—	22±3,5	0,8±0,4	3,0±0,5
PS + ketamine	0,72±0,32	1,63±0,34	38±5,2**	0,5±0,3	3,5±0,8
Haloperidol + ketamine	0,72±0,32	1,13±0,24*	26±4,0	1,0±0,5	3,9±1,0
Raclopride + ketamine	0,44±0,22	0,94±0,22*	28±3,2	0,6±0,3	3,4±0,8

Legend. *$p < 0.05$ (Mann—Whitney U test compared with PS + ketamine group). Here and in Table 3 **$p < 0.05$ (Mann—Whitney U test compared with PS + PS group). PS) Physiological saline.

injection of ketamine the intensity of stereotyped behavior was determined [5], after which the orienting-investigative activity of the experimental animals was recorded for 3 min.

EXPERIMENTAL RESULTS

A single injection of haloperidol and raclopride caused no significant change in the parameters of ^{3}H-TPC and ^{3}H-SKF binding in the rat brain. Only in the case of haloperidol was a decrease observed in the affinity of the binding site (sigma receptors). After long-term administration of both neuroleptics, however, an increase was observed in the number of ^{3}H-SKF binding sites (Table 1); this increase, moreover, was more marked 2 h after the last injection of the neuroleptics. The increase in the number of sigma binding sites 48 h after withdrawal of the neuroleptics was not statistically significant. In experiments carried out 2 h after withdrawal of haloperidol (as in the acute experiments) a decrease was observed in the affinity of the sigma binding sites. In all probability this was due to the presence of haloperidol in the brain membranes even after triple processing of the brain tissue. The density of the phencyclidine binding sites also was increased under the influence of long-term administration of haloperidol and raclopride (Table 1), but these changes were not statistically significant. Behavioral experiments conducted with ketamine demonstrated reduced activity of ketamine after cessation of long-term administration of both haloperidol and raclopride (Table 2). Ketamine ataxia and the stimulating action of ketamine on motor activity of the rats were weakened. Apomorphine stereotypy and the stimulating effect of apomorphine on the motor activity of the rats were increased after long-term administration of haloperidol, but not of raclopride (Table 3).

The results are evidence that adaptive changes take place on sigma and phencyclidine receptors under the influence of long-term administration of haloperidol and raclopride. The density of the sigma receptors immediately after discontinuation of the neuroleptics increased. The number of phencyclidine receptors in the present experiments was not increased quite so substantially as was found in the experiments of nyrd and co-workers [2]. Despite an increase in the number of sigma and

TABLE 3. Effect of 15-Day Course of Haloperidol (0.5 mg/kg) and Raclopride (1 mg/kg) on Behavioral Effects of Apomorphine (0.15 mg/kg) in Rats ($M \pm m$)

Experimental conditions	Intensity of stereotypy, points	Orienting-investigative activity during 3 min		
		number of impulses	number of rearings	number of hole sniffings
PS + PS	—	16±1,8	1,2±0,3	2,0±0,5
Haloperidol + PS	—	16±2,0	1,5±0,4	2,5±0,5
Raclopride + PS	—	22±2,5	0,8±0,4	3,0±0,5
PS + apomorphine	2,1±0,18	26±3,8	2,1±0,9	2,4±0,8
Haloperidol + apomorphine	2,9±0,20**	32±4,0*	1,0±0,5	3,0±1,0
Raclopride + apomorphine	2,1±0,25	25±3,2	0,9±0,5	1,4±07

Legend. *$p < 0.05$ (Mann–Whitney U test compared with haloperidol + PS group).

phencyclidine receptors, we did not observe increased sensitivity of the animals to ketamine, a phencyclidine receptor agonist. On the contrary, its action was weakened after long-term administration of haloperidol and raclopride. This weakening of the sensitivity of the animals to ketamine was not attributable to any change in sensitivity of the postsynaptic dopamine receptors. This conclusion was supported by the fact that long-term administration of only haloperidol, and not raclopride, caused increased sensitivity of postsynaptic dopamine receptors. Considering the close functional connection between sigma and phencyclidine receptors, on the one hand, and presynaptic dopaminergic mechanisms [1, 6, 9, 14], on the other hand, it can be tentatively suggested that the development of a depolarization blockade of dopamine neurons under the influence of long-term administration of neuroleptics [3] causes animals to become hyposensitivity to ketamine after long-term administration of haloperidol and raclopride. Dopamine neurons, inactivated by long-term administration of neuroleptics, are evidently unable to realize the effects of stimulation of sigma and phencyclidine receptors.

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