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 Ca²⁺ 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 Ca²⁺ current (I_{Ca}) and three subtypes of the outward voltage-gated K⁺ current were recorded: Ca²⁺-dependent K⁺ current (I_{K(Ca)}), delayed rectifying current (I_{RD}), 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_{RD} were inhibited (I_{K(Ca)} more strongly than I_{RD}). 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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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 Ca²⁺-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 Hermisenda 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...
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 (I₉₅₀) without affecting the three other K⁺ currents or the Ca²⁺-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) (Balestrieri 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 Ca²⁺ current. Moreover, in our previous study, we did not distinguish between I₉ (fast-inactivating K⁺ current) and I₉ificacion. In the present work, we compared nootropic effects on three different components of I₉ 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: I₉, a fast, transient, 4-AP-sensitive current; I₉ично, a delayed, sustained TEA-sensitive current; and I₉Ca, a delayed, sustained, Ca²⁺-activated K⁺ current which is both TEA- and charidotoxin-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 ganglia 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 performed 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 Ca²⁺ 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; CaCl₂, 5; MgCl₂, 4; NaHCO₃, 3; Tris–Cl, 5 (pH 7.6). In experiments studying K⁺ currents in Ca²⁺-free solution, the following ionic composition of external solution was (mM): NaCl, 100; KCl, 4; MgCl₂, 6; NaHCO₃, 3; Tris–Cl, 5 (pH 7.6). Na⁺-free solution containing K⁺-channel antagonists was used when analysing the Ca²⁺ current. This solution was composed of (mM): KCl, 4; CaCl₂, 10; MgCl₂, 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 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 (\(I\text{--}V\)) 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 \(I\text{--}V\) relationship coincided with the control curve. (b) Current traces recorded in control solution and in the presence of 30 \(\mu\)M vinpocetine, 100 \(\mu\)M 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 \(\pm\) 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 \(I\text{--}V\) 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 \(I\text{--}V\) 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 \(I\text{--}V\) 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
tested: vinpocetine, piracetam and GVS-111. The threshold concentrations were: vinpocetine, 10 µM; piracetam, 100 µM, and GVS-111, 1 nm. The peak amplitude of $I_{K(Ca)}$ decreased by approx. 50% in the presence of 30 µM vinpocetine ($49 ± 5\%$, $n = 17$, $p < 0.005$), 100 µM piracetam ($58 ± 6\%$, $n = 13$, $p < 0.005$), or 10 nm GVS-111 ($53 ± 6\%$, $n = 13$, $p < 0.005$) (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 $22 ± 10\%$ ($n = 5$, $p < 0.05$) with 30 µM vinpocetine, by $18 ± 8\%$ ($n = 6$, $p < 0.05$) with 100 µM piracetam, and by $19 ± 8\%$ ($n = 8$, $p < 0.025$) with 10 nM GVS-111 (Figure 2).

In contrast to $I_{K(Ca)}$ and $I_{KDP}$, the $I_\lambda$ was not suppressed by nootropics. Moreover, this current was enhanced in the presence of 30 µM vinpocetine by $22 ± 7\%$ ($n = 6$, $p < 0.025$). 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 $I_\lambda$ (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
233
Nootropics suppress K⁺ current 150 100
50 0
(a)
Control
Vinpocetine (30 µM)
Piracetam (100 µM)
GVS-111 (10 nM)
Washout
Normalized current (%)
−20 0 20 40 60 80 100 V (mV)
(b)
Control
Vinpocetine
Washout
Piracetam
Washout
GVS-111
Washout
20 nA
500 ms
Figure 3. The study of the influence of the nootropics on fast-inactivating K⁺ current (Iₚ). (a) Current–voltage (I–V) relations for the peak Iₚ (5 cells). Vinpocetine augmented the amplitude of Iₚ, while both piracetam and GVS-111 did not affect this current. (b) Recordings of Iₚ 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 inhibitory effect of high concentrations of nootropics on the IₖCa

As mentioned above, low concentrations of nootropics did not affect IₖCa. However, application of the drugs in higher concentrations led to a noticeable decrease in the amplitude of IₖCa. This current was inhibited by 37 ± 8% (n = 7, p < 0.005) with 600 µM vinpocetine (Figure 5), by 12 ± 5% (n = 6, p < 0.05) with 1000 µM piracetam (Figure 6), and by 32 ± 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 IₖCa 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 (\(I_{\text{Ca}}\)). The neurons were bathed in Na\(^+\)-free medium containing 95 mM TEA and 5 mM 4-AP. (a) Current–voltage (\(I-I\)) relations for the peak inward current (6 cells). There were no noticeable changes in the amplitude of \(I_{\text{Ca}}\) in the presence of any of the nootropic drugs examined. (b) The recordings of the inward \(I_{\text{Ca}}\) of the same cell obtained in control solution and in the presence of 30 \(\mu\)M vinpocetine, 100 \(\mu\)M 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 \(\mu\)M, but piracetam, at 100 \(\mu\)M, 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 \(\mu\)M (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 \(\mu\)M vinpocetine, 100 \(\mu\)M 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 \(\mu\)M of GVS-111 (FW = 318), and 30 \(\mu\)M 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,
Nootropics suppress $K^+$ current

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 $I_{K(D)}$ 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 inward current ($I_A$) was $-60$ and $+20$ mV, respectively. Holding potential and test potential for the outward current ($I_{K(Ca)}$, $I_{K(D)}$, and $I_{Ca}$) was $-50$ and $+30$ mV, respectively. $\text{I}_A$, $\text{I}_{Ca}$; $\text{I}_{K_{Ca}}$; $\text{I}_{K_{D}}$.

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 $10–1000 \mu M$, moderately inhibited $I_{K_{D}}$ at $10–1000 \mu M$, diminished $I_{Ca}$ only at $1000 \mu M$, and augmented $I_A$ only at $1000 \mu M$. The outward current ($I_{K_{Ca}}$, $I_{K_{D}}$, and $I_{Ca}$) was measured at $+30$ mV, and the inward current ($I_A$) was recorded at $+20$ mV. $\text{I}_A$, $\text{I}_{Ca}$; $\text{I}_{K_{Ca}}$; $\text{I}_{K_{D}}$.

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 $10–100 \text{nM}$, moderately inhibited $I_{K_{D}}$ at $10–1000 \text{nM}$, diminished $I_{Ca}$ at $100–1000 \text{nM}$, and partially blocked $I_A$ at $1000 \text{nM}$ only. The outward current ($I_{K(Ca)}$, $I_{K_{D}}$, and $I_{Ca}$) was measured at $+30$ mV, and the inward current ($I_A$) was recorded at $+20$ mV. $\text{I}_A$, $\text{I}_{Ca}$; $\text{I}_{K_{Ca}}$; $\text{I}_{K_{D}}$; $\text{I}_{K_{Ca}}$.

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 $\beta$-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 $\beta$-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.

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