EFFECT OF CARBIDINE* ON DOPAMINE AND SEROTONIN RECEPTORS OF THE CNS

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By its clinical spectrum of action and some pharmacologic tests characterizing neuroleptic activity, carbidine differs from other neuroleptics which are phenothiazine or butyrophenone derivatives. Carbidine is most effective when depressively delusional symptoms predominate in different forms of schizophrenia [1]. In experiments on animals carbidine, unlike other neuroleptics, potentiated amphetamine stereotypy [2]. The mechanism of action of carbidine has received little study.

A characteristic feature of most neuroleptics is their inhibitory influence on the dopaminergic and serotoninergic systems of the brain, and accordingly, in the investigation described below, the action of carbidine on dopamine and serotonin receptors of the CNS was studied. The effect of carbidine was compared with that of other known neuroleptics: the butyrophenone derivative haloperidol, and an atypical neuroleptic, a benzamide derivative, sulpiride.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 18-24 g and on male Wister rats weighing 200-250 g.

The effect of the neuroleptics on stereotypy induced by injection of apomorphine (1 mg/kg body weight, subcutaneously), a stimulator of dopamine receptors, was studied in behavioral experiments on rats. The intensity of stereotypy was estimated in points [5] 30 min after injection of apomorphine. Its duration also was measured in minutes. To assess the effect of the neuroleptics on serotoninergic processes, the head shaking syndrome in mice induced by intraperitoneal injection of the serotonin precursor 5-hydroxytryptophan (5-HTP), in a dose of 200 mg/kg, was used. Shaking was counted visually in the course of 1 min, 20 min after injection of 5-HTP [3]. The neuroleptics were injected intraperitoneally 2 h before the experiment. Binding of [^{3}H]-spiperone with membranes of the corpus striatum and cerebral cortex of the rat in vitro was carried out by the method in [10]. [^{3}H] Spiperone (20 Ci/mmole, from Amersham Corporation, England), in a concentration of 0.5 nM, was incubated with a twice washed membrane suspension in the presence of different concentrations of the neuroleptic, in a total volume of 1 ml in the course of 30 min at 25°C. The bound ligand was separated by filtration through CF/B filters (Whatman, England). Radioactivity was measured by means of the LS-7500 liquid scintillation counter (Beckman, USA), with counting efficiency of 37-39%. Specific binding was determined as the difference between binding of [^{3}H]spiperone in the absence and in the presence of 1 μM of unlabeled spiperone. Binding of [^{3}H]spiperone in vivo was carried out in experiments on mice by the method in [7], in the writers' modification. In these experiments [^{3}H]spiperone with specific radioactivity of 17 Ci/mmole was used and was injected intraperitoneally in a dose of 6 μg/kg. The animals were decapitated 2 h after the injection, the corpus striatum and frontal cortex were isolated in the cold, dissolved in BTS-4 tissue solubilizer (Beckman), and introduced into flasks for scintillation counting.

*3,6-Dimethyl-1,2,3,4,4a,9a-hexahydro-γ-carboline di-HCl.

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TABLE 1. Effect of Neuroleptics on Binding of [^{3}H] Spiperone in Vitro (rats) and in Vivo (mice) and on Dopamine-Sensitive Adenylate Cyclase Activity in Rat Corpus Striatum

Substance	Binding in vitro (IC_{50}), nM		Binding in vivo (ED_{50}), mg/kg		Dopamine-sensitive adenylate cyclase (IC_{50}), μm
	corpus striatum	cortex	corpus striatum	cortex	corpus striatum
Carbidine	1400±220	28,0±5,0	18,4±2,6	0,8±0,3	100
Haloperidol	5,0±2,0	12,0±2,0	0,7±0,3	1,0±0,4	8,5±2,3
Sulpiride	750±120	>1000	120±32	>150	>100

Legend. IC_{50}) Concentration inhibiting binding or stimulation of adenylate cyclase activity in the presence of 50 μM dopamine by 50%; ED_{50}) dose inhibiting binding in vivo by 50%. In binding experiments in vitro and to determine adenylate cyclase activity results of three experiments in triplicate are shown. For binding experiments in vivo results of four to five experiments are shown.

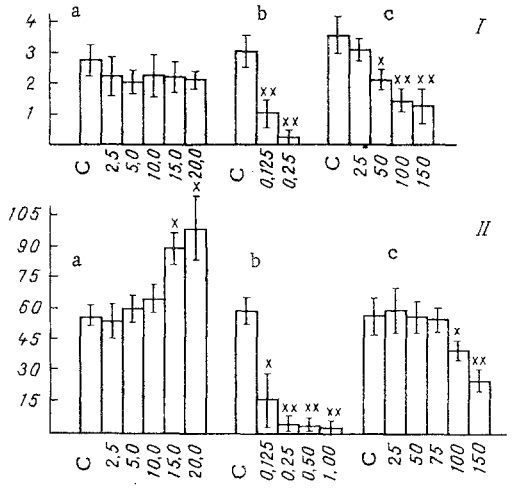

Fig. 1. Effect of neuroleptics on intensity and duration of apomorphine (1.0 mg/kg) stereotypy in rats. Abscissa, dose of neuroleptics (in mg/kg); ordinate: I) intensity of stereotypy (in points); II) duration of stereotypy (in min). a) Carbidine, b) haloperidol, c) sulpiride. C) Control. $^{\times}P < 0.05$, $^{\times\times}P < 0.01$.

Activity of dopamine-sensitive adenylate cyclase in homogenates of the rat corpus striatum was determined by the method in [8]. The cAMP content was determined by the method in [6], using standard kits from Amersham Corporation.

Protein was determined by the method in [9]. The numerical results were subjected to statistical analysis by the Mann–Whitney U and Student's t tests. Inhibiting concentrations (IC_{50}) and doses (ED_{50}) were calculated by logit-probit analysis. The following substances were used in the experiments: haloperidol (from Gedeon Richter, Hungary), sulpiride (from De Lagrange, France), apomorphine hydrochloride and 5-HTP (from Sigma, USA).

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EXPERIMENTAL RESULTS

Unlike haloperidol, carbidine did not affect the intensity of apomorphine stereotypy and actually increased its duration (Fig. 1). Sulpiride inhibited stereotypy only in relatively high doses (100-150 mg/kg). Carbidine, like haloperidol, inhibited the head-shaking syndrome induced by injection of 5-HTP over the whole range of

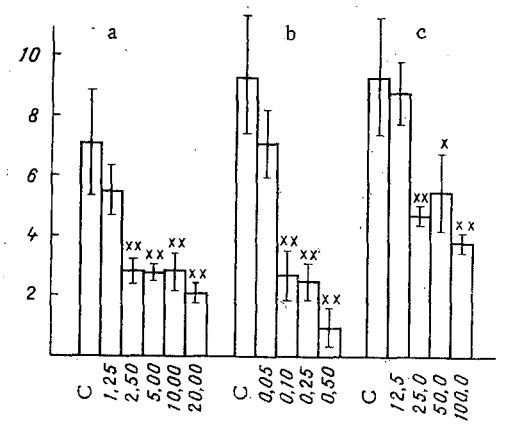

Fig. 2. Effect of neuroleptics on head-shaking syndrome induced in mice by injection of 5-HTP (200 mg/kg). Ordinate, number of shakings. Remainder of legend as to Fig. 1.

doses tested (1.25-20 mg/kg) (Fig. 2). The inhibitory action of sulpiride was weaker and was exhibited starting from a dose of 25 mg/kg. Definite differences in the action of carbidine on dopamine and serotonin receptors were observed in experiments with radioligand binding. In experiments in vitro carbidine displaced [^{3}H]spiperone in the corpus striatum only in micromolar concentrations, whereas in the cerebral cortex marked displacement of the radioligand was observed under the influence of carbidine in concentrations as low as nanomolar (Table 1).

A similar pattern was observed in the binding experiments in vitro, in which carbidine also displaced [^{3}H]-spiperone only in the cerebral cortex. Haloperidol displaced [^{3}H]spiperone from binding sites in the corpus striatum and cortex by about equal degrees, whereas sulpiride exhibited low affinity for binding sites of [^{3}H]-spiperone in the corpus striatum and had virtually no effect on binding in the cortex (Table 1).

Unlike haloperidol, carbidine had little effect on dopamine-sensitive adenylate cyclase activity in homogenates of the rat corpus striatum (Table 1).

Investigations in recent years have shown that [^{3}H]spiperone is bound in the corpus striatum mainly with dopamine (D-2) receptors, but in the cerebral cortex mainly with serotonin (S-2) receptors [11].

On this basis it can be postulated that carbidine, unlike the classical neuroleptics, does not affect post-synaptic D-2 receptors or D-1 receptors bound with adenylate cyclase. The results of the present experiments show that its action is evidently realized by selective inhibition of cortical serotonin (S-2) receptors. The atypical nature of carbidine in pharmacologic tests and clinical practice may perhaps be explained by its serotonin-blocking action. However, the possibility cannot be ruled out that carbidine may have some influence on presynaptic dopamine receptors. Evidence in support of the possibility of such an action is given by results obtained by other workers [4], who found a very small increase in the concentration of the dopamine metabolites, homovanillic acid, in the rat corpus striatum after injection of carbidine.

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