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STIMULATION OF [^{3}H]SPIROPERIDOL BINDING AFTER PROLONGED NEUROLEPTIC THERAPY BY THE CHOLECYSTOKININ OCTAPEPTIDE ANALOG CERULEIN

É. É. Vasar, A. M. Nurk,
M. O. Maimets, and L. Kh. Allikmets

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There is evidence that peptides of the cholecystokinin series, in low concentrations, modulate interaction of spiroperidol with dopamine$_2$- and serotonin$_2$-receptors [2]. Studies of behaviorial reactions have shown that cholecystokinin and its analogs, given by intracerebral and peripheral routes, have an action similar to that of both neuroleptics [4, 15] and apomorphine [1]. Parallel with behavioral changes, cholecystokinin and its analogs also cause depression of dopamine and serotonin metabolism in structures of the forebrain [1, 7]. Evidence has recently been obtained that cholecystokinin and its analog cerulein have a marked antipsychotic action on patients with schizophrenia who are resistant to neuroleptics [10, 11].

These facts explain the practical interest of a study of the effect of cerulein, a high-affinity analog of the octapeptide cholecystokinin [15], on binding of [^{3}H]spiroperidol *in vivo*. Considering the apormorphine-like action of cerulein, this biochemical analysis was undertaken in the form of a comparative study with N-propylnorapomorphine (NPA), a high-affinity analogy of apomorphine.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 20-25 g. The animals were given haloperidol (0.25 mg/kg, Gedeon Richter, Hungary), pirenperone (0.25 mg/kg, Janssen Pharmaceutica, Belgium), or physiological saline twice a day for 2 weeks. Binding experiments *in vivo* were carried out 72 h after the last dose of this prolonged course: Six animals from each group (physiological saline, haloperidol, and pirenperone) were given a subcutaneous injection of [^{3}H]spiroperidol alone, in a dose of 5 µg/kg (specific radioactivity 17 Ci/mmole, Amersham Corporation, England), and they were decapitated 20 min later. The remaining mice of the same groups (six animals in each group) were treated with displacing agents before receiving the labeled spiroperidol. Haloperidol was injected intraperitioneally in a dose of 2.5 mg/kg 40 min before injection of the labeled ligand, and cerulein, in a dose of 0.4 mg/kg (subcutaneously, Farmitalia, Italy), and MPA in doses of 5 and 50 µg/kg (Research Biochemicals Inc. USA) were injected 15 min before [^{3}H]spiroperidol. After decapitation of the animals the brain was quickly removed on ice and deep forebrain structures (limbic system and striatum) and the frontal cortex were dissected. The isolated structures were homogenized in 25 volumes of Tris-HCl buffer (50 mM, pH 7.4, 20°C). The samples were then centrifuged at 9000 rpm for 10 min. The supernatant was decanted and the residue carefully washed several times with cold Tris-HCl buffer. The radioactivity of the samples (five parallel tests) was determined in Bray's scintillator on an Ultro-Beta 1210 β-counter (LKB, Sweden). The experiments were repeated three times.

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TABLE 1. Effect of Haloperidol (2.5 mg/kg), NPA (5 and 5 μg/kg), and Cerulein (0.4 mg/kg) on Binding of [^{3}H]Spiroperidol (5 μg/kg) in Experiments *in vivo* after Prolonged Administration of Haloperidol (0.25 mg/kg) and Pirenperone (0.25 mg/kg)

Substance	Physiological saline		Haloperidol		Pirenperone	
	deep brain structures	frontal cortex	deep brain structures	frontal cortex	deep brain structures	frontal cortex
Haloperidol, 2.5 mg/kg	9 050±840	11 100±860	9 700±780	11 200±740	9 400±850	5 500±890*
NPA:						
50 μg/kg	8 600±760	14 400±890	12 400±800*	11 800±1 020*	12 300±790*	10 750±1 010
5 μg/kg	7 300±670	12 250±1 010	1 100±400†	3 750±520†	2 700±600*	3 350±650†
Cerulein, 0.4 mg/kg	1 150±300	7 300±610	+3 600±400*	+100±250*	+1 100±480*	+2 450±400†

Legend. Mean values (number of counts per gram tissue) between groups receiving [^{3}H]spiroperidol alone or [^{3}H]spiroperidol after the displacing agents are given. +) Stimulating effect on binding of [^{3}H]spiroperidol. *$P < 0.05$, †$P < 0.02$ compared with control (physiological saline).

EXPERIMENTAL RESULTS

The displacing action of a high dose of haloperidol (2.5 mg/kg) was substantially unchanged after prolonged administration of haloperidol (0.25 mg/kg) and pirenperone (0.25 mg/kg) compared with values observed in the group of animals receiving physiological saline (Table 1). Only after frequent injections of the serotonin$_2$-antagonist pirenperone was its displacing effect in the frontal cortex reduced. Unlike haloperidol, the effect of NPA was appreciably modified after prolonged administration of haloperidol and pirenperone. In a dose of 50 μg/kg NPA displaced [^{3}H]spiroperidol much more strongly in the deep brain structures after prolonged injections of both haloperidol and pirenperone. The displacing effect of NPA in the frontal cortex in this dose was actually weakened a little after prolonged injection of the neuroleptics. This difference in the action of NPA in two regions of the brain can evidently by explained by its weaker agonistic action on serotonin$_2$-receptors than on dopamine receptors. Only high doses of apomorphine, it has been shown, can induce behavioral effects similar to those of the hallucinogenic serotoninomimetics [14] and high concentrations of apormorphine can displace [^{3}H]ketanserine, an antagonist of serotonin$_2$-receptors, from binding sites in the prefrontal cortex [8]. However, tolerance to the displacing action of a small dose of NPA (5 μg/kg) developed after prolonged administration of haloperidol and pirenperone (Table 1). This fact is evidence that [^{3}H]spiroperidol interacts more strongly with dopamine and serotonin recpetors on particular binding sites after prolonged administration of neuroleptics. The results of this investigation agree in many respects with data in the literature [5], according to which neuroleptics and apormorphine interact unequally with dopamine$_2$-receptors. Interaction of apomorphine with [^{3}H]spiroperidol on dopamine$_2$-receptors has been shown to take place through low- and high-affinity binding sites, whereas neuroleptics have only high-affinity binding sites on these receptors [5]. Dissociation constants of these two binding sties for apomorphine were found to differ by about 10 times. Considering the different action of different doses of NPA after prolonged administration of haloperidol and pirenperone, it can be postulated that the affinity of the neuroleptics for these two binding sites for apomorphine is modified unequally. Affinity of [^{3}H]spiroperidol for low-affinity binding sites for apomorphine is evidently reduced, whereas binding of [^{3}H]spiroperidol on high-affinity binding sites is significantly increased. Probably the change in sensitivity of the low-affinity binding sites reflects the development of hypersensitivity to dopaminomimetics and serotoninomimetics, and weakening of the different effects of neuroleptics, whereas the increase in affinity of [^{3}H]spiroperidol for high-affinity binding sites for apomorphine is in all probability connected with the development of an antipsychotic action in the course of prolonged administration of the neuroleptics.

After prolonged administration of the neuroleptics only the action of NPA and also that of cerulein, a high-affinity analog of the octapeptide cholecystokinin [15], was unchanged. The view is held that some of the effects of cholecystokinin and its analogs, if administered peripherally, are realized through afferent mechanisms of the vagus nerve [9, 13]. However, our investigations indicate that cerulein penetrated into the brain and displaced [^{3}H]spiroperidol from its binding sites in the control animals (Table 1). After administration of haloperiodol and pirenperone for 2 weeks, however, cerulein began to have the opposite action. Cerulein did not displace, but stimulated binding of spiroperidol in both regions of the forebrain studied. After prolonged administration of pirenperone, and antagonist of serotonin$_2$-

receptors the stimulating action of cerulein, moreover, was stronger in the frontal cortex (Table 1), whereas after haloperidol, which interacts mainly with dopamine receptors, this action of cerulein was stronger in deep brain structures. These data are in agreement with the results of investigations [12] which showed that binding sites for neuroleptics in the frontal cortex are mainly associated with serotonin$_2$-receptors, whereas in deep brain structures interaction is mainly with dopamine receptors. Considering that prolonged administration of haloperidol (2-3 mg/kg) almost doubles the density of cholecystokinin receptors [3], it can be postulated that the increase in [^{3}H]spiroperidol binding on high-affinity binding sites for apomorphine is in fact due to the stronger action of the endogenous cholecystokinin octapeptide after prolonged administration of neuroleptics.

The results thus indicate that, after prolonged administration of neuroleptics, their interaction with high-affinity binding sites for apomorphine on dopamine$_2$- and serotonin$_2$-receptors is intensified. This mechanism evidently lies at the basis of the antipsychotic action of neuroleptics. However, as experimental [6] and clinical [10] studies have shown, this action of neuroleptics is realized only in the presence of adequate concentrations of cholecystokinin octapeptide. In schizophrenic patients resistant to neuroleptics, a low cholecystokinin concentration is found in the limbic structures after death.

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