PHARMACOLOGY AND TOXICOLOGY

Effect of Amiridine and Tacrine on the Functional Degeneration of the Isolated Neuron

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Amiridine and tacrine are found to have a concentration-dependent effect on the spontaneous activity of an isolated neuron from crawfish. Amiridine in a concentration of 1 μM reliably prolongs the lifetime of the neuron, whereas lower concentrations are inactive and a high concentration (10 μM) reduces spontaneous activity. Tacrine is unable to prolong the lifetime of the neuron. It is suggested that, unlike tacrine, the therapeutic effect of amiridine stems from its ability to prolong neuronal functioning.

Key Words: *amiridine; tacrine; action potential; isolated neuron; spontaneous activity*

Amiridine, a new preparation highly effective in the treatment of cognitive disorders of various etiology including Alzheimer's senile dementia (ASD), has been synthesized at the Russian Research Center for the Safety of Bioactive Compounds [1,8]. Tacrine, an agent structurally similar to amiridine, is used in the West for the treatment of ASD [11]. Experiments with various models of memory disorders have demonstrated that both amiridine and tacrine possess pronounced antiamnestic activity [4,6,9]. Amiridine weakens behavioral signs of Parkinson's disease in monkeys induced by neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) [2]. Degradation of cholinergic and dopaminergic neurons is known to be a characteristic feature of ASD and Parkinson's disease, respectively [12,13]. Hence, the use of a spontaneously degrading isolated neuron as a model of a key pathogenetic element could well elucidate the mechanism of action of drugs used in the treatment of neurodegenerative diseases.

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The aim of the present study was to investigate the effect of amiridine and tacrine on the spontaneous functional degradation of an isolated mechanoreceptive neuron from crawfish. This cell is similar to central neurons with respect to its electrophysiological, biochemical, and structural characteristics [10] and it is able to generate action potentials (AP) of a defined frequency for a long time, thus being an appropriate object for such a study.

MATERIALS AND METHODS

The experiments were performed on isolated mechanoreceptive neurons of crawfish *Astacus astacus*. AP were recorded extracellularly using clamping electrodes, a UU-90 amplifier (Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg) and an N-338 high-speed recorder (Instrument Plant, Krasnodar). Two symmetrical neurons taken from the same abdominal segment were tested in parallel: one in physiological saline (control) and another in physiological saline containing a test substance. Spike frequency was determined using a two-channel analog meter and N-339 recorders (Instrument Plant). At the beginning of the experiment

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TABLE 1. Effect of Amiridine and Tacrine on Spontaneous Activity (LT and Number of AP) of Degenerating Isolated Neuron of Crawfish

Concentration, μM	No. of experiments	LT of neurons, h			Total number of impulses		
		control	experiement	ratio	control	experiement	ratio
Amiridine							
0.1	7	9.1±1.2	9.7±1.4	1.06±0.09	412±81	435±79	1.06±0.10
0.25	4	9.1±0.5	9.6±0.5	1.05±0.05	315±55	280±34	0.94±0.15
1.0	15	6.2±0.6	7.6±0.6*	1.22±0.09	260±55	319±68*	1.29±0.12
10.0	4	11.3±2.3	2.2±1.3*	0.19±0.12	548±20	107±0.47*	0.19±0.09
Tacrine							
0.01	6	13.7±1.4	12.6±1.0	0.91±0.08	447±78	412±66	0.90±0.09
0.1	11	10.5±0.6	12.0±1.1	1.14±0.10	352±45	432±51	1.38±0.19
0.4	6	11.2±1.1	10.0±1.5	0.94±0.06	288±54	322±65	1.13±0.05
1.0	4	10.0±1.1	606±1.1*	0.66±0.11	309±90	1.77±40	0.63±0.12

Note. *$p<.05$ in comparison with the control (native neuron)

an initial pulse frequency (10-15 pulses/sec) was established by varying the muscle tension. After one hour of steady work of the neurons, either amiridine (Latvbiofarm) or tacrine (Aldrich) was added to one bath in concentrations of 0.01, 0.1, 0.25, 0.4, 1.0, and 10 μM and the pulse frequency was recorded until it decayed altogether. The duration of spontaneous activity or lifetime (LT) of the neurons and the total number of AP were determined from these records. The efficacy of the preparations was judged from the ratio of experimental to control values calculated in each experiment. Then the mean ratio was calculated, which made it possible to reveal the effect despite the large scatter of values for different neurons. The reliability of the effect of the preparations was statistically evaluated using the Student *t* test as well as nonparametric sign tests and the Wilcoxon matched-pairs signed-ranks test [7].

RESULTS

Spontaneous irreversible decay of impulse activity of an isolated neuron proceeded as follows: slight fluctuations of spike frequency occurred after a few hours of steady work, and then slower rhythms with a higher amplitude and interspike periods of a few minutes were observed. The mean spike frequency gradually decreased and then more and more impulses in the relatively regular sequence were skipped. The impulse activity became chaotic and rapidly decayed. The effect of amiridine and tacrine on the spontaneous activity (LT) of neurons and the total number of impulses in comparison with the control is illustrated in Table 1.

Amiridine in a concentration of 1 μM reliably prolonged LT of neurons and increased the number of impulses by 22 and 29%, respectively. Lower doses of the preparation (0.1 and 0.25 μM) were inactive, whereas increasing the dose to 10 μM reduced the spontaneous activity of neurons. No such prolongation of LT was noted for tacrine in the studied concentration range (0.01-1.0 μM), and in fact in the dose of 1.0 μM the agent reliably shortened LT and reduced the number of impulses by 26 and 38%, respectively.

Thus, in the present study we compared for the first time the effect of amiridine and tacrine, drugs used in the treatment of neurodegenerative disorders, on the duration of functioning of a spontaneously degrading isolated neuron. Amiridine was found to affect the LT of the neuron in a concentration-dependent manner, functioning being prolonged only with a low concentration of the preparation (1 μM). The same concentration of tacrine, a drug structurally similar to amiridine, inhibited spontaneous impulse activity, whereas lower concentrations were inactive and higher doses decreased LT. These findings are in conformity with previous data on the antiamnestic effect of low doses of amiridine [4,6,9] and the suppression of the conditioned response in intact rats caused by high doses of the preparation [5]. Moreover, this experiment corroborates the importance of our previous observation that low concentrations of amiridine and tacrine (<1 μM) shift the region of activation of calcium currents in electroexcitable membranes toward hyperpolarization of the membrane potential, and ultimately to a more reliable generation of the nervous impulse [3].

The above data suggest that the therapeutic efficacy of amiridine, but not of tacrine, may be attributed to its ability to inhibit neuron degradation.

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Delayed Damaging Effects of Anthracycline Antibiotics on the Reproductive System of Rats

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Destructive changes and disturbances of spermatogenesis are found to occur in the testes of Wistar rats 1 and 3 months after a single injection of the anthracycline antibiotic pharmorubicin in the maximum permissible dose. The morphological picture normalizes 6 months postinjection. Disturbances in reproductive function are observed as early as 3 months after treatment. The number of dominant lethal mutations rises one month postinjection.

Key Words: *anthracycline antibiotic pharmorubicin; delayed effects; testis*

Anthracycline antibiotics are widely used in the chemotherapy of malignant neoplasms [3,4]. However, their strong toxic effect on healthy organs and tissues with a high proliferative activity calls for scrupulous experimental studies of these preparations [6]. The reproductive organs, bone marrow and gastrointestinal epithelium are among the organism's systems which renew themselves rapidly.

Anthracycline antibiotics induce disturbances of spermatogenesis in rats shortly after injection [2,5]. In veiw of the fact that these drugs can sometimes considerably prolong the life of cancer patients, their delayed toxic effects on the reproductive system assume great importance.

The present study was aimed at investigating the state of the reproductive system of male Wistar rats in the long term after treatment with anthracycline antibiotics. For our experimental model we used pharmorubicin (PR).

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MATERIALS AND METHODS

The experiments were performed on 90 male and 120 female Wistar rats weighing 150-200 g, 45 males and 60 females of which were included in the control groups. The males received a single intravenous injection of PR (Farmitalia Carlo Erba) in the maximum permissible dose (MPD) of 7.5 mg/kg, calculated by the methods of graphic probit-analysis over a 30-day observation period [1]. The control animals were injected with an equivalent volume of vehicle. For the study of morphological alterations in the reproductive organs males were sacrificed by cervical dislocation 1, 3, and 6 months after injection of PR (5 rats in the control and experimental groups per point). The testes were removed and fixed in Carnoy fluid. Paraffin sections (5 μ) were prepared, stained with hematoxylin and eosin, and used for morphologi-

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