EFFECT OF AMIRIDINE AND TACRINE, DRUGS EFFECTIVE IN ALZHEIMER'S DISEASE, ON MONOAMINE OXIDASE A AND B ACTIVITY

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Besides disturbances of the cholinergic system [7, 8], other neurotransmitter systems (noradrenergic [5, 9], serotoninergic [7, 8, 10], and dopaminergic [16]) also are damaged in the CNS of patients with senile dementia of Alzheimer type (SDAT). These lesions are expressed as a fall in the concentrations of the transmitters and their metabolites in the CNS. Increased activity of type B monoamine oxidase (MAO-B) has been observed in SDAT [6, 15, 16].

The aim of this investigation was to compare the effect of amiridine (9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta(B)choline hydrochloride hydrate) [2-4] and of tacrine, both effective in Alzheimer's disease, with physostigmine, a cholinesterase inhibitor used in the treatment of dementias [14], and also with piracetam, on activity of monoamine oxidase A (MAO-A) and MAO-B.

EXPERIMENTAL METHOD

Experiments were carried out on membrane-bound mitochondrial MAO, obtained from the brain of 20 male albino rats weighing 180-200 g. Mitochondria were obtained by differential centrifugation of rat brain homogenate [12], lyophilized, and kept at −10°C. MAO activity was determined as the quantity of ammonia released, by Conway's method followed by nesslerization [3, 11], using serotonin as the substrate for MAO-A and benzylamine as the substrate for MAO-B. The final concentration of the substrates was $2.5 \cdot 10^{-2}$ M.

Samples with a final volume of 1.0 ml contained 5 mg/ml protein in 0.01 M phosphate buffer, pH 7.4, and one of the substrates in saturating concentration. The samples were incubated in a water bath for 60 min at 37°C in an atmosphere of air. After the end of incubation, 0.2 ml of 50% TCA was added to the samples and the precipitate thus formed was removed by centrifugation.

The drugs studied were preincubated with the enzyme for 45 min at room temperature, after which the substrate was added.

Evidence of inhibition of enzyme activity was obtained by the use of pargyline (10^{-7}-10^{-5}), an inhibitor of MAO-B, and nialamide (10^{-7}-10^{-5} M), an inhibitor of MAO-A and MAO-B, which were added to the incubation mixture immediately before the enzyme reaction was triggered. Enzyme activity was expressed in nanomoles ammonia/mg protein/min. Protein was determined by Lowry's method [13]. The results were subjected to statistical analysis by Student's test at $p < 0.05$ and $p < 0.01$ levels; $n = 4$-6.

Serotonin creatinine-sulfate was obtained from Reanal (Hungary), pargyline and nialamide from Serva (West Germany), and benzylamine was of Soviet origin.

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TABLE 1. Effect of Piracetam, Amiridine, Tacrine, and Physostigmine on Rat Brain MAO Activity in Vitro (M ± m)

Substance	Concentration, M	MAO activity, per cent	
		serotonin	benzylamine
Control		100±16	100±18
Piracetam	10^{-7}	115±10	115±10
	10^{-6}	120±8	130±8
	5×10^{-5}	103±12	107±13
	10^{-4}	128±8*	145±7*
	5×10^{-4}	163±4**	170±5**
	10^{-3}	186±11**	194±9**
Amiridine	10^{-8}	101,7±5,0	105,2±5,8
	10^{-7}	106,5±4,4	107,5±7,3
	10^{-6}	100,2±4,3	111,4±8,0
	5×10^{-6}	98±13	104±8
	5×10^{-5}	102±10	88±12
	5×10^{-4}	90±7	74±6*
Tacrine	10^{-8}	95,3±5,6	99,2±6,8
	10^{-7}	110,7±4,2	115,4±9,9
	10^{-6}	109,2±6,8	104,0±7,8
	5×10^{-6}	101±12	97±11
	5×10^{-5}	83±7	81±11
	5×10^{-4}	74±12*	78±9
Physostigmine	10^{-7}	100±10	115±5
	10^{-6}	77±7	130±10
	5×10^{-6}	97±19	100±9
	5×10^{-5}	96±11	95±7
	5×10^{-4}	92±7	91±15
Pargyline	10^{-7}	N.D.	71±6*
	10^{-6}	N.D.	55±4**
	10^{-5}	N.D.	31±2**
Nialamide	10^{-7}	79±9	85±12
	10^{-6}	58±5**	73±8*
	10^{-5}	40±3**	59±17**

Legend. 100% MAO activity was taken to be 4.92 ± 1.2 and 1.61 ± 0.39 nmoles ammonia/mg protein/min for serotonin and benzylamine respectively. Results of 4-6 experiments shown; N.D.) not determined; *$p < 0.05$; **$p < 0.01$.

EXPERIMENTAL RESULTS

As Table 1 shows, piracetam in a concentration of 10^{-4} M caused statistically significant dose-dependent activation of rat brain MAO-A and MAO-B by 28 and 45%, and in millimolar concentration by 86 and 94% respectively. The results are in agreement with data in the literature [4], showing that piracetam in vitro activated MAO-A and MAO-B in the rat hypothalamus.

Physostigmine in concentrations of $5\cdot10^{-6}$-$5\cdot10^{-4}$ M had no effect on the rate of deamination of serotonin and benzylamine. Amiridine was tested in concentrations of $5\cdot10^{-6}$-$5\cdot10^{-4}$ M. In micromolar concentrations amiridine did not affect MAO-A activity, and only in a concentration of 500 μM did it give weak inhibition (by 26%) of MAO-B, evidence of the nonspecific character of interaction with the enzyme.

Unlike amiridine, tacrine in a concentration of $5\cdot10^{-4}$ M statistically significantly inhibited MAO-A (by 26%), but it had no statistically significant effect (Table 1) on the velocity of benzylamine deamination (MAO-B activity).

The unselective MAO inhibitor nialamide, in our experiments, reduced the velocity of serotonin and benzylamine deamination, in a concentration of 0.01 mM, by 60% and 41% respectively; its affinity for MAO-A in these experiments was higher than that with MAO-B. The selective MAO-B inhibitor pargyline effectively inhibited MAO-B in concentrations of 10^{-7}-10^{-5} M by 29-69% respectively (Table 1).

Our investigation of amiridine, tacrine, and physostigmine demonstrate that these drugs, in appropriate pharmacologic doses, have no effect in vitro on MAO-A and MAO-B activity in the rat brain. Consequently, it can be concluded that the therapeutic effect of amiridine and tacrine is unconnected with any influence they may have on the work of the monoamine degradation enzyme monoamine oxidases, types A and B.

Unlike the other preparations mentioned above, piracetam activated both forms of the enzyme. Considering the pharmacologically effective doses of piracetam causing a specific pharmacologic effect (improvement of memory in experiments on animals in doses starting with 250 mg/kg), it can be postulated that in the period of exhibition of the specific pharmacologic effect of piracetam both enzymes – MAO-A and MAO-B – are activated. However, considering the clinical evidence of the ineffectiveness of piracetam in the treatment of the symptom-complex of disorders of the cognitive functions, characteristic of SDAT, it can be concluded that stimulation of monoamine oxidase in the CNS in order to obtain a therapeutic effect in these cases cannot be justified.

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