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Relaxing effects induced by the soluble guanylyl cyclase stimulator BAY 41-2272 in human and rabbit corpus cavernosum

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Abstract

5-Cyclopropyl-2-[1-(2-fluoro-benzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-4-ylamine (BAY 41-2272) is a potent soluble guanylyl cyclase stimulator in a nitric oxide (NO)-independent manner. The relaxant effect of BAY 41-2272 was investigated in rabbit and human corpus cavernosum in vitro. BAY 41-2272 (0.01–10 μM) relaxed both rabbit ($pEC_{50} = 6.82 \pm 0.06$) and human ($pEC_{50} = 6.12 \pm 0.10$) precontracted cavernosal strips. The guanylyl cyclase inhibitor (ODQ, 10 μM) caused significant rightward shifts in the concentration–response curves for BAY 41-2272 in rabbit (4.7-fold) and human (2.3-fold) tissues. The NO synthesis inhibitor (*N*-nitro-L-arginine methyl ester (L-NAME), 100 μM) also produced similar rightward shifts, revealing that BAY 41-2272 acts synergistically with endogenous NO to elicit its relaxant effect. The results also indicate that ODQ is selective for the NO-stimulated enzyme, since relaxations evoked by BAY 41-2272 were only partly attenuated by ODQ. The present study shows that both BAY 41-2272 and sildenafil evoke relaxations independent of inhibition of haem in soluble guanylate cyclase. Moreover, there is no synergistic effect of the two compounds in corpus cavernosum.

Keywords: Corpus cavernosum; BAY 41-2272; Soluble guanylyl cyclase; Sildenafil; Nitric oxide (NO)

1. Introduction

Erectile function depends upon an intricate balance and coordination among parasympathetic, sympathetic and nitrergic nerves, neurotransmitters, blood vessels and cavernous muscles (Andersson and Wagner, 1995; Bivalacqua et al., 2000; Andersson, 2001). Nitric oxide (NO) is considered to play a critical role in the control of erectile function, by activating soluble guanylyl cyclase in cavernosal smooth muscle to generate cGMP, which, in turn, promotes relaxation leading to penile erection (Ignarro et al., 1990; Rajfer et al., 1992). The modulation of erectile function by the NO-soluble guanylyl cyclase-cGMP pathway has been supported by (1) the inhibitory effect of both NO synthase and soluble guanylyl cyclase inhibitors on NO-mediated corpus cavernosum relaxation, and (2) the relaxant effects of phosphodiesterase inhibitors, which augment cGMP-induced relaxation (Andersson and Wagner, 1995; Bivalacqua et al., 2000; Andersson, 2001).

NO and NO donors, including glyceryl trinitrate and sodium nitroprusside, are well-known soluble guanylyl cyclase activators (Christ et al., 1995; Feelisch, 1998). The relaxations caused by these substances are enhanced in the presence of sildenafil, which increases cGMP levels by inhibiting phosphodiesterase type 5, the enzyme that degrades cGMP (Moreland et al., 1998; Corbin and Francis, 1999; Corbin et al., 2002). Furthermore, a novel class of soluble guanylyl cyclase stimulators is represented by 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1), which has been reported to directly activate soluble guanylyl cyclase in platelets, vascular smooth muscle and corpus cavernosum (Ko et al., 1994; Friebe et al., 1996; Wegener et al., 1997; Friebe and Koesling, 1998; Mizusawa et al., 2002; Nakane et al., 2002).

The compound 5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-4-ylamine (BAY 41-2272) is a soluble guanylyl cyclase stimulator with similar characteristics to YC-1, however, with a distinctly higher potency and devoid of phosphodiesterase inhibitory activity (Stasch et al., 2001). BAY 41-2272 is a potent vasodilator of aortic rings in vitro and reduces the mean arterial blood pressure in normal and hypertensive rats (Stasch et al., 2001), likely through NO-independent stimulation of the

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enzyme and sensitization of soluble guanylyl cyclase towards NO.

Recently, BAY 41-2272 has been shown to induce corpus cavernosum relaxations in vitro (Kalsi et al., 2003) and to cause penile erection in vivo by synergizing with sodium nitroprusside (Bischoff et al., 2003). In the present work, we studied the mechanisms underlying the relaxing effect of BAY 41-2272 in both rabbit and human corpus cavernosum. In particular, we investigated the resulting effects of pharmacological inhibition of the enzymes involved in the NO-signaling cascade on relaxations induced by BAY 41-2272 as well as the involvement of the cAMP pathway in these responses. The implications of the present findings for the experimental analysis are discussed.

2. Materials and methods

2.1. Corpus cavernosum preparation

Specimens of the proximal part of human corpus cavernosum from 11 patients, aged between 16 and 55 years, who underwent multiple organ donation were used after appropriated informed consent was obtained. The protocol was approved by the University Hospital Ethics Committee (UNICAMP-Campinas, Brazil). Male New Zealand white rabbits (2–3 kg) were anaesthetized with pentobarbital sodium (Hypnol®, 40 mg/kg, i.v.) and exsanguinated via the carotid artery and the penis removed. The protocol was approved by the University Ethics Committee for Experimental Research. Both rabbit and human cavernosal tissues were immediately placed in chilled Krebs solution of the following composition (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_47H_2O$, 1.17 and $CaCl_22H_2O$, 2.5. Corpus cavernosum preparations were obtained, following dissection of the tunica albuginea and surrounding connective tissues.

2.2. Isometric tension recording

Strips were mounted under tension (10 and 20 mN for rabbit and human tissues, respectively) in 10-ml organ chambers as previously described (Teixeira et al., 2003). Isometric tension was recorded using a PowerLab 400™ data acquisition system (software Chart, version 4.0, AD Instruments, MA, USA). The cavernosal strips were allowed to equilibrate for 60 min, and phenylephrine (10 μM) was added in order to increase the basal tone. The generation of prostanoids was inhibited by adding indomethacin (5.6 μM) to the bathing medium.

2.3. Experimental protocols

After the equilibration period, viability of the cavernosal muscle was confirmed following addition of phenylephrine (10 μM). Concentration–response curves were constructed by adding BAY 41-2272 cumulatively (0.01–10 μM) in corpus cavernosum, and relaxations were plotted as percentages of the contraction induced by phenylephrine. One concentration–response curve for BAY 41-2272 was obtained in each strip, due to the lower contractile effect of phenylephrine observed in the subsequent curve. Hence, control cavernosal smooth muscle strips were run in parallel with experimental tissues, where the effects of BAY 41-2272 were studied in presence of either *N*-nitro-L-arginine methyl ester (L-NAME, 100 μM), 1*H*-[1,2,4] oxadiazolo [4,3,-*a*]quinoxalin-1-one (ODQ, 10 μM), sildenafil (0.1 μM) or rolipram (10 μM); these inhibitors were incubated with the preparations for 30 min before a concentration–response curve for BAY 41-2272 was obtained. Similar protocols were performed for sildenafil and forskolin.

Concentration–response curves for glyceryl trinitrate (0.01–10 μM) were constructed in precontracted preparations. Thereafter, the tissues were washed and similar curves were performed 30 min after the addition of either L-NAME (100 μM), ODQ (10 μM) or sildenafil (0.1 μM). Similar protocols were carried out for acetylcholine.

2.4. Drugs and chemicals

Acetylcholine, L-arginine, forskolin, indomethacin, *N*-nitro-L-arginine methyl ester (L-NAME), 1*H*-[1,2,4] oxadiazolo [4,3,-*a*]quinoxalin-1-one (ODQ), phenylephrine and rolipram were purchased from Sigma (St. Louis, USA). Glyceryl trinitrate (Nitronal®, 50 ml clear glass vials filled with colourless isotonic solution containing 1 mg/ml glyceryl trinitrate) was acquired from Lipha Pharmaceuticals (London, UK). Pentobarbital sodium (Hypnol®) and sildenafil citrate were obtained from Laboratorios Cristalia (Itapira, Brazil). The compound 5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-4-ylamine (BAY 41-2272) was provided by Pharma Research Center, Bayer (Wuppertal, Germany). All other reagents used were of analytical grade. Stock solutions of acetylcholine, L-arginine, glyceryl trinitrate, L-NAME and phenylephrine were prepared in deionized water and stored in aliquots at −20 °C; dilutions were made up immediately before use. BAY 41-2272, forskolin, ODQ, rolipram and sildenafil were initially prepared as stock solutions in dimethyl sulphoxide (DMSO) at −20 °C and further diluted in deionized water just before use. Indomethacin was dissolved in 5% sodium carbonate. The final concentration of DMSO did not exceed 0.1%. Preliminary experiments ascertained the lack of response of cavernosal smooth muscle to DMSO in the concentrations employed.

2.5. Statistical analysis

Experimental values were calculated relative to the maximal changes from the contraction produced by phenyleph-

rine in each tissue, which was taken as 100%. Calculations are based on the number of experiments performed in each individual. Data are shown as the percentage of relaxation of *n* experiments, expressed as the mean ± S.E.M. Analysis of variance and Student's paired *t*-test were employed to evaluate the results. A *P* value less than 0.05 was considered to indicate significance. A program package was used for the statistical analysis of all data (GraphPAD Instat, 1997, version 3.00, GraphPAD Software, USA).

3. Results

3.1. Relaxing activity of BAY 41-2272

Phenylephrine (10 μM) caused a sub-maximal contraction in corpus cavernosum preparations from both rabbits (34.7 ± 5.2 mN; $n = 70$) and humans (55.9 ± 9.8 mN; $n = 74$). The cumulative addition of BAY 41-2272 (0.01–10 μM) produced long-lasting and concentration-dependent relaxations in contracted tissues with pEC_{50} values of 6.82 ± 0.06 ($n = 19$) in rabbit and 6.12 ± 0.10 ($n = 25$) in human tissues, with maximal responses of 116 ± 2% and 100 ± 3%, respectively.

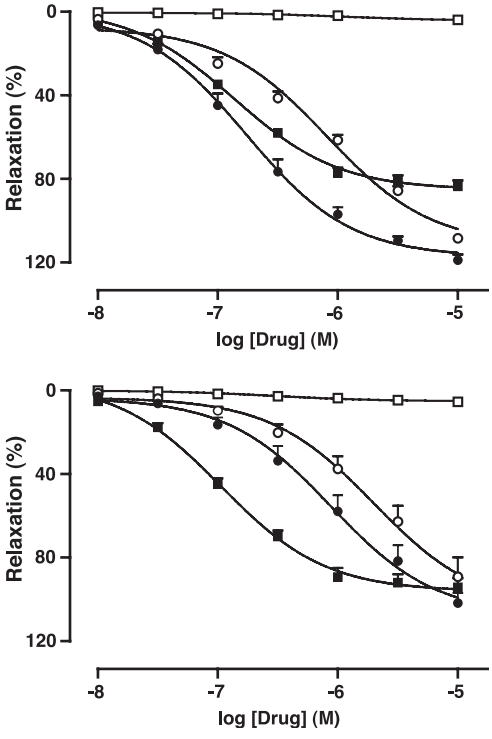

Fig. 1. Effects of the soluble guanylyl cyclase (sGC) inhibitor 1*H*-[1,2,4] oxadiazolo [4,3,-*a*]quinoxalin-1-one (ODQ, 10 μM) on the relaxations induced by the sGC stimulator BAY 41-2272 (0.01 – 10 μM; circles) and the NO donor glyceryl trinitrate (GTN, 0.01 – 10 μM; squares) in both rabbit (top panel) and human (bottom panel) corporeal smooth muscle strips contracted by phenylephrine (10 μM). Addition of ODQ fully prevented the relaxing activity of GTN. The pEC_{50} values for BAY 41-2272 were of 6.76 ± 0.05 and 6.06 ± 0.06 in rabbit and human strips in control conditions, respectively, and of 6.09 ± 0.13 and 5.70 ± 0.07 in rabbit and human strips in the presence of ODQ, respectively ($P < 0.01$). Experimental values were obtained in the absence (filled symbols) and the presence (open symbols) of ODQ, and calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%. Data represent the mean ± S.E.M. of *n* experiments.

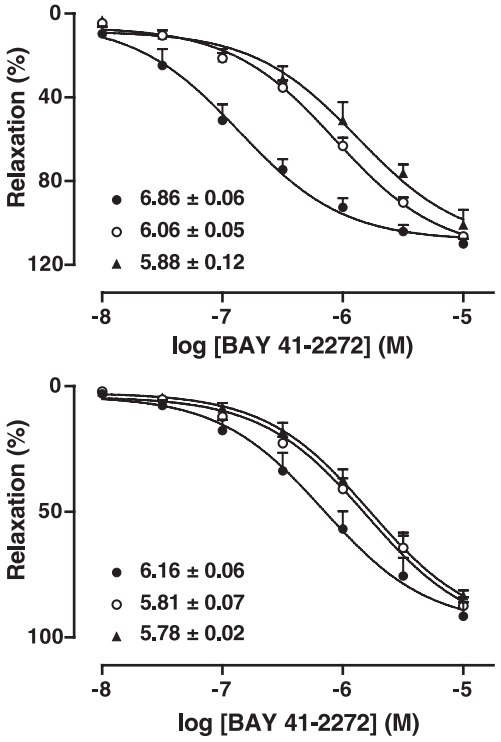

Fig. 2. Effects of the NO synthesis inhibitor *N*-nitro-L-arginine methyl ester (L-NAME, 100 μM) and soluble guanylyl cyclase (sGC) inhibitor 1*H*-[1,2,4] oxadiazolo [4,3,-*a*]quinoxalin-1-one (ODQ, 10 μM) on the relaxations induced by the sGC stimulator BAY 41-2272 (0.01 – 10 μM) in rabbit (top panel) and human (bottom panel) corporeal smooth muscle strips contracted by phenylephrine (10 μM). Experimental values were obtained in the absence (filled circles) and presence of L-NAME (open circles) or L-NAME plus ODQ (filled triangles), and calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%. The pEC_{50} values for BAY 41-2272 in the absence and presence of these inhibitors are shown in the corresponding panels. Data represent the mean ± S.E.M. of *n* experiments.

3.2. Effect of ODQ and L-NAME on BAY 41-2272-induced relaxations

The soluble guanylyl cyclase inhibitor ODQ (10 μM; Fig. 1; $n = 6–7$) and the NO synthesis inhibitor L-NAME (100 μM; Fig. 2; $n = 4–5$) increased the tone of the preparations and caused significant rightward shifts ($P < 0.01$) in the concentration–response curves for BAY 41-2272 in both rabbit (4.7-fold and 5.9-fold for ODQ and L-NAME, respectively) and human corpus cavernosum (2.3-fold and 2.5-fold for ODQ and L-NAME, respectively). The application of ODQ to the bathing medium in tissues pretreated with L-NAME (Fig. 2;

Table 1
Effects of the NO synthesis inhibitor *N*-nitro-L-arginine methyl ester (L-NAME, 100 μM) and soluble guanylyl cyclase inhibitor 1*H*-[1,2,4] oxadiazolo [4,3,-*a*]quinoxalin-1-one (ODQ, 10 μM) on maximal relaxations induced by sildenafil (SILD, 0.01–10 μM), glyceryl trinitrate (GTN, 0.01–10 μM) and acetylcholine (ACh, 0.01–100 μM) in rabbit and human corpus cavernosum (CC) preparations contracted by phenylephrine (10 μM)

	Maximal response (E_{max})							
	Rabbit CC				Human CC			
	Control	L-NAME	Control	ODQ	Control	L-NAME	Control	ODQ
SILD	105 ± 3	93 ± 8	100 ± 6	92 ± 3	97 ± 4	90 ± 4	107 ± 5	93 ± 4
GTN	91 ± 2	90 ± 4	83 ± 2	4 ± 1[a]	89 ± 5	84 ± 4	92 ± 3	5 ± 1[a]
ACh	56 ± 3	2 ± 1[a]	53 ± 4	3 ± 1[a]	65 ± 3	7 ± 1[a]	65 ± 2	7 ± 1[a]

Experimental values were calculated as percentages of relaxation relative to the maximal changes from the contraction produced by phenylephrine. Data represent the mean ± S.E.M. of *n* experiments.
[a] $P < 0.01$ compared to the respective controls.

n = 4) had no significant effect on the concentration–response curve for BAY 41-2272 in either rabbit or human cavernosal preparations.

Cumulative addition of the phosphodiesterase type 5 inhibitor sildenafil (0.01–10 μM) concentration-dependently relaxed rabbit and human corpus cavernosum with pEC_{50} values of 6.73 ± 0.08 (*n* = 13) and 6.58 ± 0.09 (*n* = 16), respectively. As observed with BAY 41-2272, ODQ (*n* = 4) and L-NAME (*n* = 4) also caused significant rightward shifts ($P < 0.01$) in the concentration–response curves for sildenafil (4.2-fold and 6.0-fold for ODQ and L-NAME, respectively) in the rabbit corpus cavernosum. In addition, both inhibitors also caused a minor rightward shift ($P < 0.05$) in the concentration–response curves for sildenafil obtained in human strips (2.6-fold and 2.8-fold for ODQ and L-NAME, respectively; *n* = 4–5). Surprisingly, ODQ and L-NAME failed to affect maximal relaxations elicited by sildenafil (Table 1). As expected, ODQ abolished the relaxations induced by acetylcholine (0.01–100 μM; *n* = 4) and glyceryl trinitrate (0.01–10 μM; *n* = 6) in both cavernosal tissues, whereas L-NAME solely affected the relaxant responses mediated by the former (Table 1).

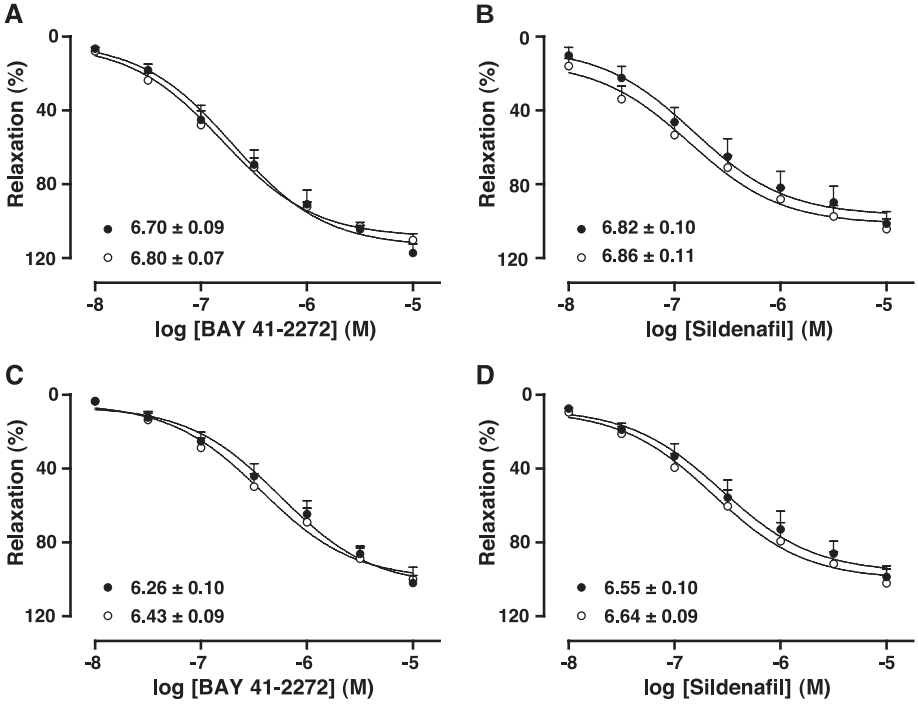

Fig. 3. Effects of the phosphodiesterase type 5 inhibitor sildenafil (0.1 μM) on the relaxations induced by the soluble guanylyl cyclase stimulator BAY 41-2272 (0.01–10 μM) and effects of BAY 41-2272 (0.1 μM) on relaxations induced by sildenafil (0.01–10 μM) in rabbit (top panels) and human (bottom panels) corporeal smooth muscle strips contracted by phenylephrine (10 μM). Experimental values were obtained in the absence (filled circles) and presence (open circles) of BAY 41-2272 (panels B and D) or sildenafil (panels A and C), and calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%. The pEC_{50} values for each set of experiments are shown in the corresponding panels. Data represent the mean ± S.E.M. of *n* experiments.

3.3. Additive effect of BAY 41-2272 and sildenafil

Sildenafil (0.1 μM; $n=4$) caused significant leftward shifts in the concentration–response curves for either glyceryl trinitrate (pEC_{50} values of 6.95 ± 0.04 in the absence and 7.32 ± 0.04 in the presence of sildenafil, respectively; $P<0.01$) or acetylcholine (pEC_{50} values of 5.46 ± 0.08 in the absence and 6.11 ± 0.08 in the presence of sildenafil, respectively; $P<0.01$) in human corpus cavernosum, with similar effects in rabbit preparations.

However, sildenafil had no significant effect on concentration–response curves for BAY 41-2272 in both rabbit ($n=4$) and human ($n=5$) preparations (Fig. 3). Moreover, Fig. 3 also shows that incubation of rabbit ($n=4$) or human ($n=5$) corpus cavernosum with BAY 41-2272 (0.1 μM) did not significantly alter the relaxations elicited by sildenafil. On the other hand, the relaxant effects of sildenafil and BAY 41-2272 were additive (3–30 nM; $n=6$), as attested by the fact that either compound summate, rather than synergize, when added simultaneously in rabbit corpus cavernosum (Fig. 4).

3.4. Effect of rolipram on BAY 41-2272-induced relaxations

In order to investigate a possible involvement of the adenylyl cyclase-cAMP pathway in the relaxations induced by BAY 41-227, we have used the selective phosphodiesterase type 4 inhibitor rolipram (10 μM). The addition of rolipram caused a slight decrease in the tone of the preparations and shifted the concentration–response curve for the adenylyl cyclase stimulator forskolin (0.001–10 μM; $n=4$) to the left both in rabbit as well as in human cavernosal tissues ($P<0.01$; Table 2). Rolipram had no significant effect on the concentration–response curves obtained for BAY 41-2272 either in rabbit or human corpus cavernosum, since the parameters of potency and maximal response were not affected by this inhibitor (Table 2; $n=4$).

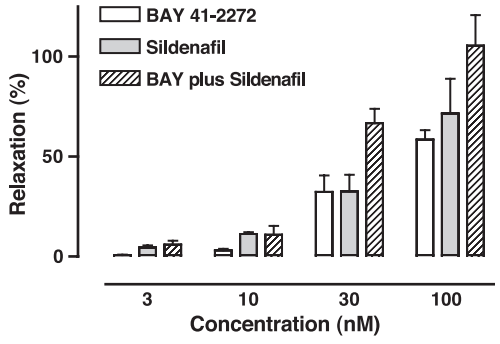

Fig. 4. Additive effect of the soluble guanylyl cyclase stimulator BAY 41-2272 (3–100 nM) and the phosphodiesterase type 5 inhibitor sildenafil (3–100 nM) in rabbit corporeal smooth muscle strips contracted by phenylephrine (10 μM; $n=6$). Experimental values were calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%. Data represent the mean ± S.E.M. of n experiments.

Table 2
Effects of the phosphodiesterase type 4 inhibitor rolipram (10 μM; $n=4$) on the relaxations induced by forskolin (0.001–10 μM) and BAY 41-2272 (0.01–10 μM) in rabbit and human corpus cavernosum (CC) preparations contracted by phenylephrine (10 μM)

	Relaxant potency (pEC_{50})			
	BAY 41-2272		Forskolin	
	Rabbit CC	Human CC	Rabbit CC	Human CC
Control	6.98 ± 0.05	5.99 ± 0.09	7.12 ± 0.02	6.17 ± 0.05
+ Rolipram	6.88 ± 0.08	6.00 ± 0.07	7.58 ± 0.05[a]	6.51 ± 0.02[a]

Experimental values were calculated relative to the maximal changes from the contraction produced by phenylephrine, and represented as-log of the molar concentration to produce 50% of the maximal relaxation elicited by forskolin and BAY 41-2272 in contracted tissues. Data represent the mean ± S.E.M. of n experiments.

[a] $P<0.01$ compared to the respective controls.

4. Discussion

Our results demonstrated that BAY 41-2272 causes sustained relaxant responses in precontracted rabbit and human cavernosal tissues, although differences in pharmacological parameters, such as potency and maximum response, rendered BAY 41-2272 to more effectively relax the rabbit corpus cavernosum. It is noteworthy mentioning that the human corpus cavernosum strips used in a previous study addressing some of the issues presented herein were obtained from patients undergoing transsexual operations, who have been exposed to high levels of estrogens (Kalsi et al., 2003), whereas we have used cavernosal tissue from healthy multiple organ donors. This aspect might account for the different potency of BAY 41-2272 in the present investigation compared to the reported by Kalsi et al. (2003).

ODQ inhibits NO-stimulated soluble guanylyl cyclase activity (Garthwaite et al., 1995) and has been extensively used to study the function of the NO-cGMP transduction pathway. NO donors such as glyceryl trinitrate elicit their relaxing effects through a common mediator, NO (Feelisch, 1998), that binds to the haem site of soluble guanylyl cyclase, activating the enzyme and catalyzing the conversion of GTP to cGMP (Lucas et al., 2000). The inhibitory effect of ODQ on NO-stimulated soluble guanylyl cyclase is due to changes in the oxidation state of the haem moiety, without adverse effects in the catalytic activity of the enzyme (Zhao et al., 2000). Indeed, the corpus cavernosum relaxations induced by exogenous or endogenous NO were abolished by ODQ in the present investigation, since the responses elicited by glyceryl trinitrate and by acetylcholine, which releases NO from the sinusoidal endothelium, were fully prevented by this inhibitor.

Although ODQ has been described to oxidize the haem moiety of soluble guanylyl cyclase, it may also have an allosteric mechanism, by interfering with the binding of BAY 41-2272, as the result of changes in the secondary structure of the recombinant enzyme when ODQ is added to the NO-stimulated soluble guanylyl cyclase (Kosarikov et al., 2001). Nevertheless, it is interesting to observe that in both rabbit and human corpus cavernosum, the relaxations elicited by BAY 41-2272 were only partially affected by ODQ. It is worth pointing out that even in the purified enzyme, ODQ failed to abolish the BAY 41-2272-induced soluble guanylyl cyclase stimulation (Stasch et al., 2001).

Incubation with L-NAME produced a rightward shift in the curve to BAY 41-2272 in rabbit and human corpus cavernosum, indicating that NO release from the endothelium has a synergistic effect with BAY 41-2272, as observed in the purified enzyme (Stasch et al., 2001). Our results cannot discriminate whether this result reflects basal release of NO or else BAY 41-2272 induces NO release. However, the BAY 41-2272 precursor YC-1 does cause NO release from endothelial cells in a Ca^{2+}-dependent manner (Wohlfart et al., 1999). The finding that no further inhibition is noticed in tissues treated with L-NAME and subsequently incubated with ODQ strongly suggests that the rightward shift caused by ODQ on BAY 41-2272-induced relaxation is mainly the result of soluble guanylyl cyclase stimulation by endogenously released NO. These results imply that BAY 41-2272 elicits cavernosal relaxation through a secondary pathway independent on increases of cGMP, or else, ODQ only inhibits NO-induced activation of the enzyme. The finding that the rightward shifts caused by L-NAME were similar to those induced by ODQ further supports the latter hypothesis.

Drugs that stimulate adenylyl cyclase such as forskolin, prostaglandin E_1 and vasoactive intestinal polypeptide (VIP) cause relaxation of corpus cavernosum (Hedlund et al., 2000; Mizusawa et al., 2001). Phosphodiesterase type 4 inhibitors, such as rolipram, enhance the relaxing responses evoked by these agents by preventing cAMP hydrolysis (Sparwasser et al., 1994; Bivalacqua et al., 1999). Indeed, our results showed that rolipram does cause a leftward shift in the concentration–response curve for forskolin. However, the finding that rolipram did not affect the relaxations induced by BAY 41-2272 suggests that the cAMP pathway is not involved in these responses.

Phosphodiesterase type 5 inhibitors, such as sildenafil, relax the cavernosal tissue by preventing cGMP breakdown (Corbin et al., 2002). Thus, soluble guanylyl cyclase activity is essential for the relaxing effect of phosphodiesterase type 5 inhibitors to occur. The relaxant responses mediated by glyceryl trinitrate and acetylcholine were significantly enhanced by sildenafil but unexpectedly, the relaxations evoked by sildenafil were just partly attenuated by treatment with either L-NAME or ODQ in the present investigation. Our hypothesis that ODQ is a selective inhibitor of NO-induced activation of soluble guanylyl cyclase is further strengthened by its partial inhibitory effect on sildenafil-induced relaxations, raising interesting possibilities for other endogenous stimulators of soluble guanylyl cyclase.

Several investigations have identified the presence of soluble guanylyl cyclase in a variety of cell types by immunohistochemistry using antibodies directed against the α_1 and β_1 subunits (Heinrich et al., 2000; Burette et al., 2001; Fathian-Sabet et al., 2001; Teunissen et al., 2001). Most importantly, studies on the subcellular distribution of the soluble guanylyl cyclase $\alpha_1\beta_1$ isoform revealed that a substantial portion of the total content of the enzyme is associated with the cellular membrane from several cell types in a state of enhanced sensitivity to NO (Zabel et al., 2002). Another report also demonstrated the targeting of the $\alpha_2\beta_1$ isoform to synaptic membranes through interaction with postsynaptic density protein 95 (PSD-95) in rat brain (Russwurm et al., 2001). In the present investigation, no leftward shift was noticed in the concentration–response curve for BAY 41-2272 when the cavernosal tissues were pre-incubated with sildenafil and vice versa, bringing up the hypothesis of distinct pools of soluble guanylyl cyclase in the corpus cavernosum smooth muscle. Moreover, this idea is further supported by the demonstration that the relaxant effects of both substances are purely additive, in addition to the spatial confinement studies on soluble guanylyl cyclase subcellular location (Zabel et al., 2002).

In conclusion, our results demonstrated that BAY 41-2272 potently relaxes both rabbit and human corpus cavernosum, synergistically with NO released from the sinusoidal endothelium. The findings that ODQ completely prevented the relaxations induced by glyceryl trinitrate and acetylcholine, but not those evoked by BAY 41-2272 or sildenafil, indicate that ODQ solely inhibits the NO-stimulated enzyme. In addition, the observation that the effects of BAY 41-2272 and sildenafil are merely additive suggests the existence of distinct pools of soluble guanylyl cyclase in cavernosal smooth muscle.

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References

Andersson, K.E., 2001. Pharmacology of penile erection. Pharmacol. Rev. 53, 417–450.

Andersson, K.E., Wagner, G., 1995. Physiology of penile erection. Physiol. Rev. 75, 191–236.

Bischoff, E., Schramm, M., Straub, A., Feurer, A., Stasch, J.P., 2003. BAY 41-2272: a stimulator of soluble guanylyl cyclase induces nitric oxide-dependent penile erection in vivo. Urology 61, 464–467.

Bivalacqua, T.J., Champion, H.C., Rajasekaran, M., Sikka, S.C., Kadowitz, P.J., Doherty, P.C., Hellstrom, W.J., 1999. Potentiation of erectile re-

sponse and cAMP accumulation by combination of prostaglandin E1 and rolipram, a selective inhibitor of the type 4 phosphodiesterase (PDE4). J. Urol. 162, 1848–1855.

Bivalacqua, T.J., Champion, H.C., Hellstrom, W.J., Kadowitz, P.J., 2000. Pharmacotherapy for erectile dysfunction. Trends Pharmacol. Sci. 21, 484–489.

Burette, A., Petrusz, P., Schmidt, H.H., Weinberg, R.J., 2001. Immunohistochemical localization of nitric oxide synthase and soluble guanylyl cyclase in the ventral cochlear nucleus of the rat. J. Comp. Neurol. 431, 1–10.

Christ, G.J., Kim, D.C., Taub, H.C., Gondre, C.M., Melman, A., 1995. Characterization of nitroglycerine-induced relaxation in human corpus cavernosum smooth muscle: implications to erectile physiology and dysfunction. Can. J. Physiol. Pharm. 73, 1714–1726.

Corbin, J.D., Francis, S.H., 1999. Cyclic GMP phosphodiesterase-5: target of sildenafil. J. Biol. Chem. 274, 13729–13732.

Corbin, J.D., Francis, S.H., Webb, D.J., 2002. Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. Urology 60, 4–11.

Fathian-Sabet, B., Bloch, W., Klotz, T., Niggemann, S., Jacobs, G., Addicks, K., Engelmann, U., 2001. Localization of constitutive nitric oxide synthase isoforms and the nitric oxide target enzyme soluble guanylyl cyclase in the human bladder. J. Urol. 165, 1724–1729.

Feelisch, M., 1998. The use of nitric oxide donors in pharmacological studies. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 113–122.

Friebe, A., Koesling, D., 1998. Mechanism of YC-1-induced activation of soluble guanylyl cyclase. Mol. Pharmacol. 53, 123–127.

Friebe, A., Schultz, G., Koesling, D., 1996. Sensitizing soluble guanylyl cyclase to become a highly CO-sensitive enzyme. EMBO J. 15, 6863–6868.

Garthwaite, J., Southam, E., Boulton, C.L., Nielsen, E.B., Schmidt, K., Mayer, B., 1995. Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one. Mol. Pharmacol. 48, 184–188.

Hedlund, P., Aszodi, A., Pfeifer, A., Alm, P., Hofmann, F., Ahmad, M., Fassler, R., Andersson, K.E., 2000. Erectile dysfunction in cyclic GMP-dependent kinase I-deficient mice. Proc. Natl. Acad. Sci. U. S. A. 97, 2349–2354.

Heinrich, U., Maurer, J., Koesling, D., Mann, W., Forstermann, U., 2000. Immuno-electron microscopic localization of the alpha(1) and beta(1)-subunits of soluble guanylyl cyclase in the guinea pig organ of corti. Brain Res. 885, 6–13.

Ignarro, L.J., Bush, P.A., Buga, G.M., Wood, K.S., Fukuto, J.M., Rajfer, J., 1990. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem. Biophys. Res. Commun. 170, 843–850.

Kalsi, J.S., Rees, R.W., Hobbs, A.J., Royle, M., Kell, P.D., Ralph, D.J., Moncada, S., Cellek, S., 2003. BAY41-2272, a novel nitric oxide independent soluble guanylate cyclase activator, relaxes human and rabbit corpus cavernosum in vitro. J. Urol. 169, 761–766.

Ko, F.N., Wu, C.C., Kuo, S.C., Lee, F.Y., Teng, C.M., 1994. YC-1, a novel activator of platelet guanylate cyclase. Blood 84, 4226–4233.

Kosarikov, D.N., Lee, J.M., Uversky, V.N., Counts-Gerber, N., 2001. Role of conformational changes in the heme-dependent regulation of human soluble guanylate cyclase. J. Inorg. Biochem. 87, 267–276.

Lucas, K.A., Pitari, G.M., Kazerounian, S., Ruiz-Stewart, I., Park, J., Schulz, S., Chepenik, K.P., Waldman, S.A., 2000. Guanylyl cyclases and signaling by cyclic GMP. Pharmacol. Rev. 52, 375–414.

Mizusawa, H., Hedlund, P., Hakansson, A., Alm, P., Andersson, K.E., 2001. Morphological and functional in vitro and in vivo characterization of the mouse corpus cavernosum. Br. J. Pharmacol. 132, 1333–1341.

Mizusawa, H., Hedlund, P., Brioni, J.D., Sullivan, J.P., Andersson, K.E., 2002. Nitric oxide independent activation of guanylate cyclase by YC-1 causes erectile responses in the rat. J. Urol. 167, 2276–2281.

Moreland, R.B., Goldstein, I., Traish, A., 1998. Sildenafil, a novel inhibitor of phosphodiesterase type 5 in human corpus cavernosum smooth muscle cells. Life Sci. 62, 309–318.

Nakane, M., Hsieh, G., Miller, L.N., Chang, R., Terranova, M.A., Moreland, R.B., Kolasa, T., Brioni, J.D., 2002. Activation of soluble guanylate cyclase causes relaxation of corpus cavernosum tissue: synergism of nitric oxide and YC-1. Int. J. Impot. Res. 14, 121–127.

Rajfer, J., Aronson, W.J., Bush, P.A., Dorey, F.J., Ignarro, L.J., 1992. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N. Engl. J. Med. 326, 90–94.

Russwurm, M., Wittau, N., Koesling, D., 2001. Guanylyl cyclase/PSD-95 interaction: targeting of the nitric oxide-sensitive alpha2beta1 guanylyl cyclase to synaptic membranes. J. Biol. Chem. 276, 44647–44652.

Sparwasser, C., Drescher, P., Will, J.A., Madsen, P.O., 1994. Smooth muscle tone regulation in rabbit cavernosal and spongiosal tissue by cyclic AMP- and cyclic GMP-dependent mechanisms. J. Urol. 152, 2159–2163.

Stasch, J.P., Becker, E.M., Alonso-Alija, C., Apeler, H., Dembowsky, K., Feurer, A., Gerzer, R., Minuth, T., Perzborn, E., Pleiss, U., Schroder, H., Schroeder, W., Stahl, E., Steinke, W., Straub, A., Schramm, M., 2001. NO-independent regulatory site on soluble guanylate cyclase. Nature 410, 212–215.

Teixeira, C.E., Ifa, D.R., Corso, G., Santagada, V., Caliendo, G., Antunes, E., De Nucci, G., 2003. Sequence and structure–activity relationship of a scorpion venom toxin with nitrergic activity in rabbit corpus cavernosum. FASEB J. 17, 485–487.

Teunissen, C., Steinbusch, H., Markerink-van Ittersum, M., Koesling, D., de Vente, J., 2001. Presence of soluble and particulate guanylyl cyclase in the same hippocampal astrocytes. Brain Res. 891, 206–212.

Wegener, J.W., Gath, I., Forstermann, U., Nawrath, H., 1997. Activation of soluble guanylyl cyclase by YC-1 in aortic smooth muscle but not in ventricular myocardium from rat. Br. J. Pharmacol. 122, 1523–1529.

Wohlfart, P., Malinski, T., Ruetten, H., Schindler, U., Linz, W., Schoenafinger, K., Strobel, H., Wiemer, G., 1999. Release of nitric oxide from endothelial cells stimulated by YC-1, an activator of soluble guanylyl cyclase. Br. J. Pharmacol. 128, 1316–1322.

Zabel, U., Kleinschnitz, C., Oh, P., Nedvetsky, P., Smolenski, A., Muller, H., Kronich, P., Kugler, P., Walter, U., Schnitzer, J.E., Schmidt, H.H., 2002. Calcium-dependent membrane association sensitizes soluble guanylyl cyclase to nitric oxide. Nat. Cell Biol. 4, 307–311.

Zhao, Y., Brandish, P.E., DiValentin, M., Schelvis, J.P., Babcock, G.T., Marletta, M.A., 2000. Inhibition of soluble guanylate cyclase by ODQ. Biochemistry 39, 10848–10854.