BIOPHYSICS AND BIOCHEMISTRY

Effects of the Nootropics Piracetam and GVS-111 on Voltage-Dependent Ion Channels of Neuronal Membranes

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The effect of two nootropics, piracetam and N-phenylacetyl-L-prolylglycine ethyl ester (GVS-111), is studied by measuring high-threshold K^+ and Ca^{2+} currents in isolated snail neurons using a two-microelectrode patch-clamp technique. Piracetam and GVS-111 are shown to reduce the amplitude of both the K^+ and the Ca^{2+} (to a lesser extent) current. The threshold concentrations for GVS-111 and piracetam are 10^{-9}-10^{-8} M and 1-5×10^{-4} M, respectively. It is assumed that the antiamnestic effect of the nootropics is partially mediated by a blockade of ion channels of the neuronal membrane.

Key Words: *high-threshold Ca^{2+} channels; high-threshold K^+ channels; nootropic drugs; snail neurons*

Numerous biochemical and electrophysiological effects of nootropics of the piracetam family suggest numerous targets of their action [10,12]. Voltage-dependent ion channels of the neuronal membrane responsible for the generation of nerve impulses can presumably be counted among these targets. This assumption is based on the positive therapeutic effect of ion channel antagonists in cognitive disorders [12]. In light of this it is obviously very important to study the effect of nootropics on ion channel conductance. However, little is known about the effect of the drugs on ion currents in the neuronal membrane, there just being a few contradictory reports on the effect of piracetam nootropics on Ca^{2+} channels. Both inhibitory [8,9,16] and stimulating [16] effects of nootropics on the voltage-dependent Ca^{2+} current have been observed. At the same time the effect of piracetam nootropics on voltage-dependent Na^+ and K^+ channels has not yet been studied. Tacrine, a drug used in the treatment of Alzheimer's senile dementia, is the only antiamnestic whose effect on Na^+- and K^+ channels has been reported [2,6,11,13].

The aim of the present study was to investigate the effect of nootropics of the piracetam family on high-threshold Ca^{2+} and K^+ currents in the neuronal membrane. The effects of two drugs were studied: the standard nootropic piracetam and an original nootropic compound synthesized at the Research Institute of Pharmacology, N-phenylacetyl-L-prolylglycine ethyl ester (GVS-111) [14]. Previous behavioral experiments have shown that GVS-111 is far superior to piracetam in terms of its ability to normalize learning disorders brought about by various adverse factors [14]. The threshold doses of GVS-111 and piracetam in the be-

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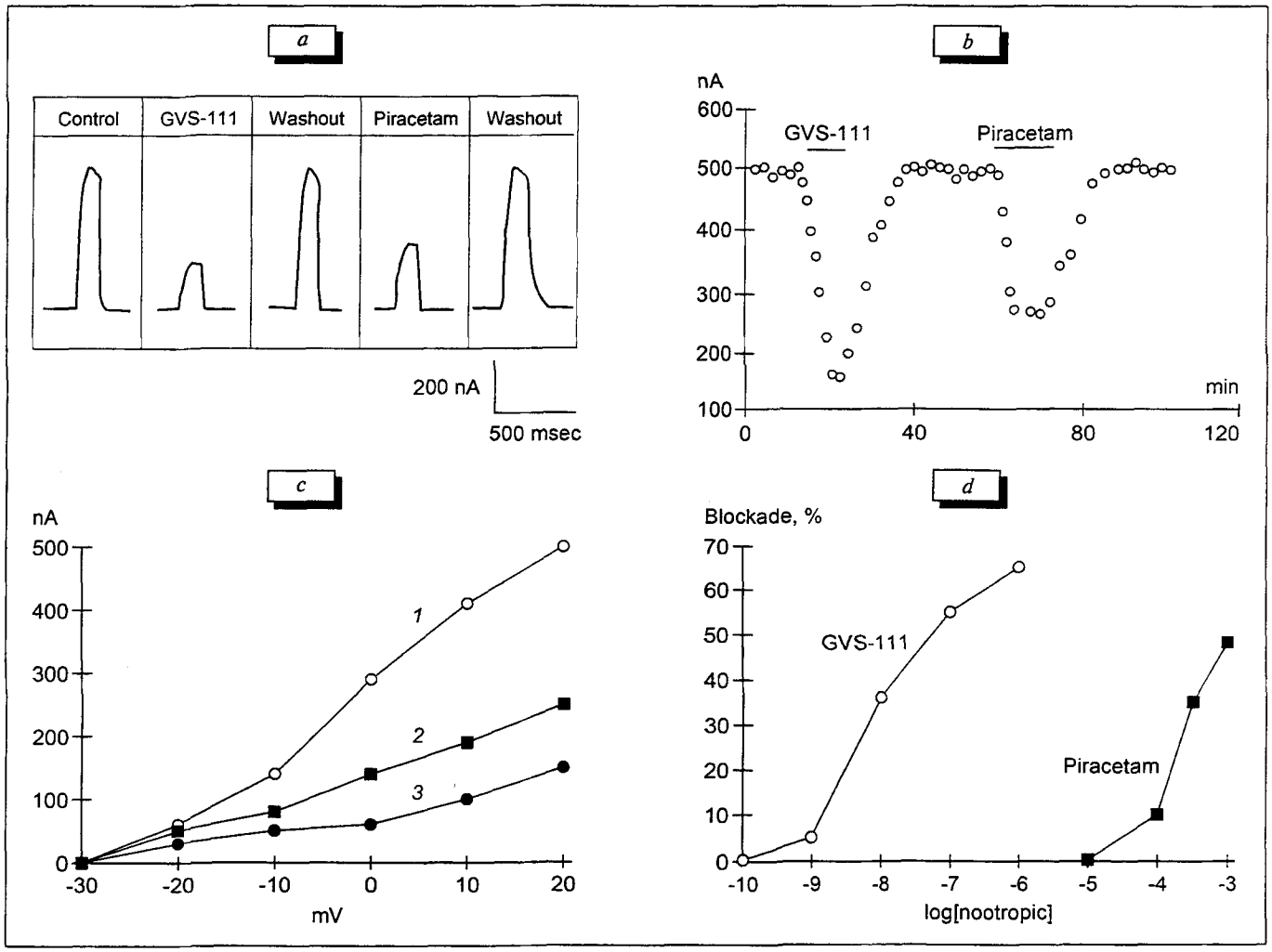

Fig. 1. Effect of nootropics on a high-threshold K^+ current in snail neurons. *a*) from left to right: K^+ current at 20 mV in the control solution, 10 min after application of 1 μM GVS-111, after a 25-min washing free of GVS-111 with the control solution, 10 min after application of 1 mM piracetam, and after a 25-min washing free of piracetam with the control solution; *b*) time-course of K^+ current amplitude; *c*) volt-ampere characteristics of K^+ current in the control solution (*1*) and in the presence of 1 mM piracetam (*2*) and 1 μM GVS-111 (*3*); *d*) concentration dependence of blocking effect of GVS-111 and piracetam on K^+ current.

havioral experiments were 0.1-0.5 and 200-300 mg/kg, respectively.

In the present study we used snail neurons as a model. Such neurons were successfully used earlier by other scientists to study the effects of tacrine on voltage-dependent Na^+ and K^+ currents [6], tacrine and piracetam on an acetylcholine-sensitive current [1,4], and oxytocin and vasopressin (peptides thought to be involved in memory formation) on a voltage-dependent Ca^{2+} current [3].

MATERIALS AND METHODS

The experiments were carried out on isolated neurons from the visceral ganglion of *Helix pomatia L.* For the recording of a high-threshold K^+ current the neurons were placed in circulating solutions containing (in mM): 100 NaCl, 4 KCl, 5 $CaCl_2$, 4 $MgCl_2$, and 5 Tris-HCl, pH 7.6. The Ca^{2+} current was recorded in a Na-free solution containing calcium channel blockers. The solution contained (in mM): 10 $CaCl_2$, 4 KCl, 4 $MgCl_2$, 95 tetraethylammonium bromide, and 5 4-aminopyridine, pH 7.6. The potential was fixed with two intracellular microelectrodes filled with 2 M potassium citrate. The experiments were performed on a Nihon Kohden standard device for microelectrode studies. The holding voltage was set at -60 mV. Depolarizing test pulses were of 150-500 msec duration. Piracetam and GVS-111 were added to the medium when the flow was stopped. Both nootropics were applied to the same neuron in a random sequence.

RESULTS

The effect of the nootropics on a high-threshold K^+ current was studied on 13 cells. In 11 of the 13 cells

both piracetam (10^{-4}-10^{-3} M) and GVS-111 (10^{-9}-10^{-6} M) rapidly reduced the amplitude of the K^+ current in a dose-dependent manner. This effect does not depend on the voltage of the test-pulses and may be reversed by washout. The steady and leakage currents remained unchanged. The maximal blockade of the K^+ current by piracetam (10^{-3} M) and GVS-111 (10^{-6} M) constituted 48±18% and 66±21%, respectively. Figure 1 shows a typical effect of nootropics on the high-threshold K^+ current recorded in one particular cell. A record of the K^+ current during test stimulation shifting the membrane potential to 20 mV is depicted in Fig. 1, *a*. The current was recorded in the control solution and in the presence of nootropics, which are seen to reduce the amplitude of the K^+ current considerably. The current amplitude as a function of time is plotted on Fig. 1, *b*. As is seen from the curve, the effect of both piracetam and GVS-111 developed after a 1-min latency, attained the maximum after 10 min, and disappeared after a 15-20-min washing with the control solution. The volt-ampere characteristics (VAC) of the K^+ current in the control solution and in the presence of piracetam (10^{-3} M) and GVS-111 (10^{-6} M) are presented on Fig. 1, *c* (leakage currents are subtracted). These curves suggest that the effects of the nootropics do not depend on the voltage of the test pulse. Figure 1, *d* shows the dose-dependence of K^+ current blockade. The effect increased with an increase of the dose of the nootropics within the concentration range used, GVS-111 being 4-5 orders of magnitude more effective than piracetam.

The effect of GVS-111 and piracetam on a high-threshold Ca^{2+} current was studied on 20 cells. Piracetam (5×10^{-4}-2×10^{-3} M) and GVS-111 (10^{-8}-2×10^{-6} M) added to the medium markedly affected the Ca^{2+} current in 11 of the 20 cells. The effect of the drugs on the Ca^{2+} current consisted in a rapid, dose-dependent reduction of amplitude, reversible upon washout. The maximal blockade of the Ca^{2+} current by piracetam (2×10^{-3} M) and GVS-111 (2×10^{-6} M) constituted 14±7% and 37±15%, respectively. Figure 2 shows a typical effect of the nootropics on high-threshold Ca^{2+} current recorded in one particular cell. Figure 2, *a* shows the maximal Ca^{2+} current recorded in the control solution and in the presence of the nootropics. Current amplitude as a function of time is shown in Fig. 2, *b*. The effect of both piracetam and GVS-111 is seen to have developed during the first 1-2 min after their addition; it attained the maximum after 10-15 min and disappeared after a 20-30-min washing of the cell with the control solution. Figure 2, *c* shows the VAC of the peak Ca^{2+} current recorded in the control solution and in the presence of 2×10^{-3} M piracetam and 2×10^{-6} M GVS-111. Piracetam is seen not to shift the VAC of the Ca^{2+} current, while GVS-111 shifted it by 15 mV toward more negative voltage values. Dose-effect curves of the nootropic-induced blockade of the Ca^{2+} current are presented in Fig. 2, *d*. Within the concentration range used the inhibition of the Ca^{2+} current increased in parallel with the drug dose. Similarly to the inhibition of the K^+ current, in these experiments GVS-111 was again 4-5 orders of magnitude more effective than piracetam, but both nootropics inhibited the Ca^{2+} current by one order of magnitude less effectively than they did the K^+ current.

In the present report we have described two effects of the nootropics, piracetam and GVS-111 on voltage-dependent ion channels of the neuronal membrane: inhibition of high-threshold K^+ and Ca^{2+} currents. In our opinion, these effects may be at the root of the antiamnestic action of piracetam and GVS-111, an assumption which is confirmed by the fact that the concentrations of piracetam (10^{-4}-10^{-3} M) and GVS-111 (10^{-9}-10^{-6} M) in our experiments are comparable with the doses which have been shown to improve cognitive activity in behavioral experiments [14]. It is important to note that, as in behavioral experiments, GVS-111 was a much more effective blocker of voltage-dependent ion currents than piracetam (by several orders of magnitude).

The blockade of a high-threshold K^+ current by nootropics of the piracetam family observed by us has not been reported before. At the same time there are some data on the effect of tacrine, another antiamnestic, on voltage-dependent K^+ channels. A tacrine-induced K^+ current blockade has been demonstrated on various experimental models [6,11,13]. However, the majority of authorities believe that this effect is hardly related to the antiamnestic activity of tacrine, since blockade of the K^+ current is produced by doses of tacrine (1-200 μM) far surpassing the doses which are known to improve cognitive activity (0.02 μM). Meanwhile, another effect of tacrine has been described [2], namely, reduction of the activation threshold of the K^+ current produced by concentrations comparable to therapeutic doses of the drug (0.01-1 μM).

Another effect of piracetam and GVS-111 observed in our experiments was a reduction of the high-threshold Ca^{2+} current. This did not differ from the previously reported effect of other nootropics of the piracetam family on Ca^{2+} channels. Blockade of a high-threshold Ca^{2+} current by such nootropics as aniracetam, bifemelan, idebenon, and vinpocetine in a concentration of 100 μM has been shown earlier on various experimental models [8,9,16]. This effect is thought to underlie the antiamnestic activity of the drugs. The new nootropic GVS-111 used in our experiments was a more effective blocker of Ca^{2+} channels (by several orders of magnitude) than other known nootropics.

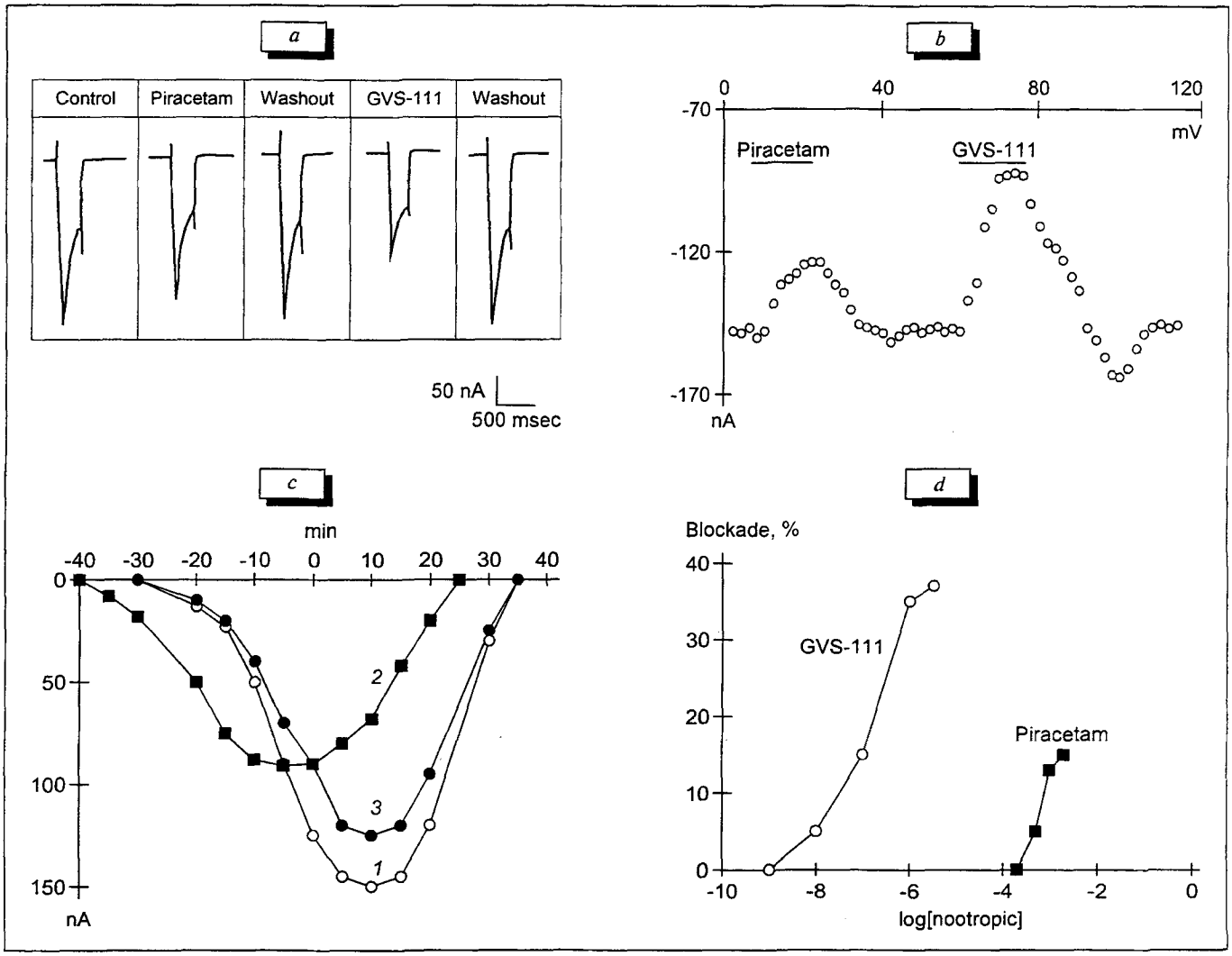

Fig. 2. Effect of nootropics on a high-threshold Ca^{2+} current in snail neurons. *a*) from left to right: maximal Ca^{2+} current in the control solution, 10 min after application of 2 mM piracetam, after a 30-min washing free of piracetam with the control solution, 10 min after application of 2 μM GVS-111, and after a 30-min washing free of GVS-111 with the control solution; *b*) time course of Ca^{2+} current amplitude; *c*) volt-ampere characteristics of Ca^{2+} current in the control solution (*1*) and in the presence of 2 μM GVS-111 (*2*) and 2 mM piracetam (*3*); *d*) concentration dependence of blocking effect of GVS-111 and piracetam on K^+ current.

It is important that the blockade of K^+ and Ca^{2+} channels by GVS-111 surpassed not only the effect of known antiamnestics, but also the effect of classical antagonists of high-threshold K^+ and Ca^{2+} channels [5,7,15].

The physiological importance of the observed nootropic-induced changes in the functioning of ion channels may consist in changes in Ca^{2+} inflow during the action potential. Nootropics reduce Ca^{2+} entry by blocking Ca^{2+} channels and, on the contrary, stimulate Ca^{2+} entry by blocking K^+ channels due to extension of the tail of the spike. Thus, in our experiments nootropics exert opposite effects with respect to Ca^{2+} entry. This may result in opposite changes in the cytoplasm of various cells depending on the prevailing type of blocked channels. However, on the whole the nootropics should apparently be recognized as stimulating Ca^{2+} entry in neurons. This follows from a comparison of the effect of the drugs on Ca^{2+} and K^+ channels. Our experiments demonstrated that they block K^+ more effectively than Ca^{2+} channels. Nootropics block the K^+ current in a greater number of cells, at lower threshold concentrations, and with a more pronounced maximal effect in comparison with their effect on the Ca^{2+} current. The stimulating effect on Ca^{2+} entry may underlie the currently discussed long-term improvement of synaptic transmission [12] achieved with nootropics.

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Significance of Various Adrenoreceptors for Brain Resistance to Total Ischemia

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In contrast to the β-agonist isoprenaline and the α_1-agonist phenylephrine, the α_2-agonists clonidine, guanobenz, and methyldopa are highly effective against total brain ischemia. Epinephrine in itself is inactive but displays protective activity against the background of the β-antagonist propranolol. The α_2-antagonist rauwolscine, but not α_1-antagonists, abolish the protective effect of clonidine.

Key Words: *adrenoreceptors; brain protectors; brain ischemia*

Protectors with receptor activity are highly effective in various critical states, including brain ischemia (BI) [2]. The effects of the adenosine receptor agonists [4,9] in ischemia are well known, whereas those of the agonists of adrenoreceptors have not been studied in sufficient detail. In focal [8] and incomplete BI [6,7], the α_2-agonists clonidine [6] and dexmedetomidine [7,8] improve neurological status [6,7] and reduce histologically revealed damage to the brain cortex [8]. The data for the hippocampus and caudal nuclei are ambiguous [6-8] and evidence on the effects of naturally occurring catecholamines is controversial. It is thought that in incomplete BI catecholamines actually aggravate neurological status and have no effect on total ischemia [6]. The adrenoreceptor agonists have not been compared, and the effects of a_2-agonists have not been investigated. The present study was designed to address these topics.

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MATERIALS AND METHODS

Experiments were performed on 350 mice of both sexes weighing 16-25 g. The following preparations were used: R(-)-epinephrine hydrotartrate, RS-isoprenaline hydrochloride, anapriline (propranolol) (both from the Kharkov Plant of Endocrine Preparations), phenylephrine (Serva), α-methylhydroxyphenylalanine (methyldopa), corynanthine (both from Sigma), rauwolscine (ICN), clonidine and prazosin (both from the Chemico-

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