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Direct modulation of Ca^{2+}-activated K^+ current by H-89 in rabbit coronary arterial smooth muscle cells

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Abstract

The effects of H-89, a potent and selective inhibitor of protein kinase A (PKA) on Ca^{2+}-activated K^+ (BK_{Ca}) channels in coronary arterial smooth muscle cells were examined using a patch-clamp technique. In inside-out configuration, H-89 increased the NP_o of the BK_{Ca} channel, but it reduced the dwell time of BK_{Ca} currents. In whole-cell configuration, H-89 markedly increased BK_{Ca} currents in a concentration-dependent manner. The EC_{50} was 0.470±0.0741 μM based on dwell time, 0.582±0.0691 μM based on the NP_o, and 0.519±0.0295 μM based on the whole-cell current, respectively. H-85, which is an inactive form of H-89, increased BK_{Ca} currents, similar to the result of H-89. The other PKA inhibitors (Rp-8-CPT-cAMPs and KT 5720) and protein phosphatase inhibitor (okadaic acid, 1 μM) had little effect on BK_{Ca} currents and did not significantly alter the stimulatory effects of 1 μM H-89. These findings suggest that H-89 increases the BK_{Ca} current independently of PKA.

Keywords: Ca^{2+}-activated K^+ channel; H-89; Coronary artery; Protein kinase A

1. Introduction

Although multiple classes of K^+ channels are expressed at varying densities in different vascular beds, the Ca^{2+}-activated K^+ (BK_{Ca}) channel is the predominant K^+ channel species in most arteries (Nelson and Quayle, 1995). The opening of BK_{Ca} channels induces hyperpolarization and limits Ca^{2+} entry through voltage-dependent Ca^{2+} channels, providing a negative feedback mechanism for smooth muscle depolarization and vasoconstriction (Nelson and Quayle, 1995).

Vasodilators that act via protein kinase A (PKA) can activate BK_{Ca} channels in vascular smooth muscle (Standen and Quayle, 1998). One method of studying the role of PKA in BK_{Ca} channels is to inhibit PKA activity by incubation with membrane-permeable PKA inhibitors. Among the PKA inhibitors, H-89 has been reported to be a selective PKA inhibitor, with no effect on the activities of other protein kinases (Chijiwa et al., 1990). The K_i of H-89 for PKA inhibition in an *in vitro* system was reported as 0.048±0.008 μM, and the maximum effective concentration for PKA inhibition was 1 μM (Chijiwa et al., 1990; Konda et al., 1994). Therefore, in general, to block the effects of PKA completely, the concentration of H-89 used ranged from 1 to 50 μM. However, the usefulness of H-89 has been limited by its unexpected effect, as it acts on other proteins in addition to PKA. For example, H-89 directly blocks Kv1.3 channels stably expressed in Chinese hamster ovary cells, rat epithelial Na^+

Abbreviations: 4-AP, 4-aminopyridine; BK_{Ca}, Ca^{2+}-activated K^+ channel; BSA, bovine serum albumin; CASMCs, coronary arterial smooth muscle cells; DTT, dithiothreitol; H-89, (*N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide); IbTX, iberiotoxin; KB, Kraft–Brühe; PKA, protein kinase A.

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channels, and sarcoplasmic reticulum Ca^{2+}-ATPase isolated from ferret ventricular myocytes, independent of PKA activity (Hussain et al., 1999; Niisato et al., 1999; Choi et al., 2001).

Considering the importance of PKA in vascular smooth muscle function, it is essential to verify the unexpected effects of H-89 before experimental data can be interpreted accurately. Therefore, in the present study, we investigated the effects of H-89 on the BK_{Ca} currents in freshly isolated coronary arterial smooth muscle cells (CASMCs). We found, for the first time, that H-89 increased BK_{Ca} currents by a mechanism that is independent of PKA.

2. Methods

2.1. Cell preparation

New Zealand White rabbits (~2.0 kg) of either sex were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) and were injected simultaneously with heparin (100 U kg^{-1}) into the ear vein. The hearts were removed immediately and were immersed in ice-cold normal Tyrode solution. The left descending coronary arteries were dissected. Each artery was cut open along the longitudinal axes and the outer and inner surfaces were cleaned of the adventitia and endothelium, respectively. Thereafter, single cells were obtained using enzymatic digestion of the arteries as follows. The arteries were incubated for 20–25 min in Ca^{2+}-free normal Tyrode solution containing (in mg ml^{-1}) 1.0 papain, 1.5 bovine serum albumin (BSA), and 1.5 dithiothreitol (DTT). The coronary artery was then further digested for 15–20 min in Ca^{2+}-free normal Tyrode solution containing (in mg ml^{-1}) 2.8 collagenase, 1.5 BSA, and 1.5 DTT. Following the aforementioned enzyme treatment, each artery was rinsed with Kraft–Brühe (KB) solution. Single smooth muscle cells were obtained by gentle trituration with a fire-polished Pasture pipette, stored at 4 °C, and used on the day of preparation.

2.2. Solution

Normal Tyrode solution contained (in mM): NaCl, 140; KCl, 5.4; NaH_2PO_4, 0.33; $CaCl_2$, 1.8; $MgCl_2$, 0.5; HEPES, 5; glucose, 16.6; adjusted to pH 7.4 with NaOH. The bath and

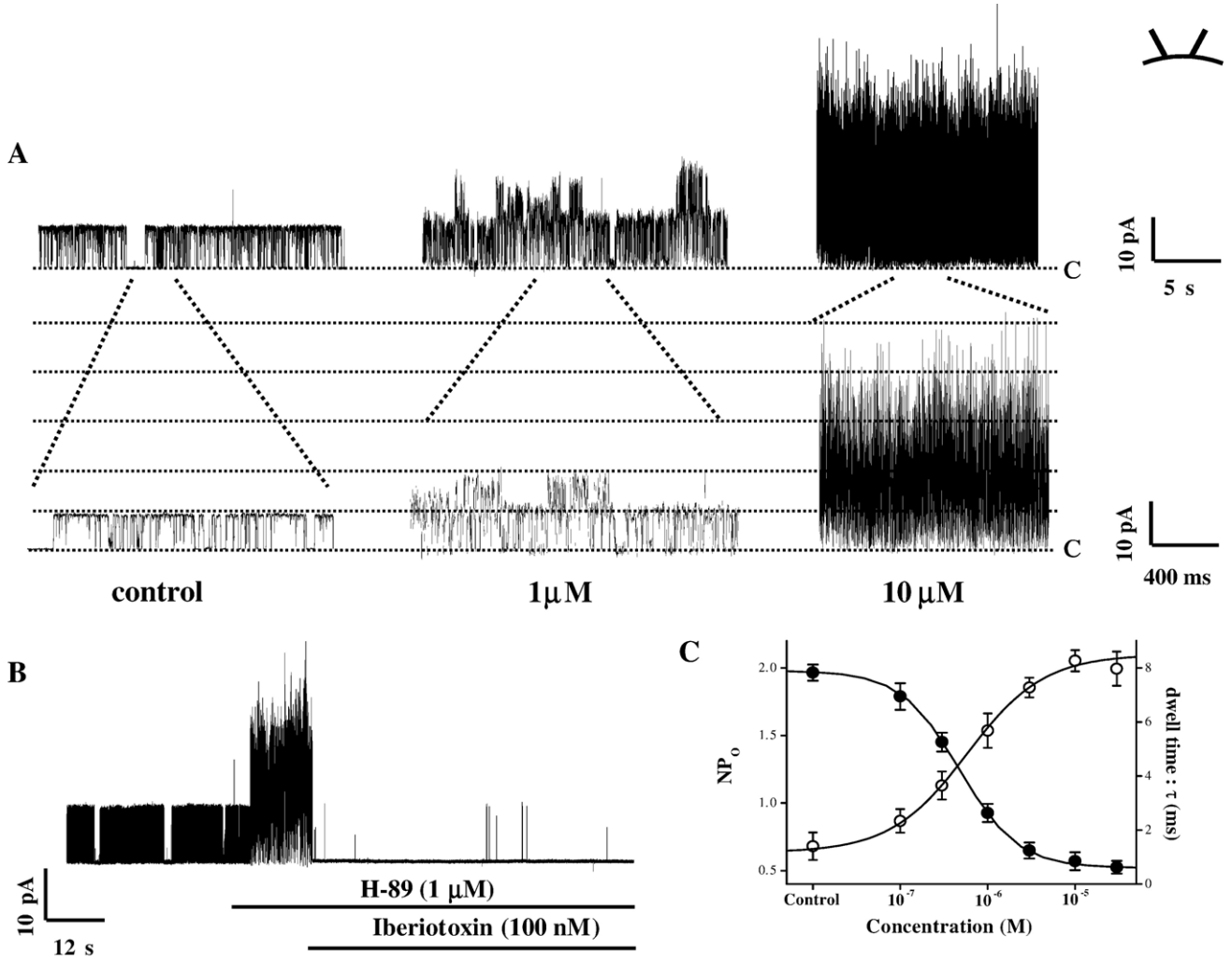

Fig. 1. The effect of H-89 on the BK_{Ca} channels of inside-out patches from CASMC. A: The traces of the single-channel currents obtained in the absence (left panel) and presence of 1 (middle panel) and 10 μM (right panel) H-89. B: The effect of 100 nM iberiotoxin on H-89-induced increase of BK_{Ca} currents ($n=4$). C: Group data (all $n=5$) for concentration-dependent effect of H-89 on BK_{Ca} channel NP_o (○) and dwell time (●).