EXPERIMENTAL STUDY OF THE EFFECT OF AMIRIDINE AND TACRINE ON LEARNING AND MEMORY

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A new product for the treatment of nervous and mental diseases has been developed at the All-Union Research Center for Safety of Biologically Active Substances, namely amiridine (9-amino-2,3,5,6,7-hexahydro-1H-cyclopenta[b]quinoline monohydrate hydrochloride). This preparation is highly effective in the treatment of various diseases of the peripheral nervous system connected with a disturbance of neuromuscular transmission, and also in lesions of the CNS accompanied by memory disturbances of varied genesis (senile feeblemindedness, senile dementia, craniocerebral trauma, cerebrovascular disturbances) [1, 9]. In 1981 the preparation tacrine (9-amino-1,2,3,4-tetrahydroacridine monohydrate hydrochloride), similar to it in structure and clinical properties in relation to senile feeblemindedness, was described [11]. The mechanism of action of tacrine has been linked with its anticholinesterase properties [5, 12], for senile dementia of Alzheimer type is characterized by a functional deficit of the cholinergic system [2].

The aim of this investigation was to compare the effect of amiridine and tacrine on learning and memory in rats and mice in the conditioned passive avoidance reaction (CPAR) test in normal animals and on a model of amnesia induced by injection of scopolamine (Sc), a central acetylcholine receptor blocker.

EXPERIMENTAL METHOD

Behavioral Tests. Experiments were carried out on 240 noninbred male albino rats weighing 180-200 g and 480 male mice weighing 18-22 g. The animals were kept was used at 21-22°C, on a standard schedule of 12 h of daylight and 12 h of darkness was used. Food and water were allowed ad libitum. A CPAR method based on the inborn attraction of dark spaces to rodents [3]. The rat was placed in the lit compartment of a two-compartment chamber. For 180 sec the animal could move freely between the two compartments. After 180 sec it was exposed in the dark compartment to inescapable nociceptive stimulation by five consecutive pulses of alternating current (5 mA, each pulse 1 sec in duration, intervals of 2 sec between pulses). Animals which did not visit the dark compartment in the course of 1 min were eliminated from the experiment. During testing (by replacing the animal in the illuminated compartment after 24 h), observations were kept on the animals for 3 min. Under these conditions of testing, the ability of the rats to learn in the control series averaged 50%. The effect of the drugs was assessed as the change in percentage of animals not visiting the dark compartment. The test drugs were injected intraperitoneally: amiridine (0.1, 0.2, 1.0, and 5.0 mg/kg), tacrine (0.1 mg/kg) 20 min before training, piracetam (250 mg/kg) 1 h before training; animals of the control group received the corresponding volume of physiological saline. To each of the six experimental groups there was a corresponding control, and all groups consisted of 20 animals.

The mice were trained in the CPAR under the same conditions as rats, but the series of stimuli, similar to that described in the experiments on rats, was applied immediately after the first movement of the animal from the light into the dark compartment. Mice not visiting the dark compartment in the course of 30 sec were eliminated from the experiment. Testing was carried out after 24 h by the method described above for 180 sec. On application of the current to the

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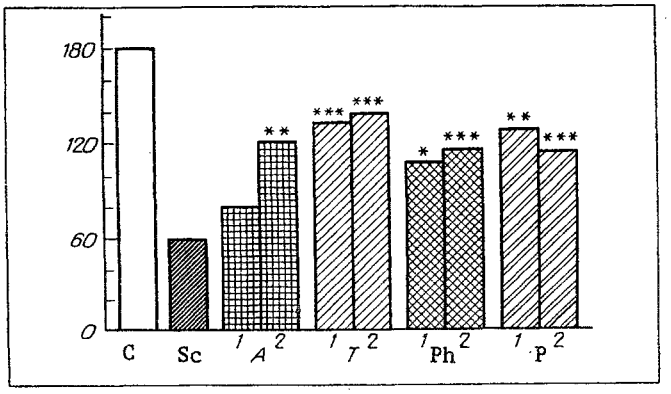

Fig. 1. Effect of test drugs on disturbances of conditioned-reflex activity of mice caused by injection of Sc, C) Animals of control group, Sc) group of animals receiving Sc (1 mg/kg). A) Group of animals receiving amiridine (0.1 mg/kg), T) group of animals receiving tacrine, Ph) group of animals receiving physostigmine, P) group of animals receiving piracetam. 1) Before training in CPAR. 2) Immediately after training in CPAR. Ordinate, latent period (in sec). *p < 0.05, **p < 0.01, **p < 0.001 compared with Sc group.

grid floor after the animal's first movement from the light to the dark compartment, ability to learn in the control series was taken as 100%. Amnesia was induced by injection of scopolamine intraperitoneally in a dose of 1 mg/kg 15 min before training [13]. The antiamnesic activity of the drugs was estimated as the change in latent period of movement of the mice from the light into the dark compartment, in seconds. The test drugs were injected intraperitoneally in accordance with two schedules: the first consisted of amiridine, tacrine, and physostigmine in a dose of 0.1 mg/kg 20 min before training and 5 min, correspondingly, before the injection of scopolamine, the second consisted of amiridine, tacrine, and physostigmine in a dose of 0.1 mg/kg and piracetam in a dose of 500 mg/kg immediately after training. Each series of experiments was accompanied by a corresponding parallel control: instead of the test substances and scopolamine, animals of one control group received physiological saline, a second control group received seopolamine and physiological saline instead of the test substances in accordance with the corresponding schedules and in the same volumes. Each of the eight experiment and four control groups consisted of 40 animals.

Biochemical Tests. To determine aeetyleholine esterase (AChE) activity the animals in experiments in vivo were decapitated 20 min after injection of the test compounds, the brain was removed and, in the cold, the cortex was separated, and tissue homogenates were prepared by the standard method using 0.1 M phosphate buffer, pH 7.0. Activity of the enzyme was determined spectrophotometrically by Ellman's method [6]. In the experiments in vitro, AChE was obtained from human blood erythrocytes, and produced by the Perm Research Institute of Vaccines and Sera.

The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Amiridine was shown to improve learning and memory of the experimental animals when initially untrained; the effect, moreover, was comparable with that of piracetam: the percentage of trained animals after injection of amiridine in doses of 0.1 and 0.2 mg/kg was 95 and 80 respectively, compared with 90 in animals receiving piracetam 250 mg/kg, and 50 in the control. When the dose of amiridine was increased to 5 mg/kg inhibition of the conditioned-reflex activity of the experimental animals was observed. Since LD_{50} of tacrine and amiridine is similar, and amounts to 35 and 52 mg/kg respectively, when the effect of tacrine on untrained animals was used the preparation was given in a dose of 0.1 mg/kg, but was ineffective.

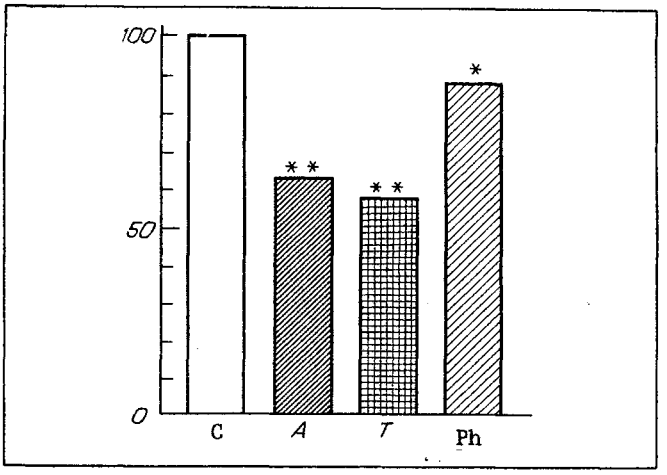

Fig. 2. Effect of amiridine, tacrine, and physostigmine on AChE activity of rat cerebral cortical homogenates, C) Control group of animals, A) group of animals receiving 30 mg/kg amiridine, T) group of animals receiving 5 mg/ml tacrine, Ph) group of animals receiving 0.1 mg/kg physostigmine, Ordinate, AChE activity (in %). *$p < 0.005$, **$p < 0.01$, ***$p < 0.001$ compared with control.

A study of the antiamnesic activity of amiridine on a model of Sc-amnesia in mice showed that administration of 0.1 or 1.5 mg/kg of the drug before training was ineffective. The antiamnesic activity of amiridine was observed after injection of a dose of 0.1 mg/kg immediately after training of the mice in the CPAR test. All the comparison preparations were active irrespective of the program of administration (Fig. 1).

The effect of a wide range of doses of the test substances (0.05-30 mg/kg) on AChE activity of the rat cerebral cortical homogenates was studied in experiments in vivo. They showed that amiridine significantly inhibited the enzyme only in a dose of 30 mg/kg, tacrine did so starting with a dose of 5 mg/kg, and physostigmine in a dose of 0.1 mg/kg (Fig. 2). It is interesting to note that in experiments in vitro all the drugs caused marked inhibition of AChE: K_i for amiridine, tacrine, and physostigmine was $(3.86 \pm 0.66) \cdot 10^{-7}$, $(3.61 \pm 0.15) \cdot 10^{-7}$, and $(0.76 \pm 0.19) \cdot 10^{-7}$ M respectively. Scopolamine and piracetam had no anticholesterase activity in vitro or in vivo.

The therapeutic efficacy of amiridine, tacrine, and physostigmine in the treatment of dementia is linked with their central anticholinesterase properties [1, 9, 12, 13]. We found in experiments in vivo that physostigmine has antiamnesic activity in a dose inhibiting brain AChE, in agreement with data in the literature. Tacrine did not exhibit activity on a model of Sc-amnesia in a dose of 0.1 mg/kg, i.e., in a dose in which it does not possess anticholinesterase activity. It can therefore be tentatively suggested that this property of the drug is not dependent on inhibition of the enzyme. A similar view is held by Levy [8], who analyzed the results of the clinical use of tacrine to treat patients with Alzheimer's disease. Amiridine in doses ineffective against AChE, unlike tacrine, improves the ability of initially untrained rats to learn, in the same way as the nootropic agent piracetam, and it also possesses antiamnesic activity when given to mice before training, just like piracetam and tacrine. On this basis it can be postulated that realization of the antiamnesic effect of amiridine is not connected with inhibition of brain AChE.

Thus the view widely held in the literature that the therapeutic efficacy of tacrine and amiridine in the treatment of senile dementia is linked with their anticholinesterase properties [5, 10, 12] was not confirmed by the present experiments.

Considering that tacrine and amiridine are still the only preparations improving the state of patients with senile dementia of Alzheimer type, and that they differ in principle in this respect from nootropic drugs, there is good reason to regard pharmacologic agents of this type separately from the nootropic group of drugs.

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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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