PHARMACOLOGY AND TOXICOLOGY

Second Messengers Contribute to Cholinoceptor Modulation by Amiridine in *Helix lucorum* Neurons

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UDC 612.822.3.014.46:615.31:547.295.96.08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 11, pp. 470-473, November, 1994
Original article submitted April 6, 1994

The effect of amiridine on the local inward acetylcholine current and its volt-ampere characteristic are studied by the two-electrode method of membrane voltage clamp in identified RPa3 and LPa3 *Helix lucorum* neurons pretreated with forskolin, sodium nitroprusside, A23187, and EGTA. The results suggest that second messengers (Ca^{2+}, NO, cGMP, and cAMP) are implicated in the amiridine-mediated regulation of cholinoceptors in *Helix lucorum* neurons.

Key Words: *amiridine; forskolin; sodium nitroprusside; A23187 Ca^{2+} ionophore; EGTA; cholinoceptors; Helix lucorum neurons*

Alzheimer's senile dementia, manifesting itself in progressive dotage and in the formation of senile plaques in the brain, is accompanied by degeneration of the cholinergic neurons [9,11]. This results in the development of a stable deficiency of the brain cholinergic structures [6]. The new Russian-manufactured preparation amiridine has proved to be highly efficient in the treatment of senile dementia [1,4]. In the USA a preparation structurally similar to amiridine, tacrine, is used in the treatment of this disease [8,12].

Our previous findings demonstrated that amiridine and tacrine are able to restore the cholinergic transfer, modulating the activity and plasticity of the cholinoceptors in *Helix lucorum* neurons; this is likely to be due to the direct action of the preparations on the receptors and on the ion channels controlled by them [2]. Recently, regulators of cholinoceptor plasticity have been discovered among well-known second messengers [3]. Reportedly, changes occur in the second messenger systems in dementia [5,7], and modulators of the second messenger system, notably staurosporin, a protein kinase C inhibitor, exhibit an antiamnestic activity [10].

We suggested that second messengers are implicated in the amiridine-mediated regulation of cholinoceptors in *Helix lucorum* neurons. The aim of the present study was to explore the effects of amiridine under the influence of modulators of the second messenger systems.

MATERIALS AND METHODS

The experiments were carried out on identified RPa3 and LPa3 *Helix lucorum taurica* Kryn neurons in an isolated ganglion preparation. The earlier described two-electrode method of membrane voltage clamp was used [2].

In our study we used amiridine (Latvbiofarm, Latvia) and the following modulators of the sec-

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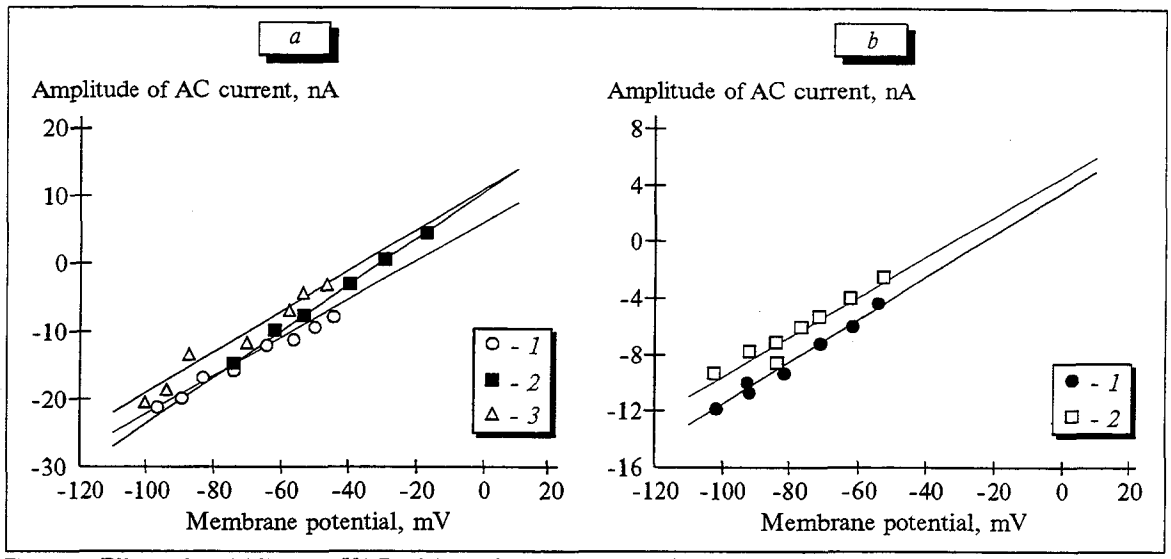

Fig. 1. Effect of amiridine on VAC of inward AC current in RPa3 neurons. Intracellular microelectrodes are filled with potassium chloride (*a*) and potassium acetate (*b*). *1*) VAC before exposure to pharmacological preparations; *2* and *3*) VAC for exposure to 70 (*a*) and 40 (*b*) μM amiridine for 10–80 (*2*) and 90–150 (*3*) min. The reversion potential was determined at the intersection of VAC (its linear extrapolation) and the abscissa.

ond messenger systems: the adenylate cyclase activator forskolin (Sigma), the source of free-radical NO ions and the guanylate cyclase activator sodium nitroprusside (Reakhim, Russia), the Ca^{2+} ionophore A23187 (Calbiochem), and the Ca^{2+}-ion chelator ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA, Serva). We analyzed the effect of extracellular pretreatment of neurons with 20-60 μM forskolin, 20-60 μM sodium nitroprusside, and 3-8 μM A23187 and the effect of 35-50 μM amiridine after pretreatment of neurons with these preparations. EGTA was injected into a neuron by passing a negative current (1-13 nA, 5-10 min) via the recording microelectrode filled with 0.2 M EGTA and 0.5 M potassium acetate. The membrane potential of cells constituted -60.4±1.8 mV. The statistical significance of the effect of the test substances was assessed from the nonparametric Wilcoxon test determined using designated STADIA software.

RESULTS

In accord with our previous findings, amiridine (>10 μM) modulated the acetylcholine (AC) current, increasing its amplitude and duration (Table

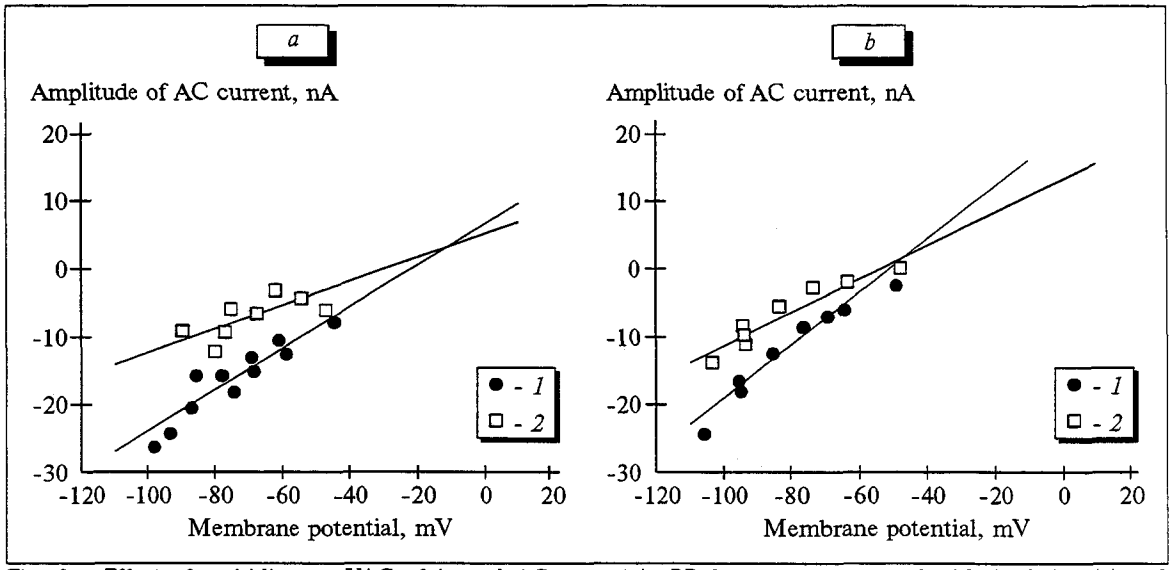

Fig. 2. Effect of amiridine on VAC of inward AC current in RPa3 neurons pretreated with forskolin (*a*) and sodium nitroprusside (*b*). *1*) VAC for exposure to 20 μM forskolin (*a*) and 50 μM sodium nitroprusside (*b*); *2*) VAC after exposure to 40 μM amiridine. Here and in Fig. 3: other designations are the same as in Fig. 1.

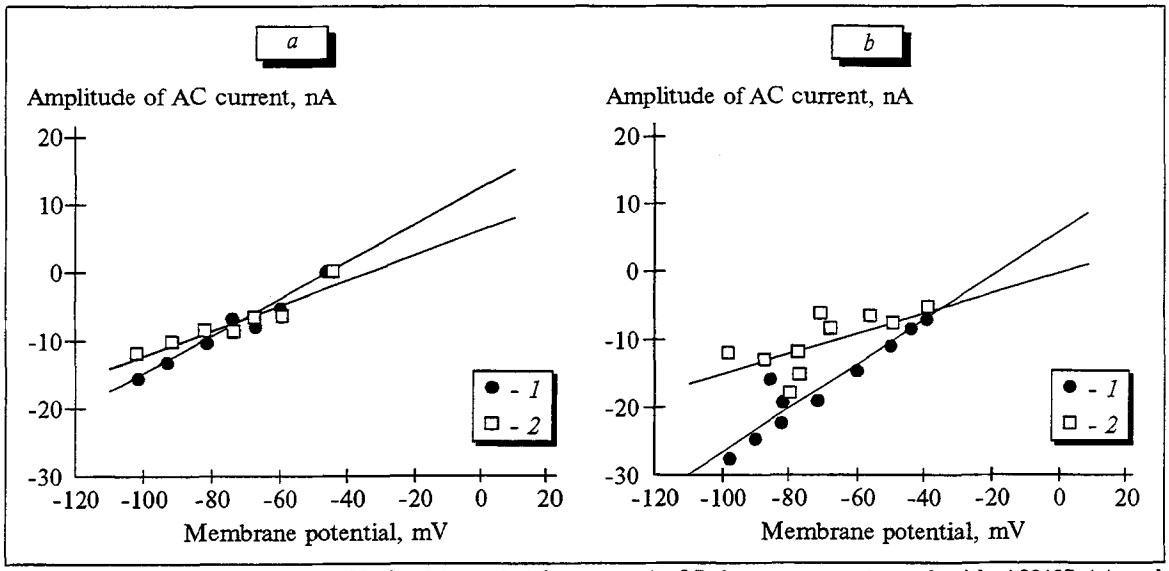

Fig. 3. Effect of amiridine on VAC of inward AC current in LPa3 neurons pretreated with A23187 (*a*) and EGTA (*b*). *1*) VAC for exposure to 8 μM A23187 and EGTA (intracellular microionophoretic injection: 2 nA, 10 min); *2*) VAC after exposure to 40 μM amiridine.

1). The preparation caused biphasic shifts in the volt-ampere characteristic (VAC), attended by a progressive negative shift of the reversion potential of the AC current in all neurons. The onset of the first phase of changes in the VAC was observed after 10-80 min of exposure to the preparation, manifesting itself in an increased slope of the VAC (Fig. 1, *a*, *2*). A longer exposure caused a negative parallel shift of the VAC (Figs. 1, *a*, *3* and 1, *b*, *2*). The amiridine-induced shift of the VAC of the AC current did not depend on the composition of the electrolyte in the intracellular microelectrodes.

We found that forskolin, an adenylate cyclase activator, lowered the amplitude of the AC current by 35.4±10.4 ($p<0.01$), without affecting its duration, and attenuated the effect of amiridine on the AC current (Table 1). Forskolin did not cause any statistically reliable shifts in the reversion potential and did not interfere with the ability of amiridine to change the slope of the linear VAC of the AC current; however, it blocked the amiridine-induced shift of the reversion potential (Fig. 2, *a*).

Sodium nitroprusside, a modulator of the NO and cGMP (cyclo-guanosine monophosphate) systems, was found to lower the amplitude of the AC current by 53.7±4.8 ($p<0.01$), without affecting its duration, and statistically reliably attenuated the amiridine-induced modulation of the AC current (Table 1). Like amiridine, sodium nitroprusside caused a negative shift of the reversion potential and did not affect the ability of amiridine to change the slope of the linear VAC of the AC current; however, it inhibited the amiridine-induced shift of the reversion potential (Fig. 2, *b*). In other words, amiridine and sodium nitroprusside caused similar negative shifts of the reversion potential, but this effect disappeared when they were used in combination. This phenomenon may be attributed to possible competitive binding of the two substances to the same receptor site.

We demonstrated that the Ca^{2+} ionophore A23187 lowered the amplitude of the AC current by 25.8±8.2 ($p<0.001$), without affecting its duration or its VAC (Table 1). A23187 blocked the effect of amiridine, inhibiting the amiridine-induced increase of the amplitude and duration of the AC current. Although A23187 did not affect the reversion potential per se, it reduced the ability of amiridine to increase the slope of the linear VAC of the AC current and blocked the effect of amiridine on the reversion potential. After pretreatment with A23187, amiridine caused a slight positive shift of the VAC (Fig. 3, *a*).

The Ca^{2+} chelator EGTA was found to increase the amplitude of the AC current by 32.1±9.6 ($p<0.05$), without affecting its duration, and caused a statistically reliable shift of the reversion potential of the AC current (Table 1). After pretreatment with EGTA, amiridine statistically reliably lowered the amplitude and slightly increased the duration of the AC current. EGTA reversed the effect of amiridine on the reversion potential of the AC current: after pretreatment with EGTA, amiridine caused a positive shift of the VAC (Fig. 3, *b*).

TABLE 1. Effect of Amiridine on the Amplitude, Duration, and Reversion Potential of the AC Current of *Helix lucorum* Neurons Pretreated with Modulators of the Second Messenger Systems ($M \pm m$)

Substance, number of experiments	Effect of amiridine on amplitude (numerator) and duration (denominator) of AC current, %	Reversion potential of AC current, mV		Shift of reversion potential, mV
		before exposure to amiridine	after exposure to amiridine	
Amiridine without pharmacological pretreatment, $n=12$	$\frac{+97.2\pm15.3^{***}}{+104.8\pm10.2^{***}}$	-28.3 ± 4.8	$-44.0\pm3.2^{**}$	$-14.0\pm3.6^{**}$
Forskolin + amiridine, $n=7$	$\frac{+53.6\pm19.9^{*}}{+75.9\pm14.1^{*}}$	-35.2 ± 4.2	-37.0 ± 3.6	-5.6 ± 5.3
Sodium nitroprusside + amiridine, $n=5$	$\frac{+14.7\pm8.1^{*}}{+60.9\pm13.3^{*}}$	$-48.6\pm2.5^{**}$	$-45.7\pm2.3^{**}$	$+0.7\pm1.5$
A23187 + amiridine, $n=7$	$\frac{+15.2\pm11.9^{**}}{+54.1\pm18.5^{**}}$	-34.3 ± 8.3	-27.7 ± 9.2	$+8.2\pm2.5$
EGTA + amiridine, $n=10$	$\frac{-23.3\pm5.6^{*}}{+39.8\pm6.9^{***}}$	$-11.1\pm7.0^{*}$	$+0.4\pm8.3^{**}$	$+11.6\pm2.5^{*}$

Note. One, two, and three asterisks indicate statistically reliable differences of parameters from the control for $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Thus, forskolin, a modulator of the cAMP second messenger system, and sodium nitroprusside, a modulator of the NO and cGMP systems, attenuated the amiridine-induced increase of the parameters of the AC current and blocked the effect of the preparation on the VAC. It is worth noting that the effect of sodium nitroprusside per se on the reversion potential of the AC current was similar to the effect of amiridine, this probably being due to competitive binding of amiridine and sodium nitroprusside to the same site of the cholinoceptor.

Studies of the effect of the Ca^{2+} ionophore A23187 for its extracellular application demonstrated only a lowering of the amplitude of the AC current, providing indirect evidence of an increased Ca^{2+} concentration in the neuron. In the presence of amiridine the amplitude and duration of the AC current slightly increased, which may be indicative of a drop of the intracellular Ca^{2+} level. In the light of published data attesting to an elevated Ca^{2+} level in neurons of Alzheimer's patients [7], amiridine may be assumed to lower the Ca^{2+} level in the neuron when the intracellular Ca^{2+} concentration is elevated.

We showed that the calcium chelator EGTA, which lowers the level of free Ca^{2+} in the neuron, increased the amplitude of the AC current and caused a statistically reliable positive shift of the reversion potential. After pretreatment of neurons with this substance, amiridine lowered the amplitude of the AC current and caused a positive shift of the reversion potential, i.e., the effects of amiridine were entirely reversed. We suggested that under these experimental conditions the effect of amiridine could manifest itself in an increase of the intracellular calcium level. Our findings lead to the conclusion that amiridine is able to regulate the level of calcium ions in the neuron within physiologically normal limits.

Thus, it was established that second messengers such as Ca^{2+}, NO, cGMP, and cAMP are implicated in the mechanism underlying the effect of amiridine on the cholinoceptors of *Helix lucorum* neurons.

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