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YC-1 potentiates the nitric oxide/cyclic GMP pathway in corpus cavernosum and facilitates penile erection in rats

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Abstract

The aim of present study was to characterize the in vitro and in vivo pharmacological effects of YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole), a soluble guanylate cyclase activator, on corpus cavernosal smooth muscle and penile erectile activity. YC-1 relaxed phenylephrine precontracted cavernosal smooth muscle (EC_{50} = 4.4 μM) and this effect was partially antagonized by 1H-[1,2,4]oxadiazole [4,3-a]quinoxalin-1-one (ODQ). ODQ is a selective soluble guanylate cyclase inhibitor that completely blocked the relaxation induced by sodium nitroprusside, suggesting that YC-1 binds to soluble guanylate cyclase at a different site from nitric oxide (NO). Both YC-1 and sodium nitroprusside, but not sildenafil (1–100 μM) caused concentration-dependent increases in cyclic GMP levels in cultured rabbit cavernosal smooth muscle cells and produced synergistic effects. Intraperitoneal administration of YC-1 (10 μmol/kg) evoked penile erection in rats with 70% incidence. More importantly, YC-1 was able to significantly augment the pro-erectile effects of a suboptimal dose of apomorphine. These results suggest that the soluble guanylate cyclase activator YC-1 increases cyclic GMP levels, leading to relaxation of cavernosal smooth muscle. These biochemical events may be related to the pro-erectile properties of YC-1 in vivo.

Keywords: YC-1; Guanylate cyclase; Nitric oxide (NO); Apomorphine; Penile erection; Corpus cavernosum

1. Introduction

Corpus cavernosum smooth muscle tone is regulated by complex biochemical events coordinated at the level of the peripheral and central nervous system (Andersson, 2001; Moreland et al., 2001). Nitric oxide (NO), the prevalent relaxant factor involved in penile erection, is released from nonadrenergic-noncholinergic nerve terminals and from cholinergic-activated corpus cavernosum endothelial cells and diffuses into the adjacent smooth muscle cells. It activates soluble guanylate cyclase and catalyzes the conversion of guanosine 5′-triphosphate in to cyclic GMP that in turn leads to the relaxation of cavernosum smooth muscle of the penis (Brioni et al., 2002). Cyclic GMP is an important intracellular mediator that is responsible for the cavernosal smooth muscle relaxation.

Nitrovasodilators (organic nitrates and NO donors) exert their therapeutic effects on vascular smooth muscle by the same mechanism attributed exclusively through spontaneous or metabolic liberation of NO (Artz et al., 2001; Carvajal et al., 2000). In the penis, cyclic GMP is degraded to GMP primarily by phosphodiesterase 5 and pharmacological inhibition of this enzyme is effective in the treatment of erectile dysfunction in humans (Moreland et al., 2001). YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole), a chemically synthesized benzylindazole derivative initially identified as an inhibitor of platelet aggregation (Ko et al., 1994), is a direct soluble guanylate cyclase activator (Mulsch et al., 1997; Wu et al., 1995). YC-1 causes activation of soluble guanylate cyclase by modulating the catalytic rate of enzyme, decreasing the K_m for GTP and increasing the V_{max} of cyclic GMP formation (Lee et al., 2000; Nakane et al., 2002). In addition, a synergistic action is observed by the combination of YC-1 with NO (Friebe and Koesling, 1998; Schmidt et al., 2001; Stone and Marletta, 1998). YC-1 induces a concentration-dependent relaxation (EC_{50} = 2.9 μM) in rat aortic rings precontracted with phenylephrine, and the

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effects of YC-1 can be blocked by soluble guanylate cyclase inhibitor ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) (Wegener et al., 1997). Unlike NO, YC-1 exerts an allosteric regulation but does not affect the heme spectrum of soluble guanylate cyclase (Friebe and Koesling, 1998). This suggests that ligands at this allosteric site may represent a novel class of drugs that exert beneficial effects by sensitizing soluble guanylate cyclase toward its physiological activator, NO. YC-1 has since been widely used as an important research tool to characterize soluble guanylate cyclase and to probe for the involvement of cyclic GMP in various biological processes (Denninger and Marletta, 1999; Hobbs, 1997; Lucas et al., 2000).

While YC-1 has been suggested to relax corpus cavernosum tissue strips in vitro (Liu et al., 2000), it has yet to be demonstrated if these relaxations can lead to penile erection in mammals. The present studies were designed to characterize the biochemical events of YC-1 in relaxing corpus cavernosal tissues in vitro and to investigate the pro-erectile effects of YC-1 in vivo in a conscious rat penile erection model.

2. Materials and Methods

2.1. Chemicals and reagents

YC-1, ODQ, and sodium nitroprusside were purchased from Sigma (St. Louis, MO). Sildenafil citrate was synthesized at Abbott Laboratories (Abbott Park, IL). Apomorphine was obtained from Aldrich Chemical (Milwaukee, WI). All other reagents, unless indicated otherwise, were obtained from Sigma.

2.2. Corpus cavernosal tissue bath experiments

The in vitro regulation of smooth muscle tone by compounds investigated in this study was assessed on isolated rabbit cavernosum smooth muscle strips mounted in organ bath chamber and precontracted by phenylephrine as previously reported (Hsieh et al., 2001a). Corpus cavernosum tissues were prepared from adult male New Zealand white rabbits (Covance Research Production, Kalamazoo, MI), weighing ~ 3.5 kg, after euthanasia by intravenous pentobarbital sodium injection. The two corpus cavernosum strips were carefully dissected under an illuminated magnifier (a slit was made in the proximal end of the tunica and extended distally and the corpus cavernosum was sharply dissected free of the tunica albuginea and surrounding connective tissues). Each tissue strip was longitudinally cut into three strip preparations measuring approximately $2 \times 2 \times 7$ mm (unstretched length, mean weight ~ 30 mg).

The strips were transferred and mounted in organ baths (10 ml) containing Kreb–Henseleit buffer solution (pH 7.4) maintained at 37 °C by a thermoregulated water circuit. The buffer solution contained D-glucose 11.1 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, KCl 4.7 mM, NaCl 118 mM (Sigma) plus $CaCl_2 \cdot 2H_2O$ 2.5 mM and $NaHCO_3$ 25 mM and was continuously aerated with a mixture of 95% O_2–5% CO_2. The tissue strips were loaded with a resting tension of 2 g and equilibrated for 90 min. During equilibration, the bath solution was replaced every 10–15 min. Changes in isometric tension of muscle tissues were measured using a force–displacement transducer (Grass FT03 model, Grass Instruments, Quincy, MA) and recorded on a Grass 7D model polygraph. Repeated adjustment of tension was performed if necessary. No changes in tension were made after the experiment was begun.

The relaxation effects of YC-1 and sodium nitroprusside were assessed in rabbit corpus cavernosum preparations precontracted with phenylephrine. The concentrations of the phenylephrine used corresponded to approximate EC_{50} values (1.5 μM, Fig. 1) in tissue organ bath preparations. At stable tension, compounds to be tested were added cumulatively, and a new concentration was not added until the response to the previous one had reached a steady level. YC-1, ODQ, or sodium nitroprusside was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution. Further diluted with ethanol before adding into the buffer solutions in tissue bath chamber so that the final total solvent concentrations in the assays was less than 0.5% (v/v) which itself did not alter the smooth muscle tone. Stock solutions of phenylephrine were prepared in saline included 0.1% ascorbic acid as an antioxidant.

In another set of experiment, tissue preparations were contracted with phenylephrine 1.5 μM and subsequently treated with 3–30 μM ODQ for 15 min. The inhibitors were added after the phenylephrine-induced contraction reached steady state. Cumulative concentration–response curves were constructed for YC-1 and sodium nitroprusside as described above.

2.3. Corpus cavernosal smooth muscle cell cultures and cyclic GMP measurement

Corpus cavernosal smooth muscle tissues were prepared from adult male New Zealand white rabbits as described

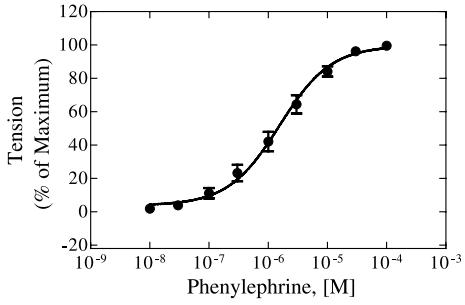

Fig. 1. Cumulative concentration–response curve for phenylephrine in rabbit corpus cavernosal tissue strips. The maximum tension of phenylephrine-induced contraction was 3899 ± 473 mg. Data are expressed as mean ± S.E.M. ($n = 7$).

above. Explant cavernosal tissues were placed in small petri dishes in Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum, antibiotics, and amphotercin B. The explants were kept undisturbed for 3 to 4 days in a humidified 5% CO_2–95% air incubator at 37 °C. Smooth muscle cells migrated out from the tissues and underwent proliferation within 7 days. The explants were removed and the medium was changed every 3 days. Once confluent, cells were detached with trypsin–EDTA and subcultured at density of 0.5×10^4 cells/well in 24-well culture plates. All experiments were performed in cavernosum cells that had been grown for 48 h (>90% confluent).

Immediately before each experiment the culture medium was changed and washed with Hank's balanced salt solution (BSS) three times. The cells were incubated with pharmacological agents, with or without the nonselective phosphodiesterase inhibitor 100 μM 3-Isobutyl-1-methylxanthine (IBMX) prepared in Hanks' BSS, for 10 min. Cells were lysed with 0.5% (w/v) dodecyl triammonium bromide in 0.05 M sodium acetate buffer (pH 5.8). Total cellular cyclic GMP levels were determined using a commercially available enzyme immunoassay (EIA) kit (RPN 226; Amersham Pharmacia Biotech, Piscataway, NJ) after acetylation of cyclic GMP with a reagent solution containing 1 acetic anhydride: 2 triethylamine (v/v). All samples were assayed in duplicate. Cyclic GMP levels are expressed as fmol/well (mean ± S.E.M.) for the indicated number of separate experiments. The significance of drug effects was assessed by analysis of variance followed by Dunnett's test and a p value <0.05 was regarded as significant.

2.4. In vivo rat penile erection

Male adult Wistar rats (Charles River Laboratories, Wilmington, MA), weighing ~300 g were used as an animal model to study penile erection in vivo. The animals were housed in groups of four to five under a controlled 12-h light–dark cycle, with light on at 6:00 a.m., and were allowed free access to water and standard food ad libitum. All experiments were carried out between 9:00 a.m. and 3:00 p.m. Animals were allowed to adapt to a diffusely illuminated testing room with red light for 1 h before the beginning of experiments. Rats were placed individually into a transparent Plexiglas cage (20 × 30 × 30 cm) immediately after the drug injection. A mirror was placed behind and under the observation cages to facilitate observation of the animals. Each rat was used only once. A penile erection was considered to occur when the following behaviors were presented: repeated pelvic thrusts immediately followed by an upright position, an emerging, engorged penis, which the rat proceeds to groom. Apomorphine was freshly prepared in ascorbic acid (added as an antioxidant) solution in saline (1 mg/ml) and was subcutaneously injected into the back neck area at an injection volume of 1 ml/kg. YC-1, dissolved in 5% ethanol and subsequently diluted with a vehicle containing 30% hydroxypropyl-beta-cyclodextrin (50% w/v in H_2O) and 65% saline (v/v), was administered i.p. (1–10 μmol/kg, 2 ml/kg) to rats. In another serious of in vivo study, YC-1 was given i.p. 10 min before injection of suboptimal dose of apomorphine (0.01 μmol/kg s.c.).

The penile erection episodes were recorded by direct observation for a period of 60 min, and the number of animals exhibiting one or more erections during the observation period was expressed as incidence (%). Statistical evaluation of the results was performed by Chi-Square test.

3. Results

3.1. Effects of YC-1 and sodium nitroprusside on phenylephrine contracted corpus cavernosal strips

Rabbit corpus cavernosal tissue strips contracted in response to phenylephrine in a concentration-dependent manner with an EC_{50} of approximately 1.5 μM (Fig. 1). Phenylephrine-induced contraction was determined by cumulatively adding phenylephrine (1×10^{-8} to 1×10^{-4} M) into tissue bath preparations. Corpus cavernosum strips were precontracted with phenylephrine (1.5 μM) and treated with increasing concentrations of YC-1 (10^{-8}–10^{-4} M). YC-1 elicited a concentration-dependent relaxation on smooth muscle strips and the potency determined from cumulative concentration–response curve was $EC_{50} = 4.4$ μM (Fig. 2). YC-1 at ≥30 μM concentrations produced complete relaxation of the phenylephrine-induced contractions. Relaxation was partially antagonized by inhibition of soluble guanylate cyclase using ODQ at concentrations up to 30 μM. As shown in Fig. 2, YC-1-induced relaxation was only partially reversed by ODQ; a 40% of maximum relaxation response for 100 μM YC-1 on 30 μM ODQ pretreatment. Schild plot analysis indicated that ODQ is a noncompetitive inhibitor (slope = 0.7). ODQ, an inhibitor of soluble guanylate cyclase, is a useful tool for characterizing drugs that act via this enzyme (Tseng et al., 2000; Zhao et al., 2000).

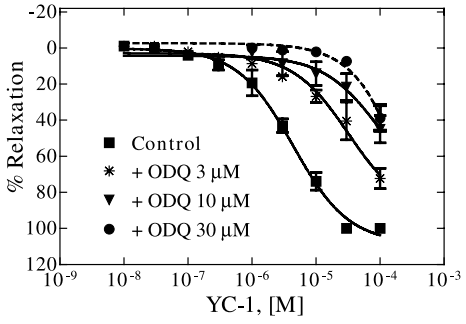

Fig. 2. The relaxing effect of YC-1 on phenylephrine-precontracted rabbit corpus cavernosal tissue strips in the absence and presence of ODQ. The total volume of the solvents (to prepare YC-1 and ODQ stock solutions) added in tissue bath chamber was 50 μl. Data are expressed as mean ± S.E.M. ($n = 4$).

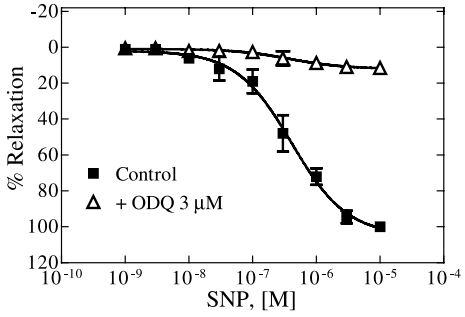

Fig. 3. The relaxing effect of sodium nitroprusside (SNP) on phenylephrine-precontracted rabbit corpus cavernosum in the absence and presence of ODQ. Data are expressed as mean ± S.E.M. ($n = 4$).

Sodium nitroprusside (10^{-9}–10^{-5} M) produced a concentration-dependent relaxation (Fig. 3) with an EC_{50} value of 0.4 μM which was more potent than that caused by YC-1. A faster onset of the relaxant response of sodium nitroprusside was observed and the concentrations at ≥10 μM completely relaxed the smooth muscle tissues contracted by 1.5 μM phenylephrine. Pretreatment with ODQ (3 μM) completely abolished the relaxation effects caused by the soluble guanylate cyclase activator sodium nitroprusside (Fig. 3). ODQ potently inhibited the relaxation of soluble guanylate cyclase activator sodium nitroprusside, but partially affected the relaxing effects of YC-1. YC-1 is reported to activate soluble guanylate cyclase at a different site from the heme (NO binding site) (Brioni et al., 2002).

3.2. Effects of YC-1 and sodium nitroprusside on intracellular cyclic GMP levels in primary cultures of corpus cavernosal cells

To examine whether the relaxing effects of YC-1 and sodium nitroprusside on cavernosal tissue strips coincided with elevated cyclic GMP levels in smooth muscle cells in vitro, we determined the effect of YC-1 and/or sodium nitroprusside on intracellular concentrations of cyclic GMP in a primary culture of rabbit corpus cavernosum smooth muscle cells. Fig. 4 shows that the incubation of corpus cavernosal cells with YC-1 or sodium nitroprusside led to a concentration-dependent increase in cyclic GMP from a basal level of 2 fmol up to 109.1 and 54.4 fmol/well, respectively. As expected, treatment of the cells with phosphodiesterase 5 inhibitor sildenafil (1–100 μM) did not significantly trigger cyclic GMP accumulation since this agent does not activate soluble guanylate cyclase.

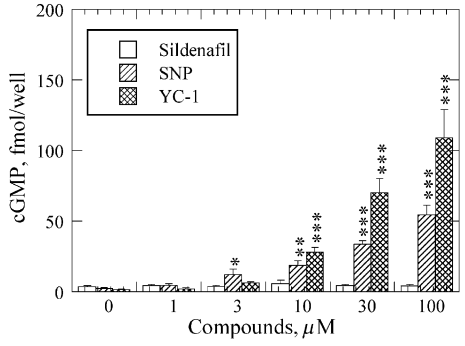

Fig. 4. Cyclic GMP levels in cultured rabbit corpus cavernosal cells after incubation with YC-1 and sodium nitroprusside (SNP), and phosphodiesterase inhibitor sildenafil. Data are expressed as mean ± S.E.M. ($n = 8$); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. vehicle.

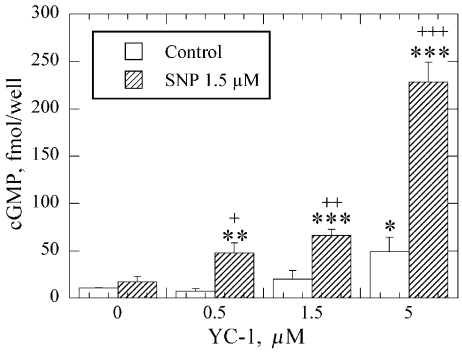

Fig. 5. Sodium nitroprusside (SNP) and YC-1 synergistically increase intracellular cyclic GMP in cultured smooth muscle cells from rabbit corpus cavernosum. Cells were incubated with soluble guanylate cyclase activators in the presence of a nonselective phosphodiesterase inhibitor 100 μM 3-Isobutyl-1-methylxanthine (IBMX) for 10 min. Data are expressed as mean ± S.E.M. ($n = 5$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. vehicle; $^{+}p < 0.05$, $^{++}p < 0.05$, $^{+++}P < 0.01$ vs. control.

In the presence of a suboptimal concentration of sodium nitroprusside 1.5 μM, YC-1 at 0.5–5 μM (which when applied alone exhibited only minor elevation of cyclic GMP accumulation as compared with basal value) markedly increased cyclic GMP concentrations (Fig. 5). These potentiating effects of YC-1 were approximately up to 25-fold of basal cyclic GMP, indicating that sodium nitroprusside and YC-1 synergistically elevated intracellular cyclic GMP levels in rabbit corpus cavernosum smooth muscle cells.

3.3. In vivo rat penile erection studies

The characterization of the conscious rat penile erection was established with apomorphine as a reference standard as

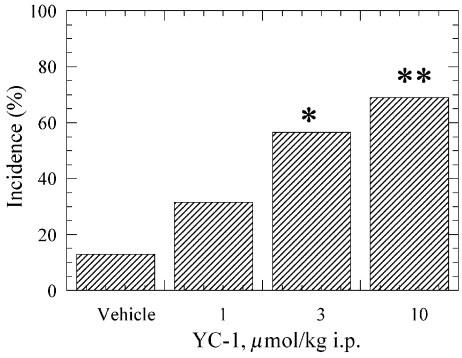

Fig. 6. Pro-erectile effects of the soluble guanylate cyclase activator YC-1 in rats after i.p. administration. $^{*}p < 0.05$, $^{**}p < 0.05$ vs. vehicle.

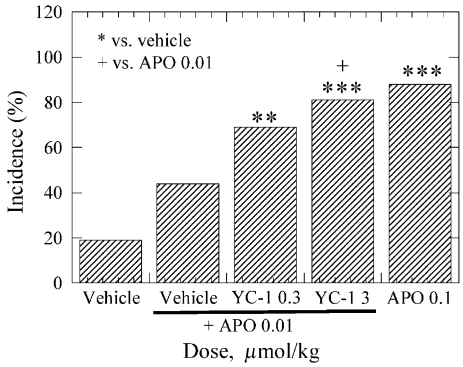

Fig. 7. YC-1 potentiates the effect of a suboptimal dose (0.01 μmol/kg s.c.) of apomorphine (APO) in the conscious rat penile erection model ($n=16$). YC-1 was administered i.p. 10 min before apomorphine dosing. Apomorphine (0.1 μmol/kg s.c.) was included as a positive control. $^{**}p<0.01$, $^{***}p<0.001$, vs. vehicle; $^{+}p<0.05$ vs. control.

this dopaminergic receptor agonist has pro-erectile properties in human and animals (Heaton, 2000). Subcutaneous injection of apomorphine (0.003–1.0 μmol/kg) induced a dose-dependent facilitation of penile erection in rats with maximum effects at 0.1 μmol/kg (Hsieh et al., 2001b). Intraperitoneal injection of YC-1 facilitated penile erection in rats with significant effects at 3–10 μmol/kg (Fig. 6). More importantly, pretreatment (10 min before) with YC-1 was able to increase the incidence of erection in rats, from 44% to 81% after a suboptimal dose of apomorphine (0.01 μmol/kg s.c.). Under this situation YC-1 amplified the effect of apomorphine as erection was presented in >80% of the animals, an efficacy similar to the maximal effects induced by 0.1 μmol/kg apomorphine (Fig. 7).

4. Discussion

The present study demonstrates that the soluble guanylate cyclase activator YC-1 effectively relaxes rabbit corpus cavernosal tissue strips precontracted with phenylephrine in vitro and that the relaxing effect is only partially blocked by ODQ. YC-1 synergistically activates soluble guanylate cyclase in the presence of NO as indicated by increases in intracellular cyclic GMP levels in cavernosal smooth muscle cells. The in vivo systemic administration of YC-1 significantly facilitates penile erection in the conscious rat model.

YC-1 was initially reported as an NO-independent activator of platelet soluble guanylate cyclase that led to the increase of cyclic GMP (Wu et al., 1995). Subsequent work with a soluble guanylate cyclase purified from mammal cells found that YC-1 causes a pronounced sensitization of the enzyme for stimulation by NO as well as an increase in maximal enzyme activity. The cyclic GMP-increasing effect of YC-1 has been reported in vascular smooth muscle cells and an increase in responsiveness toward NO has been shown to be substantially higher in intact cell than in cell-free purified enzyme system (Mulsch et al., 1997). Stimulation of the vascular endothelial cells with YC-1 increased cyclic GMP accumulation up to 100-fold, an effect that was further potentiated by NO (Schmidt et al., 2001). Using an aortic rings organ bath preparation, O'Reilly et al. (2001) also demonstrated that a non-vasorelaxant concentration of YC-1 enhanced the ability of organic nitrates to relax vascular smooth muscle and elevate intravascular cyclic GMP levels.

In order to evaluate the potency and efficacy of YC-1 in the corpus cavernosum, a series of in vitro studies were conducted. YC-1 produced a complete relaxation of phenylephrine-precontracted cavernosal tissue strips. Relaxation was partially (40%) antagonized by the selective soluble guanylate cyclase inhibitor ODQ at concentrations up to 30 μM, whereas the relaxation by the NO-donor sodium nitroprusside was fully abolished by ODQ (Figs. 2 and 3), suggesting that YC-1 may activate soluble guanylate cyclase at a different site from the NO binding site in corpus cavernosal tissues. Expression of soluble guanylate cyclase subunits α1/β1 and α2/β1, the isoforms which are sensitive to activation by NO and YC-1, in corpus cavernosum tissues has been reported (Behrends et al., 2000; Nakane et al., 2002). ODQ binds to soluble guanylate cyclase in an NO-competitive manner and inhibits NO-stimulated activity by oxidizing the ferrous heme in the first complex to a five liganded ferric intermediate (Tseng et al., 2000; Zhao et al., 2000). This prevents both binding and activation by NO, leaving basal activity unchanged. Several investigators have reported that YC-1 binds to soluble guanylate cyclase at a different site from the heme (Friebe and Koesling, 1998; Lee et al., 2000; Mulsch et al., 1997; Schmidt et al., 2001; Stone and Marletta, 1998). Studies carried out in our laboratory have also indicated that YC-1 can act as an allosteric activator in the absence of NO and lead to even more activation in the presence of NO (Nakane et al., 2002).

The relaxing effect of YC-1 in rabbit cavernosal tissue strips is in correlation with the accumulated cyclic GMP level in cavernosal smooth muscle cells (Fig. 4). An intriguing finding reported here is the significant increase in the cellular cyclic GMP levels in cultured cavernosal smooth muscle cells upon treatment with a combination of suboptimal concentrations of YC-1 and NO. These synergistic responses to cyclic GMP are in agreement with the direct synergistic action of YC-1 and NO on the soluble guanylate cyclase enzyme (Nakane et al., 2002). Fig. 4 shows that YC-1 produces a bigger increase in cyclic GMP levels than sodium nitroprusside, even though the latter is a more potent relaxant of the smooth muscle strips. This can be explained by a recent report that YC-1 not only activates soluble guanylate cyclase, but also affects the overall effects on cyclic GMP metabolisms, as it inhibits both cyclic GMP breakdown in rabbit aortic tissue extracts and the activity of phosphodiesterase (Galle et al., 1999).

Cyclic GMP is a major intracellular mediator responsible for the cavernosal smooth muscle relaxation, which in turn

leads to penile erection (Andersson and Wagner, 1995). The increase in cyclic GMP can influence several cellular events that result in smooth muscle relaxation: (1) activation of cyclic GMP-dependent protein kinase G (PKG); (2) activation of cyclic GMP-regulated ion pumps that reduce intracellular Ca^{2+} via Ca^{2+} sequestration and/or extrusion; (3) opening of K^{+} channels causing hyperpolarization of corpus cavernosum smooth muscle cells; and (4) activation of myosin light chain phosphatases (Carvajal et al., 2000; Lucas et al., 2000). Cyclic GMP activity is terminated by hydrolysis of the 3′—5′ bond by the type-5 phosphodiesterase. The documented clinical success of the phosphodiesterase 5 inhibitor sildenafil (Moreland et al., 2001) has further solidified the importance of the peripheral NO/cyclic GMP pathway in the modulation of erection and the etiology of erectile dysfunction.

Results from in vivo behavioral experiments in conscious rats demonstrated that the intraperitoneal injection of YC-1 evoked erectile response with significant effects at 3–10 μmol/kg (Fig. 6), further supporting the important role of cyclic GMP for penile erection. Studies conducted in an anesthetized rat model (Mizusawa et al., 2002) showed that YC-1 elicites dose-dependent increases in intracavernosal pressure when administered intracavernously (1, 3, and 10 μmol/kg), data that is in agreement with our findings.

Apomorphine is a dopaminergic agonist that activates erectile pathways in the brain. As this agent has been extensively studied in human clinical trials (Heaton, 2000), comparisons can be drawn directly between human and animal data. In a conscious rat model established in our laboratory, apomorphine elicited an inverted U-shape dose response in facilitating penile erection after subcutaneous injection. The maximum effect was observed with 0.1 μmol/kg (Hsieh et al., 2001b). Penile erectile activity occurs in response to the NO release mediated by apomorphine (Melis et al., 1996), possibly at both peripheral and central sites, and that modulation of various aspects of sexual physiology and penile erection has been ascribed to the NO/cyclic GMP pathway (Andersson, 2001; Garthwaite and Boulton, 1995; Giuliano and Allard, 2001; Krukoff, 1999; Sato et al., 1999). YC-1 augments the pro-erectile effect of suboptimal dose (0.01 μmol/kg s.c.) of apomorphine as erection was presented in >80% of the animals (Fig. 7), suggesting that stimulation of an NO/cyclic GMP activity by YC-1 may activate pro-erectile pathways and thus facilitate the penile erection in vivo.

In conclusion, the present study demonstrates that YC-1 stimulates soluble guanylate cyclase and synergistically increases cyclic GMP in the presence of NO which in turn leads to relaxation of cavernosal smooth muscle. These biochemical events may be related to the pro-erectile activity in vivo. Agents like YC-1 that binds allosterically to soluble guanylate cyclase to amplify the effects of endogenous NO in an NO/cyclic GMP pathway may represent a novel approach to study penile function.

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