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

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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.

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TABLE 1. Free Calcium Concentration (in nM) in Synaptosomes Suspended in Various Media (M ± m)

Group of animals	Medium		
	A	B	C
WKY (1)	62,3±14,0	115,6±28,1	83,3±22,4
SHR (2)	85,6±10,5	148,9±13,8	111,1±25,6
p_{1-2}	0,15	0,26	0,37

Legend. Here and in Tables 2 and 3, medium A (in mM): NaCl 132, KCl 5, $MgCl_2$ 1.3, NaH_2PO_4 1.2, glucose 10, Tris-HCl 20 (pH 7.4). Medium B: NaCl 66, KCl 71, the rest as in medium A. Medium C: choline chloride 132, KCl 5, the rest as in medium A.

TABLE 2. Initial Increase in Free Calcium Concentration (in nM) in Synaptosomes on Addition of KCl to 50 mM in Media A and C (M ± m)

Group of animals	Medium A	Medium C
WKY (1)	50,0±6,8	35,9±6,3
SHR (2)	73,9±8,7	66,3±10,2
p_{1-2}	<0,05	<0,025

EXPERIMENTAL METHOD

Experiments were carried out on eight male spontaneously hypertensive Kuoto-Wistar rats (SHR) aged 16-18 weeks, with a blood pressure (BP) of 170/200 mm Hg, and eight rats of the control group (normotensive Kyoto-Wistar rats) (WKY) of the same age and sex, with BP of 90/120 mm Hg.

Synaptosomes were isolated from the cerebral hemispheres of each rat by the method in [8], and EGTA was added to the homogenate in a final concentration 1 mM. A solution of quin-2 A/M (Amersham International, England) was added to the suspension of synaptosomes (1.5-2 mg protein/ml) in medium A (Table 1) up to a final concentration of 80 μM, and the sample was incubated for 60 min in a shaker at 37°C. After incubation the synaptosomes were sedimented by centrifugation for 5 min at 10,000 g and washed once with the same medium. The washed residue was resuspended in medium A to the original protein concentration and kept on ice. Aliquots of 0.08 ml were added to 0.72 ml of the corresponding medium — A, B, C (Table 1) without Ca^{++}. Calcium was titrated in a microcuvette (2 × 10 mm) for fluorescence measurements (Fig. 1), for which the JI-3CS spectrofluorometer was used. After fluorescence (F) of synaptosomes loaded with quin-2 had been recorded, they were disintegrated by the addition of sodium dodecyl sulfate solution to a final concentration of 0.1%, after which the maximal intensity (F_{max}) was measured. Subsequent binding of Ca^{++} with an excess of EGTA (10^{-2} M, pH 8.5) gave the minimal intensity (F_{min}). The free cytoplasmic calcium concentration (in mM) was calculated by the equation [14]:

$$Ca^{2+}_{in} = 115 \cdot \frac{F - F_{min}}{F_{max} - F}$$

with a correction for autofluorescence, measured on synaptosomes without the addition of the fluorescent probe.

EXPERIMENTAL RESULTS

Depolarization of the synaptolemma with 71 mM KCl (replacing NaCl in equimolar proportions) led to an increase in the Ca^{++} in concentration in the synaptosomes by 40-50% (Table 1) on account both of opening of the calcium channels and activation of Na-dependent entry of Ca^{++} as a result of its electrogenicity. In the present case the first hypothesis is evidently valid, for even the complete replacement of sodium by choline in the incubation medium led to an increase of only 25-35% in the Ca^{++}_{in} concentration. It can accordingly be suggested that loading the synaptosomes with quin-2 was accompanied by inactivation of Na^+/Ca^{++} exchange. In fact, we showed that after replacement of sodium by choline and in the absence of quin-2, uptake of $^{45}Ca^{++}$ by synaptosomes increased by an order of magnitude (results not given). Experiments on the giant axon showed that addition of EGTA [5] for 300 μM of quin-2 [4] to the perfusion fluid led to a decrease (by 90%) in both the Ca^{++}_{in}-dependent outflow of 22 Na^+ and of the Na^+_{in}-dependent inflow of $^{45}Ca^{++}$.

In all three incubation media (Table 1) the steady-state Ca^{++}_{in} concentration in synaptosomes of SHR was 20-30% higher than in animals of the control group. However, these differences in this sample were not significant.

TABLE 3. Fall of Free Calcium Concentration (in nM) in Synaptosomes during 85 sec after Potassium Depolarization (M ± m)

Group of animals	Medium A	Medium C
WKY	17,3±3,7	19,3±3,4
SHR	18,1±2,6	25,9±6,3

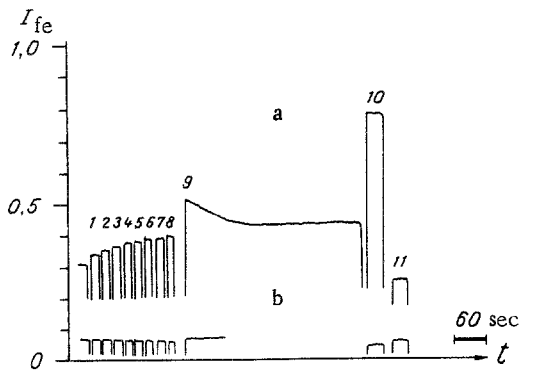

Fig. 1. Fluorescence and autofluorescence of synaptosomes, loaded (a) and not loaded (b) with quin-2. Effect of exogenous calcium and of potassium depolarization. Measurements made at 37°C in medium A (composition of medium, see Table 1). Additions to cuvette (up to final concentrations): 1-8) $CaCl_2$ (0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 mM, respectively); 9) KCl (50 mM); 10) sodium dodecyl sulfate (0.1%); 11) EGTA (10^{-2} M, pH 8.5). λ_{exc} = 340 nm (slit width 10 nm), λ_{rec} = 500 nm (slit width 20 nm).

Addition of KCl (final concentration 50 mM) to synaptosomes in media containing sodium or choline led to a rapid increase in the Ca^{++}_{in} concentration followed by a fall to a new steady state (Fig. 1). In the case of SHR, this increase was significantly higher (by 50-80%) than in animals of the control group (Table 2).

During K^+-depolarization the inflow of Ca^{++} into the synaptosomes took place through two types of voltage-dependent channels: "fast" Ca-channels, inactivated as early as 10 sec after depolarization, and "slow" channels, on which prepolarization has virtually no effect [10]. Under the present experimental conditions the steady-state level of Ca^{++}_{in} after K^+-induced depolarizaton was reached not earlier than after 2-5 min (Fig. 1). It can therefore be tentatively suggested that the kinetics of the fall of the Ca^{++}_{in} level was determined not by the channels but by activity of the Ca-pumps of the synaptolemma and of structures located inside the nerve ending. No differences were found in the rate of fall of the Ca^{++}_{in} level after K^+-depolarization between SHR and WKY (Table 3), and the most likely cause of the increased rise of Ca^{++}_{in} in the synaptosomes of SHR during K^+-induced depolarization may therefore be the greater number of "open" Ca-channels, due, for example, to differences in the conditions of phosphorylation by protein kinase A [1] or C [6]. Information on disturbance of cAMP and polyphosphinositide metabolism in primary hypertension is quite abundant and is summarized in the review [13].

Disturbances of regulation of free intracellular calcium discovered in this investigation, together with slowing of reuptake of neurotransmitters in the synaptosomes of SHR [1] can be regarded as the direct cause of activation of the sympathetic nervous system in the initial and chronic stages of primary hypertension.

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EFFECT OF FUNCTIONAL GROUPS OF TOCOPHEROL MOLECULES ON MICROVISCOSITY OF MITOCHONDRIAL LIPIDS

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Despite the great interest of many investigators in the role of natural antioxidants (AO) in the regulation of lipid peroxidation, their effect on the structural lability of biological membranes has received little study. This is an urgent problem also because there is no general agreement on the mechanism of the stabilizing action of AO on membrane structures.

The aim of this investigation was to study changes in viscosity of mitochondrial membrane lipids depending on the concentration of α-tocopherol (TP) and of its synthetic analog without the phythyl side chain, 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMHC), which has antiradical activity virtually equal to that of TP [2], in the mitochondria (MCh).

EXPERIMENTAL METHOD

MCh, isolated by differential centrifugation from mouse liver [4] were suspended in incubation medium containing TP or PMHC within the concentration range $2.3 \cdot 10^{-6}$-$2.3 \cdot 10^{-3}$ M, 0.25M sucrose, and 2 mM $MgCl_2$, pH 7.2-7.4. AO were added in the form of ethanol solutions (the ethanol concentration did not exceed 3%), after which the incubation medium was sonicated in the cold for 3-5 min by means of the UZDN-2T ultrasonic disintegrator at 44 kHz and 0.5 A. The MCh were kept in the incubation medium for 1 h at 4°C, with periodic shaking. They were then sedimented by centrifugation, and washed twice with 0.25M sucrose (pH 7.2-7.4), contain-

TABLE 1. Structural Transformation Temperature and Effective Activation Energy Values in Mitochondrial Lipids after Incubation with TP and PMHC

Concentration of TP and PMHC in incubation medium, M	T_s, °C		E_a, kcal/mole		
			temperature range		
	transformation I	transformation II	10—25 °C	25—40 °C	40—60 °C
control	23	42	3,1	0,41	1,93
TP:					
$2,3 \cdot 10^{-6}$	26	43	4,0	0,72	1,93
$2,3 \cdot 10^{-5}$	31	47	3,3	0,74	1,47
$2,3 \cdot 10^{-4}$	32	45	2,35	1,07	1,50
$2,3 \cdot 10^{-3}$	34	47	2,57	0,95	1,77
PMHC:					
$4,5 \cdot 10^{-6}$	42		3,53	—	1,5

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