The Calcium Channel Antagonist Diltiazem Effectively Blocks Two Types of Potassium Channels in the Neuronal Membrane

Yu. V. Bukanova and E. I. Solntseva

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Effect of the Ca^{2+}-channel antagonist diltiazem on potential-operated Ca^{2+} and K^+ currents was studied on isolated edible snail neurons by a two-microelectrode patch-clamp technique. Diltiazem in a concentration of 0.1 mM inhibits Ca^{2+} current, high-threshold Ca^{2+}-dependent K^+ current, and Ca^{2+}-independent K^+ current and has no effect on low-threshold K^+ current and leakage current. It is suggested that therapeutic effect of diltiazem is mediated through blockade of Ca^{2+} and K^+ channels.

Key Words: *K^+ channels; Ca^{2+} channels; Ca^{2+} antagonists; diltiazem; snail neurons*

Calcium channel antagonists (dihydropyridines, phenylalkylamines, and benzothiazepines) usually applied in cardiology [2] are now gaining wide acceptance in other fields of medicine, for instance, in the treatment of psychoneurological disorders, since these drugs possess antidepressant, anticonvulsant, anxiolytic, antidopaminergic, and nootropic activities [1, 10,12,14].

There are new data on the mechanisms of their action. The traditional concept that the effect of calcium channel antagonists is mediated only through blockade of voltage-dependent calcium channels is now out of date. The presence of other targets for these drugs on the neuronal membrane becomes evident.

For instance, it has been shown that dihydropyridines effectively inhibit not only calcium but also potassium channels of different types in the neuronal membrane [8,11,17].

The effect of two other classes of Ca^{2+} antagonists (phenylalkylamines and benzothiazepines) on voltage-dependent K^+ channels of the neuronal membrane remains unstudied. However, it has been reported that verapamil (phenylalkylamine) and diltiazem (DT, benzothiazepine) block K^+ channels in other cells: lymphocytes [7], epitheliocytes [6], and muscle cells [15].

The aim of the present study was to compare the effect of the Ca^{2+} antagonist DT [3,9,18] on calcium channels and different types of potassium channels in the neuronal membrane.

MATERIALS AND METHODS

Experiments were carried out on isolated neurons from snail *Helix pomatia L.* The neurons (30-50 μ) were isolated from the right and left parietal and visceral ganglia. The potential was fixed and transmembrane current was recorded using two intracellular microelectrodes filled with 2 mM potassium citrate. The cell was placed into a 1-ml chamber with circulating physiological saline. Normal Ringer solution for recording the low- and high-threshold potassium currents and leakage currents contained (in mM): 100 NaCl, 4 KCl, 5 $CaCl_2$, 4 $MgCl_2$, and 5 Tris-HCl. Calcium-free solution for recording the potassium currents contained 100 NaCl, 4 KCl, 9 $MgCl_2$, and 5 Tris-HCl. Solution for recording the calcium currents contained 10 $CaCl_2$, 4 KCl, 4 $MgCl_2$, 5 Tris-HCl, and 95 tetraethylammonium bromide

Laboratory of Functional Synaptology, Institute of the Brain, Russian Academy of Medical Sciences, Moscow

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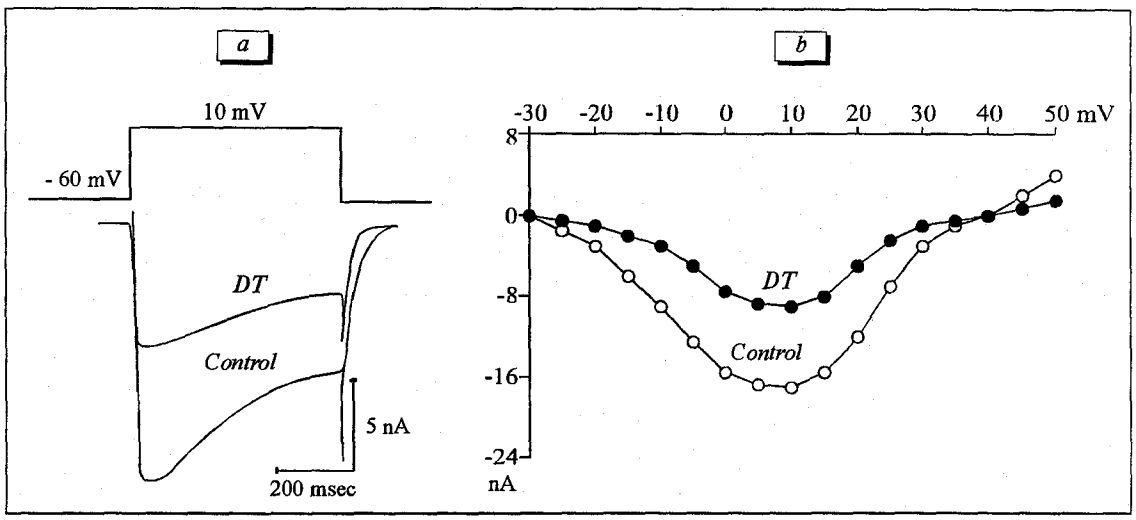

Fig. 1. Effect of diltiazem (*DT*) on high-threshold calcium current (I_{Ca}). A record (*a*) and volt-ampere characteristics (*b*) of I_{Ca} in the absence and presence of 0.1 mM DT.

(TEA). The pH of these solutions was adjusted to 7.6 with HCl. DT was dissolved in the control solution and added to the chamber when the flow was stopped. The experiments were carried out in a Nihon Kohden standard device for microelectrode studies. The holding voltage for recording the calcium and potassium currents was set at -60 mV and -50 mV, respectively. The duration of depolarizing test pulses was 150-500 msec.

RESULTS

The effect of DT on high-threshold calcium currents (I_{Ca}) was studied on 8 cells. In 6 of them, DT reversibly blocked the peak amplitude of I_{Ca} and had no appreciable effect on the kinetics of its activation and inactivation, the threshold concentration being 0.01 mM. At a concentration of 0.1 mM DT inhibited I_{Ca} by 17-100%. This effect took 3-6 min to develop and was completely abolished by 30-min washout with the control solution. The inhibiting effect of 0.1 mM DT on I_{Ca} in a one particular cell is shown on Fig. 1. It should be noted that DT inhibits not only inward Ca^{2+} currents but also residual (TEA-resistant) inward currents recorded at high-voltage test stimuli (50, 60 mV).

The low-threshold potassium currents were recorded (n=5) at a holding voltage of -50 mV after the hyperpolarizing stimulation shifting the membrane potential to -130 mV had been stopped. Diltiazem was added to the incubation medium in concentrations of 0.01-1 mM. It was found that DT has no effect on the amplitude of the low-threshold potassium current (data not shown).

The leakage currents were recorded during hyperpolarizing stimulation shifting the membrane potential from -50 to -80 mV (n=10). Diltiazem (0.01-1 mM) had no effect on the amplitude of these currents (data not shown).

The high-threshold potassium current was recorded against the background of depolarizing stimulation shifting the membrane potential from -50 to -30 mV and then stepwise to 100 mV (by 10 mV). The activation threshold for the inward currents constituted -20 mM. The parameters of the high-threshold potassium current varied from cell to cell due to the presence of different types of potassium channels in these cells [4,16]. The cells were separated in two groups.

In group 1 cells (n=4), the outward current was characterized by rapid activation and inactivation kinetics, had the same parameters in calcium-free medium, and its amplitude gradually increased along with the magnitude of the test stimulus. These characteristics suggest that this current does not depend on Ca^{2+} inward current ($I_{K(V)}$) [4,16]. Diltiazem in a concentration of 0.1 mM reduced the peak amplitude of $I_{K(V)}$ in all test cells. The blockade took 2-5 min to develop and was completely reverted by a 20-30-min washout with the control solution. This inhibition varied from cell to cell and constituted 10-94%. Figure 2 demonstrates the effect of DT on $I_{K(V)}$ recorded in one particular cell.

In group 2 cells (n=4), the outward current was characterized by slow activation and inactivation kinetics, its amplitude was considerably suppressed in calcium-free medium, and its volt-ampere characteristics was N-shaped. This suggests the presence of a Ca^{2+} entry-dependent component $I_{K(Ca)}$ in the outward current in these cells [4,16]. In all cells tested, DT in a concentration of 0.1 mM reduced the peak amplitude of $I_{K(Ca)}$ by 14-100%. The effect

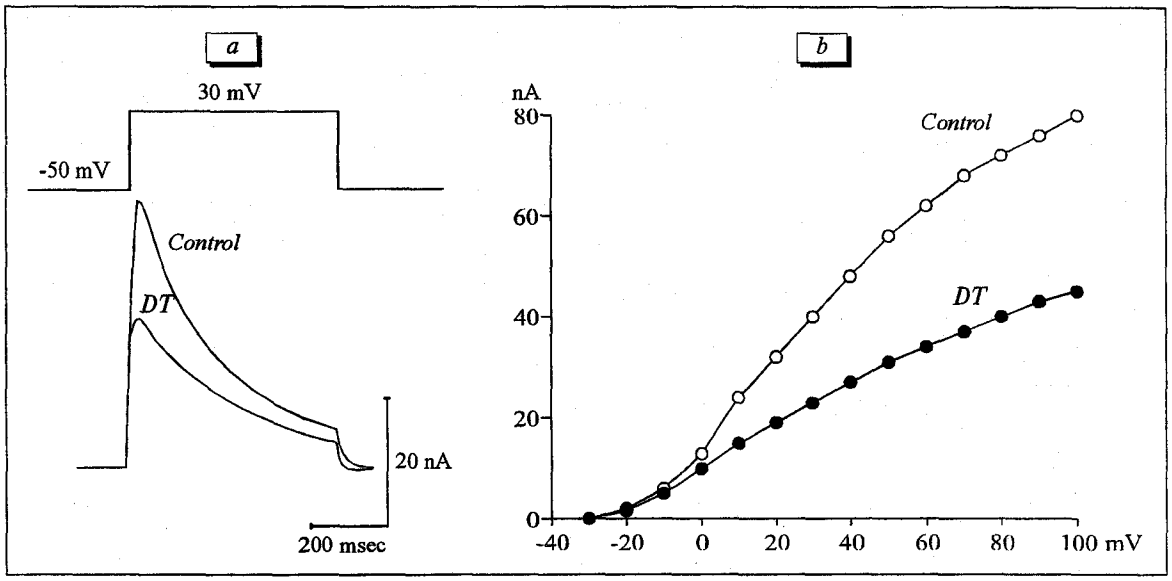

Fig. 2. Effect of diltiazem (*DT*) on high-threshold Ca^{2+}-independent potassium current ($I_{K(V)}$). A record (*a*) and volt-ampere characteristics (*b*) of $I_{K(V)}$ in the absence and presence of 0.1 mM DT.

rapidly developed (2-3 min) and disappeared after a 30-min washout with the control solution. DT-induced inhibition of $I_{K(Ca)}$ in one particular cell is shown in Fig. 3.

Thus, our experiments showed that apart from the high-threshold calcium current the Ca^{2+} channel antagonist DT inhibits two types of high-threshold potassium current ($I_{K(Ca)}$ and $I_{K(V)}$) without affecting the low-threshold potassium current and leakage currents.

The effective potassium current blockade was achieved at a DT concentration of 0.1 mM. This concentration is close to that used by us and other investigators [9] for the blockade of Ca^{2+} channels in the neuronal surface membrane.

Interestingly, the effect of DT varies in different cells. This can be attributed to the fact that integral K^+ current recorded on the total cell surface is mediated through various K^+ channel subclasses (about 10 subclasses have been discovered [5]) with different pharmacological properties [13]. It can be assumed that the population of DT-sensitive potassium channels considerably varies in different cells.

Diltiazem blocks two types of potassium currents: Ca^{2+}-dependent ($I_{K(Ca)}$) and Ca^{2+}-independent ($I_{K(V)}$) potassium currents. We assume that this block-

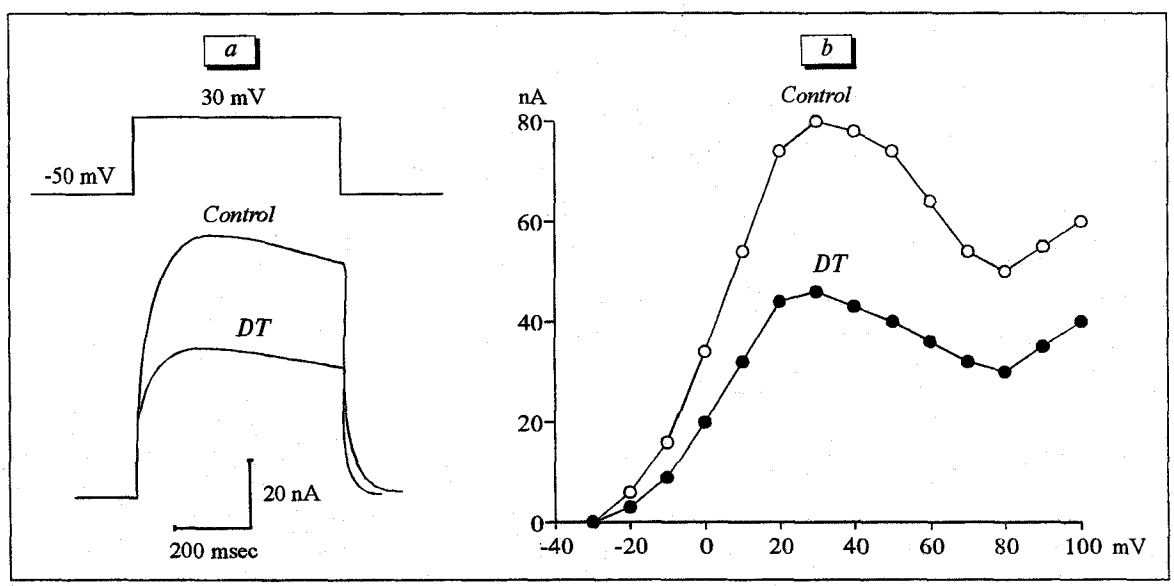

Fig. 3. Effect of diltiazem (*DT*) on high-threshold Ca^{2+}-dependent potassium current ($I_{K(Ca)}$). A record (*a*) and volt-ampere characteristics (*b*) of $I_{K(Ca)}$ in the absence and presence of 0.1 mM DT.

ade is mediated through different mechanisms. The blockade of $I_{K(Ca)}$ is effected through the blockade of voltage-dependent Ca^{2+} channels and inhibition of the calcium entry rather than through direct influence on the Ca^{2+}-dependent K^+ channels. Conversely, the blockade of $I_{K(V)}$ implies a possibility of direct interaction between DT and K^+ channels in the neuronal membrane.

Our experiments demonstrating the DT-induced inhibition of K^+ currents through the neuronal membrane supplement the data on the interaction between Ca^{2+} channel antagonists and K^+ channels. This effect of DT has been previously demonstrated in lymphoid [7] and muscular [15] cells. Similar effects of verapamil, a phenylalkylamine agent, and dihydropyridines were previously observed on epitheliocytes [6] and nerve cells [8,11,17], respectively. At present, there is ample evidence that Ca^{2+} channel antagonists also interact with K^+ channels. Consequently, the term Ca^{2+} channel antagonists now is definitely out of date.

The physiological importance of K^+ channel blockade may lie in prolongation of the action potential followed by enhanced calcium entry. Thus, in terms of the cytoplasmic level of free calcium, blockade of K^+ and Ca^{2+} channels has opposite effects, while the resultant effect of DT on free calcium level in each individual cell depends on what type of channels is predominantly blocked.

This fine balance of Ca^{2+} homeostasis and, consequently other Ca^{2+}-dependent processes (synaptic transduction, hormone release, enzyme activity, muscle fiber contraction, etc.) presumably underlies the broad therapeutic spectrum of Ca^{2+} antagonists.

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