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ANTIAMNESIC ACTIVITY OF AMIRIDINE ON A MODEL OF THE AMNESIC SYNDROME

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Amiridine is a drug which has evoked considerable scientific interest because of its efficacy in the treatment of senile dementia and disturbances of cognitive functions of varied genesis. However, the mechanism of its action remains unexplained, and this has delayed the search for more effective substances with a similar direction of therapeutic activity [2].

The investigation described below is a continuation of the experimental study of the neurochemical mechanisms of the antiamnesic activity of amiridine, a new product developed at the All-Union Research Center for Safety of Biologically Active Substances, Ministry of the Medical Industry of the USSR

EXPERIMENTAL METHOD

Behavioral Tests. Tests were carried out on noninbred male rats weighing 180-200 g. Each group consisted of 30 animals. The rats were kept at a temperature of 21-22°C with standard exposure to 12 h of daylight and 12 h of darkness. Food and water were allowed ad libitum. The compounds were injected intraperitoneally in the course of 20 days: amiridine and tacrine in a dose of 1 mg/kg, physostigmine in a dose of 0.1 mg/kg, and piracetam in a dose of 250 mg/kg. The drugs were dissolved in 0.5 ml of physiological saline, and animals of the control group received physiological saline only. The rats were trained in the conditioned passive avoidance reaction (CPAR) 24 h after the end of administration of the drugs,

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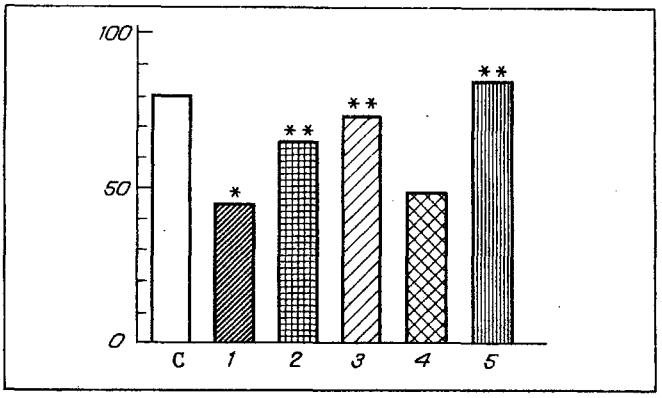

Fig. 1. Effect of amiridine and comparison drugs on disturbances of conditioned reflex activity of rats induced by repeated injections of scopolamine. C) Control, 1) animals receiving scopolamine (experimental group), 2) experimental group of animals treated with piracetam (250 mg/kg), 3) experimental groups of animals treated with amiridine (1 mg/kg), 4) experimental group of animals treated with physostigmine (0.1 mg/kg), 5) experimental group of animals treated with tacrine (1 mg/kg). *$p < 0.01$, Significant differences compared with control, **$p < 0.01$ compared with untreated animals, receiving scopolamine Ordinate, number of correct responses (in %).

by the standard method described previously [2]. The effect of the drugs was judged by the change in latent period of the reflex. An amnesic syndrome was produced by intraperitoneal injection of scopolamine in a dose of 1 mg/kg for 20 days. To assess the antiamnesic action the drugs were injected for 10 days in the above-mentioned doses immediately after the end of the course of scopolamine. Training in the CPAR was carried out 24 h after the end of the course of drugs.

Biochemical Tests. The animals were decapitated immediately after testing of the CPAR, when the brain was removed and the cortex isolated in the cold. Tissues of the rats' cerebral cortex were homogenized and synaptosomes isolated by De Robertis' method [5] and lipids were extracted by Kates' method [3]. Total lipids and phospholipids were fractionated by unidimensional ascending thin-layer chromatography-on silica-gel followed by their quantitative determination. The fatty acid composition was estimated by gas—liquid chromatography [3] on a Chrom-5 chromatograph (Czechoslovakia). The index of unsaturation of the fatty-acid composition (IUS), the cholesterol — phospholipids ratio (ChS/PL), and the phosphatidylethanolamine/phosphatidylcholine ratio (PEA/PCh), the principal parameters of flowability of the lipid composition of membranes [1, 4], were determined.

Acetylcholinesterase (AChE) activity in cerebral cortical homogenates from the rats was determined by the spectrophotometric method of Ellman et al. [6]. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

After repeated injections of scopolamine, a long period of worsening of the conditioned-reflex activity of the experimental animals was observed, lasting for at least 10 days after its withdrawal (Fig. 1). During a subsequent 10-day course of amiridine (1 mg/kg), tacrine (1 mg/kg), and piracetam (250 mg/kg) these drugs exhibited considerable antiamnesic activity, but physostigmine was ineffective.

It is interesting to note that repeated injections of amiridine, tacrine, and piracetam for 20 days into healthy rats in doses used to treat animals with an amnesic syndrome did not affect their ability to be trained in the CPAR. Regular injections of physostigmine led to inhibition of conditioned reflex formation. The latent period of the reflex in the control was 94.56 ± 17.62 sec, but after repeated injected of physostigmine it was 32.20 ± 10.05 sec ($p < 0.01$).

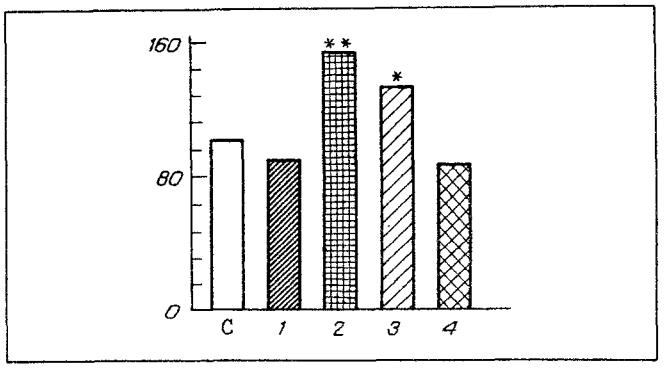

Fig 2. AChE activity of cerebral cortical homogenates from rats after repeated injections of the test drugs. C) Control, 1) group of animals receiving piracetam (250 mg/kg), 2) group of animals receiving physostigmine (0.1 mg/kg), 3) group of animals receiving tacrine (1 mg/kg), 4) group of animals receiving amiridine. *$p < 0.01$, **$p < 0.001$ compared with control Ordinate, AChE activity (in %).

The results showing the effect of single injections of the test drugs on AChE activity were fully described previously [2]. After injection of the drugs for 20 days, a statistically significant increase in brain AChE activity, which at first glance was unexpected, was observed in groups of animals receiving physostigmine and tacrine. Amiridine and piracetam did not affect activity of the enzyme (Fig. 2). Repeated injections of scopolamine were not accompanied by changes in activity of the enzyme, and subsequent courses of injections of the test drugs likewise did not affect AChE activity of the rat cerebral cortex. A single injection of the drugs in doses used in the repeated injection schedules did not induce significant changes in the fatty-acid phospholipid composition of the synaptosomes. The parameters of flowability of the lipid composition of the cerebral cortical membranes of the rats under these experimental conditions are given in Table 1. Repeated injections of scopolamine were accompanied by a significant reduction of IUS by 17% and an increase in the ChS/PL ratio by 54% on account of reduction of PL. These changes continue for 10 days after withdrawal of the amnesia-inducing agent. As a result of a course of treatment with amiridine and tacrine, of animals receiving scopolamine, complete normalization of all the parameters of flowability of the lipid composition of the synaptosomes were restored. Piracetam also restored normal IUS and a normal ChS/PL ratio, but this drug caused a statistically significant increase in the PEA/PCh ratio by more than twice due to a decrease in the PCh content. Physostigmine did not affect the changes in parameters of flowability characteristic of the model of the amnesic syndrome. Repeated injections of amiridine and tacrine into healthy rats caused no changes in IUS; piracetam significantly increased this parameter by 17.7% whereas physostigmine reduced it by 13.5%. Furthermore, tacrine and piracetam were able to reduce the ChS/PL ratio on account of an increase in the PL fraction.

Scopolamine, a blocker of central acetylcholine receptors, is widely used in neuropharmacological research to create of model of memory disturbances and, in particular, of Alzheimer's disease [7, 10]. Usually the compound is given in a single dose, but we gave a systematic course of injections lasting 20 days. The persistent worsening of the conditioned-reflex activity of the animals which we found even 10 days after the end of the scopolamine injections was accompanied by lowering of IUS and an increase in the ChS/PL ratio. This trend of changes in the parameters of the lipid composition of the rat brain synaptosomes characterizes the depression of membrane flowability [1, 4] which, on the whole, correlates with the membrane hypothesis of aging [9], linking age-related cerebral disturbances with changes in the composition and functions of neuronal membranes. It is interesting to note that we found no changes in the lipid composition of the synaptosomes in amnesia caused by a single injection of scopolamine. The results suggest that amnesia caused by repeated injection of scopolamine is based on deeper disturbances of the neurochemical mechanisms of memory processes, possibly simulating aging to some degree.

It was found that a course of treatment with all the test drugs except physostigmine statistically significantly improved learning and memory of the experimental animals in the CPAR test. The antiamnesic action of amiridine, tacrine, and piracetam was accompanied under these circumstances mainly by normalization of the parameters of flowability of the lipid composition of the synaptosomes. Our results are in agreement with views held by many workers regarding the connection between pharmacological activity of nootropic drugs and their influence on membrane flowability in the central

TABLE 1. Parameters of Flowability of Lipid Composition of Synaptosomes from Experimental Rats Treated with Test Drugs

Compound	Dose, mg/kg	Duration of course, days	IUS, %	ChS/PL	PEA/PCh
				%	
Control	—	—	47,5±2,5	59,3±5,1	46,5±8,7
Piracetam (P)	250	20	55,9±2,1*	41,6±3,8*	60,5±6,3
Amiridine (A)	1	20	42,3±2,7	53,2±3,8	57,6±4,8
Physostigmine (Ph)	0,1	20	41,4±0,6*	61,2±2,7	41,9±4,1
Tacrine (T)	1	20	46,7±2,4	47,2±1,4*	58,7±6,3
Scopolamine (Sc)	2	20	39,6±2,5*	91,3±4,8**	66,5±8,9
Sc + physiological saline	2	20+10	35,2±2,4**	86,6±3,9**	31,1±4,6
Sc + P	2±250	20+10	47,1±1,4	68,8±2,1	94,5±5,5**
Sc + A	2+1	20+10	42,5±1,2	72,6±3,4	58,1±3,8
Sc + Ph	2±0,1	20+10	38,1±1,0**	85,1±3,6**	38,6±4,5
Sc + T	2+1	20+10	48,9±1,0	72,8±3,4	55,9±4,8

Legend. *$p < 0.05$, **$p < 0.01$.

nervous system [8, 11], although amiridine and tacrine cannot be classed in the group of nootropic agents [2]. It must be pointed out that repeated injection of amiridine and tacrine, like piracetam, into healthy animals did not affect conditioned reflex formation.

Physostigmine, an AChE inhibitor with central action, was used under experimental conditions to elucidate the role of the anticholinesterase effect in the realization of the antiamnesic activity of the drug. The worsening of conditioned reflex activity of the healthy rats which was revealed after repeated injections of the drug, accompanied by reduction of membrane flowability (IUS), and also the total inefficacy of physostigmine on a model of the amnesic syndrome are evidence in support of the earlier hypothesis [2] that the antiamnesic properties of amiridine and tacrine are unrelated to their antianticholinesterase action. We also observed considerable activation of AChE, evidently compensatory in nature, 24 h after the last injection of the drug (0.1 mg/kg), whereas a single injection of this dose caused a significant decrease in activity of the enzyme [2].

This investigation thus showed that repeated injections of scopolamine led to persistent worsening of conditioned-reflex activity of the animals in the CPAR test, accompanied by changes in the lipid composition of the synaptosomes, evidence of reduction of membrane flowability. The antiamnesic activity of amiridine, tacrine, and piracetam correlated with their normalizing effect on the lipid composition of the synaptosomes. It must be pointed out that under experimental conditions the pharmacological activity of a drug and, in particular, of amiridine was exhibited only on models of pathology. The opposite direction of the effects of physostigmine, on the one hand, and amiridine and tacrine on the other hand, suggests that amiridine and tacrine cannot be included in the class of anticholinesterase agents, traditionally used in Alzheimer's disease. Their effect on cognitive functions is evidently based on different neurochemical mechanisms.

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