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ARTICLE

Selective suppression of the slow-inactivating potassium currents by nootropics in molluscan neurons

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Abstract

The role of the voltage-gated K^+ channels in the effect of some nootropics was investigated. Earlier, the multiple effect of high concentrations of two nootropics, piracetam and its peptide analogue GVS-111 [Seredenin et al. (1995), US Patent No. 5,439,930], on Ca^{2+} and K^+ currents of molluscan neurons was shown [Solntseva et al. (1997), *General Pharmacology 29*, 85–89]. In the present work, we describe the selective effect of low concentrations of these nootropics as well as vinpocetine on certain types of K^+ current. The experiments were performed on isolated neurons of the land snail *Helix pomatia* using a two-microelectrode voltage-clamp method. The inward voltage-gated Ca^{2+} current (I_{Ca}) and three subtypes of the outward voltage-gated K^+ current were recorded: Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$), delayed rectifying current (I_{KD}), and fast-inactivating K^+ current (I_A). It has been found that I_{Ca} was not changed in the presence of 30 μM vinpocetine, 100 μM piracetam or 10 nM GVS-111, while slow-inactivating, TEA-sensitive $I_{K(Ca)}$ and I_{KD} were inhibited ($I_{K(Ca)}$ more strongly than I_{KD}). In contrast, the fast-inactivating, 4-AP-sensitive K^+ current (I_A) was not diminished by low concentrations of piracetam and GVS-111, while vinpocetine even augmented it. A possible role of slow-inactivating subtypes of the K^+ channels in the development of different forms of dementia is discussed.

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Key words: GVS-111, K^+ current, molluscan neurons, piracetam, vinpocetine.

Introduction

Voltage-gated K^+ channels are strongly involved in the inhibitory function in the nervous system. The process of learning is associated with the enhancement of the neurons' excitability and is accompanied by an inhibition of K^+-channel functioning. Thus, Disterhoft et al. (1996) have shown that Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$) decreased as a result of conditioning in hippocampal neurons of young animals, but increased in ageing animals unable to learn. Alkon (1989) has found that classical conditioning of the mollusc *Hermissenda* was specifically correlated with the reduction of voltage-gated K^+ current across the soma membrane of identified neurons. On the other hand, K^+-channel antagonists, for example, aminopyridines and apamin, were shown to improve learning in cognitive behavioural studies (Barnes et al., 1989; Davis et al., 1983; Deschaux et al., 1997; Messier et al., 1991). In electrophysiological experiments, K^+-channel blockers, mast cell-degranulating peptides (Kondo et al., 1992) and tetraethylammonium (TEA) (Aniksztein and Ben-Ari, 1991; Ramakers et al., 2000), were found to induce long-term potentiation (LTP) in the mammalian hippocampus.

The idea that K^+-channel blockers might be administered for treatment of senile dementia, especially for its most serious form, Alzheimer's disease (AD), has been debated in the literature during the past two decades (Lavretsky and Jarvik, 1992). In most clinical studies, fast-inactivating A-type K^+-channel blockers, such as 4-aminopyridine (4-AP) or its analogues, were examined, and the results were contradictory. Some investigators describe the amelioration of behavioural deficits (Wesseling et al., 1984), while the others observe no significant effect of 4-AP on any particular symptoms (Davidson et al., 1988; Huff, 1996).

What subtypes of the K^+ channels are to be preferably blocked in patients with dementia is a question of great interest. One of the appropriate approaches to this question is the investigation of the potency of known nootropic agents to block different subtypes of voltage-gated K^+ currents. Kraliz and Sing (1997) studied the ability of tacrine, the well-known anti-dementia drug, to

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modulate different types of ionic currents in the larval muscles of *Drosophila*. They have found that this drug, at concentrations as low as 10 μM, selectively blocked the delayed rectifying K^+ current (I_{KD}) without affecting the three other K^+ currents or the Ca^{2+}-channel current in these cells.

The goal of the present work was to examine the effects of several cognitive enhancers on different types of voltage-gated K^+ current in isolated neurons of the mollusc *Helix pomatia*. The group of drugs included: vinpocetine (cavinton) (Balestreri et al., 1987; Paroszai et al., 1998), piracetam (nootropil) (Gouliaev and Senning, 1994) and a peptide analogue of piracetam, an ethyl ester of *N*-phenyl-acetyl-L-prolyl-glycine (GVS-111) (Gudasheva et al., 1996; Seredenin et al., 1995).

This work is the development of our previous study (Solntseva et al., 1997) where we used relatively high concentrations of the drugs (1–2 mM of piracetam and 0.1–2 μM of GVS-111). These concentrations appeared to block multiple ionic currents, including different types of K^+ and Ca^{2+} current. Moreover, in our previous study, we did not distinguish between I_A (fast-inactivating K^+ current) and I_{KD}. In the present work, we compared nootropic effects on three different components of I_K and revealed the selective effects of low concentrations of nootropics on slow-inactivating K^+ currents.

Voltage-gated K^+ currents in molluscan neurons have been well characterized and completely resolved into three distinct components (Hermann and Erxleben, 1987; Lux and Hofmeier, 1982; Nick et al., 1996; Thompson, 1977). These include: I_A, a fast, transient, 4-AP-sensitive current; I_{KD}, a delayed, sustained TEA-sensitive current; and $I_{K(Ca)}$, a delayed, sustained, Ca^{2+}-activated K^+ current which is both TEA- and charibdotoxin-sensitive.

Electrophysiological and pharmacological properties of these components of high- threshold K^+ current in molluscan neurons look similar to corresponding types of voltage-gated K^+ currents in mammalian neurons (Catterall, 1995; Kaczorowski and Garcia, 1999). Functional similarity of the K^+ channels from different species is believed to result from high ($> 60\%$) amino-acid sequence identity of channel protein across the species (Pfaffinger et al., 1991; Temple et al., 1988). Such an evolutionary conservation allows us to believe that the mechanisms of drug interaction with K^+ channels in invertebrate animals are similar to those in higher animals.

Materials and methods

Cell isolation

The experiments were performed on isolated neurons of the left and right parietal ganglions of the land snail (*Helix pomatia*). Neurons were isolated with the help of perfect needles without any pretreatment of the ganglia with proteolytic enzymes. The neurons under study were usually approx. 20–40 μm in diameter. They were pipetted into a recording chamber of approx. 1 ml volume and continuously perfused with a standard Ringer solution, feeding by gravity.

Voltage-clamp

A two-microelectrode voltage-clamp technique was used. The microelectrodes were filled with potassium citrate solution (2 M). The experiments were perfomed using a MEZ 7101 micro-electrode amplifier and a CEZ 1100 voltage-clamp amplifier (Nihon Kohden, Japan). Voltages and currents were recorded using a RJG 4024 4-channel pen-recorder with a bandwidth of up to 40 kHz. Voltage-gated K^+ currents were evoked by depolarizing test pulses of 100–550 ms applied from a -50 mV holding potential. Test pulses varied from -30 to $+100$ mV with increments of 10 mV. Voltage-gated Ca^{2+} currents were triggered by depolarizing 150–550 ms test pulses applied from the holding potential of -60 mV. In tracing the *I–V* curves, the current responses to equivalent hyperpolarizing pulses were added to cancel linear leakage.

Experimental solutions

The high-threshold K^+ current was recorded in normal Ringer solution containing (mM): NaCl, 100; KCl, 4; $CaCl_2$, 5; $MgCl_2$, 4; $NaHCO_3$, 3; Tris–Cl, 5 (pH 7.6). In experiments studying K^+ currents in Ca^{2+}-free solution, the following ionic composition of external solution was (mM): NaCl, 100; KCl, 4; $MgCl_2$, 6; $NaHCO_3$, 3; Tris–Cl, 5 (pH 7.6). Na^+-free solution containing K^+-channel antagonists was used when analysing the Ca^{2+} current. This solution was composed of (mM): KCl, 4; $CaCl_2$, 10; $MgCl_2$, 4; TEA–Br, 95; 4-AP, 5; Tris–Cl, 5 (pH 7.6).

Drug application

Vinpocetine (Sigma), piracetam (Sigma) and GVS-111 (Institute of Pharmacology, Moscow) were dissolved in the extracellular solution and introduced into the bath medium. The duration of cell exposure to a drug was 5–10 min, and washing time was 20–30 min. The same cell could be used to study of the effects of different drugs. The sequence of the application of different drugs was irregular.

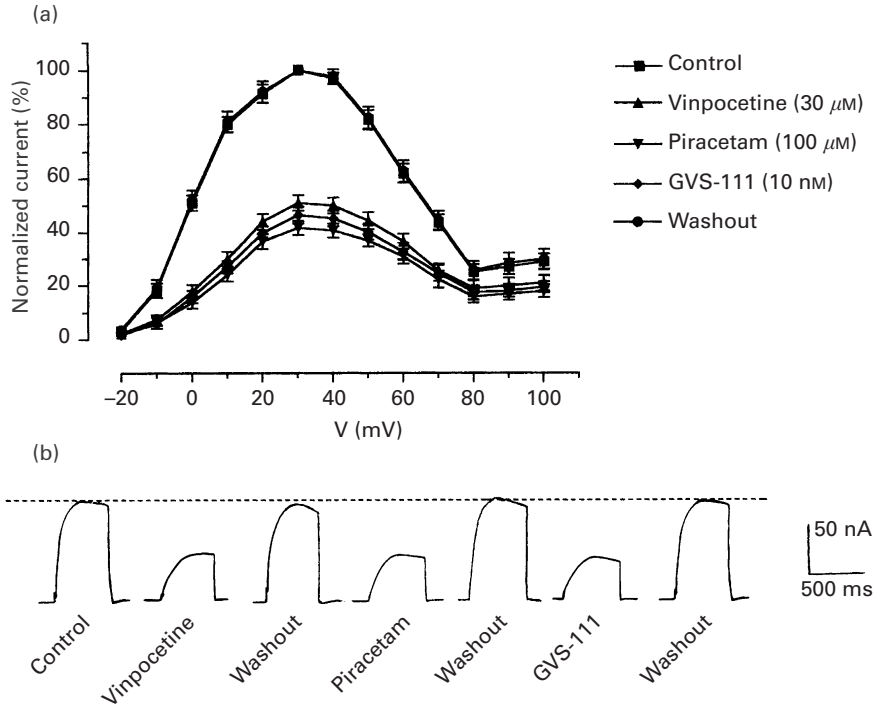

Figure 1. The effects of nootropics on Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$). (a) Current-voltage (I–V) relations for the peak $I_{K(Ca)}$ obtained from 7 cells. Control curves were bell-shaped. Vinpocetine, piracetam and GVS-111 significantly decreased outward current mainly in the 0–60 mV potential region. The washout I–V relationship coincided with the control curve. (b) Current traces recorded in control solution and in the presence of 30 μM vinpocetine, 100 μM piracetam or 10 nM GVS-111. The recordings were obtained from the same cell. The cell was washed with control solution for approx. 30 min between application of the drugs. Holding potential, −50 mV; test potential, +30 mV.

Data analysis

Group data are presented as means ± S.E. Statistical tests of drug effects were performed using paired Student's t tests. A t value producing $p < 0.05$ was considered to be significant.

Results

Three types of high-threshold K^+ current in Helix *neurons*

Three major types of high-threshold K^+ current were recorded in our experiments: (1) an $I_{K(Ca)}$ with slow kinetics of activation and inactivation, that disappeared in a Ca^{2+}-free solution and demonstrated a bell-shaped I–V curve with a maximum of approx. +30 mV (Figure 1). This $I_{K(Ca)}$ was insensitive to 0.1–1 mM 4-AP, but was almost completely blocked by 1 mM TEA; (2) an I_{KD} also with slow gate kinetics and high sensitivity to TEA. In contrast to $I_{K(Ca)}$, this delayed rectifying K^+ current was sustained in Ca^{2+}-free solution and demonstrated a smooth I–V curve (Figure 2); (3) an A-type K^+ current (I_A) showing fast kinetics of activation and inactivation. This A-type K^+ current was resistant in Ca^{2+}-free solution and had a smooth I–V curve (Figure 3). Its pharmacological properties were different from those for $I_{K(Ca)}$ and I_{KD}: 1 mM 4-AP reduced the I_A peak by 50–70%, and TEA did not affect this current. These characteristics of $I_{K(Ca)}$, I_{KD} and I_A are in line with the data of other authors (Hermann and Erxleben, 1987; Lux and Hofmeier, 1982; Nick et al., 1996; Thompson, 1977). The outward current in different cells could contain one, two or all three components.

Our experiments were performed on those cells, whose outward K^+ current contained one predominant component: $I_{K(Ca)}$, I_{KD} or I_A.

The effects of low concentrations of nootropic drugs on K^+ current

The $I_{K(Ca)}$ appeared to be the most sensitive to nootropics. This current was strongly inhibited by all three substances

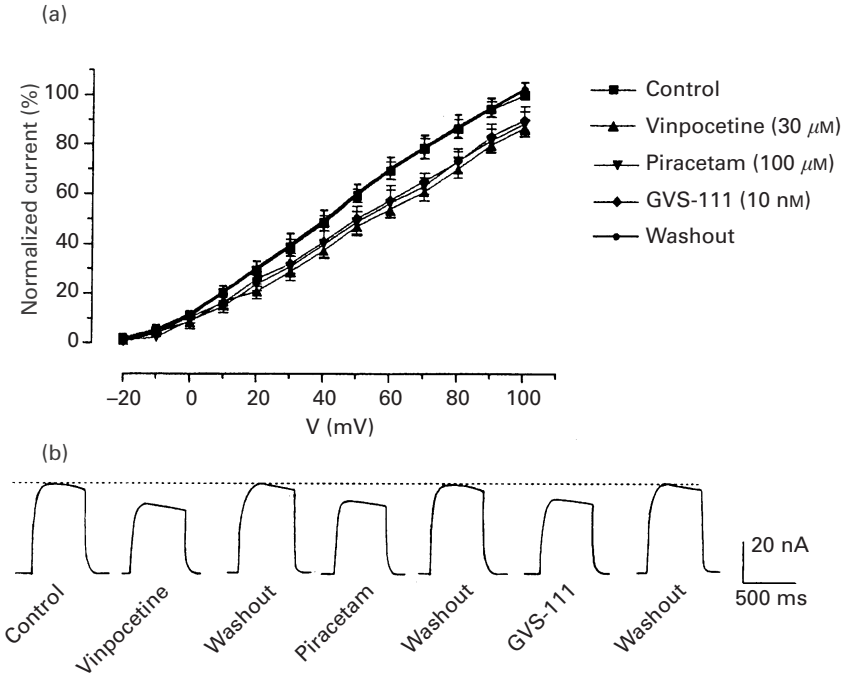

Figure 2. The effects of nootropics on a delayed rectifying K^+ current (I_{KD}). (a) Current–voltage (I–V) relations for the peak I_{KD} obtained from 5 cells. Vinpocetine, piracetam and GVS-111 diminished the amplitude of I_{KD} by approx. 20%, on average. The effects of nootropics were the same in all test potentials used. (b) Current traces recorded from the same cell in control solution and during exposure of the cell to 30 μM vinpocetine, 100 μM piracetam, or 10 nM GVS-111. The reversibility of the effects of the drugs was observed after washing the cell with control solution for 20–30 min. Holding potential, −50 mV; test potential, +30 mV.

tested: vinpocetine, piracetam and GVS-111. The threshold concentrations were: vinpocetine, 10 μM; piracetam, 10 μM, and GVS-111, 1 nM. The peak amplitude of $I_{K(Ca)}$ decreased by approx. 50% in the presence of 30 μM vinpocetine ($49 \pm 5\%$, $n = 17$, $p < 0.005$), 100 μM piracetam ($58 \pm 6\%$, $n = 13$, $p < 0.005$), or 10 nM GVS-111 ($53 \pm 6\%$, $n = 13$, $p < 0.005$) (Figure 1). The effect appeared 1–2 min after drug application, reached its maximum in 5–7 min and was washed out in 15–20 min.

Other subtypes of K^+ current were less affected by the same concentrations of the drugs (30 μM vinpocetine, 100 μM piracetam and 10 nM GVS-111) than $I_{K(Ca)}$.

The I_{KD} was moderately inhibited by nootropics. The peak amplitude of this current was suppressed by $22 \pm 10\%$ ($n = 5$, $p < 0.05$) with 30 μM vinpocetine, by $18 \pm 8\%$ ($n = 6$, $p < 0.05$) with 100 μM piracetam, and by $19 \pm 8\%$ ($n = 8$, $p < 0.025$) with 10 nM GVS-111 (Figure 2).

In contrast to $I_{K(Ca)}$ and I_{KD}, the I_A was not suppressed by nootropics. Moreover, this current was enhanced in the presence of 30 μM vinpocetine by $22 \pm 7\%$ ($n = 6$, $p < 0.025$). Two other drugs applied in low concentration, namely, 100 μM piracetam ($n = 9$) and 10 nM GVS-111 ($n = 6$), did not change the amplitude of the I_A (Figure 3).

Low concentrations of nootropics did not affect inward Ca^{2+} current (I_{Ca})

In the same cells where $I_{K(Ca)}$ was investigated, high-threshold I_{Ca} was measured after substitution NaCl by TEA–Br in perfusing media (see Materials and methods section). It was found that I_{Ca} was resistant to nootropics applied in low concentrations. The amplitude of this current recorded in the presence of 30 μM vinpocetine ($n = 8$), or 100 μM piracetam ($n = 6$), or 10 nM GVS-111 ($n = 6$) had no significant differences from control values (Figure 4). Results allow us to conclude that the blockade of $I_{K(Ca)}$ by nootropics could hardly be explained by changes in the Ca^{2+} influx into the cell.

The effects of high concentrations of nootropics on K^+ currents

Two approaches were employed to test the dose dependence of the drugs' effects. In the first series of experiments, the applications of different concentrations of a drug were separated by 20–30 min washing of the cell with a control solution. In the second series, the cell was exposed to cumulatively increasing concentrations of

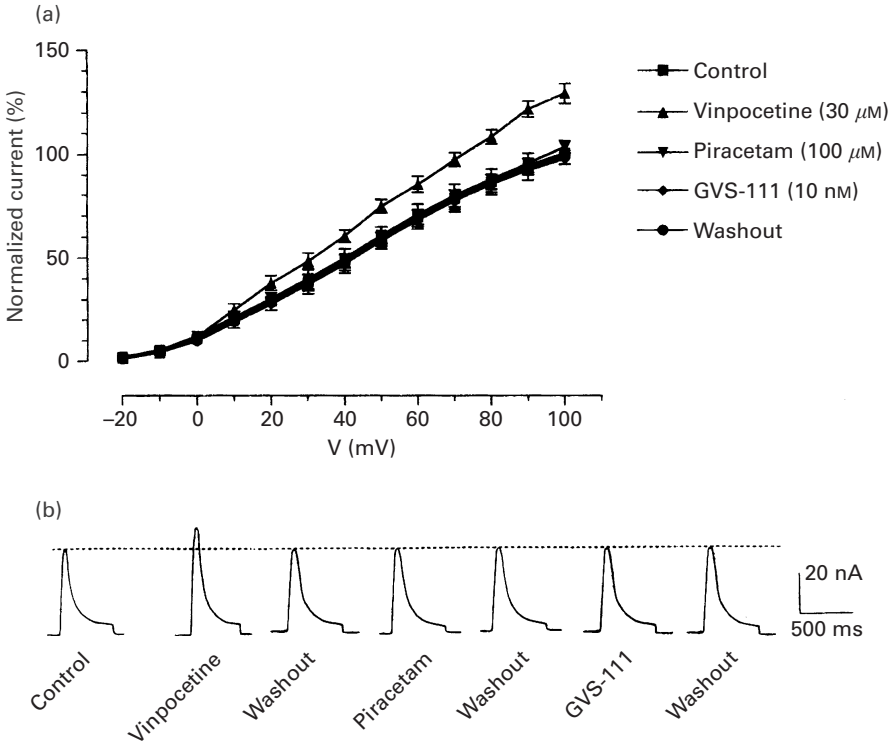

Figure 3. The study of the influence of the nootropics on fast-inactivating K^+ current (I_A). (a) Current–voltage (I–V) relations for the peak I_A (5 cells). Vinpocetine augmented the amplitude of I_A, while both piracetam and GVS-111 did not affect this current. (b) Recordings of I_A obtained from the same cell and made in control solution during the treatment with 30 μM vinpocetine, or 100 μM piracetam, or 10 nM GVS-111. The cell was washed with control solution for 20–30 min between application of the drugs. Holding potential, −50 mV; test potential, +30 mV.

the respective drug. For each nootropic agent, the results obtained from these two groups of experiments were combined.

The dose–effect curves for vinpocetine are shown in Figure 5. The highest concentration of the drug used in the study of K^+ currents was 100 μM. This concentration strongly suppressed $I_{K(Ca)}$ ($55 \pm 12\%$, $n = 7$, $p < 0.005$), moderately inhibited I_{KD} ($28 \pm 8\%$, $n = 6$, $p < 0.01$) and upregulated I_A ($30 \pm 13\%$, $n = 8$, $p < 0.05$). There were no statistically significant differences between the effects caused by 30 and 100 μM vinpocetine.

Figure 6 shows the dose–effect relationships for piracetam. As one can see from the figure there are no significant differences between the effects of 100 and 1000 μM piracetam both on $I_{K(Ca)}$ and on I_{KD}. However, the influence of these two concentrations on the I_A are different. While 100 μM of the drug does not change the amplitude of the current, 1000 μM increases it by $25 \pm 10\%$ on average ($n = 8$, $p < 0.025$).

The dose–effect relationship for GVS-111 is shown in Figure 7. The drug at 100 nM reduced the amplitude of $I_{K(Ca)}$ by $58 \pm 10\%$ ($n = 9$, $p < 0.005$), and this effect had no significant difference from that evoked by 10 nM. The I_{KD} was suppressed by $32 \pm 7\%$ with 100 nM GVS-111 ($n = 8$, $p < 0.005$), and it was stronger than the I_{KD} reduction caused by 10 nM of the substance. Neither 10 nor 100 nM GVS-111 affected I_A. This current was only changed with a high concentration of the drug (1000 nM). The effect was manifest in the decrease of the current amplitude by $18 \pm 8\%$ ($n = 6$, $p < 0.05$).

The inhibitory effect of high concentrations of nootropics on the I_{Ca}

As mentioned above, low concentrations of nootropics did not affect I_{Ca}. However, application of the drugs in higher concentrations led to a noticeable decrease in the amplitude of I_{Ca}. This current was inhibited by $37 \pm 8\%$ ($n = 7$, $p < 0.005$) with 600 μM vinpocetine (Figure 5), by $12 \pm 5\%$ ($n = 6$, $p < 0.05$) with 1000 μM piracetam (Figure 6), and by $32 \pm 8\%$ ($n = 7$, $p < 0.005$) with 1000 nM GVS-111 (Figure 7). A more detailed description of the effects of high concentrations of nootropics on I_{Ca} can be found in our previous work (Solntseva et al., 1997).

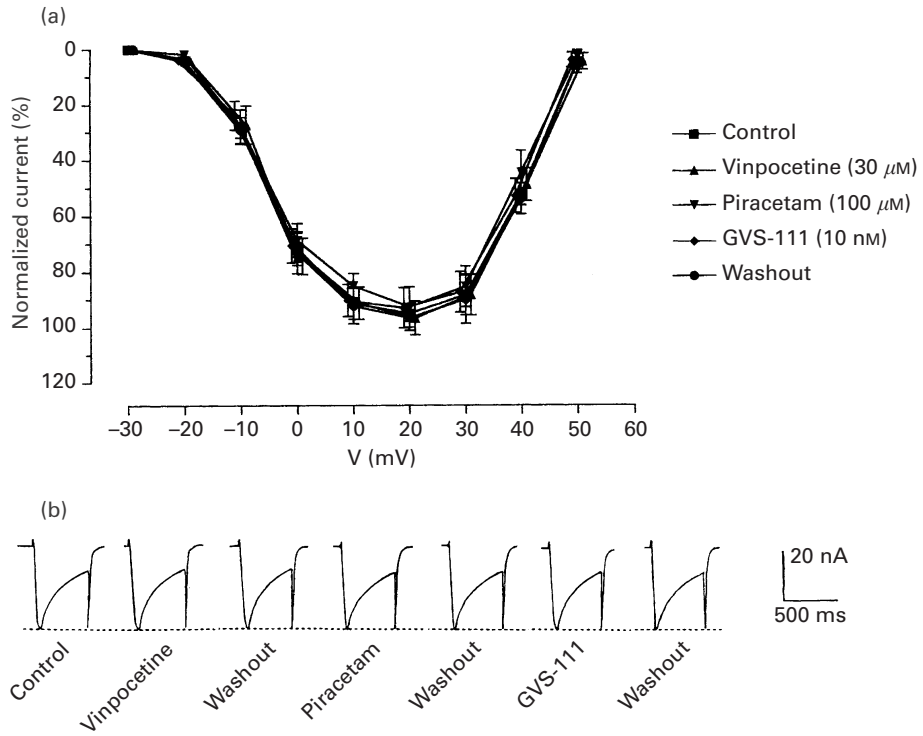

Figure 4. The absence of the effects of low concentrations of nootropics on the inward Ca^{2+} current (I_{Ca}). The neurons were bathed in Na^+-free medium containing 95 mM TEA and 5 mM 4-AP. (a) Current–voltage (I–V) relations for the peak inward current (6 cells). There were no noticeable changes in the amplitude of I_{Ca} in the presence of any of the nootropic drugs examined. (b) The recordings of the inward I_{Ca} of the same cell obtained in control solution and in the presence of 30 μM vinpocetine, 100 μM piracetam, or 10 nM GVS-111. The cell was washed with control solution for 20–30 min between application of the drugs. Holding potential, −60 mV; test potential, +20 mV.

Discussion

In the available literature we were not able to find any data on the interaction of vinpocetine, piracetam and GVS-111 with K^+ channels, whereas the influence of vinpocetine and piracetam on the inward voltage-gated Ca^{2+} and Na^+ currents was described. Thus, vinpocetine inhibited Ca^{2+} current with an IC_{50} value of 100 μM, but piracetam, at 100 μM, did not affect this current in *Xenopus* oocytes injected with brain mRNA (Kaneko et al., 1990). Vinpocetine blocked Na^+ current in rat cortical neurons with an IC_{50} value of 44 μM (Molnar and Erdo, 1995), and piracetam was not sufficiently potent to suppress Na^+ current in rat pyramidal neurons at concentrations as high as 3 mM (Kopanitsa et al., 2000).

Our study, conducted in isolated molluscan neurons, revealed that the nootropics vinpocetine, piracetam and GVS-111, used in low concentrations, inhibit slow-inactivating, TEA-sensitive subtypes of K^+ currents, i.e. $I_{K(Ca)}$ and I_{KD}. The $I_{K(Ca)}$ appeared to be the most sensitive current to all the drugs examined in our work. This current was reduced by approx. 50% in the presence of 30 μM vinpocetine, 100 μM piracetam, or 10 nM GVS-111. The I_{KD} was inhibited by approx. 20% with the same concentrations of the substances. In contrast, the fast-inactivating, 4-AP-sensitive K^+ current (I_A) was not diminished by low concentrations of nootropics. Piracetam and GVS-111 did not affect this current, while vinpocetine even augmented it. The I_{Ca} persisted in the presence of nootropics.

The effective concentrations of the compounds seem to be in the relevant range with their effective anti-amnesic doses revealed in the behavioural experiments: piracetam, 200–300 mg/kg; GVS-111, 0.1–0.5 mg/kg (Gudasheva et al., 1996; Seredenin et al., 1995); vinpocetine, 10 mg/kg (Paroczai et al., 1998). Having been converted into a molar scale, these values approximately correspond to 1.5–2 mM of piracetam [FW (formula weight) = 142], 0.3–2 μM of GVS-111 (FW = 318), and 30 μM of vinpocetine (FW = 350).

The question about the participation of the different K^+ subtypes in brain functions is debated in the literature. Different K^+ channels were shown to have distinct spatial and temporal patterns of appearance in the brain. The localization of specific subtypes of K^+ channels was found to have both subcellular (Pongs, 1999; Tan and Llano,

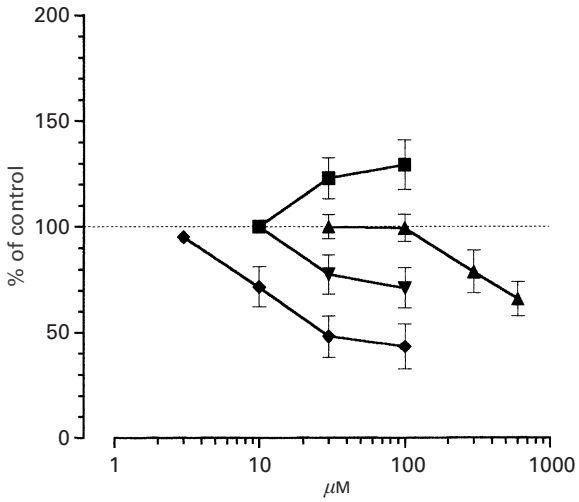

Figure 5. Dose–response curves of vinpocetine effects on the ionic currents of molluscan neurons. The logarithmic concentration of vinpocetine is plotted vs. the mean amplitude of the ionic current, expressed in % of the control amplitude. Vinpocetine inhibited both $I_{K(Ca)}$ and I_{KD} in low and moderate concentrations, augmented I_A in low and moderate concentrations, and suppressed I_{Ca} only in high concentrations. Holding potential and test potential for the outward current ($I_{K(Ca)}$, I_{KD} and I_A) was −50 and +30 mV, respectively. Holding potential and test potential for the inward current (I_{Ca}) was −60 and +20 mV, respectively. ■, I_A; ▲, I_{Ca}; ▼, I_{KD}; ◆, $I_{K(Ca)}$.

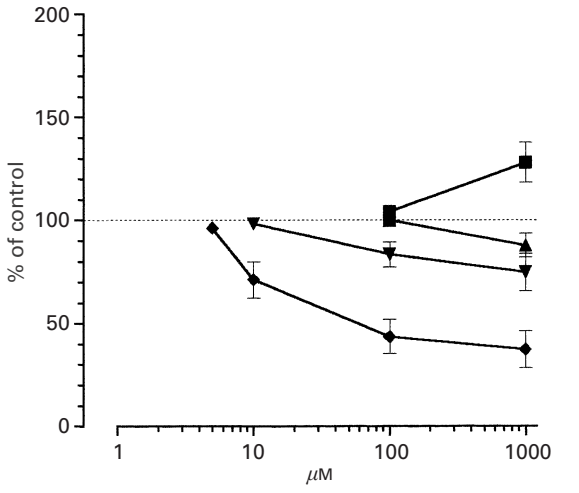

Figure 6. Dose–response curves of the effects of piracetam on the ionic currents. The mean amplitude of the respective current, expressed in % of the control amplitude, is plotted vs. the logarithmic concentration of the drug. Piracetam strongly inhibited $I_{K(Ca)}$ at 10–1000 μM, moderately inhibited I_{KD} at 10–1000 μM, diminished I_{Ca} only at 1000 μM, and augmented I_A only at 1000 μM. The outward current ($I_{K(Ca)}$, I_{KD} and I_A) was measured at +30 mV, and the inward current (I_{Ca}) was recorded at +20 mV. ■, I_A; ▲, I_{Ca}; ▼, I_{KD}; ◆, $I_{K(Ca)}$.

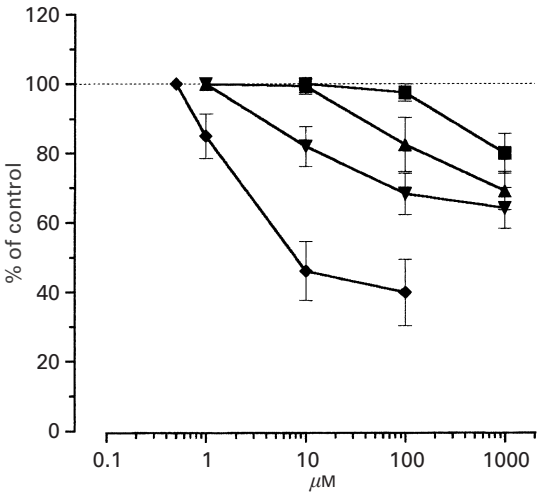

Figure 7. Dose–response curves of GVS-111 effects on the ionic currents. The amplitude of respective current, expressed in % of the control amplitude, is plotted vs. the drug concentration. GVS-111 strongly suppressed $I_{K(Ca)}$ at 10–100 nM, moderately inhibited I_{KD} at 10–1000 nM, diminished I_{Ca} at 100–1000 nM, and partially blocked I_A at 1000 nM only. The outward current ($I_{K(Ca)}$, I_{KD} and I_A) was measured at +30 mV, and the inward current (I_{Ca}) was recorded at +20 mV. ■, I_A; ▲, I_{Ca}; ▼, I_{KD}; ◆, $I_{K(Ca)}$.

1999) and brain (Drewe et al., 1992; Gehlert and Gackenheimer, 1993) regional differences, and selective expression of specific K^+ channels has been described during maturation of neuronal excitability (Drewe et al., 1992; Nick et al., 1996) and with ageing (Disterhoft et al., 1996; Tribut et al., 1999).

The study of molecular mechanisms of associative memory and learning in animal models, both vertebrates and invertebrates, has revealed that a long-lasting reduction of specific K^+ currents is critical for the acquisition and storage of associative learning (Alkon, 1989; Disterhoft et al., 1996; Milner et al., 1998). The blockade of different subtypes of K^+ channels causes LTP in the hippocampus with different strength and duration. Thus, TEA, the antagonist of slow-inactivating K^+ channels, was found to be more potent to induce LTP than 4-AP, the antagonist of the fast-inactivating K^+ channels (Aniksztein and Ben-Ari, 1991).

K^+-channel dysfunction was discovered to occur in AD patients and was suggested to be involved in the mechanisms of memory loss in AD (Etcheberrigaray and Alkon, 1997). It was found that the 113-pS K^+ channel was consistently missing in fibroblasts from AD patients, and this omission was shown to be due to an action of β-amyloid, a peptide that is the main component of neurotic plaques and is widely believed to play a critical role in the pathophysiology of AD. Good et al. (1996) studied the

effect of β-amyloid on the ionic currents of rat hippocampal neurons and reported that only the I_A was clearly and most significantly affected by treatment. The results obtained by Kraliz and Singh (1997) in larval muscle of *Drosophila* are in agreement with the observations mentioned above. The authors have determined that tacrine, an agent used in treatment of AD, selectively blocks the slow-inactivating I_{KD} without affecting I_A.

Therefore, the results of the present work taken together with data in the literature allow us to suggest that the blockade of slow-inactivating subtypes of K^+ channels can be important in treatment of memory impairment.

It is notable that K^+-channel blockade may cause some side-effects. It should be kept in mind that K^+ antagonists increasing Ca^{2+} influx into the cell, can cause not only the strengthening of synaptic efficacy, but neurotoxic side-effects also. In some forms of dementia (e.g. ischaemia), where excitotoxic mechanisms play a major role in the progression of the disease, the enhancement of excitability is not necessarily good.

Acknowledgements

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