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Short communication

Differential effects of the BK_{Ca} channel openers NS004 and NS1608 in porcine coronary arterial cells

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Abstract

The effects of newly claimed BK_{Ca} channel openers NS004 (5-trifluoromethyl-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-2*H*-benzimidazole-2-one) and NS1608 (*N*-(3-(trifluoromethyl)phenyl)-*N'*-(2-hydroxy-5-chlorophenyl)urea) were investigated on whole-cell K^+ current (I_K) in enzymatically isolated porcine coronary arterial cells using patch-clamp technique with a double holding potential protocol. When cells were held at 0 mV, I_K was augmented by NS004 in a concentration-dependent manner. With a holding potential of −60 mV, however, I_K was moderately inhibited by NS004 between 0.5 and 10 μM, but robustly stimulated by 50 μM NS004 at highly depolarized potentials. The effects of NS1608 on I_K did not differ due to change in holding potential. At −60 mV and 0 mV, NS1608 activated I_K with bell-shaped concentration-response curves peaked between 5 and 10 μM. The differential mode of action of the two compounds suggested an involvement of mechanism(s) other than an opening of BK_{Ca} channel.

Keywords: Coronary arterial cell; BK_{Ca} channel; Patch-clamp; (Whole-cell recording); (Holding potential)

1. Introduction

K^+ channel opener, a new class of smooth muscle relaxant, acts to open plasmalemmal K^+ channels which leads to membrane hyperpolarization and cell inhibition. Among diverse sets of K^+ channels, most of the currently known K^+ channel openers interact primarily with a K^+ channel subtype referred to as the ATP-sensitive K^+ channel (K_{ATP}). It has recently been reported that a group of compounds, typified by NS004 (5-trifluoromethyl-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-2*H*-benzimidazole-2-one, Oleson and Watjen, 1992) and NS1608 (*N*-(3-(trifluoromethyl)phenyl)-*N'*-(2-hydroxy-5-chlorophenyl)urea, Oleson, 1994a), activates a different subtype of K^+ channel, the large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}, Oleson, 1994b, 1995). This novel type of opener is of particular interest because of the wide distribution of BK_{Ca} channels in a variety of tissue (Lattore et al., 1989) and the important roles of BK_{Ca} channels in modulating cellular excitability and maintaining Ca^{2+} homeostasis.

In the present study, we investigated the effects of NS004 and NS1608 on whole-cell K^+ current (I_K) in smooth muscle cells from porcine coronary artery. The results revealed differential concentration-dependent activities of these two compounds.

2. Materials and methods

Single smooth muscle cells were isolated from porcine circumflex and left anterior descending coronary arteries by enzymatic dissociation. The endothelium-denuded media intima were incubated in nominally Ca^{2+}-free saline containing 0.16% collagenase, 0.12% papain, and 0.04% DL-dithiothreitol for 55 min. Single cells were obtained by gentle agitation of digested tissue and stored at 4°C for 1 h before testing.

Whole-cell I_K in porcine coronary arterial cells was recorded using standard patch-clamp technique (Hamill et al., 1981) in response to step depolarizing pulses (1 s in duration) from a holding potential of −60 mV or 0 mV. The bath solution contained (mM) NaCl (140), KCl (5), $CaCl_2$ (1), $MgCl_2$ (1), glucose (5) and Hepes (10). The pipette solution contained (mM) KCl

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(140), $MgCl_2$ (1), $CaCl_2$ (0.1), EGTA (0.6), Na_2UDP (2), glucose (5), K_2ATP (2) and Hepes (10), in which the free intracellular Ca^{2+} concentration was calculated to be 10 nM. The junction potential between electrodes and the bath solution was compensated using the DC offset on the amplifier. No leak current subtraction was applied. In on-line data acquisition, I_K was amplified with a List EPC-7 amplifier, filtered at 1 kHz, digitized at a sampling rate of 4 kHz with a TL-1-125 DMA interface, and stored on a Compaq Deskpro/66M microcomputer for future analysis with pClamp version 6.0. The amplitude of the steady-state I_K was measured as the mean value of data points near the end of a given test pulses. All experiments were performed at 22°C.

NS004 and NS1608 were synthesized in the Research Department of Ciba-Geigy Corp. The drugs were first dissolved in 95% ethyl alcohol to form a stock solution of 50 mM, which was diluted with saline to the desired concentrations shortly before testing. In this study, the maximal concentration of ethyl alcohol was 0.1%, which had no discernible electrophysiological effect on I_K.

3. Results

In porcine coronary arterial cells, step depolarizing pulses from a holding potential of −60 mV generated a family of noisy, high-threshold (−15 to −20 mV), and non-inactivating whole-cell I_K (Figs. 1A and 2A), of which 75–80% was blocked by 5 mM tetraethylammonium and 80 nM iberiotoxin (Hu et al., 1994), indicating a prominent contribution of the BK_{Ca} channel activity.

At a holding potential of −60 mV, NS004 at 0.5 and 5 μM caused a moderate inhibition of I_K (Fig. 1, column A). The steady-state I_K at 60 mV was reduced to a respective 0.79 ± 0.04 and 0.62 ± 0.06 fold (n = 7) of the control. NS004 at 50 μM further suppressed I_K at test potentials below 40 mV while robustly stimulating I_K at 40 and 60 mV. The current at 60 mV was increased by 2.59 ± 0.61 fold (n = 7). Typical current recordings in control, 0.5, 5, and 50 μM NS004 and concentration-response (amplitude of I_K) relationship at test potentials of 20, 40 and 60 mV are shown in Fig. 1A (top to bottom).

The effects of NS004 on BK_{Ca} current (I_{BK}) were examined when activation of other voltage- and time-dependent I_K were minimized by clamping the cells at a holding potential of 0 mV. As shown in Fig. 1, column B, NS004 induced activation of I_{BK} in a concentration-dependent manner. Upon exposure to NS004 at 0.5, 5 and 50 μM, the amplitude of I_{BK} at 60 mV was, respectively, 1.16 ± 0.07, 1.42 ± 0.04, and 3.44 ± 0.82 fold (n = 5) of the control. No inhibitory action

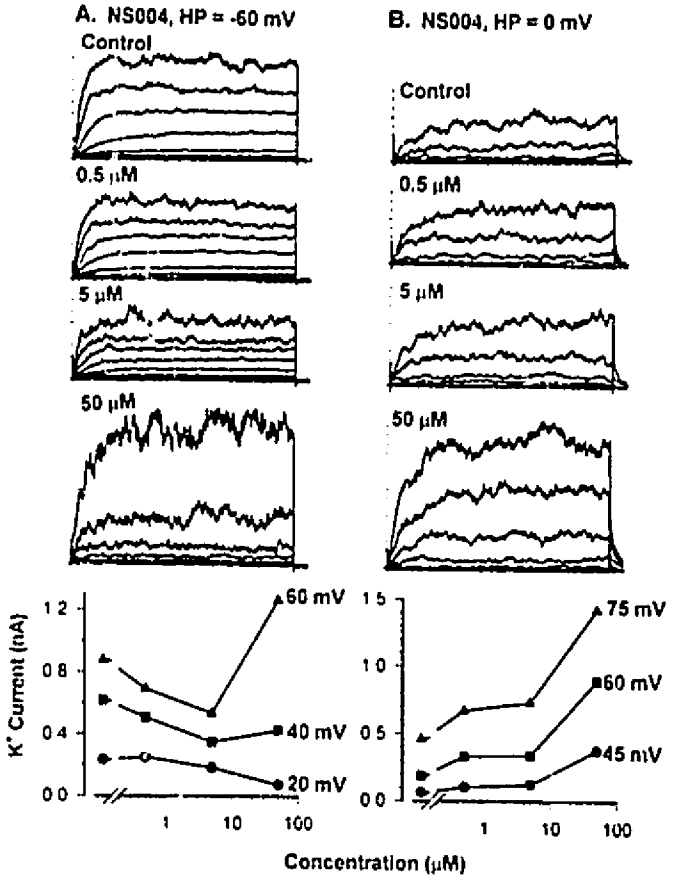

Fig. 1. Effects of NS004 on whole-cell I_K at holding potentials of −60 mV (A) and 0 mV (B). All test potential had a duration of 1 s. In the concentration-current curves at the bottom of each column, the ordinate is the steady-state I_K (nA), and the abscissa is NS004 concentration (μM) in logarithmic scale. Column A (top to bottom): family of I_K in the presence of 0, 0.5, 5 and 50 μM NS004 (holding potential = −60 mV, test potentials from −60 mV to +60 mV in 20 mV increment) and the concentration-current curves at 20 mV (circle), 40 mV (square) and 60 mV (triangle). Column B (top to bottom): family of I_K in the presence of 0, 0.5, 5 and 50 μM NS004 (holding potential = 0 mV, test potentials from −30 mV to +75 mV in 15 mV increment) and the concentration-current curves at 45 mV (circle), 60 mV (square) and 75 mV (triangle).

was observed. The concentration-response curves for I_{BK} at 45, 60 and 75 mV are shown at the bottom of Fig. 1B.

In contrast, the effects of NS1608 on I_K did not vary qualitatively with changes in holding potential (compare Fig. 2, column A to B). In the presence of 0.5, 5 and 50 μM NS1608, the steady-state I_K at 60 mV was, respectively, 0.95 ± 0.13, 3.09 ± 0.98, and 1.06 ± 0.40 fold of the control (n = 5, Fig. 2, column A) when cells were held at −60 mV; and 1.11 ± 0.11, 2.97 ± 0.16, and 0.78 ± 0.24 fold (n = 6, Fig. 2, column B) when cells were held at 0 mV. In both cases, the stimulatory effect of NS1608 on I_K reached a maximum at the concentration between 5 and 10 μM. Prolonged exposure (> 5 min) to 50 μM NS1608 resulted in an attenuation of I_K and retardation of the current activation kinetics. These effects were more pronounced with a

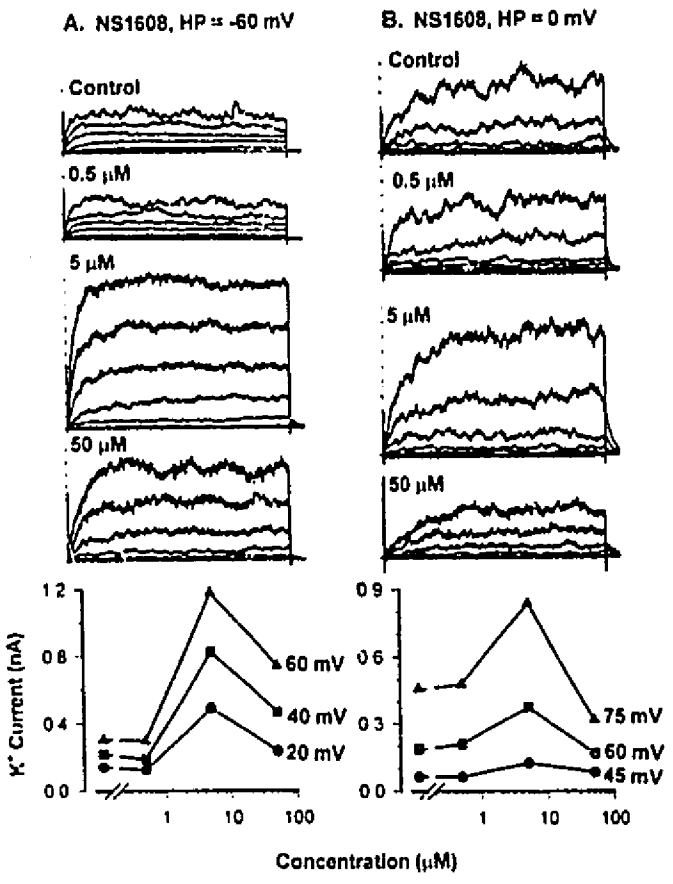

Fig. 2. Effects of NS1608 on whole-cell I_K at holding potentials of −60 mV (A) and 0 mV (B). All test potential had a duration of 1 s. In the concentration-current curves at the bottom of each column, the ordinate is the steady-state I_K (nA), and the abscissa is NS1608 concentration (μM) in logarithmic scale. Column A (top to bottom): family of I_K in the presence of 0, 0.5, 5 and 50 μM NS1608 (holding potential = −60 mV, same voltage protocol as in Fig. 1A) and the concentration-current curves at 20 mV (circle), 40 mV (square) and 60 mV (triangle). Column B (top to bottom): family of I_K in the presence of 0, 0.5, 5 and 50 μM NS1608 (holding potential = 0 mV, same voltage protocol as in Fig. 1B) and the concentration-current curves at 45 mV (circle), 60 mV (square) and 75 mV (triangle).

holding potential of 0 mV (Fig. 2B). Thus, the effects of NS1608 on I_K had bell-shaped concentration-response curves (bottom of Fig. 2A and 2B).

4. Discussion

In this study, the effects of NS004 and NS1608 on total I_K (at a holding potential of −60 mV) were compared to those on I_{BK} (at a holding potential of 0 mV) in porcine coronary arterial cells. When cells were held at −60 mV, I_K elicited by depolarizing steps was generally composed of all voltage- and time-dependent I_K (e.g., I_V and I_{BK}). Clamping cells at 0 mV led to the inactivation of I_V, and optimized the condition for the detection of I_{BK}. The observed suppression of total I_K (Fig. 1A) contrasted with the activation of I_{BK} (Fig. 1B) by NS004 over a same range of concentration could be conceived, to some extent, by assuming that the compound produced a substantial inhibitory action on I_V, a result with NS1619 has been reported by Edwards et al. (1994) and Xu et al. (1994). In our study, however, a reduction of total I_K (from a holding potential of −60 mV) by 5 μM NS004 reached 38%, a number greater than what would be expected if 75–80% of the whole-cell I_K, inhibitable by iberiotoxin and tetraethylammunium, is associated with I_{BK}. In other words, an inhibition of the iberiotoxin-resistant I_V (20–25% of total I_K) alone was not sufficient to account for the inhibitory effect of NS004 on total I_K. It is possible that the suppression of total I_K by NS004 also resulted from an indirect inhibition of I_{BK} subsequent to its strong inhibition of the L-type of Ca^{2+} channel (Hu et al., 1994; Edwards et al., 1994). The effect of NS004 at a holding potential of −60 mV may, therefore, be a net result from a direct activation of I_{BK}, an inhibition of I_V, and an indirect suppression of I_{BK} secondary to blockade of I_{Ca}. When cells were held at 0 mV, L-type Ca^{2+} channel and I_V were inactivated allowing the direct stimulatory effect of NS004 on I_{BK} to be unmasked.

NS004 and its analog NS1619 were also reported to have inhibitory effect on the K_{ATP} channel in vascular smooth muscle cells (Edwards et al., 1994; Hu et al., 1994; Xu et al., 1994). In this study, no attempt was made to see the effect, since the pipette solution contained 2 mM ATP, considerably higher than 40–50 μM, the concentration generally found to half-maximally inhibit the K_{ATP} channel.

The lack of the dependence on holding potential of the effects of NS1608 suggests that it has little effect either on I_V or L-type Ca^{2+} channel. In a separate study, we found that NS1608 did not significantly inhibit K_{ATP} as was observed with NS004. Thus, NS1608 at lower concentration (< 10 μM) is a more specific BK_{Ca} channel opener than NS004. The overall inhibition of I_{BK} by NS1608 at higher concentrations (> 50 μM) might be associated with its effect on other types of ion channel. It is also speculated that NS1608 exerted a dual action (stimulatory and inhibitory) on I_{BK} depending on drug concentration. The mechanisms involved will be clarified in a future study.

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