RELATIONSHIP OF BRAIN SYNAPTOSOMAL Na,K-ATPase TO DOPAMINE RECEPTORS

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Dopamine (DA), which activates presynaptic D_2 or D_1-dopamine receptors, in brain slices and synaptosomes modulates (inhibits or facilitates) the secretion of many mediators evoked by electrical stimulation or by K^+ ions: DA, noradrenalin, serotonin, acetylcholine, and GABA [10]. Since exocytosis of mediators by nerve endings depends on the degree of membrane depolarization, and since the level of depolarization, in turn, is largely determined by activity of electrogenic Na,K-ATPase [1], changes evoked in the activity of this enzyme by DA [2, 3, 7] may be essential to the modulation of mediator secretion. In this connection it is important to determine whether DA changes Na,K-ATPase activity through DA-receptors [7] or whether it acts independently on the enzyme.

The aim of this investigation was to study the effect of neuroleptics which are known to be DA antagonists [5, 9] on DA-evoked changes in synaptosomal Na,K-ATPase activity in the rat caudate nucleus.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 220 g. The animals were decapitated, the brain removed, and the caudate nuclei shelled out of both hemispheres. Synaptosomes were obtained by the method in [4], by pooling homogenate of 20 caudate nuclei. The residue of synaptosomes was suspended in 0.32 M sucrose in the presence of 3 mM EDTA, washed, and kept at 0°C for 1-2 days in 10 mM Tris-HCl (pH 7.4).

Na,K-ATPase activity was determined as the difference between total and Mg-ATPase activity. The former was measured in medium containing 120 mM NaCl, 20 mM KCl, 1 mM $MgCl_2$, and 50 mM Tris-HCl. In the series of experiments without Ca^{++} 1 mM EGTA was added to the media, whereas in the other series $CaCl_2$, in a final concentration of 0.25 mM, was added instead of EGTA. Neuroleptics in concentrations of IC_{16} were added to the reaction mixture 5 min before DA (10^{-7}-3×10^{-4} M), and 5 min later, the reaction was started by the addition of ATP-Na_2 (final concentration 1 mM). The inorganic phosphorus concentration (P_i) was determined by the method in [6]. The ATP-Na_2 and Tris-HCl were obtained from Reanal (Hungary), the DA-HCl from Ferak (West Berlin), fluphenazine, chlorpromazine, and trifluoperazine were of USSR origin, the haloperidol was of Hungarian and the thioridazine of Polish origin, and sulpiride was used as an ampul solution of Eglonil (Yugoslavia).

EXPERIMENTAL RESULTS

All the neuroleptics (six substances) used reduced synaptosomal Na,K-ATPase activity. In the absence of Ca^{++} (in the presence of EGTA) the inhibitory action of the neuroleptics on the enzyme was significantly weaker than in the presence of Ca^{++} in a concentration of 0.25 mM (Table 1).

In the absence of Ca^{++} in the incubation medium, DA had a biphasic action on synaptosomal Na,K-ATPase activity (Table 1), for it increased the activity of the enzyme (by 15% at most; $p < 0.5$) in concentrations of 3×10^{-7}-3×10^{-6} M, and reduced (at most by 24-25%; $p < 0.05$) its activity in higher concentrations (3×10^{-5}-3×10^{-4} M). These effects of DA were described by the writers previously [3].

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TABLE 1. Synaptosomal Na,K-ATPase Activity (in μmoles P_i/mg protein/h) of the Rat Caudate Nucleus in the Presence of DA in Various Concentrations and in the Presence and Absence of Neuroleptics in Concentrations of IC_{16} (M ± m, n = 5)

Series of experiments	Neuroleptics	Dose, μM	DA concentration, M								
			0	10^{-7}	$3\cdot10^{-7}$	10^{-6}	$3\cdot10^{-6}$	10^{-5}	$3\cdot10^{-5}$	10^{-4}	$3\cdot10^{-4}$
I	—	—	12,01±0,20	12,02±0,16	12,35±0,07	13,37±0,1	13,82±0,07	12,19±0,2	11,41±0,1	9,91±0,09	9,13±0,05
	Fluphenazine	0,32	—	5,76*	**6,61***	**8,77***	**9,85***	**7,69***	**6,97***	**5,04***	**4,20***
	Chlorpromazine	0,70	—	8,23*	**9,19***	**11,11***	**12,07***	**10,33***	**9,43***	**7,54***	**6,73***
	Trifluoperazine	1,00	—	9,37*	**10,33***	**12,19***	**13,03***	**11,17***	**10,29***	**8,57***	**7,69***
II	—	—	13,56±0,08	13,59±0,06	13,97±0,04	15,05±0,05	15,59±0,06	13,76±0,1	12,88±0,1	11,19±0,1	10,30±0,08
	Haloperidol	0,56	—	8,27*	**9,49***	**11,83***	**12,88***	**10,85***	**9,76***	**7,92***	**6,79***
	Sulpiride	1,6	—	9,89*	**11,12***	**13,22***	**14,24***	**13,75**	**12,87**	**11,21**	**10,31**
	Thioridazine	4,0	—	12,07*	**12,07***	**14,10***	**15,19***	**13,09***	**12,14***	**10,03***	**9,02***
III	—	—	6,70±0,06	5,76±0,08	5,53±0,04	5,06±0,05	4,86±0,05	4,39±0,04	4,15±0,04	3,69±0,04	3,48±0,04
	Fluphenazine	0,025	—	3,28*	3,02*	2,61*	2,41*	2,01*	1,81*	1,4*	1,2**
	Chlorpromazine	0,050	—	3,89*	3,65*	3,22*	3,02*	2,58*	2,38*	1,94*	1,74*
	Trifluoperazine	0,063	—	4,09*	3,89*	3,45*	3,23*	2,81*	2,58*	2,14*	1,94*
	Haloperidol	0,035	—	3,62*	3,42*	2,98*	2,78*	2,35*	2,14*	1,74*	1,54*
	Sulpiride	0,071	—	4,56*	4,29*	3,89*	3,65*	3,25*	3,02*	2,58*	2,35*
	Thioridazine	0,079	—	5,16*	4,96*	4,49*	4,25*	3,85*	3,62*	3,18*	2,95*

Legend. Statistically significant differences from effect of DA in a concentration of 10^{-7} M are printed in bold type. Series of experiments: I and II) in the absence, and III) in the presence of 0.25 mM $CaCl_2$.
*$p \leq 0.05$ compared with control (in absence of neuroleptic).

TABLE 2. Comparison of Different Types of Activity of Neuroleptics

Combination of neuroleptics (in μM) with DA	1. Na,K-ATPase activity (in μmoles P_i/mg protein/h) with DA in concentration of		2. Change in Na,K-ATPase activity (activation), %	3. Constant of inhibition of DA-stimulated adenylate cyclase activity, nM	4. IC_{50} for binding of ^3H-spiperone, nM
	0 μM	3 μM			
DA	12,01±0,2*	13,82±0,07	15	—	—
	13,56± ,08**	15,59±0,06	15	—	—
Fluphenazine (0.32) + DA	10,08*	9,85	—2,3 (1)	4,3 (1)	2—32 (1)
Chlorpromazine (0.70) + DA	10,08*	12,07	19,6 (3)	48,0 (3)	27—171 (4)
Trifluoperazine (1.0) + DA	10,08*	13,03	29 (5)	19,0 (2)	28 (3)
Haloperidol (0.56) + DA	11,40**	12,88	13 (2)	220,0 (5)	4,8—34 (2)
Sulpiride (1.6) + DA	11,40**	14,24	25 (4)	1000 (6)	250—870 (6)
Thioridazine (4.0) + DA	11,40**	15,19	33 (6)	130,0 (4)	40—220 (5)
Rank correlation coefficients with data:					
3			0,28 ($p<0,05$)		
4			0,72 ($p<0,05$)		

Legend. *) Experiments of series II, **) of series II. Neuroleptic, in concentration specified, inhibits Na,K-ATPase by 16%. 3) Values taken from [8], 4) from [9]. Ranking order of neuroleptic activity (from 1 to 6) shown in parentheses.

In the presence of neuroleptics (IC_{16}) and in the absence of Ca^{++} in the medium, the character of the action of DA on the enzyme remained biphasic. The exception was sulpiride, which only activated the enzyme. However, in concentrations in which the neuroleptics themselves inhibit activity of the enzyme by 16%, in the presence of DA they inhibited Na,K-ATPase significantly more strongly. Even with the minimal concentration of DA (10^{-7} M) the neuroleptics depressed activity of the enzyme (thioridazine by 19% and fluphenazine by 52%). This same pattern was observed for the majority of neuroleptics in the presence of high DA concentrations, such as 3×10^{-4} M. In the presence of the majority of neuroleptics the activating effect of DA on Na,K-ATPase also was intensified. Whereas in a concentration of 3×10^{-6} M DA increased activity of the enzyme by 15%, in the presence of chlorpromazine, trifluoperazine, sulpiride, and thioridazine it increased it by 19-33% (Table 2).

Since neuroleptics, being blockers of DA-receptors, do not diminish, but rather potentiate the activating effect of DA on Na,K-ATPase, there is reason to suppose that DA and neuroleptics have different sites of action (fixation), and consequently, DA may have a direct effect on the enzyme, independent of interaction with DA-receptors. Evidence in support of this view is given by the ability of DA to potentiate the inhibitory effect of the neuroleptics on the enzyme and the absence of correlation between the ability of neuroleptics to counteract the activating effect of DA on Na,K-ATPase and their inhibitory effect on DA-stimulated adenylate cyclase, or the degree of opposition to the specific binding of ^3H-spiperone with membrane fragments of the caudate nucleus (Table 2).

In the presence of Ca^{++} in the medium DA had an exclusively inhibitory action on Na,K-ATPase (Table 1), which the writers established previously [3]. Under these conditions, just as in the absence of Ca^{++}, DA potentiates the inhibitory effect of neuroleptics on the enzyme. Meanwhile Ca^{++} ions potentiate the inhibitory effect of DA. For instance, DA in a concentration of 3×10^{-4} M (in the presence of neuroleptics in concentrations blocking the enzyme by 16%), reduced Na,K-ATPase activity in the absence of Ca^{++} in the medium by not more than 27%, but in the presence of 0.25 mM Ca^{++} in the medium, by 37-57% (Table 1). The absence of antagonism between DA and neuroleptics when Ca^{++} was present in the medium may also be evidence that the inhibitory effect of DA on Na,K-ATPase is independent of its action on DA-receptors. Although the possibility that different isoforms of Na,K-ATPase exist and that the methods of regulation of the enzyme are different in pre- and postsynaptic membranes, cannot be ruled out, it is most probable that neuroleptics modify Na,K-ATPase by exerting a membranotropic action, which also indirectly modifies interaction of DA with its receptors.

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DETERMINATION OF ^{3}H-SEROTONIN REUPTAKE IN SYNAPTOSOMES: CORRECT APPRAISAL OF THE CONTRIBUTION OF NONSPECIFIC UPTAKE

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Interest in the study of the mechanisms of active serotonin (5-hydroxytryptamine, 5-HT) reuptake is due primarily to changes in the kinetic characteristics of this process in various diseases [3, 8, 9]. Inhibition of 5-HT transport into the interior of the serotoninergic terminal is regarded as the central mechanism of action of tricyclic (imipramine) and some new heterocyclic (zimelidine) antidepressants. The study of the mechanism of regulation of 5-HT reuptake is also promising in connection with the discovery of endogenous inhibitors of 5-HT reuptake in the rat brain [4] and in human blood plasma [2, 6].

Experimental studies of 5-HT reuptake in different brain regions have been undertaken most frequently on suspensions of synaptosomes. However, the methods used for this purpose have significant differences, which are concerned mainly with assessment of the contribution of nonspecific 5-HT uptake to its total uptake by synaptosomes. Nonspecific 5-HT uptake by synaptosomes is usually determined at a low temperature [10], in the presence of micromolar concentrations of inhibitors of active transport [4].

The aim of this investigation was to study the effect of these conditions on the experimentally determined nonspecific 5-HT uptake, and it showed that these methods give an estimate of the contribution of nonspecific 5-HT uptake to its total transport that is too low. The writers suggest a method based on the sensitivity of active 5-HT transport to Na^+ ions, capable of giving the most accurate assessment of nonspecific 5-HT uptake by synaptosomes, in consequence of which it enables the parameters of serotonin reuptake to be determined for accuracy.

EXPERIMENTAL METHOD

Male Wistar rats weighing 120-160 g were used. A partially purified synaptosomal fraction (P2) was obtained by the method in [11]. To determine the total uptake of ^{3}H-5-HT (14 Ci/mmole, Amersham International, England) aliquots of the synaptosomal suspension were added in a volume of 100 µl to preincubated samples (5 min, 37°C) 0.5 ml in volume, containing different concentrations of ^{3}H-5-HT (from 0.07 to 3 µM) in incubation buffer (buffer A) in the presence of 150 mM NaCl. The composition of buffer A was: 50 mM Tris-HCl, 1.3 µM KH_2PO_4, 2.8 mM $CaCl_2$, 0.6 mM $MgCl_2$, 7.7 mM glucose, and 0.2% EDTA (pH 7.5). The samples were incubated for 30 sec at 37°C with continuous shaking. Nonspecific ^{3}H-5-HT uptake was determined in samples containing different concentrations of this ligand, in incubation buffers of the

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