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SPECIAL REPORT

The Rho-kinase inhibitor Y-27632 and the soluble guanylyl cyclase activator BAY41-2272 relax rabbit vaginal wall and clitoral corpus cavernosum

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The effects of Y-27632, a Rho-kinase inhibitor and BAY41-2272, a soluble guanylyl cyclase activator, on the tone and nitrergic responses of rabbit vaginal wall and clitoral corpus cavernosum were investigated. Y-27632 and BAY41-2272 (10 nM – 10 μM) elicited concentration-dependent relaxation of phenylephrine-induced tone in both tissues. IC_{50} values of Y-27632 for vaginal and clitoral tissues were 370 ± 30 nM, and 467 ± 14 nM, respectively. BAY41-2272 had IC_{50} values of 478 ± 54 nM and 304 ± 38 nM respectively. The effect of the Y-27632 on the tissue tone was not affected by an inhibitor of nitric oxide synthase (L-NAME; 500 μM). However, L-NAME reduced the potency of BAY41-2272 in the clitoral corpus cavernosum but not in the vaginal wall. BAY41-2272 enhanced nitrergic relaxation responses only in the clitoral corpus cavernosum. Y-27632 had no effect on nitrergic relaxations in either tissue. These results demonstrate that Y-27632 and BAY41-2272 elicit relaxation of the rabbit vaginal wall and clitoral corpus cavernosum.
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Keywords: Rho-kinase; soluble guanylyl cyclase; female sexual arousal disorder; clitoris; vagina; nitric oxide; female sexual dysfunction

Abbreviations: EFS, electrical field stimulation; FSAD, female sexual arousal disorder; NANC, non-adrenergic non-cholinergic; NO, nitric oxide; sGC, soluble guanylyl cyclase

Introduction Female sexual arousal disorder (FSAD) has been defined as persistent or recurrent inability to attain, or to maintain until completion of sexual activity, an adequate lubrication-swelling response of sexual excitement, causing personal distress (American Psychiatric Association, 2000). The disorder is characterized by diminished vaginal lubrication, decreased clitoral engorgement and lack of vaginal wall relaxation (Goldstein, 2000), all of which result from a lack of smooth muscle relaxation. Studies on diabetic female rats have suggested that a defective nitric oxide (NO)-cGMP pathway might be responsible for the decreased smooth muscle relaxant responses (Giraldi *et al*., 2001). Therefore treatment modalities which do not depend on intact NO production are required for FSAD. Two groups of compounds: Rho-kinase inhibitors (Chitaley *et al*., 2001; Rees *et al*., 2001) and soluble guanylyl cyclase (sGC) activators (Kalsi *et al*., 2003; Mizusawa *et al*., 2002), which do not require endogenous NO to relax smooth muscle, have been proposed as treatments for male erectile dysfunction. To my knowledge the effects of such compounds have not been investigated in the vaginal wall and clitoral corpus cavernosum.

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Methods Female New Zealand white rabbits (3.5–4.0 kg, Harlan, U.K.) were killed by an overdose of pentobarbitone (Euthatal, Rhône Merieux, U.K.) injected into the ear marginal vein. The vaginal canal, including the clitoris, was excised down to the pubic bone and transferred to modified Krebs' solution consisting of (mM): NaCl 136.9, KCl 2.7, $MgSO_4$ 0.6, $NaHCO_3$ 11.9, KH_2PO_4 0.5, $CaCl_2$ 1.8, glucose 12.5, dexamethasone 0.01, indomethacin 0.01. The modified Krebs' solution was kept at room temperature and gassed with 95% O_2/5% CO_2. Four strips of vaginal wall and two strips of clitoral corpus cavernosum were isolated as described previously (Cellek & Moncada, 1998; Ziessen *et al*., 2002). The ends of the strips were tied with silk sutures and mounted horizontally between two platinum electrodes in superfusion chambers continually perfused at 1 ml min^{-1} with modified Krebs' solution at 37°C. One end of the preparation was tied to a Grass FT03C force-displacement transducer connected to a Linearcorder WR3101 (Graphtec, U.K.) for measurement of isometric changes in tension. The mechanical responses were also recorded on a computer running specialized software (Axotape, Axon Instruments, U.S.A.). The preparations were stretched to approximately their *in situ* length by applying tension of 0.4 g and allowed to equilibrate for 90 min without stimulation. After the equilibration period the tissue strips were stimulated with electrical field stimulation (EFS; 5 s trains of rectangular pulses of 50 V, 0.3 ms pulse duration, 0.5–10 Hz, delivered by Grass S88 stimulators). Noradrenergic and cholinergic responses were blocked by addition of guanethidine (10 μM) and scopolamine (10 μM) respectively, and the tissue tone was raised with a sub-maximal concentration of phenyl-

ephrine (5–10 μM), revealing non-adrenergic non-cholinergic (NANC) relaxant responses. Drugs were introduced by addition to the reservoir feeding the superfusion chamber. The Rho-kinase inhibitor (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate (Y-27632) was purchased from Calbiochem, U.K. The sGC activator 5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1*H*-pyrazolo[3,4-*b*]pyridine-3-yl]-pyrimidin-4-ylamine (BAY41-2272) was a gift from Bayer AG, Germany. All other chemicals were purchased from Sigma, U.K.

In one set of experiments concentration-response curves to BAY41-2272 and Y-27632 were obtained in phenylephrine-contracted tissues in the absence or presence of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 500 μM). In another set of experiments NANC relaxation responses to various frequencies of EFS (0.5–10 Hz) were recorded in the absence or presence of the two drugs at sub-threshold concentrations that did not alter the tone (30 nM for each).

Results are expressed as mean values ± standard error of mean from a number (*n*) of animals. Statistical analyses were performed using GraphPad Prism software (Version 3.0; GraphPad Software Inc, U.S.A.). IC_{50} values were calculated from concentration-response curves using Microcal Origin Software (Version 4.10; Microcal Software Inc., U.S.A.). The relaxation responses elicited with EFS at various frequencies were measured as the area under the curve (AUC) using Clampfit Software (Version 8.2; Axon Instruments, U.S.A.) in order to assess the effect of drugs on both the magnitude and duration of the response. Data were compared by Student's unpaired *t*-test. *P* values of less than 0.05 were considered significant.

Results The Rho-kinase inhibitor Y-27632 and the sGC activator BAY41-2272 (each at 10 nM–10 μM) caused concentration-dependent reductions in phenylephrine-induced tone in both the vaginal wall and the clitoral corpus cavernosum (Figure 1 and Table 1). Y-27632 was more potent in the vaginal wall than in the clitoral corpus cavernosum (Table 1). BAY41-2272 was more potent in the clitoral corpus cavernosum than in the vaginal wall (Table 1). In the presence of L-NAME (500 μM), the potency of Y-27632 in either tissue did not differ from the control values (Table 1). The IC_{50} value for BAY41-2272 was unchanged in the vaginal wall but was significantly higher in the clitoral corpus cavernosum in the presence of L-NAME (Table 1).

The duration and magnitude of nitrergic relaxation responses were not affected in the presence of a sub-threshold concentration (30 nM) of Y-27632 in either tissue (not shown). In the presence of 30 nM BAY41-2272, however, the nitrergic responses were augmented in the clitoral corpus cavernosum (Figure 2) but not in the vaginal wall (not shown).

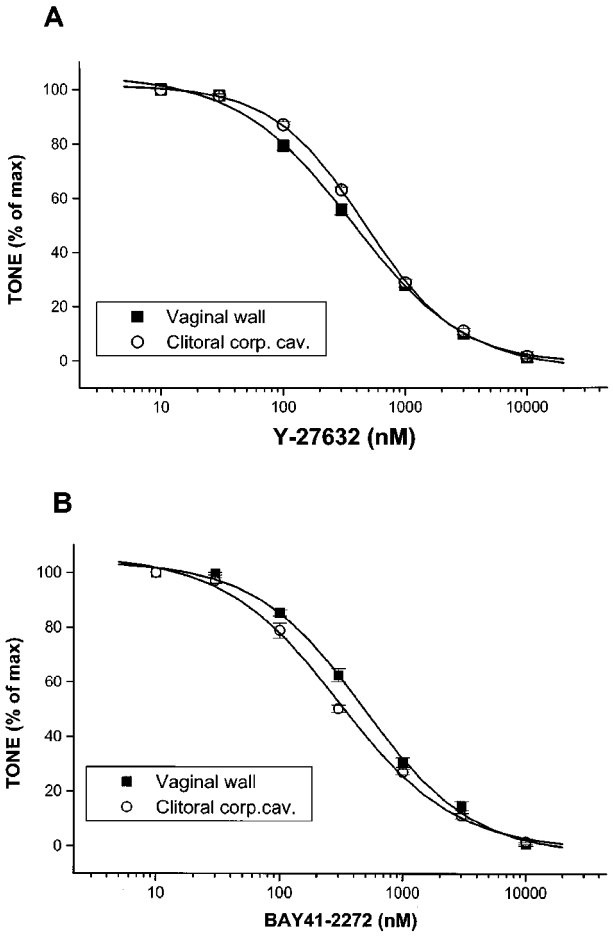

Figure 1 The effect of Y-27632 (A) and BAY41-2272 (B) on phenylephrine-induced tone of rabbit vaginal wall (black squares) and clitoral corpus cavernosum (open circles). $n=4$.

Discussion The contraction of smooth muscle is regulated by mechanisms involving an increase in intracellular calcium concentrations $[Ca^{2+}]_i$ and/or increasing Ca^{2+}-sensitivity without changing $[Ca^{2+}]_i$. One of the calcium-sensitizing pathways involves RhoA, a small, monomeric G-protein that activates Rho-kinase. Activated Rho-kinase phosphorylates the regulatory subunit of smooth muscle myosin phosphatase (SMPP-1M). Inhibitory phosphorylation of SMPP-1M leads to sensitization of myofilaments to Ca^{2+} (for review see Somlyo & Somlyo, 2000). A specific inhibitor of Rho-kinase, Y-27632 (Uehata *et al*., 1997), has been shown to relax vascular (Uehata *et al*., 1997) and penile cavernosal (Rees *et al*., 2001) smooth muscle. Intracavernosal application of Y-27632 has been shown to induce erection in anaesthetized rats in an NO-independent manner (Chitaley *et al*., 2001).

NO-independent activators of sGC include a benzylindazole derivative, YC-1, which has been shown to relax vascular (Mulsch *et al*., 1997) and cavernosal (Mizusawa *et al*., 2002) smooth muscle. However, it has also been reported to have inhibitory activity on phosphodiesterase (PDE; Friebe *et al*., 1998) and to stimulate the synthesis and release of NO (Wohlfart *et al*., 1999). Recently, a pyrazolopyridine derivative, BAY41-2272, has been synthesized and shown to stimulate sGC in a NO-independent manner (Stasch *et al*., 2001). It has been shown to have a distinctly higher potency than YC-1 and no PDE inhibitory activity (Stasch *et al*., 2001). BAY41-2272 has been shown to induce vasodilatation (Stasch *et al*., 2001) and to relax human and rabbit penile corpus cavernosum (Kalsi *et al*., 2003).

In this study I have shown that both Y-27632 and BAY41-2272 cause concentration-dependent relaxation of vaginal and clitoral smooth muscle. The IC_{50} of Y-27632 in reducing phenylephrine-induced tone in the rat and rabbit penile corpus cavernosum has been found to be in the region of

Table 1 IC_{50} values (nM) of Y-27632 and BAY41-2272 in rabbit vaginal wall and clitoral corpus cavernsum for lowering of phenylephrine-induced tone

	Vaginal wall		*Clitoral corpus cavernosum*	
	Control	*+L-NAME*	*Control*	*+L-NAME*
Y-27632	370±30	363±22	467±14*	472±23
BAY41-2272	478±54	475±46	304±38*	1826±444†

EC_{50} values are mean±s.e.mean of four separate experiments. *$P<0.05$ vs the same compound on vaginal wall in the absence of L-NAME. †$P<0.05$ vs the same compound on clitoral corpus cavernosum in the absence of L-NAME.

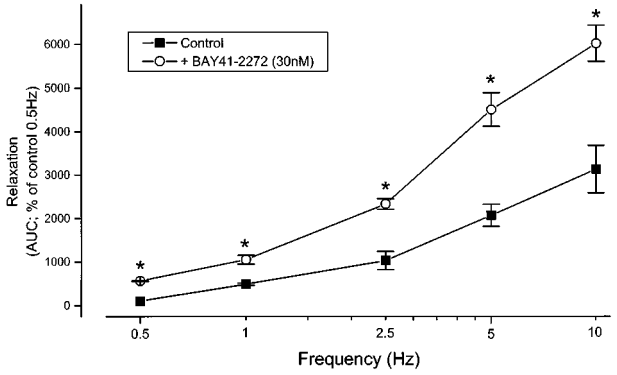

Figure 2 The effect of a sub-threshold concentration (30 nM) of BAY41-2272 on NANC relaxation responses of the clitoral corpus cavernosum elicited by EFS (50 V, 0.3 ms duration, for 5 s) at varying frequencies (0.5–10 Hz). Relaxation responses were calculated as area under the curve (AUC) and expressed as the percentage of relaxation of the same tissue at 0.5 Hz in the absence of BAY41-2272. *$P<0.05$ vs control; $n=4$.

1 μM (Chitaley *et al*., 2001; Rees *et al*., 2001). In the present study the IC_{50} value for Y-27632 was around 370 nM in the vaginal wall and 470 nM in the clitoral corpus cavernosum. The higher potency of the compound in the female genital smooth muscle may be due to higher expression of RhoA in female genital tissues than in the male corpus cavernosum. Alternatively the diffusion of the compound into smooth muscle cells might be different in female and male tissues. Indeed, the expression of RhoA has recently been found to be 17 times greater in penile cavernosal smooth muscle than in other smooth muscle (Wang *et al*., 2002). The levels of expression of RhoA in male and female genital smooth muscle are currently under investigation.

Y-27632 was also found to be more potent in the vaginal wall than in the clitoral corpus cavernosum in this study (Table 1). Again this may be due to different expression levels of RhoA in the two genital tissues or to a difference in the diffusion rates.

The potency of Y-27632 was unaltered in the presence of an inhibitor of NO synthase, suggesting that the compound can induce smooth muscle relaxation in a NO-independent manner. Furthermore, sub-threshold concentration of Y-27632 did not alter nitrergic relaxations, suggesting that Y-27632 does not interact with endogenous NO.

The potency of BAY41-2272 in reducing phenylephrine-induced tone in female genital smooth muscle was similar to that in penile cavernosal smooth muscle (this study and Kalsi *et al*., 2003). Interestingly BAY41-2272 was more potent in the clitoral corpus cavernosum than in the vaginal wall. This might be due to the fact that NO mediates only 30% of the NANC relaxation responses in the vaginal wall whereas NANC relaxation responses are completely nitrergic in the clitoral corpus cavernosum (Ziessen *et al*., 2002). Therefore one would assume there would be more NO available in the clitoral cavernosum than in the vaginal wall. Since the effect of BAY41-2272 on sGC is known to be potentiated by NO (Stasch *et al*., 2001), the higher NO content in the clitoral corpus cavernosum might be augmenting the effect of the sGC activator. Indeed, such a potentiating effect of endogenous NO on BAY41-2272-induced relaxation responses has previously been reported in the penile corpus cavernosum (Kalsi *et al*., 2003). Furthermore L-NAME altered the potency of BAY41-2272 in the clitoral corpus cavernosum but not in the vaginal wall, further indicating that the amount of available endogenous NO is greater in the clitoral corpus than in the vaginal wall. Moreover BAY41-2272, at a sub-threshold concentration that did not affect the tone (30 nM), was able to enhance the magnitude and the duration of nitrergic responses in the clitoral corpus cavernosum but not in the vaginal wall, further confirming the above.

In conclusion, the Rho-kinase inhibitor Y-27632 and the sGC activator BAY41-2272 can induce relaxation in the female genital smooth muscle. Thus Rho-kinase and sGC are therapeutic targets for the treatment of FSAD.

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