Effect of the Aspartic Acid Derivatives N-Acetyl-Aspartate and Its Phosphonic Analog PIR-87-6-0 on the Release of Dopamine from Rat Striatum During *In Vitro* Perfusion

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N-acetyl-aspartate and its phosphonic analog PIR-87-6-0 significantly increase basal dopamine release from isolated rat striatum in a neurochemical model *in vitro*. It is found that NMDA glutamate heteroreceptors are involved in the dopamine-mobilizing effect of these compounds, which is confirmed by behavioral studies.

Key Words: *NMDA receptors; striatum; dopamine; release*

The neurospecific agent N-acetyl-aspartate (NAA) and its phosphonic analog PIR-87-6-0 stimulate learning and mnestic processes in behavioral tests [5-7,10] as well as exploring and motor activities [6,10].

NAA and PIR-87-6-0 produce a pronounced antiamnestic effect on impaired conditioned passive avoidance reaction induced by MK-801, an antagonist of N-methyl-D-aspartate (NMDA) glutamate receptors [10], implying that these receptors participate in the realization of the effects of these compounds.

The interaction of NNA and PIR-87-6-0 with glutamate receptors may be associated with increased locomotor activity in the open field test [6,10]. This hypothesis in based on the observation that stimulation of glutamate heteroreceptors of dopaminergic terminals potentates the release of dopamine (DA) from the nigrostriatal tract endings [9,11]. Dopamine has been regarded as a marker of motor activity in animals [14]. However, this hypothesis was not confirmed by neurochemical evidence.

Our goal was to examine the putative heteroreceptor-dependent effect of NAA and PIR-87-6-0 on DA release.

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MATERIALS AND METHODS

The release of DA was studied in the isolated striatum model [4]. The striatum was isolated at 4°C from the brain of male Wistar rats (body weight 240-270 g) and placed in carbogenized buffer containing 111 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.64 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11 mM glucose, 50 mg/liter ascorbic acid, and 20 mg/liter Na-EDTA. The striatum from each rat was studied in an individual chamber. After a 1-h incubation, the buffer was replaced, and a 10-min incubation was carried out in the presence or absence of the test compounds. The incubation medium was collected, and the specimens were incubated in a medium with a high content of K^+ (20 mM) and an equimolar content of Na^+. After a 10-min incubation, both control and experimental samples reflecting basal and K^+-stimulated DA release, respectively, were precipitated on aluminum oxide. The DA content was measured using high-efficiency liquid chromatography with electrochemical detection [3,4].

NAA and PIR-87-6-0 were kindly provided by Dr. V. A. Sazhin (Institute of Pharmacology, Volgograd). Their effects on DA release were studied in the concentration range of 1-100 μM. 2-Carboxy-

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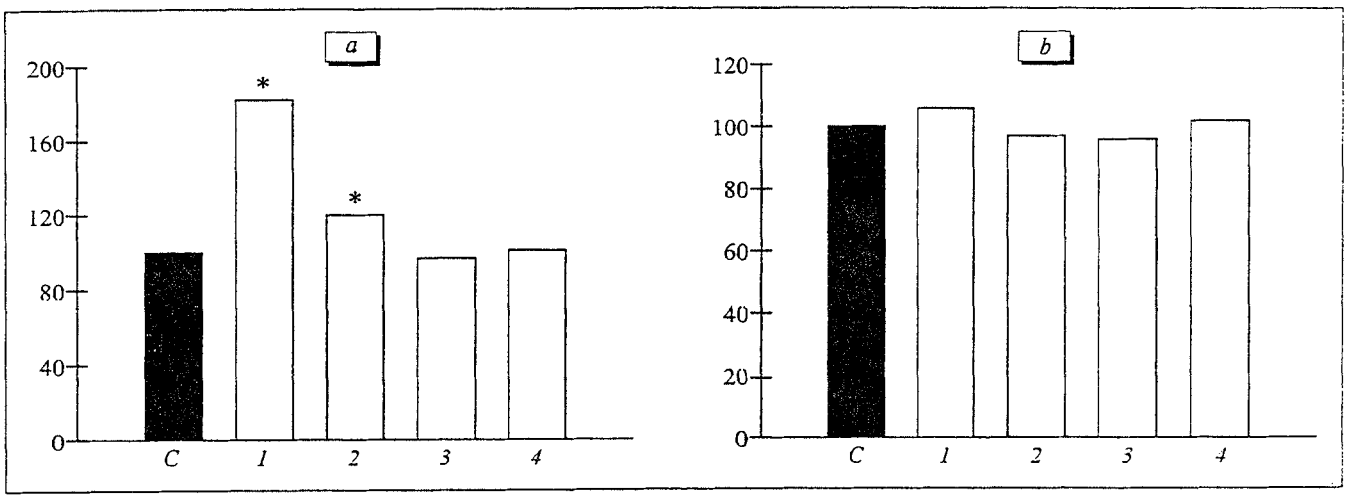

Fig. 1. Effect of N-acetyl-aspartate (NAA, *1*, *3*) and PIR-87-6-0 (*2*, *4*) on basal (*a*) and K^+ stimulated (*b*) dopamine release from rat striatum under condition of *in vitro* perfusion. Concentrations: *1* and *2*) 100 μM; *3* and *4*) 1 μM. *C*) controls: 54.7±5.1 and 238±10 pmol/min/mg tissue for baseline and K^+-stimulated DA release, respectively. $p<0.05$: *compared with *C*, °compared with *1*. Here and in Fig. 2: ordinate: dopamine release, % of control.

piperazine-4-propylphosphonic acid (CPP, RBI) and glutaric acid diethyl ester (GADE) were kindly provided by Dr. L. B. Piotrovskii (Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg). These compounds are specific antagonists of NMDA and quisqualate glutamate receptors, respectively. They were used for pharmacological analysis of the effects produced by NAA and PIR-87-6-0. The antagonists were added to the buffer simultaneously with the test compound in concentrations that did not affect DA release. Statistical analysis of results was performed using the Student's *t* test.

RESULTS

Psychotropic agents are known to modulate the mechanisms underlying synaptic plasticity [8]. It has been suggested that the antiamnestic effects of nootropics are due primarily to the optimization of the aspartate-glutamate neurotransmission [1,8,12].

On the other hand, modulation of the brain DAergic system may also contribute to the nootropic effects [12,13].

Thus, it was interesting to assess the ability of NAA and PIR-87-6-0 to stimulate DA release from isolated rat striatum in a neurochemical model. NAA (100 μM) and PIR-87-6-0 (100 μM) elevated basal extracellular DA content by 84 and 20%, respectively (Fig. 1, *a*), compared with the control.

The glutamatergic selectivity of the effect was studied using specific glutamate antagonists.

Experiments with NAA and glutamate antagonists showed that only the NMDA antagonist CPP blocks the effect of NAA on the basal release of DA (Fig. 2), while the quisqualate antagonist GADE was ineffective. The effect of PIR-87-6-0 was also CPP-dependent (Fig. 2).

These data suggest that NMDA receptors participate in the realization of the pharmacological effects of NAA and PIR-87-6-0. This is consistent with the results of behavioral experiments in which the aspartate derivatives exhibited the most pronounced activity in impaired conditioned passive avoidance reaction modeled with MK-801, a noncompetitive antagonist of NMDA receptors [10].

At a concentration of 10^{-4} M both compounds had no effect on K^+-stimulated DA release, which may be associated with the peculiarities of hetero-

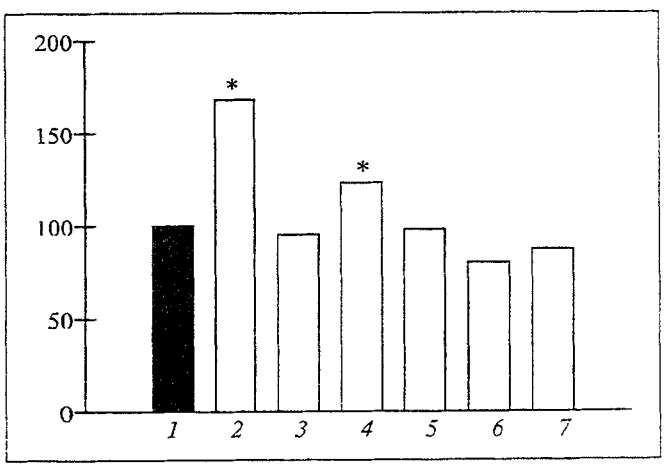

Fig. 2. Analysis of dopamine-stimulating effect of N-acetyl-aspartate (100 μM) and PIR-87-6-0 (100 μM) on isolated striatum during perfusion *in vitro*. *1*) control (54.7±5.1 pmol/min/mg tissue); *2* and *4*) against the background of GADE (100 μM); *3* and *5*) against the background of CPP (100 μM); *6*) effect of GADE (100 μM); *7*) effect of CPP (100 μM). *$p<0.05$ compared with the control (*1*).

receptor modulation of DA release under the chosen experimental conditions. Previously, it was shown that NMDA modulates only basal DA release [2], whereas much lower concentrations (10^{-6} and 10^{-7} M) of neuroleptics acting via DA receptors elevate the extracellular DA content under conditions of K^+ stimulation [4].

It should be noted that PIR-87-6-0 was more effective in the open field test than NAA [10], although the effect of NAA on DA release from rat striatum was more pronounced in comparison with that of its phosphonic analog (Fig. 1, *a*). Different neurochemical and behavioral activities indicate that phosphonic radical, which performs the transport function in the PIR-87-6-0 molecule, enhances psychotropic activity of the agent by improving its pharmacokinetic rather than pharmacodynamic characteristics. In the chosen neurochemical model, the rate of hydrolytic cleavage of the transport unit from the biologically active moiety is low, providing a lower ligand concentration in the buffer compared with that *in vivo*.

Thus, our findings show that NAA and PIR-87-6-0 stimulate the DA release from the striatum and outline a possible neurochemical mechanism responsible for this effect.

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