PHARMACOLOGY AND TOXICOLOGY

Membranotropic Effect of 2(Chloroethoxy)-Para-N-Dimethylaminophenyl Phosphinylacetyl Hydrazide (CAPAH)

I. G. Semina, I. I. Semina, D. A. Faizyllin, N. A. Baichurina, E. A. Stupishina, N. N. Vylegzhanina, R. I. Tarasova, V. D. Fedotov, R. S. Garaev, and V. A. Pavlov

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Membranostabilizing effect of CAPAH is demonstrated *in vivo* and *in vitro*. The preparation decreases experimental osmotic hemolysis and prevents elevation of blood enzymes in rats exposed to hypoxia. Infrared spectroscopy and electron paramagnetic resonance show that CAPAH is located in the superficial hydrophilic layer of modeled phosphatidylcholine membranes and interacts with lipid molecules.

Key Words: *CAPAH; membrane-stabilizing effect; modeled membranes*

According to current views, changes in the physicochemical state of lipid domains in the neuronal membrane caused by exogenous agents modulating synaptic transmission and enzyme and receptor activity underlie pharmacological, in particular, psychotropic effects of these agents [1]. Membranotropic effect is a characteristic feature of nootropics and an essential component of their pharmacological activity.

The aim of the present study was to investigate the membranotropic effect of 2(chloroethoxy)-para-N-dimethylaminophenyl phosphinylacetyl hydrazide (CAPAH), which exhibited nootropic activity in our previous experiments [5].

MATERIALS AND METHODS

Membranotropic activity of CAPAH was assessed by its effect on the erythrocyte resistance to hypotonic shock [8]. A suspension of rat erythrocytes (4×10^8 cells/ml) was used. Hemoglobin content in the supernatant was measured at 543 nm in an SF-46 spectrophotometer. Hemolysis in control samples (hypotonic solution without CAPAH) was taken as 100%. Control and experimental samples were processed in triplicates; CAPAH was added in concentrations of 10^{-2}-10^{-9} M. Serum alanine and asparagine transaminases, alkaline phosphatase, creatine phosphokinase, and lactate dehydrogenase activities in rats subjected to hypoxia were determined using a Cobas mira Plys analyzer and monotests (Hoffman-La Roche).

The mechanism of the membranotropic effect of CAPAH was studied in modeled phosphatidylcholine membranes: hydrated reversed micelles with different volume of hydrated phase (P), but the same hydratation degree [7] and simple bilayer liposomes dispersed in heavy water (D_2O) [2]. The interaction between CAPAH and the membranes was assessed by electron paramagnetic resonance (EPR) and infrared (IR) spectroscopy. Phospholipids with

Kazan' State Medical University; Institute of Biology, Russian Academy of Sciences, Kazan'

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TABLE 1. Effect of CAPAH on Osmotic Resistance of Erythrocytes (% of Control, *M±m*)

Concentration of CAPAH, M	% hemolysis
Control	100.0±4.4
10^{-4}	66.7±7.4*
10^{-5}	80.4±9.7
10^{-6}	76.4±6.8*
10^{-7}	64.6±17.1*
10^{-8}	65.6±0.29*
10^{-9}	76.2±10.3*

Note. Here and in Table 2: $p<0.05$: *compared with the control, **compared with hypoxia.

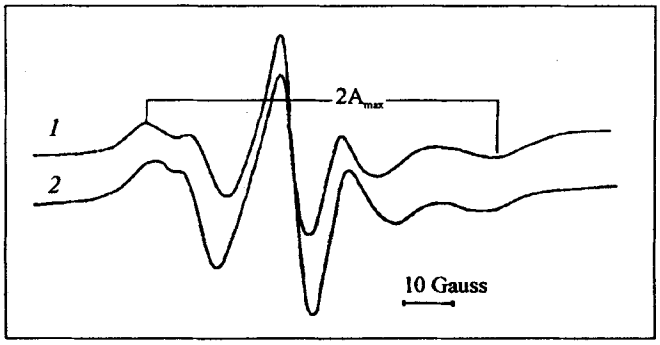

Fig. 1. Electron paramagnetic resonance spectra of 5-doxylphospholipid-labeled microemulsion with P=0.27. *1*) control ($2A_{max}$=53.3 Gauss); *2*) in the presence of 10^{-5} M CAPAH ($2A_{max}$=46.8 Gauss).

a paramagnetic (doxyl) ring at C_5 or C_7 of the acyl sn-2 chain (5- and 7-doxylphospholipids, respectively) were used as spin probes. EPR spectra were recorded in an RE-1306 radiospectrometer in the temperature range of 0-35°C (from high to low temperatures) [6], IR spectra were recorded in an M-80 IR spectrometer (Karl Zeiss Jena).

RESULTS

In all concentrations used CAPAH protected rat erythrocytes from osmotic hemolysis, the effect reaching the maximum at concentrations of 10^{-7}-10^{-8} M (Table 1). Being injected in a dose of 1 mg/kg 40 min before hypoxia, CAPAH reduced blood activity of aspartate aminotransaminase, lactate dehydrogenase, and alkaline phosphatase (Table 2). This attests to a membrane-stabilizing effect of CAPAH, since hypoxia-induced rise of serum enzyme activity is associated with the damage to cell membranes and a release of cell enzymes into circulation [4]. These changes were not observed in intact animals injected with CAPAH.

Figure 1 shows typical EPR spectra of spin-labeled phospholipids. These spectra display a 5-component hyperfine structure characteristic of a low probe motion in the sample. The amplitude of the maximum superfine splitting ($2A_{max}$) was used for spectral analysis, since this parameter reflects structural ordering in the paramagnetic group surrounding [3].

For 5-doxylphospholipid-labeled microemulsion with P=0.27, $2A_{max}$ in the presence of CAPAH was lower than in the control (Fig. 2, *a*). Thus, CAPAH reduced membrane ordering at the depth of C_5 of fatty acid chains.

In analogous experiments with 7-doxylphospholipid-labeled microemulsions, CAPAH had no effect on EPR spectra. These data suggest that CAPAH interacts with polar phospholipid heads and its effect decreases toward the fatty acid tails. It should be noted that CAPAH had practically no effect on a microemulsion with a low dispersed phase content (P=0.087, Fig. 2, *b*). IR spectra of CAPAH-con-

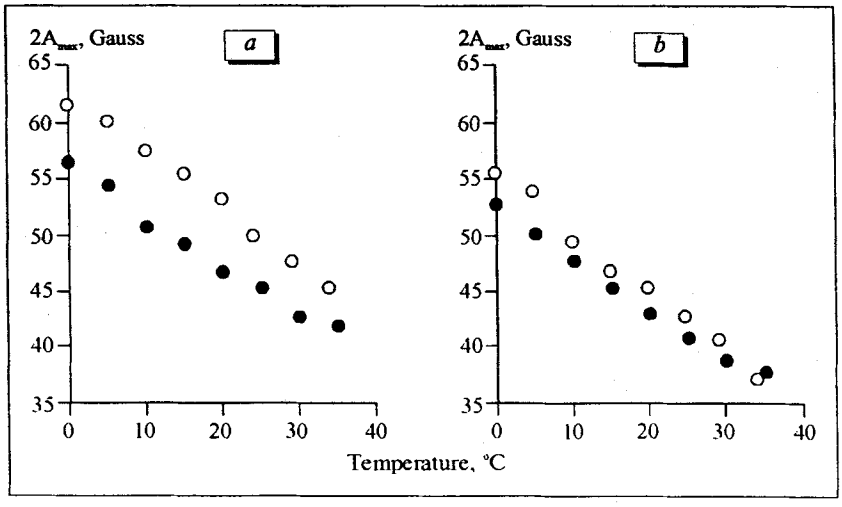

Fig. 2. Amplitude of hyperfine splitting ($2A_{max}$) of electron paramagnetic resonance spectrum for 5-doxylphospholipid-labeled microemulsions with P=0.27 (*a*) and P=0.087 (*b*) as a function of temperature in control (open circles) and in the presence of 10^{-5} M CAPAH.

TABLE 2. Effect of CAPAH Pretreatment on Serum Enzyme Activity 3 h after Hypobaric Hypoxia (U/liter, *M±m*)

Group	Alanine aminotrans-aminase	Aspartate aminotrans-aminase	Lactate dehydrogenase	Alkaline phosphatase	Creatine phosphokinase	γ-Glutamyl transpeptidase
Control	61.7±7.5	185.5±23.5	2209.6±228.6	24.7±1.4	703.9±42.3	2.22±0.36
Hypoxia	87.1±6.9*	275.5±29.3*	3085.6±326.7*	44.8±3.5*	873.0±228.7	3.25±0.50
CAPAH, 1 mg/kg	68.4±8.1	201.4±34.1	2577.8±279.4	26.0±2.7	705.6±195.6	2.56±0.53
CAPAH, 1 mg/kg+ hypoxia	73.4±8.8	195.4±18.8**	2306.0±76.2**	28.9±3.5**	783.0±263.1	2.27±0.41
Piracetam, 100 mg/kg+hypoxia	87.4±12.9	213.6±49.7	2552.3±148.3	27.3±4.9**	597.7±125.0	2.31±0.39

taining liposomes display a downward shift (from 1730 to 1725 cm^{-1}) of carbonyl (C=O) band of phosphatidylcholine ester group, attesting to the formation of a hydrogen bond between phosphatydylcholine carbonyl and hydrazide ($NHNH_2$) group in the CAPAH molecule. Hence, CAPAH interacts with polar phospholipid heads in the membrane.

This assumption is confirmed by the absence of characteristic IR shifts of the hydrocarbon band (2900-2800 cm^{-1}). IR spectroscopy these data are consistent with EPR findings.

Thus, our experiments revealed a membrane-stabilizing effect of CAPAH both *in vivo* and *in vitro*. It is shown that CAPAH located in the surface hydrophilic layer interacts with membrane lipids, and the mobility of hydrophobic layer under these conditions increases. In light of modern concept of biomembranes it can be hypothesized that the interaction between membrane lipids and CAPAH in physiological concentrations (10^{-4}-10^{-7} M) modulates functional activity of membrane proteins, in particular, monoamine carriers, receptors, and enzymes, which probably underlies the nootropic effect of CAPAH.

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