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Activation of soluble guanylyl cyclase by YC-1 in aortic smooth muscle but not in ventricular myocardium from rat

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1 The effects of YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole), an activator of soluble guanylyl cyclase, on tension, levels of cyclic GMP and cyclic AMP, and cardiac L-type Ca^{2+}-current ($I_{Ca(L)}$) were investigated in aortic smooth muscle and ventricular heart muscle from rat.

2 YC-1 (0.1–30 μM) induced a concentration-dependent relaxation in aortic rings precontracted with phenylephrine (3 μM). The relaxant effects of YC-1 were reversed by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (30 μM; ODQ), potentiated by zaprinast (10 μM) and antagonized by Rp-8-Br-cGMPS (100 μM).

3 In ventricular heart muscle strips, YC-1 (30 μM) exhibited no effects on force of contraction (F_c) in the absence or presence of either zaprinast (10 μM) or 3-isobutyl-1-methylxanthine (30 μM). F_c was slightly increased by YC-1 (30 μM) in the presence of isoprenaline (100 nM), but this effect was not influenced by ODQ (30 μM).

4 Cardiac $I_{Ca(L)}$ was not significantly affected by YC-1 (30 μM), either in the absence or presence of isoprenaline (30 nM).

5 In aortic rings, cyclic GMP levels were increased almost 3 fold by YC-1 (30 μM); this effect was abolished by ODQ (30 μM). In isolated ventricular cardiomyocytes, cyclic GMP levels were not affected by YC-1 (30 μM) but almost doubled by activation of particular guanylyl cyclase with atriopeptin II (100 nM).

6 YC-1 (30 μM) did not increase cyclic AMP levels either in aortic rings or in ventricular cardiomyocytes. In contrast, isoprenaline (3 μM) increased cyclic AMP levels about two fold in both tissues. In cardiomyocytes, the effect of isoprenaline (3 μM) was slightly enhanced by YC-1 (30 μM).

7 It is concluded that relaxation of smooth muscle preparations by YC-1 is mediated mainly by activation of soluble guanylyl cyclase and subsequent increase in cyclic GMP levels. The failure of YC-1 to affect cardiac F_c, levels of cyclic GMP, and $I_{Ca(L)}$ suggests that soluble guanylyl cyclase is not influenced by YC-1 in rat heart muscle or only barely present in this tissue.

Keywords: Cyclic GMP; tension; L-type Ca^{2+} current; aortic smooth muscle; heart muscle; cardiomyocytes

Introduction

Soluble guanylyl cyclase (sGC) is considered the key enzyme mediating vascular relaxation induced by nitric oxide ($NO^{\bullet}$) and $NO^{\bullet}$-releasing compounds that increase guanosine 3′:5′-cyclic monophosphate (cyclic GMP) levels (for review see Ignarro, 1990). The increase in cyclic GMP content in smooth muscle is associated with an activation of cyclic GMP-dependent protein kinase and subsequent phosphorylation of numerous intracellular proteins (Murad, 1994). Given the broad chemistry of $NO^{\bullet}$ and its redox-activated forms (NO^{-} and NO^{+}), it is not surprising that a number of other proteins, besides sGC, can also be affected by this molecule (Stamler *et al*., 1992). For instance, $NO^{\bullet}$ has been shown to activate directly Ca^{2+}-dependent K^{+}-channels in vascular smooth muscle (Bolotina *et al*., 1994). Therefore, the biological role of sGC can be more exactly identified by the use of direct activators and/or inhibitors of this enzyme. Methylene blue and LY83583 (6-anilino-5,8-quinolinedione) have been used as inhibitors of sGC (Gruetter *et al*., 1981; Mülsch *et al*., 1988). However, neither compound is specific in inhibiting sGC; non-specific effects include the generation of superoxide anions and inhibition of NO-synthases (Kontos & Wei, 1993; Mayer *et al*., 1993). More recently, the introduction of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a potent and selective inhibitor of sGC (Garthwaite *et al*., 1995), has helped to identify more precisely the sGC-mediated effects in various tissues (Brunner *et al*., 1996; Cellek *et al*., 1996; Hebeiss & Kilbinger, 1996; Moro *et al*., 1996; Wegener & Nawrath 1997). In addition, two activators of sGC have recently been described, isoliquiritigenin (Yu & Kuo, 1995) and YC-1 (Ko *et al*., 1994; Wu *et al*., 1995). Several studies have shown that the pharmacological effects of YC-1, but not of isoliquiritigenin (Wegener & Nawrath 1997), are inhibited by ODQ and, therefore, probably exclusively explained by the activation of sGC and the subsequent increase in cyclic GMP levels (Wu *et al*., 1995; Friebe *et al*., 1996; Mülsch *et al*., 1997). In the present study, YC-1 and ODQ were used to investigate the effects of selective sGC activation in aortic smooth and ventricular heart muscle, independent of $NO^{\bullet}$.

Methods

Preparations

Sprague-Dawley rats (200–300 g) of either sex were anaesthetized with ether and bled from the carotid arteries. The heart and the thoracic aorta were quickly removed and immersed in warmed and oxygenated Tyrode 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). The aorta was cut into rings of 3–5 mm in width. For the determination of levels of cyclic GMP and cyclic AMP, the endothelium-intact aortic rings were transferred to microtubes containing buffered salt solution (in mM): NaCl 137, KCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, glucose 5, HEPES 10; pH was adjusted to 7.4 with NaOH. For tension measurements, the aortic endothelium was removed by gently rubbing the luminal surface with a wooden stick. The aortic rings were then tied with silk ligatures after the connective tissue had been removed. Cardiac ventricular strips were prepared from the right ventricle and supplied at either end with silk ligatures. Single ventricular

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cardiomyocytes were isolated as described previously (Wegener & Nawrath, 1995). Briefly, the hearts were enzymatically digested by perfusion with a collagenase-containing buffer solution via the aorta by use of the Langendorff-setup. Single myocytes were obtained from ventricular tissue pieces by mechanical dispersion. The suspension of cardiomyocytes was passed through a 250 μm nylon gaze, centrifuged ($25 \times g$, 15 min), and resuspended in the buffered salt solution. This cell suspension was centrifuged through a gradient of 0.6% Ficoll and washed three times with bath solution ($25 \times g$, 15 min). The final cell pellet contained 50–80% rod-shaped cardiomyocytes with cross striations. Microscopically, no other cell types were identified in the pellet.

Measurement of tension

Aortic rings and cardiac ventricular strips were mounted vertically in organ baths (5 ml) containing oxygenated Tyrode 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 the output of which was fed to a carrier frequency preamplifier (Carrier amplifier/TA2000, Gould, Cleveland, Ohio, U.S.A.). Resting tension of either tissue was set to 10 mN. Aortic rings were precontracted with phenylephrine (3 μM) to produce about 90% of maximal contraction in response to the agonist. Removal of endothelium was verified by the lack of any relaxation in the presence of carbachol (3 μM). Ventricular strips were mounted next to two platinum electrodes built in a muscle holder and electrically stimulated by square wave voltage pulses at 3 Hz (Grass S4, 1 ms duration, voltage 20% above threshold). Drugs were added from stock solutions to the organ bath as single or repeatedly applied doses to achieve the final concentrations as indicated.

Measurement of L-type Ca^{2+}-current

Electrophysiological experiments were performed on rod-shaped myocytes with clear cross striations using the whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981). The experimental equipment used is described elsewhere (Wegener & Nawrath, 1995). During the experiments, the myocytes were voltage-clamped at a holding potential of -80 mV. To inactivate the fast sodium current, a 15 ms prepulse to -40 mV was set before the Ca^{2+}-current was activated. L-type Ca^{2+}-currents ($I_{Ca(L)}$) were elicited by 180 ms depolarizing voltage pulses to 0 mV at 0.2 Hz. The experiments were performed at 36 ± 1°C.

Determination of cyclic AMP and cyclic GMP levels

Preparations of aortic rings and cardiomyocytes were treated with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 100 μM) for 15 min in microtubes to inhibit breakdown of the cyclic nucleotides. After this period, the preparations were incubated with test drugs for 10 min. The duration of the treatment was sufficient to affect tension in aortic rings pre-contracted with phenylephrine (3 μM), as determined in preliminary experiments. At the end of the incubation, the preparations were immediately frozen in liquid nitrogen and stored at -20°C. The cyclic nucleotides were extracted by addition of four volumes 1 N $HClO_4$ followed by sonification and centrifugation (15 min, $13000 \times g$). The pellets were used for protein determination according to Bradford (1976). The supernatants were neutralized with KOH, centrifuged again (2 min, $3000 \times g$) and used for determination of cyclic GMP and cyclic AMP by a radioimmunoassay with ^{125}I-labelled compounds according to Steiner *et al.* (1972).

Chemicals

Na_2 GTP was obtained from Boehringer (Mannheim, Germany), [^{125}I]-cyclic GMP-TME and [^{125}I]-cyclic AMP-TME from Biotrend (Köln, Germany), zaprinast from Calbiochem (Bad Soden, Germany), and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) from Tocris Cookson (Bristol, U.K.). YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole) was a gift from Hoechst AG (Germany). All other chemicals used were at least p.a. grade and purchased from Sigma (St Louis MO, U.S.A.). Stock solutions of isobutyl methylxanthine (IBMX), YC-1, ODQ and zaprinast were prepared in dimethylsulphoxide (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 the amount of the solvent DMSO being significantly increased. The final amount of DMSO in test solutions did not exceed 1% (v/v) and this did not significantly affect the parameters measured.

Evaluation of results

Data are presented as original recordings or expressed as means ± s.e.mean (as % of control values or % of maximal effects). Changes in aortic tension were expressed as % of phenylephrine-induced tension. Cardiac F_c was measured as the difference between resting and peak tension. Cardiac $I_{Ca(L)}$ was measured as the difference between the peak inward and steady-state current values at the end of the voltage pulse. Concentration–response curves were fitted by sigmoidal functions (correlation coefficient >0.99) by use of GraphPad

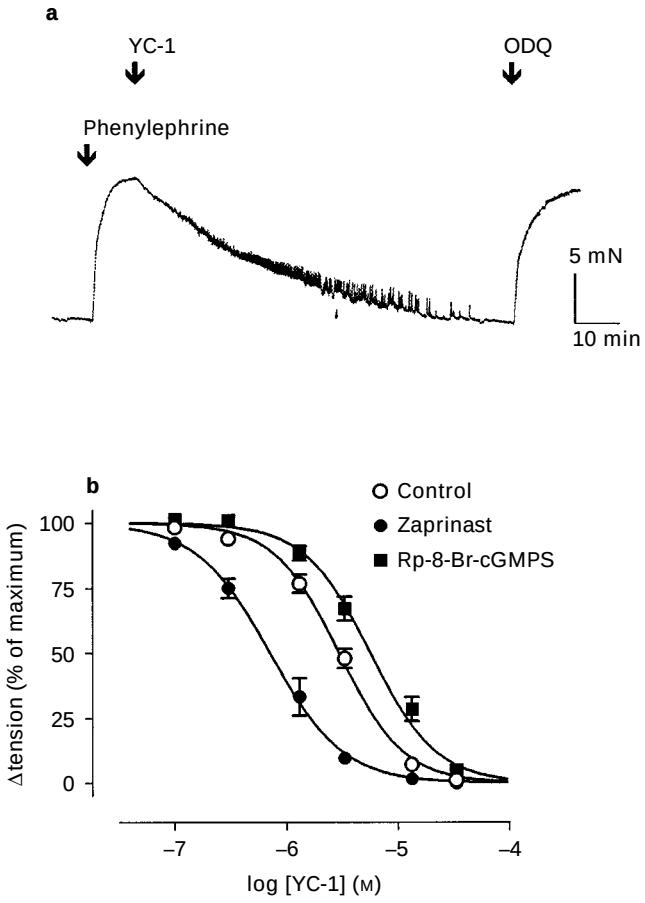

Figure 1 Effects of YC-1 on tension in rat aortic rings. (a) Time course of tension in an aortic ring. Resting tension was set at 10 mN. Phenylephrine (3 μM), YC-1 (10 μM) and ODQ (30 μM) were added at the times indicated by the arrows. (b) Concentration-dependent effects of YC-1 on tension under control conditions and in the presence of either zaprinast (10 μM) or Rp-8-Br-cGMPS (100 μM). Zaprinast or Rp-8-Br-cGMPS were applied 30 min before application of YC-1. The EC_{50} values of YC-1 were 2.9 μM under control conditions, 0.7 μM in the presence of zaprinast and 5.6 μM in the presence of Rp-8-Br-cGMPS, respectively. Points represent means and vertical lines show s.e.mean ($n \geqslant 6$ each).

Prism 2.0 (GraphPad Software Inc., San Diego, CA, U.S.A.). Statistical analysis was performed by either paired or unpaired Student's t test. P values <0.05 were considered to be significant.

Results

Effects of YC-1 on aortic tension

YC-1 (10 μM) completely relaxed rat aortic rings precontracted with phenylephrine (3 μM; Figure 1a). The relaxant effect of YC-1 was reversed by the application of ODQ (30 μM), an inhibitor of soluble guanylyl cyclase (sGC; Garthwaite *et al*., 1995; Figure 1a). Zaprinast (10 μM), a selective inhibitor of type V (cyclic GMP specific) phosphodiesterase (Lugnier *et al*., 1986), potentiated the relaxant effects of YC-1 (Figure 1b). Rp-8-Br-cGMPs, a cyclic GMP analogue that inhibits protein kinase G (Zhuo *et al*., 1994), antagonized the relaxant effects of YC-1 (Figure 1b). The EC_{50} values of YC-1 were 2.9 μM under control conditions, 0.7 μM in the presence of zaprinast (10 μM) and 5.6 μM in the presence of Rp-8-Br-cGMPS (100 μM).

Effects of YC-1 on cardiac force of contraction and L-type Ca^{2+}-current

In rat cardiac ventricular strips, YC-1 did not affect force of contraction (F_c) at concentrations up to 30 μM ($P=0.85$; Figure 2a,b) which were fully effective in relaxing rat aortic smooth muscle. Also, the time courses of single contractions were not affected by YC-1 (30 μM; Figure 2a inset). In further experiments, the effects of YC-1 on F_c were investigated after inhibition of phosphodiesterase activity by either zaprinast or IBMX, a non-selective phosphodiesterase inhibitor (Beavo, 1988). In the presence of zaprinast (10 μM) or IBMX (30 μM), YC-1 (30 μM) did not affect F_c ($P=0.57$ and $P=0.34$, respectively; Figure 2b). Further addition of isoprenaline (1 μM) increased F_c 3 fold and 1.5 fold, respectively ($P<0.01$; not shown). In addition, the effects of YC-1 were studied on the L-type Ca^{2+}-current ($I_{Ca(L)}$) in isolated cardiomyocytes. At concentrations maximally effective on aortic smooth muscle (10–30 μM), YC-1 did not affect $I_{Ca(L)}$ ($P=0.81$ and 0.73, respectively; Figure 2c and d).

Effects of YC-1 on F_c and cardiac $I_{Ca(L)}$ in the presence of isoprenaline

In another series of experiments, the possible interaction of YC-1 with the cardiac cyclic AMP pathway was investigated. For this purpose, the effect of YC-1 on F_c and $I_{Ca(L)}$ was studied after stimulation with a half-maximally effective concentration of isoprenaline (100 and 30 nM, respectively). In cardiac ventricular strips, addition of isoprenaline (100 nM) doubled F_c to 209±11% of control ($n=9$). Further addition of YC-1 (30 μM) increased F_c to 250±24% of control ($n=5$; $P=0.046$; Figure 3a, b). The inhibitor of sGC, ODQ, was used to clarify whether activation of this enzyme was involved in the

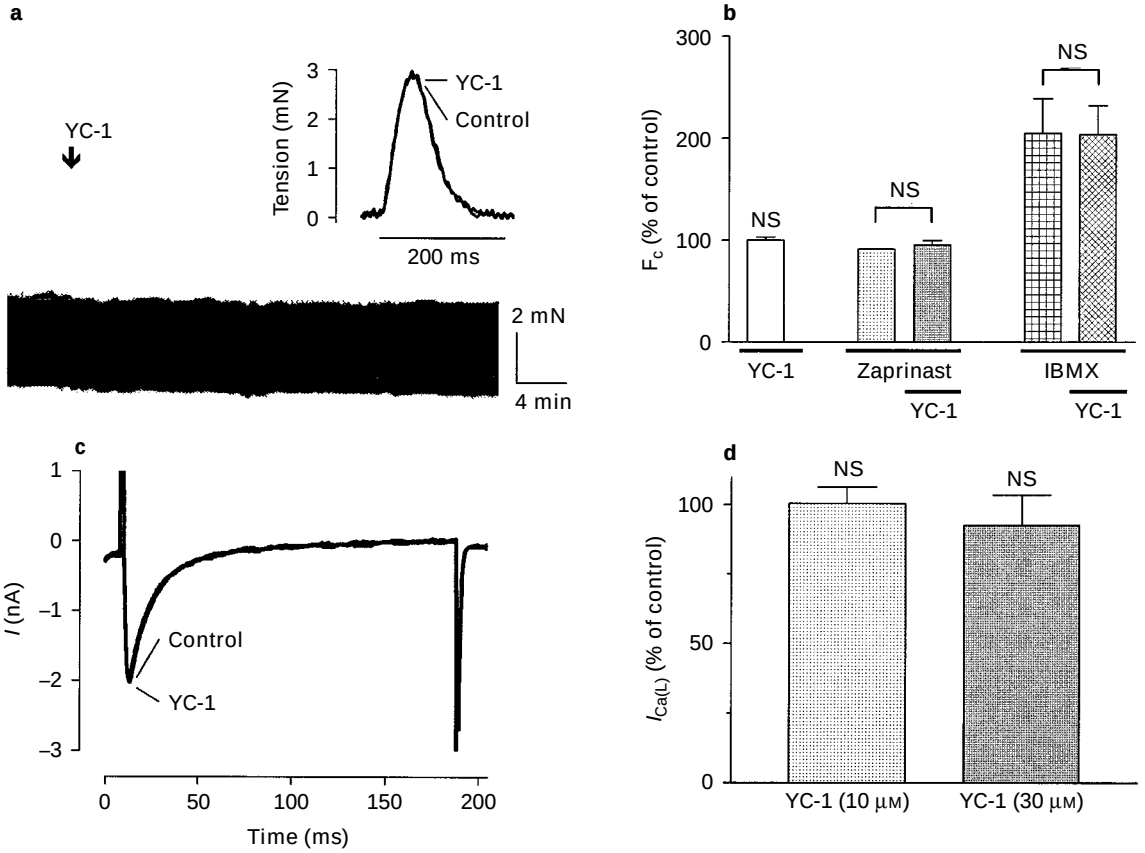

Figure 2 Effects of YC-1 on F_c and $I_{Ca(L)}$ in rat heart muscle. (a) Time course of F_c in a cardiac ventricular strip. The arrow indicates the time of addition of YC-1 (30 μM). Single contractions were superimposed under control conditions and in the presence of YC-1 (30 μM, 5 min) in the inset. (b) Effects of YC-1 on F_c under control conditions and in the presence of either zaprinast or IBMX (as % of control). Control values of F_c were 2.6±0.2 mN ($n=22$). In the presence of YC-1 (30 μM), F_c was 100±3% ($n=16$). In the presence of zaprinast (10 μM; 12 min), F_c was 94±5% ($n=3$), whereas in the presence of IBMX (30 μM; 12 min), F_c increased to 205±34% ($n=3$). After application of YC-1 (30 μM, 10 min), F_c was 96±4% in the presence of zaprinast ($n=3$) and 204±29% in the presence of IBMX ($n=3$). (c) Original recordings of $I_{Ca(L)}$. Current traces were superimposed under control conditions and after application of YC-1 (30 μM, 3 min). (d) Effects of YC-1 on $I_{Ca(L)}$ (as % of control). Control values of $I_{Ca(L)}$ were 2±0.1 nA ($n=15$). In the presence of YC-1 (2 min), $I_{Ca(L)}$ was 100±6% at 10 μM YC-1 ($n=11$) and 93±10% at 30 μM YC-1 ($n=4$). Columns represent means±s.e.mean. Lack of statistically significant differences is marked by NS.

positive inotropic effect of YC-1 in the presence of isoprenaline. In the presence of both ODQ (30 μM) and isoprenaline (100 nM), YC-1 increased F_c to 254±41% of control ($n=4$; $P=0.02$; Figure 3b). The positive inotropic effect of YC-1 (30 μM) in the presence of isoprenaline (100 nM) was not different in the absence and presence of ODQ (30 μM; $P=0.97$). ODQ (30 μM) alone had no significant effect on F_c which amounted to 98±2% of control ($n=6$; $P=0.64$; not shown) and to 212±6% of control in the presence of isoprenaline (100 nM; $n=4$; $P=0.23$; Figure 3b). In isolated cardiomyocytes, application of isoprenaline (30 nM) almost doubled $I_{Ca(L)}$ to 193±21% of control ($n=5$; Figure 3c, d). Further application of YC-1 (30 μM) slightly increased $I_{Ca(L)}$ to 203±14% of control ($n=5$), but this increase was not significant compared to the increase induced by isoprenaline alone ($P=0.43$).

Effects of YC-1 on cyclic GMP levels in aortic rings and isolated cardiomyocytes

In aortic rings, stimulation with YC-1 (30 μM) increased the cyclic GMP levels about 3 fold to 14.4±1.7 pmol mg^{-1} wet weight ($n=9$; $P<0.001$); control values amounted to 4.0±0.2 pmol mg^{-1} wet weight ($n=15$; Figure 4a). The increase in cyclic GMP levels by YC-1 was abolished in the presence of ODQ (30 μM). Under these conditions, the cyclic GMP levels amounted to 2.4±0.7 pmol mg^{-1} wet weight ($n=8$; $P<0.001$). ODQ (30 μM) alone reduced the cyclic GMP levels to 1.9±0.4 pmol mg^{-1} wet weight ($n=9$; $P<0.001$) compared to the control values which are probably due to sGC activity induced by NO•-release from the endothelium.

In isolated cardiomyocytes, stimulation with YC-1 (30 μM) did not change significantly the cyclic GMP levels, which amounted to 6.1±0.5 pmol mg^{-1} protein ($n=24$) under control conditions and to 5.9±0.6 pmol mg^{-1} protein ($n=16$) after stimulation with YC-1 (30 μM; $P=0.79$; Figure 4b). Inhibition of sGC by ODQ resulted in cyclic GMP levels of 6.5±0.5 pmol mg^{-1} protein ($n=10$) which were not different from unstimulated preparations ($P=0.75$). In parallel experiments, atriopeptin II (100 nM), an activator of particular guanylyl cyclase (McCall & Fried, 1990), increased the cyclic GMP levels to 11.5±1.4 pmol mg^{-1} protein ($n=9$) in the isolated cardiomyocytes ($P<0.001$; Figure 4b),

Effects of YC-1 on cyclic AMP levels in aortic rings and isolated cardiomyocytes

In both aortic rings and ventricular cardiomyocytes, YC-1 (30 μM) did not significantly influence the content of cyclic AMP ($P=0.44$ and 0.57, respectively; Figure 5). In the aortic rings, the levels of cyclic AMP were 248±31 pmol mg^{-1} wet

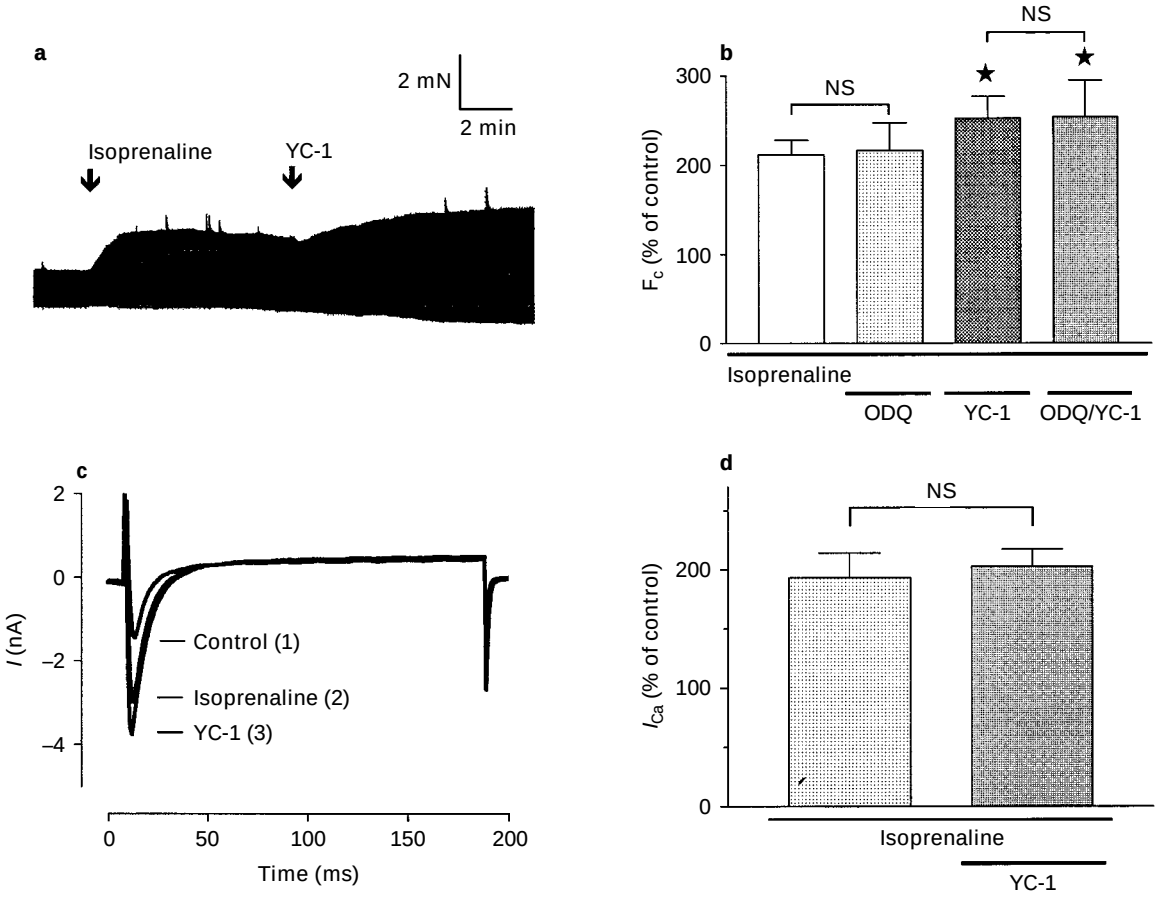

Figure 3 Effect of YC-1 on F_c and $I_{Ca(L)}$ in the presence of isoprenaline. (a) Time course of F_c in a ventricular strip. Isoprenaline (100 nM) and YC-1 (30 μM) were added at the times indicated by the arrows. (b) Effects of YC-1 on F_c in the presence of isoprenaline and of both isoprenaline and ODQ (as % of control). Control values of F_c reached 2.5±0.2 mN ($n=18$). Isoprenaline (100 nM) increased F_c to 212±16% within 2 min ($n=9$). In the presence of isoprenaline (100 nM), YC-1 (30 μM) increased F_c to 250±24% within 5 min ($n=5$). In the presence of both isoprenaline (100 nM) and ODQ (30 μM), YC-1 (30 μM) increased F_c to 254±41% within 5 min ($n=4$). There was no significant difference between the effects of YC-1 on F_c in the presence of isoprenaline and in the presence of both isoprenaline and ODQ ($P=0.97$). (c) Original recording of $I_{Ca(L)}$. Current traces were superimposed under control conditions, in the presence of isoprenaline (30 nM, 2 min), and in the presence of both isoprenaline (30 nM) and YC-1 (30 μM, 3 min). (d) Effects of YC-1 on $I_{Ca(L)}$ in the presence of isoprenaline (as % of control). Control values of $I_{Ca(L)}$ were 1.9±0.1 nA ($n=5$). Isoprenaline (30 nM) increased $I_{Ca(L)}$ to 193±21% within 2 min ($n=5$). In the presence of isoprenaline (30 nM) and YC-1 (30 μM), $I_{Ca(L)}$ amounted to 203±14% within 3 min ($n=5$). Columns represent means±s.e.mean. Statistically significant differences ($P<0.05$) are indicated by asterisks, whereas non-significant differences are marked by NS.

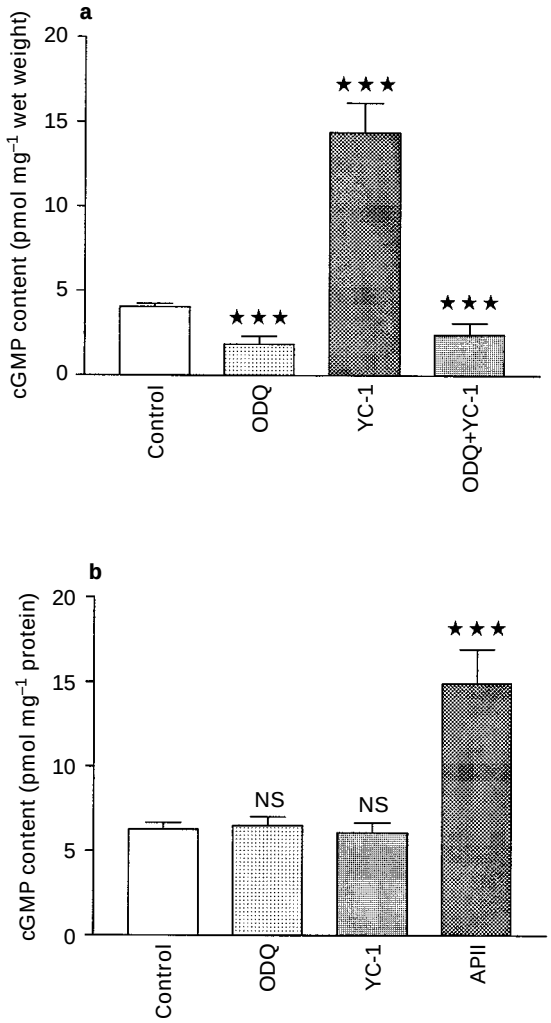

Figure 4 Effects of YC-1 on cyclic GMP levels in aortic rings and cardiomyocytes. (a) Cyclic GMP levels in aortic rings with intact endothelium. The cyclic GMP levels (in pmol mg^{-1} wet weight) reached 4.0 ± 0.2 ($n=15$) under control conditions, 1.9 ± 0.4 ($n=9$) in the presence of ODQ (30 μM), 14.4 ± 1.7 ($n=9$) in the presence of YC-1 (30 μM), and 2.4 ± 0.7 ($n=8$) in the presence of both ODQ (30 μM) and YC-1 (30 μM). (b) Cyclic GMP levels in isolated cardiomyocytes. The cyclic GMP levels (in pmol mg^{-1} protein) were 6.1 ± 0.5 ($n=24$) under control conditions, 6.5 ± 0.5 ($n=10$) in the presence of ODQ (30 μM), 5.9 ± 0.6 ($n=16$) in the presence of YC-1 (30 μM) and to 11.5 ± 1.4 ($n=9$) in the presence of atriopeptin II (APII; 100 nM). Columns represent means $\pm$ s.e.mean. Statistically significant differences ($P<0.05$) are marked by asterisks, whereas non-significant differences are marked by NS.

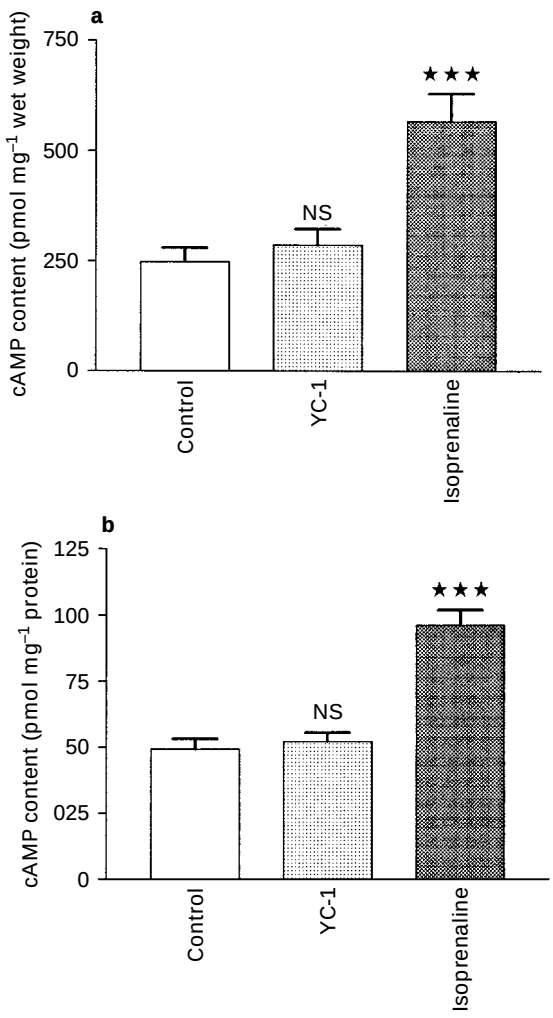

Figure 5 Effects of YC-1 on cyclic AMP levels in aortic rings and cardiomyocytes. (a) Cyclic AMP levels in aortic rings with intact endothelium. The cyclic AMP levels (in pmol mg^{-1} wet weight) reached 248 ± 31 ($n=8$) under control conditions, 286 ± 36 ($n=8$) in the presence of YC-1 (30 μM) and 566 ± 62 ($n=9$) in the presence of isoprenaline (3 μM). (b) Cyclic AMP levels in isolated cardiomyocytes. The cyclic AMP levels (in pmol mg^{-1} protein) were 49 ± 4 ($n=10$) under control conditions, 52 ± 3 ($n=12$) in the presence of YC-1 (30 μM) and 96 ± 6 ($n=12$) in the presence of isoprenaline (3 μM). Columns represent means $\pm$ s.e.mean. Statistically significant differences ($P<0.05$) are marked by asterisks, whereas non-significant differences are marked by NS.

weight ($n=8$) and 286 ± 36 pmol mg^{-1} wet weight ($n=8$) under control conditions and after stimulation with YC-1 (30 μM), respectively. In the cardiomyocytes, the corresponding values were 49 ± 4 pmol mg^{-1} protein ($n=10$) and 52 ± 3 pmol mg^{-1} protein ($n=10$), respectively. In parallel experiments, isoprenaline (3 μM) increased the cyclic AMP levels to 566 ± 62 pmol mg^{-1} wet weight ($n=9$) in the aortic rings and to 96 ± 6 pmol mg^{-1} protein ($n=12$) in the cardiomyocytes.

In another series of experiments with ventricular cardiomyocytes, the preparations were not treated with IBMX. Instead, cyclic AMP levels were measured 2 min after stimulation with isoprenaline (3 μM) in the absence and presence of YC-1 (30 μM). In response to isoprenaline (3 μM), the cyclic AMP content was slightly but significantly larger ($P=0.03$) after 10 min incubation with 30 μM YC-1 (24.7 ± 0.5 pmol mg^{-1} protein; $n=5$) than without YC-1 (22.5 ± 0.5 pmol mg^{-1} protein; $n=4$; data not shown). Under control conditions, the cyclic AMP levels were 14.7 ± 1.2 pmol mg^{-1} protein ($n=5$; data not shown).

Discussion

YC-1 completely relaxed rat aortic smooth muscle precontracted with phenylephrine. This effect was fully reversed by ODQ confirming that YC-1 exerts its effects through activation of soluble guanylyl cyclase (sGC; Ko *et al.*, 1994; Wu *et al.*, 1995). The relaxing effects of YC-1 were potentiated by zaprinast, a selective inhibitor of type V (cyclic GMP specific) phosphodiesterase (Lugnier *et al.*, 1986) and antagonized by Rp-8-Br-cGMPS, an inhibitor of cyclic GMP-dependent protein kinase (Zhou *et al.*, 1994). Both findings demonstrate the key role of cyclic GMP in mediating the effects of YC-1.

In rat ventricular heart muscle, YC-1 did not change the time course of magnitude of contraction, either alone or in the presence of zaprinast or IBMX, a non-selective inhibitor of phosphodiesterases (Beavo, 1988). In β-adrenoceptor-stimu-

lated preparations, YC-1 exerted a moderate positive inotropic effect, accompanied by a minor increase in the magnitude of $I_{Ca(L)}$. However, these effects were not influenced by ODQ, indicating that they were unrelated to sGC activity. The most likely explanation of these moderate effects would be a partial direct inhibition of phosphodiesterase following high concentrations of YC-1. In fact, YC-1 at 100 μM has been observed to inhibit purified human phosphodiesterase isozymes III, IV and V (personal communication, Dr Schindler, U., Hoechst AG, Germany). Correspondingly, a slight potentiation by YC-1 of the effect of isoprenaline on cyclic AMP levels was observed in ventricular cardiomyocytes (this paper).

These findings raise the question as to why YC-1 can fully relax aortic smooth muscle, whereas myocardial functions remain virtually unchanged. Measurements of cyclic GMP levels in preparations from both tissues showed that YC-1 significantly increased cyclic GMP levels in smooth muscle but not in isolated myocytes from the heart. In contrast, activation of particular guanylyl cyclase with atriopeptin II (McCall & Fried, 1990) significantly increased the cyclic GMP levels in the isolated cardiomyocytes. These findings suggest that sGC can be activated by YC-1 in aortic smooth muscle but not in heart muscle. In addition, inhibition of basal sGC activity by ODQ increased tension (unpublished observations) and decreased cyclic GMP levels in endothelium-containing aortic rings, but not in heart muscle. Two major possibilities exist to explain these discrepancies. Either the sGC isoform in heart muscle cannot be stimulated by YC-1 and inhibited by ODQ or it is only barely present in heart muscle as compared to smooth muscle.

Cyclic GMP measurements in whole heart preparations cannot resolve this question, because of the presence of both smooth muscle and heart muscle tissue. However, several studies in isolated cardiomyocytes with nitric oxide ($NO^{\bullet}$), or $NO^{\bullet}$-liberating substances as activators of sGC are of interest in this context. Stein *et al.* (1993) have shown that sodium nitroprusside increases cyclic GMP levels in guinea-pig cardiomyocytes. However, this effect was not influenced by methylene blue. Kojda *et al.* (1995, 1996) showed increases in cyclic GMP levels in rat ventricular myocytes in response to nitrovasodilators, whereas the influence of inhibitors of sGC on these effects were not shown. Further experiments in isolated cardiomyocytes seem necessary to determine to what extent sGC activity induced by $NO^{\bullet}$ determines the level of cyclic GMP in heart muscle.

The lack of effects of YC-1 in heart muscle is also of interest in another context. Numerous pharmacological effects of $NO^{\bullet}$ and related substances in the heart have been commonly ascribed to activation of sGC (Hare & Colucci, 1995). However, some of these effects are controversial; for instance, in various cardiac tissues, $NO^{\bullet}$ and related substances have been found either to reduce (Smith *et al.*, 1991; Brady *et al.*, 1993) or to have no effect on cardiac contractility (Weyrich *et al.*, 1994; Nawrath *et al.*, 1995). The use of substances which selectively activate and/or inhibit sGC may help to clarify some of these discrepancies.

The present study shows that the activator of sGC, YC-1, completely relaxes rat vascular smooth muscle but leaves myocardial function unchanged. This property would be desirable in a clinical setting where vasodilatation is required without effects on the myocardium. YC-1 differs from NO-donor compounds which lack selectivity for sGC activation and may influence the myocardium under certain conditions (Hare & Colucci, 1995; Kelly *et al.*, 1996).

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