Comparative Analysis of Neuroprotective Activity of New Chemical Agent Vp and Piracetam

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The effect of new agent Vp (9-butylamine-3,3-dimethyl-3,4-dihydroacridine-1(2H) hydrochloride) on lifetime of isolated mechanoreceptive crayfish neurons was evaluated by the duration of its impulse activity. Vp significantly and dose-dependently prolonged the lifetime of both spontaneously degrading neurons and neurons degrading under conditions of inhibition of various stages of the energy metabolism: glycolysis and oxidative phosphorylation. The effect of Vp in a concentration of 10^{-7} M surpassed that of amiridine. Piracetam also prolonged the lifetime of spontaneously degrading neurons, but only in very high concentration (10^{-2} M). It is concluded that Vp possesses a neuroprotective activity.

Key Words: *aminoacridine derivatives; piracetam; mechanoreceptive crayfish neurons; potential effects*

Recent studies showed the possibility of treating neurodegenerative diseases, including Alzheimer's disease (AD) with aminoacridine and tacrine derivatives [9,12,13]. The therapeutic effect of these drugs can be due to their neuroprotective activity manifested in inhibition of neuronal degeneration [4,15]. Isolated crayfish mechanoreceptive neurons are a convenient experimental model of neuronal degeneration, which makes it possible to study the dynamics and mechanisms of neuronal death and the effect of various test agents. Crayfish neurons are similar to central neurons by their ultrastructure, electrophysiological and biochemical properties, and can generate action potentials (AP) at chosen frequency for a long time [6,11]. It was recently shown that amiridine, but not tacrine, inhibits spontaneous death of isolated crayfish neurons induced by axotomy or hypoxia induced by inhibition of oxidative phosphorylation with sodium azide [4,15]. Recently, a new agent Vp (9-butylamino-3,3-dimethyl-3,4-dihydroacridine-1(2H) hydrochloride) was synthesized at the Research Center for Safety of Bioactive Compounds. This compound possesses antiamnestic effect surpassing that of amiridine and tacrine, while is less toxic [2].

Our aim was to compare the potency of Vp to inhibit degeneration of isolated neurons with that of amiridine and tacrine. Nootropic piracetam was used as the reference drug.

MATERIALS AND METHODS

The study was carried out on slowly adapting mechanoreceptive neurons isolated from crayfish *Astacus leptodactilus*. Action potentials were recorded extracellularly with patch-clamp electrodes, amplified with an UU-90 amplifier (Institute of Experimental Medicine, St. Petersburg) coupled with an N-338 recorder (Measuring Instruments Plant, Krasnodar). Simultaneously, the discharge frequency was measured with a dual-channel MFU-1 frequency meter (Institute of Experimental Medicine, St. Petersburg) and recorded with an N-339 recorder (Measuring Instruments Plant, Krasnodar). Two symmetrical neurons (control and experimental) isolated from the same abdominal segment were tested simultaneously in 2-ml baths filled with physiological saline. At the beginning of the experiments, receptor muscles were stretched so as to induce approximately identical pulse activity of both

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neurons (10-15 Hz). After 1-h recording of the baseline AP frequency, Vp and piracetam in different concentrations were added to bathing solutions. Impulse activity of both neurons was recorded until its complete cessation. In the experiments with inhibitors, the experimental neuron fired persistently during 1 h in the presence of Vp in a concentration of 0.1 μM optimally prolonging neuron lifetime, and then sodium azide (0.5 mM), an inhibitor of oxidative phosphorylation, or sodium monoiodoacetate (1 mM), an inhibitor of glycolysis, were added to both control and experimental neurons, and the dynamics of AP frequency was recorded. The results were compared with previous data on the effect of amiridine and tacrine [6, 11]. The results were analyzed statistically using parametrical and non-parametrical tests [1,7].

RESULTS

When applied in concentrations of 10^{-8} and 10^{-6} M, Vp produced no significant changes of the lifetime of isolated neuron kept after axotomy in small volume of physiological saline (2 ml), although there was a tendency to increase in this parameter (by 10%) and decrease by 12%, respectively (Table 1). However, the intermediary concentration (10^{-7} M) significantly increased the neuron lifetime by 33% ($p<0.01$). Amiridine in a higher concentration of 10^{-6} M less markedly prolonged the lifetime (by 23%) [6,11], hence our experiments demonstrated a more pronounced and dose-depended neuroprotective effect of Vp, which attained the maximum at a concentration of 10^{-7} M. In higher concentrations ($\geq 10^{-5}$ M) Vp drastically and irreversibly blocked impulse activity within a few seconds, which occurred without preliminary increase in spike frequency characteristic of well-known depolarization or cathodic block [7]. In this concentration range, the increase in Vp concentration rapidly decreased the lifetime of the neurons: from 107 min for 10^{-5} M to 19 and 1 min for concentrations of $2\text{-}4\times10^{-5}$ M and 2×10^{-4} M, respectively.

Piracetam also significantly prolonged the lifetime of isolated neuron (by 57%), although in notably higher concentrations (Table 1).

To evaluate the role of energy processes in the maintenance of neuron activity in the presence of pharmacological agents, we studied the effect of Vp on the lifetime of isolated neurons under conditions of glycolysis and oxidative phosphorylation inhibition. The effect of Vp was compared to that of amiridine and tacrine [6]. Similarly to amiridine and tacrine [6], Vp did not affect the lifetime of neuron in the presence of the glycolysis inhibitor sodium monoiodoacetate (Table 2). However, Vp in a concentration of 10^{-7} M, which is optimal to delay spontaneous neuronal death, almost 2-fold (by 88%) prolonged the lifetime of neurons treated with sodium azide (1 mM) (Table 2). Under these conditions, amiridine (10^{-6} M) produced a less pronounced effect (by 42%), while tacrine was ineffective [6].

It can be assumed that during the long-term activity of isolated neuron, which generates hundreds

TABLE 1. Effect of Vp, Piracetam, Amiridine, and Tacrine on the Lifetime of Spontaneously Degrading Isolated Mechanoreceptive Crayfish Neurons (*M±m*)

Substance	Concentration, M	Number of experiments	Lifetime, h		Changes, %
			control	experiment	
Vp	10^{-8}	6	7.9±1.3	8.9±1.2	+10
	10^{-7}	10	9.1±1.0	12.1±1.7*	+33
	10^{-6}	5	9.5±2.0	8.3±2.0	-12
Piracetam	10^{-3}	3	7.6±2.7	9.2±3.0**	+22
	10^{-2}	4	8.3±1.7	13.1±1.9**	+57
	10^{-1}	3	10.3±1.5	10.1±1.2	-2
Amiridine	10^{-7}	7	9.1±1.2	9.7±1.4	+6
	2.5×10^{-7}	4	9.1±0.5	9.6±0.5	+5
	10^{-6}	15	6.2±0.5	7.6±0.6**	+23
	10^{-5}	4	11.3±2.3	2.2±71.3**	-81
Tacrine	10^{-8}	7	13.1±1.5	12.0±1.1	-8
	10^{-7}	17	9.8±0.5	10.4±0.9	+5
	4×10^{-7}	6	11.1±1.1	10.0±1.5	-10
	10^{-6}	5	9.5±0.9	6.7±0.9**	-29

Note. *$p<0.01$, **$p<0.05$ compared to the control. Data on amiridine and tacrine are taken from previous reports [6,11].

TABLE 2. Effect of Vp (0.1 M), Amiridine (1.0 M), and Tacrine (0.1 M) on the Lifetime of Isolated Neurons in the Presence of Inhibitors of Energy Metabolism (*M±m*)

Inhibitor	Substance	Lifetime, min		Mean changes, %
		control	experiment	
Sodium monoiodoacetate, 1 mM	Vp	62±6 (30)	58±7 (10)	-6
	Amiridine	62±6 (30)	74±7 (19)	+19
	Tacrine	62±6 (30)	76±16 (11)	+23
Sodium azide, 1 mM	Vp	64±10 (16)	120±21* (16)	+88
	Amiridine	115±6 (14)	163±26* (27)	+42
	Tacrine	86±12	93±13	+8

thousands spikes, the energy resources are exhausted, and the cell becomes unable to maintain the high transmembrane potential and reduce intracellular Ca^{2+} concentration. The release of Ca^{2+} ions from mitochondria [10] reduces excitability and functional activity of neurons [8]. Further increase in the intracellular Ca^{2+} concentration triggers cascade reactions leading to cell death [14]. Vp and amiridine can affect these stages of the cell death, for example, they stimulate glycolytic ATP production in such a way that inhibition of oxidative phosphorylation by metabolic toxins (sodium azide) is not fatal for the cell. Another possibility is deceleration of the rise of internal Ca^{2+} concentration as was observed for amiridine [3].

Finally, in comparison with amiridine, Vp is a more efficient neuroprotector acting at lower concentration (10^{-7} M). Piracetam also increased the lifetime of isolated neuron, but only in very high concentrations (10^{-2} M).

REFERENCES

1. I. P. Ashmarin, N. N. Vasil'ev, and V. A. Ambrosov, *Prompt Statistical Analysis and Planning of Experiments* [in Russian], Leningrad (1975).
2. Yu. V. Burov, S. B. Goncharenko, T. N. Robakidze, *et al., Byull. Izobret.*, No. 3 (1994).
3. Yu. V. Burov, A. S. Pivovarov, and T. N. Robakidze, *Byull. Eksp. Biol. Med.*, **118**, No. 11, 470-473 (1994).
4. Yu. V. Burov, A. B. Uzdenskii, and T. N. Robakidze, *Ibid.*, **121**, No. 1, 52-54 (1996).
5. B. M. Vladimirskii, *Mathematical Methods in Biology* [in Russian], Rostov-on-Don (1983).
6. O. B. Il'inskii, *Physiology of Sensory Systems.* Part 3: *Physiology of Mechanoreceptors,* [in Russian], Leningrad (1975).
7. B. I. Khodorov, *General Physiology of Excitable Membranes* [in Russian], Leningrad (1975).
8. P. F. Baker, *Fed. Proc.*, **35**, No. 14, 2589-2595 (1976).
9. Y. Burov, L. Kadysheva, T. Robakidze, *et al., Eur. J. Pharmacol.*, **183**, 1464 (1990).
10. E. Carafoli, *Biochimie,* **55**, 755-762 (1973).
11. *E. Giacobini,* in: *Neuroscience Researches,* Vol. 2, New York (1969), pp. 111-202.
12. E. Lavretskaya, K. Shimada, and T. Yoshida, *Dementia*, **3**, 97-107 (1989).
13. W. Summers, L. Majowsre, G. Marsh, *et al., N. Engl. J. Med.*, **315**, 1241-1245 (1986).
14. B. F. Trump, I. K. Berezevsky, T. Sato, *et al., Environ. Health Perspect.*, **57**, 281-287 (1984).
15. A. Uzdensky, Y. Burov, and T. Robakidze, in: *Neurochemistry,* Eds. A. Teelken and J. Korf, New York (1997), pp. 345-349.