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Involvement of K^+ channels in the relaxant effects of YC-1 in vascular smooth muscle

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Abstract

This study addresses the question whether K^+ channels are involved in the vasorelaxant effects of 3-(5′-hydroxymethyl-2′-furyl)-1-benzyl-indazole (YC-1). In rat aorta, guinea pig aorta, and guinea pig a. carotis, YC-1 inhibited contractions induced by phenylephrine (3 μM) more potently than those induced by K^+ (48 mM). In rat aorta, tetraethylammonium (10 mM), charybdotoxin (0.2 μM), and iberiotoxin (0.1 μM), but not glibenclamide (10 μM), attenuated the relaxant effects of YC-1. In guinea pig a. carotis, YC-1 (30 μM) induced a hyperpolarisation which was antagonised by 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ; 50 μM). In rat aorta, YC-1 (30 μM) increased the rate constant of ^{86}Rb-efflux. The effect of YC-1 was potentiated by zaprinast (10 μM), but inhibited by ODQ (50 μM) or charybdotoxin (0.2 μM). In smooth muscle cells from rat aorta, YC-1 (10 μM) increased BK_{Ca} channel activity. It is suggested that YC-1-induced vasorelaxation is partially mediated by the activation of K^+ channels.

Keywords: Relaxation; Cyclic GMP; Guanylyl cyclase, soluble; BK_{Ca} channel; Smooth muscle, vascular

1. Introduction

The activation of soluble guanylyl cyclase (EC 4.6.1.2.) by nitric oxide (NO) is considered a major key mechanism in regulating vascular smooth muscle tone (Moncada et al., 1991). Under physiological conditions, NO is released from endothelial cells and diffuses to adjacent vascular smooth muscle cells where it causes vasodilation by activating soluble guanylyl cyclase and increasing intracellular cyclic GMP levels (Ignarro, 1990). The mechanisms by which increases in cyclic GMP levels induce vasorelaxation are complex. (1) Activation of protein kinase G regulating various phosphoproteins, (2) activation or (3) inhibition of phosphodiesterases, (4) a desensitisation of the contractile elements to Ca^{2+} have all been discussed as major mechanisms (McDaniel et al., 1994).

In vascular preparations, a membrane hyperpolarisation in response to NO or NO-related substances has been demonstrated (e.g., Tare et al., 1990; Yuan et al., 1996). NO-induced membrane hyperpolarisation is related to the opening of K^+ channels. Several types of K^+ channels, including voltage-dependent (K_v), ATP-sensitive (K_{ATP}), and large-conductance, Ca^{2+}-sensitive (BK_{Ca}) channels, have been identified in smooth muscle cells and proposed to be the target of NO signalling (Archer et al., 1994; Murphy and Brayden, 1995; Yuan et al., 1996). The effects of NO and related substances on K^+ channels seem to be mediated by an increase in intracellular cyclic GMP levels and/or result from a direct interaction of NO molecules with K^+ channel proteins. For example, NO has been shown to enhance the activity of BK_{Ca} channels both directly (Bolotina et al., 1994; Abderrahmane et al., 1998), and indirectly via cyclic GMP and a cyclic GMP-dependent protein kinase (Robertson et al., 1993; Archer et al., 1994; Carrier et al., 1997).

In this study, the effects of 3-(5′-hydroxymethyl-2′-furyl)-1-benzyl-indazole (YC-1) on tension, membrane po-

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tential, ^{86}Rb efflux, and BK_{Ca} channel activity were investigated in vascular smooth muscle. YC-1 activates soluble guanylyl cyclase directly, independent from NO (Wu et al., 1995; Friebe and Koesling, 1998) and relaxes smooth muscle due to a more selective effect on intracellular cyclic GMP levels than NO (Mülsch et al., 1997; Wegener et al., 1997). Preliminary accounts of this work have been presented (Wegener et al., 1999a,b).

2. Methods

2.1. Preparations

Sprague–Dawley rats and guinea pigs (both 200–300 g) of either sex were anaesthetised with ether and killed by cervical dislocation. The thoracic aorta and the carotid arteries were quickly removed and immersed in warmed and oxygenated Tyrode's solution (containing in mM: NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 12, NaH_2PO_4 0.42, glucose 5.6; bubbled with 95% O_2 + 5% CO_2; pH 7.4). After the connective tissue had been removed, the vascular preparations were cut into rings of variable width dependent on the experimental requirements.

Vascular smooth muscle cells were isolated by enzymatic treatment according to Minowa et al. (1997). Briefly, aortic rings were transferred to a nominally Ca^{2+}-free solution (containing in mM: NaCl 135, KCl 5.4, KH_2PO_4 1.2, $MgCl_2$ 1, glucose 30, HEPES 10; pH was adjusted to 7.4 with NaOH). After 5 min, collagenase (80 units/ml) and elastase (1.2 units/ml) were added to this solution. The rings were incubated at 37°C for 50–60 min and then gently passed through the mouth of a wide-bore glass pipette in a freshly prepared, nominally Ca^{2+}-free solution until a sufficient number of single cells were released. The isolated cells were stored at 8°C and used for electrophysiological experiments within 4 h.

2.2. Measurement of tension

Vascular rings were mounted vertically in organ baths (5 ml) containing oxygenated Tyrode's solution at 36 ± 1°C. One end was fixed to a hook of a muscle holder, while the other end was connected to an inductive force-displacement transducer whose output was fed to a carrier frequency preamplifier (Carrier amplifier/TA2000, Gould, Cleveland, OH, USA). Resting tension was set to 10 mN (aortic preparations) or 5 mN (carotid preparations). The rings were precontracted either by phenylephrine (3 μM) or by a high extracellular K^+ solution (containing in mM: NaCl 94, KCl 48, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 12, NaH_2PO_4 0.42, glucose 5.6; aerated with 95% O_2 + 5% CO_2; pH 7.4). Drugs were added from stock solutions to the organ bath as single or repeatedly applied doses to achieve the final concentrations as indicated.

2.3. Determination of ^{86}Rb-efflux

Rat aortic rings of 15–20 mm in width were transferred into 10 ml Tyrode's solution to which $^{86}Rb^+$ (250 μCi) had been added. After 3 h incubation at 37°C, the rings were transferred to organ baths containing 5 ml buffer solution. Then, the preparations were partially depolarised by using a modified Tyrode's solution containing 27 mM K^+ (containing in mM: NaCl 115, KCl 27, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 12, NaH_2PO_4 0.42, glucose 5.6; aerated with 95% O_2 + 5% CO_2; pH 7.4) in order to increase the driving force for Rb^+ to leave the cells. After an initial washing period of 45 min, the buffer solution was changed in 5-min intervals and counted for radioactivity using a γ-counter (Cobra, Canberra-Packard, Dreieich, Germany).

2.4. Recording of membrane potential

The carotid arteries from guinea pigs were cut into rings of about 4–7 mm in width. Both ends were ligated with a fine silk suture. The preparations were mounted in a 2-ml organ bath which was built into a perspex block containing also a main reservoir of 100 ml Tyrode's solution heated to 36 ± 1°C. Communication between both compartments was provided by connecting pores through which the fluids were driven by gas (95% O_2 and 5% CO_2). Both ends of the preparation were connected to stainless steel wires. Recording of intracellular potentials was performed using microelectrodes filled with 3 M KCl (resistance 20–40 MΩ). Voltage signals were recorded by a voltage follower with input capacitance compensation. All signals were displayed on an oscilloscope and digitally stored on floppy disks using a Nicolet 310 (Nicolet Instr., Madison, WI, USA). Drugs were applied at maximally effective concentrations, since stable recordings (up to 10 min) in smooth muscle cells are notoriously difficult to obtain.

2.5. Recording of single-channel activity

Single vascular smooth muscle cells were voltage-clamped, and membrane currents were recorded using the cell-attached configuration of the patch-clamp technique (Hamill et al., 1981). Pipette and bath solution consisted of (in mM) 140 KCl, 2 $MgCl_2$, 2.29 $CaCl_2$, 3 EGTA, 10 HEPES; pH was adjusted to 7.4. The micropipettes were made of borosilicate glass capillaries and had a resistance of 5–10 MΩ. Voltage clamp potentials were applied to membrane patches, and membrane currents were recorded using an Axopatch amplifier (200A, Axon Instruments, Foster City, CA, USA). The current signals were filtered at 2 kHz, digitized at 5 kHz using an A/D–D/A-converter (DigiData 1200 system, Axon Instr.) and stored on a

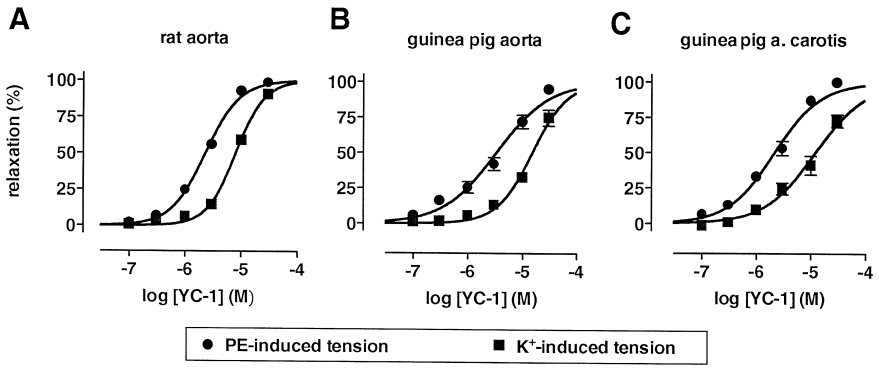

Fig. 1. Concentration-dependent effects of YC-1 on rat aorta (A), guinea pig aorta (B), and guinea pig carotid artery (C). Each preparation was precontracted with either phenylephrine (PE; 3 μM; ●) or high extracellular K^+ (48 mM; ■). The pairs of data sets obtained from each preparation were statistically different evaluated by a two-way analysis of variance (repeated-measurements design). The corresponding EC_{50} values of YC-1 are shown in Table 1. Data represent means ± S.E.M. ($n \geq 6$ each).

486-microprocessor-based computer, running pClamp 6 software (Axon Instr.) which was additionally used for the data analysis. Single-channel currents were calculated by Gaussian distributions fitted to current amplitude histograms. The channel open probability was calculated as the fraction of time in which the channel was in the open state in relation to the total time analysed (1 s or 1 min).

2.6. Chemicals

S-nitroso-*N*-acetylpenicillamine and zaprinast were obtained from Calbiochem (Bad Soden, Germany), ^{86}RbCl (0.8 mCi/μmol) from Amersham (Little Chalfont, UK), charybdotoxin and iberiotoxin from Alomone Labs (Jerusalem, Israel), and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) from Tocris Cookson (Bristol, UK). YC-1 was a gift from Hoechst (Germany). All other chemicals used were at least of reagent grade and purchased from Sigma (St. Louis, MO, USA). The effects of charybdotoxin and iberiotoxin were investigated in the presence of 0.01% (v/v) bovine serum albumine which did not affect the parameters measured. Stock solutions of YC-1, ODQ and zaprinast were prepared in dimethylsulfoxide (DMSO) and further diluted to achieve the final bath concentration. Concentrations of YC-1 higher than 30 μM were not used, since the drug was not soluble in the buffer solutions used without increasing significantly the amount of the solvent DMSO. The final amount of DMSO in test solutions did not exceed 0.7% (v/v) which did not significantly affect the parameters measured.

Table 1
EC_{50} values of YC-1 in vascular preparations precontracted with either phenylephrine (3 μM) or high extracellular K^+ (48 mM)
Data represent means ± S.E.M. ($n \geq 6$).

Tissue	Phenylephrine-induced tension [EC_{50} (μM)]	K^+-induced tension [EC_{50} (μM)]
Rat aorta	2.3 ± 0.4	8 ± 1.3[a]
Guinea pig aorta	3.3 ± 1.8	15 ± 3[b]
Guinea pig a. carotis	2 ± 1	12 ± 3[b]

[a] $P < 0.001$ vs. values obtained in preparations precontracted with phenylephrine.
[b] $P < 0.01$ vs. values obtained in preparations precontracted with phenylephrine.

2.7. Evaluation of results

Data are presented as original recordings or expressed as means ± S.E.M. Changes in aortic tension were expressed in percentage of either phenylephrine-induced or K^+-induced tension. ^{86}Rb-efflux data were expressed as the efflux rate constant which expresses the radioactivity released per minute in relation to the radioactivity which has remained in the tissue. Data from intracellular record-

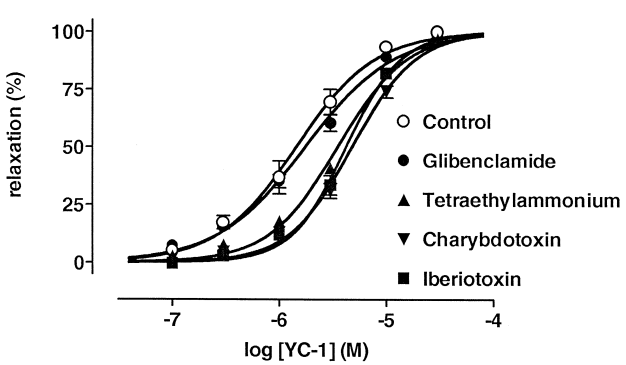

Fig. 2. Concentration-dependent effects of YC-1 on rat aorta in the presence of K^+ channel inhibitors. Rat aortic rings were precontracted with phenylephrine (3 μM). The effects of YC-1 were assessed in the presence of glibenclamide (10 μM), tetraethylammonium (10 mM), charybdotoxin (0.2 μM), or iberiotoxin (0.1 μM). Data sets obtained in the presence of tetraethylammonium, charybdotoxin, or iberiotoxin, but not in the presence of glibenclamide, were statistically different to the control values evaluated by a two-way analysis of variance (repeated measurements design). The corresponding EC_{50} values are shown in Table 2. Data represent means ± S.E.M. ($n \geq 6$ each).

Table 2
EC_{50} values of YC-1 under control conditions and in the presence of either glibenclamide, tetraethylammonium, charybdotoxin, or iberiotoxin in rat aorta precontracted by phenylephrine (3 μM)
Data represent means ± S.E.M. ($n \geq 6$).

YC-1 induced relaxation in the presence of:	EC_{50} (μM)
Vehicle	1.5 ± 0.4
Glibenclamide (10 μM)	1.7 ± 0.5
Tetraethylammonium (10 mM)	3.6 ± 0.7[a]
Charybdotoxin (0.2 μM)	4.8 ± 1[b]
Iberiotoxin (0.1 μM)	4.7 ± 1.2[b]

[a] $P < 0.05$ vs. vehicle.
[b] $P < 0.01$ vs. vehicle.

ings were stored on an IBM-compatible computer and evaluated using GraphPad Prism 2.0 (GraphPad Software, San Diego, CA, USA). Concentration–response curves were fitted by sigmoidal functions (correlation coefficient > 0.99) using GraphPad Prism. Statistical analysis was performed using either paired or unpaired Student's *t*-test or by a two-way analysis of variance (repeated-measurements design). Statistically significant differences were marked by asterisks (* *P*-values < 0.05; ** *P*-values < 0.01; *** *P*-values < 0.001), whereas no significance was marked by N.S.

3. Results

The relaxant effects of YC-1 on vascular preparations from rat and guinea pig are shown in Fig. 1. Vascular rings from rat aorta, guinea pig aorta and from guinea pig a. carotis were precontracted either by phenylephrine (3 μM) or by high extracellular K^+ (48 mM). In all preparations investigated, YC-1 induced relaxation of the precontracted rings in a concentration-dependent manner; YC-1 showed an about fourfold higher potency to relax contractions induced by phenylephrine as compared to those induced by K^+ (see the EC_{50} values in Table 1).

The obvious dependence of the effects of YC-1 on the transmembrane K^+ gradient suggests that K^+ channel currents are involved. Therefore, the effects of YC-1 in rat aortic rings, precontracted by phenylephrine, were studied in the presence of K^+ channel inhibitors (Fig. 2). In the presence of either tetraethylammonium (10 mM; Castle et al., 1989) or the more selective BK_{Ca} channel inhibitors charybdotoxin (0.2 μM; Miller et al., 1985) and iberiotoxin (0.1 μM; Galvez et al., 1990), the potency of YC-1 was about twofold lower as in control conditions (see EC_{50} values in Table 2). In the presence of the selective K_{ATP} channel inhibitor glibenclamide (10 μM; Ashcroft, 1988), the effects of YC-1 remained unchanged.

These results suggest that the relaxing effects of YC-1 in vascular smooth muscle are, at least partially, related to the activation of K^+ channels. Therefore, the effects of YC-1 on the membrane potential of smooth muscle cells were studied. In guinea pig carotid arteries, YC-1 (30 μM) induced a hyperpolarisation of about 5 mV which was antagonised by ODQ (50 μM; Fig. 3), a selective inhibitor of soluble guanylyl cyclase (Garthwaite et al., 1995).

To study ion fluxes through K^+ channels more directly, the effects of YC-1 on ^{86}Rb-efflux were investigated in rat

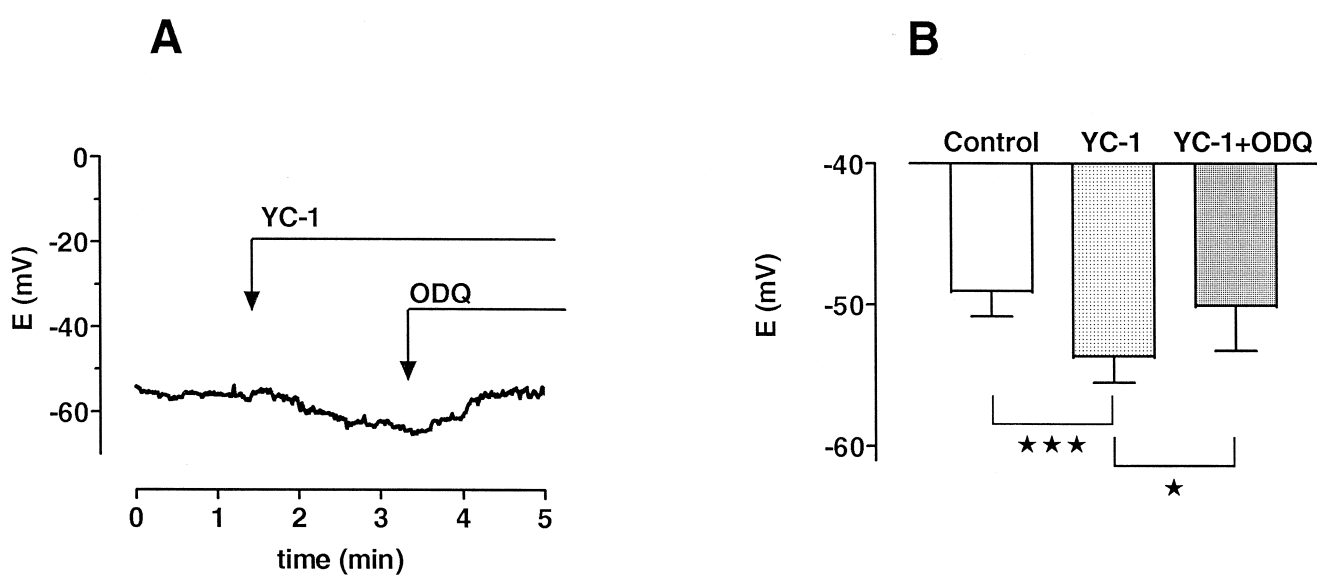

Fig. 3. Effects of YC-1 on membrane potential in smooth muscle cells from guinea pig carotid artery. (A) Original recording. Arrows indicate the application of YC-1 (30 μM) and ODQ (50 μM). (B) Mean values ± S.E.M. The membrane potentials were −49 ± 2 mV ($n = 14$) under control conditions, −54 ± 2 mV ($n = 14$) after application of YC-1 (30 μM), and −50 ± 3 mV ($n = 6$) after addition of ODQ (50 μM). Asterisks indicate statistically significant differences evaluated by a paired Student's *t*-test.

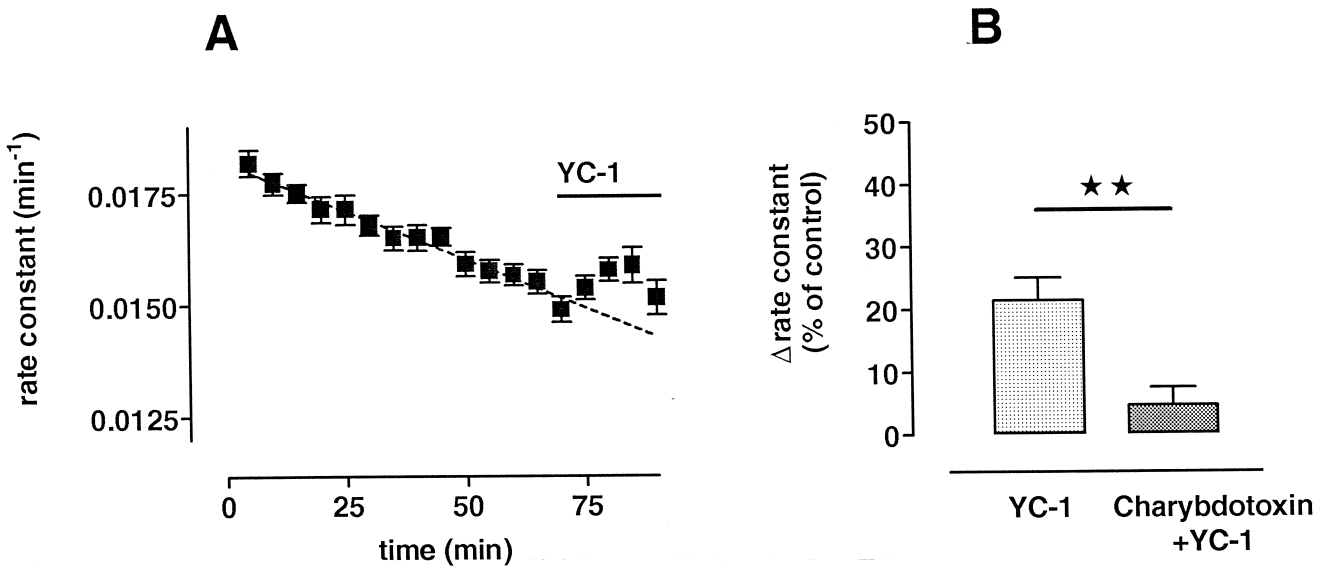

Fig. 4. Effects of YC-1 on $^{86}Rb^{+}$-efflux in rat aorta. (A) Rate constants of $^{86}Rb^{+}$-efflux. The rate constants were determined in time intervals of 5 min. After 70 min, YC-1 (30 μM) was added to the preparations. Data represent means ± S.E.M. ($n \geq 12$). The dotted line represents a linear fit using the data points obtained during the first 60 min. (B) $\Delta_{\text{rate constants}}$ (effect-control) of $^{86}Rb^{+}$-efflux as affected by charybdotoxin. YC-1 (30 μM) increased the rate constant by 21 ± 4% under control conditions and by 5 ± 3% in the presence of charybdotoxin (0.2 μM). Data represent means ± S.E.M. ($n \geq 6$ each). Asterisks indicate statistically significant differences evaluated by an unpaired Student's *t*-test.

aortic rings. YC-1 (30 μM) increased the rate constant of $^{86}Rb^{+}$efflux by about 20% (Fig. 4). This effect of YC-1 was inhibited by charybdotoxin (0.2 μM) suggesting the involvement of BK_{Ca} channels. The increase in the rate constant of $^{86}Rb^{+}$ efflux induced by YC-1 was potentiated by zaprinast (10 μM), a selective inhibitor of phosphodiesterase V (Lugnier et al., 1986), and inhibited by ODQ (50 μM; Fig 5).

For comparison, the effects of other substances involved in cyclic GMP or cyclic AMP signalling on $^{86}Rb^{+}$-efflux were investigated (Fig. 6). 8-Br-cyclic GMP (100 μM), *S*-nitroso-*N*-acetylpenicillamine (100 μM), and forskolin (10 μM) increased the rate constant of $^{86}Rb^{+}$-efflux in rat aorta by about 15%, 25%, and 41%, respectively.

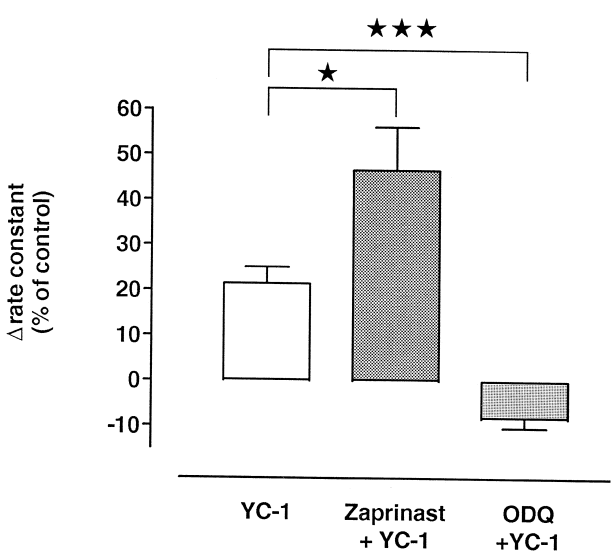

Fig. 5. Effects of YC-1 on the $\Delta_{\text{rate constants}}$ (effect-control) of $^{86}Rb^{+}$-efflux in rat aorta under control conditions and in the presence of zaprinast or ODQ. Under control conditions, YC-1 (30 μM) increased the rate constant by 21 ± 4%. In the presence of zaprinast (10 μM), YC-1 (30 μM) changed the rate constant to 47 ± 9%, whereas the rate constant was −8 ± 2% in the presence of both YC-1 (30 μM) and ODQ (50 μM). Data represent means ± S.E.M. ($n \geq 6$ each). Asterisks indicate statistically significant differences evaluated by an unpaired Student's *t*-test.

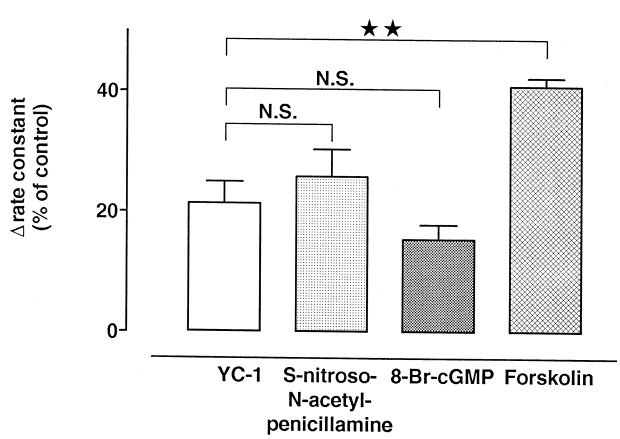

Fig. 6. Effects of YC-1, *S*-nitroso-*N*-acetylpenicillamine, 8-Br-cyclic GMP, and forskolin on the $\Delta_{\text{rate constants}}$ (effect-control) of $^{86}Rb^{+}$-efflux in rat aorta. The rate constants of $^{86}Rb^{+}$-efflux were increased (in percentage) by YC-1 (30 μM), *S*-nitroso-*N*-acetylpenicillamine (100 μM), 8-Br-cyclic GMP (100 μM), and forskolin (10 μM) to 21 ± 4, 26 ± 5, 15 ± 2, and 41 ± 1, respectively. Data represent means ± S.E.M. ($n \geq 6$ each). Asterisks indicate statistically significant differences evaluated by an unpaired Student's *t*-test. N.S. indicates no statistically significant difference.

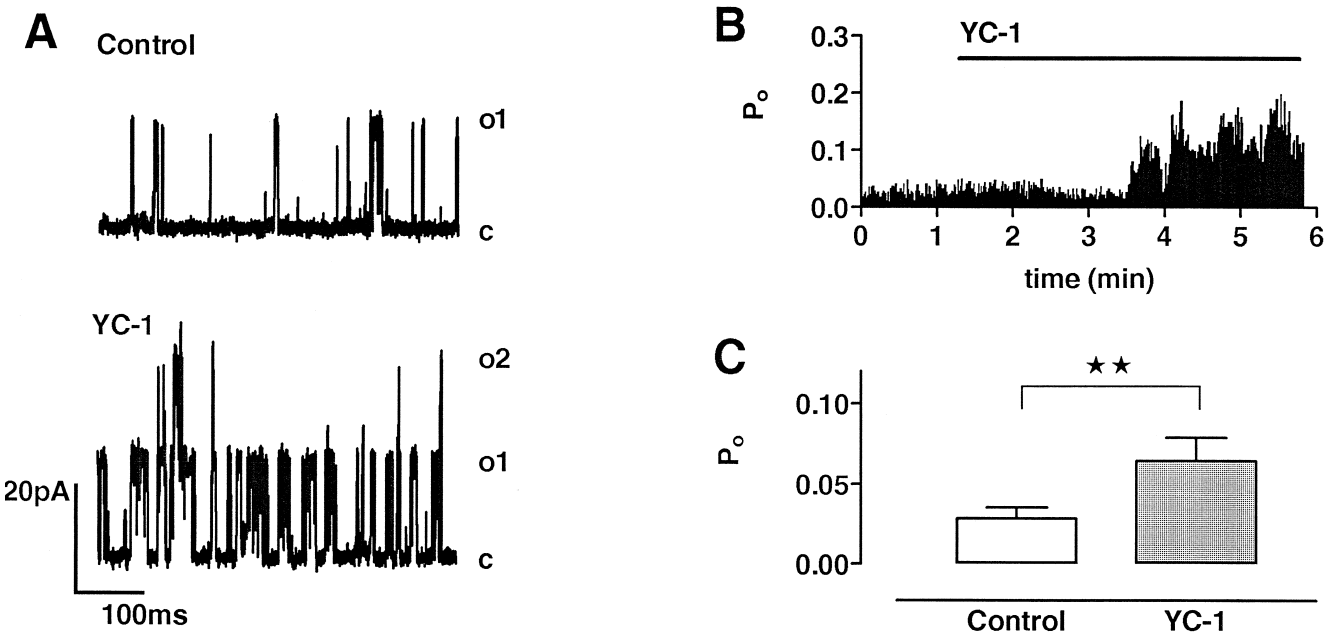

Fig. 7. Effects of YC-1 on K^+ channel activity in smooth muscle cells from rat aorta at a pipette potential of −70 mV. (A) Single-channel recordings in the cell-attached configuration under control conditions (above) and 3 min after the application of YC-1 (10 μM; below). The patch contained 2 K^+ channels which displayed a conductance of 245 pS, respectively. C indicates the closed level, whereas o1 and o2 indicate the open levels of one or both channels, respectively. (B) Time course of the open probability (P_o) of the K^+ channels in (A). P_o was calculated in intervals of 1 s. The bar indicates the presence of YC-1 (10 μM). (C) Mean values of the open probability of K^+ channels calculated in intervals of 1 min. P_o was 0.03 ± 0.007 under control conditions and changed to 0.07 ± 0.014 in the presence of YC-1 (10 μM) within 3–5 min. Data represent means ± S.E.M. ($n = 13$). Asterisks indicate a statistically significant difference evaluated by a paired Student's *t*-test.

In the last series of experiments, the effects of YC-1 on K^+ channel activity was investigated in isolated smooth muscle cells from rat aorta. In cell-attached recordings, the activity of a K^+ channel was frequently observed which exhibited a conductance of 238 ± 6 pS ($n = 25$). In 13 out of 25 experiments, YC-1 (10 μM) increased the open probability of this channel type from 0.03 ± 0.007 to 0.07 ± 0.014 ($P < 0.01$) within 3–5 min at a pipette potential of −70 mV (Fig. 7).

4. Discussion

YC-1 is a direct activator of soluble guanylyl cyclase (Wu et al., 1995; Friebe and Koesling, 1998), which has been shown to produce relaxation in vascular smooth muscle (Mülsch et al., 1997; Wegener et al., 1997). The relaxant effects of YC-1 are abolished by selective inhibition of soluble guanylyl cyclase activity (Mülsch et al., 1997; Wegener and Nawrath, 1997) and potentiated by zaprinast (Wegener et al., 1997) illustrating that they are mediated by cyclic GMP. The relaxant effects of YC-1 were also attenuated by inhibition of K^+ channel activity and by decreasing the transmembrane K^+ gradient demonstrating the involvement of K^+ channels (this paper). In addition, YC-1 hyperpolarised smooth muscle cells and increased the Rb^+ efflux. Furthermore, YC-1 elevated the activity of a K^+ channel type which displayed a conductance of about 240 pS. This type of K^+ channel is, therefore, supposed to belong to the family of large-conductance, Ca^{2+}-sensitive K^+ channels (BK_{Ca} channel) present in vascular smooth muscle cells (Kuriyama et al., 1995). These findings represent more direct evidence that relaxation by activation of the soluble guanylyl cyclase/cyclic GMP signalling pathway is linked to the regulation of BK_{Ca} channel activity.

The endogenous activator of soluble guanylyl cyclase, NO, has been reported to mediate the activation of both BK_{Ca} channels and K_v channels in the bovine coronary artery and the rat pulmonary artery (Li et al., 1997; Zhao et al., 1997), BK_{Ca} channels in rabbit middle cerebral artery (Dong et al., 1998), and K_{ATP} channels in pig pial artery (Armstead, 1996) and in rabbit mesenteric artery (Murphy and Brayden, 1995). Therefore, the subtypes of K^+ channels involved in NO-mediated relaxation of vascular smooth muscle appear to be both tissue- and species-dependent. Our results on rat aorta indicate that the effects of soluble guanylyl cyclase activation by YC-1 are mediated by changes in BK_{Ca} channel activity. Tetraethylammonium attenuated the relaxant effects of YC-1 to the same extent as the more selective BK_{Ca} channel inhibitors charybdotoxin or iberiotoxin (Miller et al., 1985, Galvez et al., 1990). Glibenclamide, a selective inhibitor of K_{ATP} channels (Ashcroft, 1988), failed to inhibit the relaxant

effects of YC-1. It is, therefore, suggested that BK_{Ca} channels rather than K_{ATP} or other K^+ channel types participate in soluble guanylyl cyclase/cyclic GMP-mediated relaxation in rat aorta.

Several studies have presented evidence for the involvement of BK_{Ca} channels in vascular relaxation induced by cyclic GMP elevating substances. For example, relaxation induced by acetylcholine or NO-related substances has been shown to be attenuated by charybdotoxin or iberiotoxin (Kitazono et al., 1997; Zhao et al., 1997; Jiang et al., 1998). In addition, the ANP-induced relaxation by activation of particular guanylyl cyclase has also been reported to involve activity of BK_{Ca} channels (Tanaka et al., 1998). Recently, relaxation induced by 8-Br-cyclic GMP has also been shown to be attenuated by iberiotoxin (Price and Hellermann, 1997; Tanaka et al., 1998), further supporting the view that BK_{Ca} channels are a target of cyclic GMP signalling. However, this channel type is also supposed to be involved in vascular relaxation mediated by cyclic AMP elevating agents (Paterno et al., 1996; Price et al., 1996; Satake et al., 1998).

In this study, forskolin increased $^{86}Rb^+$ efflux in rat aorta to a significant larger extent than YC-1. Therefore, it seems likely that cyclic AMP-dependent activation of K^+ channels may play a more prominent role in regulating membrane potential than do cyclic GMP-mediated mechanisms. It also cannot be excluded that a part of the soluble guanylyl cyclase/cyclic GMP-induced effects on K^+ channel activity is mediated by the cyclic AMP level which can be increased by cyclic GMP-dependent inhibition of cyclic AMP-degrading phosphodiesterase III present in smooth muscle cells (Delpy et al., 1996).

Finally, the fact that K^+ channel inhibitors or high extracellular K^+ did not fully abolish the YC-1-induced vascular relaxation suggests that the activation of K^+ channels is not the only mechanism responsible for soluble guanylyl cyclase/cyclic GMP-induced relaxation. Other mechanisms may include stimulation of sarcolemmal and sarcoplasmic Ca^{2+} ATPases leading to a decrease in $[Ca^{2+}]_i$ (Lincoln and Cornwell, 1993), inhibition of Ca^{2+}-permeable nonselective cation channels (Minowa et al., 1997), and desensitisation of the contractile elements by cyclic nucleotide sensitive phosphatases (Kotlikoff and Kamm, 1998). The relative contribution of the various effects to the relaxation cannot be precisely estimated at this time.

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