Comparative Study of the Effect of Amiridine on Biological Membranes

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The effects of amiridine and of the comparable drugs tacrine and piracetam on synaptosomes and membranes of sarcoplasmic reticulum were studied by electron paramagnetic resonance; in addition,the effects of these drugs on the activity of Ca^{2+}, Mg^{2+}-dependent ATPase regulating calcium transport in neurons were investigated. In concentrations of 10^{-7} to 10^{-5} M the drugs did not affect the structure of synaptosomal membranes of rat brain. Amiridine and tacrine in a concentration of 0.1 mM reduced the rate of calcium ion transport across the sarcoplasmic reticulum membrane by inhibiting the function of Ca^{2+}, Mg^{2+}-dependent ATPase and induced marked changes of the structural rigidity of the protein part of the membrane.

Key Words: *amiridine; tacrine; piracetam; synaptosomes; sarcoplasmic reticulum Ca-ATPase*

Intensive studies of the mechanisms of the antiamnestic effect of amiridine, a drug designed at the All-Russian Research Center for the Safety of Biologically Active Compounds, are in progress. The drug has proved to be highly effective in the treatment of dementias of different genesis, including Alzheimer's senile dementia [8]. Previous studies using molecular probes and gas-liquid and thin-layer chromatography demonstrated that in some models of memory disorders the antiamnestic activity of amiridine was paralleled by its normalizing effect on the microviscosity of synaptosomal lipids [2,3,9]. Single or repeated injections of the drug to intact rats did not cause changes in microviscosity [3].

An antiamnestic activity of dihydropyridine calcium channel blockers has been revealed [10], suggesting that these substances act upon the structure of the biological membranes participating in the regulation of the Ca^{2+} ion concentration in neurons. One of the principal enzymes regulating Ca^{2+} transport in neurons and influencing neuronal activity is Ca^{2+}, Mg^{2+}-dependent ATPase of sarcoplasmic reticulum (SPR) [11-13].

This study was aimed at comparing the effects of amiridine on SPR synaptosomes and membranes by electron paramagnetic resonance (EPR) spectroscopy and its effects on the activity of SPR Ca^{2+}, Mg^{2+}-dependent ATPase with those of tacrine, which is structurally similar to amiridine and is used for the treatment of senile dementia, and the nootropic agent piracetam.

MATERIALS AND METHODS

The membranotropism of amiridine, tacrine, and piracetam was studied by EPR using spin labels and probes. A nitroxyl derivative of para-bromacetamide (PBA) was used as the paramagnetic label. The variation of a structural parameter - the τ correlation - was assessed, that is, the variation of the rotation of the spin label (nsec) attached to the biological membrane [4], as a function of the concentration of the test compounds. 5-Doxylstearic acid was used as the paramagnetic probe. The distance between the external extrema of the EPR spectrum was assessed, which defines the mobility

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TABLE 1. Effects of Drugs on Magnitude of A_{zz} of Probe Bound to SPR Membranes

Drug in SPR+5-doxylstearic acid system	A_{zz} (Gs) at concentrations of substances, M	
	10^{-6}	10^{-4}
Amiridine	31.5±0.1	31.25±0.1*
Tacrine	31.5±0.1	31.25±0.1*
Piracetam	31.7±0.1	31.75±0.1

Note. A_{zz} of native SPR preparation is 31.75±0.1 Gs; *$p<0.01$ in comparison with native SPR preparation.

of lipid fragments in the bilayer (A_{zz}), and the parameter $I_{bound}/1+I_{free}$, representing the ratio of the intensity of the EPR signal of the probe bound to the membrane to that of the free one. EPR spectra were recorded with a SEIX 2547 EPR spectrometer (Radiopan).

Experiments were carried out on rat synaptosomes. In addition, SPR membranes from rabbit muscles were used, with due consideration for the slight differences in the structure of the plasma membranes of muscle and brain tissues [7].

Synaptosomes were isolated from rat brain by gradient centrifugation [5] and stored in liquid nitrogen. SPR membranes were isolated from the muscles of rabbit hind limbs by centrifuging in a sucrose density gradient [6]. Spin label was applied by adding paramagnetic label and the probe to the membrane suspension in 0.05 M Tris-HCl buffer, pH 7.4, containing 20 mg/ml protein. Spin-labeled biological membranes were incubated with the test drugs for 20 min at 20±2°C.

ATPase activity of Ca^{2+}, Mg^{2+}-dependent SPR ATPase was determined by pH-metry [5] from changes in the concentrations of protons forming as a result of ATP substrate hydrolysis in a medium containing 4 mM $MgCl_2$, 2.5 mM imidazole, 10.0 mM NaCl, 5 mM sodium oxalate, 0.2 mg protein (SPR preparation), and 2 mM ATP. The velocity of Ca^{2+} transport was assessed according to the time it took for a known amount of Ca^{2+} ions in the incubation medium to be completely absorbed by the SPR vesicles, this leading to the arrest of ATP hydrolysis.

Drug concentrations of 10^{-7} to 10^{-4} M were used in all experiments.

The results were statistically processed using Student's test.

RESULTS

None of the drugs affected the binding of the paramagnetic probe to rat brain synaptosomes in the studied concentrations, because no statistically significant changes were observed in parameters A_{zz} and $I_{bound}/1+I_{free}$.

Drug effects on SPR membranes of rabbit muscles were studied. Table 1 presents the results of measuring the changes of the A_{zz} value of the paramagnetic probe adsorbed on the SPR membrane under the effect of the studied compounds. Only in a concentration of 10^{-4} M did amiridine and tacrine slightly (by 1.6%) lower A_{zz}, which may be due to a reduced microviscosity of the membrane lipid bilayer, whereas piracetam was inactive.

The results of a study of the effects of the drugs on SPR membrane structure in the region of thiol protein groups to which paramagnetic PBA label binds are presented in Table 2. All the studied compounds in concentrations of 10^{-7} to 10^{-5} M caused virtually no changes in this part of the SPR membrane, except for tacrine, which increased the membrane structural rigidity by 23% when used in a concentration of 10^{-7} M. Tacrine and amiridine in a concentration of 10^{-4} M statistically reliably reduced the $\tau_{corr.}$ value of PBA rotation by 34.6 and 23%, respectively, this indicating a lessening of the structural rigidity of the SPR membrane.

In the next series of experiments we investigated the effects of the drugs on the function of Ca-ATPase enzyme inserted in the SPR membranes (Table 3). Amiridine and tacrine inhibited the enzyme by decelerating ATP hydrolysis and Ca^{2+} transport only when used in a concentration of 10^{-4} M, whereas piracetam was not active at all.

Hence, *in vitro* experiments using the EPR method revealed that amiridine, tacrine, and piracetam in the studied concentrations did not affect the synaptosomal membranes of rat brain. These results are in good correlation with our previous data obtained *in vivo* for synaptosomes of intact rats

TABLE 2. Effects of Studied Drugs on $\tau_{corr.}$ of Paramagnetic Label Bound to SPR Membranes

Drug in SPR+PBA system	$t_{corr.}$ (nsec) at concentrations of substances, M			
	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Amiridine	0.28±0.014	0.25±0.012	0.29±0.014	0.2±0.01*
Tacrine	0.32±0.016*	0.24±0.013	0.25±0.012	0.17±0.008**
Piracetam	0.25±0.015	0.25±0.012	0.28±0.014	0.29±0.015

Note. $\tau_{corr.}$ of native SPR preparation is 0.26±0.013 nsec; *$p<0.05$; **$p<0.01$ in comparison with native SPR preparation.

TABLE 3. Drug Effects on Activity of SPR Ca–ATPase

Drug	Concentration, M	SPR Ca-ATPase activity, %	
		Ca^{2+} transport	ATP hydrolysis
Control	-	100±9	100±8
Amiridine	10^{-7}	82±4	105±1
	10^{-6}	80±4	100±10
	10^{-5}	92±8	100±10
	10^{-4}	72±7*	75±7*
Tacrine	10^{-7}	100±10	95±9
	10^{-6}	85±8	89±8
	10^{-5}	87±8	100±10
	10^{-4}	70±8*	72±6*
Piracetam	10^{-7}	90±10	90±8
	10^{-6}	85±8	86±8
	10^{-5}	102±8	106±8
	10^{-4}	100±8	102±8

Note. The activity of SPR Ca–ATPase preparations is 1200 nM inorganic phosphate per mg of protein in 1 min. *$p<0.05$; $n=4-6$.

[3] and indicate that the drugs in question do not interfere with the normal functioning of neuronal membranes.

The effects of these drugs on SPR membranes of rabbit muscles were also weakly pronounced. Only in the protein part of the membrane was a marked reduction of its structural rigidity observed under the influence of very high amiridine and tacrine concentrations. Interestingly, it was in this very concentration (10^{-4} M) that the drugs reduced Ca^{2+} transport across the SPR membrane by inhibiting Ca-ATPase. Since the Ca^{2+} blockers include compounds improving the memory processes, for example, nimodipine [10], we believe that amiridine and tacrine, in contrast to piracetam, owe their antiamnestic activity to their capacity to act upon the energy-dependent "calcium pump" of the endoplasmic reticulum of the neurons, a component of which is Ca-ATPase.

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