ACTION OF DOPAMINE AND DOPAMINOMIMETICS ON SYNAPTOSOMAL Na, K-ATPase IN THE CORPUS STRIATUM

I. V. Komissarov, G. I. Zholob, and R. N. Glebov

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The physiological action of dopamine (DA) on neurons is realized with the aid of at least two types of membrane receptors: P_1-DA and P_2-DA [7]. Whereas receptors of the first type are linked with adenylate cyclase, the pathways by which activation of P_2-DA is transformed into a change in ionic permeability of the neuronal membranes are unknown [7, 14]. One of the probable mechanisms whereby catecholamines can change the degree of polarization of the excitable membranes is a change in activity of Na,K-ATPase of the synaptic membranes, mediated through a generalized structural reorganization of the membrane induced by interaction between transmitter and receptor [1, 4].

Information on the effect of DA on Na,K-ATPase activity of nerve tissue membranes is contradictory. These contradictions are due in part to the fact that the effect of DA has been studied on homogenates or on synaptosomes obtained from the cerebral or cerebellar cortex [5, 9, 11-13, 15], the neurons of which have no dopaminergic innervation. However, for brain regions rich in DA-containing terminals and DA-sensitive neurons (the corpus striatum), both an activating [15] and an inhibitory [2, 8] effect of DA has been described on Na,K-ATPase activity of the synaptosomes. In some cases the action of DA was observed to be dependent on Ca^{++} [2, 8, 12, 13], whereas in others no such dependence was observed [11, 15].

This paper describes the results of an investigation of the effect of DA and dopaminomimetics on synaptosomal Na,K-ATPase in the corpus striatum and the role of Ca^{++} and cAMP in this effect of DA.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 180-260 g. After decapitation of the animals the brain was removed, and the caudate nuclei were shelled out of the two hemispheres. Synaptosomes were obtained by the method in [6], and homogenates from 20 to 24 caudate nuclei were pooled. The residue of synpatosomes was suspended in 0.32M sucrose, pH 7.4, and kept at 0° C for 1-3 days. In some cases 1 mM EGTA was added.

ATPase activity was determined by measuring hydrolysis of ATP in medium (1 ml) containing 120-150 μg synpatosomal protein; protein was determined by Lowry's method. Na,K-ATPase activity was measured by two methods, based on the difference between total and Mg-ATPase activities. In the first case the synaptosomes were incubated (10 min, 37°C) in medium containing (in mM): NaCl 120, KCl 20, $MgCl_2$ 0.25-4, Tris-HCl 50, pH 7.4 (total ATPase), and in the same medium containing also 1 mM ouabain (Mg-ATPase activity). In the second case Mg-ATPase activity was determined in medium containing (in mM): NaCl 140, $MgCl_2$ 1, Tris-HCl 50, pH 7.4. In every case the reaction was started by addition of ATP-Na_2 to a final concentration of 1 mM and it was stopped by addition of 1 ml of cold 10% TCA. The inorganic phosphate concentration (P_i) was determined by the method in [10] with certain modifications [3]. DA, dopaminomimetics, cAMP, or $CaCl_2$ was added to the incubation medium 5 min before addition of ATP (final concentrations are given in the text). In both cases, when activity of Na,K-ATPase was determined its value was virtually the same, namely about 70% of the total activity.

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TABLE 1. Changes in Synaptosomal Na,K-ATPase Activity of Corpus Striatum (in per cent of corresponding control, taken as 100) under the Influence of DA and under Different Experimental Conditions (M ± m)

Experimental conditions		Control	DA concentration, M						
			$3 \cdot 10^{-7}$	$3 \cdot 10^{-6}$	$6 \cdot 10^{-6}$	$1 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	$3 \cdot 1^{-4}$	$1 \cdot 10^{-3}$
Native synaptosomes	1	1,80±0,01	106,0±0,8*	118,0±0,5*	—	—	77,0±0,8*	17,0±0,6*	—
Synaptosomes + 1 mM EGTA	1	9,53±0,12	103,0±1,2	115,0±1,0*	—	—	92,0±1,1*	76,0±0,7*	—
	2	11,80±0,15	—	104,0±1,3*	112,0±1,2*	135,0±0,8*	101,0±0,7	84,0±1,0*	79,0±1,0*
Synaptsomes + 1 mM EGTA + 2,5 mM Ca^{++}	1	1,38±0,01	93,0±0,7*	73,0±1,5*	—	—	59,0±0,7*	41,0±0,7	—
	2	1,63±0,02	86,0±1,0*	72,0±0,6*	—	—	56,0±1,2*	42,0±0,7	28,0±0,6*
Synaptsomes + 1 mM EGTA + 50 μM cAMP	1	10,33±0,15	115,0±0,9*	96,0±0,8*	—	—	75,0±1,0*	55,0±0,2	—
	2	15,5±0,14	117,0±1,1*	115,0±1,2*	—	—	97,0±1,6	—	—

Legend. 1) activity determined in absence of ouabain (n = 5), 2) in presence of oubain (n = 10). ATP concentration in samples 1 mM. cAMP in the concentration indicated has no effect by itself on Na,K-ATPase.
*P < 0.05 compared with control. Here and in Table 2, enzyme activity in control given in micromoles P_i/mg protein/h.

TABLE 2. Effect of DA and Dopaminomimetics on Synaptosomal Na,K-ATPase Activity (in per cent of control, taken as 100) in Rat Corpus Striatum (M ± m)

Substance tested	Control	Concentration of substance, M				
		$3 \cdot 10^{-7}$	$3 \cdot 10^{-6}$	$3 \cdot 10^{-5}$	$3 \cdot 10^{-4}$	$1 \cdot 10^{-3}$
DA	9,53±0,12	103,0±1,2	115,0±1,0	92,0±1,1	76,0±0,7	—
Apmorphine	7,9±0,12	102,0±0,7	119,0±1,8	98,0±0,8	87,0±0,8	75,0±0,9
Amantadine	5,52±0,11	102,0±0,6	109,0±0,7	93,0±0,7	79,0±0,8	63,0±0,9
Piribedil	6,4±0,14	102,0±0,5	108,0±0,6	96,0±0,8	83,0±0,9	—

Legend. Enzyme activity tested in absence of ouabain (n = 5), and in the presence of 1 mM Mg-ATP and 1 mM EGTA.

The ATP-Na_2, cAMP, and Tris-HCl used in the experiments were obtained from Reanal (Hungary), DA-HCl from Ferak (Berlin), Piribedil was from Lab. Servier (France), EGTA and ouabain from Sigma (USA), and the apomorphine, amantadine (midantan), and other reagents were of Soviet origin.

EXPERIMENTAL RESULTS

In the absence of EGTA the original Na,K-ATPase activity of the native suspension of the synaptosomes was only 1/5-1/6 of activity determined in the presence of 1 mM EGTA (Table 1). Considering that the suspension of synaptosomes contained Ca^{++} as an impurity, Na,K-ATPase activity was measured in the presence of 1 mM EGTA. Under these conditions activity of the enzyme increased with an increase in substrate concentration (0.25-4.0 mM), to reach a maximum in the presence of 3 mM ATP. With a further increase in ATP concentration, the rate of its hydrolysis declined.

Addition of Ca^{++} (2.5 mM) to medium with 1 mM EDTA, as might be expected, caused a sharp fall in Na,K-ATPase activity (Table 1).

In the experiments with DA and dopaminomimetics, activity was determined without ouabain, and with the presence of ATP in a concentration of 1 mM. DA was found to act on Na,K-ATPase in two distinct phases: it activated the enzyme in low concentrations (micromolar) and inhibited it in high concentrations ($\geqslant$30 μM). The effect of DA on Na,K-ATPase activity in the corpus striatum evidently depends on substrate concentration, for it was observed in [2, 8] that, with an optimal ATP concentration (3 mM), DA had an exclusively inhibitory effect on Na,K-ATPase. The essential fact is that dopaminomimetics apomorphine, amantadine, and piribedil have a biphasic effect on Na,K-ATPase activity, like the action of DA. It will be clear from Table 1 that the activating effect of DA disappeared completely, whereas the inhibitory effect became stronger in the presence of Ca^{++}, and that against the background of cAMP (50 μM), which by itself has no effect in this concentration on synaptosomal Na,K-ATPase in the corpus striatum, the maximum of enzyme activation by DA is shifted (why this is so is

not yet clear) toward lower concentrations of mediator, although under these circumstances the inhibitory effect is preserved. It will be noted that the activating effect of DA is probably functionally important, for it depends on the action of universal intracellular regulators of cell metabolism: cAMP and Ca^{++}.

When the "ouabain" method of determination of Na,K-ATPase activity is used (as it is quite frequently, incidentally, when the action of mediators and other substances is studied), it should be noted that what is in fact studied is the combined action of ouabain and mediators on the enzyme. It was found that all the general principles pointed out above still applied to the action of DA, but in the presence of ouabain the maximum of the activating effect of DA was shifted toward higher concentrations (Table 1). In other words, blocking Na,K-ATPase by ouabain reduces its sensitivity to the activating action of DA on the enzyme. These facts indicate that DA activates Na,K-ATPase by modifying its potassium active center. In addition, these facts are evidence of a change in sensitivity of synaptosomal Na,K-ATPase to ouabain in the presence of DA.

Thus DA and dopaminomimetics have a biphasic effect — activating in low and inhibitory in high ($\geqslant$ 30 μM) concentrations — on synaptosomal Na,K-ATPase in the corpus striatum. It is difficult at present to estimate which of these effects is mediated by which type of DA-receptors. cAMP facilitates the activating effect of DA on Na,K-ATPase, whereas Ca^{++}, on the contrary, prevents the activating effect of DA on the enzyme, which leads to inhibition of the enzyme during the action of DA over a wide range of its concentrations. The effect of cAMP and of Ca^{++} on Na,K-ATPase is perhaps interconnected: for example, by the formation of a complex of Ca^{++} with calmodulin, followed by activation of Ca-dependent phosphodiesterase and a fall in the intracellular cAMP level.

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