COMPARATIVE CONDITIONED PASSIVE AVOIDANCE TEST STUDY OF THE EFFECT OF AMIRIDINE ON LEARNING AND MEMORY IN OLD RATS

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Amiridine is a new original preparation developed at the All-Union Research Center for Safety of Biologically Active Substances (NCSBAS), which is being effectively used for the treatment of senile dementia and, in particular, that of Alzheimer type (SDAT). The mechanisms of its antiamnesic action are currently being vigorously studied in NCSBAS. The effects of amiridine were investigated previously and compared with those of piracetam and tacrine on memory and learning processes in rats aged 3 months. The aim of the present investigation was to study the effect of amiridine, tacrine, and piracetam on learning and memory of old rats (aged 18 months) and also to study some molecular mechanisms accompanying the pharmacologic effects of these substances.

EXPERIMENTAL METHOD

Behavioral tests: tests were carried out on noninbred male rats aged 18 months (weight 500-600 g); control I comprised male rats aged 3 months and weighing 180-200 g. Each group contained 30 animals. The rats were kept at a temperature of 21-22°C with standard alternation of 12 h daylight and 12 h darkness. Access to food and water was ad libitum. The animals were kept in cages with a floor area of 2145 cm^2. In accordance with international requirements, the rats weighing 180-200 g were kept 10 in a cage, those weighing 400 g and over, 5 to a cage. The drugs were injected once a day intraperitoneally (IP) for 20 days in doses of: amiridine and tacrine 1 mg/kg, piracetam 250 mg/kg. The substances were dissolved in 0.5 ml physiological saline and control animals received physiological saline alone in the same volume. The rats started to be taught a conditioned passive avoidance reaction (CPAR) by the standard method described previously [1] 24 h after the end of the course of treatment. The effects of the substances was judged from the change in latent period of the reflex after 24 h and 7 days.

Biochemical Tests. The animals were decapitated immediately after testing the CPAR, when the brain was removed and the cortex isolated in the cold. The lipid composition was analyzed, changes in microviscosity noted, and acetylcholinesterase (AChE) activity determined by standard methods described by the writers previously [2].

EXPERIMENTAL RESULTS

As Table 1 shows, ability to learn the CPAR test was greatly impaired in the 18-month-old rats compared with rats aged 3 months: the latent period of the conditioned reflex in the control group of old rats was statistically significantly lower than in the control group of young animals, when tested both after 24 h and after 7 days. After a 20-day course of injections of amiridine, tacrine, and piracetam, the latent period of CPAR in animals aged 18 months was statistically significantly longer than in the group of old rats, and was indistinguishable from the latent period of the conditioned reflex in the groups of animals aged 3 months, when tested both after 24 h and after 7 days.

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TABLE 1. Effect of Repeated Injections of Test Drugs on Learning and Memory of 18-Month-Old Rats in CPAR Test

Preparation	Age, months	Dose, mg/kg	Latent period of changing from light compartment to dark (sec), tested after	
			24 h	7 days
Control I	3	—	151,3±19,2	109,6±24,7
Control II	18	—	64,8±12,7*	47,5±13,3*
Amiridine	18	1	130,7±25,1**	116,1±16,9**
Piracetam	18	250	136,5±17,8**	120,0±24,3**
Tacrine	18	1	147,6±21,6**	163,4±16,5**

Legend. Difference from control I statistically significant (*$p < 0.05$); differences from control II statistically significant (**$p < 0.05$).

TABLE 2. Microviscosity of Synaptosomal Membranes and Some Parameters Characterizing Course of Lipid Composition

Preparation	Age, months	Changes in m microviscosity	USI, %	Ch/PL, %	PEA/PCh, %
Control I	3	1	47,5±2,5	52,7±2,8	56,6±2,7
Control II	18	1,32***	41,3±0,9*	86,8±3,1**	49,7±3,8
Amiridine	18	1,08	52,3±2,1	68,6±3,6*	78,7±3,5*
Piracetam	18	1,07	53,6±2,0	62,3±3,3	71,7±4,3*
Tacrine	18	0,92	50,4±1,3	88,3±1,7**	66,8±2,4*

Legend: *$p < 0.05$ compared with control I; **$p < 0.01$ compared with control I; ***$p < 0.001$ compared with control I.

AChE activity of cerebral cortical homogenates from old rats was statistically significantly lower than in animals aged 3 months, by 19.6% (Fig. 1). After administration of amiridine, AChE activity was unchanged, but after tacrine, there was a further statistically significant increase of 13.0% in the degree of lowering of this parameter, and the use of piracetam led to an increase in AChE activity up to the level observed in 3-month-old animals.

The results of the study of the lipid composition of the synaptosomal membranes in old rats are given in Table 2. Aging of the rats was found to be accompanied by a decrease in the percentage content of short-chain fatty acids (C 14:0 and C 15:0) and a decrease in the content of mono-(C 18:1) and polyunsaturated (C 20:4) fatty acids, which led to a decrease in the unsaturation index (USI). An increase also was found in the Ch/PL ratio as a result of a statistically significant increase of 10% in the cholesterol concentration and reduction of 11% in the phospholipid fraction. Measurement of microviscosity showed that the flowability of the synaptosomal membranes of the old rats was significantly less than in the experimental 3-month-old animals.

Repeated injections of amiridine, piracetam, and tacrine restored the normal IN and microviscosity of the synaptosomal membranes. The use of all the test preparations led to an increase in the PEA/PCh ratio compared with 3-month-old rats, due to a fall in the phosphatidyl choline level. Amiridine also normalized the Ch/PL ratio in synaptosomes of the old rats through a decrease in the cholesterol concentration.

The investigation just described thus revealed disturbances of learning and memory in 18-month-old rats, in agreement with data in the literature [7]. Significant changes in the lipid composition of phospholipids and in the content of unsaturated fatty acids and an increase in cholesterol were observed in these animals. All these disturbances were reflected in changes in the basic characteristics of the course of the lipid composition of the synaptosomes, namely, a decrease in USI and an increase in Ch/PL, evidence of increased microviscosity of the membranes. We confirmed this fact also by another method, namely by direct measurement of microviscosity with the aid of the molecular fluorescent probe, pyrene. The results correlate with the "membrane hypothesis of aging," which, in particular, explains age-related cerebral disturbances, including changes in cognitive functions, by an increase in microviscosity of neuronal membranes [3, 5, 9]. We also found that aging is accompanied by a decrease in brain AChE activity, also in agreement with published data [4].

We showed that after a course of amiridine, tacrine, and piracetam, learning and memory processes in old rats are restored to normal. The result achieved, moreover, is sufficiently stable, for a positive effect was noted not only 24 h, but also 7 days after learning, i.e., more than a week after discontinuation of the drugs.

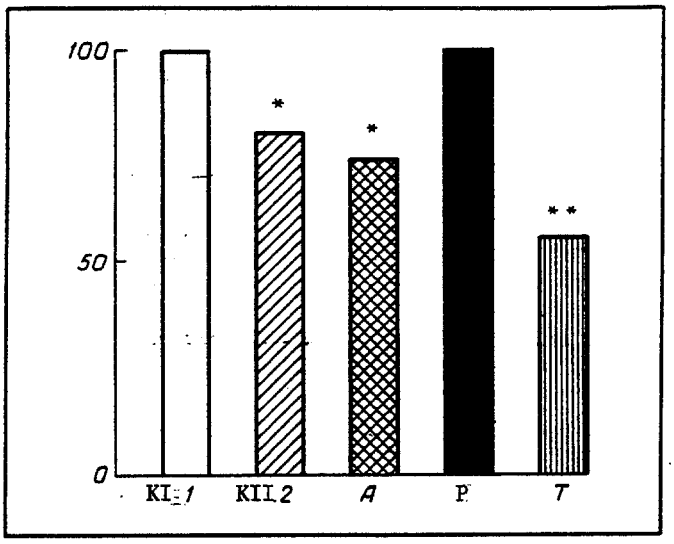

Fig. 1. Effect of amiridine, tacrine, and piracetam on brain AChE activity after their repeated injection into old rats. KI) control (3-month-old rats); KII) control (18-month-old rats); A) amiridine; P) piracetam; T) tacrine; *p < 0.05 compared with KI; **p < 0.05 compared with KII; ordinate, AChE activity, % cent; abscissa, KI, KII, A,P,T.

Meanwhile, under the influence of amiridine, tacrine, and piracetam, both the microviscosity and the fatty-acid composition of the lipids (USI) of the brain synaptosomes of old rats are restored to normal. In this respect our findings obtained for piracetam agree with views of those research workers who link the pharmacologic activity of nutropic drugs with their effect of membrane flowability in the CNS [6, 10], but the data obtained for amiridine and tacrine are new. The increase in the PEA/PCh ratio found in synaptosomes of old rats on account of a significant fall in the phosphatidylcholine content under the influence of all these compounds must be noted. It is the content of this phospholipid which, according to the "cannibalism theory," is significantly lowered in the brain of patients with SDAT, for it is utilized by the body to synthesize acetylcholine [11]. This may perhaps be a side effect for amiridine, tacrine and piracetam, and it is evidence of an undesirable action of these three drugs on neuronal membranes.

The marked selectivity of the action of these preparations on AChE activity characterizes their possible effect on the cholinergic system. The absence of changes in AChE activity under the influence of amiridine confirms our previous suggestion that its antiamnesic properties are unconnected with an anticholinesterase action [1]. Inhibition of brain AChE in old rats against the background of tacrine is evidence that it exerts its pharmacological effect in several ways including through its anticholinesterase properties, in agreement with data in the literature [8]. The normalization of AChE activity under the influence of piracetam evidently takes place through nonspecific activation of the central cholinergic system, or in other words, piracetam is a neurometabolic stimulator.

The present investigation thus shows that improvement of the conditioned-reflex activity of old rats following a course of treatment with amiridine, tacrine and piracetam, was accompanied by a mainly normalizing effect of these substances on the lipid matrix of the synaptosomal membranes of the cerebral cortex of these animals. However, the character of the changes in AChE activity under the influence of the preparations points to differences in the mechanisms of their antiamnesic activity.

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BIOKINETICS OF A NEW PROTODRUG HYDAZEPAM AND ITS METABOLITE

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Hydazepam (I) [1-(hydrazinocarbonyl)-7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one] is a new original therapeutic preparation of benzodiazepine structure, recommended for clinical use as a tranquilizer and combining an anxiolytic and anticonvulsant action with mildness of its side effects and with low toxicity. In experimental animals I undergoes intensive N^1-dealkylation with the formation of a physiologically active metabolite, namely 7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one (II) and products of its further oxidation [5]. The affinity of II for benzodiazepine receptors in the CNS is higher (by more than 3 orders of magnitude) than that of I. Accordingly, I can be regarded as a protodrug, and II as a drug-metabolite [4].

The aim of this investigation was to substantiate methods of effector analysis of the pharmacokinetics of I, and to determine the biokinetic features of the protodrug (I) and to compare them with the corresponding parameters of its physiologically active metabolite (II).

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice weighing 18-22 g – intact mice and mice receiving various doses (0.7-22.4 mg/kg) of 2-^{14}C-I (0.27 Ci/mole) intraperitoneally within a time interval (0.083-24 h) of the experiment. Minimal effective doses of metrazol (mg/kg) inducing the development of clonicotonic convulsions (DCTC) and tonic extension (DTE) [8, 11], by intravenous infusion (0.01 ml/sec) of a 1% solution into the caudal vein, were determined as the recorded parameters of the pharmacodynamics. Parallel determinations were made of concentrations of ^{14}C-products in the animals' brain. The methods of determination of ^{14}C-compounds in the mouse brain during simultaneous recording (±1 min) of the values of DCTC and DTE, were described previously [3, 6]. Concentrations of ^{14}C-products in the blood plasma and brain of the mice were determined within the interval of 0.017-24 h and 0.017-6 h of the experiment after intraperitoneal injection of ^{14}C-I and ^{14}C-II (0.70 and 0.78 Ci/mole) intraperitoneally into the animals in doses of 1.4 mg/kg, by methods described in [5, 7]. The experimental data were analyzed using algorithms described in [2, 10].

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