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Nitric oxide and relaxation of pig lower urinary tract

[1]Katarina Persson & Karl-Erik Andersson

Department of Clinical Pharmacology, Lund University Hospital, 221 85 Lund, Sweden

1 We studied the non-adrenergic, non-cholinergic (NANC) nerve-mediated relaxation induced by electrical stimulation in pig isolated lower urinary tract smooth muscle, and the possible involvement of the L-arginine (L-ARG)/nitric oxide (NO) pathway in this response.

2 Trigonal strips, precontracted by noradrenaline (NA), carbachol or endothelin-1 (ET-1), relaxed frequency-dependently in response to electrical stimulation. Maximum relaxation was obtained at 6–8 Hz, and amounted to 56 ± 2%, 77 ± 3% and 62 ± 6% of the agonist-induced tension in preparations contracted by NA, carbachol, or ET-1, respectively. Exposure to N^G-nitro-L-arginine (L-NOARG; 10^{-7}–10^{-5} M) concentration-dependently reduced the relaxant response in preparations contracted by NA. L-NOARG (10^{-6} M) reduced the maximal response to 51 ± 8% of control. L-NOARG (10^{-5} M) abolished all relaxation, and unmasked a contractile component; D-NOARG had no effect. Also in trigonal preparations, where the tension had been raised by carbachol or ET-1, L-NOARG (10^{-5} M) markedly reduced relaxations evoked by electrical stimulation.

3 In trigonal preparations contracted by NA, maximal relaxation was increased after pretreatment with L-ARG (10^{-3} M), and the inhibitory effect of L-NOARG (10^{-6} M) was prevented. Incubation of the trigonal strips with methylene blue had no effect on relaxations elicited at frequencies <6 Hz, but a small inhibition was observed at higher frequencies.

4 Administration of NO (present in acidified solution of $NaNO_2$) induced concentration-dependent relaxations in trigonal preparations contracted by NA, carbachol, or ET-1. L-NOARG (10^{-5} M) and L-ARG (10^{-3} M) had no effect on these relaxations. However, methylene blue (10^{-5} M) significantly shifted the concentration-response curve for NO to the right. NANC-relaxation and NO-induced relaxation of trigonal preparations were both inhibited by oxyhaemoglobin (10^{-5} M) and pyrogallol (10^{-4} M).

5 In urethral preparations precontracted by NA, electrical stimulation caused frequency-dependent relaxations. A maximum relaxation of 73 ± 4% was obtained at 10 Hz. Also in the urethra, NANC-relaxation was blocked by L-NOARG (10^{-5} M), and a contractile response generally appeared.

6 Detrusor strips treated with α-β methylene ATP (10^{-5} M) and atropine (10^{-6} M), and then contracted by ET-1, showed relaxations (19 ± 3% of the induced tension) in response to electrical field stimulation (2–20 Hz) only when the tension was high. No response at all, or small contractions, were found in response to electrical stimulation in K^+ (35 mM)-contracted detrusor strips. Detrusor preparations contracted by carbachol were concentration-dependently relaxed by exogenously administered NO, SIN-1 (NO-donor), and isoprenaline, whereas vasoactive intestinal polypeptide had minor effects. NO and SIN-1 induced maximal relaxations of 63 ± 3% and 70 ± 4%, respectively, of the tension induced by carbachol. Isoprenaline produced an almost complete relaxation (96 ± 4%).

7 The results suggest that NANC-nerve mediated relaxation, involving the L-ARG/NO pathway, can be demonstrated consistently in the pig trigonal and urethral, but not in detrusor smooth muscle. The importance of this pathway for lower urinary tract physiology and pathophysiology remains to be established.

Keywords: Non-adrenergic non-cholinergic nerves; nitric oxide; urinary tract; N^G-nitro-L-arginine

Introduction

Previous investigations have shown that isolated, contracted urethral smooth muscles from rabbit, sheep, pig and man respond to transmural stimulation with a relaxant response mediated by a non-adrenergic, non-cholinergic (NANC) mechanism (Klarskov *et al.*, 1983; Andersson *et al.*, 1983; 1991; 1992; Garcia-Pascual *et al.*, 1991). Tetrodotoxin (TTX) blocks the response, suggesting the involvement of transmitter(s) released from nerves. The electrically evoked relaxation could be completely blocked by N^G-nitro-L-arginine (L-NOARG), which inhibits the synthesis of nitric oxide (NO) from L-arginine (L-ARG) (Mülsch & Busse, 1990). The effect was enhanced by L-ARG, whereas no effects were obtained with D-NOARG or D-ARG (Andersson *et al.*, 1991; 1992; Garcia-Pascual *et al.*, 1991). In addition, NO (present in acidified $NaNO_2$ solution) relaxed the preparations, even in the presence of L-NOARG. These results suggest that NO (or a NO-containing compound) is a main mediator of urethral relaxation.

A NANC-mediated relaxation in pig detrusor muscle, preceded by a contraction, and which could be blocked with TTX, was demonstrated by Klarskov (1987). He also claimed to have found an occasional NANC-mediated relaxation in the human detrusor, but the evidence for this was not convincing. Recently, however, James *et al.* (1991) reported that human isolated, atropinized detrusor muscle, depolarized with 20 mM K^+, relaxed on electrical stimulation. The relaxation was TTX-insensitive, but could be blocked with L-NOARG, suggesting the involvement of the L-ARG/NO pathway.

Speakman *et al.* (1988) showed that electrical stimulation of strips of human superficial trigone produced relaxation in 40% of the preparations. This response could not be blocked by propranolol, and it was enhanced by atropine and phentolamine, which suggested that it was NANC-mediated.

[1] Author for correspondence.

Similar relaxant effects had previously been demonstrated in human and pig trigonal muscle (Klarskov *et al.*, 1983; Hills *et al.*, 1984). The mechanism of this relaxant effect has not been clarified.

The present study was performed, in order to study further NANC-mediated relaxation in lower urinary tract smooth muscle, particularly in the trigone and the detrusor, and the possible involvement of the L-ARG/NO pathway in this response. Pig lower urinary tract was chosen for a comparison between the detrusor, trigone, and urethra, partly because of their similarity to corresponding regions in man, partly because the tissues were readily available.

Methods

Tissue preparations and recording of mechanical activity

The bladder and urethra from female pigs were removed in a slaughterhouse shortly after the animals had been killed. The tissue was transported to the laboratory in cold Krebs solution (for composition, see below). The bladder and urethra were opened longitudinally and smooth muscle strips ($1 \times 2 \times 5$ mm) stripped of mucosa were prepared. Detrusor strips were taken from the anterior wall of the dome, and urethral strips were cut transversally from the proximal (2–3 cm distal to the bladder neck) part of the urethra. The trigonal strips were taken in an oblique direction from the internal urethral orifice toward one of the orifices of the ureter.

Tissue preparations were investigated on the same day as the tissue was obtained, or stored for 24 h at 4°C in Krebs solution before investigation. All experiments with electrical field stimulation were made on the first day. There were no differences in the responses to drugs between preparations investigated on the first day and those studied the day after.

The preparations were transferred to 5 ml organ baths containing Krebs solution maintained at 37°C by a thermoregulated water circuit. The Krebs solution was bubbled with a mixture of 95% O_2 and 5% CO_2, maintaining pH at 7.4. The strips were mounted between two L-shaped hooks by means of silk ligatures. One of the hooks was connected to a Grass Instrument FT03C force-displacement transducer for registration of isometric tension and the other was attached to a movable unit. By varying the distance between the hooks the tension could be adjusted. The transducer output was recorded on a Grass Polygraph model 7D or E. During an equilibration period of 45–60 min, the preparations were stretched until a stable tension of 4–6 mN was obtained.

When subjected to electrical field stimulation, the preparations were mounted between two parallel platinum electrodes (3 mm long and 4 mm apart) in the organ baths. Transmural stimulation of nerves was performed with a Grass S48 or S88 stimulator delivering single square pulses (duration 0.8 ms) at supramaximal voltage. The train duration was 5 s and the stimulation interval 120 s.

Experimental procedure

After the equilibrium period, each experiment was started by exposing the preparations to a K^+ (124 mM) Krebs solution (for composition, see below), until two reproducible contractions (difference $<10\%$) had been obtained. The mean of these contractions amounted to 51 ± 3 mN ($n = 67$, N = 23), 15 ± 1 mN ($n = 127$, N = 29) and 7.8 ± 1.2 mN ($n = 10$, N = 6), in detrusor, trigonal, and urethral preparations, respectively.

Relaxant responses to electrical stimulation and the agonists NO, SIN-1, vasoactive intestinal polypeptide (VIP) and isoprenaline were studied in precontracted preparations. SIN-1 is the active metabolite of molsidomine, and believed to liberate NO non-enzymatically (Reden, 1990). Contractions of detrusor and trigonal preparations were evoked by carbachol 5×10^{-7}–10^{-6} M or endothelin-1 (ET-1) 10^{-8}–10^{-7} M. Noradrenaline (NA) 3×10^{-6}–10^{-5} M was used to induce contractions in the urethra and the trigone. These concentrations gave submaximal contractions of approximately the same relative amplitude (60–90% of the tension induced by K^+ 124 mM).

First, frequency-response curves (electrical stimulation, 1–16 Hz) or concentration-response curves (agonists) were constructed. Next, L-NOARG (10^{-7}–10^{-5} M), D-NOARG (10^{-5} M), L-ARG (10^{-3} M), or methylene blue (10^{-5}–3×10^{-5} M) were given at least 15 min before the preparations were once again subjected to electrical stimulation or exposed to agonists. The relaxant effects of NO (10^{-6}–3×10^{-3} M), SIN-1 (10^{-7}–3×10^{-4} M), VIP (10^{-9}–10^{-6} M) and isoprenaline (10^{-9}–10^{-5} M) were studied by cumulative addition. The concentration was increased only after the response to the previous addition had reached a maximal level.

The effects of oxyhaemoglobin (10^{-5} M) and pyrogallol (10^{-4} M) on trigonal relaxation induced by electrical stimulation (2 and 8 Hz), NO (10^{-4} M) and SIN-1 (10^{-4} M) were subsequently studied on the same NA-induced contraction.

In some experiments, the effects of SIN-1 on spontaneous myogenic activity (in the trigone and urethra) and Bay K 8644-induced myogenic activity (in the detrusor) were studied.

The effect of electrical stimulation on detrusor strips, contracted by K^+ (35 mM) or ET-1, were studied after pretreatment with α-β methylene ATP (10^{-5} M), and in the presence of atropine (10^{-6} M).

Drugs and solution

The following drugs were used: (−)-noradrenaline hydrochloride, carbamylcholine chloride, vasoactive intestinal polypeptide (VIP), isoprenaline, propranolol hydrochloride, tetrodotoxin, atropine sulphate, methylene blue, N^G-nitro-L-arginine (L-NOARG), L-arginine hydrochloride, α-β methylene ATP, pyrogallol, haemoglobin (human) (Sigma, U.S.A.), N^G-nitro-D-arginine (D-NOARG), (Bachem, Germany), endothelin-1 (Peptide Institute, Japan), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl phenyl)-pyridine-5-carboxylate), nifedipine (Bayer, Germany) phentolamine methane sulphonate (Ciba-Geigy, Switzerland). SIN-1 (3-morpholino-sydnonimin hydrochloride) was a gift from Dr Kunstmann, Cassella AG, Germany. Stock solutions were prepared and then stored at − 70°C. Subsequent dilutions of the drugs were made with 0.9% NaCl, and when appropriate, 1 mM ascorbic acid was added as an antioxidant. Bay K 8644, nifedipine and SIN-1 were kept in dark vessels in order to minimize light-induced degradation. ET-1 was diluted with 0.9% NaCl containing 0.05% human serum albumin (Behringwerke, Germany) to counteract the adhesive tendency of the peptide. Acidified solutions (pH 2) of $NaNO_2$ (Sigma, U.S.A.) were prepared immediately before use and protected from atmospheric oxygen by covering the tubes with plastic caps. Separate experiments showed that the vehicle had no relaxant effect *per se*. Oxyhaemoglobin was prepared as described by Martin *et al.* (1985).

The reported concentrations are the calculated final concentrations in the bath solution. The Krebs solution used had the following composition (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 11. K^+-Krebs solutions (124 mM and 35 mM) were prepared by replacing NaCl with equimolar amounts of KCl.

Analysis of data

The effects of electrical field stimulation and drugs are expressed either as percentage relaxation of the agonist-induced tension, or as percentage of the maximal response obtained in control experiments before drug treatment. Statistical determinations were performed by use of Student's two-tailed *t* test. A probability level <0.05 was accepted as significant.

n denotes the number of preparations and N the number of animals. When the number of preparations and animals is identical, only n is given. Results are given as mean values ± s.e.mean.

Results

In all detrusor preparations, tension decreased during the period of equilibration, and the preparations had to be stretched repeatedly. The majority of trigonal and urethral strips were only stretched initially, since the tension increased spontaneously to a steady level during the recovery period. Trigonal strips (92%) developed spontaneous contractions with large amplitudes (Figure 1) during the period of equilibration. Also urethral strips (80%) displayed spontaneous, myogenic activity, although the amplitudes were smaller than in the trigone. In contrast, spontaneous activity was not regularly present in strips from the detrusor (26%), but could be induced by Bay K 8644. The spontaneous activity persisted after addition of atropine (10^{-6} M), phentolamine (10^{-6}M), and TTX (10^{-6} M), but was abolished by nifedipine (10^{-6} M). SIN-1 reduced or abolished spontaneous contractions in the trigone ($n = 7$, Figure 1) and urethra ($n = 6$), and was also able to reduce Bay K 8644-induced contractile activity in the detrusor ($n = 11$).

The trigone

Trigonal strips, precontracted by NA, carbachol or ET-1, caused TTX-sensitive relaxations in response to electrical stimulation. Maximum relaxation was obtained at 6–8 Hz, and amounted to 56 ± 2% ($n = 46$, N = 15), 77 ± 3% ($n = 9$, N = 7) and 62 ± 6% ($n = 5$) in preparations contracted by NA, carbachol, or ET-1, respectively (Figure 2). Exposure to L-NOARG (10^{-7}–10^{-5} M) concentration-dependently reduced the relaxant response to electrical stimulation in preparations contracted by NA (Figure 3). In untreated control preparations, there was no difference in response between the first and the second period of electrical stimulation. Thus, the second stimulation averaged 104 ± 5% ($n = 5$) of the maximal response during the first period of stimulation. At the lowest concentration of L-NOARG used, 10^{-7} M, no inhibition was obtained (data not shown). L-NOARG 10^{-6} M reduced the maximal response to 51 ± 8% ($n = 5$, $P < 0.01$) of control, and L-NOARG 10^{-5} M totally abolished all relaxation (Figure 3), and a contractile component was generally unmasked. D-NOARG (10^{-5} M) had no effect (Figure 3). Also in trigonal preparations where the tension had been raised by carbachol or ET-1, L-NOARG (10^{-5} M) markedly reduced relaxations evoked by electrical stimulation (data not shown).

The maximal relaxation obtained by electrical stimulation in preparations contracted by NA, was increased to 132 ± 10% ($n = 5$, $P < 0.05$) after pretreatment with 10^{-3} M L-ARG (Figure 4), and the inhibitory effect of L-NOARG (10^{-6} M) was prevented (Figure 4). Incubation of the trigonal strips with methylene blue (10^{-5} and 3×10^{-5} M; $n = 5$ in each case) had no effect on relaxations elicited at frequencies < 6 Hz, but a small inhibition (< 25%) was observed at higher frequencies (data not shown).

L-NOARG did not affect the basal tension of the trigonal strips. However, in about 15% of the preparations L-NOARG produced a small further increase in tension when added to preparations contracted by an agonist.

Administration of NO (present in acidified solution of $NaNO_2$) induced concentration-dependent relaxations in trigonal preparations contracted by NA, carbachol, or ET-1. The maximal relaxation obtained at 3×10^{-3} M amounted to 87 ± 4% ($n = 20$, N = 8), 97 ± 2% ($n = 8$) and 86 ± 7% ($n = 5$) of the agonist-induced contraction, respectively (data not shown). L-NOARG (10^{-5} M) and L-ARG (10^{-3} M) had no effects on the relaxations induced by exogenously applied NO ($n = 5$, in each case; Figure 5). However, methylene blue (10^{-5} M) significantly ($n = 5$, $P < 0.05$) shifted the concentration-response curve for NO to the right (Figure 5).

Figure 6 illustrates that pyrogallol (10^{-4} M) and oxyhaemoglobin (10^{-5} M) significantly inhibited relaxations in response to both nerve-stimulation (2 and 8 Hz) and exogenously applied NO (10^{-4} M). Neither pyrogallol nor oxyhaemoglobin inhibited the relaxation produced by SIN-1 (10^{-4} M).

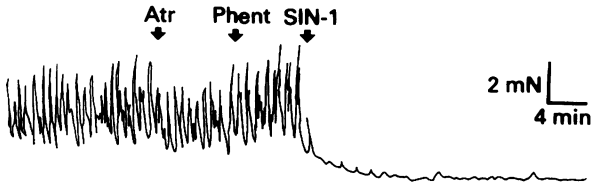

Figure 1 Original tracing from an isolated preparation of the pig trigone showing the effects of atropine (Atr 10^{-6} M), phentolamine (Phent, 10^{-6} M) and SIN-1 (3-morpholino-sydnonimin HCl, 10^{-6} M) on spontaneous myogenic activity.

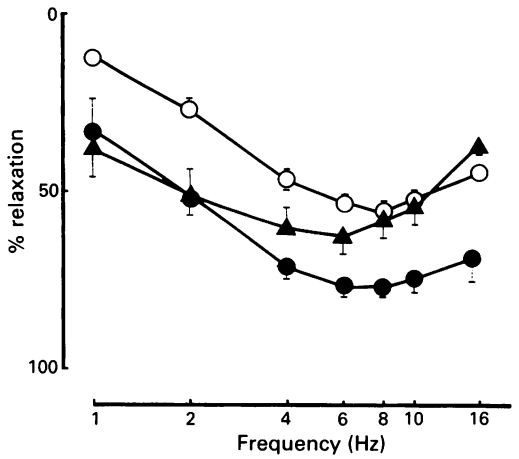

Figure 2 Frequency-response relations for electrically induced relaxations in pig isolated trigonal preparations contracted by (○) noradrenaline (3×10^{-6}–10^{-5} M, $n = 46$, N = 15), (●) carbachol (5×10^{-7}–10^{-6} M, $n = 9$, N = 7) or (▲) endothelin-1 (ET-1, 10^{-8}–10^{-7} M, $n = 5$). Each point is expressed as percentage relaxation of the agonist-induced tension, and represents mean with s.e.mean shown by vertical bars.

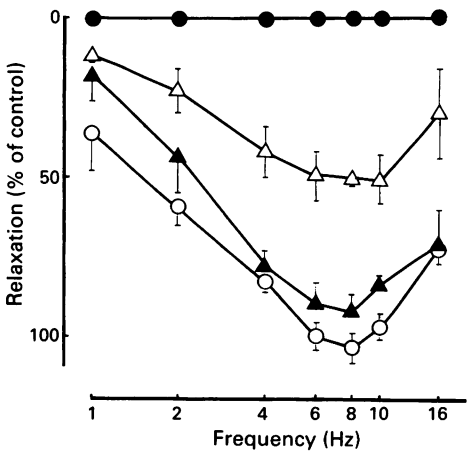

Figure 3 Frequency-response relations for electrically induced relaxations in pig isolated trigonal preparations contracted by noradrenaline (3×10^{-6}–10^{-5} M). Responses were recorded in controls (○) or after pretreatment with N^G-nitro-L-arginine (L-NOARG) 10^{-6} M (△), 10^{-5} M (●) or D-NOARG 10^{-5} M (▲). Each point is expressed as percentage of the maximal response before treatment, and represents mean ($n = 5$) with s.e.mean shown by vertical bars.

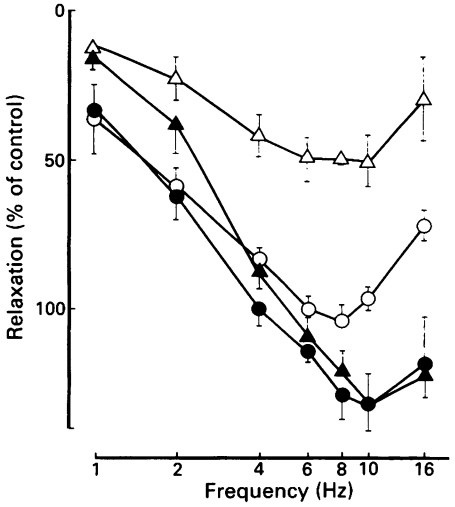

Figure 4 Frequency-response relations for electrically induced relaxations in pig isolated trigonal preparations contracted by noradrenaline (3×10^{-6}–10^{-5} M). Responses were recorded in controls (○) or after pretreatment with N^G-nitro-L-arginine (L-NOARG) 10^{-6} M (△), L-arginine (L-ARG) 10^{-3} M (●) or L-NOARG 10^{-6} M + L-ARG 10^{-3} M (▲). Each point is expressed as percentage of the maximal response before treatment, and represents mean ($n = 5$) with s.e.mean shown by vertical bars.

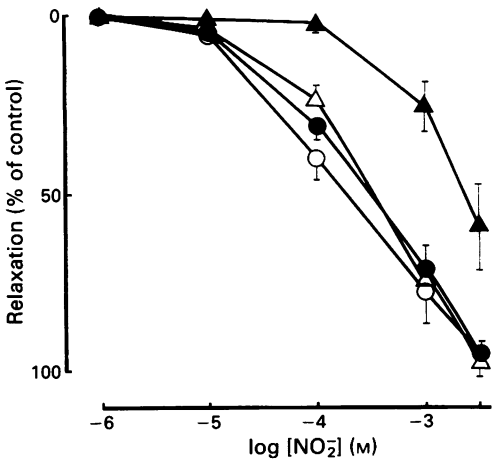

Figure 5 Concentration-response relations for nitric oxide (NO, present in acidified solution of $NaNO_2$) in pig isolated trigonal preparations contracted by noradrenaline (3×10^{-6}–10^{-5} M). Responses were recorded in controls (○) or after pretreatment with L-NOARG 10^{-5} M (△), L-ARG 10^{-3} M (●) or methylene blue 10^{-5} M (▲). Each point is expressed as percentage of the maximal response before treatment, and represents mean ($n = 5$) with s.e.mean shown by vertical bars.

The urethra

In urethral preparations, precontracted by NA, electrical stimulation caused frequency-dependent relaxations that were sensitive to TTX. A maximum relaxation of 73 ± 4% ($n = 10$, N = 6) was obtained at 10 Hz. Also in the urethra, NANC-relaxation was blocked by L-NOARG (10^{-5} M), and a contractile response generally appeared (Figure 7). L-NOARG had no effect on relaxations induced by exogenously applied NO (Figure 7).

The detrusor

In an attempt to suppress contractions induced by potential mediators released by electrical stimulation, detrusor strips

Figure 6 Relaxation induced by electrical stimulation (2 and 8 Hz), nitric oxide (NO, 10^{-4} M, present in acidified solution of $NaNO_2$) and 3-morpholino-sydnonimin HCl (SIN-1, 10^{-4} M) in pig isolated trigonal preparations contracted by noradrenaline (NA, 3×10^{-6}–10^{-5} M). The preparations were pretreated with oxyhaemoglobin (10^{-5} M, hatched columns), pyrogallol (10^{-4} M, solid columns) or remained untreated (open columns). Results are expressed as percentage relaxation of the NA-induced tension, and each column represents mean from 4–5 experiments; s.e.mean shown by vertical bars.

were pretreated with α-β methylene ATP (10^{-5} M) and atropine (10^{-6} M), and then contracted by ET-1 (10^{-8}–3×10^{-8} M) or K^+ (35 mM). ET-1 (10^{-8}–3×10^{-8} M) and K^+ (35 mM) produced contractions amounting to 52 ± 6% ($n = 6$) and 17 ± 3% ($n = 5$) of the K^+ 124 mM-induced response, respectively. α-β methylene ATP *per se* caused transient contractions which were abolished after repeated addition. Electrical field stimulation (2–20 Hz) in no case induced relaxation. In preparations contracted by ET-1 (10^{-8} M–3×10^{-8} M) contractions were observed (Figure 8). However, when the ET-1 concentration was increased to 6×10^{-8}–10^{-7} M (87 ± 4% of the K^+ 124 mM-induced response), 9 out of 11 preparations showed relaxation (19 ± 3% of the induced contraction) in response to electrical stimulation, provided that the preparations were pretreated with atropine (10^{-6} M). L-NOARG abolished the relaxation, but part of the response, particularly that obtained at higher frequencies, persisted after TTX treatment. No response at all, or small contractions, were obtained in K^+ (35 mM)-contracted detrusor strips (Figure 8).

Detrusor preparations contracted by carbachol were concentration-dependently relaxed by NO (10^{-6}–3×10^{-3} M), SIN-1 (10^{-7}–3×10^{-4} M) and isoprenaline (10^{-9}–10^{-5} M), whereas VIP (10^{-9}–10^{-6} M) had only minor effects (Figure 9). NO and SIN-1 produced maximal relaxations of 63 ± 3% ($n = 10$, N = 5) and 70 ± 4% ($n = 10$, N = 5), of the tension induced by carbachol, respectively. Isoprenaline produced an almost complete relaxation (96 ± 4%, $n = 5$, Figure 9). Propranolol (10^{-6} M) did not affect relaxations evoked by NO and SIN-1. Furthermore, NO and SIN-1 also elicited relaxations of detrusor strips precontracted by ET-1 (data not shown). There was no difference in the maximal relaxation produced by NO or SIN-1 in ET-1-contracted compared to carbachol-contracted preparations. Methylene blue (10^{-5} M) inhibited ($P < 0.01$) NO-induced, but not SIN-1-induced relaxations in detrusor strips ($n = 5$, in each case; Figure 10).

Discussion

L-NOARG, an inhibitor of NO synthesis from L-ARG (Mülsch & Busse, 1990), inhibited NANC-relaxation induced by electrical stimulation in smooth muscle preparations of the pig trigone and urethra. The failure of D-NOARG to inhibit the relaxation supports the stereospecific effect of L-NOARG observed on e.g. endothelium-derived relaxing factor (EDRF)-mediated relaxation of vascular smooth mus-

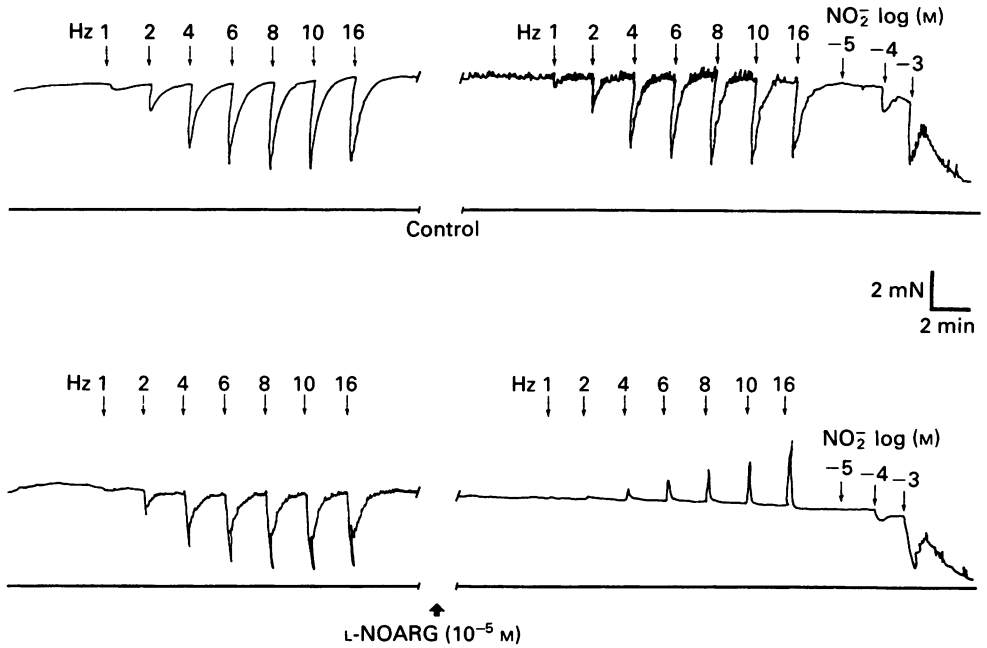

Figure 7 The effect of N^G-nitro-L-arginine (L-NOARG (10^{-5} M) on relaxation induced by electrical stimulation in pig isolated urethra. Precontraction above baseline (shown as a solid line) was induced by noradrenaline (10^{-5} M). The upper panel shows a time-matched control preparation. NO (present in acidified solution of $NaNO_2$) was applied at the end of the experiment.

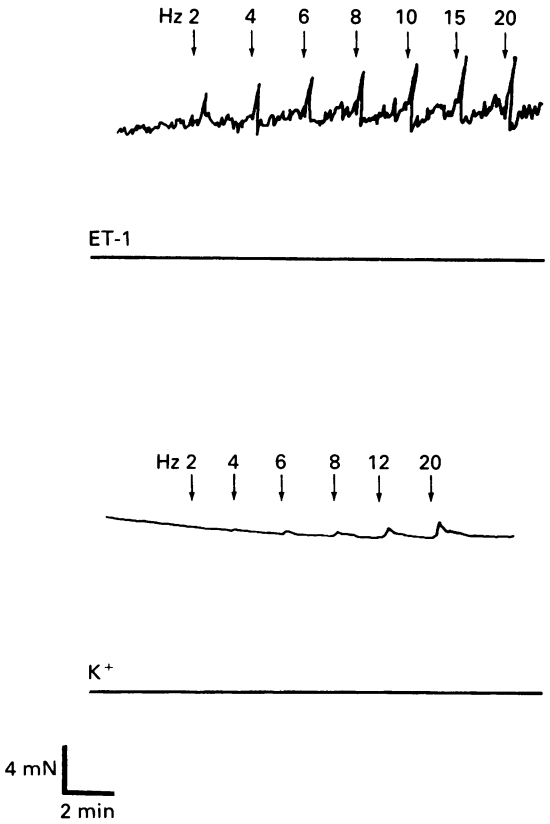

Figure 8 The response to electrical stimulation in pig isolated detrusor preparations contracted above baseline (shown as a solid line) by 10^{-8} M endothelin-1 (ET-1) (upper panel) or K^+ (35 mM) (lower panel). The experiments were performed after pretreatment with α-β methylene ATP (10^{-5} M), and in the presence of atropine (10^{-6} M).

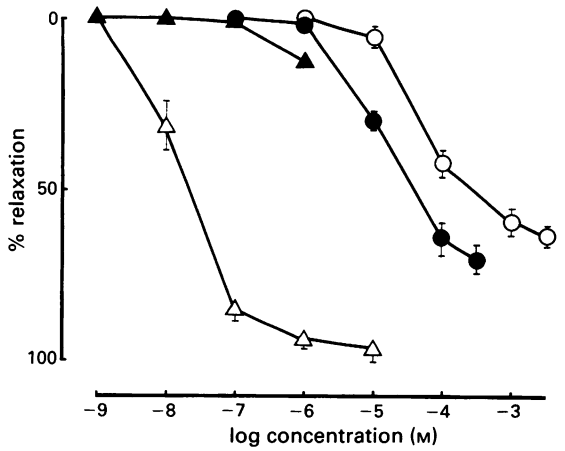

Figure 9 Concentration-response curves obtained by addition of nitric oxide (NO, present in acidified solution of $NaNO_2$) (○, $n = 10$, N = 5), (SIN-1, ●, $n = 10$, N = 5), VIP (▲, $n = 5$) and isoprenaline (△, $n = 5$) to pig isolated detrusor preparations contracted by carbachol (5×10^{-7}–10^{-6} M). Each point is expressed as percentage relaxation of the carbachol-induced tension, and represents mean ± s.e.mean.

cle (Rees *et al.*, 1989). In support of the view that L-ARG is the substrate for the NO-yielding enzyme, it was found that the nerve-induced response increased after pretreatment with L-ARG. L-ARG was also able to reverse the inhibitory effect of L-NOARG, suggesting that L-NOARG is acting competitively. Furthermore, L-NOARG and L-ARG had no effects on the response to exogenously added NO, confirming that their site of action is prejunctional. Taken together, it seems that NO or a NO-containing substance is involved in NANC-relaxation in the pig trigone and urethra. It is not known if NO is released from nerves directly, or whether an unknown nerve-released transmitter causes the generation of NO. However, NO synthase has recently been localised to peripheral neurones (Bredt *et al.*, 1990) and to NANC-nerves

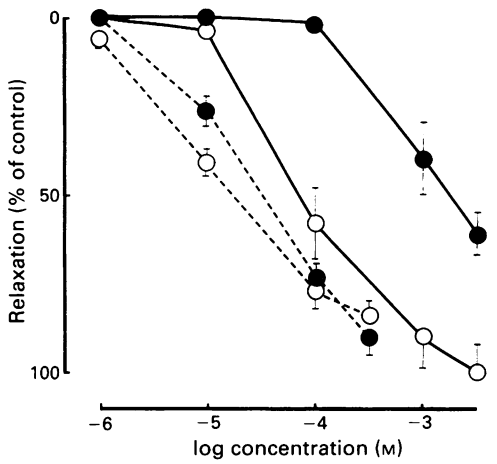

Figure 10 Concentration-response curves obtained by addition of nitric oxide (NO, present in acidified solution of $NaNO_2$) (continuous line) and 3-morpholino-sydnonimin (SIN-1, dashed line) to pig isolated detrusor preparations contracted by carbachol (5×10^{-7}–10^{-6} M) in the absence (○) or presence (●) of methylene blue (10^{-5} M). Each point is expressed as percentage of the maximal response before treatment, and represents mean ($n = 5$) with s.e.mean shown by vertical lines.

(Mitchell *et al.*, 1991) suggesting that NO synthesis by nerves is possible. The fact that exogenously applied NO mimics the response to nerve stimulation gives further support to the view that NO is involved. Thus, the present study extends the previous proposal (Garcia-Pascual *et al.*, 1991; Andersson *et al.*, 1991; 1992) that the L-ARG/NO pathway may have a role in regulation of urethral smooth muscle relaxation, and also suggests a similar mechanism for trigonal relaxation.

NANC-relaxation involving the L-ARG/NO pathway has now been recognized in e.g. the gastrointestinal tract (Bult *et al.*, 1990; Li & Rand, 1990; Boeckxstaens *et al.*, 1991), the oesophageal sphincter (Tøttrup *et al.*, 1991; De Man *et al.*, 1991), tracheal smooth muscle (Tucker *et al.*, 1990; Li & Rand, 1991), the anococcygeus muscle (Gillespie *et al.*, 1989; Hobbs & Gibson, 1990) and in the corpus cavernosum (Ignarro *et al.*, 1990; Holmquist *et al.*, 1991; 1992). Thus, it seems that the L-ARG/NO pathway represents a common pathway mediating NANC-relaxation in a number of tissues.

In this study, the presence of spontaneous contractile activity was clearly dependent on the region from where the strips had been taken. This is in agreement with the results of Sibley (1984), who also found that spontaneous activity in the pig bladder was regularly present in the trigone region, but sparse in the dome. The spontaneous activity in detrusor, trigone and urethral preparations was not affected by TTX, atropine or phentolamine, but depressed by SIN-1. This suggests that the spontaneous activity is of myogenic origin, and that it can be modulated by drugs believed to act as NO-donors.

In trigonal strips, L-NOARG did not affect basal tension. However, when the preparations were contracted, addition of L-NOARG occasionally caused a further increase in tension. This is similar to findings in rabbit urethral preparations where it was found that L-NOARG caused a further increase of the NA-induced tension in about 50% of the strips (Andersson *et al.*, 1992). These results suggest that the L-ARG/NO pathway is not activated during resting conditions, but that NO can be released by contractile activation.

Methylene blue, an agent believed to inhibit the activation of soluble guanylate cyclase (Martin *et al.*, 1985), had only minor effects on the nerve-induced relaxation in the trigone. On the other hand, the relaxation induced by exogenously applied NO was reduced by methylene blue. This result suggests that the mediator of NANC-relaxation is not authentic NO, or alternatively, that the mechanisms for guanylate cyclase activation for exogenous NO and the NANC-mediator may be different. In fact, recent data (Myers *et al.*, 1990) has shown that EDRF is more likely to be a nitrosylated compound such as a nitrosothiol than authentic NO. Oxyhaemoglobin, which binds to NO in the extracellular space (Martin *et al.*, 1985), and pyrogallol, which generates superoxide anion (Moncada *et al.*, 1986), should both be expected to reduce the response to NO. In fact, inhibitory effects of oxyhaemoglobin and pyrogallol were found on relaxations induced by electrical stimulation of NANC-nerves and on relaxations induced by exogenous NO. This further strengthens the view that the NANC-transmitter closely resembles NO. The fact that the effects of SIN-1 were insensitive to methylene blue, pyrogallol, and oxyhaemoglobin may argue against liberation of NO as the predominant mechanism of SIN-1 action in the lower urinary tract. Alternatively, SIN-1 may liberate NO first after cellular metabolism, and in that case be insensitive to extracellular degradation by pyrogallol and oxyhaemoglobin.

Little is known of the mechanisms that keep the detrusor relaxed during urine storage. In the cat, Edvardsen (1968) found an increased sympathetic activity during bladder filling. However, the role of β-adrenoceptor-mediated relaxation during this phase has been questioned. In a previous study (Klarskov, 1987), a NANC-mediated, TTX-sensitive, relaxation in response to electrical stimulation was observed in the pig detrusor after pretreatment with several contractile antagonists. The relaxation was always preceded by a contraction, and was not dependent on the stimulation frequency used. Generally, the NANC-mediated relaxations in the urethra of several species have had a frequency maximum below 10–12 Hz (Klarskov *et al.*, 1983; Andersson *et al.*, 1983; 1991; 1992; Garcia-Pascual *et al.*, 1991). We had difficulties in demonstrating a relaxant effect in our detrusor preparations. Only when the preparations were contracted to a high tension and after pretreatment with α-β methylene ATP and atropine, was a small relaxant response found. The discrepancy in results may be due to differences in experimental approach.

A TTX-insensitive response to electrical stimulation in human detrusor strips, which could be blocked with L-NOARG, has recently been described (James *et al.*, 1991). The preparations were pretreated with atropine, and then contracted by K^+ (20 mM). The relaxation obtained had a maximum at a stimulation frequency of 10 Hz. The authors speculated that NO might be generated by the detrusor muscle itself, and that defects in this system may play a role in the pathophysiology of detrusor instability. We were not able to reproduce the results reported by James *et al.* (1991) in the pig detrusor. This may be due to differences in the experimental approach (we used 35 mM K^+ for contraction) or to species differences. However, the relaxant response we found in some detrusor preparations was abolished by L-NOARG, and a detrusor relaxation involving NO can therefore not be excluded. Considering the difficulties in evoking nerve-induced detrusor relaxations (e.g. blockade of muscarinic receptors was necessary), the physiological relevance of this response might be questioned. In addition, the relaxation of the pig detrusor showed inconsistent responses to TTX, and therefore no conclusion can be drawn about the nature of the relaxation found. Although no endogenous source for NO could be confirmed in the detrusor in this study, it was found that both SIN-1 and exogenous NO had relaxant effects. The possibility that NO generated in other bladder structures (e.g., the urothelium) can exert relaxant effects on the detrusor muscle can therefore not be excluded. The maximal relaxant effect of SIN-1 and NO in the detrusor was, on the other hand, not as pronounced as in the trigone. However, the existence of a functionally important relaxation of detrusor muscle, mediated by the L-ARG/NO pathway, remains uncertain.

Thus, the results show that NANC-nerve mediated relaxation, involving the L-ARG/NO pathway, can be demon-

strated consistently in the trigonal and urethral, but not in the detrusor smooth muscle. The importance of this pathway for lower urinary tract physiology and pathophysiology remains to be established.

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