Cromakalim, a vasodilator, differentially inhibits Ca^{2+} currents in NG108-15 neuroblastoma × glioma hybrid cells

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Extracellular perfusion with the antihypertensive agent cromakalim produced an inhibition of 22–66% in the low-threshold transient Ca^{2+} (T-like) current in NG108-15 hybrid cells. Cromakalim suppressed the high-threshold and long-lasting Ba^{2+} current (L-like Ca^{2+} current) by 29–73%, but had almost no effect on the high-threshold and inactivating Ba^{2+} current (N-like Ca^{2+} current). IC_{50} for T-like and L-like currents was the same at about 100 μM. The inhibitory effect developed relatively fast and was reversible. These results indicate that cromakalim can selectively inhibit the activity of inward Ca^{2+} currents.

Cromakalim; Patch clamp; Ca^{2+} inward current; T-type; L-type; (NG108-15 cell)

1. INTRODUCTION

Cromakalim (BRL 38227: (-)-*trans*-6-cyano-3,4-dihydro-2, 2-dimethyl-4-(2-oxo-1-pyrrolidinyl)-2H-1-benzo-[b]pyran-3-ol]) is a novel class of antihypertensive drugs which cause relaxation in smooth muscle preparations [1]. The principal site of action of cromakalim is thought to be K^+ channels [2]: stimulation of the opening of ATP-sensitive K^+ channels [3,4], leading to cell membrane hyperpolarization. In addition, it has recently been reported that cromakalim has a direct effect on inward Ca^{2+} currents in smooth muscle cells of the rat portal vein [5]. Ca^{2+} channels are subdivided into 3 distinct types, namely T-, N-, and L-types originally reported in sensory ganglion cells [6]. The low-threshold and transient (T-type) current and the high-threshold Ca^{2+} currents consist of the inactivating (N-type) current and the non-inactivating (L-type) current. The N-type Ca^{2+} current has not been found in muscle cells of the vascular system but has been recorded in neuronal cells [7]. NG108-15 neuroblastoma × glioma hybrid cells, neuronal model cells [8], have been reported to possess two types of Ca^{2+} (T- and L-like) currents [9]. Recently Docherty and McFadzean found a rapidly inactivating (N-like) component of the high-threshold Ca^{2+} current in NG108-15 cells [10]. Therefore, it is worth examining NG108-15 cells to find out to which species of Ca^{2+} currents cromakalim is sensitive. In this report we show that cromakalim inhibits the T- and L-like Ca^{2+} currents without any effect on the N-like current of NG108-15 cells under the voltage-clamp condition.

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2. MATERIALS AND METHODS

Cells used in these experiments were NG108-15 mouse neuroblastoma × rat glioma hybrid cells. Hybrid cells were cultured in polyornithine-coated dishes and induced to differentiate by growing in Dulbecco's modified Eagle's medium containing 1% fetal bovine serum, 100 μM hypoxanthine, 16 μM thymidine and 0.25 mM dibutyryl cyclic AMP for 2–3 weeks, as described previously [11].

Membrane currents were recorded via a discontinuous single-electrode voltage-clamp amplifier (model 2A, Axon Instruments) in a switching mode operating at 2–6 KHz. For the low-threshold Ca^{2+} (T-like) current, membrane currents were measured with sharp microelectrodes (filled with 1 M K^+ citrate, 10 mΩ) by impaling cells soaked in Tris-HCl-buffered saline (TBS) (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.8, D-glucose 20, tetrodotoxin 0.5 μM, Tris-HCl 20, pH 7.2. The patch voltage-clamp technique was applied to the recording of high-threshold Ca^{2+} (N- and L-like) currents, with Ba^{2+} as ion charge carrier in the following extracellular solution (in mM): $BaCl_2$ 50, NaCl 30, $MgCl_2$ 1, CsCl 5, glucose 20, tetraethylammonium chloride 20, tetrodotoxin 1 μM; Hepes 10; pH 7.24 as described [9]. The patch electrodes contained a solution of the following composition (in mM): CsCl 150, $MgCl_2$ 1, EGTA 10, ATP 1, Hepes 10, pH 7.3. Cromakalim was dissolved to make a stock solution (50 mM) in 100% dimethyl sulfoxide (DMSO). Cells were superfused at 33–35°C with the buffer solution plus 1% DMSO with or without cromakalim. In order to exclude artifacts of the solvent, control experiments were carried out with cromakalim in 0.1% ethanol solution. Results obtained with the two solvents were identical, and DMSO alone did not mimic the effect of cromakalim. Cromakalim was a kind gift from Beecham Pharmaceuticals, Harlow, UK.

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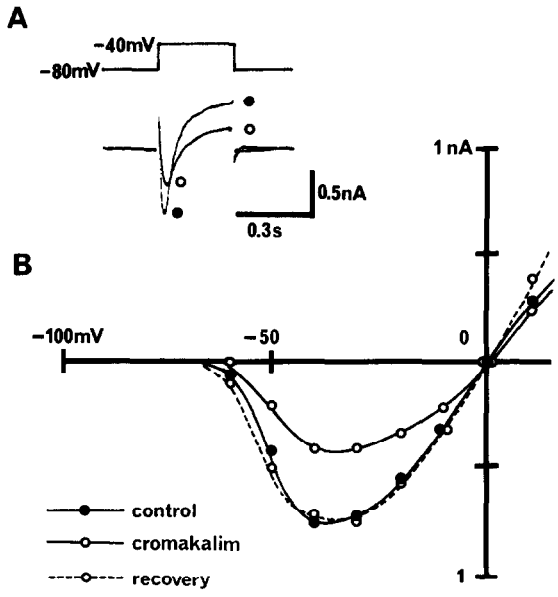

Fig.1. Effects of cromakalim on low-threshold transient calcium currents of NG108-15 cells. A. Ca^{2+} inward currents elicited in one cell, by going from a holding potential of −80 mV to a test potential of −40 mV before (●) and during (○) application of 500 μM cromakalim. B. Current-voltage plot of Ca^{2+} currents at peak before, during, and after cromakalim, as indicated.

3. RESULTS AND DISCUSSION

We examined the effects of cromakalim on 3 classes of Ca^{2+} currents in NG108-15 cells. First, the effect of cromakalim was studied on the transient inward Ca^{2+} current (the T-component).

In a typical voltage-clamp experiment, the cells were subjected to 0.3 s depolarizing voltage steps (to −40 mV) from a holding potential of −80 mV in the presence of 0.5 μM tetrodotoxin. As illustrated in fig.1A, such voltage steps evoked a fast transient inward current followed by a sustained outward current. Cromakalim caused a decrease in the inward current of 42 ± 5% (n = 10), as well as in the outward current, but had no effect on the resting current at −80 mV. The inhibition in the transient inward Ca^{2+} current amplitude caused by cromakalim occurred at all potentials tested as shown in the current-voltage relationships (fig.1B).

The whole cell patch-clamp recording from NG108-15 cells perfused with 50 mM $BaCl_2$ revealed two types of Ba^{2+} currents that are due to activation of Ca^{2+} channels by depolarization steps. Usually in hybrid cells depolarized to 0–+10 mV from a holding potential of −80 mV, this current was maximal immediately after a voltage step (the N-like current) and was inactivated progressively thereafter (fig.2A). The degree of such inactivation varied among cells (58 ± 5% inactivation after 1 s, mean ± SEM, n = 25), and the long-lasting current remained active at the end of the 1 s depolarizing step (the L-like current). Cromakalim (100–500 μM) reduced the long-lasting current by 55 ± 4% (n = 12), although little or no effect was found in the peak current. Fig.2B shows typical current voltage curves constructed in the absence (vehicle alone) and presence of 500 μM cromakalim from a holding potential of −80 mV. It is clear that the degree of inhibition of the L-like current produced by cromakalim is depen-

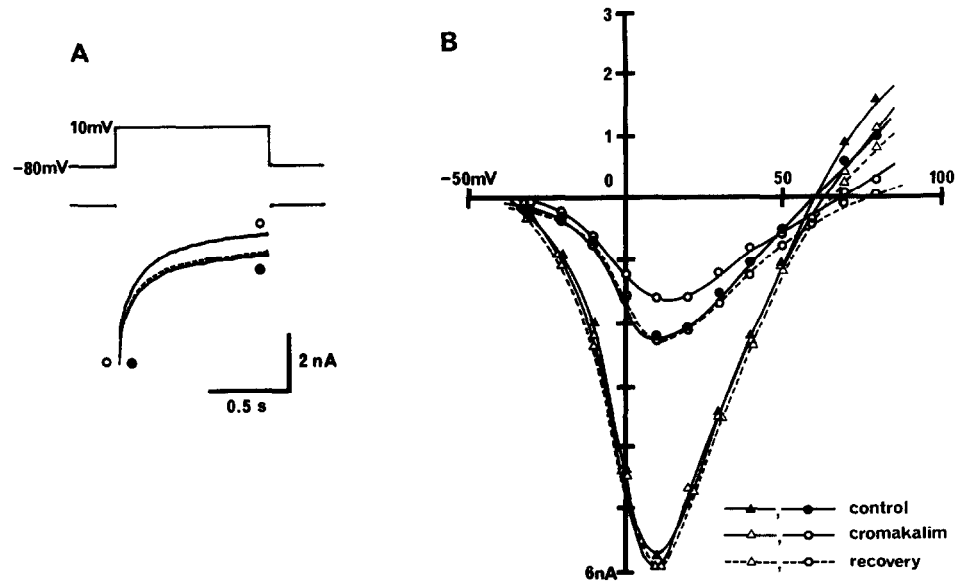

Fig.2. Effect of cromakalim on high-threshold Ba^{2+} currents of NG108-15 cells. A. The traces show Ba^{2+} currents recorded during 1 s steps from a holding potential of −80 mV to a command potential of +10 mV in the presence (○) and absence (●) of 500 μM cromakalim. Note that no decrease at the peak current (the N-component) but reduction of the long-lasting current (the L-component) at the end of the command pulse was observed. B. Current-potential plot of Ba^{2+} currents at peak (△,▲) and after 1 s (○,●) and during (△,○) cromakalim application, as indicated.

dent on the command potential. The maximal inhibition of the L-like current was obtained at potentials positive to the threshold potentials (0- + 30 mV), while no inhibition in the N-like current was observed in the wide range of potentials tested.

Steady state inactivation curves for 3 components of Ca^{2+} channel currents were obtained by measuring Ca^{2+} and Ba^{2+} currents at constant command potentials (−40 and 0 mV) from different holding potentials (n = 3, each curve). The suppression of the T-like and L-like currents was much more obvious at potentials more negative than the holding potentials of −70 mV and −10 mV, respectively, (fig.3A and C), where these currents are active. The steady state inactivation curve for the N-component was identical in the presence or absence of cromakalim (fig.3B). When curves obtained in the presence and absence of cromakalim were normalized, the control and cromakalim curves were almost identical (data not shown). These data suggest that 3 Ca^{2+} currents in the presence of cromakalim (i.e. the residual current) have time- and voltage-dependent inactivation properties similar to the control current.

The inhibition for T- and L-currents was dose-dependent (IC_{50} = 100 and 102 μM, respectively, n = 3 and 4) and reversible (data not shown). Since the unaffected Ba^{2+} peak current by cromakalim was inhibited by perfusion of acetylcholine as shown by McFadzean et al. [12], the N-like current that was measured here was a responsible component.

The effect of cromakalim on the two components of the Ca^{2+} currents was relatively fast to develop. The inhibition began after a lag period of several seconds, and a maximal response was readily obtained within 6.7 min after onset of the drug superfusion in 7 cells.

The suppression of the T-current was confirmed by generation of Ca^{2+} action potentials by constant current stimulation. The peak amplitude of Ca^{2+} action potentials in the presence of 500 μM cromakalim was smaller than the control value by 47 ± 6% (n = 8) (data not shown). Furthermore, the tail outward current evoked when 500 ms depolarizing voltage steps were stopped, the fast component of afterhyperpolarization currents (i.e. Ca^{2+}-activated K^+ currents) was also suppressed by cromakalim (45 ± 6%, n = 10). Therefore, these observations indicate that Ca^{2+} influx was depressed by cromakalim.

The results indicate that cromakalim inhibited T- and L-like Ca^{2+} currents of NG108-15 cells. Although inhibition required a relatively high amount of the drug, the IC_{50} of cromakalim for Ca^{2+} currents is the same order as that for potentiation of the delayed rectifier current in hippocampal neurons [13]. Cromakalim did not inhibit the N-like Ca^{2+} current. This implies that cromakalim's action is highly selective, indicating that cromakalim may act directly on different Ca^{2+} channel molecules rather than having a non-specific action. Taking into account the previous observations that

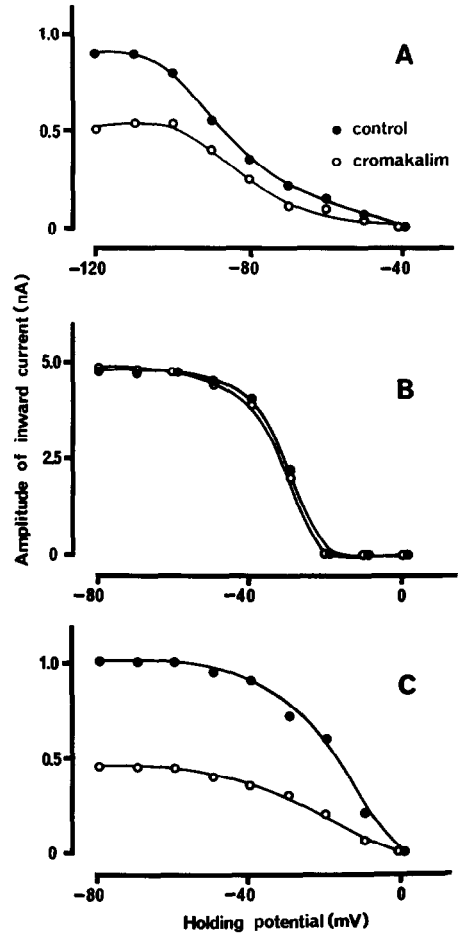

Fig.3. Steady state inactivation curves for 3 components of Ca^{2+} currents constructed in the absence (●) and presence (○) of 500 μM cromakalim. Each panel shows T-like Ca^{2+} currents (A) and the N-like (B) or L-like (C) Ba^{2+} currents. Note that cromakalim reduced the amplitude of Ca^{2+} (A) and Ba^{2+} (C) currents. Command potential = −40 (A) and 0 mV (B and C).

cromakalim is capable of reducing the slow inward current in the portal vein [5], cromakalim seems to target voltage-dependent Ca^{2+} channels, as well as K^+ channels [2].

In summary, voltage-clamp recordings from neuroblastoma hybrid cells treated with cromakalim enabled us to speculate that the drug would impair the Ca^{2+} currents. The results suggest that blocking the Ca^{2+} entry through the L- and T-type channels may also contribute to a cromakalim-induced relaxation of vascular tissues.

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