Erratum:

Inhibition of Ion Currents in Membrane of Sensory Neuron by the Antiarrhythmic Drug BK 129 and Selected Ca^{2+} Entry Blockers

S. ŠTOLC

Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 16 Bratislava, Slovakia

Abstract. 1) The inhibitory effects of the prospective antiarrhythmic drug BK 129 on Ca^{2+} and Na^+ inward currents (I_{Ca} and I_{Na}, respectively) and on fast inactivating and slow noninactivating K^+ outward currents (I_{Kf}, I_{Ks}, respectively) were tested in young rat sensory neurons by modified whole cell voltage clamp technique *in vitro*. The effects were compared to those of nifedipine, verapamil and local anesthetic carbisocaine. Both BK 129 and carbisocaine are basic carbanilates. 2) At a frequency of test pulses of 0.2 Hz apparent dissociation constants (pD_2) of BK 129 to channels conducting I_{Ca}, I_{Na}, I_{Kf}, and I_{Ks} were 5.313, 4.429, 3.985, and 4.154, of carbisocaine 5.428, 5.896, 3.992, and 4.091, and of verapamil 4.249, 4.093, 3.839, and 4.453, respectively. In nifedipine only the pD_2 for I_{Ca} inhibition could be measured (5.624). This drug failed to exert any appreciable effect on the other currents up to the highest concentration used (15 μmol/l). Higher concentrations could not be tested because of the interfering effect of nifedipine solubilizer (ethanol). 3) The inhibiting effect of verapamil on I_{Ca} revealed slight potential dependence which however, could not account for the observed low specificity of this drug. Frequency of calcium channel activations might be more important determinant of the verapamil induced I_{Ca} inhibition rather than the holding potential. 4) The weak inhibiting effect of ω-conotoxin GVIA (5 μmol/l) and Ni^{2+} (100 μmol/l) as well as the strong effect of Cd^{2+}, Co^{2+} (both 5 mmol/l) and nifedipine on I_{Ca} indicated that it was mainly the L type Ca^{2+} channel that conducted this current. 5) The differential effect of Cd^{2+} and Co^{2+} (5 mmol/l) compared to tetrodotoxin (3 μmol/l) on I_{Ca} and I_{Na} disproved the possibility that these currents would pass via identical channels. 6) While nifedipine was shown to be a highly specific inhibitor of I_{Ca} in the young rat sensory neurons the other drugs tested inhibited the currents with a much smaller selectivity, with verapamil being the least specific at low stimulus rates (0.2 Hz). BK 129 is a powerful although nonspecific blocker of the inward I_{Ca} in the neuronal membrane. It is suggested that these properties of BK 129 might participate in its antiarrhythmic effect.

Key words: Sensory neuron membrane — Ca^{2+} current — Na^+ current — K^+ currents — BK 129 — Ca^{2+} entry blockers — Antiarrhythmics — Local anesthetics — Cell dialysis — Voltage-clamp

Introduction

The drug BK 129 (hydrochloride of 1-methoxymethyl-2-[1-perhydroazepinyl]ethyl-ester of 2-[n]-pentyloxycarbanilic acid) was prepared in an extensive search for new antiarrhythmics and local anesthetics in group of basic carbanilates (Beneš et al. 1983; Búčiová et al. 1987). The drug was able to prevent epinephrine induced heart rhythm disturbances in guinea pigs (Babuľová and Buran 1983). It suppressed the ouabain induced ventricular tachycardia as well as the ectopic activity occurring following coronary artery ligation in dogs (Gibala et al. 1987a). Spontaneous activity of sino-atrial and idioventricular pacemakers as well as intra-atrial, atrio-ventricular and intraventricular conduction were suppressed by BK 129 (Gibala et al. 1987b). Carbisocaine (hydrochloride of 1-methyl-2-diethylaminoethylester of 2-[n]-heptyloxycarbanilic acid), is a close congener of BK 129. This powerful local anesthetic (Beneš et al. 1978; Štolc and Stankovičová 1986) was shown to inhibit Na^+ and K^+ currents in rat dorsal root ganglion neurons (Štolc 1988).

The aim of the present study was to analyze the effect of BK 129 on selected transmembrane currents passing through voltage-operated ion channels with a special attention to Ca^{2+} current. The currents analyzed were Ca^{2+} and Na^+ inward currents (I_{Ca} and I_{Na}, respectively) and fast inactivating and slow noninactivating K^+ outward currents (I_{Kf} and I_{Ks}, respectively). The effect of BK 129 was compared with that of carbisocaine as well as with that of verapamil, nifedipine, and some other Ca^{2+} antagonists (Cd^{2+}, Co^{2+}, Ni^{2+}, ω-conotoxin).

The results showed that BK 129 inhibited the ion currents studied in the neuronal membrane in a similar way as carbisocaine, however, with preferential affinity to Ca^{2+} channels. While verapamil blocked the currents in a non-specific manner, nifedipine and Cd^{2+} or Co^{2+} inhibited the I_{Ca} with high specificity in this preparation and under the used experimental conditions.

Part of the results has been published in abstract form (Štolc and Nemček 1990).

Materials and Methods

Materials

BK 129 and carbisocaine hydrochloride were kindly supplied by dr.L.Beneš (Inst. Exp. Pharmacol., Slovak Acad. Sci., Bratislava). The other drugs used were: nifedipine (Drug Res. Inst., Modra, Slovakia), verapamil (Lääketehdas, Orion, Finland), tris-[hydroxymethyl]aminomethane puriss. p.a. (Tris; Fluka), hydrofluoric acid puriss. p.a.

(Fluka), hydrochloric acid p a (Lachema), cobalt nitrate hexahydrate p a (Lachema), cadmium hempentahydrate puriss (Lachema) nickel sulphate heptahydrate p a (Lachema), ethylenediaminetetraacetic acid disodium salt dihydrate puriss p a (EDTA, Fluka), trypsin (Trypsin® Spofa), collagenase crude (ÚSOL, Bohumile, Bohemia), ω-conotoxin GVIA, synthetic (Sigma), tetrodotoxin crystalline 3X (Sankyo), adenosine-3′,5′ cyclophosphate purum (cyclic AMP, Fluka) adenosine triphosphate purum (ATP, Lachema), Eagle's minimal essential medium (MEM, SEVAC) All other chemicals were of analytical purity

Methods

The experiments were carried out on single neurons isolated from rat dorsal root ganglia The technique was described in details elsewhere (Štolc 1988, Štolc et al 1988) Briefly, neurons dissected from the ganglion of 5-10 day old rats following enzymatic treatment (trypsin 8 mg/ml and collagenase 1000 U/ml in MEM for 15 min at 32 °C) Single cell was wedged in a conical hole made in the wall of a thin polyethylene tube The inner and outer diameters of the hole were approximately 5 and 50 μm, respectively The tube filled with intracellular medium (solution E, Table 1) was positioned in the experimental chamber containing the extracellular medium (solution A, Table 1) The tube and the chamber created the perfusion system suitable for internal dialysis of the neuron (Kostyuk et al 1981a) Following penetration of the appropriate part of the neuronal membrane the system allowed an easy control of the extracellular as well as intracellular media A conventional voltage clamp circuitry was used to control the membrane potential and to measure the evoked transmembrane current The leakage current was compensated by subtracting an appropriate fraction of command voltage from the signal representing total current passing through the pore The compensation was adjusted manually to zero deflection at an arbitrary hyperpolarizing step The effective patch of the cell membrane was approximately one half of the whole somatic surface in this arrangement If the current measured was too large and thus the potential fixation uncertain as usually the case in analysis of I_{Na} and I_{Kf} and I_{Ks}, the "reversed" system was used (Štolc et al 1988) In this arrangement the plastic tube was filled with the extracellular medium A while the intracellular medium E was present in the experimental chamber As that part of the cell membrane positioned in the wider part of the conical pore was penetrated, the effective patch and consequently the transmembrane currents were reduced The ratio of membrane and series resistances (R_M/R_S) increased approximately tenfold and the precision of voltage clamp control was substantially improved (Štolc et al 1988) No further electrical compensation of R_S was necessary

Separation of the studied ion current from the other current components was reached by replacing the all nonrelevant monovalent cations in extracellular and intracellular so lutions by Tris (Kostyuk et al 1981a,b,c) The composition of the solutions used is summarized in Table 1 Solutions A and D were used in analysis of inward I_{Na}, solutions B and E in analysis of outward I_{Kf} and I_{Ks} and solutions C and F in separation of inward I_{Ca} The solution pH was adjusted to 7 3 by the indicated acids Inward currents (I_{Ca}, I_{Na}) were recorded during series of 30 ms depolarizing pulses at frequency 0 2 Hz arranged to get voltage current relationship To evaluate the inhibitory effect of drugs only the maximal available amplitude of the current, i e I_{max} at E_{max}, were monitored in selected time intervals K^+ outward current was evoked by arbitrary depolarization to 0 mV for 300 ms The current amplitude was measured at the moment of its maximal value (I_{Kf}) and at the end of the depolarizing pulse (I_{Ks}) Holding potential was −100

Table 1. Composition of extracellular (*ec*) and intracellular (*ic*) solutions used in the neuron dialysis. Concentration of substances is expressed in mmol/l. The pH of the media was adjusted to 7.3 by the indicated acid. Solutions A and D were used for analyses of I_{Na}, B and E of I_{Kf} and I_{Ks}, and C and F of I_{Ca}.

	NaCl	$CaCl_2$	$MgCl_2$	Tris	KF	glucose	EDTA	ATP	cAMP	acid
ec										
A	110	2	2	5						HCl
B		2	2	145						HCl
C		14	2	133						HCl
ic										
D				120		20	1			HF
E				50	70	20	1			HF
F				140				2	0.05	HF

mV negative inside when measuring the I_{Ca} and −120 mV when measuring the other currents. Each recorded current was stable under the experimental conditions for more than 20 min, as continuously checked in series of control cells from each batch. If the stability of the currents was not satisfactory (±10 15 %) the batch was discarded. The tested drugs were applied into the extracellular compartment for 20 min. The drug effect usually became stabilized by this time. Concentration-effect relationship was constructed for each drug from measurements on 5–12 separate cells in each of 3–5 different concentrations. The effect of ω-conotoxin, Cd^{2+}, Co^{2+}, Ni^{2+} and tetrodotoxin developed quickly, hence the duration of drug application was shorter as indicated in the text. One cell was used in one test, only. All solutions were oxygenated and were prepared fresh daily using deionized water. The experiments were carried out at 18 °C.

Apparent dissociation constants (pD_2) i.e. negative logarithms of middle inhibitory concentrations (-log IC_{50}) and estimates of their errors were calculated from the equation of the regression line characterizing quasilinear middle part of dependence of ion currents on negative logarithms of drug concentration (Grimm 1973). Statistical significance of differences between the pD_2 values were tested according to Boxenbaum et al. (1974) while differences between mean values of the currents measured were evaluated by the unpaired Student's *t*-test.

Results

Typical I_{Ca} evoked by membrane depolarization in the rat sensory neuron is shown in Fig. 1A. This inward current revealed, comparing to the sodium one (Fig. 2A), comparatively slow activation with almost absent inactivation during 30 ms depolarization. Slight inactivation could appear during longer depolarization steps. However, as the cells deteriorated quickly under such circumstances, short depolarizations were used. only. Under the given experimental conditions I_{Ca} remained

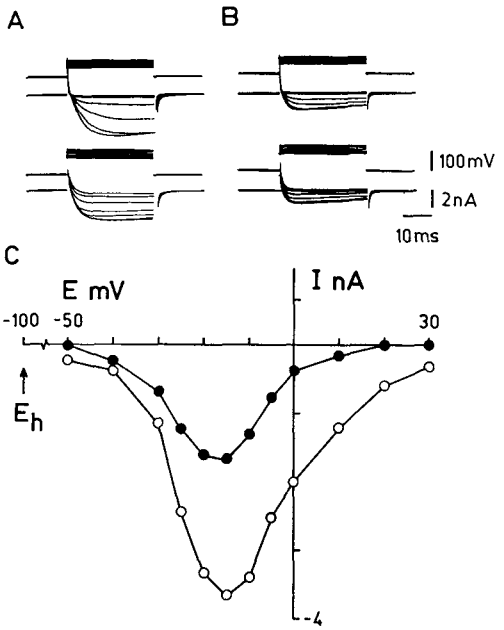

Figure 1. Effect of BK 129 on I_{Ca} in single internally dialyzed rat sensory neuron. *A* – control record. Sets of command pulses and corresponding currents for the onset and the declining part of current-voltage characteristics are shown separately. *B* – effect of BK 129 (3 μmol/l for 10 min). *C* – current-voltage plot of I_{Ca} constructed from records shown in *A* (○) and B (●). E_h – holding potential

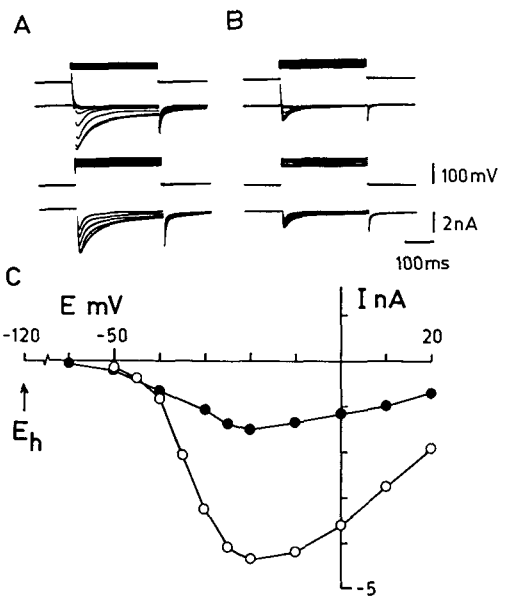

Figure 2. Effect of BK 129 (30 μmol/l for 10 min) on I_{Na}. For legend see Fig. 1.

comparatively stable over the experimental period of 20 min This might be related not only to the presence of cyclic AMP and ATP (Kostyuk et al. 1981b; Fedulova

et al. 1985) but also to the high concentration of F^- in the intracellular medium. This ion was shown to be a powerful unspecific inhibitor of phosphoproteases of type I, IIA and IIB (Shenolikar and Nairn 1991). Magnesium cations which were absent in the intracellular medium used are essential in activation of phoshoprotease IIC (Ingebritsen and Cohen 1983a,b). As these enzymes dephosphorylate many cellular proteins including ion channels (Tanabe et al. 1987; Trautwein and Hescheler 1988) the survival of calcium channels may be improved by inhibition of the phosphoproteases. Besides, control of low intracellur concentration of Ca^{2+} may be effectively improved by precipitating effect of F^-.

As demonstrated in Fig. 1*B* I_{Ca} was suppressed by BK 129. Comparison of the control and the drug-affected current-voltage characteristics shows depression with no distinct lateral shift of the maximal current observed (Fig. 1*C*). The BK 129 of concentration-effect dependence is shown in Fig. 4*A*. The apparent dissociation constant (pD_2) calculated from this relationship which was considered to be the measure of drug potency is shown in Fig. 5.

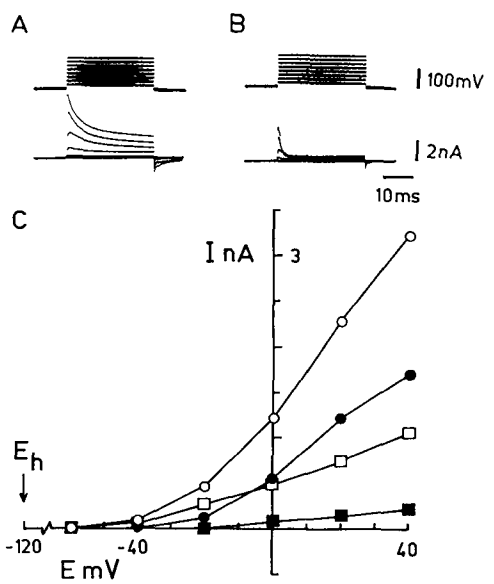

Figure 3. Effect of BK 129 (30 μmol/l for 10 min) on K^+ currents. Fast (o) and slow (□) components of I_K were evaluated separately. The former was measured at the maximal amplitude, the latter at the end of the depolarizing step. Empty symbols – controls, full symbols – effect of drug. For legend see Fig. 1.

The effect of BK 129 on Na^+ and both components of K^+ currents was tested in a similar way. Examples of the currents as well as their dependence on membrane potential are shown in Figs. 2 and 3. The two components of potassium current might be identical with the delayed rectifier and the A-current, respectively. The monotonous direction of tail currents appearing in Fig. 3 may be related to an increase in concentration of potassium ions on the external surface of somatic

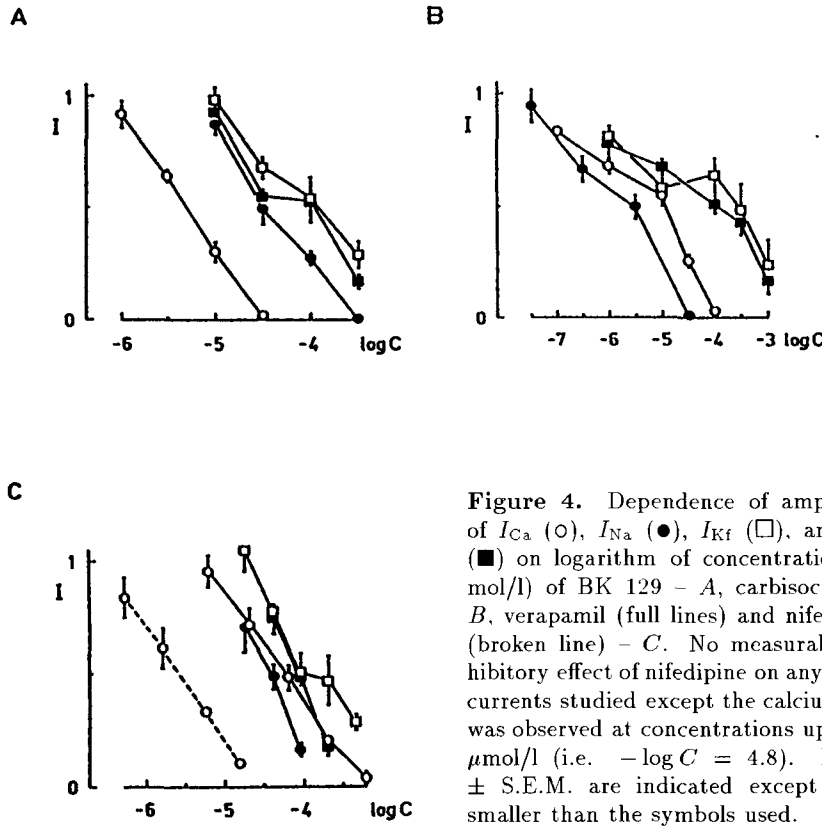

Figure 4. Dependence of amplitude of I_{Ca} (○), I_{Na} (●), I_{Kf} (□), and I_{Ks} (■) on logarithm of concentration (in mol/l) of BK 129 – *A*, carbisocaine – *B*, verapamil (full lines) and nifedipine (broken line) – *C*. No measurable inhibitory effect of nifedipine on any of the currents studied except the calcium one was observed at concentrations up to 15 μmol/l (i.e. $-\log C = 4.8$). Means ± S.E.M. are indicated except when smaller than the symbols used.

membrane occurring likely following the long depolarization pulses and to the high resting potential used. The concentration-effect plot in inhibition of these currents by BK 129 is shown in Fig. 4*A*. Comparison of the pD_2 values of BK 129 in the studied currents showed (Fig. 5) that this drug reveals by approximately one order higher affinity to Ca^{2+} conducting channels than to the other ones ($p < 0.001$).

The effect of carbisocaine, a congener of BK 129, on I_{Na}. I_{Kf} and I_{Ks} was demonstrated earlier (Štolc 1988). The concentration-effect relationship on I_{Ca} and the other currents is shown in Fig. 4*B*. Similar to BK 129, also this drug revealed higher affinity to I_{Ca} conducting channels comparing to K^+ channels ($p < 0.001$, Fig. 5). As could be expected, however, this drug being local anesthetic, is the powerful inhibitor of Na^+ channels too, with pD_2 significantly exceeding even that for Ca^{2+} channels ($p < 0.01$).

Nifedipine was used as a representative of dihydropyridine type inhibitors of I_{Ca}. Water solubility of nifedipine had to be increased by ethanol (0.005–0.15%). Measures were taken to avoid light exposure of the drug solution. Nifedipine inhi-

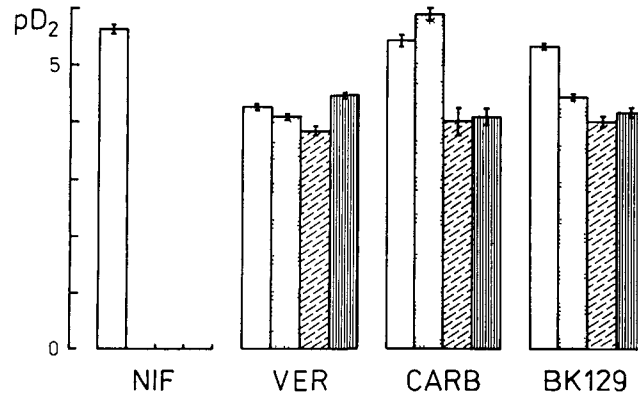

Figure 5. Apparent dissociation constants (pD_2) of nifedipine (NIF), verapamil (VER), carbisocaine (CARB) and BK 129 with channels conducting I_{Ca} (empty columns), I_{Na} (dotted columns), and I_{Kf} and I_{Ks} (oblique and upright labeled columns, respectively). Estimates of pD_2 standard errors are indicated. Nifedipine revealed specific affinity to Ca^{2+} conducting channels comparing to the other channels while verapamil showed almost no specificity. Note higher affinity of BK 129 to channels conducting I_{Ca} compared to other channels. The affinity of carbisocaine to the calcium and sodium channels was higher than to the both outward current conducting channels. For statistical significances see text.

bited I_{Ca} in a concentration dependent manner up to apparently complete blockade (Fig. 4*C*). However, no measurable effect of this drug on the other currents was observed even at the highest concentration used. Higher nifedipine concentrations were not tested because of the interfering effect of the solubilizer. Nifedipine is a highly specific Ca^{2+} entry blocker in this type of neuronal membrane (Fig. 5).

Verapamil, contrary to nifedipine, did not reveal such a selective effect. Although it was inhibiting the I_{Ca} in a concentration dependent manner the other tested currents were also inhibited by this drug in approximately the same concentration range (Fig. 4*C*). Although the pD_2 for I_{Ks} was significantly higher than that for I_{Kf} ($p < 0.001$), the pD_2 values to all the tested channels did not differ more than one half an order.

The profile of pD_2 values for each drug indicates that with the exception of nifedipine both carbanilates as well as verapamil are essentially nonspecific inhibitors of the tested channels in this type of neurons and under the used conditions. However, the higher affinity of BK 129 to Ca^{2+} conducting channels as well as carbisocaine to channels conducting both inward currents might have some pharmacological implications.

Lee and Tsien (1983) showed that block of I_{Ca} induced by verapamil can be reversed by a short lasting hyperpolarization. Consequently, the hypothesis was

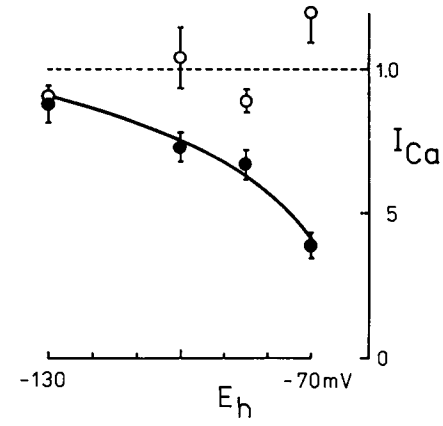

Figure 6. Dependence of I_{Ca} at the presence of verapamil (6.1 μmol/l) on holding potential (E_h). Controls – o, verapamil – •; Means ± S.E.M. are shown. Note the increasing inhibition of I_{Ca} with decreasing E_h.

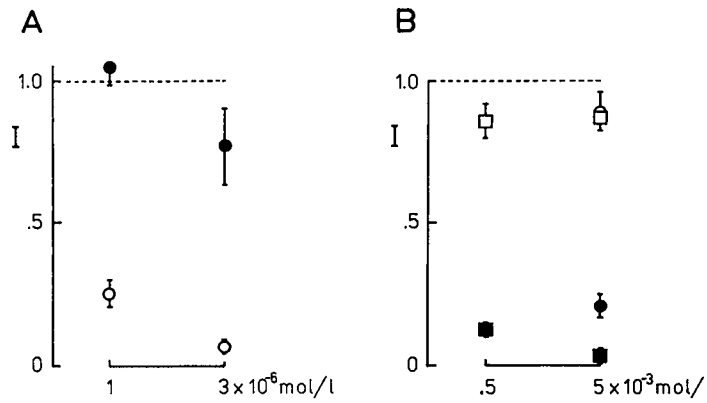

Figure 7. A – Effect of tetrodotoxin applied for 4 minutes in two different concentrations on I_{Ca} and I_{Na} (• and o, respectively). *B* – Effect of Cd^{2+} and Co^{2+} (squares and circles, respectively) applied for 6 minutes in two different concentrations on I_{Ca} and I_{Na} (full and empty symbols, respectively). The absence of tetrodotoxin effect on I_{Ca} as well as that of Cd^{2+} and Co^{2+} on I_{Na} clearly distinguish the both inward current conducting channels.

tested whether the low blocking effect of verapamil on I_{Ca} might be linked to the comparatively high resting membrane polarization (−100 mV) used in this study. The plot of I_{Ca} in the presence of verapamil (6.1 μmol/l) against the holding potential is shown in Fig. 6. When the membrane was depolarized from −130 mV to −70 mV the verapamil blocking effect of Ca^{2+} channels was enhanced only by approximately 50 %. A similar relationship was found also in blocking the Na^+ and both K^+ currents (results not presented). Thus, participation of the

high resting polarization of the membrane in the low specificity of verapamil in inhibition of the tested ion conducting channels does not seem to be probable. As only low frequency stimulation was used in this study (0.2 Hz), an analysis of use-dependence of verapamil effect could contribute to elucidation of the unexpectedly low specificity of this drug.

Although the kinetics of I_{Ca} and I_{Na} in the present experiments were remarkably different (slow activation and only very slight inactivation of I_{Ca} during 30 ms depolarizing pulse compared to fast activation and almost full inactivation of I_{Na}) it was analyzed whether Ca^{2+} ions did not pass at least partially also through Na^+ channels. The clearcut blocking effect of tetrodotoxin (1 and 3 μmol/l) on I_{Na} and the absence of such an effect in I_{Ca} (Fig. 7*A*) excluded this possibility. Moreover, the inorganic Ca^{2+} channel blockers Cd^{2+} and Co^{2+} (0.5 and 5 mmol/l) exerted a differential effect on the two currents. These cations readily inhibited I_{Ca} leaving I_{Na} almost unaffected (Fig. 7*B*). In an attempt to determine more precisely the type of channels conducting the I_{Ca} measured we observed that Ni^{2+} (0.1 mmol/l) depressed I_{Ca} to 58.35 ± 5.24 % and ω-conotoxin (5 μmol/l) to 61.4 ± 6.42 % only.

Discussion

The three separate parts can be distinguished in the BK 129 molecule, namely a polar head, a lipophilic part and an intermediary chain linking together the two parts. This structure corresponds well to the general model of local anesthetics suggested by Löfgren (1948). The incorporation of such a molecule into the lipid bilayer and/or into the channel protein is an essential step in local anesthetic effect of many drugs. A similar mechanism of action can be presumed also in BK 129. This is further supported by comparatively high lipophilicity of this drug ($P' = 2294$, octanol/phosphate buffer, pH 7.3) facilitating its incorporation into the lipophilic parts of the neuronal membrane. The capability of BK 129 to block the early inward current (I_{Na}) is in accordance with its antiarrhythmic property described by Babuľová and Buran (1983), Búčiová et al. (1987) and Gibala et al. (1987a,b). The remarkable capability of this drug to inhibit the I_{Ca} in concentrations even lower than those inhibiting I_{Na} might contribute to its antiarrhythmic effect, although Gibala et al. (1987b) denied this involvement. Neither participation of inhibition of K^+ channels similar to that known in Class III antiarrhythmics (Vaughan Williams 1989; Beatch et al. 1991) can be excluded in BK 129.

Carbisocaine, a close congener of BK 129, also corresponds well with the structure of typical local anesthetic. Accordingly, the high local anesthetic potency of this drug as well as its capability to inhibit action potential conduction has been demonstrated (Beneš et al. 1978; Štolc and Stankovičová 1986). Procaine or lidocaine, typical local anesthetics, inhibit I_{Na} and/or sodium spikes in 4–10 times lower concentrations than those required to inhibit I_{Ca} and/or calcium spikes (Bing-

mann et al. 1987; Elliot 1990). It has been found in the present study that both inward currents were almost equally sensitive to carbisocaine, while both K^+ outward currents were less sensitive to carbisocaine than Na^+ current (Štolc 1988). Among properties participating in the comparatively nonspecific profile as well as high affinity of carbisocaine to the channels conveying the currents studied, the high lipophilicity of this drug should be considered. The carbisocaine apparent partition coefficient exceeds that of procaine and lidocaine by more than 3 orders ($P' = 12000$, 8.3, and 2.9, respectively, octanol/phosphate buffer, pH 7.3).

Fedulova et al. (1985) distinguished low and high threshold voltage operated Ca^{2+} channels in the rat sensory neuron. They also found that the proportion of these channels is changing during ontogenesis. At least three types of Ca^{2+} channels, namely T, N and L should be considered in the preparation used (Hosey and Lazdunski 1988; Kostyuk et al. 1988; Tsien et al. 1988, 1991; Bean, 1989). According to Fox et al. (1987), Tsien et al. (1988, 1991), Bean (1989), and Ozawa et al. (1989) the T type Ca^{2+} channel is more sensitive to Ni^{2+} than to Cd^{2+} while the L channel is more sensitive to Cd^{2+} (Reynolds and Snyder 1987; Ozawa et al. 1989; Triggle 1989; Zelis and Moore 1989). The higher sensitivity of I_{Ca} to Cd^{2+} compared to Ni^{2+} observed in this study indicates that T type channels were not remarkably represented in the neuronal membrane studied. ω-conotoxin is considered to be a powerful inhibitor of the N type and to a smaller extent also of L type Ca^{2+} channels (Hosey and Lazdunski 1988; Tsien et al. 1988, 1991; Bean 1989; Carbone et al. 1989; Triggle 1989; Toselli and Taglietti 1990; Horne and Kemp 1991). In spite of high concentration of this substance I_{Ca} was inhibited by ω-conotoxin only partially in our experiments. The high sensitivity of L channels to dihydropyridines is well known (Hosey and Lazdunski 1988; Tsien et al. 1988, 1991; Bean 1989; Takahashi et al. 1989; Triggle 1989). The inhibition of I_{Ca} induced by nifedipine observed in this study, along with all the above mentioned findings, indicate that it is the L type Ca^{2+} channel that was dominant in our preparation. Accordingly an attempt to distinguish the channel type by analyzing the voltage-current characteristics in the studied cells did not indicate the N and/or T type channels in a remarkable proportion. Although some atypical effects of dihydropyridine Ca^{2+} entry blockers on channels other than Ca^{2+} ones were described (Yatani and Brown 1985; Yatani et al. 1988) we did not observe a similar effect of nifedipine.

It is well known that verapamil is able to inhibit Ca^{2+}as well as other transmembrane currents (Kuroda 1976; Frelin et al. 1982; Kraynack et al. 1982; DeFeudis 1988). Yet, the quantitative comparison of this effect was not available. The largest difference between pD_2 values in verapamil was that one between those observed for I_{Ks} and I_{Kf}. As the ratio of the affinities of verapamil to the channels analyzed did not exceed one order in the present experiments, the specificity of the Ca^{2+} antagonistic effect of verapamil is low in the membrane used. Since both

polar and nonpolar moieties flexibly linked together can be distinguished in verapamil molecule, an interaction with biological membranes similar to that suggested for local anesthetics might be presumed in this drug, too. The analysis of potential dependence of the verapamil inhibitory effect on I_{Ca} disclosed potentiation of this effect in depolarized membrane. However, as this potentiation was small the high holding membrane potential could not be responsible for the low specificity of this drug. The observed voltage dependence of verapamil effect might be related to some general mechanisms such as modification of drug availability at the site of action or a change in drug dissociation (Lullmann and Peters 1977; Reynolds and Snyder 1987). However, the efficacy of verapamil in inhibiting I_{Ca} might depend more remarkably on rate of channel activation. This use-dependence analysis would however, require separate experiments.

Although the effect of BK 129 on I_{Ca} and/or on I_K observed in membrane of the studied neurons cannot be readily extrapolated to myocardium, the results suggest the possibility of participation of such effects in the antiarrhythmic effect of this drug. To obtain conclusive evidence, further studies are to be made directly on myocardial cells.

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