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European Journal of Pharmacology 458 (2003) 57–59
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Short communication

Modulation of spontaneous transient Ca^{2+}-activated K^+ channel currents by cADP-ribose in vascular smooth muscle cells

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Received 12 September 2002; received in revised form 14 November 2002; accepted 19 November 2002

Abstract

Transient local releases of Ca^{2+} from the sarcoplasmic reticulum activate nearby Ca^{2+}-activated K^+ channels to produce spontaneous transient outward current (STOC) in smooth muscle cells. We examined if cADP-ribose, an endogenous mediator of Ca^{2+} release channels of the sarcoplasmic reticulum, could modify STOC activity. In freshly isolated rat tail arterial cells, cADP-ribose (5 μM) increased STOC frequency significantly from 308 ± 26.2 to 398.8 ± 28.8 per minute. The average current at a test potential of −20 mV was increased significantly from 47.8 ± 0.7 to 101.1 ± 0.7 pA in the presence of cADP-ribose. The cell permeant antagonist 8-bromo-cADP-ribose (50 μM) reduced significantly the STOC frequency to 52.5 ± 7.5 per minute and the average current to 24.7 ± 0.1 pA. The STOCs were inhibited significantly by ryanodine (1 μM) and charybodotoxin (150 nM). These findings suggest the presence of basal cADP-ribose activity in resting vascular smooth muscle cells and that STOC activity is stimulated by cADP-ribose.

Keywords: cADP-ribose; STOC (spontaneous transient outward current); Smooth muscle, vascular

1. Introduction

Spontaneous transient outward currents (STOCs) are Ca^{2+}-activated K^+ channel currents activated by local Ca^{2+} transients arising from opening of ryanodine-sensitive Ca^{2+} release channels in the sarcoplasmic reticulum (Benham and Bolton, 1986; Nelson et al., 1995). STOCs may contribute to regulation of vascular tone by hyperpolarization of vascular smooth muscle cells. It is noted that agents that deplete Ca^{2+} from the sarcosplamic reticulum also inhibit STOCs (Benham and Bolton, 1986; Nelson et al., 1995). cADP-ribose, a metabolite of nicotinamide-adenine dinucleotide, is an endogenous regulator of Ca^{2+}-induced Ca^{2+} release. It induces Ca^{2+} release from ryanodine-sensitive intracellular stores independent of inositol 1,4,5-trisphosphate in a variety of tissues (Lee, 1997). An enzymatic pathway for the production of cADP-ribose is present in vascular tissues (Li et al., 2000). cADP-ribose has been shown to activate directly reconstituted ryanodine receptors from arterial smooth muscle (Li et al., 2001). In permeabilized smooth muscle cells, cADP-ribose stimulates Ca^{2+} release from the sarcoplasmic reticulum (Kuemmerle and Makhlouf, 1995; Kannan et al., 1996). Since ryanodine receptors are functionally coupled to Ca^{2+}-activated K^+ channels in vascular smooth muscle (Perez et al., 1999), we examined if cADP-ribose could modulate STOC activity in freshly isolated cells from the rat tail artery.

2. Materials and methods

Fresh single cells from tail arteries of 12–14-week-old male rats (Charles Rivers) were isolated using a method developed in our laboratory (Bolzon and Cheung, 1989). The arteries were incubated in a Ca^{2+}-free medium containing 0.02% collagenase, 0.1% papain, and 4 mM dithiothreitol for 90–120 min at 37 °C. Single cells obtained by this method are relaxed and contract in response to stimulation by a wide variety of agonists. Patch electrodes of resistance 2–6 MΩ were used for recording of whole cell currents. Nystatin was included in the pipette for perforated-patch recording and stable currents could be recorded usually in about 10 min. Liquid junction potential, series resistance, and capacitive currents were electronically com-

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PII: S0014-2999(02)02816-9

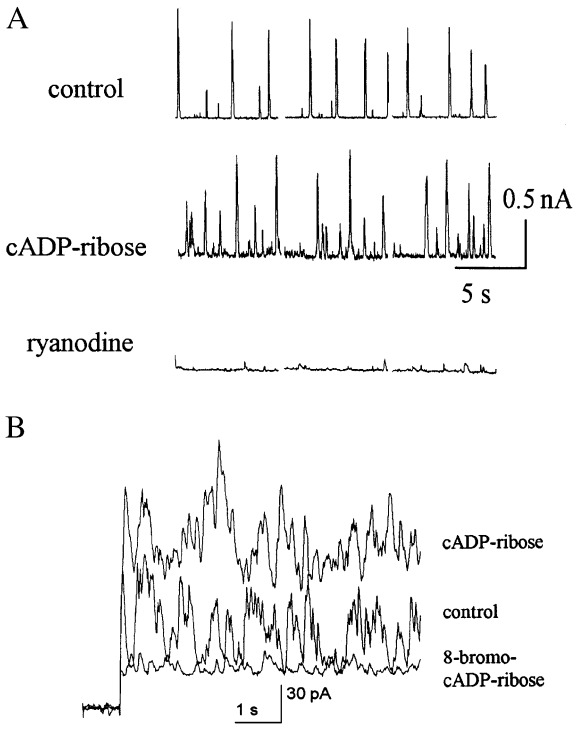

Fig. 1. (A) Typical traces showing STOC activity in control conditions and the increase in activity with cADP-ribose (5 μM). Ryanodine (1 μM) was effective in inhibiting STOC activities even in the presence of cADP-ribose. Sets of three consecutive traces recorded at a test potential of − 20 mV. (B) Signal-averaged traces showing changes in current amplitude induced by cADP-ribose (5 μM) and 8-bromo-cADP-ribose (50 μM). Control, $n = 15$ cells; cADP-ribose, $n = 17$; 8-bromo-cADP-ribose, $n = 4$.

pensated with an Axopatch 1C amplifier. From a holding potential of − 60 mV, test pulses to − 20 mV of 5 s durations were applied. All STOCs exceeding 25 pA in amplitude were counted in the study. pClamp 5.5 and 6.0 software (Axon Instruments) was used for data acquisition and analysis such as signal averaging and digitalizing of the currents. Five consecutive traces per cell were used for signal averaging of the currents. The ionic composition of extracellular solution was (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.0, $MgCl_2$ 1.0, KH_2PO_4 0.4, NaH_2PO_4 0.4, glucose 5.6, HEPES 4.2, and pH adjusted to 7.4. The pipette solution contained (in mM): KCl 130, $MgCl_2$ 1.8, Na_2 ATP 1.0, $CaCl_2$ 0.05, EGTA 0.1 and pH adjusted to 7.3. The calculated free Ca^{2+} concentration is 70 nM from the Stanford WEBMAXC programme. The experiments were carried out at room temperature. Ryanodine and charybdotoxin were obtained from Alomone Laboratories. All other chemicals were obtained from Sigma. cADP-ribose was included in the pipettes in whole-cell recordings and stable activity was recorded between 2 and 6 min. Ryanodine and 8-bromo-cADP-ribose were added to the external solution. Results are expressed as mean ± S.E.M. Statistical significance was evaluated using Student's *t*-tests for paired observations when comparing the before and after effects of drug treatments. Student's *t*-test for unpaired observations was used when comparing cADP-ribose with controls. A value of $P < 0.05$ was considered significantly different.

3. Results

The STOC frequency was 308.0 ± 26.2 per minute ($n = 15$) in control cells. Most of the STOCs (72.0 ± 2.0%) were of amplitudes less than 200 pA. In the presence of cADP-ribose (5 μM), the STOC frequency increased significantly to 398.8 ± 28.8 per minute ($n = 17$). The STOCs, even in the presence of cADP-ribose, were inhibited significantly by ryanodine (1 μM; $n = 3$) (Fig. 1A) and by charybdotoxin (150 nM; $n = 2$, data not shown). Signal averaging of the traces showed an overall increase in current amplitude in the presence of cADP-ribose (Fig. 1B). At a test potential of − 20 mV, the average current was 47.8 ± 0.7 pA for control cells. The average current increased significantly to 101.1 ± 0.7 pA with cADP-ribose. The increase in the averaged current was due to increase in the STOC frequency mainly at amplitudes below 200 pA and not due to increase in amplitude of individual STOCs (Fig. 2A). STOC activity was significantly suppressed by 8-bromo-cADP-ribose (50 μM), a cell permeant antagonist of cADP-ribose (Fig. 2B). 8-Bromo-cADP-ribose reduced the STOC frequency significantly to 52.5 ± 7.5 per minute and the average current to 24.7 ± 0.1 pA (Figs. 1 and 2). In the presence of 8-bromo-cADP-ribose,

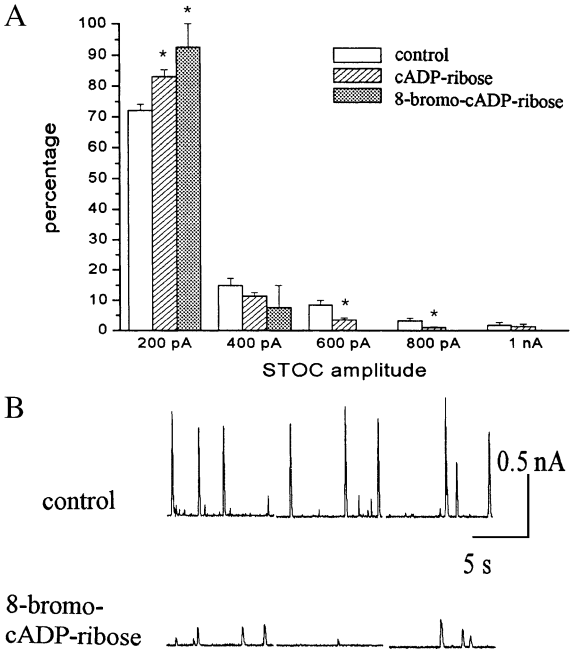

Fig. 2. (A) Percent distribution of STOCs in relation to amplitude in control ($n = 15$), cADP-ribose (5 μM, $n = 17$), and 8-bromo-cADP-ribose (50 μM, $n = 4$). *$P < 0.05$. (B) Typical traces showing the effect of 8-bromo-cADP-ribose (50 μM) on STOC activity. Sets of three consecutive traces.

92.5 ± 7.5% of the STOCs were of small amplitude and large amplitude STOCs were not observed (Fig. 2A).

4. Discussion

cADP-ribose mobilizes intracellular Ca^{2+} in a wide variety of cells by sensitizing ryanodine-sensitive Ca^{2+} release channels of the sarcoplasmic reticulum to Ca^{2+} (Lee, 1997). It activates directly reconstituted ryanodine receptors from vascular smooth msucle cells (Li et al., 2001). Since STOC is triggered by Ca^{2+} release from ryanodine-sensitive Ca^{2+} channels in the sarcoplasmic reticulum, we tested if cADP-ribose could modify STOC activity in vascular smooth muscle cells. We observed a significant increase in STOC frequency induced by cADP-ribose. The effectiveness of ryanodine in inhibiting the responses suggests that changes in STOC activity induced by cADP-ribose is mediated by Ca^{2+} release through ryanodine-sensitive channels. STOC activity could be modulated by cADP-ribose either by changes in frequency or in amplitude. The present study showed that cADP-ribose modulates STOC frequency and not amplitude. We also tested if there is a tonic cADP-ribose influence on basal STOC activity with the cell permeant antagonist 8-bromo-cADP-ribose. Endogenous levels of cADP-ribose apparently sufficient to promote release of Ca^{2+} in smooth muscle cells had been measured in rat tissues (Walseth et al., 1991). The observation that 8-bromo-cADP-ribose inhibits basal STOC activity significantly suggests the presence of tonic cADP-ribose activity. In conclusion, our findings suggest that cADP-ribose stimulates STOC activity and it could contribute to the maintenance of basal STOC activity.

Acknowledgements

This study was supported by the Heart and Stroke Foundation of Ontario. The experiments were carried out with the technical assistance of Ms. L. Xu.

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