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Relaxation of rabbit lower urinary tract smooth muscle by nitric oxide and carbon monoxide: modulation by hydrogen peroxide

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Abstract

Recent studies suggest that the body produces two gaseous messengers, nitric oxide (NO) and carbon monoxide (CO), both of which activate soluble guanylyl cyclase and thus modulate the activity of smooth muscle cells. In the present study, the effects of NO and CO on the smooth muscle of the lower urinary tract were compared. In addition, the modulation of tissue NO- and CO-induced relaxation by hydrogen peroxide was examined. NO, produced endogenously by electrical field stimulation (EFS) or applied exogenously as a solution, induced a concentration-dependent relaxation of rabbit cavernosal and urethral smooth muscle strips, but not of bladder tissues. The cavernosal tissue was found to be three times more sensitive to the actions of NO than the urethra. CO also induced relaxation of both tissue types, but with no apparent difference in sensitivity between the tissues. However, CO was much less potent than NO with respect to smooth muscle relaxation. The mechanism of action of the two mediators was cyclic guanosine monophosphate (cGMP)-dependent, as evidenced by enhanced formation of cGMP and inhibition of relaxation by the guanylyl cyclase inhibitor, oxadiazoloquinoxaline-1-one (ODQ.) The data suggests that NO is the dominant messenger in these tissues, but does not exclude a role for CO.

In the presence of hydrogen peroxide, the relaxation responses induced by both NO and CO were significantly increased, regardless of tissue type. The mechanism for this effect is unclear, but evidence points to a requirement for the activation of guanylyl cyclase and enhanced formation of cGMP, since potentiation by the peroxide was blocked by a specific guanylyl cyclase inhibitor. We suggest that H_2O_2 may play a positive role in the amplification or NO and CO-mediated responses.

Keywords: Nitric oxide (NO); Carbon monoxide (CO); Hydrogen peroxide; Corpus cavernosa; Urethra relaxation

1. Introduction

The enzyme nitric oxide synthase (NOS) has been identified by diaphorase staining in different regions of the genito-urinary tract and in a variety of different species (Andersson and Persson, 1994). The enzymatic product, nitric oxide (NO), is thought to be the facilitator of cyclic guanosine monophosphate (cGMP)-dependent relaxation of cavernosal (Ignarro et al., 1990) and urethral (Dokita et al., 1994), although this action is unclear in bladder smooth muscle. Recently, it has been suggested that carbon monoxide (CO) may also act as a cellular messenger by activation of soluble guanylyl cyclase (Stone and Marletta, 1994; Kharatinov et al., 1995). CO is formed during the degradation of haem to biliverdin by the enzyme haemoxygenase (HO) (Maines, 1988). HO exists in two isoforms designated as haemoxygenase-1, which is inducible, and haemoxygenase-2, the constitutive isoform of the enzyme (Maines, 1988). Haemoxygenase-2 and NOS are both present in the genito-urinary tract and, in some cases, appear to be co-localised (Kossen et al., 1999). NO is now considered the key physiological mediator of penile erection (Burnett, 1997) and may also play an important role in urethral smooth muscle relaxation during micturition (Dokita et al., 1994). However, the relative efficacy and physiological importance of CO in the regulation of caver-

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nosal and urethral smooth muscle relaxation is not fully understood.

Reactive oxygen species are known to modulate the biological actions of NO. Superoxide ions (O_2^-), which are produced by all aerobic cells, react with NO and reduce its biological activity (Beckman et al., 1990). This process may contribute to the pathogenesis of erectile dysfunction and alterations in the micturition process. Under physiological conditions, O_2^- is rapidly converted to hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase, which is present both intra- and extracellularly (Fridovich, 1995). Previous studies have shown superoxide dismutase to enhance NO-mediated relaxation of rabbit corpus cavernosum, suggesting that endogenous O_2^- may modulate NO activity (Chen and Lee, 1995). The mechanism responsible for this enhancement is unclear, but may involve the removal of O_2^-, which would prolong the activity of NO. Alternatively, H_2O_2 may have an effect on genito-urinary smooth muscle cell reactivity, this has been shown in other tissues responsive to NO (Burke-Wolin et al., 1991; Zembowicz et al., 1993). Previously, we have shown that H_2O_2 strongly enhances the inhibitory actions of NO on human blood platelets (Naseem and Bruckdorfer, 1995).

In the present study, the relative efficacy of CO with NO on the smooth muscle of the rabbit lower urinary tract was evaluated, by measuring functional responses and cGMP accumulation. In addition the influence of H_2O_2 on NO and CO-mediated responses in the rabbit cavernosal and urethral smooth muscle were determined.

2. Materials and methods

Atropine, methylene ATP, indomethacin, hydrogen peroxide and phenylephrine were all obtained from Sigma (Poole, Dorset, UK). N^G-nitro-arginine and oxadiazoloquinoxalin-1-one were purchased from Tocris Cookson (Bristol, UK). The NO and CO gas were provided by Lynde gas (Stoke-onTrent, Staffs., UK). Elisa kit for measurement of cGMP was from Cayman Chemicals (Ann Arbor, USA).

2.1. Tissue preparation

Age matched 3 kg male New Zealand white rabbits ($n = 12$) were killed by cervical dislocation. Cavernosal, trigone, detrusor, bladder neck and transverse proximal urethral strips were prepared on the same day and mounted vertically in organ baths containing Kreb's buffer at 37°C, as described previously (Mumtaz et al., 1999). The strips were perfused with 95% O_2 and 5% CO_2 and maintained at pH 7.4. Tissues were then placed under 1 g tension, recorded on a force displacement transducer (FT-03, Grass instruments, Quincy, MA, USA) using a Grass polygraph (model 7D). All strips were equilibrated for 1 h and were subsequently challenged with KCl (124 mM). Two reproducible contractions, varying in magnitude by less than 10%, were consistently obtained. All experiments were performed in the presence of alpha-beta methylene ATP (10 μM) and atropine (1 μM) to block the effects of purinergic and cholinergic receptors, respectively. In addition, indomethacin (1 mM) was used to abolish any prostaglandin-mediated effects.

2.2. Preparation of NO, CO and H_2O_2 solutions

NO and CO were prepared as solutions as described previously (Naseem and Bruckdorfer, 1995). Briefly, double distilled water was boiled for 15 min, allowed to cool to 60°C and pulled under vacuum into specially adapted gas sampling tubes. The water was then flushed with N_2 for 45 min and the tubes sealed with rubber septa. The appropriate amount of NO or CO gas was then injected into the gas sampling tubes using gas-tight syringes. Control solutions were prepared by following the above procedure, and then bubbling the solutions with O_2 for 1 h.

H_2O_2 was diluted from a stock solution (8.82 M) in deoxygenated water and kept in air-tight vials throughout the duration of the experiment. Control solutions for H_2O_2 were prepared by leaving diluted solutions exposed to air overnight (with stirring).

All solutions were prepared freshly on the day and kept on ice for the duration of the experiment.

2.3. Functional studies

The relaxation of cavernosal and urethral smooth muscle by EFS, or by the addition of authentic NO and CO solutions was performed on strips precontracted using phenylephrine (phenylephrine; 100 μM). EFS was performed with a Grass S88 stimulator, delivering single square waves (duration 0.8 ms) at a frequency of 0.5–20 Hz at 2-min intervals. These experiments were performed in the presence and absence of H_2O_2 (20 μM). A bolus of H_2O_2 (20 μM) was added to the organ bath immediately prior to each individual EFS assessment. In control experiments an equivalent volume of degraded H_2O_2 was used.

Concentration response curves to bolus additions of NO and CO were performed in the presence and absence of H_2O_2 (20 μM). In each case, H_2O_2 (20 μM) was added simultaneously with either NO or CO. These experiments were repeated in the presence of either N^G-nitro-L-arginine (L-NNA: 1 μM) or oxadiazoloquinoxalin-1-one (ODQ: 10 μM), inhibitors of NOS and soluble guanylyl cyclase, respectively. The concentrations of NO and CO were based on their solubility in water, and the assumption that the oxidation of these gases during addition to the organ bath was negligible. However, the oxygenated environment of the organ bath, may lead to the oxidation of the gases, particularly NO, and therefore the concentration of the gases which the tissues encounter may be lower than the concentration added to the bath.

2.4. Estimation of cGMP formation

The epidermal and connective tissue was removed from the penile samples, which were then cut into smaller segments. The tissues were incubated in 200 μl of Kreb's buffer containing isobutyl methylxanthine (IBMX; 200 μM) for 30 min before experimentation. NO or CO, in the presence or absence of H_2O_2, were then added to the tissues and incubated for 10 min at 37°C. The reaction was stopped by the addition of ice-cold trichloroacetic acid (10%). The tissues were then macerated, ultrasonicated (3×20 s bursts) (Soniprep) and centrifuged. The supernatant was collected and cGMP measured using a commercially available enzyme linked immunosorbent assay (ELISA: Cayman Chemicals).

2.5. Statistical analysis

All data are expressed as mean ± S.D. and are representative of at least five individual experiments. Statistical analysis was performed using Student's *t*-test with *P* values of less than 0.05 being taken as significant.

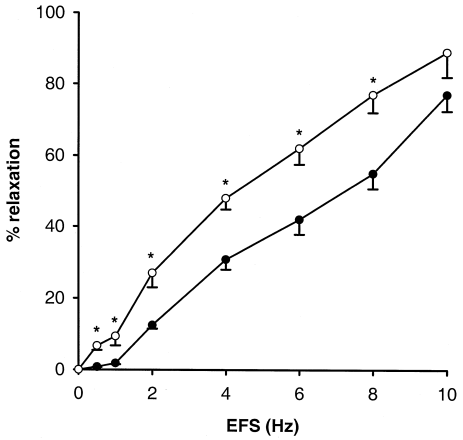

Fig. 1. Relaxation of rabbit cavernosal tissue by electrical field stimulation in the presence and absence of hydrogen peroxide. Cavernosal smooth muscle strips were mounted under 2 g tension and precontracted with phenylephrine (100 μM). Electrical field stimulation was conducted (0–10 Hz) in the form of square wave pulses for 0.8 ms. After obtaining control responses to electrical field stimulation (●), the experiments were repeated with H_2O_2 (20 μM) added immediately prior to application of electrical field stimulation (○). The data produced are expressed as percent relaxation of tissues compared to the precontracted tension and are representative of five independent experiments. $^*P < 0.05$.

3. Results

3.1. EFS of urinary tissues

Cavernosal smooth muscle strips were precontracted with phenylephrine (100 μM). After a stable tension had been achieved the application of EFS (0–10 Hz) resulted in a frequency-dependent relaxation of all strips. The maximum effect was achieved at 10 Hz, which induced a relaxation of 77 ± 4.6% (Fig. 1). These relaxation responses, which have been shown previously to be cGMP-dependent (Ignarro et al., 1990), were transient with the tension returning to the precontracted levels within 2 s. Incubating of the tissues with ODQ (10 μM), a specific guanylyl cyclase inhibitor (Garthwaite et al., 1995), caused a marked inhibition of the EFS-induced relaxations. Relaxation induced by 10 Hz was reduced to 4% in the presence of ODQ, compared to vehicle control, implicating cGMP as the key mediator of this response.

The addition of H_2O_2 to cavernosal strips during EFS, led to a significant increase in the magnitude of the relaxation responses over the whole range of frequencies tested (Fig. 1). For example, 2 Hz induced 12.5 ± 1% relaxation, this was increased to 27 ± 4% in the presence of H_2O_2 ($P < 0.05$). The experiments were repeated in the presence of ODQ (10 μM), which blocked the actions of both EFS alone and in combination with H_2O_2. As a control, H_2O_2 solutions, which had been spent by exposure to air overnight (with stirring), failed to have any effect on EFS. Furthermore, H_2O_2 alone failed to induce relaxation of the smooth muscle strips.

EFS also induced a frequency-dependent smooth muscle relaxation of urethral tissues precontracted with phenylephrine (100 μM). The enhancement of smooth muscle relaxation by H_2O_2 in the urethra was not significant. EFS, either in the presence or absence of H_2O_2, did not induce relaxation of the smooth muscle strips obtained from the trigone or detrusor of the bladder; therefore these experiments were not pursued.

3.2. Regulation of urinary tissues by NO

NO solutions induced a concentration-dependent relaxation of cavernosal smooth muscle strips precontracted with phenylephrine 100 μM (Fig. 2A). In contrast to the relaxation elicited by EFS, NO-induced responses were longer lasting, taking almost 10 s to return to the original tension. The IC_{50} for NO was 16 ± 1.2 μM and maximal relaxation was induced by 100 μM NO (Fig. 2B). In control experiments, NO solutions which had been bubbled with air prior to use did not induce a relaxation response, indicating that the active component of the solutions was NO and not nitrite or nitrate.

The simultaneous addition of H_2O_2 (20 μM) with NO to the cavernosal tissues caused a significant enhancement of the relaxation compared to that induced by NO alone (Fig. 2A). The IC_{50} for NO in the presence of H_2O_2 was reduced to 3.6 ± 0.2 μM, significantly ($P < 0.05$) less than the control value (Fig. 2B). Although, H_2O_2 increased the magnitude of the relaxation, it had no effect on the duration of the response.

NO induced concentration-dependent relaxations in all urethral smooth muscle preparations. However, these tissues were found to be less sensitive to NO than cavernosal

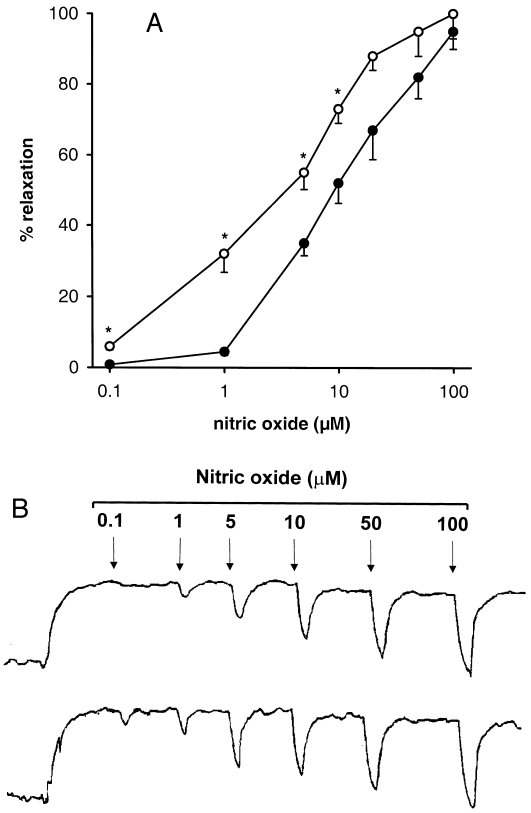

Fig. 2. (A and B) Nitric oxide-mediated relaxation of rabbit cavernosal tissues: modulation by hydrogen peroxide. (A) Cavernosal smooth muscle strips were mounted under 2 g tension and precontracted with Phenylephrine (100 μM). After a stable contraction had been achieved, the tissues were exposed to non-cumulative concentrations of NO (1–100 μM) either alone (●) or in the presence of H_2O_2 (20 μM) (○). The data are expressed as percent relaxation of tissues and are expressed as mean ± S.D. of at least five independent experiments. * $P < 0.05$. (B) The figures show representative tracings of relaxation of cavernosal tissue in presence (lower panel) or absence (upper panel) of H_2O_2, as in (A). These tracings are representative of at least five individual experiments.

tissue, with a higher IC_{50} (96 ± 13 μM). The simultaneous addition of H_2O_2 with NO caused a significant increase in the relaxation response compared to that induced by NO alone. Here the IC_{50} for NO alone being reduced 3-fold from 96 ± 1.2 μM to 31 ± 1.2 μM ($P < 0.05$) (Fig. 3). The relaxations induced by both NO alone, and NO in combination with H_2O_2 in both tissues were completely abolished by ODQ (10 μM). This implies the direct involvement of soluble guanylyl cyclase in the potentiating actions of the peroxide.

NO, in the presence or absence of H_2O_2, did not induce relaxation of the smooth muscle strips obtained from the trigone or detrusor of the bladder; therefore these responses were not investigated further.

3.3. Regulation of urinary tissues by CO

CO (0–150 μM) caused a concentration-dependent relaxation of cavernosal smooth muscle strips. However, the potency of CO as a mediator of relaxation was found to be far less than that of NO. The maximum concentration of CO (150 μM) that could be used only induced 36 ± 2.8% relaxation (Fig. 4A); NO at a similar concentration induced complete relaxation of the same tissue (Fig. 2B). Conversely, the duration of CO-mediated relaxation was more prolonged than that observed with NO, with responses taking almost 20 s to return to precontracted tension. If the tissues were washed out after a CO dose response curve, they responded normally to phenylephrine (not shown). These two observations imply that the effects of CO at these concentrations were not due to its cytotoxicity.

The simultaneous addition of H_2O_2 with CO caused a small, but significant enhancement in the magnitude of cavernosal smooth relaxation, but not the duration of the response (Fig. 4A). In the presence of H_2O_2, the relaxation induced by maximum dose of CO was increased from 36.9 ± 2.8% to 59.8 ± 6.7% ($P < 0.05$) (Fig. 4A). The relaxation of cavernosal tissue in response to CO, both in the presence and absence of H_2O_2 was completely abolished by ODQ.

In contrast to the NO studies, no difference in the sensitivity to CO was observed between cavernosal and urethral tissue. The maximal dose of CO (150 μM) induced 42.5 ± 3.5% relaxation of urethral strips, very similar to that observed with cavernosal smooth muscle. The simultaneous addition of H_2O_2 with CO led to a significant increase in the level of relaxation, except at the maximum dose of CO (Fig. 4B). CO did not induce relaxation of the smooth muscle strips obtained from the trigone or detrusor of the bladder.

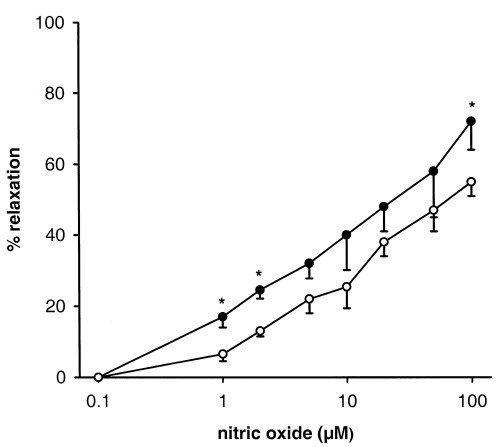

Fig. 3. The influence of hydrogen peroxide on nitric oxide-mediated relaxation of rabbit urethral tissue. Urethral smooth muscle strips were mounted under 2 g tension and precontracted with Phenylephrine (100 μM). After a stable contraction had been achieved, the tissues were exposed to non-cumulative concentrations of NO (1–100 μM) either alone (○) or in the presence of H_2O_2 (20 μM) (●). The data are expressed as percent relaxation of tissues and are expressed as mean ± S.D. of at least five independent experiments. * $P < 0.05$.

3.4. NO and CO stimulated formation of cGMP: influence of hydrogen peroxide

To study the mechanism by which H_2O_2 exerted its actions we measured the rate of cGMP formation in cavernosal tissue under different conditions. The basal level of cGMP formation in cavernosal tissue was 0.23 ± 0.05 fmol/mg per minute. In the presence of NO (10 μM), the rate of cGMP formation increased to 1.3 ± 0.2 fmol/mg per minute ($P < 0.05$ compared to basal rate). When H_2O_2 (20 μM) was added simultaneously with NO (10 μM) to the tissues, the rate of cyclic nucleotide synthesis was increased further to 2.2 ± 0.27 fmol/mg per minute ($P < 0.05$ compared to NO alone) (Fig. 5).

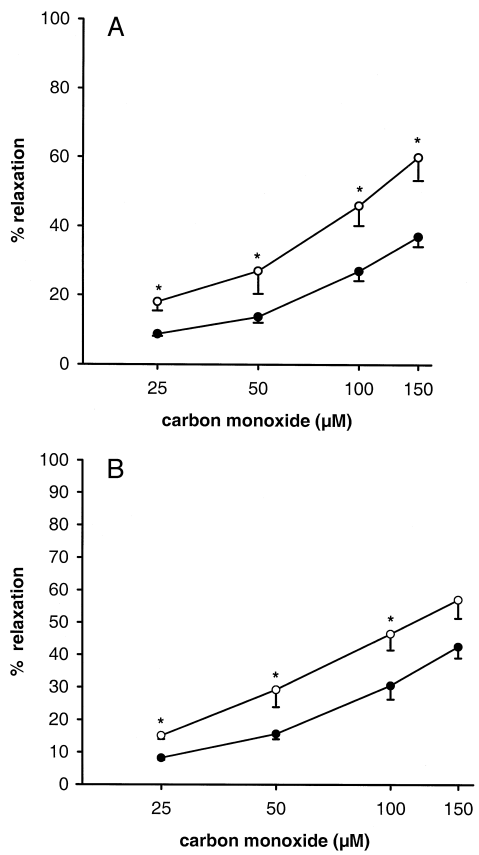

Fig. 4. (A) Carbon monoxide-induced relaxation of rabbit cavernosal tissue: modulation by hydrogen peroxide. Cavernosal smooth muscle strips were mounted under 2 g tension and precontracted with Phenylephrine (100 μM). After a stable contraction had been achieved, the tissues were exposed to non-cumulative concentrations of CO (1–150 μM) either alone (●) or in the presence of H_2O_2 (20 μM) (○). The data are expressed as percent relaxation of tissues and are expressed as mean ± S.D. of at least five independent experiments. $^*P < 0.05$. (B) Carbon monoxide-induced relaxation of rabbit urethral tissue: modulation by hydrogen peroxide. Urethral smooth muscle strips were mounted under 2 g tension and precontracted with phenylephrine (100 μM). After a stable contraction had been achieved, the tissues were exposed to non-cumulative concentrations of CO (1–150 μM) either alone (●) or in the presence of H_2O_2 (20 μM) (○). The data are expressed as percent relaxation of tissues and are expressed as mean ± S.D. of at least five independent experiments. $^*P < 0.05$.

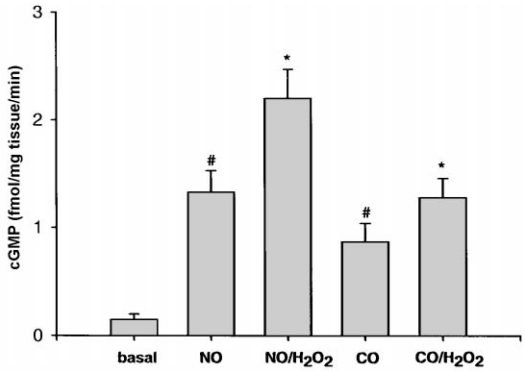

Fig. 5. Stimulation of cGMP formation in rabbit cavernosal tissue by nitric oxide and carbon monoxide: modulation by hydrogen peroxide. Smooth muscle strips were incubated with NO (10 μM) or CO (100 μM) in the presence or absence of H_2O_2 (20 μM) and the formation of cGMP estimated. All tissues had previously been incubated with IBMX (200 μM), a phosphodiesterase inhibitor, for 30 min prior to experimentation to allow accumulation of the nucleotide. The results are expressed as fmol cGMP/mg tissue per minute and represent the mean ± S.D. of three independent experiments. $^*P \leq 0.05$ compared to NO or CO alone. $\#P \leq 0.05$ compared to basal levels.

Incubation of cavernosal tissues with CO (100 μM) also increased the rate of cGMP synthesis of cGMP from 0.23 ± 0.05 fmol/mg per minute to 0.93 ± 0.17 fmol/mg per minute ($P < 0.05$ compared to basal) (Fig. 5). When taking into consideration that the concentration of CO used was 10 times that of NO, the data shows CO as a weaker stimulator of soluble guanylyl cyclase, reinforcing the results of the functional experiments. The simultaneous addition of H_2O_2 (20 μM) with CO enhanced the rate of cGMP formation by a small, but significant amount; increasing basal levels to 1.28 ± 0.18 fmol/mg per minute ($P < 0.05$ compared to basal).

4. Discussion

NO, both humoral and neurogenic, and CO are proposed regulators of smooth muscle tone in the lower genito-urinary tract (Andersson and Persson, 1994; Burnett, 1997; Werkstrom et al., 1997). Here, we compared the effects of NO and CO on different lower urinary tract tissues in the rabbit. NO induced a concentration-dependent relaxation of both urethral and cavernosal smooth muscle, but not of the detrusor, trigone or bladder neck. The cavernosal tissue was approximately three times more sensitive to NO than the urethral tissue. The role of NO in regulation of cavernosal tissue and penile erection is well documented (Burnett, 1997), but NO-mediated regulation of urethral smooth muscle is less clear. Our data confirms previous findings demonstrating that in vitro, urethral smooth muscle is responsive to both neurogenically-released and exogenously applied NO, and that this process is mediated via cGMP (Andersson et al., 1991; Dokita et al., 1994; Persson and Andersson, 1994). However, we

found urethral tissue less sensitive to NO than cavernosal tissue, which may indicate differing levels of importance for NO in the regulation of distinct areas of the lower urinary tract.

The constitutive enzyme for the synthesis of CO, haemoxygenase-2, is present in smooth muscle cells, and the neurons regulating these cells, from several different species (Maines, 1997). CO activates soluble guanylyl cyclase with a concomitant rise in cGMP, using purified enzyme (Kharatinov et al., 1995), cultured rat aortic smooth muscle cells (Ramos et al., 1989) and smooth muscle strips (Rattan and Chakder, 1993). Further strengthening this link between cGMP and CO is the observation that, in some tissues, HO-1 is colocalised with soluble guanylyl cyclase (Verma et al., 1993). We demonstrate here that CO causes a cGMP-dependent relaxation of both rabbit cavernosal and urethral smooth muscle. In contrast to NO, no differences in sensitivity to CO was observed between the two tissue types. The effective concentrations for CO was comparable to similar experiments performed using porcine urethral smooth muscle (Werkstrom et al., 1997) and rat tail artery smooth muscle (Morita et al., 1995). Importantly, CO-induced responses were reversible, suggesting that the effect was not toxic and may have a physiological role. CO was less potent than NO, both in functional studies and cGMP formation, consistent with CO being a less potent activator of soluble guanylyl cyclase than NO (Friebe et al., 1996). It has been suggested that CO-mediated relaxation is both cGMP-dependent and independent (Farrugia et al., 1993). The cGMP-independent mechanism involves the activation of K^+-channels leading to hyperpolarization of cell membranes. However, in our experiments the actions of CO were completely inhibited by the presence of the soluble guanylyl cyclase inhibitor, ODQ, indicative of a cGMP-dependent mechanism. Similar results were found in pig urethra (Werkstrom et al., 1997) and rabbit aorta (Hussain et al., 1997), which may suggest that mechanistically CO operates differently in the lower urinary tract compared with other tissues.

CO-induced relaxation of urethral and cavernosal smooth muscle was weaker and slower in onset, although the response was more persistent than NO. CO has a greater stability than NO in solution, and therefore its actions may persist for longer. This slow action of CO could also imply a secondary role for this mediator, for example, in the maintenance of penile erection, as well as urethral relaxation during micturition, as opposed to the initiation of these processes. Alternatively, the stability of CO in the aqueous environment is greater than NO. The equipotent activity of CO on the two tissue types, in contrast to that of NO, also suggests a more general role for this messenger in urinary tract function. Interestingly, NO can cause the induction of haemoxygenase-1 and hence CO production in a variety of cell types (Motterlini et al., 1996; Takahashi et al., 1996; Durante et al., 1997; Hartsfield et al., 1997). Since NOS and haemoxygenase-2 are colocalised (Kossen et al., 1999), it is reasonable to speculate that the release of NO from neurones or the urothelium may increase the production of CO, which then acts to maintain or modulate the biological actions of NO. This way, NO and CO may play ''cooperative roles'' in the regulation of urethral and cavernosal smooth muscle. However, the putative roles for CO must be analysed cautiously, since findings in this area are based on the presence of HO or the effects of exogenously applied CO.

H_2O_2, at millimolar concentrations, has been used extensively as a model for experimental oxidative stress. However, at lower concentrations (μM range) of this peroxide can induce both relaxation, and inhibition of agonist-induced contraction of rabbit and rat aortic ring preparations (Burke-Wolin et al., 1991; Mian and Martin, 1997). The mechanism is thought to involve that activation of NOS (Zembowicz et al., 1993) or soluble guanylyl cyclase (Burke-Wolin et al., 1991), or both. Previously, we have demonstrated that low concentrations of H_2O_2 can enhance the inhibitory actions of NO on platelets (Naseem and Bruckdorfer, 1995). In contrast, very little is known about the effects of H_2O_2 per se on the lower urinary tract. Here H_2O_2, at physiologically relevant concentrations, potentiated the relaxation of urethral and cavernosal tissue, induced by exogenous NO and CO, but had no effect alone. More importantly, H_2O_2 had similar enhancing effects on endogenously produced neurogenic NO. The potentiation by H_2O_2 was consistent regardless of tissue type or which gaseous mediator was used, and was mediated, at least in part, by an increased rate of cGMP formation. However, H_2O_2 does not potentiate the effects of NO on the purified guanylyl cyclase (Sabetkar, Naseem and Bruckdorfer, unpublished observations). H_2O_2 may activate other pathways in the cell leading indirectly to enhanced guanylyl cyclase activity or, act on events downstream of this. In contrast to previous studies, H_2O_2 alone had no effect on functional responses or cGMP formation, indicating that whatever the mechanism of action, a ligand for guanylyl cyclase must be present. One plausible mechanism relates to the effects of the peroxide on intracellular calcium concentrations $(Ca^{2+})_i$. H_2O_2 can raise the $(Ca^{2+})_i$ in cultured endothelial cells (Kimura et al., 1992) and rabbit aortic strips (Iesaki et al., 1996). This effect may in turn, increase the sensitivity of neuronal NOS, thereby increasing NO release.

The physiological importance of H_2O_2 as a regulator of vascular responses is yet to be fully elucidated, but may have important implications for pathophysiological states. Erectile dysfunction is associated with a reduced bioavailabity of NO, a concept validated by the clinical success of sildenafil citrate (a type V phosphodiesterase inhibitor). It is plausible that in situations of reduced NO levels the presence of H_2O_2 could act as a compensatory mechanism to promote NO-mediated actions. The formation of H_2O_2 from O_2^- by superoxide dismutase, which is present in regions of the lower urinary tract (Kim et al., 1997), may

therefore have a positive role in the intensification and prolongation of the actions of NO and CO on the cavernosal and urethral smooth muscle. Similarly, this peroxide could act to enhance the sensitivity of tissues to CO, which normally is a weak modulator of smooth muscle. NO, CO and H_2O_2 are also potential targets for therapeutic intervention.

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