Effect of Amiridine and Tacrine on Reuptake of Neurotransmitters in Experimental Amnesia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 5, pp. 512-514, May, 1995
Original article submitted June 2, 1994

It is demonstrated that a single administration of amiridine, tacrine, piracetam, and physostigmine has no effect on the reuptake of adrenalin, noradrenalin, dopamine, and glycine, or of γ-aminobutyric, glutamic, and aspartic acids. Scopolamine (single administration or a 20-day treatment) also has no effect on the reuptake of these neurotransmitters. Administration of amiridine to intact rats during a 20-day period leads to a decrease in the reuptake of dopamine and γ-aminobutyric acid. A course of amiridine therapy of rats after repeated administration of scopolamine results in a reduced reuptake of dopamine. Tacrine, piracetam, and physososstigmine exhibit no activity under the chosen experimental conditions.

Key Words: *amiridine; tacrine; amnesia; neutrotransmitters; synaptosomal uptake*

Amiridine (9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]quinoline hydrochloride monohydrate), synthesized at the Russian Research Center for the Safety of Biologically Active Substances, has proved to be effective in the treatment of dementias of various origin [7]. However, the mechanism of the antiamnestic activity of amiridine, as well as that of tacrine (9-amino-1,2,3,4-tetrahydroacridine), a preparation developed in the West which is effective in the treatment of senile dementia of the Alzheimer type and which has a structural fragment similar to that of amiridine, remains unclear.

In senile dementia of the Alzheimer type, functional disorders occur not only in the cholinergic system, but also in the catecholaminergic [8,11], serotoninergic [6,8] and glutamatergic systems [10]. Previously, we demonstrated that *in vitro* amiridine and tacrine affect the transport of brain neurotransmitters [2].

The purpose of this work was to examine the effect of amiridine and tacrine in comparison with the cholinesterase inhibitor physostigmine and the nootropic drug piracetam, which are used in the treatment of dementiae, on the reuptake of adrenalin, noradrenalin, dopamine, γ-aminobutyric acid (GABA), glutamic acid (GLU), aspartic acid (ASP), and glycine *in vivo* in normal and amnestic animals to elucidate the role of these systems in the realization of the antiamnestic effects of these drugs.

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MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 180-200 g. The animals were maintained at 21-22°C on a standard schedule of 12-h daylight in 20×55×30 cm cages, 10 rats per cage, with food and water *ad libitum*.

The effects of the preparations on the reuptake of neutrotransmitters by brain synaptosomes were studied on intact rats after single and repeated administration in doses improving learning ability [1]. The preparations were injected intraperitoneally according to the following schedules: I) amiridine (1 mg/kg), tacrine (1 mg/kg), and physostigmine (0.1 mg/kg) 20 min and piracetam (250 mg/kg) 60 min prior to sacrifice; II) amiridine, tacrine,

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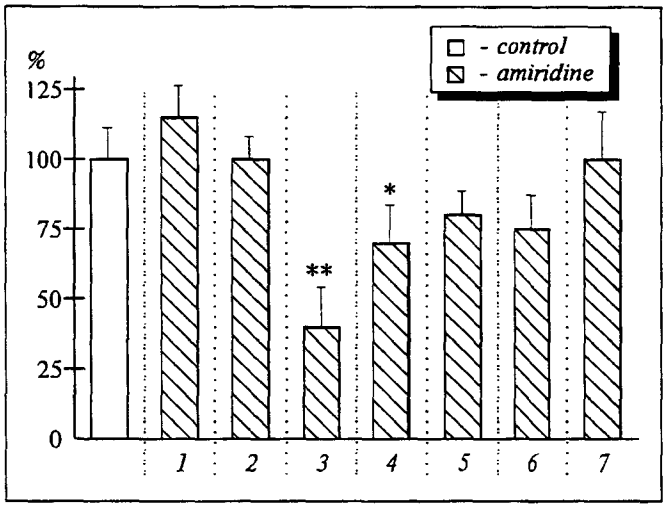

Fig. 1. Effect of repeated administration of amiridine during a 20-day period on synaptosomal uptake of neurotransmitters in intact rats. One asterisk indicates $p<0.05$ and two asterisks $p<0.01$ compared with the control. Reuptakes equal to 0.02 (noradrenalin, *1*), 0.003 (adrenalin, *2*), 0.004 (dopamine, *3*), 2.6 (GABA, *4*), 1.1 (GLU, *5*), 6.4 (ASP, *6*), and 0.11 (glycine, *7*) nmol neurotransmitter bound per mg synaptosomal protein for 5 min are taken as 100%.

physostigmine, and piracetam during a 20-day period in the same daily doses, the rats being sacrificed 24 h after the last injection. All groups consisted of 12 animals.

Amnesia was modeled using the central cholinoblocker scopolamine: single or multiple (a 20-day period) intraperitoneal injections in a dose of 2 mg/kg [1].

The effect of the preparations on the reuptake of neurotransmitters by brain synaptosomes was

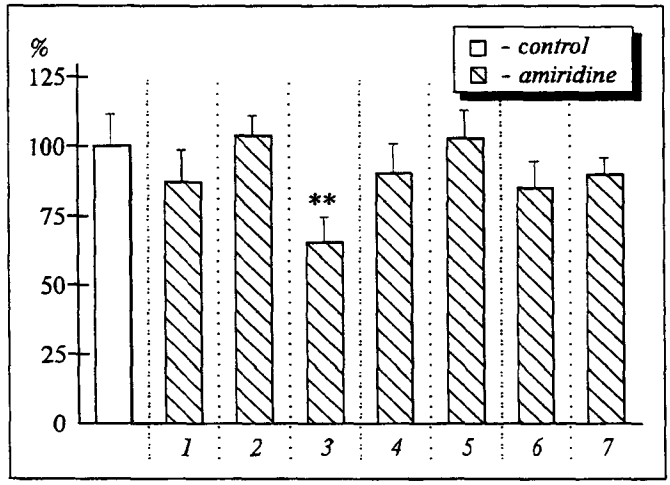

Fig. 2. Effect of repeated administration of amiridine (10 days) on synaptosomal uptake of neurotransmitters in rats given scopolamine during a 20-day period. Two asterisks indicate $p<0.01$ compared with the control. Reuptakes equal to 0.02 (noradrenalin, *1*), 0.027 (adrenalin, *2*), 0.035 (dopamine, *3*), 2.7 (GABA, *4*), 1 (GLU, *5*), 6.5 (ASP, *6*), and 0.13 (glycine, *7*) nmol neurotransmitter bound per mg synaptosomal protein for 5 min are taken as 100%.

examined in a model of scopolamine amnesia according to the following schemes: I) amiridine, tacrine, physostigmine, and piracetam were injected intraperitoneally in the above-mentioned doses 10 min after a single administration of scopolamine, and the rats were sacrificed 20 min later; II) the preparations were injected intraperitoneally during a 10-day period in the above-mentioned doses one day after the last administration of scopolamine (2 mg/kg), the rats being sacrificed 24 h after the last injection. All compounds were dissolved in 0.9% NaCl immediately before the experiment; the volume of injection was 0.5 ml. Control animals received the same volumes of normal saline.

Brain synaptosomes were isolated immediately after sacrifice as described [4]. Reuptake of neurotransmitters by synaptosomes was studied as previously [5]. The incubation medium contained (in μM): NaCl - 100, KCl - 6, $CaCl_2$ - 6, $MgCl_2$ - 3, glucose - 10, sucrose - 100, EDTA - 0.54, pargyline - 0.125, Tris-HCl (pH 7.4-30) and labeled neutrotransmitters in a concentration close to the Michaelis-Menten constant (Km), which is (in mM) for noradrenalin - 0.5, dopamine - 0.1, GABA - 10, adrenalin - 3 [5], GLU - 30 [4], ASP - 0.15, and glycine - 0.15 [9]. The following neurotransmitters were used (specific radioactivity in TBq/mmol): ^{3}H-dopamine - 1.59 (Amersham), ^{3}H-adrenalin - 0.15, ^{3}H-noradrenalin - 0.24, ^{3}H-GABA - 1.78, ^{3}H-GLU - 1.6, ^{3}H-ASP - 0.86, and ^{3}H-glycine - 0.45 (Izotop). Radioactivity was measured in a liquid scintillation β-counter and expressed in decays per minute. Each experiment was performed in triplicate. The data were related to protein measured by the method of Lowry.

The results were statistically analyzed with calculation of the mean values and the confidence limits at $p=0.05$.

RESULTS

Single administration of the drugs to intact rats had no effect on reuptake of the neurotransmitters.

Repeated administration of amiridine in a dose of 1 mg/kg to intact rats for a 20-day period resulted in a statistically significant decrease in the uptake of dopamine and GABA by 54.7 and 28.8%, respectively (Fig. 1). Tacrine, piracetam, and physostigmine had no effect under these conditions.

Single and repeated administration of scopolamine in a dose of 2 mg/kg had no effect on the uptake of the neurotransmitters by brain synaptosomes. The uptake was not influenced by subsequent single injection of any of the studied drugs.

Previously, it was demonstrated that repeated administration of scopolamine during a 20-day period leads to amnesia accompanied by alterations in the microviscosity of the lipid component of brain synaptosomes. A ten-day treatment with amiridine, tacrine, and piracetam restored the ability of the animals to develop the conditioned reflex of passive avoidance and normalized the microviscosity of the neuronal membranes [1].

A course of amiridine therapy of rats given repeated injections of scopolamine led to a statistically significant ($p<0.05$) decrease in synaptosomal uptake of dopamine by 34.1% compared with the scopolamine group (Fig. 2). In contrast to amiridine, however, tacrine, piracetam, and physostigmine had no the effect on the uptake of the studied neurotransmitters.

Thus, our findings indicate that single and repeated administration of the cholinoblocker scopolamine produces no effect on the reuptake of the neurotransmitters. Only repeated administration of amiridine diminished the reuptake of dopamine and GABA and only the reuptake of dopamine in amnesia caused by repeated administration of scopolamine. We believe that these facts indicate that in a model of cholinergic amnesia the antiamnestic activity of amiridine, tacrine, physostigmine, and piracetam is not associated with reuptake of the test neurotransmitters.

However, bearing in mind the observation [3] that repeated administration of amiridine restores to a considerable degree behavioral alterations in a monkey model of Parkinson's disease (reduced dopamine content in the striatum is the major manifestation of this disorder), one can assume that the therapeutic effect of amiridine in this case arises from inhibition of dopamine reuptake by nerve terminals with a subsequent increase in its content in the synaptic gap.

The ability of dopamine to increase the amount of dopamine and GABA due to inhibition of reuptake opens up new prospects in the search for new amiridine analogs for the treatment of neurodegenerative diseases.

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