Possible Involvement of Neurokinin Receptors in CNS and Neuromuscular Synapse in the Realization of the Effects of CAPAH and Substance P

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Nootropic non-anticholinesterase organophosphorus preparation CAPAH and substance P produced similar and dose-dependent effects on the amplitude and temporal parameters of miniature endplate potentials in mammalian neuromuscular synapse. Neurokinin receptor antagonist Win-51.708 abolished the effects of these agents. In behavioral experiments substance P moderated the mnemotropic and antidepressant effects of CAPAH. It was assumed that neurokinin receptors are the targets of CAPAH and substance P in CNS and neuromuscular synapse.

Key Words: *substance P; CAPAH; neurokinin receptors; neuromuscular synapse; CNS*

Tachykinin neurokinin (NK_1) receptors are widely spread in the central and peripheral nervous system [5,10]. The study of their interaction with agonists and antagonists helps to elucidate the physiological role of these receptors and their involvement in genesis of various diseases. It also provides theoretical basis for the search of potential drugs. Substance P (SP) modulates the release of some neurotransmitters, *e.g.* acetylcholine and dopamine via interaction with NK_1-receptors [4,7]. SP plays an important role in the processes of neuronal plasticity associated with learning and memory. Considerable attention is now focused on the search of nonpeptide agents acting similarly to neuropeptides [6]. We previously demonstrated affinity binding of CAPAH, a non-anticholinesterase organophosphorus agent possessing nootropic and antidepressant activity, to NK_1-receptors [12]. The effects of this agent in cholinergic synapse are similar to those of SP [3]. Here we studied the possible role of NK_1-receptors in the realization of the effects of SP and CAPAH in neuromuscular synapses and on the behavior of experimental animals.

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MATERIALS AND METHODS

Experiments were performed on phrenic-diaphragm preparations isolated from albino rats at 20.0±0.3°C. The preparation was perfused with physiological saline containing (in mM): 120.0 NaCl, 5.0 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 11.0 $NaHCO_3$, 1.0 NaH_2PO_4, and 11.0 glucose (pH 7.3-7.4). The solution was continuously aerated with carbogen (95% O_2 and 5% CO_2). Miniature endplate currents (MEPC) were recorded using routine two-electrode voltage clamp technique. The amplitude and temporal parameters of MEPC were analyzed using original software. The sampling time was 10 μsec.

The behavioral effects of SP and CAPAH on CNS were studied on the models of behavioral despair [9] and passive avoidance conditioning. *In vivo* experiments were performed on male mice weighing 20-22 g. SP was injected intraperitoneally in a dose of 50 nmol/kg [8]; CAPAH was injected intraperitoneally in doses of 100 mg/kg, 10 mg/kg, and 1 μg/kg 30 min before testing. For combined treatment CAPAH was injected 30 min before CP.

Specific blocker of NK_1 receptors Win-51.708 (RBI) and SP were used in the experiments. In experi-

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ments with neuromuscular preparation CAPAH hydrochloride was used.

RESULTS

In control preparation clamped at 50-60 mV, the mean amplitude of MEPC was 2.63±0.02 nA (n=15). The slope of MEPC was described by a monoexponential with a time constant τ_{MEPC}=0.99±0.05 msec (n=15). The effects of CAPAH and SP on MEPC amplitude and temporal parameters depended on their doses (Table 1). Both CAPAH and SP in low concentrations (10^{-8}-10^{-7} M) increased MEPC amplitude, in a medium concentration (10^{-6} M) produced no significant effect, and in high concentrations (10^{-5}-10^{-4} M) decreased MEPC amplitude (Table 1, Fig. 1). The latter effect attests to desensitization of the postsynaptic membrane in the presence of high doses of SP [1,2]. The increase in MEPC amplitude in the presence of CAPAH did not depend on its anticholinesterase activity, because it was not associated with prolongation of its rise and the increase in time constant (Fig. 1, *b*, *d*).

Apart from the increase in MEPC amplitude, low concentrations of CAPAH and SP increased the MEPC area (S). Since CAPAH and SP do not modify sensitivity of the postsynaptic membrane to exogenous acetylcholine, this phenomenon indicates that quantum size, *i.e.* the number of acetylcholine molecules in a vesicle, increased [10,13]. This effect was not associated with activation of cholinergic receptors, because radioligand assay showed [12] that CAPAH has no affinity to nicotinic and muscarinic (M_1 and M_2) receptors. We assumed that the effects of CAPAH and SP are mediated via NK_1 receptors. To verify this hypothesis, we used Win-51.708, a specific antagonist of NK_1 receptors.

Application of Win-51.708 (10^{-6} M) had no effect on MEPC amplitude (2.18±0.06 nA, n=6). This antagonist was used to test the effects of CAPAH and SP, which increase MEPC amplitude at low (10^{-8} M) and decrease it at higher concentration (10^{-5} M).

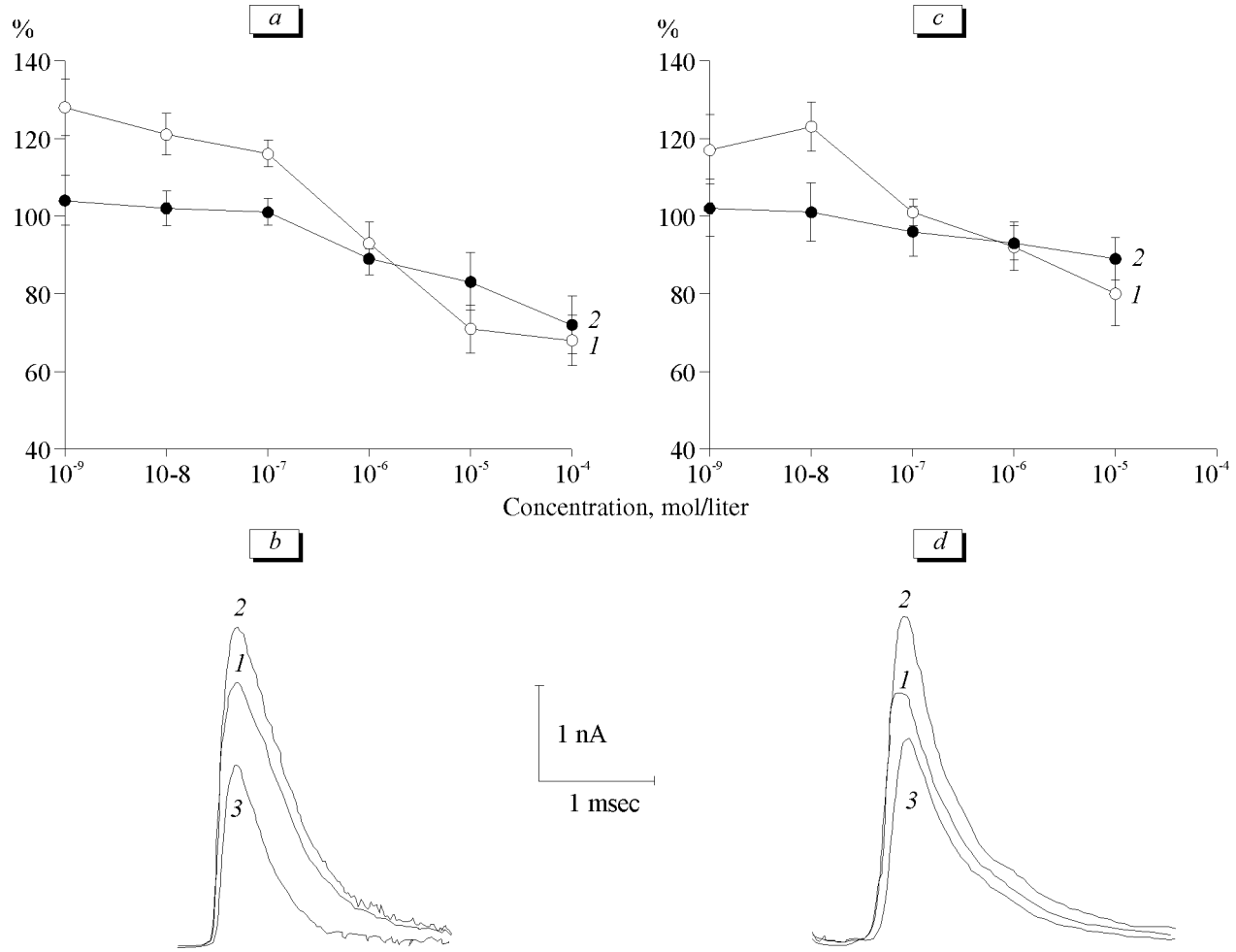

Fig. 1. Effect of CAPAH and SP on MEPC amplitude. *a, b*): ordinate: ratio of MEPC amplitudes in the presence of CAPAH (*a*) and SP (*b*) to the control value. *a*: *1*) CAPAH; *2*) CAPAH+Win-51.708 (10^{-6} M). *b*: *1*) control; *2*) CAPAH 10^{-8} M; *3*) CAPAH 10^{-5} M. *c*: *1*) SP; *2*) SP+Win-51.708 10^{-6} M. *d*: *1*) SP 10^{-8} M; *3*) SP 10^{-5} M.

TABLE 1. Effect of CAPAH and SP on MEPC Parameters ($M\pm m$, n=10)

Experiment	Amplitude, nA	RT, msec	τ, msec	S, nA×msec	n
Control	2.63±0.02	0.21±0.01	0.99±0.05	3.03±0.05	15
CAPAH, mol/liter					
10^{-8}	3.18±0.04*	0.21±0.02	1.21±0.09	4.01±0.12*	6
10^{-5}	1.88±0.05*	0.19±0.02	0.78±0.03*	2.68±0.07	5
SP, mol/liter					
10^{-8}	3.23±0.03*	0.19±0.01	1.12±0.03	4.02±0.06*	6
10^{-5}	2.12±0.03*	0.18±0.02	0.87±0.02*	2.92±0.06	5

Note. *p<0.05 compared to the control.

Win-51.708 prevented the increase in MEPC amplitude produced by SP and CAPAH in low doses (Fig. 1, *a*, *b*). The inhibitory effects of SP and CAPAH were less pronounced in the presence of Win-51.708, but did not completely disappeared.

These findings suggest that potentiation of the postsynaptic response produced by CAPAH and SP can be realized via NK_1 receptors.

Being a lipophilic agent, SP can penetrate the blood-brain barrier after intraperitoneal administration and affect memory development and consolidation. This correlates with changes in dopaminergic activity in mesolimbic structures. At low doses of SP this effect can be mediated via binding to NK_1 receptors [8].

Experiments on the model of passive avoidance conditioning showed that CAPAH (in all doses) and SP prolonged the latency of the entry into the dark compartment in the shuttle box (Fig. 2), which attests to its positive mnemotropic effect. When the agents were applied in combination, SP moderated the effect of CAPAH in a lower dose (1 μg/kg) and completely eliminated it in higher doses (10 and 100 mg/kg).

Experiments on the model of behavioral despair CAPAH (1 μg/kg, 1 mg/kg, and 10 mg/kg) produced an antidepressant effect and shortened freezing time (Fig. 2, *b*). SP produced no antidepressant effect and moderated the effect of CAPAH.

Thus, our findings suggest that NK_1 receptors in CNS and neuromuscular synapses are the target of CAPAH and SP. These data extend our knowledge on the functional role of SP in the neuromuscular synapse and shed light on the mechanism of nootropic action of CAPAH. These data can be useful in targeted synthesis of agents interacting with NK_1 receptors.

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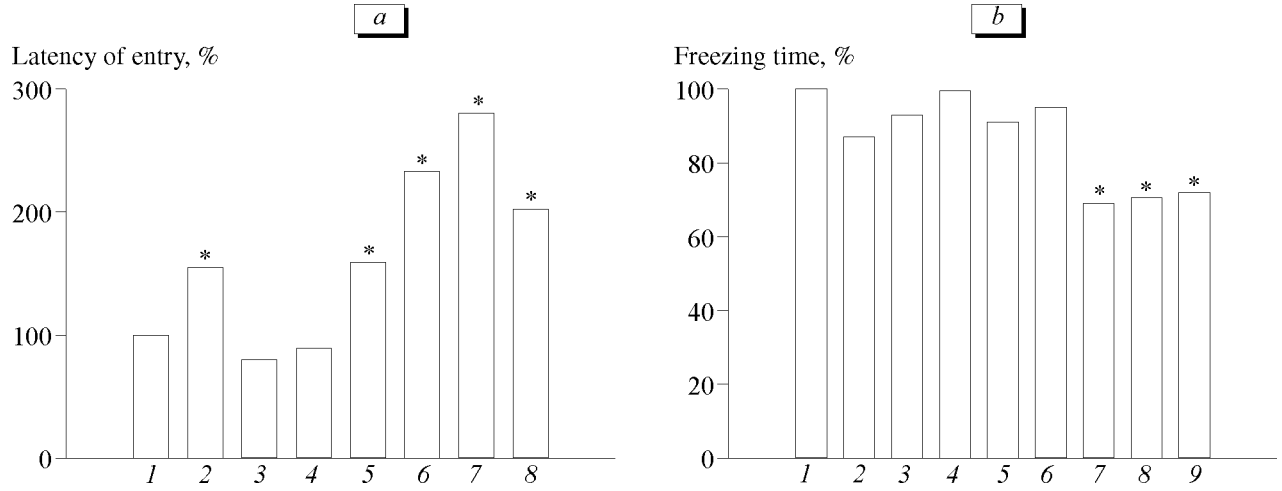

Fig. 2. Effect of CAPAH and SP on memory and learning in conditioned passive avoidance test (*a*) and behavioral despair test (*b*). *1*) control; *2*) SP, 50 nM; *3*) CAPAH (100 mg/kg)+SP; *4*) CAPAH (10 mg/kg)+SP; *5*) CAPAH (1 μg/kg)+SP; *6*) CAPAH, 100 mg/kg; *7*) CAPAH, 10 mg/kg; *8*) CAPAH, 1 μg/kg; *9*) CAPAH, 1 μg/kg. *p<0.05 compared to the control (100%).

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