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Ca^{2+}-induced Ca^{2+} release activates K^+ currents by a cyclic GMP-dependent mechanism in single gastric smooth muscle cells

Dessislava B. Duridanova *, Hristo S. Gagov, Kiril K. Boev

Institute of Biophysics, Bulgarian Academy of Sciences, Georgi Bonchev Street, Block 21, 1113 Sofia, Bulgaria

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Abstract

The participation of sarcoplasmic reticulum Ca^{2+} release channels in the activation of Ca^{2+}-sensitive K^+ currents ($I_{K(Ca)}$) by cyclic dibutyryl GMP was investigated in smooth muscle cells from the circular layer of guinea-pig gastric fundus. All experiments were performed in the presence of 3 μM nicardipine into the bath and low Ca^{2+} buffering capacity of the pipette-filling solution (pCa 7.4). Ruthenium red (10 μM) as well as its combination with 10 μM heparin abolished the cyclic GMP-induced activation of $I_{K(Ca)}$, while 10 μM heparin remained ineffective. Ryanodine (10 μM) and the subsequently added 1 μM thapsigargin induced a relatively small increase in $I_{K(Ca)}$ amplitudes. The addition of 10 μM ryanodine to 1 μM thapsigargin-containing bath solution caused a vast increase in $I_{K(Ca)}$. It is hypothesyzed that protein kinase G-induced vectorial Ca^{2+} flux from the cell bulk and sarcoplasmic reticulum Ca^{2+} stores toward the plasma membrane is realized by a spontaneous Ca^{2+}-induced Ca^{2+} release from a superficially situated Ca^{2+} store.

Keywords: Ca^{2+} store; Ryanodine; Heparin; Thapsigargin; Ruthenium red; cGMP

1. Introduction

Recently it has been suggested that in some smooth muscle cells a vectorial Ca^{2+} release mechanism transports Ca^{2+} ions from the deeply situated myoplasm toward the cell membrane (Van Breemen and Saida, 1989; Chen and Van Breemen, 1992). Stehno-Bittel and Sturek (1992) strongly support this suggestion showing that in some vascular smooth muscle cells the superficially located sarcoplasmic reticulum Ca^{2+} store spontaneously releases Ca^{2+}, which in turn causes a substantial activation of Ca^{2+}-dependent K^+ currents ($I_{K(Ca)}$).

Lincoln et al. (1994) postulated that cyclic GMP-dependent protein kinase (protein kinase G) plays a key role in the regulation of vascular smooth muscle tone due to its pleiotropic effects on intracellular Ca^{2+} redistribution. Such suggestion is widely supported by several observations, which have shown that nitrovasodilators exert their relaxing effects by altering the intracelullar Ca^{2+} homeostasis (Clapp and Gurney, 1991; Cornell and Lincoln, 1989), and Ca^{2+}-dependent ion fluxes (Magliola and Jones, 1990), especially K^+ currents (for example see Taniguchi et al., 1993; Archer et al., 1994).

Our preliminary studies have shown that nitric oxide (NO)-liberating substances and cyclic GMP analogues induced an increase in Ca^{2+}-sensitive K^+ conductivity in single smooth muscle cells of guinea-pig stomach fundus. This effect was abolished by sarcoplasmic reticulum Ca^{2+}-ATPase antagonists (Duridanova et al., 1995).

A relevant further investigation was to clarify what type of intracellular Ca^{2+} release channels were involved in the observed vectorial Ca^{2+} release. It is known that in some smooth muscles protein kinase G inhibits inositol 1,4,5-trisphosphate (IP_3) formation (Rapoport, 1986; Hirata et al., 1990). In guinea-pig gastric smooth muscle cells cyclic nucleotides were found to inhibit both IP_3 formation and IP_3-dependent Ca^{2+} mobilization (Murthy et al., 1993). Therefore IP_3-sensitive Ca^{2+} release channels do not seem to be involved in this mechanism.

Studies of the guinea-pig mesenteric artery (Buryi et al., 1994) and ileum (Gagov et al., 1993) suggest the existence of a ryanodine-sensitive Ca^{2+} store, located close to the plasma membrane. The increased Ca^{2+} concentration in subplasmalemmal space activates the Ca^{2+}-induced Ca^{2+} release mechanism by this store (Iino, 1989), which in turn contributes to the opening of Ca^{2+}-sensitive K^+ channels (Isenberg et al., 1992). Thus, the Ca^{2+}-induced Ca^{2+}

* Corresponding author. Tel.: 359-2-713-2139; fax: 359-2-730-385; e-mail: DESSYBD@IPH.BIO.ACAD.BG.

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release mechanism seems to be a suitable candidate to maintain the spontaneous superficial Ca^{2+} release (Stehno-Bittel and Sturek, 1992) in tonically contracted smooth muscles.

The aim of the present study was to determine the involvement of the two types of intracellular Ca^{2+} release channels in the cyclic GMP-induced activation of $I_{K(Ca)}$ in single smooth muscle cells isolated from guinea pig gastric fundus.

2. Materials and methods

2.1. Whole cell patch-clamp experiments

Experiments were performed on single smooth muscle cells, freshly isolated from the circular layer of a guinea-pig gastric fundus. The animals weighed 300–400 g. Isolated muscle strips were placed in physiological salt solution (PSS) and cut into small pieces. The pieces were then transferred into Ca^{2+}-free PSS, prewarmed to 37°C and containing 1 g/l collagenase (type 1A, Sigma), 0.5 g/l soybean trypsin inhibitor and 1.5 g/l bovine serum albumin. After 55–70 min of incubation at 37°C the enzyme was carefully washed out from the pieces with 20 ml prewarmed Ca^{2+}-free PSS. Single smooth muscle cells were then obtained by gentle agitation of the pieces with two Pasteur pipettes with different tip openings in 1 ml fresh modified 'KB' solution (see below) until the solution became cloudy. Cells were stored up to 12 h at 6°C in this solution. Only fully relaxed cells were used for the purpose of the study. Cells which reduced their length by more than 35% or did not contract at all after the application of 0.1 μM acetylcholine were rejected, as well as those which did not relax after the acetylcholine-induced contraction. PSS in which the drugs were diluted, was perfused continuously to the bath chamber where cells had adhered to the glass bottom.

2.2. Electrical recordings

The whole-cell mode of the patch-clamp technique was employed (Hamill et al., 1981). The patch electrodes from borosilicate glass (Jencons Scientific), when filled with the internal solution, had resistances of approximately 2.5 MΩ. Membrane currents were recorded by an EPC-7 (List Electronics) amplifier. Current signals were recorded and further analyzed on an AT 286 PC through a TL-1 DMA (AXOPATCH) interface, and Step Wave Cell Tester – version 1.0 (Georgi Shkodrov, Institute of Biophysics, Bulgarian Academy of Sciences) software, respectively.

The comparison of the absolute values of the K^+ current amplitudes, measured in different cells, is not very appropriate due to the considerable difference in the capacitive and also the visible membrane surface of single cells, isolated from the circular layer of gastric fundus smooth muscle. Therefore we found it necessary to estimate the values of the current densities, expressed as $i_K = \mu A/cm^2$ of membrane surface (assuming the specific membrane capacitance of 1 $\mu F/cm^2$ – see Hamill et al., 1981) and to plot it versus the potential applied in order to obtain comparable data for a statistical analysis. In all experiments the holding potential (V_h) was −50 mV.

2.3. Solutions

The physiological salt solution (PSS) in the experiments was of the following composition (in mM): 110 NaCl, 5.6 KCl, 10 Hepes, 20 taurine, 20 glucose, 1.2 $MgCl_2$, 1.8 $CaCl_2$, 5 Na-pyruvate, pH 7.4. The medium, used in cell isolation, consisted of 85 KCl, 30 KH_2PO_4, 5 $MgCl_2$, 20 taurine, 5 Na_2-ATP, 5 Na-pyruvate, 5 creatine, 5 oxalacetate, 1 g/l bovine serum albumin (pH 7.2). The internal solution into the recording pipette contained: 123 KCl, 5 Hepes, 4 EGTA, 0.5 $CaCl_2$, 0.5 $MgCl_2$, 5 Na-pyruvate, 5 succinic acid, 5 oxalacetic acid, 1.5 g/l Na_2-ATP, pH 7.2, pCa 7.4. The intracellular Ca^{2+} concentrations were estimated according to the program of Fabiato and Fabiato (1979).

2.4. Substances

All substances used were obtained from Sigma Chemical Co. (St. Louis, MO, USA), except ryanodine, which was from Calbiochem Corp. (La Jolla, CA, USA). Ryanodine, thapsigargin and cyclic dibutyryl GMP were added to the bath solution, while heparin and ruthenium red were added to the pipette solution.

All experiments were performed in the presence of 3 μM nicardipine to block Ca^{2+} entry (Lammel et al., 1991) at a temperature of 31 ± 2°C.

3. Results

3.1. Effects of Ca^{2+} release channel antagonists on cyclic GMP-induced changes of I_K amplitudes

Preliminary studies on single smooth muscle cells of guinea-pig gastric fundus showed that in high EGTA-containing internal solution (pCa > 7.4) cyclic dibutyryl GMP decreased the amplitudes of Ca^{2+}-dependent K^+ currents ($I_{K(Ca)}$). In the lower EGTA-containing pipette solution (pCa < 7.4) the same cyclic GMP analogue caused a considerable and sustained activation of $I_{K(Ca)}$ (Duridanova et al., 1995). Under these conditions (pCa 7.4) cells expressed a vast increase in $I_{K(Ca)}$ amplitudes 6 min after the bath application of 0.5 μM cyclic dibutyryl GMP (Fig. 1A, closed circles). The presence of 10 μM heparin in the pipette solution, added to prevent IP_3-induced Ca^{2+} release (Kobayashi et al., 1989), could not attenuate the cyclic dibutyryl GMP-induced $I_{K(Ca)}$ activation (Fig. 1A, closed squares, Fig. 1C).

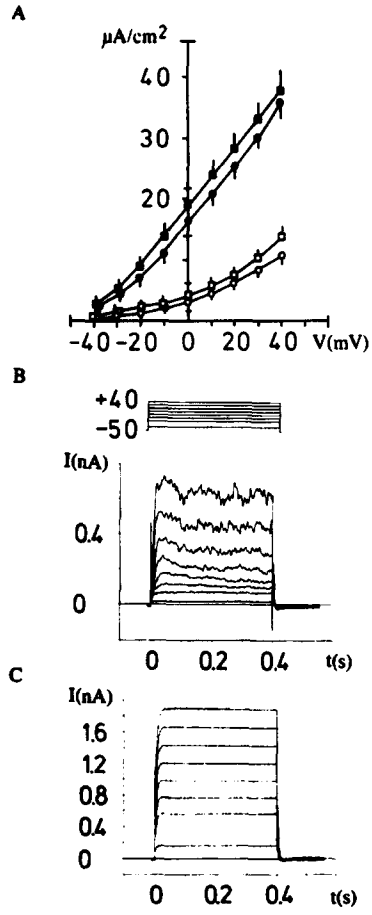

Fig. 1. Effect of 0.5 μM cyclic dibutyryl GMP on I_K amplitudes in single smooth muscle cells bathed in 3 μM nicardipine-containing PSS. $V_h = -50$ mV. Rectangular depolarizing pulses of 400 ms duration were applied at 10 mV increments. (A) Potential dependence of current density estimated by the peak I_K amplitudes, expressed in control conditions (open circles) or in the presence of 10 μM heparin in the pipette solution (open squares) and compared to the peak I_K in control (closed circles) and heparin-containing pipette solution (closed squares) 6 min after the addition of 1 μM cyclic dibutyryl GMP to the bath. Data are means ± S.E.M. for 6 cells with similar passive electrical properties. (B) Whole-cell current responses of a cell with an input impedance of 1.65 GΩ and a capacitance of 53 pF, measured in control conditions (10 μM heparin-containing pipette solution) and 9 min after the addition of 0.5 μM cyclic dibutyryl GMP to nicardipine-containing PSS (C). Standard voltage-clamp protocol is given in the right upper corner of (B).

The addition of 10 μM ruthenium red to the pipette solution did not affect significantly the net I_K amplitudes and caused only a slight increase in the amplitudes (Fig. 1A and Fig. 2A, open circles). Ruthenium red alone or its combination with heparin effectively antagonized the activating effect of cyclic dibutyryl GMP (Fig. 2) or sodium nitroprusside (not shown) on $I_{K(Ca)}$. In the presence of both heparin and ruthenium red, cyclic dibutyryl GMP (1 μM) caused a weak decrease in amplitudes of I_K, especially those of the sustained K^+ current component, measured 400 ms from the pulse onset (Fig. 2B-D).

3.2. Effects of ryanodine and thapsigargin on $I_{K(Ca)}$ amplitudes

Zholos et al. (1991) have shown that 5 mM EGTA-containing pipette solution had sufficient chelating capacity to bind the total amount of Ca^{2+}, released from the deeply located sarcoplasmic reticulum Ca^{2+} stores. In this way Ca^{2+} chelating agents impede Ca^{2+} from reaching the plasma membrane, where it usually acts as an intracellular activator of Ca^{2+}-sensitive K^+ channels. Using 4 mM EGTA-containing pipette solution we checked the possibility for the existence of a superficially located ryanodine-sensitive Ca^{2+} store in the cells investigated. Ryanodine (1–10 μM), added to the bath, was without effects on I_K up to +20 mV membrane potentials. Under more positive membrane voltages ryanodine caused a significant increase in $I_{K(Ca)}$ amplitudes (Fig. 3A). Subsequent addition of 1 μM thapsigargin to ryanodine- and nicardipine-containing PSS caused a substantial increase in I_K amplitudes, measured at positive membrane voltages (Fig. 3A,C).

Thapsigargin (0.1–1 μM), applied alone, was without effects on $I_{K(Ca)}$. Subsequent addition of 10 μM ryanodine caused a large time-dependent $I_{K(Ca)}$ increase (Fig. 4).

4. Discussion

Under our experimental conditions the effectiveness of cyclic dibutyryl GMP as $I_{K(Ca)}$ activator was found to

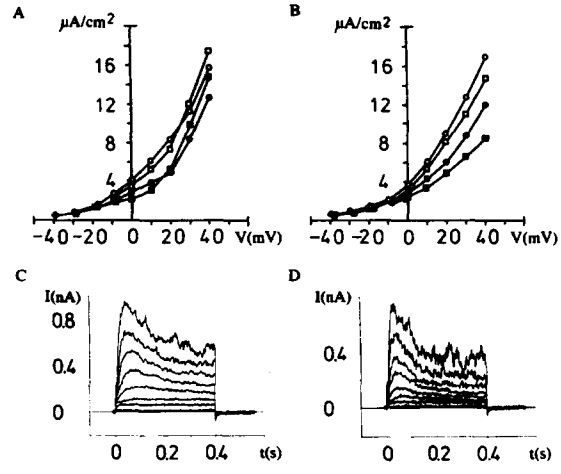

Fig. 2. Effect of 1 μM cyclic dibutyryl GMP on the whole-cell currents. $V_h = -50$ mV. Single cells were bathed in 3 μM nicardipine-containing PSS. Potential dependence of current density estimated by the peak (open circles) and sustained (closed circles) I_K as compared to the peak (open squares) and sustained (closed squares) I_K obtained 9 min after the addition of 1 μM dibutyryl cyclic GMP to the bath in cells dialysed with 10 μM ruthenium red-containing (A) or with 10 μM heparin- and 10 μM ruthenium red-containing pipette solution (B). Data are means ± S.E.M. for 7 cells (A) and 5 cells (B). Error bars were omitted for clarity. Typical I_K waveforms obtained 9 min after the addition of 1 μM cyclic dibutyryl GMP to the bath solution in cells dialysed with 10 μM ruthenium red-containing pipette solution (C) or with heparin- and ruthenium red-containing (10 μM) pipette solution (D).

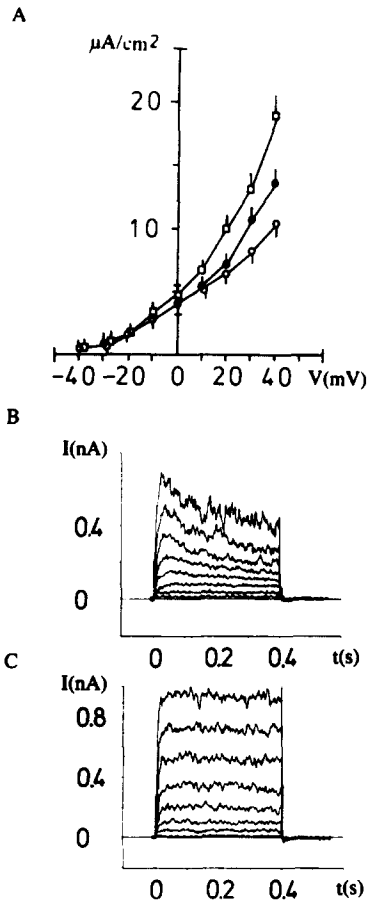

Fig. 3. Effect of subsequent addition of 10 μM ryanodine and 1 μM thapsigargin on peak I_K amplitudes as measured in 3 μM nicardipine-containing PSS. $V_h = -50$ mV. (A) Potential dependence of current density estimated by the peak I_K amplitudes (open circles) as compared to those obtained at the 7th min of drug exposure (closed circles). I_K increased a further 8 min after subsequent addition of 1 μM thapsigargin (open squares). Data are means ± S.E.M. for 4 cells. (B) Typical I_K waveforms, obtained from a cell, dialysed with 10 μM ryanodine-containing solution. (C) I_K waveforms, obtained from the same cell 8 min after the bath application of 1 μM thapsigargin to ryanodine- and nicardipine-containing PSS.

depend on the presence of ruthenium red in the pipette solution. Ruthenium red, as well as its combination with heparin, abolished the cyclic GMP-induced activation of $I_{K(Ca)}$, while heparin, known to be the most specific inhibitor of IP_3-sensitive Ca^{2+} release channels (Kobayashi et al., 1989), remained ineffective.

The effects of ryanodine and thapsigargin on the net K^+ currents were found to depend on the sequence, in which drugs were applied. Ryanodine induced only a slight increase in $I_{K(Ca)}$ amplitudes in the presence of an intact intracellular Ca^{2+} uptake mechanism, which could effectively counteract the outflow of Ca^{2+} in the direction of the plasma membrane. However, Ca^{2+} ions which left the pool toward the cell bulk could not be pumped back because of their binding to the chelator. Thus, the submembrane-located ryanodine-depleted Ca^{2+} pool could not release sufficient amounts of Ca^{2+} to enrich the adjacent subplasmalemmal space up to the levels required for the activation of the whole cell $I_{K(Ca)}$. That is why the thapsigargin-evoked increase in $I_{K(Ca)}$ amplitudes was relatively small in ryanodine-treated cells.

On the other hand, the addition of ryanodine to the thapsigargin-containing bath solution caused a large time-dependent and sustained increase in $I_{K(Ca)}$ amplitudes. The latter is a result of the Ca^{2+} depletion of the preloaded pool, located near the plasma membrane, which led to a several-fold increase in subplasmalemmal Ca^{2+} concentration in the absence of the intracellular Ca^{2+} uptake mechanism.

These results present evidence for the existence of ryanodine-sensitive sarcoplasmic reticulum Ca^{2+} store situated closely to the plasma membrane of the gastric smooth muscle cells. As a result of the ryanodine-induced depletion in the presence of a sarcoplasmic reticulum Ca^{2+}-ATPase blocking drug or due to the spontaneous release after the protein kinase G-induced preloading, Ca^{2+} ions leave this store through Ca^{2+}-activated release channels to enrich the adjacent subplasmalemmal space with Ca^{2+}, needed for the activation of $I_{K(Ca)}$.

In conclusion, we consider that the last step of the protein kinase G-induced vectorial Ca^{2+} flux directed from the cell bulk and sarcoplasmic reticulum Ca^{2+} stores to the plasma membrane is realized by a spontaneous

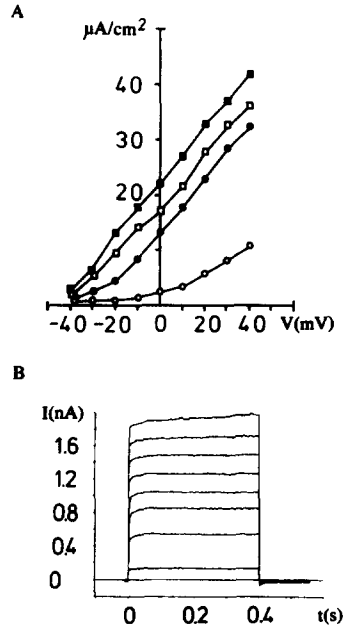

Fig. 4. Effect of subsequent addition of thapsigargin and ryanodine on peak I_K amplitudes, obtained in 3 μM nicardipine-containing PSS. $V_h = -50$ mV. (A) Potential dependence of current density, estimated by the peak I_K amplitudes in 1 μM thapsigargin-containing solution (open circles) and its increase, as observed 6 min (closed circles), 9 min (open squares), and 12 min (closed squares) after bath application of 10 μM ryanodine. Data are means ± S.E.M. for 5 cells. (B) Typical I_K waveforms expressed in cells bathed in nicardipine- and thapsigargin-containing PSS, 9 min after ryanodine application.

Ca^{2+}-induced Ca^{2+} release from a superficially situated sarcoplasmic reticulum Ca^{2+} store.

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