EFFECT OF AMIRIDINE AND TACRINE, DRUGS EFFECTIVE IN ALZHEIMER'S DISEASE, ON SYNAPTOSOMAL NEUROTRANSMITTER UPTAKE

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UDC 615.217.32.03:616.894-053.9].015.4

KEY WORDS: tacrine, amiridine, neurotransmitter uptake, synaptosomes.

The mechanism of action of amiridine and tacrine, substances improving cognitive functions in patients with senile dementias of Alzheimer type (SDAT), is linked with their anticholinesterase properties [2, 10, 13]. However, the action of these compounds may also be effected through other neurotransmitter systems, for it has been shown that functions of other neurotransmitter systems such as catecholaminergic [7, 15], serotoninergic [5, 7], and glutamatergic [14], are disturbed in SDAT. In particular, it has been shown that tacrine and its analogs affect catecholamine transport [7, 8].

The aim of this investigation was to compare the action of preparations effective in Alzheimer's disease, namely amiridine [9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta (B)choline hydrochloride hydrate] and tacrine with that of the cholinesterase inhibitor physostigmine, which is also used in the treatment of dementias, and the nootropic drug piracetam, on reuptake of adrenalin (AD), noradrenalin (NA), serotonin (5-HT), dopamine (DA), γ-aminobutyric acid (GABA), glutamic acid (GLU), aspartic acid (ASP), and glycine (GLY) by rat brain synaptosomes.

METHODS

The coarse synaptosomal fraction was obtained by the method described previously [3]. For the experiments 20 μliters of a suspension of the coarse synaptosomal fraction (on average 1.5 mg protein) was added to medium (0.5 ml) containing (in mM) NaCl — 100, KCl — 6, $CaCl_2$ — 6, $MgCl_2$ — 3, glucose — 10, sucrose — 100, bisodium salt of ethylenediaminetetra-acetic acid — 0.54, pargyline — 0.125, Tris-HCl buffer, pH 7.4 — 30, together with one of the following labeled drugs (with specific radioactivity): ^{3}H-5-HT (344 GBq/mmole), ^{3}H-DA (1.59 TBq/mmole) (from Amersham, England); ^{3}H-AD (0.15 TBq/mmole), ^{3}H-NA (0.24 TBq/mmole), ^{3}H-GABA (1.78 TBq/mmole), ^{3}H-GLU (1.6 TBq/mmole, ^{3}H-ASP (0.86 TBq/mmole, and ^{3}H-GLY (0.45 TBq/mmole)(from Izotop, Leningrad). Incubation was carried out at 37°C and 0°C for 3 min. Synaptosomal uptake of the neurotransmitters was stopped by filtration of 0.2 ml of the incubation medium through "Millipore" membrane filters 25 mm in diameter (pore size 0.45 M), followed by rinsing three times, each time with 5 ml of incubation medium at room temperature. The filters were dried and the residues dissolved in 10 ml of scintillation fluid containing 7 ml toluene, 3 ml of the methylene ester of ethylene-glycol, 0.5% 2,5-diphenyloxazole (PPO), and 0.01% bis-[2-(5-phenyloxazolyl)]benzene (POPOP). Radioactivity was measured on a "beta-analyzer" scintillation counter (USSR) with calculation of the mean number of counts per minute. Each experiment was conducted in three parallel determinations. Protein was measured by Lowry's method. The results were subjected to statistical analysis with calculation of the mean values and their confidence limits at $p = 0.05$.

RESULTS

The effect of the drugs on reuptake of the neurotransmitters was studied with a concentration of the latter

All-Union Research Centers for Safety of Biologically Active Substances. (Presented by Academician I. P. Ashmarin, Academy of Medical Sciences.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 4, pp. 379-381, April, 1992. Original article submitted September 17, 1991.

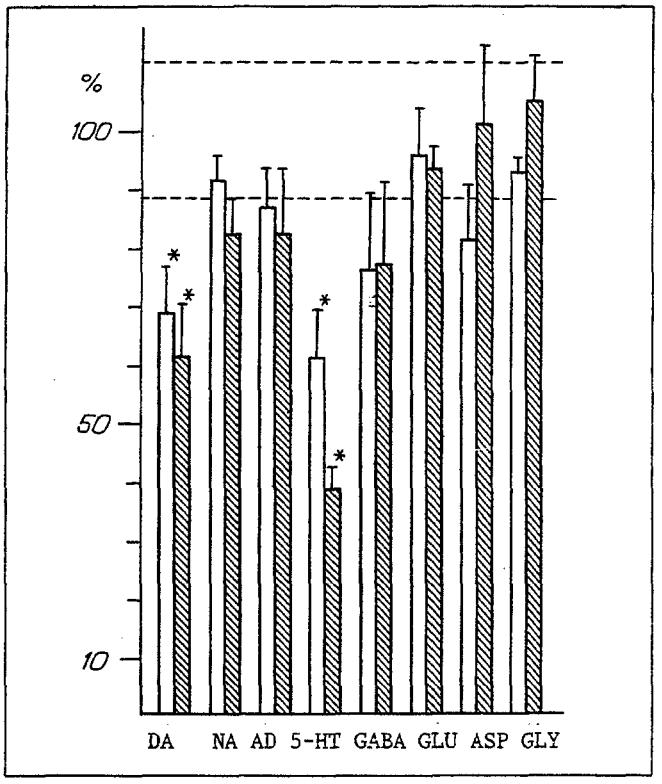

Fig. 1. Effect of amiridine and tacrine on synaptosomal uptake of neurotransmitters. Abscissa) effect of amiridine (unshaded columns) and tacrine (shaded columns) in a concentration of $5 \cdot 10^{-5}$ M on synaptosomal uptake of dopamine (DA), noradrenalin (NA), adrenalin (AD), serotonin (5-HT), aminobutyric acid (GABA), glutamic acid (GLU), aspartic acid (ASP), and glycine (GLY) (100% + 10 — uptake without drug, control); ordinate) synaptosomal uptake of neurotransmitters as percentage of control, taken as 100% (absolute values shown in Table 1). Asterisk indicates statistically significant difference from control at $p < 0.05$.

close to the Michaelis—Menten constant (K_M), i.e., the concentration of the neurotransmitter at which the rate of its synaptosomal uptake was half the maximal level, namely 0.5 μM for NA, 0.1 μM for DA and 5-HT, 10 μM for GABA [1], 30 μM for GLU [4], 0.15 μM for ASP and GLY [11], and according to our own data, 3 μM for AD.

Neurochemical spectra of the action of amiridine and tacrine in a concentration of $5 \cdot 10^{-5}$ M are given in Fig. 1. Clearly tacrine inhibits synaptosomal uptake of DA and 5-HT statistically significant, in agreement with data in [7]. Amiridine ($5 \cdot 10^{-5}$ M) also inhibits synaptosomal uptake of DA and 5-HT statistically significantly. There was no difference between amiridine and tacrine in the degree of their inhibition of synaptosomal uptake of DA and 5-HT. Inhibition of NA reuptake by synaptosomes by 50% by amiridine and tacrine was observed in the region of $5 \cdot 10^{-4}$ M concentration, in agreement with results in [8]. In a concentration of $5 \cdot 10^{-4}$ M amiridine and tacrine also suppressed synaptosomal uptake of AD and GABA, whereas tacrine, unlike amiridine, had the same action of GLU, ASP, and GLY. The investigation thus showed for the first time that amiridine, in therapeutically effective doses, inhibits synaptosomal uptake of DA- and 5-HT, and in higher doses, of AD and GABA also.

Table 1 shows that physostigmine, an anticholinesterase drug used for the treatment of Alzheimer's disease [11], inhibits only the synaptosomal uptake of 5-HT, in a concentration of $5 \cdot 10^{-4}$ M. Piracetam, a nootropic drug,

had no effect on uptake of any of the neurotransmitters tested, even in a concentration of $5 \cdot 10^{-3}$ M, in agreement with results in [6]. As was shown in [14] piracetam, by intraperitoneal injection in a dose of 75-400 mg/kg in the course of 15 days, did not change the uptake or release, and also synthesis of GABA in brain regions receiving a GABA-ergic innervation. It can be postulated that piracetam does not affect transport of AD, NA, DA, 5-HT, GABA, GLU, ASP, and GLY in nerve endings both in vitro and in vivo.

The results are evidence that the mechanisms of action of amiridine and tacrine differ from those of piracetam and the anticholinesterase drug physostigmine. According to data in [7], IC_{50} of tacrine for synaptosomal uptake of noradrenalin, dopamine, and serotinin, was 1.7 and 2 μM, respectively, a little different from our own values. This may be explained by differences in the experimental conditions, and in particular by the use of different concentrations of the neurotransmitter. Incidentally, the action of amiridine and tacrine on acetylcholinesterase is exhibited in concentrations of 10^{-7}-10^{-6} M [2, 10], much lower than concentrations which effectively inhibit synaptosomal neurotransmitter uptake (10^{-5}-10^{-4} M). On the basis of the foregoing facts it can be concluded that the effect on reuptake of neurotransmitters into nerve endings is not the dominant stage in the mechanism of the therapeutic action of amiridine and tacrine. However, there is no doubt that the neurochemical spectrum of action of amiridine and tacrine differs from that of the nootropic drug piracetam and the acetylcholinesterase inhibitor physostigmine.

The results are evidence that elements characteristic of antidepressants are present in the neurochemical spectrum of action of amiridine and tacrine [4]. These properties of the drugs can evidently explain why their use can lead to some alleviation of the state of depression accompanying the course of Alzheimer's disease [9]. The neurochemical spectrum of action of amiridine and tacrine (drugs effective in the treatment of Alzheimer's disease) is evidently different from that of piracetam and physostigmine. There is therefore good reason to regard the effect of drugs of this kind separately from drugs of the nootropic group.

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