Regulation of intracellular Ca\(^{2+}\) release in corpus cavernosum smooth muscle: synergism between nitric oxide and cGMP

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THE TONE OF VASCULAR SMOOTH MUSCLE in the corpus cavernosum regulates penile tumescence: tonic contraction maintains the flaccid state, and relaxation leads to erection. Contraction of penile smooth muscle cells (SMCs) is modulated by various neurotransmitters and locally produced vasoactive substances. Considerable evidence suggests that norepinephrine is primarily responsible for tonic contraction of corpus cavernous SMCs through activation of \(\alpha\)-adrenergic receptors (1). Agonist binding to \(\alpha\)-adrenergic receptors activates a G protein-coupled pathway increasing intracellular inositol 1,4,5-trisphosphate (IP\(_3\)) and activation of IP\(_3\) receptors, followed by Ca\(^{2+}\) release (3).

Nitric oxide (NO), which is released by both endothelial cells and nonadrenergic, noncholinergic nerves, leads to relaxation of corpus cavernous and is necessary for erection (6, 28). NO has a variety of cellular effects including direct effects on ion channels and activation of soluble guanylyl cyclase (sGC), which converts GTP to cGMP (4, 26). cGMP in turn activates cGMP-dependent ion channels, cGMP-dependent protein kinase (PKG), and cGMP-regulated phosphodiesterases (PDEs). It has been suggested that, in vascular smooth muscle, most of the cGMP effects are mediated by PKG, because in PKG-1-deficient mice, aortic and corpus cavernosum smooth muscles fail to relax upon activation of the NO/cGMP pathway (16, 27). PKG has a variety of effects in smooth muscle, including inhibition of the IP\(_3\) receptor (30) and regulation of the Ca\(^{2+}\) sensitivity of contractile proteins (23). Specific PKG mechanisms contributing to the regulation of SMC tone almost certainly vary from tissue to tissue, and the identity of specific targets involved in relaxation of corpus cavernosum SMCs remains uncertain.

PDE types 2, 3, 5, and 11 are expressed in corpus cavernosum SMCs, although the main PDE activity is due to PDE5, which hydrolyzes cGMP (5, 10, 25). Sildenafil citrate (Viagra) acts by enhancing NO-mediated smooth muscle relaxation by competitive inhibition of PDE5, thereby maintaining elevated intracellular cGMP levels (2, 5).

We investigated the effect of NO on adrenergically stimulated changes in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in freshly isolated corpus cavernous SMCs and found that NO and cGMP act synergistically to reduce Ca\(^{2+}\) release from intracellular stores. Reduction of intracellular Ca\(^{2+}\) release may contribute to relaxation of the corpus cavernosum, leading to erection.

MATERIALS AND METHODS

**Rat cell isolation.** Male Sprague-Dawley rats weighing 300–550 g were killed by injection of euthanyl (400 mg/kg ip). The penis was removed, and the smooth muscle tissue was isolated from the crura and dissociated using (in mg/ml) 1 papain, 2.4 BSA, 0.18 L-dithiothreitol, and 1.2 Sigma blend collagenase type F. Tissues were placed in a gently shaking water bath at 31°C for 30–60 min and dispersed by trituration for immediate use or stored overnight at 4°C. The following day, tissues were warmed to room temperature for 30 min and then dispersed. All cells were studied within 8 h. Procedures for animal handling were carried out in accordance with the guidelines of the Canadian Council on Animal Care. Experiments were carried out in rat corpus cavernosum cells unless specifically stated otherwise.

**Human tissue.** Tissue collection was carried out in accordance with guidelines of the University Review Board for Research Involving Human Subjects and conformed to the Helsinki Declaration. Fragments of corpus cavernosum were retrieved during reconstructive surgery and implant of penile prostheses. Tissue was obtained from...
patients with neurological damage following surgery for cancer or from patients with Peyronie's disease in which the corpus tissue is unaffected. Two samples were obtained from patients with diabetes. No differences in the responses to phenylephrine (PE) or S-nitroso-N-acetylpenicillamine (SNAP) and sildenafil were observed among the cells from different sources. Segments of cavernosal tissue (~1 mm²) were dissociated as described previously (20).

Measurement of \([Ca^{2+}]_i\). Cells were loaded with 0.2 \(\mu M\) fura-2 acetoxymethyl ester (AM) for 20–40 min at room temperature and allowed to settle on a glass perfusion chamber. The chamber was mounted on a Nikon inverted microscope and perfused with solution at 1–3 mL/min at room temperature. Cells were relaxed and contracted reversibly upon stimulation with phenylephrine. Cells were illuminated with alternating 345- and 380-nm light using a Deltascan system (Photon Technology International, London, ON, Canada), with the 510-nm emission detected using a photometer. \([Ca^{2+}]_i\) was calibrated using the methods of Grynkiewicz et al. (14).

Solutions and chemicals. Solutions used for tissue retrieval and dissociation have been described previously (20). To minimize changes in cell membrane potential and reduce \(Ca^{2+}\) influx, we used a bath solution for \([Ca^{2+}]_i\), measurements containing (in mM) 135 KCl, 20 HEPES, 10 \(\beta\)-glucose, 1 CaCl₂, and 1 MgCl₂ (pH 7.4 with KOH). For zero-\(Ca^{2+}\) bath solution, \(Ca^{2+}\) was replaced with 0.5 mM EGTA. Similar basal \([Ca^{2+}]_i\), and \(Ca^{2+}\) transients were observed for cells bathed in Na-HEPES solution (NaCl replaced 130 mM KCl). Sodium nitroprusside (SNP) and 8-(4-chlorophenylthio)-cGMP (8-pCPT-cGMP) were obtained from Sigma (St. Louis, MO); SNAP, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1), 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), and 8-bromo-cGMP (8-Br-cGMP) were from Calbiochem (San Diego, CA); and chemicals for the bath solutions were from BDH Limited (Toronto, ON, Canada). Sildenafil citrate was from Pfizer. Fura-2 AM (Molecular Probes, Eugene, OR) was prepared in dimethyl sulfoxide. PE was applied focally to cells by pneumatic ejection from a micropipette attached to a Picospritzer (General Valve, Fairfield, NJ) while the bath was constantly perfused.

Statistical analysis. Values are provided as means ± SE, with error bars in the figures representing SE and with \(n\) indicating the number of cells studied. For each treatment group, cells were obtained from at least three rats. Statistical comparisons were made using either repeated-measures ANOVA with the Tukey-Kramer post hoc analysis or paired Student’s \(t\)-test. \(P < 0.05\) indicates significance.

RESULTS

**PE induces release of \(Ca^{2+}\) from intracellular stores.** Enzymatic dissociation of corpus cavernosum yielded spindle-shaped SMCs, which contracted reversibly in response to the \(\alpha_1\)-adrenergic agonist PE (see Fig. 8). \([Ca^{2+}]_i\) was monitored in rat corpus cavernosum SMCs, and PE was applied at 5-min intervals to allow for recovery between stimulations. In bath solution containing 1 mM \(Ca^{2+}\), \(\alpha_1\)-adrenergic stimulation with PE (10 \(\mu M\)) induced a rapid and transient elevation in \([Ca^{2+}]_i\), by 382 ± 19 nM (\(n = 109\)) from a basal level of 126 ± 3 nM (\(n = 109\)). The peak did not decrease significantly following repetitive stimulation; the fifth stimulation was 83 ± 20% (\(n = 5\)) of the first (Fig. 1, A and C). In Ca²⁺-free solution, the response to PE persisted, although there was gradual decline in the peak response, consistent with depletion of intracellular stores. The third stimulation in Ca²⁺-free solution was 46 ± 10% of control (\(n = 5\), Fig. 1, B and C).

We investigated the contribution of \(Ca^{2+}\) stores by using cyclopiazonic acid (CPA; 10 \(\mu M\)) to block the sarcoplasmic reticulum Ca²⁺-ATPase (SERCA). Addition of CPA in Ca²⁺-free solution to eliminate store-operated Ca²⁺ influx led to an increase in basal \([Ca^{2+}]_i\), and a rapid reduction in response to PE (10 \(\mu M\)), indicative of store depletion (\(n = 24\), data not shown). Inhibition of SERCA by CPA was accompanied by a marked reduction in the rate of decline of \([Ca^{2+}]_i\). When the rate of [\(Ca^{2+}]_i\) decline was measured (31), the maximal slope of the decay under control conditions was 5 ± 2 nM/s and with CPA was 1.2 ± 0.5 nM/s (\(n = 7\)), indicating that SERCA contributes to the restoration of basal Ca²⁺ levels.

**Attenuation of the PE-induced [\(Ca^{2+}]_i\) transient by NO and sildenafil.** NO mediates relaxation of corporal smooth muscle necessary for erection (6, 28). Sildenafil citrate enhances NO-mediated smooth muscle relaxation by inhibiting hydrolysis of cGMP by PDE5 (5). We therefore examined effects of NO and sildenafil on intracellular Ca²⁺ levels. The NO donor SNAP (10 \(\mu M\)), applied with sildenafil (10 \(\mu M\)) for 3 min, had no effect on basal Ca²⁺ levels. In contrast, SNAP and sildenafil significantly reduced the PE-induced Ca²⁺ transient. From a

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**Fig. 1.** Phenylephrine (PE) causes release of \(Ca^{2+}\) from intracellular stores in rat corpus cavernosum cells. A: there was no significant decline in the response to PE (100 \(\mu M\)) upon repetitive stimulation in solution containing 1 mM \(Ca^{2+}\). PE application is indicated by horizontal bars below the \([Ca^{2+}]_i\) trace (\(n = 5–6\) cells measured). B: response to PE gradually declined in \(Ca^{2+}\)-free solution, consistent with a depletion of intracellular stores, and was partially reversible upon a return to 1 mM \(Ca^{2+}\) solution (\(n = 5–7\)). C: summarized data from A and B. [\(Ca^{2+}]_i\), intracellular Ca²⁺ concentration. *\(P < 0.05\) vs. stimulation 1.
resting level of 98 ± 5 nM, PE elicited a rise of 350 ± 49 nM, compared with 252 ± 43 nM after SNAP and sildenafil application (27 ± 7% reduction; n = 21, P < 0.005). Recovery to 356 ± 48 nM was evident within 5 min (n = 21, Fig. 2). SNAP and sildenafil markedly delayed the rise of Ca²⁺ (Fig. 2C). By contrast, vehicle treatment had no effect on the PE-induced transients (Fig. 2B). In addition, another NO donor, SNP (100 µM), applied with sildenafil (10 µM), also significantly attenuated the Ca²⁺ transient, indicating a common effect of SNAP and SNP. The PE-induced peak under control conditions was 298 ± 42 nM compared with 166 ± 48 nM after SNP and sildenafil application and recovered to 286 ± 49 nM (46 ± 8% reduction; n = 16, P < 0.05).

**NO donors alone do not attenuate the PE-induced transient.** NO has been shown by many groups to relax corpus cavernosum tissue (8, 16, 28). We therefore tested the effect of SNAP and SNP in the absence of sildenafil citrate on our isolated cells. Surprisingly, application of the NO donors SNAP (10 µM, n = 6) and SNP (100 µM, n = 6) without sildenafil had no significant effect on basal Ca²⁺ levels or PE-induced Ca²⁺ transients (Fig. 3). To confirm these results, we examined the effect of SNAP in experiments in which we first established that the cell was responsive to SNAP and sildenafil. The PE transient following SNAP alone (10 µM) was 96 ± 5% of control, compared with 72 ± 10% of control following SNAP and sildenafil (both 10 µM; n = 17, Fig. 3). Increasing the concentration of SNAP to 250 µM also had no significant effect (n = 5, Fig. 3C). One possible explanation for these results is that the cGMP generated in our isolated cell model is degraded too quickly by PDE5 to effect a change, in line with previous reports on human corpus cavernosum (21), as considered in DISCUSSION.

**Human corpus cavernosum cells.** The effect of NO donors and sildenafil was examined on freshly isolated human corpus cavernosum SMCs. Following the protocol developed for rat

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**Fig. 2.** A: S-nitroso-N-acetylpenicillamine (SNAP) with sildenafil citrate (both 10 µM) had no effect on basal Ca²⁺, but the subsequent PE-induced transient was reversibly reduced (10 µM PE). B: there was no decline in the response to PE after vehicle treatment, whereas there was a clear reduction in response to PE after SNAP and sildenafil in the same cell. C: transients from B, indicated as i, ii, and iii, are superimposed to compare responses. D: histogram showing that the Ca²⁺ transient was significantly reduced by SNAP and sildenafil (n = 21). Sil, sildenafil citrate (Viagra). *P < 0.05.

**Fig. 3.** Nitric oxide (NO) donors alone have no effect on the height of the PE-induced Ca²⁺ transient. A: application of 10 µM SNAP alone had no effect on the subsequent Ca²⁺ transient. Data are representative of 6 cells. B: SNAP with sildenafil (both 10 µM) reduced the Ca²⁺ transient, whereas in the same cell, 10 µM SNAP alone had no effect. Data are representative of 17 cells. C: summary of experiments shown in B, as well as individual experiments with 250 µM SNAP and 100 µM sodium nitroprusside (SNP). Numbers in parentheses indicate number of cells measured. *P < 0.05.
cells, we applied PE at 5-min intervals in the presence and absence of extracellular Ca\(^{2+}\). The response to PE persisted in Ca\(^{2+}\)-free solution, consistent with release from intracellular stores (n = 3, Fig. 4A). Furthermore, SNAP and sildenafil attenuated the PE-induced Ca\(^{2+}\) transient by 55 ± 15% without affecting basal [Ca\(^{2+}\)] (n = 9, P < 0.05, Fig. 4). Thus the PE-induced transient is primarily due to Ca\(^{2+}\) release from intracellular stores in both human and rat SMCs. The average elevation in Ca\(^{2+}\) in response to PE in human cells was 380 ± 77 nM (n = 12), which was not significantly different from that in rat cells. Furthermore, both human and rat SMCs respond in a similar manner to NO donors by decreasing release of Ca\(^{2+}\) from intracellular stores.

**NO and sildenafil regulate release from intracellular Ca\(^{2+}\) stores.** To characterize the mechanism of inhibition, we quantified the rates of rise and decay of the PE-induced transient. To minimize cell-to-cell variability, we applied SNAP and sildenafil as well as SNAP alone to the same cell. Under control conditions, the average time to peak [Ca\(^{2+}\)] was significantly increased by 144 ± 39% after treatment with SNAP and sildenafil (n = 17, P < 0.01). In contrast, the time to peak was not different after SNAP alone (Fig. 5A). The rise to peak after PE application was also significantly delayed in the presence of another NO donor, SNP, with sildenafil (n = 16, data not shown).

NO is reported to activate SERCA in other vascular muscles, reducing the peak and increasing the rate of decay of the Ca\(^{2+}\) transient (9, 19). In our experiments, the rapid decay phase began 2.5 ± 0.2 s (n = 112) from the end of PE application and was well fit with a single exponential (Fig. 5C). Neither SNAP alone nor SNAP with sildenafil had any significant effect on the rate of decay (n = 16, Fig. 5D). Our data therefore are not consistent with NO activating SERCA in corpus cavernosum.

To examine whether SNAP and sildenafil regulate Ca\(^{2+}\) influx from the bath or Ca\(^{2+}\) release from intracellular stores, we repeated experiments in Ca\(^{2+}\)-free solution. Results were essentially the same as those obtained in 1 mM Ca\(^{2+}\) bath solution. SNAP and sildenafil reduced the PE-induced transient by 79 ± 8% (1 μM PE; n = 10, P < 0.01) of control measured in zero-Ca\(^{2+}\) solution (Fig. 5E). By comparison, in Ca\(^{2+}\)-containing solution, SNAP and sildenafil reduced the PE-induced transient by 54 ± 8% (1 μM PE; n = 24, P < 0.01). The time to peak in zero-Ca\(^{2+}\) solution was increased by 200 ± 46% after SNAP and sildenafil (n = 10, P < 0.01, Fig. 5F). Thus, even in the absence of extracellular Ca\(^{2+}\), SNAP and sildenafil inhibited the Ca\(^{2+}\) transient, indicating regulation of Ca\(^{2+}\) release from intracellular stores.

**Role of cGMP in attenuation of PE-induced [Ca\(^{2+}\)] transient.** To understand the signaling cascade mediating the reduction of agonist-induced Ca\(^{2+}\) release, we applied sildenafil, cGMP analogs with sildenafil, and an sGC inhibitor to the cells. As expected, sildenafil alone had no effect on basal Ca\(^{2+}\) or on the PE-induced transient (n = 10, data not shown). To verify the involvement of sGC, we tested the effect of ODQ, a selective sGC inhibitor. SNAP and sildenafil reduced the PE-induced transient (1 μM PE) by 63 ± 12% of control, whereas in the presence of 10 μM ODQ in the same cells, SNAP and sildenafil reduced the transient by only 35 ± 15% of control (n = 7, P < 0.05, Fig. 6). To control for nonspecific effects, we tested ODQ in the absence of SNAP and sildenafil and found that ODQ alone did not affect the transient (n = 11).

The effect of membrane-permeant cGMP analogs on PE-induced transients was then examined. Because the results from NO donors suggested that PDE5 was very active, sildenafil was included with the cGMP analogs to exclude any possibility of degradation. Application of either 8-Br-cGMP (n = 19, Fig. 7A) or 8-pCPT-cGMP (n = 11) in the presence of sildenafil had no significant effect on basal Ca\(^{2+}\) or PE-induced transients. We next investigated whether application of YC-1 (100 μM), a nitric oxide-independent activator of sGC, could mimic the effect of NO donors. YC-1 with sildenafil caused a slight rise in basal Ca\(^{2+}\) but had no significant effect on PE-induced transients (n = 13, Fig. 7A). These findings demonstrate that neither NO nor cGMP alone was sufficient to reduce the PE-induced Ca\(^{2+}\)-transient.

The ineffectiveness of cGMP analogs to mimic SNAP and sildenafil suggested that both NO and cGMP were necessary to mediate the reduction of Ca\(^{2+}\) release. We confirmed that SNAP with 8-Br-cGMP but without sildenafil significantly

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**Fig. 4.** PE-induced Ca\(^{2+}\) release in human corpus cavernosum. A: repetitive stimulation with 10 μM PE in Ca\(^{2+}\)-free solution continued to elicit Ca\(^{2+}\) transients, indicating release from intracellular stores. Data are representative of 3 cells. B: SNAP with sildenafil (both 10 μM) caused no change in basal Ca\(^{2+}\) but attenuated the transient. C: histogram showing that SNAP and sildenafil reduced Ca\(^{2+}\) transients in human corpus cavernosum cells (n = 9). *P < 0.05.
reduced the rise of Ca\textsuperscript{2+}, although this effect was not readily reversible. After application of SNAP and 8-Br-cGMP, the PE-induced transient was reduced by 42 ± 9\% of control (1 μM PE; \(n = 22\), \(P < 0.01\), Fig. 7B). This was not significantly different from the reduction by SNAP and sildenafil (54 ± 8\% reduction, 1 μM PE; \(n = 24\)). We also applied 10 μM SNAP with 30 μM YC-1, which also significantly reduced the PE-induced transient (by 96 ± 8\%; \(n = 5\), \(P < 0.01\), Fig. 7C).

**Attenuation of PE-induced contraction by NO and sildenafil.** The functional significance of attenuating the PE-induced [Ca\textsuperscript{2+}], transient was tested by monitoring contraction of single cells. PE was applied at 5-min intervals as before, and cell length was measured from video images of the cells (Fig. 8). Spindle-shaped cells contracted briskly, reducing cell length by 26 ± 6 μm (32 ± 7\%; \(n = 5\)) 15–20 s after application of PE. Upon washout of PE, the cells relaxed to 90 ± 3\% of their original length. We attribute this degree of relaxation to a lack of restorative tension, as would occur in intact tissue. SNAP and sildenafil were applied for 3 min and had no effect on the resting cell length; the subsequent PE application led to only minimal shortening (12 ± 6 μm, 17 ± 5\%; \(n = 5\)). This effect was significant and reversible (Fig. 8B). Similar experiments were performed with SNAP alone (no sildenafil), and no significant effect of SNAP was observed. Under control conditions, PE led to a contraction of 17 ± 3\%, whereas after SNAP, PE led to a contraction of 14 ± 2\% (\(n = 13\)). Thus the reduction of the PE-induced Ca\textsuperscript{2+} transient by SNAP and sildenafil is reflected in a reduction of the contractile response.

**DISCUSSION**

NO has a variety of cellular effects, including direct modulation of ion channels and activation of sGC (4, 26). In smooth muscle, NO is believed to mediate relaxation primarily by activation of sGC, increasing intracellular cGMP level (26).
We have demonstrated that NO donors in the presence of sildenafil dramatically reduce PE-induced Ca\textsuperscript{2+}/H\textsubscript{11001} release from intracellular stores. This was reflected functionally as a reduction in PE-induced contraction. We suggest that relaxation of the corpus cavernosum, leading to erection, may involve down-regulation of agonist-induced contraction by inhibition of Ca\textsuperscript{2+}/H\textsubscript{11001} release from stores.

**Synergism of NO and cGMP.** In other smooth muscles, NO or cGMP alone has been shown to regulate Ca\textsuperscript{2+} release. In aortic SMCs, NO inhibits vasopressin- and angiotensin-induced rise of intracellular Ca\textsuperscript{2+} level, and the effect is mimicked by the cGMP analog 8-Br-cGMP (9, 11, 15). Similarly, in tracheal SMCs, SNAP inhibits Ca\textsuperscript{2+} release induced by acetylcholine, and 8-Br-cGMP mimics the response (18). In corpus cavernosum, PDE5 has been shown to be abundant and to be the predominant PDE enzyme in this tissue (5, 10). One possible explanation for our results is that in an isolated cell preparation, the cGMP generated is degraded too quickly by PDE5 to exert its full effect. There is support for this possibility from the studies of Kim et al. (21). They did not observe any significant changes in cGMP content compared with control after treatment with 10 μM SNP alone on their cultured corpus cavernosum cells, whereas SNP with sildenafil increased cGMP levels 2.8-fold (21). Thus the difference between our results and those previously published may be due to NO producing sufficient cGMP in other SMCs, whereas in corpus cavernosum, the effect of PDE5 cannot be overcome.

Although NO has been shown by many groups to relax intact tissue from corpus cavernosum (8, 16, 28), few studies have investigated the mechanism of NO on acutely isolated corpus cavernosum cells. Tissue strips, in contrast to isolated cells, contain other cell types, including neuronal and endothelial cells (for review, see Ref. 1). It is possible that these other cell types contribute to the tissue response (1, 12). Escrig et al. (12) demonstrated that after electrical stimulation of cavernosal nerves in anesthetized rats, the rise in NO outlived the increase in intercavernosal pressure by several minutes. This finding led those authors to suggest that NO is necessary but not sufficient for the maintenance of penile erection and that some additional factor may be involved in mediating relaxation. In this regard, there are several vasodilators that act through cAMP, and interactions between cGMP- and cAMP-mediated mechanisms have been demonstrated in vascular as well as corporal smooth muscle (21, 22). Thus sildenafil may substitute for a vasodilator co-mediator not present in our isolated single-cell preparation.
Unexpectedly, we found that membrane-permeant cGMP analogs were ineffective in attenuating the PE-induced rise in Ca\(^{2+}\). However, we did find that 8-Br-cGMP or YC-1, when combined with an NO donor, decreased the PE-induced transient. The failure to observe reversibility of these combinations probably reflects kinetic parameters different from those of sildenafil, which is readily reversible (2, 13). Our results demonstrate an interesting feature of corpus cavernosum cells, whereby NO and cGMP act synergistically to reduce PE-induced Ca\(^{2+}\) release from stores.

**Mechanism of NO and cGMP inhibition of Ca\(^{2+}\) release.** Studies of PKG-1-deficient mice have revealed that a major target of cGMP in corpus cavernosum is PKG. The corpus cavernosum of these mice fails to relax upon activation of the NO/cGMP pathway, resulting in erectile dysfunction (16). Our results demonstrate an interesting feature of corpus cavernosum cells, whereby NO and cGMP act synergistically to reduce PE-induced Ca\(^{2+}\) release from stores.

The role of NO, apart from activating sGC, in reducing the PE-induced Ca\(^{2+}\) transient is unclear. However, NO is known to have diverse effects on proteins, interacting with metal and thiol groups, and conceivably could directly modulate signaling molecules of the Ca\(^{2+}\) release pathway (33). Cohen et al. (9) suggested that NO leads to increased Ca\(^{2+}\) uptake via activation of SERCA because the rate of decay of the IP\(_3\)-mediated Ca\(^{2+}\) rise in aortic SMCs was increased; however, it was not determined whether this was a direct effect of NO or occurred via cGMP. Others have shown that phosphorylation of the regulatory protein phospholamban by PKG is associated with reduced sensitivity of phospholamban to phosphorylation, leading to a reduction in Ca\(^{2+}\) release.
with activation of SERCA (19). Because NO and sildenafil did not affect baseline Ca\(^{2+}\) levels or the rate of decay of the Ca\(^{2+}\) transient, our data also do not support the involvement of SERCA in mediating the effects in corpus cavernosum.

**Effects of NO and cGMP on PE-induced contraction.** The functional significance of the NO and cGMP effect was demonstrated in single corpus cavernous cells by measuring PE-induced contraction after application of SNAP with sildenafil. SNAP alone had no effect on PE-induced contraction, whereas SNAP with sildenafil reduced PE-induced contraction in addition to reducing PE-induced Ca\(^{2+}\) release. Both the concentration of intracellular Ca\(^{2+}\) and the sensitivity of the contractile proteins to Ca\(^{2+}\), called Ca\(^{2+}\) sensitization, regulate contraction in vascular smooth muscle. Ca\(^{2+}\) sensitization is a result of phosphorylation of the myosin light chain, leading to increased muscle tension for a given Ca\(^{2+}\) concentration (32). In this regard, RhoA/Rho-kinase and PKG both have been shown to regulate Ca\(^{2+}\) sensitization/desensitization in smooth muscles (23, 32, 36).

The NO/cGMP pathway has been suggested to control relaxation of corpus cavernosum by acting in two ways: by lowering intracellular Ca\(^{2+}\) and by inhibiting Rho-kinase (24). RhoA is highly expressed in corpus cavernosum, and several groups have shown that inhibition of Rho-kinase promotes relaxation of corpus cavernosum tissue and erection (7, 29, 36).

**Effects of sildenafil on the relaxation of human corpus cavernosum AM.**

**REFERENCES**


**GRANTS**

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