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The Effects of Piracetam and Its Novel Peptide Analogue GVS-111 on Neuronal Voltage-Gated Calcium and Potassium Channels

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ABSTRACT. 1. With the use of the two-microelectrode voltage-clamp method, three types of voltage-activated ionic currents were examined in isolated neurons of the snail *Helix pomatia*: high-threshold Ca^{2+} current (I_{Ca}), high-threshold Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$) and high-threshold K^+ current independent of Ca^{2+} ($I_{K(V)}$).

2. The effect of bath application of the nootropics piracetam and a novel piracetam peptide analog, ethyl ester of N-phenyl-acetyl-L-prolyl-glycine (GVS-111), on these three types of voltage-activated ionic currents was studied.

3. In more than half of the tested cells, I_{Ca} was resistant to both piracetam and GVS-111. In the rest of the cells, I_{Ca} decreased 19±7% with 2 mM of piracetam and 39±14% with 2 μM of GVS-111.

4. $I_{K(V)}$ in almost all cells tested was resistant to piracetam at concentrations up to 2 mM. However, $I_{K(V)}$ in two-thirds of the cells was sensitive to GVS-111, being supressed 49±18% with 1 μM GVS-111.

5. $I_{K(Ca)}$ appeared to be the most sensitive current of those studied to both piracetam and GVS-111. Piracetam at 1 mM and GVS-111 at 0.1 μM decreased the amplitude of $I_{K(Ca)}$ in most of the cells examined by 49±19% and 69±24%, respectively.

6. The results suggest that piracetam and GVS-111 suppression of voltage-activated calcium and potassium currents of the neuronal membrane may regulate (both up and down) Ca^{2+} influx into neurons. GEN PHARMAC 29;1:85–89, 1997. **© 1997 Elsevier Science Inc.**

KEY WORDS Acylprolyl-containing dipeptide, calcium current, molluscan neurone, nootropic drug, piracetam, potassium current

INTRODUCTION

A question of interest in the field of investigations of the mechanisms of action of the nootropic drugs is their possible interaction with potential-operated ionic channels of neuronal membranes [for review, see Benesova (1994); Gouliaev and Senning (1994) and Sarter (1991)]. This interest is based on the understanding that, by changing the conductance of calcium and potassium ionic channels, it is possible to regulate the cytosolic Ca^{2+} level in presynaptic and postsynaptic cell regions. Such regulaton is of great importance in view of recent research that showed both a deficit in neurotransmission (Benesova, 1994) and neuronal damage induced by Ca^{2+} accumulation (Benesova, 1994; Khachaturian, 1994) in the brains of demented patients.

This interest is also supported by recent discoveries that antagonist of calcium channels, nimodpine, as well as antagonist of potassium channels, aminopyridines, display the nootropic activity (Sarter, 1991). It is supposed that the therapeutic effect of calcium channel antagonists is based on the prevention of Ca^{2+} entry into the cell body and on the protection of the cell from Ca^{2+} overload (Scriabin *et al.*, 1989; Triggle, 1994). On the other hand, potassium channel antagonists induce an increase in calcium influx into presynaptic nerve terminals, resulting in the enhancement of neurotransmission (Lavretsky and Jarvik, 1992).

The results of previous studies of the effects of nootropics on voltage-operated calcium channels are not homogeneous. Both a suppression and an enhancement of Ca^{2+} current were observed (Kaike *et al.*, 1993; Kaneko *et al.*, 1990; Yoshii and Watabe, 1994). Surprisingly, both these effects were supposed to be relevant to antiamnestic actions of these drugs.

The effects of piracetam and its analog on voltage-operated potassium channels were not studied. From a great number of nootropic agents, only tetrahydroaminoacridine (tacrine) is reported to exert an action on potassium channels. Inhibitory (Drukarch *et al.*, 1987; Rogawski, 1987; Schauf and Sattin, 1987) as well as stimulatory (Burov *et al.*, 1993) effects were described.

In the present work, we studied the effects of piracetam and its novel peptide analog, ethyl ester of N-phenyl-acetyl-L-prolyl-glycine (GVS-111), on the high-threshold Ca^{2+} currents and high-threshold K^+ current of neuronal membranes. GVS-111 was designed and synthesized at the Institute of Pharmacology of the Russian Academy of Medical Sciences (Gudasheva *et al.*, 1996). This compound was found to be four–five orders stronger as a cognitive en-

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hancer than the prototype (Ostrovskaya *et al.*, 1994; Seredenin *et al.*, 1995). Threshold doses of GVS-111 and piracetam in behavioral experiments were revealed to be 0.1–0.5 mg/kg and 200–300 mg/kg, accordingly.

Isolated neurons of the snail *Helix pomatia* were used as a model. This model has already been employed by other authors for studying the nootropic drugs, tacrine and piracetam (Drukarch *et al.*, 1987; Pivovarov *et al.*, 1987).

MATERIALS AND METHODS

The experiments were performed on the isolated neurons of left and right parietal ganglia of the land snail *H. pomatia*. The isolation of the neurons was made without pretreatment of the ganglia with proteolytic enzymes. The diameter of the neurons studied was usually 20–40 μm. The volume of the solution in the experimental chamber was about 1 ml.

For K^+-current recording, the cells were kept in a flow of normal Ringer solution with the following ionic composition (in mM): 100 NaCl, 4 KCl, 5 $CaCl_2$, 4 $MgCl_2$, 3 $NaHCO_3$, 5 Tris-Cl (pH=7.6). In experiments studying K^+ current in Ca^{2+}-free solution, the following ionic composition of external solution was (mM): 100 NaCl, 4 KCl, 6 $MgCl_2$, 3 $NaHCO_3$, 5 Tris-Cl (pH=7.6). Ca^{2+} current was registrated in Na^+-free solution containing K^+-channel antagonists. It was composed of (mM): 4 KCl, 10 $CaCl_2$, 4 $MgCl_2$, 95 tetraethylammonium bromide (TEA), 5 4-aminopyridine (4-AP), 5 Tris-Cl (pH=7.6).

The neurons were voltage clamped by using two intracellular microelectrodes filled with potassium citrate solution (2 M). The assays were carried out with the use of equipment supplied by Nihon Kohden (Japan). Voltages and currents were recorded by using a four-channel pen recorder, RJG-4024, with a bandwidth of up 40 kHz. Piracetam (Sigma) and GVS-111 (Institute of Pharmacology, Moscow) were employed by bath application.

RESULTS

The effects of piracetam and GVS-111 on high-threshold Ca^{2+} current (I_{Ca})

Experiments were performed on 21 neurons. I_{Ca} was measured in Na^+-free solution containing high doses of K^+ current antagonists. To evoke I_{Ca}, depolarizing test pulses of 150–500 ms were applied from a holding potential of −60 mV. Test pulses varied from −30 mV to +40 mV in 5-mV increments. Piracetam and GVS-111 were added to the experimental chamber at concentrations of 0.1–2.0 mM and 0.001–2.0 μM, accordingly. I_{Ca} in 12 cells of 21 tested was found to be resiatant to both piracetam and GVS-111. However, the treatment of the other nine cells with piracetam or GVS-111 led to a decrease in I_{Ca} amplitude. The threshold concentration was 0.5 mM for piracetam and 0.01 μM for GVS-111. The effect of nootropic drugs on I_{Ca} was found to enhance with an increase in the dose. On average, I_{Ca} block at 2 mM piracetam and at 2 μM GVS-111 was 19±7% and 39±14%, respectively. Fig. 1 illustrates the blocking effect of piracetam and GVS-111 on I_{Ca} in one of these cells. The effect of nootropics began initially after 1–2 min, reached a maximum in 5–10 min and disappeared after 15–20 min of washing the cell with control solution. Fig. 2 shows voltage-current relations of peak inward current constructed in normal solution and in the presence of 2 mM of piracetam or 2 μM of GVS-111. One may see that the I-V curve shifts along the potential axis in the direction of the negative potential by 15 mV after exposure to GVS-111.

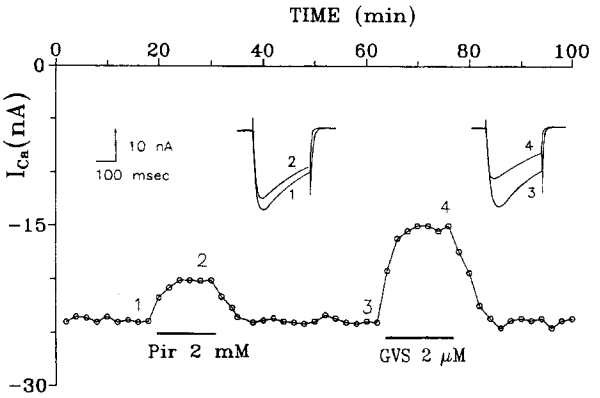

FIGURE 1. Effect of piracetam (Pir) and GVS-111 (GVS) on high-threshold Ca^{2+} current of snail neuron. The neuron was bathed in Na^+-free medium containing 95 mM TEA and 5 mM 4-AP. Time course of changes in the peak of maximal I_{Ca} is shown. The inward current was evoked at 2-min intervals. Piracetam and GVS-111 applications are indicated by bars. At 5 min, an application of piracetam evoked a 16% decrease in Ca^{2+} current. This response is shown in the left inset, in which record 1 corresponds to the control Ca^{2+} current and record 2 corresponds to the piracetam-induced Ca^{2+} current inhibition. An application of GVS-111 evoked a 39% decrease in Ca^{2+} current. The recordings in the right inset were obtained before (record 3) and during (record 4) GVS-111 application. Holding potential, −60 mV; test potentials, +10 mV (everywhere except GVS-111 application); test potentials during GVS-111 application, −5 mV.

Various types of high-threshold K^+ current in different cells

High-threshold K^+ current was measured in 32 isolated neurons. To prevent contamination with low-threshold K^+ currents, we held the cells at −50 mV. Test pulses of 150–500 ms varied from −30 mV to +100 mV in 10-mV increments. Similar to the literature data (Baxter and Byrne, 1989; Thompson, 1977), activation and inactivation kinetics of high-threshold K^+ current varied from cell to cell. In 15 neurons, slow outward currents were generated whose time of activation was shown to be 220–280 ms and inactivation was 0–17% within 500 ms. The current diminished by 50–70% in Ca^{2+}-free solution and demonstrated an N-shaped I-V curve. These features cor-

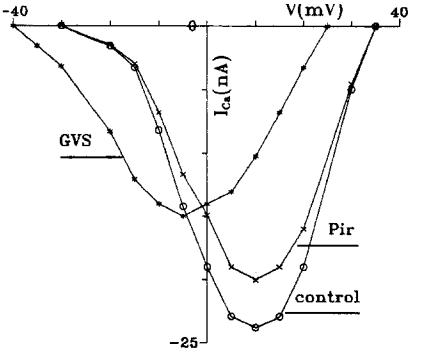

FIGURE 2. Current-voltage relation for peak inward current from the same cell as that of Fig. 1, constructed in normal solution (circles) and in the presence of 2 mM of piracetam (crosses) or 2 μM GVS-111 (asterisks). The I-V curve has shifted along tbe potential axis in the direction of negative potentials at 15 mV after exposure to GVS-111.

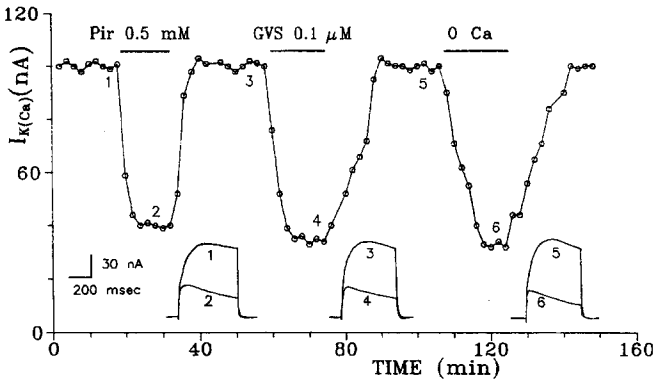

FIGURE 3. Effect of piracetam (Pir) and GVS-111 (GVS) on slow Ca^{2+}-dependent outward current [$I_{K(Ca)}$] of snail neuron bathed in normal Ringer solution. Time course of changes in the peak $I_{K(Ca)}$ is shown. The outward current was evoked at 2 min intervals. Application of 0.5 mM of piracetam caused a 60% decrease in peak of outward current (left inset). Application of 0.1 μM of GVS-111 reversibly decreased the outward current by 63% (middle inset), and the removal of Ca^{2+} from the external medium supressed the outward current by 68% (right inset). Holding potential, −50 mV; test potential, +30 mV.

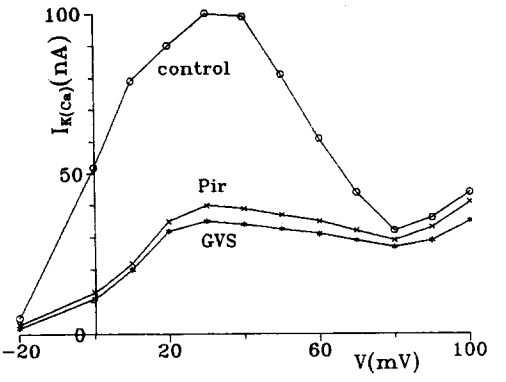

FIGURE 4. Current-voltage relation for the peak slow Ca^{2+}-dependent outward current [$I_{K(Ca)}$] of the same cell as that of Fig. 3. Control curve was N-shaped (circles). 0.5 mM of piracetam (crosses) and 0.1 μM of GVS-111 (asterisks) decreased an outward current mainly in the 0–60 mV potential region.

respond to the characterstics of high-threshold Ca^{2+}-dependent K^+ current described in molluscan neurons by other researchers (Baxter and Byrne, 1989; Thompson, 1977). We have concluded that a significant component of a slow outward current is provided by Ca^{2+}-dependent K^+ current, which is why it was designated as $I_{K(Ca)}$.

In the remaining 17 cells, a high-threshold outward current showed faster kinetics of activation and inactivation. This current was activated within 20–50 ms and inactivated by 50–80% within 500 ms. It was resistant in Ca^{2+}-free solution and had a smooth I-V curve. This current resembled a voltage-dependent and Ca^{2+}-independent K^+ current observed by other authors in molluscan neurons (Baxter and Byrne, 1989; Thompson, 1977) and may be designated as $I_{K(V)}$. Further, we will show that nootropic drugs affect $I_{K(Ca)}$ and $I_{K(V)}$ in a different way.

The effects of piracetam and GVS-111 on $I_{K(Ca)}$

The effects of piracetam and GVS on $I_{K(Ca)}$ were studied on 15 cells. Only two of them appeared to be insensitive both to 0.05–1.0 mM of piracetam and to 0.001–1.0 μM of GVS-111. In the remaining 13 cells, both piracetam and GVS-111 induced a suppression of the outward current amplitude in a fast, reversible and dose-dependent manner. Threshold concentrations of piracetam and GVS-111 were revealed to be 0.1 mM and 0.001 μM, respectively. 1 mM of piracetam decreased the amplitude of $I_{K(Ca)}$ by 49±19%, and 0.1 μM of GVS-111 by 69±24%. Fig. 3 illustrates the blocking effect of piracetam and GVS-111 on $I_{K(Ca)}$. It is obvious from current recordings that the currents that remained in the presence of nootropics have faster kinetics of activation and inactivation.

Figure 4 demonstrates the I-V relations of the peak amplitudes of outward currents of the same cell. The control curve is N-shaped with a maximum near +30 mV. Study of the dependence of the effects of nootropics on the test-stimulus potential shows that the effect displays mainly in the 0–70 mV potential region. Taking into consideration, that a bell-shaped increase of outward current at 0–70 mV potentials is provided by $I_{K(Ca)}$ activation, and that the outward current at 80–100 mV potentials does not depend on Ca^{2+} (Meech and Standen, 1975, Thompson, 1977), we may conclude that suppression of the slow outward current by nootropics is mainly achieved by inhibition of $I_{K(Ca)}$.

To elucidate the possible dependence of $I_{K(Ca)}$ suppression by nootropics on I_{Ca} inhibition, we investigated the effects of nootropics on $I_{K(Ca)}$ and I_{Ca} in the same cells ($n=4$) changing the ionic composition of the external solution as described in the section on materials and methods. No correlation was found between the effect of nootropics on $I_{K(Ca)}$ and that on I_{Ca}. Although in all four cells both piracetam and GVS-111 effectively suppressed $I_{K(Ca)}$, only in one cell did they inhibit I_{Ca} and this effect was weak (<10%). In the other three cells, I_{Ca} appeared to be resistant to nootropics. The results obtained suggest that a downregulation of $I_{K(Ca)}$ by nootropic drugs cannot be explained by the inhibition of I_{Ca}.

The effects of piracetam and GVS-111 on $I_{K(V)}$

The effects of piracetam and GVS-111 on $I_{K(V)}$ were studied in 15 cells. Most of the cells examined (13 of 15) were found to be resistant to 0.5–2.0 mM of piracetam. However, GVS-111 applied to the cells generating $I_{K(V)}$ gave other results. Only six cells were resistant to 0.1–1.0 μM of GVS-111, whereas, in the other nine cells $I_{K(V)}$ was reversibly reduced by 47±18% at 1 μM of GVS-111. The effect of GVS-111 on $I_{K(V)}$ is illustrated in Fig. 5. Fig. 6 shows the I-V relation for $I_{K(V)}$ constructed in control solution and after exposure of the cell to 1 mM of piracetam or to 1 μM of GVS-111.

DISCUSSION

The present study revealed two independent effects of the nootropic drugs piracetam and GVS-111 on voltage-gated ionic channels of the neuronal membrane: the suppression of a high-threshold Ca^{2+} current and of a high-threshold K^+ current. The effective concentrations of piracetam and GVS-111 were as follows: 0.05–2.0 mM and 0.001–2.0 μM, respectively. This relation was close to that revealed *in vivo* experiments for cognitive-enhancing doses, which were shown to be 200–300 mg/kg and 0.2–0.5 mg/kg, respectively (Seredenin *et al.*, 1995). These correlations allow us to suppose the involvement of ionic channel blocking in the mechanism of antiamnestic action of piracetam and GVS-111.

The suppression of I_{Ca} by piracetam and GVS-111 observed in our experiments resembles an effect on I_{Ca} of some other nootropics described elsewhere. It was shown in various models that such noo-

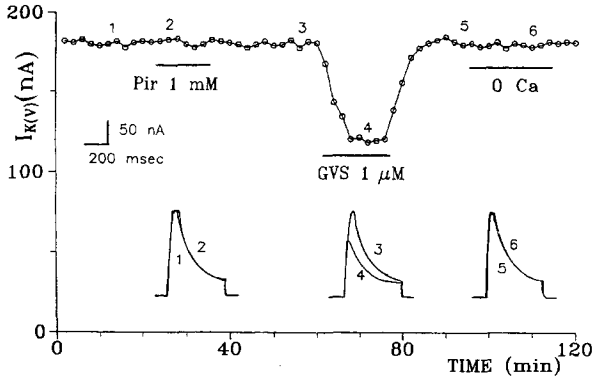

FIGURE 5. The effects of piracetam (Pir) and GVS-111 (GVS) on fast high-threshold outward current [$I_{K(V)}$] of a neuron bathed in normal Ringer solution. Application of 1 μM of GVS-111 reversibly decreased the outward current by 30% (middle inset). Neither 1 mM of piracetam (left inset) nor Ca^{2+}-free solution (right inset) changed $I_{K(V)}$. Holding potential, −50 mV; test potential, +20 mV.

tropic agents as aniracetam, bifemelane, idebenone and vinpocetine, applied at 100 μM, decreased I_{Ca} (Kaike *et al.*, 1993; Kaneko *et al.*, 1990; Yoshii and Watabe, 1994). It must be noted that this dose of nootropic agents capable of blocking I_{Ca} is several orders higher than the dose of the novel nootropic agent GVS-111 shown to be active in our experiments.

Another effect of piracetam and GVS-111 discovered in our work is a decrease of voltage-activated K^+ current. Two types of high-threshold K^+ current, Ca^{2+}-dependent K^+-current having slow kinetics [$I_{K(Ca)}$] and a faster K^+ current independent of Ca^{2+} [$I_{K(V)}$], were studied. According to our findings, $I_{K(Ca)}$ appeared to be more sensitive to nootropics than did $I_{K(V)}$. A comparative study of nootropic effects on $I_{K(Ca)}$ and on I_{Ca} in the same cells led to a conclusion about the independence of $I_{K(Ca)}$ suppression on those of I_{Ca}. Thus, Ca^{2+} channels and K^+ channels in neuronal membranes might be regarded as two independent targets for nootropics.

For the present, there are no data in the literature concerning the effects of piracetam or its analog on K^+ currents. We succeeded in finding only data about the effects of tacrine, an antiamnestic widely used for the treatment of Alzheimer disease, on the total high-threshold K^+ current. The majority of investigators observed the inhibitory effect of tacrine at 1–200 μM on K^+ current (Drukarch *et al.*, 1987; Rogawski, 1987; Schauf and Sattin, 1987). However, the authors did not consider this effect to be related to the antiamnestic effect of tacrine action, because the therapeutic concentration of the drug was known to be much smaller (0.02 μM).

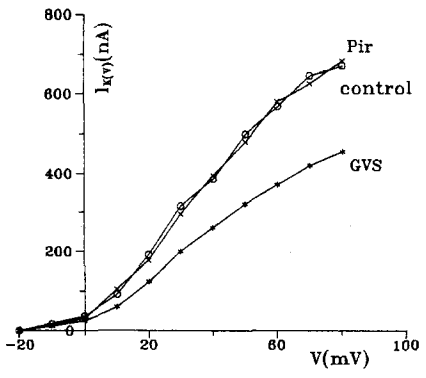

FIGURE 6. Current-voltage relation for peak fast high-threshold outward current [$I_{K(V)}$] of the same neuron as that of Fig. 5. 1 μM of GVS-111 (asterisks) decreased $I_{K(V)}$ by approximately 30% in all test potentials used. 1 mM of piracetam (crosses) had no effect on $I_{K(V)}$.

The physiological significance of the changes in the calcium and potassium channels' functioning may consist in a change in the Ca^{2+} entry into the cell during an action potential. Our results suggest that piracetam and GVS-111 are capable both of decreasing and of increasing the concentration of intracellular Ca^{2+}. Blocking Ca^{2+} channels, they reduce the Ca^{2+} cytoplasmic level, whereas a blockade of the K^+ channels causes its elevation owing to an action potential prolongation. Taking into considiration that $I_{K(Ca)}$ is much more sensitive to nootropics than I_{Ca}, one can suppose that, in those cells that possess K(Ca) channels, cytosolic Ca^{2+} will be elevated by the effects of nootropics. On the contrary, nootropics (piracetam>GVS-111) will decrease an intracellular Ca^{2+} in the cells possessing K(V) channels.

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