and it justifies the recommendation that LE activity under these circumstances be tested both in liver tissue and in blood serum.

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EFFECT OF POLYMETHYLENE DERIVATIVES OF 4-AMINOPYRIDINE ON HIPPOCAMPAL NEURON FUNCTION

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Among drugs used previously to correct cognitive disorders in senile dementia of Alzheimer type (SDAT), the most effective has proved to be tacrine [13], which is an aminoacridine derivative of 4-aminopyridine, a substance well known as blocker of potassium channels of a particular type [14]. It has been suggested that the high efficacy of tacrine (tetrahydroaminoacridine) may be due to its special pharmacologic spectrum, in which anticholinesterase activity is combined with ability to block potassium channels [13]. A similar spectrum is possessed also by substances belonging to a different class of polymethylene derivatives of 4-aminopyridine, namely the aminoquinoline class [1], among which special attention has been drawn to amiridine [9-amino-2,3,5,6,7,7'-hexahydro-IH-cyclopenta(B)-quinoline], a compound synthesized in the USSR and recommended for clinical use as a stimulator of conduction of excitation in nerve and muscle tissue. Amiridine has been shown to facilitate the learning process in animals with models of congenital and acquired memory disturbances [8]. Neurochemical changes linked with SDAT are most marked

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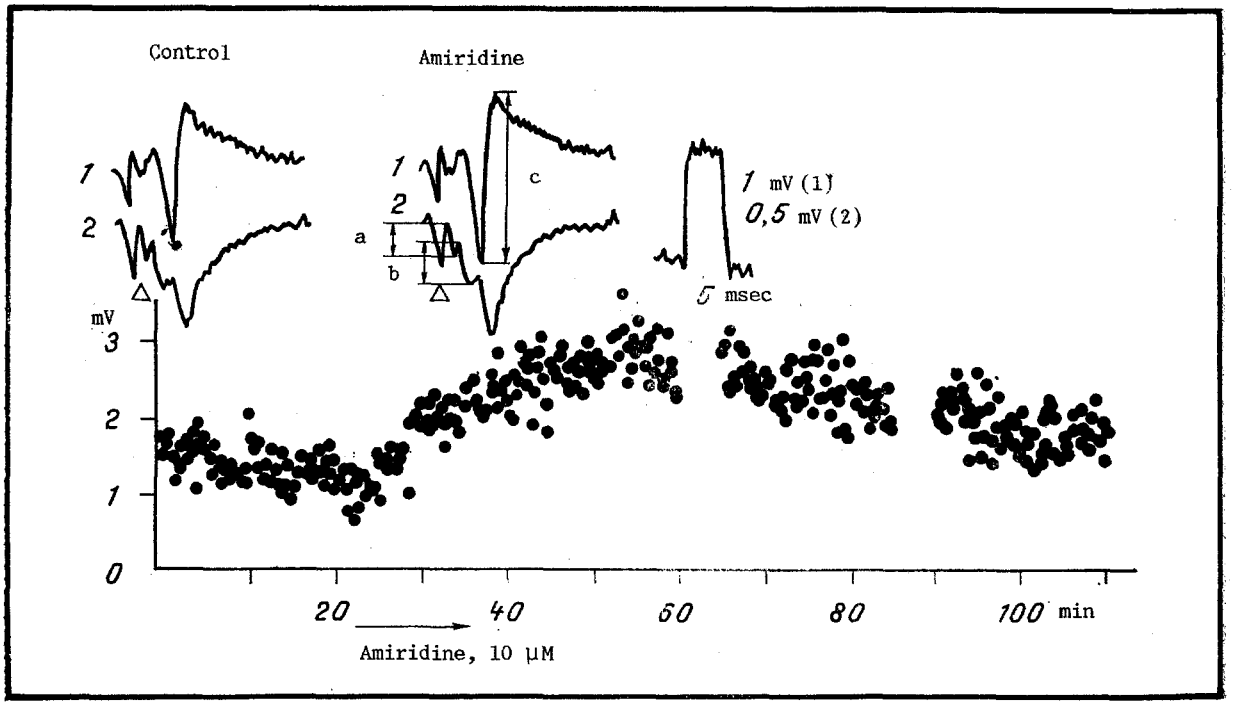

Fig. 1. Prolonged increase in amplitude of pop-spike under the influence of amiridine. Top traces: 1) field potential recorded in pyramidal layer during stimulation of Schaffer's collaterals; 2) field potentials recorded in radial layer; a) fiber potential; b) leading edge of pop-EPSP, relative to which amplitude or rate of rise of pop-EPSP was measured; c) pop-spike. Graph showing running amplitude of pop-spike, measured as indicated in c. Gaps in curve correspond to interruptions in recording of potentials.

in the hippocampus [5, 6, 9], a key structure in the organization of memory. It was considered important to compare the action of amiridine and tacrine on function of hippocampal neurons. This paper describes the study of the action of these substances and of 4-aminopyridine on CAI field potentials in rat hippocampal slices.

EXPERIMENTAL METHOD

Transverse slices of the hippocampus were cut from the brain of Wistar rats aged 4-8 weeks and transferred to a continuous-flow chamber filled with oxygenated medium, heated to 30-31°C, and fixed on a nylon grid, located 1-1.5 mm below the level of the liquid. The medium was a modified Krebs–Ringer solution of the following composition (in mM): NaCl 124, KCl 3, $CaCl_2$ 2.5, $MgSO_4$ 2.4, KH_2PO_4 1.25, $NaHCO_3$ 26, D-glucose 10. The solution was saturated with a mixture of 95% O_2 and 5% CO_2 and its pH was 7.2-7.4. The rate of flow was 1.5-2 ml/min, ensuring complete replacement of the medium in the chamber every 2-3 min. Recording microelectrodes filled with 3 M NaCl were located on the pyramidal layer of area CAI in order to record the population (pop) spike of the pyramidal neurons, and on the radial layer in order to record the fiber potential and the population (pop) EPSP. Bipolar stimulating electrodes were located on the radial layer to activate Schaffer collaterals and commissural fibers. Recording of electrical activity began 1.5-2 h after preparation of the slices. Field potentials evoked by single periodic (1/15 sec) stimulation by square pulses (0.1 msec) were recorded and analyzed on a Labtam microcomputer. The action of substances on reactivity was assessed from the curve of the running amplitude of the pop-spike and averaged field potentials (pop-spike and pop-EPSP). The substances were dissolved in the perfusion medium and added to the chamber by switching the flow system to the corresponding reservoir for 10 min. The amiridine used in the experiments was synthesized by the All-Union Research Institute of the Nitrogen Industry of the USSR and by the firm of "Nikken" (Japan), the tacrine was obtained from "Aldrich Chemicals Limited" and the 4-aminopyridine from "Sigma."

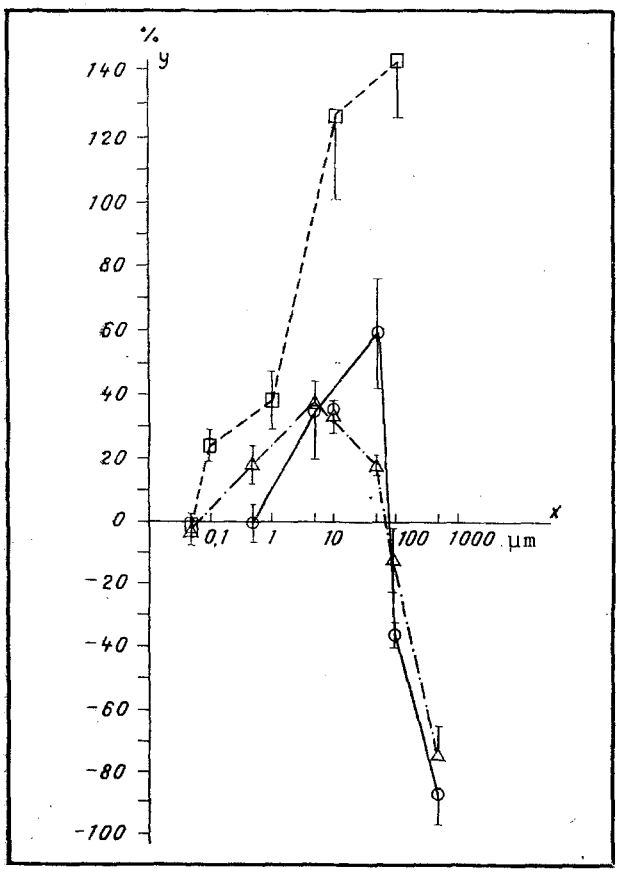

Fig. 2. Dependence of effect of amiridine, tacrine, and 4-aminopyridine on amplitude of pop-spike on concentration of substance. X axis) concentration of substance on a logarithmic scale; Y axis) relative changes in mean value of pop-spike (in % of control). Circles – experiments with amiridine, triangles – with tacrine, squares – with 4-aminopyridine (mean values and standard errors of means).

EXPERIMENTAL RESULTS

Perfusion of the slices with a solution containing 5-50 μM amiridine caused an increase in amplitude of the pop-spike which appeared 7-10 min after the beginning of perfusion and reached its maximum after 15-20 min, when the solution had already been replaced by normal. Recovery of the initial magnitude of the response required prolonged rinsing (more than 60 min), but the effects were not always completely reversible. The typical time course of the development of the response to the average concentration (10 μM) of amiridine is shown in Fig. 1.

The mean effect was an almost linear function of the amiridine concentration between 0.5 and 50 μM (Fig. 2). At a concentration of 100 μM and higher, instead of being facilitated the pop-spike was inhibited, and completely blocked at a concentration of about 1 mM. Strong inhibition of the pop-spike was associated with inhibition of all components of the field potential, including the fiber potential. This inhibition was reversible, and after restoration of the original value, further additional growth of the amplitude of the pop-spike was observed.

At concentrations facilitating the pop-spike the characteristics of the fiber potential showed no appreciable change, but the amplitude of the pop-EPSP was reduced a little on average (Table 1).

Tacrine also caused similar changes to the pop-spike (Fig. 2), the only difference being that facilitation of the pop-spike appeared at a concentration as low as 0.5 μM, and its inhibitory action appeared at concentrations of 10-50 μM. Tacrine also had a long after-effect: the small increase in the pop-spike could continue for 1.5 h. At low

TABLE 1. Effect of Amiridine, Tacrine, and 4-Aminopyridine on Amplitude of Pop-EPSP (relative changes in % of control); M ± m

Concentration, μM	Amiridine	Tacrine	4-Aminopyridine
0,05		12,5±8,9 (2)	3,0±4,7 (3)
0,1			2,9±3,8 (5)
0,5	—9,0±5,2 (4)	7,3±6,4 (3)	
1,0			2,0±1,3 (5)
5,0	—5,0±4,3 (4)	—1,3±2,4 (4)	
10,0	—7,4±4,5 (5)	—4,7±3,8 (3)	47,8±10,6 (4)
50,0	—6,4±5,1 (4)	—7,8±3,9 (6)	
100,0	—3,0±2,5 (3)	—1,0±7,8 (3)	54,0±13,5 (2)
500,0	—86,0±9,9 (2)	—31,7±13,8 (3)	74,0±18,4 (2)

Legend. Number of preparations for which the action of the given concentration of the substance was measured is shown between parentheses.

concentrations (0.05 and 0.5 μM) tacrine caused a small increase, whereas at higher concentrations it caused a small decrease in the mean amplitude of the pop-EPSP (Table 1).

Comparison of the action of amiridine and tacrine with that of 4-aminopyridine revealed significant differences between them in both the efficacy and character of their action: 4-aminopyridine, which caused marked facilitation of the pop-spike at a concentration as low as 0.1 μM, potentiated facilitation when the concentration was increased to 100 μM (Fig. 2). A further increase in concentration led to transformation of the response into epileptiform (multiple pop-spikes of high amplitude, followed by a high-amplitude slow wave) and to the appearance of spontaneous epileptiform activity (about 1 mM). Growth of the pop-spike was accompanied by an increase in the mean amplitude of the pop-EPSP (Table 1). At concentrations of over 500 μM, widening of the fiber potential and lengthening of LP of peak amplitudes of the pop-EPSP and pop-spike were observed. Like its polymethylene derivatives, 4-aminopyridine had a very long aftereffect.

Changes in field potentials observed following administration of 4-aminopyridine corresponded to those described previously for hippocampal slices [2] and were in good agreement with existing views regarding increased release of mediator as the principal mechanism of its action, coupled with blocking of potassium conduction and an increase in the inflow of calcium [14]. Since facilitation of the pop-spike by amiridine and tacrine was not accompanied by an increase in amplitude of the pop-EPSP, it was evidently different in nature. This is also indicated by inhibition of field potentials in response to an increase in concentration of the substances.

It was shown recently that tacrine blocks not only fast potassium conduction, sensitive to 4-aminopyridine, but also fast inward (sodium) and slow outward (potassium) currents [11]. Changes in reactivity which we observed could be the result of these changes of conductivity. Facilitation of spike activity (and the small decrease of the pop-EPSP) at low concentrations could be caused by weak depolarization as a result of blockade of slow potassium currents. Tacrine in fact induced depolarization of pyramidal cells, accompanied by a decrease of conductivity [12]. Inhibition of field potentials may be connected with blocking of sodium conduction. The similarity of the changes in field potentials evoked by amiridine and tacrine suggests common mechanisms of their action on transmembrane currents.

The long trace effects are an interesting aspect of the action of amino pyridine and its derivatives. On the one hand, they can be explained by accumulation of the substances in cellular structures and their very slow elution. However, the possibility cannot be ruled out that the long aftereffect may reflect the ability of the test substances to activate processes enabling prolonged maintenance of enhanced reactivity through an increase in, for example, the inflow of calcium into the neurons during depolarization, i.e., by

means of the same mechanism as that which acts as the trigger for development of long-lasting potentiation of synaptic efficacy in the hippocampus during electrical stimulation [3]. Although activation of calcium processes by amiridine or tacrine has not yet been described, 4-aminopyridine is known to stimulate calcium currents and to facilitate calcium spikes in neurons of several CNS structures [7, 10, 15].

To conclude, therapeutically effective serum tacrine concentrations in patients with SDAT lie within the range 5-70 mg/ml [13], i.e., 0.02-0.3 μM. Minimal effective concentrations of tacrine in the present experiments (0.05 μM for the pop-EPSP and 0.5 μM for the pop-spike) were close to these values, suggesting that prolonged facilitation of reactivity of hippocampal pyramidal cells is an essential component of the therapeutic action of tacrine. The similarity of the effects of amiridine and tacrine on evoked activity in the hippocampus supports the view that effective paliative treatment of SDAT with amiridine is possible [8].

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