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Extracellular Ca^{2+}-activated K channel in coronary artery smooth muscle cells and its role in vasodilation

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An extracellular Ca^{2+}-activated K channel (K_R channel), having a conductance of 30 pS, was identified in isolated single smooth muscle cells from porcine coronary artery. The K_R channel was active at $>10^{-5}$ M Ca^{2+}, and was blocked by 4-aminopyridine (4AP). At $<10^{-6}$ M Ca^{2+}, the K_R channel became inactive, but could be activated by 2-nicotinamidethyl nitrate (nicorandil), or by 4-acetamide-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) applied to the pipette solution. It was found that there is a close correlation between the K_R channel activity and cell contraction: cells contracted under conditions in which the K_R channel became inactive, but were relaxed when the K_R channel was active. As the K_R channel is highly active in cells in physiological saline, we suggest that it controls the tonus of the coronary artery, as an endogenous dilating factor.

K channel; Ca^{2+}, external; Smooth muscle; Vasodilator

1. INTRODUCTION

In contractile cells, contraction is regulated by cytoplasmic free Ca^{2+} in the order of 10^{-7} M [1–3]. Ca^{2+} channels are known to play important roles in controlling contraction [4,5]. In vascular smooth muscle cells, mechanisms that prevent the cells from contracting are also important in maintaining the physiological function of blood vessels. The present study suggests that a newly identified K^+ channel in porcine coronary artery smooth muscle cells acts as a vasodilating mechanism under physiological conditions.

2. METHODS

2.1. *Cell preparation*

Coronary arteries were excised from fresh porcine heart, and cut into small pieces in normal Tyrode's solution after removing the endothelial tissue. The pieces were then explanted in culture dishes filled with medium 199 (Nissui Chem. Japan) containing 10% fetal bovine serum (Gibco, USA), and stored in a CO_2 incubator (5% CO_2, 37°C) [6]. Three or four glass coverslips were placed on the bottom of each culture dish. Single smooth muscle cells that came out from the tissues and adhered to the coverslips during primary culture for 5–7 days were used for experiments. No proteolytic enzyme was used for isolation of single cells.

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2.2. *Solutions and chemicals*

Tyrode's solution consisted of 137 mM NaCl, 2.7 mM KCl, 7.5 mM Na-Mops buffer (pH 7.2), and 5.5 mM glucose. High K^+ solution consisted of 140 mM KCl (or K-aspartate) in 5 mM K-Mops buffer (pH 7.2). $CaCl_2$ or $MgCl_2$ was added to these solutions. Normal Tyrode's solution contained 1.2 mM $CaCl_2$. EGTA-Ca^{2+} buffer was used for adjusting low Ca^{2+} concentrations $<5 \times 10^{-6}$ M. Free Ca^{2+} concentrations were determined with K_d = 87 nM. Nicorandil was provided by Chugai Pharmaceutical Co. (Japan).

2.3. *Determination of cytoplasmic free Ca^{2+} concentration*

The cells were loaded with fura 2 in culture medium containing 3 μM fura 2-AM (Wako) for 30 min. The solution was then switched to normal Tyrode's solution, and fluorescence measurements made by the dual-wavelength excitation method [7,8]. Cytoplasmic free Ca^{2+} concentrations were determined by the ratio method [8].

2.4. *Single channel recordings*

Single channel currents were recorded with a home-made patch clamp amplifier. Soft glass patch pipettes having a heat-

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polished tip were used without Sylgard coating [9]. The electric resistance of the patch pipettes used was 5–7 MΩ. All experiments were carried out at a temperature of 35–37°C.

3. RESULTS AND DISCUSSION

3.1. *Low-Ca^{2+} contraction of smooth muscle cells*

The isolated smooth muscle cells contracted when exposed to solutions of low concentrations of divalent cation (Ca^{2+} or Mg^{2+}). In low Ca^{2+} (or Mg^{2+}) medium this contraction occurred after a certain latent period. Once a cell started contracting, it contracted rapidly for 1–2 min, becoming 1/3–1/5 of its relaxed length (fig.1A). Subsequently it contracted slowly until it became globular. This mode of full contraction is distinct from caffeine-induced or high K^+-induced contraction, which results in formation of a rough cell surface and only a small decrease in size. In fig.1B, average values (with ± SD) for the fraction of cells that underwent contraction within 5 min are plotted against log values of the Ca^{2+} concentration of the Mg^{2+}-free bathing media. When the bathing medium was Tyrode's solution (open circles), the plot represented a dose-response curve with an apparent ED_{50} of 50 μM Ca^{2+}. A similar dose-response curve was obtained for Mg^{2+} concentration in Ca^{2+}-free Tyrode's solution with an apparent ED_{50} of 200 μM Mg^{2+} (not shown). This contraction was named 'low-Ca^{2+} contraction'.

The low-Ca^{2+} contraction was completely inhibited by addition of 20–100 μM nicorandil or of 1–10 μM SITS to the bathing Tyrode's solutions (fig.1B). Conversely, addition of 4AP (5 mM) induced the full contraction of nearly all the cells at all Ca^{2+} (or Mg^{2+}) levels studied. Verapamil (1 μM) and nicardipine (1 nM) did not inhibit the low-Ca^{2+} contraction.

The fast phase of the low-Ca^{2+} contraction and that of the 4AP-induced contraction were accompanied by transient elevation of cytoplasmic free [Ca^{2+}] (fig.1C, a and b). After this transient rise, the cytoplasmic free Ca^{2+} level became lower than that initially, yet the cell continued to contract

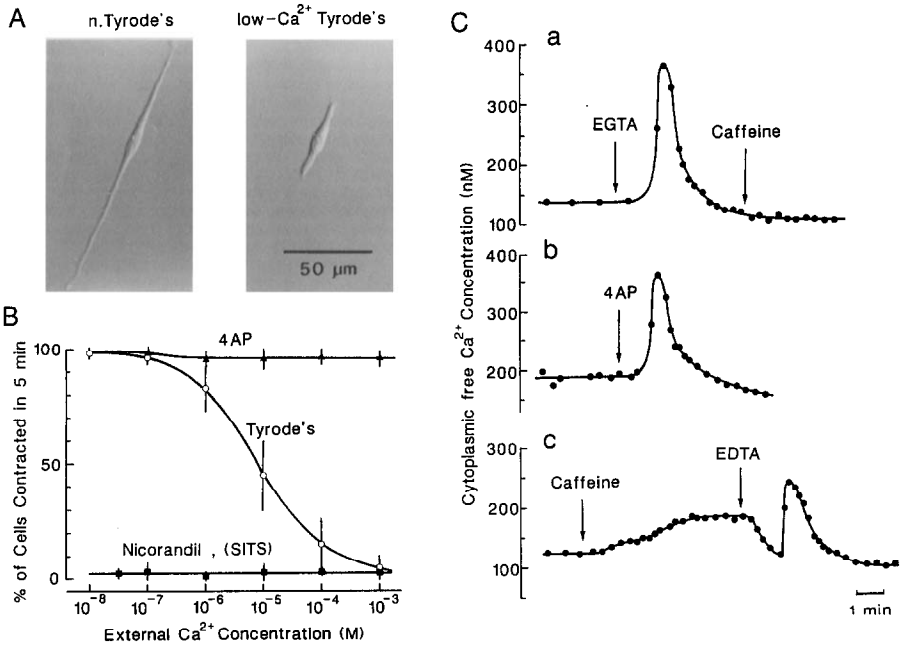

Fig.1. (A) A smooth muscle cell isolated from a porcine coronary artery observed with Nomarski optics before (left) and after (right) the fast phase of contraction produced by lowering the Ca^{2+} concentration of the bathing Tyrode's solution from 1.2 mM to 10^{-7} M. (B) Fractions of smooth muscle cells that underwent contraction within 5 min at various extracellular Ca^{2+} concentrations. Each symbol represents a mean value with SD ($n \geq 5$). (C) Time courses of change in the cytoplasmic free Ca^{2+} concentration measured with fura 2. Effects of 5 mM EGTA and then 10 mM caffeine (a), effect of 5 mM 4AP (b), and effects of 10 mM caffeine and then 5 mM EDTA (c). Control data recorded before application of chemicals were obtained in normal Tyrode's solution.

slowly. No subsequent cytoplasmic Ca^{2+} rise was observed either on repeated change in the external Ca^{2+} concentration or external application of 10 mM caffeine. When a cell was pretreated with caffeine, which caused a small increase in the cytoplasmic free Ca^{2+} level due to the mechanism of Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum [10,11], transient cytoplasmic Ca^{2+} rise could be induced by lowering the external divalent cation concentration (fig.1C, c).

These results strongly suggest that the low-Ca^{2+} contraction is unrelated to Ca^{2+} permeation across the cell membrane or to Ca^{2+}-induced Ca^{2+}-release mechanism. It should be noted that the drugs that affected the low-Ca^{2+} contraction are known as K^+ channel modifiers; 4AP blocks K^+ conductances [12,13], nicorandil increases the K^+ conductance of some vascular smooth muscles [14,15], and SITS is a K^+ channel opener [16]. We therefore postulated that the low-Ca^{2+} contraction is related to a K^+ channel which is activated by extracellular Ca^{2+}.

3.2. *Extracellular Ca^{2+} activated K channel*

When a giga-seal cell attached patch was produced in normal Tyrode's solution, transient activities of large conductance channels (50–200 pS) were recorded in the first 10–30 s, followed by a steady-state channel activity of only one or two types of small conductance channels (20–30 pS). Even at the steady state, the channel activity was too high to demonstrate clear single channel currents when the pipette solution contained the normal extracellular concentration of Ca^{2+} (1.2 mM). Single channel currents became distinct when the Ca^{2+} concentration in the pipette was lowered to 10^{-4} M (records a and b in fig.2A). The channels were still highly active at 10^{-4} M Ca^{2+}, and showed similar open-close kinetics for both outward- and inward-directed currents at different pipette voltages (V_p). In fact, no appreciable voltage dependency of the open channel fraction could be detected within the voltage region tested (± 60 mV from the resting potential). The mean value of the open channel fraction at 10^{-4} M Ca^{2+} was 80% (SD ± 24%; n = 12). The open time fraction decreased remarkably as the Ca^{2+} concentration was lowered (records b–e). At 10^{-5} M Ca^{2+}, the open channel fraction became 17 ± 5.3% (n = 4), and at 10^{-6} M Ca^{2+}, it decreased to 2.0 ± 2.5% (n = 6). When the Ca^{2+} concentration was lowered to 3×10^{-8} M, the steady-state channel activity could no longer be detected from 22 cell-attached patches examined. Even at 3×10^{-8} M Ca^{2+}, however, a large amount of single-channel currents could be recorded from all cell-attached patches when the pipette solution contained 20–100 μM nicorandil (n = 15) (record f), or 1–10 μM SITS (n = 7) (record g). On the other hand, single-channel currents which were observed at 10^{-4} M Ca^{2+} under an inside out configuration, with a bath medium of 140 mM K-aspartate and 1 mM EGTA, disappeared after addition of 5 mM 4AP to the bath (record h).

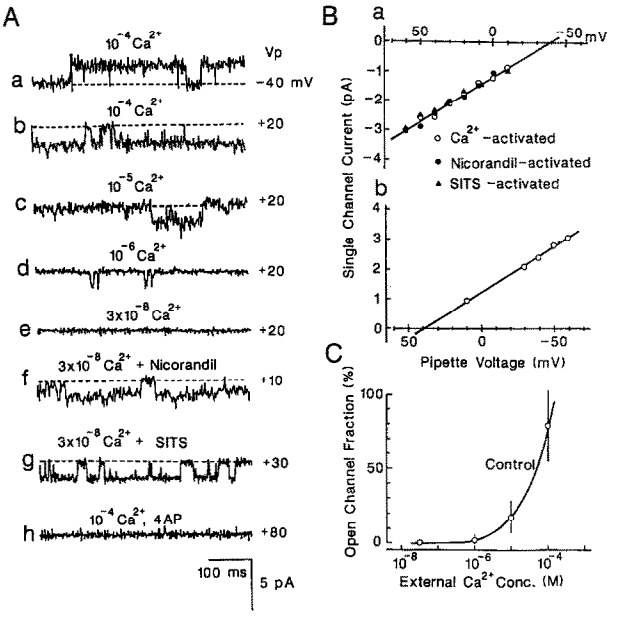

Fig.2. (A) Steady-state single channel current traces demonstrating the extracellular Ca^{2+} activated ionic channel (records a–e), nicorandil-activated channel (record f), SITS-activated channel (record g), and blocking of the channel activity by 4AP (record h). An upward direction indicates outward-directed transmembrane current, and the dashed line shows the zero current level. (B) Current-voltage relations of the major steady-state single-channel component at different K^+ concentrations of the pipette solution. (C) Mean values of the open channel fraction of the K_R channel at various extracellular Ca^{2+} concentrations. Vertical bars indicate ± SD.

The unit conductance of the major extracellular Ca^{2+}-activated channel was 30.5 pS for the inward currents measured with a cell-attached patch pipette filled with 140 mM K^+ solution (fig.2B, a),

and was 30.0 pS for the outward currents measured with Tyrode's solution which contained 2.7 mM K^+ as the pipette solution (fig.2B, b). The reversal pipette potentials obtained from the current-voltage relations in fig.2B, a and b were −39 mV and +40 mV, respectively. The magnitude of the reversal potential shift with change in the K^+ concentration in the pipette from 140 to 2.7 mM was thus 79 mV, which indicates that this channel was a K^+ selective channel. We have named this channel the 'K_R channel'. As shown in fig.2B, a, both the nicorandil-activated and the SITS-activated channels had the same slope, conductance and reversal potential as the K_R channel. (Longer applications of SITS activated at least 4 types of K^+ channels other than the K_R channel.)

In fig.2C, the open channel fraction of the K_R channel is plotted against log values of the extracellular Ca^{2+} concentration. The Ca^{2+} dependency of the channel is well correlated with that of the low-Ca^{2+} contraction (fig.1B).

The physiological saline contains both Ca^{2+} and Mg^{2+}, and the concentration of these cations is high enough to maintain the K_R channel in a highly active state, which relaxes the smooth muscle cells or prevents them from contracting.

A K^+ channel (K_M channel) having a large unit conductance (180 pS) which is sensitive to external Ca^{2+} concentration has been found in rabbit portal vein smooth muscle cells [17]. Judging from the great difference in the unit conductances, the K_M channel is obviously different from the K_R channel. Nevertheless, we observed similar contractions induced by lowering the external Ca^{2+} concentration or adding ATP in single smooth muscle cells prepared from rabbit portal veins, mesenteric arteries, and aortae, although nicorandil had only a weak effect upon these contractions. Therefore, it seems possible that, although their unit conductance and drug sensitivities may differ, extracellular Ca^{2+}-activated K^+ channels may exist in various vascular smooth muscle cells and play an important role in controlling vascular tone.

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