EFFECT OF NEUROLEPTICS ON ACETYLCHOLINE METABOLISM IN THE BASAL GANGLIA

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Neuroleptics (haloperidol, clozapine, pimozide, chlorpromazine) caused a decrease in the level of the free (functionally active) form of acetylcholine (ACh) and also, to some extent, of the bound form of ACh, inconsistent changes in the content of loosely bound (vesicular) form of ACh, and a weak effect on choline acetyltransferase activity in the basal ganglia of rats 5-30 min after injection. By contrast with their inhibitory action on acetylcholinesterase (AChE) activity, in experiments in vitro most neuroleptics (except clozapine) increased ACh activity in vivo. These results show that neuroleptics activate ACh metabolism and evidently stimulate cholinergic structures in the basal ganglia; AChE activity can be used as a criterion of this stimulating action of the neuroleptics.

KEY WORDS: acetylcholine; acetylcholinesterase; choline acetyltransferase; basal ganglia; neuroleptics.

The characteristic effect of dopaminergic drugs on acetylcholine (ACh) metabolism in the basal ganglia (BG) has recently been demonstrated and, on this basis, the existence of close functional connections has been postulated between the dopaminergic and cholinergic systems in the corpus striatum, with a predominantly inhibitory character of the influence of dopaminergic structures on cholinergic [1, 3, 5, 9, 14]. The action of neuroleptics widely used in clinical practice [2] is characterized by a decrease in motor activity in animals, usually linked with blocking of dopamine receptors in the brain, above all in BG, and it is accompanied by activation of dopamine metabolism in these structures [4] and also by activation of cholinergic structures [3, 9, 14]. However, no combined neurochemical investigations of ACh metabolism in BG during the action of neuroleptics have so far been undertaken.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-220 g. BG (caudate nucleus, corpus striatum, putamen, globus pallidus) were isolated by the method of Glowinski and Iversen [8]. The level of free, loosely bound, and firmly bound ACh was determined [11]; choline acetyltransferase (ChA) was isolated and purified by Street's method [12]. Activity of the enzyme was estimated from the quantity of ACh (in micrograms) synthesized per gram wet weight of tissue per hour. Acetylcholinesterase (AChE) activity was determined by Ellman's method [7] and expressed in micromoles thiocholine per gram tissue per minute. The ACh concentration was determined by a biological method on the ventricle of the frog's heart isolated by Straub's method [6, 13]. The drugs other than pimozide were injected intraperitoneally in doses giving a marked effect on the dopamine system and producing a behavioral effect: Haloperidol 2 mg/kg, clozapine 10 mg/kg, chlorpromazine 10 mg/kg; pimozide was injected into the lateral ventricles in a dose of 10 μg per rat.

EXPERIMENTAL RESULTS

Haloperidol, a powerful neuroleptic, stimulated the liberation of ACh in BG during the early stages after its injection, with a decrease in the level of the free and bound forms of the mediator (Fig. 1), and also with activation of AChE (Table 1). Meanwhile in experiments in vitro, haloperidol inhibited AChE activity somewhat (Table 2).

Clozapine, 5 min after injection, also activated liberation of ACh (Fig. 1). Unlike the other neuroleptics, in the experiments in vivo clozapine inhibited AChE activity (Table 1), probably on account of its direct effect

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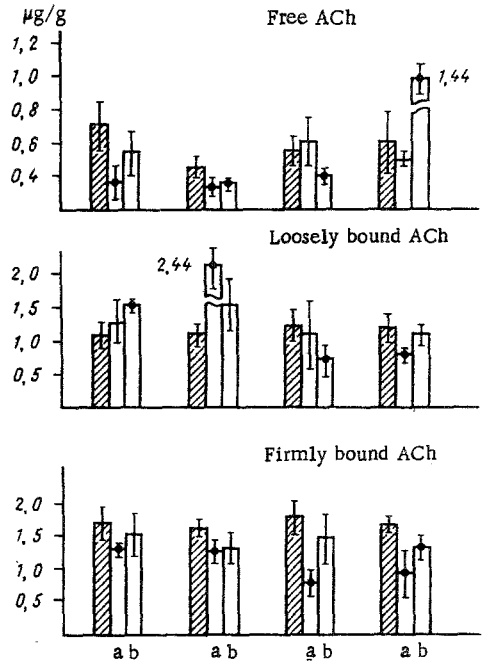

Fig. 1. Changes in ACh concentration (in μg/g wet weight of tissue) by fractions in BG of rats under influence of neuroleptics: 1) haloperidol, after 5 (a) and 30 (b) min; 2) clozapine, after 5 (a) and 30 (b) min; 3) pimozide, after 5 (a) and 30 (b) min; 4) chlorpromazine, after 5 (a) and 90 (b) min. Shaded columns represent control, unshaded columns experiments.

TABLE 1. AChE Activity (in μmoles thiocholine/g tissue/min) in BG of Rats After Administration of Neuroleptics

Drug	Dose	Time, min	Control (n-5)	Experiment (n-5)	P
Haloperidol	2 mg/kg	5	700,0±10,9	740.0±6,3	<0,05
		30	816,0±11,7	1392.0±32,3	<0,001
Clozapine	10 mg/kg	5	826,0±23,9	448.0±36,2	<0,001
		30	804,0±27,3	770.0±33.8	>0,1
Pimozide	10 μg per rat	30	708,0±5,8	1280.0±18.7	>0,001
Chlorpromazine	10 mg/kg	5	730,0±5,5	840.0±28.9	<0,05
		90	730,0±5,5	488.0±5,8	<0,001

TABLE 2. AChE Activity (in μmoles thiocholine/g tissue/min) in Homogenates of BG of Rats After Injection of Neuroleptics (M ± m)

Drug	Control (n-5)	Experiment (n-6) concentration of drugs		
		$1 \cdot 10^{-4}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-6}$
Haloperidol, M	809,0±12,7	816,0±4,0	672.0±11,6*	768,0±26,3*
Clozapine g/ml	818,0±15.9	250,0±6,9*	248,0±8,0*	622,0±25,0*
Pimozide g/ml	818,0±15,9	580,0±32,7*	502.0±5,8*	698,0±10,2*
Chloropromazine, M	720,0±8,3	598,0±36,2*	614.0±28,9*	680,0±21,7

*P < 0.05.

on the enzyme (Table 2). By the 90th minute of action of the drug a phase of ACh accumulation was observed, on account of an increase in the level of both the free and bound forms (Fig. 1).

Pimozide, which blocks predominantly postsynaptic dopaminergic structures, led to a decrease in the levels of both the free and the loosely bound ACh 30 min after its injection (Fig. 1). ACh activity in this case fell from 696.0 ± 10.3 to 598 ± 10.8 units, but AChE activity increased (Table 1), despite some degree of inhibitory action of the drug on activity of the enzyme in vitro (Table 2).

Chlorpromazine, in the early periods after its injection, lowered the level of loosely bound and firmly bound ACh (Fig. 1) and increased AChE activity. After 90 min of action of chlorpromazine a phase of accumulation of mediator in the free form began and was accompanied by depression of AChE activity (Table 1).

All the neuroleptics investigated, in the early stages after injection, thus led to stimulation of liberation of ACh in BG of the rats, and this was accompanied by a fall in the level of the free, physiologically active, form of the mediator and, evidently, induction of increased AChE activity. An increase in AChE activity in BG of rats in the early stages of their action is characteristic of most neuroleptics. The unique action of chlozapine on AChE activity in BG can be explained by the direct and powerful inhibitory effect of the drug on the activity of the enzyme. Meanwhile, all the neuroleptics investigated were able to inactivate AChE by a greater or lesser degree in vitro, in agreement with earlier results [10].

Besides the decrease in the level of free ACh, a decrease in the level of the bound form of the mediator was usually observed under the influence of the neuroleptics also, whereas variations in the content of loosely bound (vesicular) ACh were inconsistent. Neuroleptics (other than pimozide) had no significant effect on ChA activity in BG. As investigations in which haloperidol was given after actinomycin D (15 μg per rat injected into the lateral ventricle 30 min before the neuroleptic) showed, activation of AChE by haloperidol is evidently mixed in character: Both an increase in protein synthesis de novo and allosteric activation of the enzyme are possible.

It can be concluded from these results that measurement of AChE activity is a relatively simple and adequate method of assessing the activity of cholinergic structures in BG during the action of neuroleptics. The combined assessment of changes in the content of individual ACh fractions and activity of the enzymes of synthesis and hydrolysis of the mediator gives evidence of the activating influence of the neuroleptics on ACh metabolism and also, evidently, on the functional activity of cholinergic structures in BG. The results indicate that neuroleptics, even while they differ very considerably in their chemical structure, are effective regulators (activators in the early stages after administration) of ACh metabolism in BG. This action of the neuroleptics is evidently due to their primary blocking effect on central dopaminergic structures.

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