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European Journal of Pharmacology 394 (2000) 35–40

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Modulation of Ca^{2+}-dependent K^+ currents in mesenteric arterial smooth muscle cells by adenosine

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Received 8 February 2000; accepted 15 February 2000

Abstract

The effect of adenosine on Ca^{2+}-dependent K^+ currents was studied in freshly isolated smooth muscle cells from rat mesenteric artery. Perforated-patch recordings showed that adenosine induced transient outward currents and an overall increase in the averaged currents at higher depolarizing potentials. The changes in current activity induced by adenosine could be blocked by iberiotoxin. The transient outward currents were not dependent directly on external Ca^{2+} and could be induced after brief exposure to Ca^{2+}-free solutions. In conventional whole-cell recordings, transient outward currents were also induced by adenosine when a low EGTA concentration of 0.1 mM was included in the pipette solution. Adenosine was not effective in inducing increases in outward currents when a higher concentration of 5.0 mM EGTA was used. Ryanodine and thapsigargin were also effective in blocking the effect of adenosine. These observations suggest that adenosine may activate Ca^{2+}-dependent K^+ currents by inducing Ca^{2+} release from ryanodine-sensitive channels in the sarcoplasmic reticulum of rat mesenteric arterial cells.

Keywords: Smooth muscle, vascular; Adenosine; K^+ channel, Ca^{2+}-dependent

1. Introduction

Voltage-sensitive K^+ channels of large conductance that are activated by Ca^{2+} (K_{Ca}) can be found in vascular smooth muscle cells. The activation of these channels results in membrane hyperpolarization and a decrease in vascular tone. The activity of K_{Ca} channels are directly modulated by intracellular Ca^{2+}. Recent studies indicate that the sarcoplasmic reticulum may act as an important source in providing Ca^{2+} for the activation of K_{Ca} channels in vascular smooth muscle (Benham and Bolton, 1986; Desilets et al., 1989; Ganitkevich and Isenberg, 1990). Immunofluorescence studies showed that ryanodine receptors, which are the Ca^{2+} release channels, are present at the cell periphery of vascular smooth muscle cells (Lesh et al., 1998). Local Ca^{2+} release at these sites elicits elementary Ca^{2+} signals known as Ca^{2+} sparks (Nelson et al., 1995; Knot et al., 1998). Nearby K_{Ca} channels are activated by Ca^{2+} sparks to cause transient outward currents (Benham and Bolton, 1986; Nelson et al., 1995). There is evidence indicating that vasoactive agents may activate K_{Ca} channels by inducing Ca^{2+} release from ryanodine-sensitive stores. In rabbit cerebral artery (Kang et al., 1995) and hog carotid artery (Desilets et al., 1989), histamine induced increases in transient outward K_{Ca} currents. In guinea-pig coronary smooth muscle cells, similar increases were induced by acetylcholine (Ganitkevich and Isenberg, 1990). Increases in transient outward currents induced by agents such as forskolin and nicorandil were also reported in rat coronary and cerebral arteries (Porter et al., 1998). In all cases, the transient outward currents were abolished by ryanodine and thapsigargin.

In the present study, we tested the effect of adenosine on whole cell outward currents from freshly isolated rat mesenteric arterial cells. We also examined if ryanodine-sensitive Ca^{2+} stores were involved in the potentiation of K_{Ca} currents by adenosine. In order not to disturb the intracellular environment, perforated-patch recording was used in most of the experiments (Horn and Marty, 1988). Results from these experiments were compared to those obtained from conventional whole-cell recordings in which the cellular Ca^{2+} buffering capacity was modified by a low and a high concentration of EGTA.

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00142-4

2. Materials and methods

2.1. Cell isolation

Single cells were isolated from sections of the primary branches (200–300 μm in diameter) of mesenteric arteries of male Wistar rats (10–12 weeks old, Charles River) with a technique similar to those previously developed in our laboratory (Xiong et al., 1993). The arteries were treated with a Ca^{2+}-free HEPES buffer containing 0.02% collagenase, (Sigma, St. Louis, MO, USA), 0.1% papain (Sigma) and 4 mM dithithreitol (Sigma) for 2 h. Most of the single cells isolated were relaxed. They contracted in response to stimulants such as high potassium and noradrenaline. Only relaxed cells were used in the experiments.

2.2. Electrophysiology

Currents from freshly isolated cells were recorded using either perforated-patch or conventional whole-cell recording mode with an Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA). For perforated-patch recording, nystatin freshly dissolved in dimethylsulphoxide at a final concentration of 100 μg/ml was included in the pipette solution (Horn and Marty, 1988; Xiong et al., 1993). Patch pipettes were pulled from borosilicate glass with a Flaming–Brown P-80 puller (Sutter Instruments, Novato, USA). Data acquisition and analysis were performed using pClamp 5.5 and 6.1 software (Axon Instruments). Recordings were made after the formation of gigaohm seals. Series resistance and cell capacitance were electronically compensated. Leak currents were relatively small and were not subtracted from the records. Recordings from cells showing changes in leak currents with time were discontinued and discarded. Signals were digitalized at 3–5 kHz and filtered at 2 kHz with an eight-pole Bessel filter. The cells were maintained at a holding potential of −80 mV and currents were elicited by 500-ms test pulses from −80 to +60 mV. The currents stabilized within 4 min after establishing whole-cell configuration. They remained constant for more than 50 min, well within the time course of the experiments. Figures and curve-fitting were made with SigmaPlot 5.0 software (Jandel Scientific, San Rafael, USA). For construction of current–voltage curves, currents were normalized to current density (current/cell capacitance) to take into consideration possible variations in current with cell size. Taking into consideration the large fluctuations in the currents, 3–4 consecutive sweeps of current segments elicited from 100 to 500 ms of the test pulses were signal-averaged to obtain a more accurate measure of the currents.

The ionic composition of the extracellular solution was (in mM): NaCl 137, KCl 5.5, $CaCl_2$ 1.8, $MgCl_2$ 1.0, KH_2PO_4 0.4, $NaHCO_3$ 4.2, glucose 5.6, and pH adjusted to 7.4 with 10 mM HEPES. The intracellular solution consisted of (in mM): KCl 150, $MgCl_2$ 1.0, Na_2 ATP 1.0, and pH adjusted to 7.2 with HEPES. Free Ca^{2+} concentration was adjusted to 155 nM with either 0.1 or 5.0 mM of EGTA. Preliminary studies showed that transient outward current activities in these cells at a Ca^{2+} concentration of 155 nM and low EGTA more resembled those recorded with perforated-patch recording. Adenosine, iberiotoxin, ryanodine, and thapsigargin were obtained from Sigma and were introduced by adding to the perfusate. Recordings were made only after the currents had reached a stable level with each addition of the agent. All experiments were performed at room temperature. The data were plotted as mean ± S.E.M. Student's *t*-test was used for statistical analysis of the data. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Effects of adenosine

Perforated-patch recording of whole-cell currents from freshly isolated mesenteric cells showed outward currents superimposed with transient outward currents, which increased in amplitude with depolarization (Fig. 1A). The size of these transient currents varied amongst smooth

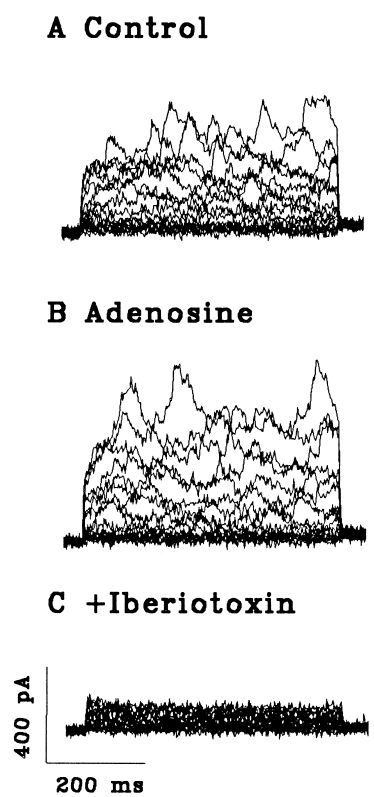

Fig. 1. Effects of adenosine and iberiotoxin on currents from a rat mesenteric arterial cell. (A) From a holding potential of −80 mV, test pulses from −80 to +60 mV elicited outward currents. (B) Adenosine (10 μM) caused increases in the currents. (C) Currents were inhibited by the addition of iberiotoxin (150 nM). Perforated-patch recordings.

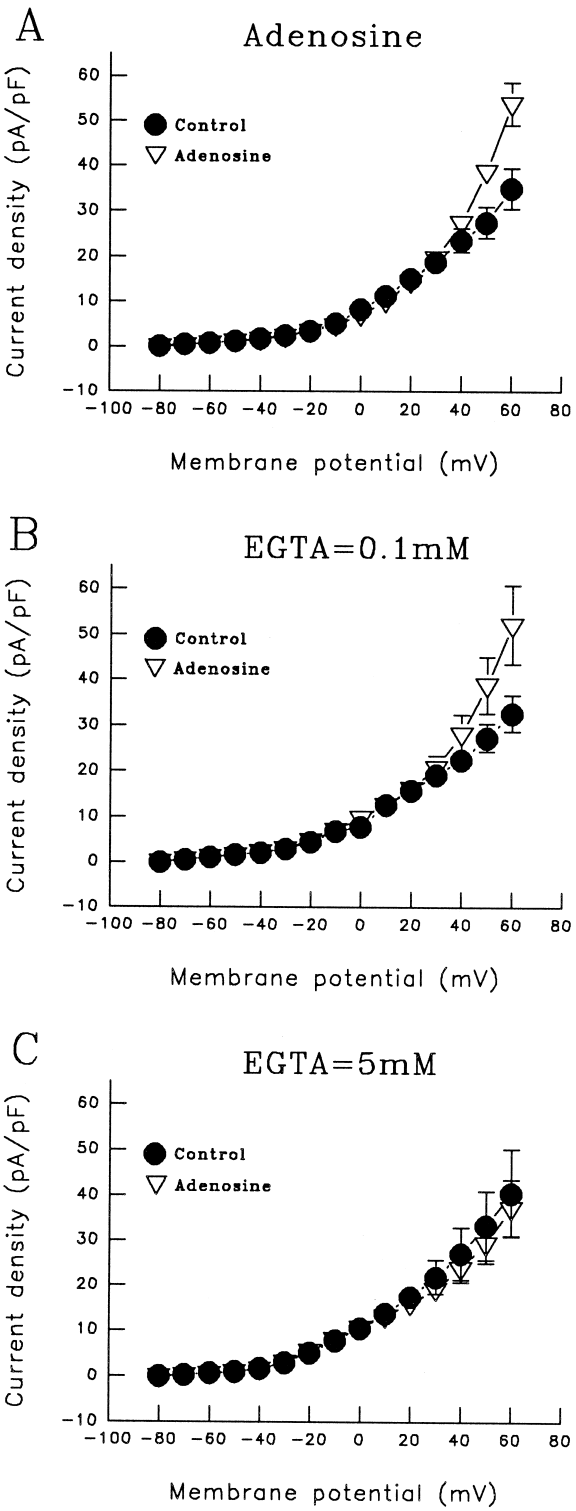

Fig. 2. Current–voltage relationships showing the effect of adenosine (10 μM) on currents elicited with test potentials from −80 to +60 mV from a holding potential of −80 mV under various experimental conditions. (A) Data obtained using perforated-patch recording. (B) Data obtained using whole-cell recording and EGTA = 0.1 mM in the pipette solution. (C) Data obtained using whole-cell recording and EGTA = 5 mM in the pipette solution. $n = 4–7$.

muscle cells. Adenosine (10 μM) increased the amplitude of the transient outward currents (Fig. 1B). Adenosine also increased significantly the averaged currents, but only the high-threshold component of the currents elicited at more positive potentials (Fig. 2A). At +60 mV, the current density increased significantly to 53.77 ± 4.73 pA/pF from a control value of 34.95 ± 4.42 pA/pF ($n = 7$). The transient outward currents and the high-threshold component of the currents were inhibited by iberiotoxin (150 nM), suggesting that they were mediated by K_{Ca} channels (Fig. 1C).

3.2. Effects of external Ca^{2+}

The removal of external Ca^{2+} resulted in a gradual reduction of the outward currents upon repeated stimula-

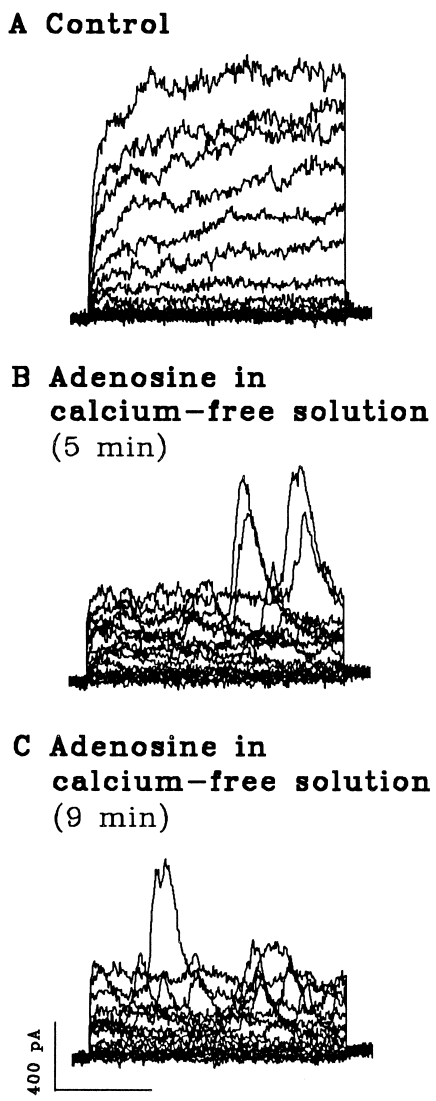

Fig. 3. Effect of Ca^{2+}-free solution on adenosine-induced responses in a mesenteric arterial cell. (A) Control currents elicited in normal solution. (B) After exposing to Ca^{2+}-free solution for 5 min, adenosine (10 μM) could still induce transient outward currents in this cell. (C) Transient outward currents elicited after 9 min exposure to Ca^{2+}-free solution. Holding potential = −80 mV, test potentials from −80 to +60 mV.

tion. In Ca^{2+}-free solutions, large transient outward currents could still be triggered by adenosine (10 μM) even though the basal outward currents may have declined (Fig. 3). The ability of adenosine to induce large transient outward currents decreased with prolonged exposure to Ca^{2+}-free solution and with repeated stimulation. In one preparation, large transient outward currents could still be triggered after exposure to Ca^{2+}-free solution for 9 min (Fig. 3C). In the other five preparations tested, the adenosine effect was not sustained and was lost more quickly in Ca^{2+}-free solutions.

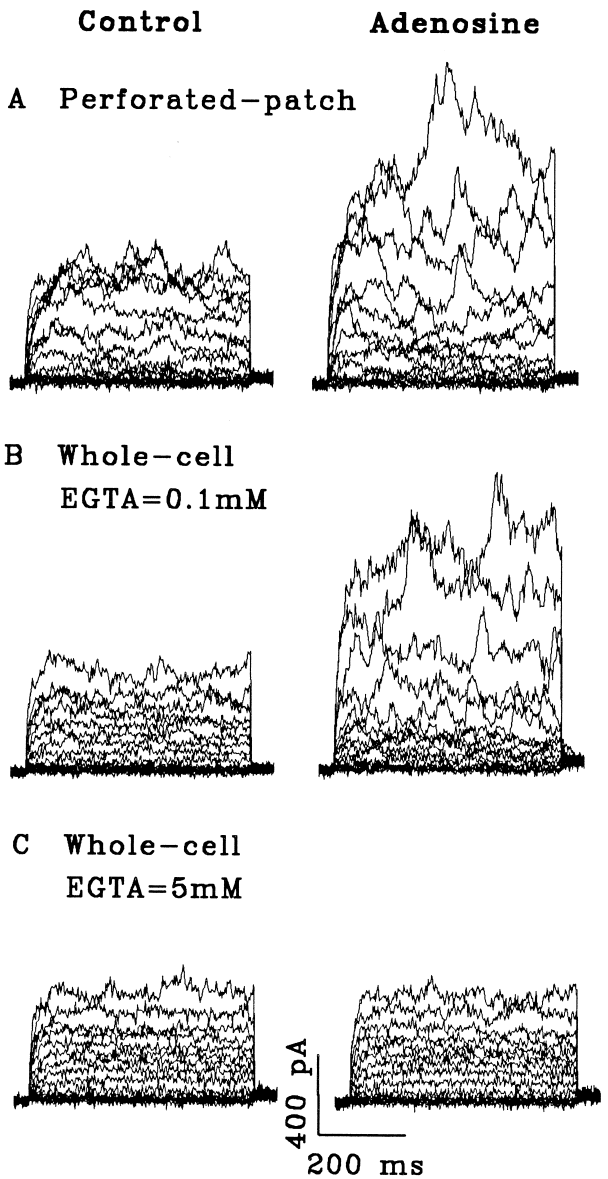

Fig. 4. Effect of EGTA on adenosine-induced currents. (A) Adenosine (10 μM) induced increases in outward currents. Perforated-patch recording. (B) Similar potentiation of the outward currents by adenosine using whole-cell recording with the pipette solution containing 0.1 mM EGTA. (C) No increases in currents were observed when 5 mM of EGTA was included in the pipette solution. Holding potential = −80 mV, test potentials from −80 to +60 mV.

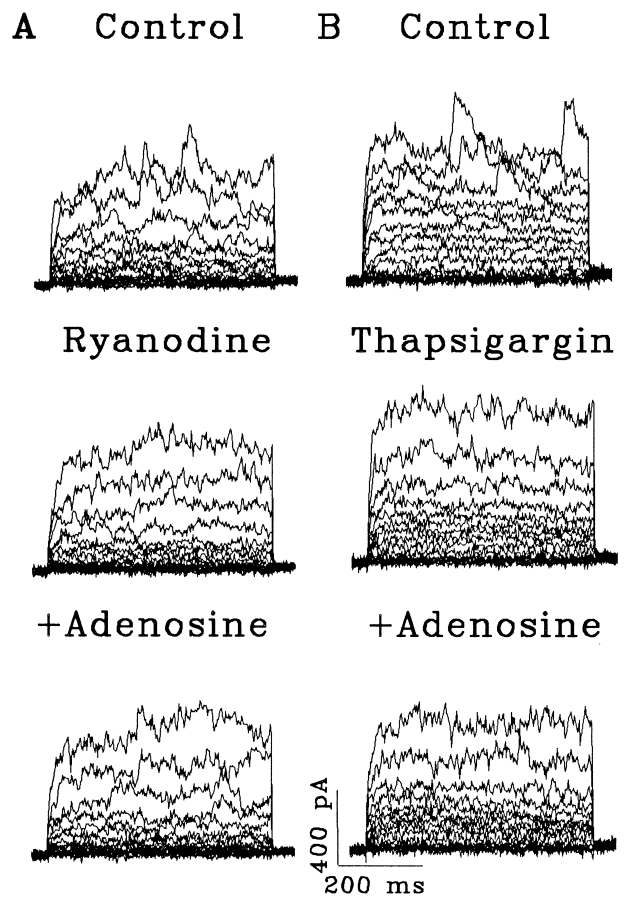

Fig. 5. Effects of ryanodine and thapsigargin on adenosine-induced currents. (A) In the presence of ryanodine (1 μM), adenosine (10 μM) failed to induce increases in the currents. (B) Adenosine also did not cause increases in the currents in the presence of thapsigargin (10 μM). Perforated-patch recordings. Holding potential = −80 mV, test potentials from −80 to +60 mV.

3.3. Effects of internal Ca^{2+} buffering

Using conventional whole-cell recording, the effects of EGTA at 0.1 and 5.0 mM in the intracellular solutions on adenosine-induced responses were compared. In these experiments, the free intracellular Ca^{2+} concentration remained adjusted to 155 nM with the two EGTA concentrations. With low internal EGTA concentration, a similar potentiation of the currents by adenosine as those from perforated-patch recordings was observed (Fig. 4). Adenosine (10 μM) induced large transient outward currents and an overall increase in the amplitudes of the averaged currents. At a test potential of +60 mV, the current density increased significantly from a control value of 40.18 ± 3.69 to 62.24 ± 2.78 pA/pF ($n = 5$). The current–voltage relationship of the currents recorded in low EGTA concentration was also similar to those recorded with perforated-patch recordings (Fig. 2B). When 5.0 mM EGTA was included in the intracellular solution, no significant changes in the currents were observed with adenosine

application (Fig. 4). Adenosine failed to induce large transient outward currents or increases in the overall current amplitude. Thus a current density of 37.27 ± 6.33 pA/pF elicited at +60 mV in the presence of adenosine was not significantly different from the control value of 40.62 ± 9.53 pA/pF ($n = 4$). However, the basal control currents recorded in 5.0 mM EGTA were similar to those recorded in 0.1 mM EGTA and from perforated-patch recordings (Fig. 2C).

3.4. Effects of ryanodine and thapsigargin

Ryanodine (1 μM) and thapsigargin (10 μM) had no significant effect on the averaged control currents from perforated-patch recordings. However, transient currents of larger amplitude were less conspicuous in the presence of ryanodine (Figs. 5A and 6A). Similarly, large transient currents were not prominent in the presence of thapsigargin (Fig. 5B). Adenosine was ineffective in inducing current increases in the presence of ryanodine or thapsigargin (Figs. 5 and 6).

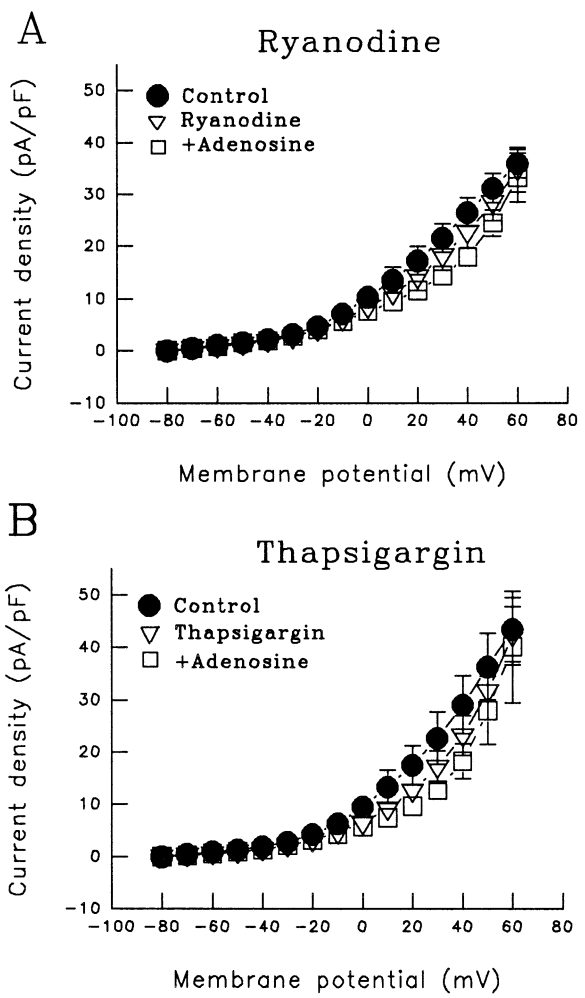

Fig. 6. Current–voltage relationships showing the effect of ryanodine and thapsigargin on currents elicited with test potentials from −80 to +60 mV from a holding potential of −80 mV. (A) Ryanodine and the subsequent addition of adenosine (10 μM) did not alter the currents significantly. (B) Thapsigargin and the subsequent addition of adenosine also failed to alter the currents significantly. $n = 4–5$.

4. Discussion

The spontaneous and agonist-induced transient outward currents associated with opening of K_{Ca} channels have been well documented in vascular smooth muscle cells (Benham and Bolton, 1986; Desilets et al., 1989). The transient outward currents have been associated with Ca^{2+} release from the sarcoplasmic reticulum and could be inhibited by ryanodine and thapsigargin (Ganitkevich and Isenberg, 1990; Kang et al., 1995; Nelson et al., 1995). Recent studies using digital fluorescence imaging techniques showed that the transient K_{Ca} currents are activated by Ca^{2+} sparks, which are local increases in Ca^{2+} from peripheral sarcoplasmic reticulum close to the surface membrane (Nelson et al., 1995; Knot et al., 1998). By activating nearby K_{Ca} channels, Ca^{2+} sparks could induce vasodilation indirectly through membrane hyperpolarization. Therefore, induction of Ca^{2+} release from the sarcoplasmic reticulum to activate K_{Ca} may provide a mechanism for some vasoactive substances to effect vasodilation.

It has previously been noted that adenosine could increase the amplitude and frequency of transient K_{Ca} currents in cerebral arterial cells (Porter et al., 1998). In the present study, we examined if Ca^{2+} release from the sarcoplasmic reticulum is involved in the potentiation of K_{Ca} currents by adenosine in rat mesenteric arterial cells. Perforated-patch recordings were used in some experiments in order not to disturb the cellular environment. Under these conditions, outward currents at more depolarized potentials were superimposed with transient outward currents. Adenosine caused increases in the transient and total outward currents. These currents were inhibited by the specific K_{Ca} blocker iberiotoxin. The changes induced by adenosine were most likely mediated by Ca^{2+} release from the sarcoplasmic reticulum since both ryanodine and thapsigargin were effective in abolishing the current increases.

Increasing the EGTA buffering of cellular Ca^{2+} is known to abolish spontaneous and agonist-induced transient outward currents in other vascular smooth muscle cells (Benham and Bolton, 1986; Desilets et al., 1989). We also tested the effect of intracellular Ca^{2+} buffering with different concentrations of EGTA in whole-cell recordings. At a low EGTA concentration of 0.1 mM, current activities in control and in response to adenosine were similar to those obtained using perforated-patch recording. When buffered to a higher EGTA concentration of 5.0 mM, the basal control currents were not significantly affected. This was not unexpected since the free Ca^{2+} concentration

under the two EGTA conditions was adjusted similarly to 155 nM. However, no further increases in the currents were observed with the application of adenosine. This is consistent with the notion that the adenosine responses were mediated by Ca^{2+} sparks and that local transient increases in Ca^{2+} induced by adenosine could be better buffered by a higher EGTA concentration.

The transient outward currents were modified indirectly by external Ca^{2+}. The removal of external Ca^{2+} resulted in the eventual abolition of the transient currents. However, the effect of Ca^{2+} removal was not immediate and transient currents could still be induced up to a few minutes after exposure to Ca^{2+}-free solutions. A similar effect of Ca^{2+}-free solutions on spontaneous and agonist-induced transient outward currents had been observed in other vascular smooth muscle cells (Benham and Bolton, 1986; Desilets et al., 1989; Kang et al., 1995).

There are suggestions that Ca^{2+} sparks and transient K_{Ca} currents may be modulated by cyclic nucleotides (Porter et al., 1998). Adenosine is known to increase cGMP levels without changing the cAMP levels in some arteries (Kurtz, 1987; Moritoki et al., 1990). We have not examined if the adenosine effect in rat mesenteric arterial cells was mediated by cyclic nucleotides. However, a previous study on the rat mesenteric artery showed that adenosine caused relaxation without concomitant changes in cAMP or cGMP levels (Vuorinen et al., 1992). Therefore, it is not likely that cyclic nucleotides were involved in the adenosine responses observed in the present study. Nevertheless, our study is consistent with the notion that increases in transient and outward currents induced by adenosine are mediated by Ca^{2+} release from the sarcoplasmic reticulum.

Acknowledgements

This work was supported by a grant from the Heart and Stroke Foundation of Ontario.

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