Biochemical Pharmacology, Vol. 41, No. 2, pp. 237–241, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

CARBON MONOXIDE INHIBITS DEPOLARIZATION-INDUCED Ca RISE AND INCREASES CYCLIC GMP IN VISCERAL SMOOTH MUSCLE CELLS

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(*Received* 1 *February* 1990; *accepted* 15 *September* 1990)

Abstract—Monocytes were isolated from the urinary bladder of the guinea-pig. By means of the voltage clamp technique, whole cells were depolarized from −65 to +10 mV in order to increase the intracellular calcium concentration $[Ca^{2+}]_i$ and to monitor this increase by means of the calcium activated potassium current $I_{K,Ca}$. Superfusion of the cells with carbon monoxide-containing solutions for 2 min inhibited the signal to about 50% of the control suggesting depression of the depolarization-induced increase in $[Ca^{2+}]_i$. The CO-mediated inhibition of $I_{K,Ca}$ was partially reversed by wash-off of CO; flashes of high light intensity accelerated the rate of recovery. Sodium nitroprusside (0.01–1 mM) depressed the depolarization-induced increase in $[Ca^{2+}]_i$ similar to CO. In multicellular preparations of the urinary bladder, CO-containing media were shown to increase the cGMP concentration by a factor of 2 in the absence and by a factor of 3 in the presence of 1 mM of the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX). According to our previous work, CO binds to and activates soluble guanylate cyclase [Brüne B and Ullrich V, *Mol Pharmacol* **32**: 497–504, 1987; Utz J and Ullrich V, *Naunyn Schmiedebergs Arch Pharmacol* **337** (Suppl): 299, 1988] and the rise in cGMP could thus effect $[Ca^{2+}]_i$ by still unknown mechanisms.

In smooth muscle cells, excitation initiates contraction via an increase in the concentration of cytosolic calcium ions $[Ca^{2+}]_i$ to which both Ca influx through sarcolemmal Ca channels and Ca-release from the sarcoplasmic reticulum (SR) contribute [1]. Relaxation of the muscle is promoted by Ca-antagonists due to their block of Ca influx, which, secondarily, is followed by depletion of the SR of Ca. Relaxation due to organic nitrate containing vasodilators is less understood. Morgan and Morgan [2] studied the effects of sodium nitroprusside (SNP) on the depolarization-induced increase in the ferret portal vein by means of the aequorin technique. Depolarization was induced by increasing the bath KCl to 30 mM. By this intervention the force and $[Ca^{2+}]_i$ had increased and subsequent addition of 10 μM SNP reduced these parameters within 2 min. Pretreatment of the muscle with 10 μM SNP largely prevented the capability of depolarization to increase $[Ca^{2+}]_i$ and force. Present knowledge links the SNP-mediated relaxation to intracellular delivery of NO, stimulation of the guanylate cyclase activity and elevation of intracellular cGMP concentrations (cited in Ref. 3). How this cGMP increase is linked to reduction of depolarization-induced $[Ca^{2+}]_i$ and force is not known up to now.

Recently, activation of the soluble guanylate cyclase and increase in cGMP have been described for platelets after treatment with carbon monoxide. These conditions strongly desensitized platelets against aggregatory stimuli. The CO effect was thought to result from a CO–heme interaction since it was reversed by light above a wavelength of 400 nm [4]. In smooth muscular tissue, CO has a relaxant effect which was explained through an inhibition of the respiratory chain with a concomitant fall in ATP levels [5] or the inhibition of a cytochrome P450 dependent vasocontracting arachidonate metabolite [6]. Since aggregation of platelets and activation of smooth muscle contraction resemble in many respects, we have put up the alternative hypothesis that relaxation of smooth muscle may be mediated by an increase in cGMP. Preliminary experiments with ileal smooth muscle showed indeed a rise of cGMP after CO exposure [7] but no data on the Ca levels could be obtained. In this study, we show the influence of CO on depolarization-induced $[Ca^{2+}]_i$-transients and cGMP levels in preparations from the urinary bladder of the guinea-pig.

The depolarization-induced increase in $[Ca^{2+}]_i$ was studied with the voltage clamp technique which was used for (i) imposing the depolarizations and (ii) measuring the current flowing through $[Ca^{2+}]_i$ activated potassium channels ($I_{K,Ca}$) in order to monitor the change in $[Ca^{2+}]_i$ [8, 9]. The $I_{K,Ca}$-signal could not be calibrated in terms of $[Ca^{2+}]_i$ thereby remaining a "qualitative" probe that can show whether a $[Ca^{2+}]_i$-transient is suppressed or not. However, $I_{K,Ca}$ is easily recorded during the voltage-clamp depolarization whereas simultaneous microfluospectrometry (Fig. 1, [10]), demands special equipment and has a low success rate.

MATERIALS AND METHODS

Myocytes were isolated from the urinary bladder

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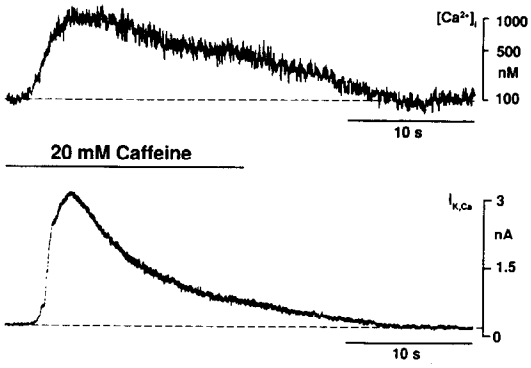

Fig. 1. $I_{K.Ca}$ increases and decreases along a similar time course as $[Ca^{2+}]_i$ does. $[Ca^{2+}]_i$ was measured by microfluospectrometry (indo-1 fluorescence) from the same isolated myocyte from which the Ca-activated K-current $I_{K.Ca}$ was recorded. The cell was loaded with 100 μM of the sodium salt of indo-1 through the patch pipette for 4 min. The myocyte was illuminated at 360 nm, and the emitted light was collected simultaneously at 405 and 480 nm for on-line data processing of $[Ca^{2+}]_{cyt}$ (ratio technique). At a constant holding potential of +10 mV, caffeine-induced SR–Ca-release induces a roughly parallel change of $[Ca^{2+}]_i$ and $I_{K.Ca}$.

of the guinea-pig by means of collagenase and pronase [11]. Briefly, chunks of muscular tissue (diameter about 2 mm) were stirred for three periods of 20 min in the following "enzyme medium": 130 mM KCl, 5 mM pyruvate, 1.2 mM KH_2PO_4, 5 mM creatine, 1 mM $MgCl_2$, 20 mM glucose, 20 mM taurine, 5 mM Hepes, adjusted with methane sulfonic acid to pH 7.1, and complemented with 1 mg/mL collagenase (Sigma, CO130) and 0.25 mg/mL pronase (Type E, Serva). The supernatant with the isolated cells was harvested. The cells were suspended in a physiological salt solution (PSS) composed of 150 mM NaCl, 5.4 mM KCl, 3.6 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, 5 mM Hepes, adjusted with NaOH to pH 7.4. For the experiment, the cells were pipetted into the experimental chamber (volume 100 μL). When the cells had settled down to the glass bottom, they were continuously superfused with the PSS which was prewarmed to give a temperature of 36°. In the chamber, the solution could be completely changed within 20 sec. For the electrical measurements we used single patch electrodes in the "whole cell clamp configuration" [12]. Electrodes with fire polished tips (inner diameter about 1 μm) were filled with a solution composed of 130 mM KCl, 5 mM oxalacetic acid, 5 mM pyruvate, 5 mM succinate, 10 mM Hepes, pCa adjusted with 0.02 mM EGTA to 7, pH adjusted with KOH to pH 7.4. The electrodes had resistances of about 3 MOhm and were connected via Ag/AgCl wires to a current injecting input amplifier. The voltage-clamp circuit settled the potential within less than 1 msec and with voltage inhomogeneities of less than 3 mV [11]. A PDP 11–23+ minicomputer generated the pulse protocol, digitized the recorded membrane pulse (1024 points of 12 bit resolution) and stored them. The figures are playbacks from the computer to an interfaced line printer.

Carbon monoxide was applied by superfusing the preparations with CO-containing PSS which was prepared by bubbling PSS in a container of about 120 mL volume with a gas mixture of 80 vol. % CO and 20 vol. % O_2 (supply rate about 120 mL/min). The PSS flowed through the chamber with a rate of 2 mL/min. To prevent any loss of CO by diffusion, gas-tight "tygon" tubings were used for both gas supply and the flow of PSS to the experimental chamber.

To measure the levels of cGMP, pieces of the urinary bladder (about 2 × 2 mm) were incubated in Erlenmeyer flasks in a shaking waterbath at 37°. The Tyrode–Hepes buffer used (148 mM NaCl, 2.68 mM KCl, 0.9 mM $CaCl_2$, 1.05 mM $MgCl_2$, 5.55 mM glucose, 5 mM HEPES, adjusted with NaOH to pH 7.4) was pregassed for 10 min with the required gas mixture after degassing under vacuum. The flasks were equipped with pierced rubber caps which allowed to maintain the gas supply through capillary tubes during the incubation. After 10 min, the pieces were quickly removed and frozen by immersion in an acetone–dry ice mixture (2–3 sec). Storage until cGMP analysis occurred at −80° and never exceeded 1 week. To extract cGMP the frozen tissue was ground in a mortar cooled with liquid nitrogen. The resulting powder was transferred to 12% TCA and after 10 min on ice the protein was precipitated by centrifugation (10 min at 10,000 g and 4°). After extraction with diethylether (4 x) and an acetylation step to increase the sensitivity of the assay, the supernatant was used for cGMP determinations by radioimmunoassay as described [13]. Counting and calculation of the calibration curve was performed by a Philips Gamma Counter PW 4800 with integrated Single Board Computer PW 4801. The pellet was redissolved in 1 N NaOH and the protein concentration was determined as described [14].

RESULTS

$I_{K.Ca}$ *as an indicator of depolarization-induced* $[Ca^{2+}]_i$*-transients*

The sarcolemma of urinary bladder cells contains several thousands of "maxi-K channels" (conductance about 180 pS, [15]) that are regulated by the intracellular Ca concentration $[Ca^{2+}]_i$. Figure 1 shows an experiment where $[Ca^{2+}]_i$ has been elevated by a short bath application of 20 mM caffeine, the increase being quantified by means of indo-1 microfluospectrometry [10]. The membrane potential was held constant (+10 mV) by the voltage clamp, and the increase in $[Ca^{2+}]_i$ induced a macroscopic current $I_{K.Ca}$ with a time course roughly in parallel to $[Ca^{2+}]_i$, i.e. both signals show a time-dependent upward deflection followed by a slower decay.

The interrelation between membrane potential $[Ca^{2+}]_i$ and $I_{K.Ca}$ is highly non-linear [15]. Therefore, $I_{K.Ca}$ was used only as a qualitative measure of $[Ca^{2+}]_i$ and only at a comparable potential of +10 or +20 mV. When the potential was set by the voltage-clamp from −65 to +10 mV, $I_{K.Ca}$ changed with a complex time course. In cells pretreated with 20 mM caffeine, depolarization-induced $I_{K.Ca}$ was largely suppressed, therefore we tentatively attributed part of it to Ca release from the SR. As expected from a signal of Ca release, optimal

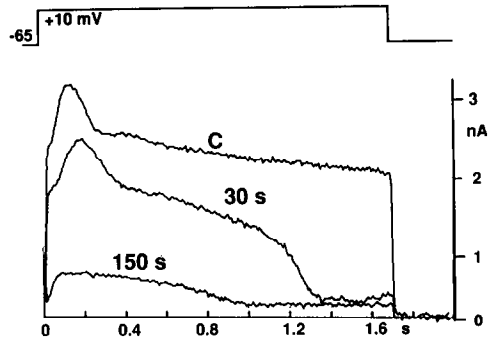

Fig. 2. The calcium activated changes in potassium current $I_{K,Ca}$ as a probe for depolarization-induced $[Ca^{2+}]_i$ as they are reduced by 50 μM sodium nitroprusside (SNP). The record labelled by C was taken before, the others 30 and 150 sec after exposure to SNP. Top: 1.7 sec long clamp-pulses depolarized the membrane from −65 to +10 mV at a rate of 2 per min. Bottom: membrane currents in response to depolarization. The tracings start with a holding current that is almost zero and insensitive to SNP. During depolarization, $I_{K,Ca}$ comes within 100 msec to a peak from which it slowly decays to the late current (measured at the end of depolarization). In the beginning of SNP-exposure, the late current is reduced (30 sec). Later on, the peak of $I_{K,Ca}$ and disappears (150 sec).

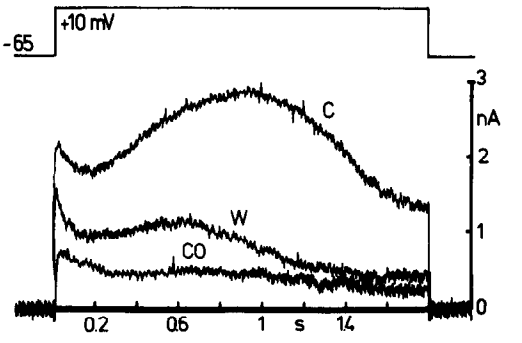

Fig. 3. $I_{K,Ca}$ indicates CO-induced reduction of the depolarization-induced $[Ca^{2+}]_i$-transient. Top: 1.8 sec long clamp steps depolarized the cell from −65 to +10 mV at a rate of 2 per min. Bottom: membrane currents as they were measured before (label C), 150 sec after superfusion of CO-containing PSS (label CO) and 60 sec after wash-off of CO (label W).

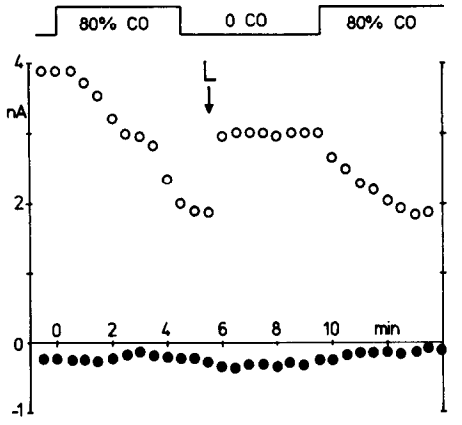

Fig. 4. Time course of the CO-effect on $I_{K,Ca}$. Pulses (1.8 sec long) from −65 to +10 mV were applied at a frequency of 2/min. A first exposure to CO reduces $I_{K,Ca}$. Within 1 min, the effect is not reversed upon washing with CO-free solution (W). Bright illumination (L) leads to rapid recovery of $I_{K,Ca}$ to 50% of the control. Open circles represent the maximal $I_{K,Ca}$ at +10 mV. The dots mark the current which is required to hold the membrane at −65 mV in between the pulses, its constant value suggests that the cell is not unspecifically damaged.

amplitude of $I_{K,Ca}$ was obtained only when the pulses were applied at the low rate of 2 per min. Amplitude and the time course of $I_{K,Ca}$ largely varied between the individual cells.

Sodium nitroprusside (SNP) reduces the depolarization-induced $[Ca^{2+}]_i$*-transients*

By means of aequorin luminescence, SNP has been shown to reduce the $[Ca^{2+}]_i$-transients as they are induced by KCl depolarization of vascular smooth muscle. In order to verify that the $I_{K,Ca}$-signals are a suitable $[Ca^{2+}]_i$-probe, we have aimed to demonstrate similar effects of SNP on the $[Ca^{2+}]_i$-transients as they are induced by voltage-clamp depolarizations. Figure 2 compares $I_{K,Ca}$-records that were taken before and after application of 50 μM SNP to the cell. The tracing obtained after 30 sec shows a continuous decrease of $I_{K,Ca}$, and 150 sec after drug application, the outward current is suppressed to about 18% of the control value. Similar reduction of $I_{K,Ca}$ by SNP was recorded from six other cells, two cells did not respond to SNP. The results are in line with the more direct $[Ca^{2+}]_i$ measurements by means of aequorin and suggest that SNP can suppress the depolarization-induced increase in $[Ca^{2+}]_i$.

CO *reduces the depolarization-induced* $[Ca^{2+}]_i$*-transients*

CO-mediated reduction of $I_{K,Ca}$ is demonstrated in Figs 3 and 4. In the experiment of Fig. 3, superfusion of CO-containing PSS for 150 sec suppressed the late current to 20% and the hump to 14% of the control. The suppression of $I_{K,Ca}$ by CO is qualitatively similar to the one described for SNP. The CO-induced suppression of $I_{K,Ca}$ is partially reversible; a distinct recovery of the delayed hump (suggesting some refilling of the SR–Ca-stores) is seen 1 min after changing to a CO-free superfusate. Similar results were obtained with four other cells, two cells did not respond to CO.

Superfusion of CO-containing PSS suppresses the current component $I_{K,Ca}$ without modifying the current which is required to hold the membrane between the depolarizing clamp steps at −65 mV. The constant appearance of the holding current excludes the possibility that the observed CO effect may have resulted from an unspecific damage or leakage of the cell.

The increase in cGMP by CO has been thought to result from a stimulatory interaction of CO with the heme in the regulatory subunit of the guanylate

Table 1. Cyclic GMP levels in urinary bladder

Gassing (% vol.)	Addition	cGMP (pmol/mg protein)
100 O_2	—	0.19 ± 0.09 (12)
80 N_2, 20 O_2	—	0.19 ± 0.05 (6)
80 CO, 20 O_2	—	0.32 ± 0.09 (14)*
100 O_2	1 mM IBMX	1.1 ± 0.7 (7)
80 CO, 20 O_2	1 mM IBMX	3.5 ± 1.6 (7)*
100 O_2	50 μM SNP	0.93 ± 0.23 (6)
80 CO, 20 O_2	50 μM SNP	5.8 ± 2.5 (6)*

Pieces of urinary bladder were incubated for 10 min at 37° under the conditions indicated. Levels of cGMP were anlaysed by radioimmunoassay.

The data shown represent mean ± SE. Statistical analysis has been performed using the Student's *t*-test. The numbers in parenthesis indicate the numbers of experiments performed.

* $P < 0.005$ compared with the corresponding control under 100% O_2.

cyclase. The CO–Fe^{2+} complex shows an absorption maximum at 422 nm [16] and is photodissociable. Therefore, we tried to accelerate the rate of reversibility during washout by illumination of the cell (white light from a slide projector). Figure 4 shows the results of an experiment which starts with a 3 min exposure to CO-containing PSS resulting in depression of the delayed hump of $I_{K,Ca}$ to about 55%. Superfusion with CO-free PSS did not restore the $[Ca^{2+}]_i$-transient within the first minute. Then, illumination was switched on (arrow L in Fig. 4), and $I_{K,Ca}$ recovered immediately (30-sec time resolution given by the interval between the pulses). Light induced recovery of $I_{K,Ca}$ reached about 75% of the control and it remained at this level. A second exposure to CO reduced the $I_{K,Ca}$ again.

CO and SNP increase the level of cyclic GMP

The effects of CO and SNP on cGMP levels of multicellular preparations are shown in Table 1. Incubation with 80% CO approximately doubles cGMP in the absence, and about trebles cGMP in the presence of 1 mM IBMX. In a control experiment using 80% N_2 it was demonstrated, that a reduction in oxygen partial pressure alone has no effect on the basal level of cGMP. Treatment with 50 μM SNP also increased the level of cGMP. The increased formation of cGMP in SNP stimulated tissue under 80% CO compared to 100% O_2 is presumably due to the decreased oxygen concentration. This leads to higher concentrations of NO, since NO readily reacts with oxygen to form nitrite and nitrate, which are only weak activators of guanylate cyclase.

DISCUSSION

The results of this paper have shown that SNP decreases the depolarization-induced $[Ca^{2+}]_i$-transients, as they are revealed by $I_{K,Ca}$ from an isolated visceral smooth muscle cell. Despite the different techniques for depolarization and $[Ca^{2+}]_i$ measurement, our results compare favorably with those of Morgan and Morgan [2] in which vascular muscle (portal vein) was depolarized with KCl and where the depolarization-induced $[Ca^{2+}]_i$-transient was monitored by means of the Ca-specific luminescence of aequorin.

The molecular events by which Ca ions activate the maxi K-channels have been extensively studied, both in myoballs [17] and in vascular myocytes [15, 18]. During activation, the channel binds two Ca ions, and the depolarization of the membrane promotes the binding step. The Ca-induced activation does not require ATP. Whereas a cyclic AMP mediated activation of the Ca-activated K-channel has been reported [19], a modulation of the channel function by cGMP-dependent phosphorylation is unknown. A later study in vascular smooth muscle cells [20] suggested that cGMP can increase the Ca sensitivity of the maxi-K channel, most likely through changes in cytosolic GMP concentration. If this effect were present in the myocytes from urinary bladder, we would have underestimated the "true" reduction of the depolarization-induced Ca-transients. It seems to be justified therefore, to attribute the SNP- or CO-induced reduction of $I_{K,Ca}$ to a reduction of $[Ca^{2+}]_i$.

The biochemical analysis of the cGMP levels in urinary bladder corroborate our findings in platelets where we established a significant increase by CO and SNP [4]. How this increase is linked to lowered Ca levels in smooth muscle is an open question. In platelets, the onset of the agonist-induced phosphatidyl-inositol cycle seemed to be delayed, which could have been the consequence of a cGMP-dependent phosphorylation of a G-protein or a still unknown gating event. In smooth muscle the physiological target of cGMP is even less obvious. A CaM-regulated sarcolemmal Ca-ATPase activation has been suggested [21, 22] but also doubted [23]. Other possibilities include an inhibition of SR-located Ca release or activation of the SR–Ca pump activity.

Since myocytes from the urinary bladder have a well-developed SR [24] the release of Ca through the SR is probably the main factor in the depolarization-induced elevation of $[Ca^{2+}]_i$. Suppression of $I_{K,Ca}$ by both SNP and CO fits with the hypothesis that Ca release from the SR has been suppressed. One could speculate that SNP could facilitate Ca extrusion into the extracellular space via cGMP-dependent stimulation of the CaM-dependent Ca-ATPase. Then the cell loses a relatively larger part of Ca that is usually taken up

by the SR. Later on, when this Ca is no longer available for SR–Ca-release, the $[Ca^{2+}]_i$-increase due to SR–Ca-release disappears.

Alternatively the changes in $I_{K,Ca}$ can as well be explained by a suppression of SR–Ca-release.

From the clearer separation of the effects on Ca-ATPases or Ca-release channels and by the possibility of photodissociating the CO-activated G-cyclase, future work may contribute to an answer to this pharmacologically highly relevant problem of cGMP-mediated smooth muscle relaxation.

REFERENCES

1. Rueegg JC, *Calcium in Muscle Activation*. Springer, Berlin, 1988.
2. Morgan JP and Morgan KG, Alterations of cytoplasmic ionized calcium levels in smooth muscle by vasodilators in the ferret. *J Physiol* **357**: 539–551, 1984.
3. Ignarro LJ and Kadowitz PJ, The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annu Rev Pharmacol Toxicol* **25**: 171–191, 1985.
4. Brüne B and Ullrich V, Inhibition of platelet aggregation by carbon monoxide is mediated by activation of guanylate cyclase. *Mol Pharmacol* **32**: 497–504, 1987.
5. Fay FS, Guinea pig ductus arteriosus. I. Cellular and metabolic basis for oxygen sensitivity. *Am J Physiol* **221**: 470–479, 1971.
6. Coceani F, Hamilton NC, Labuc J and Olley PM, Cytochrome P450-linked monooxygenase: involvement in the lamb ductus arteriosus. *Am J Physiol* **246**: H640–643, 1984.
7. Utz J and Ullrich V, Relaxation of ileal smooth muscle by carbon monoxide is mediated by activation of guanylate cyclase. *Naunyn Schmiedebergs Arch Pharmacol* **337** (Suppl): 299, 1988.
8. Klöckner U and Isenberg G, Calcium activated potassium currents as an indicator for intracellular Ca-transients. (Single smooth muscle cells from trachea and urinary bladder). *Pflügers Arch* **405**: R61, 1985.
9. Bolton TB and Lim SP, Properties of calcium stores and transient outward currents in single smooth muscle cells of rabbit intestine. *J Physiol (Lond)* **409**: 385–401, 1989.
10. Benham CD, Voltage gated and agonist-mediated rises in intracellular Ca^{2+} in rat clonal pituitary cells (GH_3) held under voltage clamp. *J Physiol (Lond)* **415**: 143–158, 1989.
11. Klöckner U and Isenberg G, Action potentials and net membrane current of isolated smooth muscle cells (urinary bladder of the guinea pig). *Pflügers Arch* **405**: 329–339, 1985.
12. Hamill OP, Marty A, Neher E, Sakmann B and Sigworth FG, Improved patch clamp technique for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* **391**: 85–100, 1981.
13. Ortmann R, Effects of PGI_2 and stable endoperoxide analogues on cyclic nucleotide levels in clonal cell lines of CNS origin. *FEBS Lett* **90**: 348–352, 1978.
14. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchonic acid. *Anal Biochem* **150**: 76–85, 1985.
15. Singer JJ and Walsh JV, Large-conductance Ca-activated K channels in freshly dissociated smooth muscle cells. *Membrane Biochem* **6**: 83–110, 1986.
16. Gerzer R, Böhme E, Hofmann F and Schultz G, Soluble guanylate cyclase purified from bovine lung contains heme and copper. *FEBS Lett* **132**: 71–74, 1981.
17. Blatz LA and Magleby KL, Ion conductance and selectivity of single Ca-activated potassium channels in cultured rat muscle. *J Gen Physiol* **84**: 1–23, 1984.
18. Benham CD, Bolton TB, Lang RJ and Takewaki T, Calcium-activated potassium channels in single smooth muscle cells of rabbit jejunum and guinea pig mesenteric artery. *J Physiol* **371**: 45–67, 1986.
19. Sodoshima JI, Akaike N, Kanaide H and Nakamura M, Cyclic AMP modulates Ca-activated K channel in cultured smooth muscle cells of rat aortas. *Am J Physiol* **255**: H754–759, 1988.
20. Williams DL, Katz GM, Roy-Contanicin L and Reuben JP, Guanosine 5′-monophosphate modulates gating of high-conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells. *Proc Natl Acad Sci USA* **85**: 9360–9364, 1988.
21. Suematsu E, Hirata M and Kuriyama H, Effects of cAMP- and cGMP-dependent protein kinases, and calmodulin on Ca-uptake by highly purified sarcolemmal vesicles of vascular smooth muscle. *Biochim Biophys Acta* **773**: 83–90, 1984.
22. Popescu LM, Foril CP, Hinescu M, Panoiu C, Cinteza M and Gherasim L, Nitroglycerin stimulates the sarcolemmal Ca-extrusion. ATPase of coronary smooth muscle cells. *Biochem Pharmacol* **34**: 1857–1860, 1985.
23. Vrolix MC, Raeymaekers L, Wuytack F, Hofmann F and Casteels R, Cyclic GMP-dependent protein kinase stimulates the plasmalemmal CA^{2+} pump of smooth muscle via phosphorylation of phosphatidylinositol. *Biochem J* **255**: 855–863, 1988.
24. Gabela G, Structural apparatus for force transmission in smooth muscles. *Physiol Rev* **64**: 455–477, 1984.