ROLE OF DOPAMINE RECEPTOR AND MUSCARINIC ACETYLCHOLINE RECEPTOR BLOCKADE IN THE ANTIAPOMORPHINE ACTION OF NEUROLEPTICS

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A particular feature of neuroleptics in experimental pharmacology is their ability to exhibit antogonism toward apomorphine stereotypy. It is considered that this behavioral effect of neuroleptics is due to their dopamine-blocking activity [3, 9]. The strength of the antiapomorphine action differs considerably in a series of neuroleptics. Some workers consider that a weak experimental antiapomorphine and cataleptogenic action and also a low extrapyramidal potential under clinical conditions, characteristic of certain neuroleptics, may be due to the fact that these substances have a blocking action on muscarinic acetylcholine receptors [2, 6, 11]. On the other hand, as experimental data show, additional administration of muscarinic acetylcholine receptor blockers has little effect on the strength of the antiapomorphine action of typical neuroleptics [5]. On this basis it has been concluded that the muscarinic acetylcholine receptor blocking action is of no essential importance in the realization of the behavioral effects of the neuroleptics. It was therefore important to analyze the role of dopamine and muscarinic acetylcholine receptor blocking components in the antistereotypic action of neuroleptics with different chemical structure.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats of both sexes weighing 200-250 g and noninbred male albino mice weighing 18-24 g. Methods of radioligand binding *in vitro* and behavioral tests of antagonism with apormorphine stereotypy in rats and arecoline tremor in mice were chosen in order to study blocking effects on dopamine and muscarinic acetylcholine receptors.

Apomorphine was injected subcutaneously in a dose of 0.5 mg/kg 60 min after injection of the neuroleptics, and the presence of stereotyped investigative behavior was determined 15 min later. Arecoline (25 mg/kg) was injected 60 min after the neuroleptics and the presence of tremor in the mice was determined 5 min later.

To determine dopamine-blocking activity *in vitro*, binding of ^{3}H-spiperone (specific activity 19-21 Ci/mmole, Amersham International, England) with membranes of the rat striatum, measured by the method in [10], was used. The incubation medium, with a volume of 1 ml, consisted of 100 µl of a solution of ^{3}H-spiperone (0.25 nM), 100 µl of solutions of the substances, and 800 µl of membrane suspension. The tubes were incubated for 60 min at 22°C and the contents filtered through GF/B glass-fiber filters (Whatman, England). Nonspecific binding was determined in the presence of 1 µM (+)-butaclamol.

To study the blocking action of the substances on muscarinic acetylcholine receptors binding of ^{3}H-quinuclidinyl benzylate (^{3}H-QNB, specific activity 39 Ci/mmole, Amersham) with brain membranes was chosen. Binding was carried out by the method in [12] with the authors' modification. After decapitation of the rat the brain was divided in the cold into two parts — the striatum and the frontal and parietal cortex. The parts of the brain were homogenized in 0.32 M sucrose in a glass homogenizer with Teflon pestle and centrifuged at 1000g for 10 min. The supernatant was diluted with ice-cold 0.1 M Na-K-phosphate buffer (pH 7.3)

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TABLE 1. Dopamine-Blocking Activity of Neuroleptics in ^{3}H-Spiperone Binding Test and Antagonism to Apomorphine

Substance	No. of experiments	Binding of ^{3}H-spiperone (IC_{50}), nM	Antiapomorphine action (ED_{50}), mg/kg
Dibenzoxazepines:			
clozapine	3	270,5±47,0	30,5
Phenothiazines:			
Chlorpromazine	3	140,0±20,0	4,5
Fluphenazine	3	75,0±14,1	0,31
γ-Carbolines:carbidine	4	1500,0±320	30,0
Butyrophenones:			
Spiperone	3	1,3±0,2	0,185
Haloperidol	3	5,0±2,0	0,325
Pipamperone	3	1250,0±112	62,5
Benzamides:			
(+)-Sulpiride	2	>5000	>100
(−)-Sulpiride	3	820,0±240	81,0
Thioxanthenes:			
cis-Flupenthixol	2	190	—
trans-Flupenthixol	2	3300	—
Unclassified:			
(+)-Butaclamol	2	3,6	0,6
(−)-Butaclamol	2	>1000	>10

TABLE 2. Muscarinic Acetylcholine Receptor Blocking Activity of Neuroleptics in ^{3}H-Quinuclidinyl Benzylate (0.2 nM) Binding Test with Rat Brain Membranes and Antagonism with Arecoline (25 mg/kg)

Substance	No. of expts.	Binding of ^{3}H-QNB (IC_{50}), μM		Antagonism with arecoline tremor (ED_{50}), mg/kg
		cortex	striatum	
Dibenzoxazepines:				
clozapine	3	0,3±0,1	0,6±0,3	17,4
Phenothiazines:				
Chlorpromazine	3	2,2±0,3	2,2±0,6	35,7
Fluphenazine	3	19,2±4,3	24,5±2,5	40,0
γ-Carbolines:				
carbidine	4	33,5±12,8	60,2±14,8	>20
Butyrophenones:				
Haloperidol	3	20,0±3,3	20,9±5,2	>20
Pipamperone	3	20,3±6,2	32,7±2,9	100
Spiperone	3	22,4±12,3	28,2±3,4	>10
Benzamides:				
(+)-Sulpiride	3	96,5±3,95	>100	>100
(−)-Sulpiride	2	>100	>100	>100
Thioxanthenes:				
cis-Flupenthixol	4	5,07±0,49	4,37±0,6	11,7
trans-Flupenthixol	4	8,09±1,09	6,82±0,8	>40
Pyrimidines:				
Pirenperone	3	1,0±0,3	1,9±0,5	20,4
Unclassified:				
(+)-Butaclamol	3	27,1±1,4	29,5	>40
(−)-Butaclamol	3	15,6±4,5	24,7	>40

to 50 volumes (w/v) and centrifuged at 3000g for 20 min. The residue was resuspended in the same buffer and recentrifuged. The final residue was homogenized in 100 volumes of 0.05 M phosphate buffer. Binding was carried out in glass test tubes to which 100 μl of ^{3}H-QNB (0.2 nM), 100 μl of the solutions of the substances in different concentrations, and 800 μl of membrane suspension were added. The samples were incubated at 25°C for 60 min and the contents of the tubes filtered through GF/B filters (Whatman). Nonspecific binding was determined in the presence of 1 μM atropine.

Radioactivity on the filters was counted on an LS-7500 liquid scintillation counter (Beckman, USA).

The following substances were generously supplied for the experiments: carbidine (Professor K. S. Raevskii), haloperidol, pipamerone, dipiperone, pirenperone (Janssen Farmaceutica, Belgium), (+)- and (–)-butaclamol (Ayerst Laboratories, Canada), cis- and trans-flupenthixol (Lundbeck Co., Denmark), (+)- and (–)-sulpiride (Ravizza, Italy), clozapine (Sandoz, Switzerland), for which the authors are grateful.

IC_{50} (the concentration inhibiting binding by 50%) was calculated by probit analysis. Coefficients of correlation were found by linear correlation analysis.

EXPERIMENTAL RESULTS

Data on the dopamine-blocking activity of the neuroleptics are given in Table 1. The strongest dopamine-blocking activity with respect to inhibition of ^{3}H-spiperone binding was exhibited by butyrophenone derivatives (spiperone and haloperidol), and also by (+)-butaclamol, and weaker activity was shown by the phenothiazines. Carbidine and (–)-sulpiride inhibited ^{3}H-spiperone binding only in micromolar concentrations. These data correlated closely with the ability of the neuroleptics to inhibit apomorphine sterotypy. The coefficient of correlation was 0.76 ($P < 0.05$). Clozapine which, despite its marked dopamine-blocking action in radioligand binding experiments, inhibited stereotypic investigative behavior, but without affecting apomorphine-induced nibbling, was an exception (Table 1).

The results of the study of the acetylcholine-blocking activity of the neuroleptics are given in Table 2. The neuroleptics inhibited ^{3}H-QNB binding only in micromolar concentrations. Clozapine and chlorpromazine had the greatest ability to inhibit ^{3}H-QNB binding (Table 2), in agreement with data given by other workers [2, 3, 8]. The butyrophenones were weaker and their IC_{50} was 60-80 times higher than that for clozapine. Carbidine had very weak acetylcholine-blocking activity (Table 2), although we know that it rarely induces extrapyramidal disorders and has no antistereotypic action [1]. The relatively strong acetylcholine-blocking action ($IC_{50} = 1.0 \pm 0.3$ μM in the cortex) of the new pyrimidine derivative pirenperone (Table 2) was established. Recent investigations have shown that pirenperone has a marked serotonin-blocking action.

Among the enantiomers of the neuroleptics, the isomers of flupenthixol differed in their effects on ^{3}H-QNB binding. Its cis-enantiomer displaced ^{3}H-QNB from binding sites rather more strongly than the trans-form. These findings contradict those of previous investigations [4] in which, during a study of chlorprothixene isomers, the trans-form was found to have greater activity. It can be postulated that the stronger acetylcholine-blocking action of the pharmacologically less active enantiomers is not a common feature of enantiomers of other neuroleptics. This conclusion is confirmed by the fact that in radioligand binding experiments the inactive (–)-butaclamol was more active than the (+)-form, and the two stereoisomers of sulpiride were virtually inactive (Table 2).

The data obtained by radioligand binding of ^{3}H-QNB correlated closely with data on antagonism with arecoline tremor in mice. For instance, the coefficient of correlation between IC_{50} of the neuroleptics with respect to displacement of ^{3}H-QNB from the binding sites in the striatum and ED_{50} for the antiarecoline action was 0.77 ($P < 0.05$).

Analysis of correlation between the antiapomorphine action of the neurolptics and their acetylcholine-blocking activity both in the radioligand binding experiments and in the arecoline tremor test showed that correlation was absent. Considering the concentrations and doses of neuroleptics blocking muscarinic acetylcholine receptors, and also the absence of correlation between the behavioral effects of the substances and their blocking activity on muscarinic acetylcholine receptors, it can be concluded that the behavioral effects of the neuroleptics depend only a little on the intensity of their muscarinic acetylcholine receptor blocking action; the decisive feature in this effect is the ability of the neuroleptics to block dopamine receptors of the CNS. The exception among the neuroleptics studied is clozapine, whose powerful acetylcholine-blocking action may be important in the absence of an antagonizing effect on apomorphine stereotypy.

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EFFECT OF THE HEXAPEPTIDE DALARGIN ON ORNITHINE DECARBOXYLASE ACTIVITY IN THE DUODENAL MUCOSA OF RATS WITH EXPERIMENTAL DUODENAL ULCER

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Previously the authors showed that several endogenous opioid peptides and their synthetic analogs have marked antiulcerative activity in rats with an experimental model of cysteamine-induced duodenal ulcer [1, 2].

The substance with the strongest antiulcerative potential was found to be a hexapeptide with the structure Tyr-D-Ala-Gly-Phe-Leu-Arg, which was synthesized in the Laboratory of Peptide Synthesis, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Director M. I. Titov), and which was called dalargin. It differs from the N-terminal fragment of dinorphine in replacement of Gly by D-Ala in position 2. Dalargin has now been successfully used for the treatment of duodenal ulcer in man [3], but the precise mechanisms of its antiulcerative action have not been established.

The aim of this investigation was to study the effect of dalargin on ornithine decarboxylase (ODC; ED 4.1.1.17) in homogenates of the duodenal ulcer from rats with experimental duodenal ulcer induced by cysteamine.

EXPERIMENTAL METHOD

Experiments were carried out on 150 male Wistar rats weighing 200-250 g. The animals were given a single subcutaneous injection of cysteamine hydrochloride (Fluka, Switzerland) in a dose of 350 mg/kg. Immediately thereafter and 12 h later the rats received an injection of dalargin in doses of 12.5 (group 1) and 5000 μg/kg (group 2) or of physiological saline (group 3), also subcutaneously, 12 h later. Group 4 consisted of animals which received a subcutaneous injection of naloxone in a dose of 1 mg/kg simultaneously with dalargin in a dose of 12.5 μg/kg. Rats of group 5 received an injection of physiological saline only. Some rats were decapitated 24 h after the beginning of the experiment, the duodenum was removed, and by means of a binocular loupe, the state of the mucosa was assessed. The parameters of ulcer formation were the ulcer index (UI), the frequency of involvement (FI), and the severity of the lesion (SL), which were determined by the method described previously [2]. Activity of ODC was then determined in homogenates prepared from scrapings of the mucosa [11]. Some animals from each group were decapitated 6 h after the beginning of the experiment and their duodenum was taken for determination of ODC activity in the mucosa before the development of ulcers. ODC activity was determined by the method described pre-

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