Ca^{++}-INDUCED POTENTIATION OF THE INHIBITORY EFFECT OF NEUROLEPTICS ON BRAIN SYMAPTOSOMAL Na,K-ATP-ASE

I. V. Komissarov, R. M. Glebov, and G. I. Zholob UDC 612.822.1.015.1.014.46:615.214.2

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Neuroleptics inhibit Na,K-ATPase in nerve tissues [1-5], skeletal muscle sarcolemma [16], and the kidneys [7]. Neuroleptics which inhibit this enzyme most strongly compete with monovalent cations Na^+ and K^+, activating the enzyme system, whereas those with weaker inhibitory action compete with Na^+ only [1]. Correlation is found between the degree of inhibition of Na,K-ATPase activity by neuroleptics and inhibition of K^+-dependent conformational changes in spin-labeled membrane preparations of the enzyme [7]. The importance of bivalent cations and, in particular, of Ca^{++}, which plays an important role in the regulation of synaptic transmission, in the inhibitory action of neuroleptics has not been specially investigated.

The present investigation showed that Ca^{++} ions potentiate the inhibitory action of neuroleptics on synaptosomal Na,K-ATPase in the caudate nucleus of the rat brain.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 200 g. The animals were decapitated, the brain was removed, and the caudate nuclei were shelled out from both hemispheres. Synaptosomes were obtained by the method in [8]. The residue of synaptosomes was suspended in 0.32M 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. Activity of the ATPases was determined by measuring hydrolysis of ATP in medium (1 ml) containing 120-150 µg synaptosomal protein. Protein was determined by Lowry's method. Total ATPase activity was measured in medium containing (in mM): NaCl 120, KCl 20, $MgCl_2$ 1, Tris-HCl 50, pH 7.4. Mg-ATPase activity was determined in medium containing (in mM): NaCl 140, $MgCl_2$ 1, Tris-HCl 50, pH 7.4. Activity of Na,K-ATPase was determined as the difference between total and Mg-ATPase activity. Inorganic phosphate (P_i) was determined by the method in [9]. The reaction was started by the addition of ATP-Na_2 (1 mM). The neuroleptics (10^{-7}-10^{-4} M) were added to the reaction medium 5 min before the substrate. In three

TABLE 1. Inhibitory Action of Neuroleptics (IC_{16} and IC_{50}, in µM) on Synaptosomal Na,K-ATPase in the Caudate Nucleus of Rats depending on the Ca^{++} Concentration in the Medium.

Neuroleptic	In presence of 1 mM EGTA, IC_{16}	In absence of EGTA and in presence of $CaCl_2$			
		0,05 mM		0,25 mM	
		IC_{16}	IC_{50}	IC_{16}	IC_{50}
Fluphenazine	0,32	0,05	10	0,025	1,1
Haloperidol	0,56	0,08	20	0,035	2,0
Chlorpromazine	0,70	0,10	32	0,050	2,8
Trifluperazine	1,00	0,13	50	0,063	4,0
Sulpiride	1,60	0,18	130	0,071	7,9
Thioridazine	4,0	0,22	—	0,079	40,0

Legend. Here and in Table 2 average values of 5-6 experiments are given.

TABLE 2. Hill's Coefficient for Interaction between Neuroleptics and Synaptosomal Na,K-ATPase in the Caudate Nucleus with Different Ca^{++} Concentrations in the Medium

Neuroleptic	In absence of Ca^{++}	In presence of Ca^{++}	
		0,05 mM	0.25 mM
Fluphenazine	0,23	0,29	0,42
Haloperidol	0,21	0,27	0,38
Chlorpromazine	0,21	0,27	0,38
Trifluperazine	0,21	0,27	0,38
Sulpiride	0,20	0,25	0,33
Thioridazine	0,15	0,21	0,27

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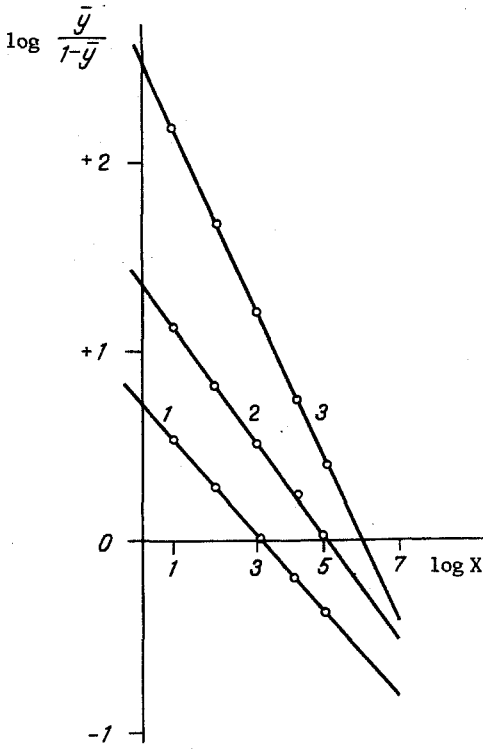

Fig. 1. Dependence of degree of saturation ($\bar{y}$) of enzyme and fluphenazine concentration on a Hill plot in absence (1) and presence of Ca^{++} in concentrations of 0.05 mM (2) and 0.25 mM (3).

series of experiments 1 mM of EGTA or $CaCl_2$ in final concentrations of 0.05 and 0.25 mM was added 5 min before the neoroleptics.

The ATP-Na_2 and Tris-HCl were obtained from Reanal, Hungary, the fluphenazine, chlorpromazine, and trifluperazine were of USSR origin, haloperidol was from Gedeon Richter, Hungary, thioridazine from Poland, and sulpiride was used in the form of a solution in ampuls, from Eglonya, Yugoslavia.

EXPERIMENTAL RESULTS

In the control series of experiments (in the presence of EGTA, without Ca^{++}) Na,K-ATPase activity was 14.5 ± 0.9 moles P_i/mg protein/h. Under these conditions the neuroleptics depressed enzyme activity by an amount which depended on concentration. The maximal reduction of activity obtained with the highest concentration of neuroleptics tested (10^{-4} M) did not exceed 23-38% of the initial level. Values of IC_{16} (a concentration producing inhibition by 16%) are given in Table 1.

The presence of Ca^{++} ions in the medium in concentrations of 0.05 and 0.25 mM reduced Na,K-ATPase activity by 39 and 48%, respectively. In the presence of Ca^{++} (in concentrations of 0.05 and 0.25 mM) the neuroleptics (0.1 mM) reduced enzyme activity by the greatest degree (by 40-65 and 55-92%, respectively). Whereas in the presence of 0.05 mM Ca^{++} the values of IC_{50} (concentration inhibiting activity by 50%) was 10-130 mM or higher for the neuroleptics, in the presence of Ca^{++} in a concentration of 0.05 mM were the values of IC_{50} comparable with those reported by other workers [1, 6]. It can be seen by comparing the values of IC_{16} that the higher the Ca^{++} concentration the stronger the inhibitory activity of the neuroleptics, and in the presence of 0.25 mM Ca^{++} their inhibiting activity was increased by 13-51 times compared with activity in the absence of Ca^{++}. Such a marked increase in the inhibitory activity of the neuroleptics in a concentration at which Ca^{++} ions inhibit Na,K-ATPase activity by not more than 50%, indicates that Ca^{++} ions potentiate the inhibitory action of neuroleptics on the enzyme.

Potentiation of the inhibitory action of the neuroleptics by Ca^{++} ions may be the result of a change in the cooperativity of interaction of the substances with the enzyme. It follows from the results that neuroleptics exhibit negative cooperativity during interactions with synaptosomal Na,K-ATPase of the rat caudate nucleus both in the absence and in the presence of Ca^{++} (0.05 and 0.25 mM; Fig. 1; Table 2). However, in the presence of Ca^{++} the negative cooperativity is reduced, although Hill's coefficient remained below unity even if Ca^{++} is present in the incubation medium in a concentration of 0.25 mM. The reduction of the nega-

tive cooperativity of interaction between neuroleptics and the enzyme by Ca^{++} ions is evidently the cause of potentiation by Ca^{++} of the inhibitory action of the neuroleptics on synaptosomal Na,K-ATPase of the caudate nucleus. The mechanisms of the direct membranotropic effect of the neuroleptics on Na,K-ATPase of presynaptic and (or) postsynaptic brain membranes remained unexplained.

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FREE CALCIUM CONCENTRATION IN BRAIN NERVE ENDINGS OF SPONTANEOUSLY HYPERTENSIVE RATS

S. N. Orlov, N. I. Pokudin, G. M. Kravtsov, Yu. V. Postnov, I. M. Okun', N. A. Shukanova, A. A. Rakovich, S. L. Aksentsev, and S. V. Konev

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One possible cause of the increase in resistance in the peripheral circulatory system in primary hypertension may be increased activity of the peripheral component of the sympathetic nervous system, which regulates smooth muscle tone, due to the release of neurotransmitters which interact with receptors of the postsynaptic membrane. The writers found previously that the equilibrium concentration of neurotransmitters such as noradrenalin, serotonin, and GABA, in brain nerve endings of spontaneously hypertensive rats (SHR) is 15-20% lower than in animals of the control group [3]. We attributed these differences to partial depolarization of the synaptolemma, leading to exocytosis of the neurotransmitters. The frequency of neurotransmitter release from the synaptic vesicles of nerve endings by exocytosis depends primarily on the free calcium concentration in the cytoplasm (Ca^{++}_{in}) [9], which is controlled by Ca-transporting and Ca-binding systems. Little information is available on the state of these systems in primary hypertension. We know, for example, that just as in the case of cells of many tissues [7, 12], the Ca-binding capacity of the synaptolemma is depressed in SHR; this difference, moreover, can be detected also in animals in the prehypertensive stage [11]. The study of $^{45}Ca^{++}$ uptake revealed an increase in the concentration of exchangeable calcium in synaptosomes of SHR [2]. Reduction of both basal and calmodulin-stimulated components of activity of the microsomal Ca pump also has been established in the brain of SHR [2].

Data on the increase Ca^{++}_{in} concentration in the synaptosomes of SHR, obtained by the use of a fluorescent indicator for Ca^{++}, namely quin-2, are given below.

Laboratory of Biophysics and Photobiology of Membranes, Institute of Photobiology, Academy of Sciences of the Belorussian SSR, Minsk.

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