Effect of Vinpocetine on Various Types of High-Threshold Potassium Currents in Snail Neurons

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A high-threshold (-20 mV) K^+ current was recorded from isolated edible snail neurons by a two-microelectrode voltage clamp technique. This current consisted of three components: fast-inactivating K^+ currents (I_A), noninactivating K^+ current (I_{KD}), and Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$). Different cells had one to three components of K^+ current. Vinpocetine increased I_A, moderately inhibited I_{KD} (by 30-50%) and strongly suppressed $I_{K(Ca)}$ (by 60-90%). Inhibition of $I_{K(Ca)}$ was not related to the effect of vinpocetine on the inward Ca^{2+} current. When K^+ current consisted of all three components, the effect of vinpocetine on the ionic current amplitude was opposite at different potentials.

Key Words: *nootropic agents; vinpocetine; A-current; delayed rectified current; Ca^{2+}-dependent K^+ current; snail neurons*

Potassium channels of neuronal membrane are considered as the targets for some drugs [9]. Specifically, the role of K^+ channels in the effect of the drugs that stimulate the cognitive functions (nootropic agents) is currently discussed [15]. This role was confirmed by electrophysiological experiments where changes in K^+ currents of neuronal membrane were observed under the effect of nootropic agents applied in the doses comparable to therapeutic ones. This effect is produced by the following nootropic agents: tacrine, amiridin [2], pyracetam, GBS-111 [13], and NIK-247 [8].

Our aim was to examine the effect of the nootropic agent vinpocetine (VP) on potassium currents of the neuronal membrane. This work continues our studies which showed that the total high-threshold K^+ current of snail neurons is changed by low doses of VP [1]. However, the specificity of VP effect on various components of high-threshold K^+ current with different kinetic and pharmacological properties [3,14] remained unclear.

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MATERIALS AND METHODS

Experiments were carried out on isolated neurons of the snail *Helix pomatia* using two-microelectrode voltage clamp technique based on a Nihon Kohden standard device for microelectrode studies. Vinpocetine (Sigma) was dissolved in physiological saline and added to the chamber when the flow was stopped. Other methodical features were described elsewhere [1,13].

RESULTS

Three types of high-threshold K^+ current (activation threshold -20 mV) were recorded in our experiments. Type 1 current was preserved in a Ca^{2+}-free solution. It had fast activation and inactivation kinetics, while its current-voltage relation (CVR) was represented by a smooth curve. These features correspond to the characteristics of A-type K^+ current (I_A) described elsewhere [3]. Type 2 current was also preserved in Ca^{2+}-free solution and had a smooth CVR. However, in contrast to type 1 current, it was activated slowly and was practically inactivated for

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500 msec. Type 2 current corresponds to delayed rectified current I_{KD} [3,14]. Type 3 current disappeared in Ca^{2+}-free solution, it had slow kinetics of activation and inactivation, and a bell-shaped CVR. Evidently, it was the Ca^{2+}-dependent K^+ current $I_{K(Ca)}$ [3,14]. Some neurons in our experiments demonstrated only one of these currents, while other neurons had potassium current of two or three types.

The effect of VP was studied on 35 neurons. Vinpocetine was added to the extracellular solution to a concentration of 30-60 μmol/liter. In 24 out of 35 cells there were marked changes in the amplitude of K^+ current, which were reversed after washout. As a rule, the effect started within the first minute after VP addition, reached the maximum after 3-8 min, and disappeared after 15-20 min of washout with the control solution. Both the sign and the degree of the effect depended on the type of potassium current.

I_A either increased (n=4/8) or remained unchanged (n=4/8) under the effect of VP. Figure 1 shows an increase in I_A amplitude caused by VP (50 μmol/liter). Clearly, potassium current of this type has fast activation and inactivation kinetics. The characteristic time of activation was 20 msec and that of inactivation (85%) was 500 msec. CVRs measured in the control solution and after application of VP attest to the independence of the current increment on membrane potential. The increment of I_A in this cell was 30%. In three other cells the VP-induced increase in I_A varied from 20 to 70%.

Vinpocetine either decreased (n=4/6) or did not affect (n=2/6) I_{KD}. The decrease in I_{KD} amplitude varied in different cells from 30 to 50%. Figure 2 shows this effect for one cell. Evidently, activation of this current developed for 130 msec, while inactivation was only 5% at the end of a 500-msec pulse. CVR plotted for VP test solution demonstrates independence of I_{KD} inhibition on membrane potential.

Vinpocetine also inhibited $I_{K(Ca)}$ (n=6/7). The effect was more pronounced in comparison with I_{KD}, and it varied in different cells from 60 to 90%. Figure 3 shows the inhibitory effect of VP on $I_{K(Ca)}$ in a neuron. The current trace shows that activation lasted for 180 sec, while inactivation was only 6% at the end of a 500-msec pulse. Vinpocetine not only decreased the amplitude of $I_{K(Ca)}$, but also slowed its activation. CVR of this current was a bell-shaped curve with the maximum at 30 mV, which corresponds to the maximum of inward Ca^{2+} current. The effect of VP was potential-dependent and it was most pronounced at potentials near 30 mV.

Taking into consideration the literature data [6] and our findings [1] on ability of VP to block potential-dependent Ca^{2+} channels, one may propose that inhibition of $I_{K(Ca)}$ by VP is coursed by inhibition of inward Ca^{2+} current (I_{Ca}). To check up this hypothesis, we tested the effects of VP on $I_{K(Ca)}$ and I_{Ca} in the same cells (n=5). They showed that a marked blockade of I_{ca} was produced by an order of magnitude higher concentrations of VP (300-600 μmol/liter) than blockade of $I_{K(Ca)}$. These data indicate that inhibition of $I_{K(Ca)}$ is not mediated by the effect of VP on Ca^{2+} channels.

In the cells where the high-threshold potassium current had two or three components (n=14) the effect of VP on each component was the same as in the above, i.e., I_A was augmented, I_{KD} was mo-

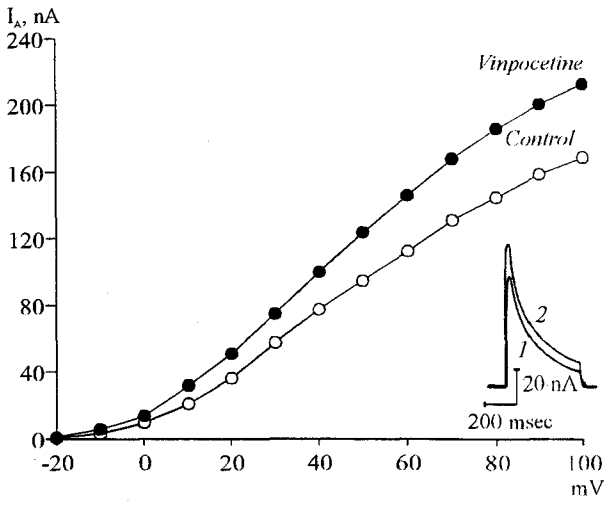

Fig. 1. Increase in the amplitudes of fast inactivating I_A caused by vinpocetine (50 μmol/liter). Here and in Figs. 2 and 3: current-voltage relations are plotted for the control solution and for the test solution with vinpocetine. The inset shows the currents evoked by test stimulation to 30 mV in (*1*) control solution and (*2*) in the test solution with vinpocetine. Holding potential was -50 mV.

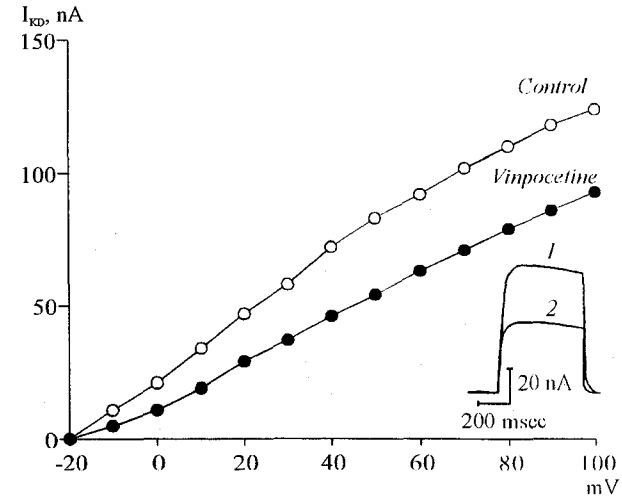

Fig. 2. A decrease in the amplitude of non-inactivating I_{KD} caused by vinpocetine (40 μmol/liter).

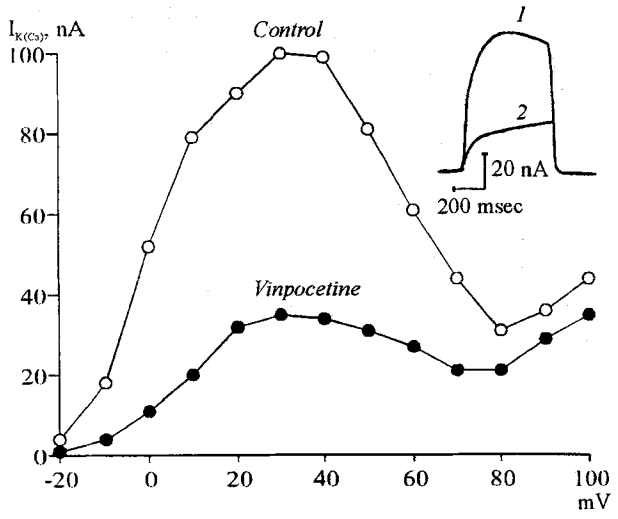

Fig. 3. A decrease in the amplitude of Ca^{2+}-dependent K^+ current $I_{K(Ca)}$ caused by vinpocetine (30 μmol/liter).

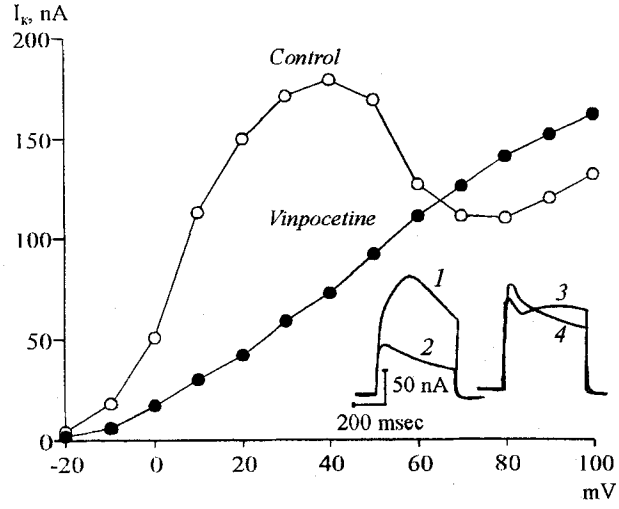

Fig. 4. Changes in the amplitude of the total potassium currents ($I_{K(Ca)}+I_{KD}+I_A$) caused by vinpocetine (60 μmole/liter). The inset shows K^+ current at test depolarization (*1*, *2*) to 30 mV and (*3*, *4*) 100 mV in control solution (*1*, *3*) and in test solution with vinpocetine (*2*, *4*).

derately inhibited, and $I_{K(Ca)}$ was strongly suppressed. The opposite effects of VP on various components of potassium currents account for the opposite changes in the total potassium current induced by VP at various potentials and the crossing of CVRs plotted for the control and test solutions. Figure 4 shows that the control CVR is *N*-shaped with a maximum near 40 mV, which attests to the availability of $I_{K(Ca)}$. The contribution of I_A and I_{KD} to the total current at potentials 0-70 mV is smaller than that of $I_{K(Ca)}$. At potentials 80-100 mV, the inward current consists only of I_A and I_{KD}. Vinpocetine completely blocked $I_{K(Ca)}$, partially inhibited I_{KD}, and augmented I_A. As a result, the total outward current was decreased in the potential range from -20 to 60 mV, and it was increased when the membrane potential varied from 70 to 100 mV. Vinpocetine modified the character of CVR from an *N*-shaped curve to a monotone plot that crossed the control CVR plot at 65 mV.

Our data show that potassium currents in snail neurons are modified by VP in micromolar concentrations. Probably, potassium channels of neuronal membrane could be the molecular targets for this nootropic agent.

The search for molecular targets of VP is currently in progress. Two effects were found which also were induced by VP in micromolar concentrations: blockade of potential-dependent Na^+ current in rat cerebral neurons [11] and inhibition of cyclic nucleotide phosphodiesterase (Ca^{2+}/calmodulin-dependent type) [4,10]. The question is still open whether K^+ and Na^+ channels, on the one hand, and phosphodiesterase, on the other, are independent targets for VP, or whether the ionic channels modify their own activity due to changes in the level of cyclic nucleotides.

Other effects of VP were induced by several hundred micromoles of this drug. They include inhibition of Ca^{2+} current [1,6] and blockade of various glutamate receptors [5,7]. Presumably, these effects do not play any important role in the therapeutic action of VP.

The physiological role of VP-induced changes in the amplitude of the high-threshold potassium current could be based on modulation of Ca^{2+} entry into the cell during the action potential. Our data indicate that VP increases and decreases Ca^{2+} entry into a cell. In the cells or cellular organelles with a large number of A-type channels VP decreases Ca^{2+} entry due to enhancement of I_A and by shortening the action potential. By contrast, if the outward current is composed of $I_{K(Ca)}$ or I_{KD}, Ca^{2+} current will be increased by VP. As the density of diverse types of K^+ channels is different in various cerebral structures and even in various parts of neuronal membrane [12], it is probable that the integral pattern of VP-evoked alterations in the intracellular Ca^{2+} in CNS will be rather complicated.

It is interesting that another widely used nootropic agent, pyracetam, known to modify K^+ currents in snail neuron [13], affects various types of K^+ currents in a different manner. For example, pyracetam inhibits $I_{K(Ca)}$, but does not modify I_A.

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