Br. J. Pharmacol. (1992), **107**, 178–184

Effects of inhibition of the L-arginine/nitric oxide pathway in the rat lower urinary tract *in vivo* and *in vitro*

*K. Persson, [1]†Y. Igawa, †A. Mattiasson & [2]*K.-E. Andersson

Departments of *Clinical Pharmacology and †Urology, Lund University Hospital, Lund, Sweden

1 The present study was performed to investigate how blockade of the L-arginine/nitric oxide (NO) pathway influences the function of the lower urinary tract *in vivo*, as studied by cystometry in conscious rats and *in vitro*, in isolated muscle preparations from the rat detrusor and urethra.

2 L-N^G-nitro arginine methyl ester (L-NAME), 10 and 20 mg kg^{-1}, administered intra-arterially, decreased micturition volume and bladder capacity, and increased spontaneous bladder contractions. D-NAME (20 mg kg^{-1}) had no effect. No changes in the urodynamic parameters were recorded if L-NAME (20 mg kg^{-1}) was administered in combination with L-arginine (200 mg kg^{-1}).

3 Cystometries performed after intra-arterial administration of sodium nitroprusside (SNP) (3 mg kg^{-1}) and 3-morpholino-sydnonimin hydrochloride (SIN-1, 2 mg kg^{-1}) showed a decrease in bladder capacity, micturition volume and threshold pressure. SIN-1, but not SNP, induced spontaneous bladder contractions.

4 Isolated precontracted urethral preparations responded to electrical stimulation with a frequency-dependent tetrodotoxin-sensitive relaxation. L-NAME (10^{-4} M), but not D-NAME, reduced the maximal relaxation to 31 ± 8% ($n = 8$) of the response prior to drug administration. The inhibition induced by L-NAME was completely reversed by L-arginine (10^{-3} M). SNP (10^{-8}–10^{-4} M), SIN-1 (10^{-6}–3×10^{-4} M) and NO (10^{-5}–10^{-3} M; present in acidified solution of $NaNO_2$), caused relaxation (93–100%) of urethral preparations. L-NAME did not affect these relaxations.

5 Detrusor strips contracted by carbachol or K^+ showed contractions in response to electrical stimulation, even when pretreated with α,β-methylene ATP and/or atropine. Small relaxations (14–41%) of detrusor strips were evoked by SNP (10^{-6}–10^{-4} M), SIN-1 (10^{-5}–3×10^{-4} M) and NO (10^{-5}–10^{-3} M). Electrically (20 Hz) induced contractions of the detrusor muscle were unaffected by addition of L-NAME (10^{-6}–10^{-4} M) or L-arginine (10^{-3} M).

6 The present results suggest that the L-arginine/NO pathway is of functional importance for the bladder outlet region, but that its role in the detrusor is questionable. They also suggest that the site of action of L-NAME for inducing bladder hyperactivity in the rat is the outlet region rather than the detrusor muscle.

Keywords: Nitric oxide; rat urinary tract; cystometry; L-N^G-nitro arginine methyl ester

Introduction

Normally, filling of the bladder occurs without any marked changes in intravesical pressure (Coolsaet, 1985). The mechanism is not known, but the behaviour has been attributed mainly to the physical properties of the detrusor muscle (Tang & Ruch, 1955; Klevmark, 1977). In addition, there may be factors which keep the detrusor muscle from being activated. Such a factor may be increased sympathetic activity and release of noradrenaline acting on the β-adrenoceptors of the detrusor (Edvardsen, 1968). However, the importance of β-adrenoceptors in human detrusor relaxation has been questioned (Klevmark, 1977; Nordling, 1983), and is not clarified.

In man, the normal pattern of voiding is characterized by an initial drop in urethral pressure, followed 5 to 15 s later by an increase in intravesical pressure (Tanagho & Miller, 1970; Asmussen & Ulmsten, 1976; Low, 1977; McGuire, 1978; Rud *et al.*, 1978). Urethral pressure variations have also been demonstrated, both in normal females and in patients with certain voiding disturbances (Ulmsten *et al.*, 1977; 1982; Kulseng-Hanssen, 1987; Low *et al.*, 1989). The mechanism(s) behind urethral relaxation and urethral pressure variations has not been clarified.

Thus, there seems to be a physiological role for a relaxation producing system in the outflow region. The involvement of such a system in voiding disturbances, such as those caused by unstable detrusor contractions may be of pathophysiological importance.

It is known that isolated, contracted urethral and trigonal smooth muscles from rabbit, pig, sheep, and man, respond to transmural stimulation of nerves with a relaxant response mediated by a non-adrenergic, non-cholinergic (NANC) mechanism (Andersson *et al.*, 1983; 1991; 1992; Klarskov *et al.*, 1983; Speakman *et al.*, 1988; Garcia-Pascual *et al.*, 1991; Dokita *et al.*, 1991; Persson *et al.*, 1991a; Persson & Andersson, 1992). This relaxant response can be reversed by N^G-L-nitro arginine (L-NOARG), but not D-NOARG, and is enhanced by L-arginine (L-Arg), suggesting involvement of the L-Arg/nitric oxide (NO) pathway (Andersson *et al.*, 1991; 1992; Garcia Pascual *et al.*, 1991; Dokita *et al.*, 1991; Persson *et al.*, 1991a; Persson & Andersson, 1992). The present study was performed to investigate how blockade of the L-Arg/NO pathway by the NO synthase inhibitor L-N^G-nitro arginine methyl ester (L-NAME; Rees *et al.*, 1990) affects micturition in the conscious rat, as reflected by cystometry, and how such blockade affects NANC-nerve-mediated relaxation of rat isolated urethral smooth muscle. A brief account of some of these results has been given previously (Persson *et al.*, 1991b).

[1] On leave from Shinshu University School of Medicine, Matsumoto, Japan.
[2] Author for correspondence.

Methods

In vivo *experiments*

Surgical procedures Female Sprague-Dawley rats weighing 180–240 g were anaesthetized with ketamine (75 mg kg^{-1}, i.m.) and xylazine (15 mg kg^{-1}, i.m.). The abdomen was opened through a midline incision and a polyethylene catheter (Clay-Adams PE-50) with a cuff was inserted into the bladder through the dome and held in place with a purse-string suture. The catheter was tunnelled subcutaneously, and an orifice was made at the back of the animal. The abdominal incision was closed and the free end of the catheter sealed. Two days after this operation, the animals were again anaesthetized and a femoral artery was exposed through an inguinal incision and a polyethylene catheter (Clay-Adams PE-10) filled with heparinised saline (30 i.u. ml^{-1}) was inserted into the vessel and advanced proximally until the tip of the catheter reached the abdominal aortic bifurcation. Both femoral arteries were ligated to increase the amount of drug reaching the bladder. After the experiment, the position of the catheter in the abdominal aorta was confirmed in each animal.

Cystometrical investigations Cystometrical investigations were performed the day after insertion of the femoral catheter. No anaesthesia was used. The conscious rat was placed in a metabolic cage which also enabled measurements of micturition volumes by means of a fluid collector connected to a Grass force displacement transducer (FT03C). The bladder catheter was connected via a T-tube to a pressure transducer (Statham P23 DC) and an infusion pump (Microinject, Bioinvent). Saline was infused into the bladder at a rate of 10 ml min^{-1}. Intravesical pressure and micturition volume were recorded continuously on a Grass polygraph. The following urodynamic variables were investigated: basal pressure (the lowest bladder pressure during cystometry), threshold pressure (bladder pressure immediately prior to micturition), micturition pressure (the maximum bladder pressure during micturition), micturition volume (volume of expelled urine), residual volume (volume of infused saline minus micturition volume), spontaneous activity (mean amplitude and frequency of bladder pressure fluctuations during two minutes prior to micturition), and bladder compliance (bladder capacity/(threshold pressure minus basal pressure)). Urodynamic parameters were analysed as described previously (Malmgren *et al.*, 1987) during a 20 min period before and a 10 min period after each intra-arterial administration of drug.

Drugs (see below) were dissolved in 0.9% saline and administered through the intra-arterial catheter followed by a flush of 0.1 ml of heparinised saline for 5 s; 0.2 ml of heparinised saline was injected intra-arterially as control, prior to drug administration.

In vitro *experiments*

Tissue preparations and recording of mechanical activity Female Sprague-Dawley rats (200–250 g) were killed by CO_2 asphyxia, and the bladder together with the urethra were dissected out. Thereafter fat and connective tissue were removed, and the detrusor and the urethra separated by a transverse cut. Circular (intact ring segments, 2 mm wide) or longitudinal (1 × 1 × 5 mm) preparations were taken from the proximal part of the urethra. Detrusor strips (1 × 1 × 5 mm) were dissected from a ring of detrusor tissue comprising the middle third of the bladder. The ring was opened and yielded 2 to 4 preparations. The mucosa was not removed from urethral and detrusor strips.

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 urethral and detrusor 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 wave pulses (duration 0.8 ms) at supramaximal voltage. The train duration was 5 s and the stimulation interval 120 s.

Experimental procedure After the equilibration 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. Relaxant responses to electrical stimulation and to NO (present in acidified solution of $NaNO_2$), SIN-1 (3-morpholino-sydnonimin hydrochloride) and sodium nitroprusside (SNP) were studied in precontracted preparations. Contractions of detrusor preparations were evoked by carbachol 10^{-6}–10^{-5} M, and noradrenaline (NA) 10^{-5} and 3×10^{-5} M was used to induce contractions in the urethra.

In urethral preparations subjected to electrical stimulation, frequency-response (0.5–30 Hz) relations were first studied in the absence of drug treatment. Thereafter L-NAME (10^{-4} M), D-NAME (10^{-4} M) or L-NAME (10^{-4} M) + L-Arg (10^{-3} M) were given at least 15 min before the preparations were once again subjected to electrical stimulation. Control preparations were run in parallel to study the reproducibility of the relaxation. The relaxing effects of NO (10^{-5}–10^{-3} M), SIN-1 (10^{-6}–3×10^{-4} M), and SNP (10^{-8}–10^{-4} M) were studied by cumulative addition. The concentration was increased only after the response to the previous addition had reached a maximal level.

The effect of electrical stimulation on detrusor strips was studied in carbachol- or K^+ (35 mM) contracted preparations. In some experiments, α,β-methylene ATP (10^{-5} M) and/or atropine (10^{-6} M) were included. The effects of L-NAME (10^{-6}–10^{-4} M) and L-Arg (10^{-3} M) on detrusor contractions evoked by electrical stimulation were investigated at a submaximal (70–80% of max) frequency (20 Hz). When the variation between three consecutive contractions was $< 10\%$ the drug investigated was applied cumulatively.

Drugs and solutions

The following drugs were used: (−)-noradrenaline hydrochloride, atropine sulphate, carbamylcholine chloride, isoprenaline, tetrodotoxin, L-N^G-nitro arginine methyl ester (L-NAME), L-arginine hydrochloride, α,β-methylene ATP, (Sigma, USA), N^G-nitro-D-arginine methyl ester (D-NAME), (Bachem, Germany), sodium nitroprusside (Nipride, Roche, 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. Sodium nitroprusside and SIN-1 were kept in dark vessels in order to minimize light-induced degradation. To study the effects of NO, an acidified solution of $NaNO_2$ was used (Furchgott *et al.*, 1988). The $NaNO_2$ was adjusted to pH 2 by addition of HCl. Separate experiments showed that the vehicle had no relaxing effect *per se*.

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 *in vitro* 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 for paired data. A probability level <0.05 was accepted as significant. Results are given as mean values ± s.e.mean. n denotes the number of preparations, and N the number of animals. When the number of preparations and animals are identical, only n is given.

Results

In vivo *experiments*

Cystometrical investigation Repeated cystometries in the same animal gave reproducible and consistent results. No spontaneous bladder contractions were recorded between each micturition (Figure 1). Intra-arterial administration of L-NAME 10 mg kg^{-1} ($n = 6$) and 20 mg kg^{-1} ($n = 11$) induced spontaneous bladder contractions (bladder hyperactivity) and decreased bladder capacity and micturition volume (Figure 1; Table 1). L-NAME, 20 mg kg^{-1} increased the frequency of the spontaneous activity from 0.23 ± 0.12 to 1.6 ± 0.3 min^{-1} ($P<0.01$) and the amplitude from 2.7 ± 1.3 to 5.8 ± 1.3 cmH_2O ($P<0.05$). The bladder capacity and micturition volume decreased from 0.90 ± 0.08 to 0.66 ± 0.08 ml ($P<0.01$) and from 0.88 ± 0.08 to 0.59 ± 0.08 ml ($P<0.001$), respectively. In addition, a decrease ($P<0.01$) in threshold pressure and an increase ($P<0.05$) in residual urine were noted (Table 1). After administration of 10 mg kg^{-1} L-NAME, similar changes in bladder capacity, micturition volume, and in the frequency of the spontaneous activity were observed (Table 1). However, the other parameters did not achieve statistical significance at this dose. The effects of L-NAME were reversible and the cystometrical parameters were restored to the starting level within less than 1 h.

Administration of L-Arg (200 mg kg^{-1}; $n = 8$) 5 min before the injection of L-NAME (20 mg kg^{-1}) antagonized the previously described effects of L-NAME (Figure 2; Table 2). Thus, a tendency toward a decrease in the amplitude and the frequency of the spontaneous activity was found. In the presence of L-Arg, L-NAME was without effect on bladder capacity and micturition volume (Figure 2; Table 2). No significant change in any cystometrical parameter was found after administration of D-NAME (20 mg kg^{-1}; $n = 4$, Table 2).

Cystometries performed after intra-arterial administration of SNP (3 mg kg^{-1}; $n = 6$) showed a decrease in bladder capacity, micturition volume, and threshold pressure. Micturition pressure was reduced in the majority of the rats after administration of SNP, but the decrease was not significant (Table 3). SIN-1 (2 mg kg^{-1}; $n = 7$) reduced bladder capacity, micturition volume, and threshold pressure. SIN-1 also increased both frequency and amplitude of the spontaneous contractions.

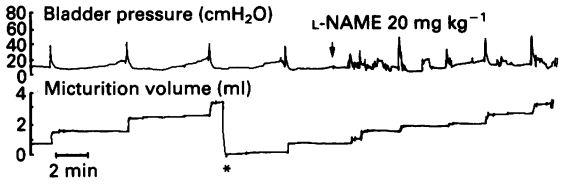

Figure 1 Original recordings of bladder pressure and micturition volume during cystometry before and after intra-arterial administration of L-N^G-nitro arginine methyl ester (L-NAME, 20 mg kg^{-1}) to an unanaesthetized rat. *indicates adjustment to baseline position.

In vitro *experiments*

Urethra All of the longitudinal urethral preparations responded to electrical stimulation with a tetrodotoxin (TTX)-sensitive relaxation. The relaxation of the longitudinal preparations was frequency-dependent. Maximal relaxation was obtained at 16 Hz and averaged 79 ± 3% ($n = 23$, N = 15) of the induced tension (Figure 3). Only 25% ($n = 12$, N = 8) of the urethral preparations taken in the circular direction showed a relaxation when stimulated electrically.

The relaxation induced by electrical stimulation of long-

Table 1 The effects of intra-arterial administration of L-N^G-nitro arginine methyl ester (L-NAME, 10 mg kg^{-1}, $n = 6$ and 20 mg kg^{-1}, $n = 11$) on cystometrical parameters in the rat

	Control	*L-NAME* (10 mg kg^{-1})
Basal pressure (cmH_2O)	10 ± 2	9.3 ± 2.0
Threshold pressure (cmH_2O)	14 ± 2	12 ± 2
Micturition pressure (cmH_2O)	51 ± 10	56 ± 10
Micturition volume (ml)	0.78 ± 0.15	0.53 ± 0.10**
Freq. Spont. activity (min^{-1})	0.32 ± 0.21	1.1 ± 0.3*
Amp. Spont. activity (cmH_2O)	2.2 ± 1.8	11 ± 4
Compliance (ml cmH_2O^{-1})	0.30 ± 0.10	0.39 ± 0.17
Bladder capacity (ml)	0.84 ± 0.17	0.60 ± 0.14**
Residual volume (ml)	0.06 ± 0.03	0.08 ± 0.04
	Control	*L-NAME* (20 mg kg^{-1})
Basal pressure (cmH_2O)	6.5 ± 0.9	6.0 ± 1.0
Threshold pressure (cmH_2O)	13 ± 2	10 ± 1**
Micturition pressure (cmH_2O)	55 ± 5	51 ± 6
Micturition volume (ml)	0.88 ± 0.08	0.59 ± 0.08***
Freq. Spont. activity (min^{-1})	0.23 ± 0.12	1.6 ± 0.3**
Amp. Spont. activity (cmH_2O)	2.7 ± 1.3	5.8 ± 1.3*
Compliance (ml cmH_2O^{-1})	0.17 ± 0.03	0.15 ± 0.02
Bladder capacity (ml)	0.90 ± 0.08	0.66 ± 0.08**
Residual volume (ml)	0.03 ± 0.01	0.08 ± 0.02*

Results are expressed as mean ± s.e.mean.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

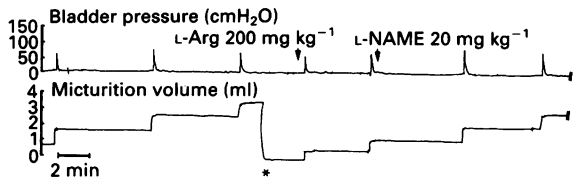

Figure 2 Original recordings of bladder pressure and micturition volume during cystometry before and after intra-arterial administration of L-arginine (L-Arg, 200 mg kg^{-1}) in combination with L-N^G-nitro-arginine methyl ester (L-NAME, 20 mg kg^{-1}) to an unanaesthetized rat. *indicates adjustment to baseline position.

itudinal preparations was reproducible. Thus, the second stimulation averaged 104 ± 6% ($n = 6$) of the maximal response during the first period of stimulation (Figure 4). In longitudinal preparations, exposure to L-NAME (10^{-4} M) significantly ($P < 0.001$) reduced the maximal relaxation to electrical stimulation to 31 ± 8% ($n = 8$) of control (Figures 4 and 5). In five of eight strips, L-NAME caused a further increase in tension (26 ± 6%) when added to the NA-induced contraction (Figure 5). L-NAME did not affect the tension when applied at baseline level. No inhibition of the electrically-induced relaxation was obtained by 10^{-4} M D-NAME ($n = 4$; Figure 4). The inhibition induced by L-NAME (10^{-4} M) was completely reversed by 10^{-3} M L-Arg (Figure 4).

Table 2 The effects of intra-arterial administration of L-arginine (200 mg kg^{-1}) + L-N^G-nitro arginine methyl ester (L-NAME, 20 mg kg^{-1}, $n = 8$) and D-NAME (20 mg kg^{-1}, $n = 4$) on cystometrical parameters in the rat

	Control	*L-Arginine* (200 mg kg^{-1}) + *L-NAME* (20 mg kg^{-1})
Basal pressure (cmH_2O)	6.5 ± 1.5	7.6 ± 2.0
Threshold pressure (cmH_2O)	11 ± 3	10 ± 2
Micturition pressure (cmH_2O)	55 ± 7	54 ± 7
Micturition volume (ml)	0.84 ± 0.08	0.86 ± 0.08
Freq. Spont. activity (min^{-1})	0.21 ± 0.08	0.09 ± 0.07
Amp. Spont. activity (cmH_2O)	1.9 ± 0.8	0.94 ± 0.66
Compliance (ml cmH_2O^{-1})	0.31 ± 0.04	0.37 ± 0.10
Bladder capacity (ml)	0.88 ± 0.08	0.90 ± 0.05
Residual volume (ml)	0.03 ± 0.01	0.04 ± 0.04
	Control	*D-NAME* (20 mg kg^{-1})
Basal pressure (cmH_2O)	6.8 ± 2.8	5.8 ± 2.2
Threshold pressure (cmH_2O)	12 ± 4	11 ± 3
Micturition pressure (cmH_2O)	55 ± 12	54 ± 16
Micturition volume (ml)	0.89 ± 0.17	0.84 ± 0.16
Freq. Spont. activity (min^{-1})	0.78 ± 0.40	0.88 ± 0.26
Amp. Spont. activity (cmH_2O)	4.5 ± 1.9	4.5 ± 0.9
Compliance (ml cmH_2O^{-1})	0.24 ± 0.06	0.23 ± 0.05
Bladder capacity (ml)	0.94 ± 0.16	0.88 ± 0.15
Residual volume (ml)	0.05 ± 0.02	0.03 ± 0.02

Results are expressed as mean ± s.e.mean.

Table 3 The effects of intra-arterial administration of sodium nitroprusside (SNP) (3 mg kg^{-1}, $n = 6$) and 3-morpholino-sydnonimin (SIN-1, 2 mg kg^{-1}, $n = 7$) on cystometrical parameters in the rat

	Control	*SNP* (3 mg kg^{-1})
Basal pressure (cmH_2O)	6.4 ± 1.3	5.2 ± 1.2
Threshold pressure (cmH_2O)	11 ± 1	7.4 ± 0.9***
Micturition pressure (cmH_2O)	49 ± 8	33 ± 6
Micturition volume (ml)	1.0 ± 0.04	0.48 ± 0.11**
Freq. Spont. activity (min^{-1})	0.46 ± 0.19	0.27 ± 0.21
Amp. Spont. activity (cmH_2O)	2.8 ± 1.0	1.7 ± 1.3
Compliance (ml cmH_2O^{-1})	0.26 ± 0.07	0.26 ± 0.08
Bladder capacity (ml)	1.1 ± 0.04	0.59 ± 0.10**
Residual volume (ml)	0.05 ± 0.01	0.12 ± 0.02
	Control	*SIN-1* (2 mg kg^{-1})
Basal pressure (cmH_2O)	5.9 ± 1.1	4.8 ± 1.0
Threshold pressure (cmH_2O)	11 ± 1	8.2 ± 1.3*
Micturition pressure (cmH_2O)	51 ± 5	46 ± 6
Micturition volume (ml)	1.0 ± 0.1	0.60 ± 0.06***
Freq. Spont. activity (min^{-1})	0.19 ± 0.11	0.69 ± 0.15**
Amp. Spont. activity (cmH_2O)	1.9 ± 1.2	5.0 ± 1.0*
Compliance (ml cmH_2O^{-1})	0.29 ± 0.06	0.24 ± 0.05
Bladder capacity (ml)	1.1 ± 0.1	0.70 ± 0.06***
Residual volume (ml)	0.07 ± 0.02	0.09 ± 0.02

Results are expressed as mean ± s.e.mean.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

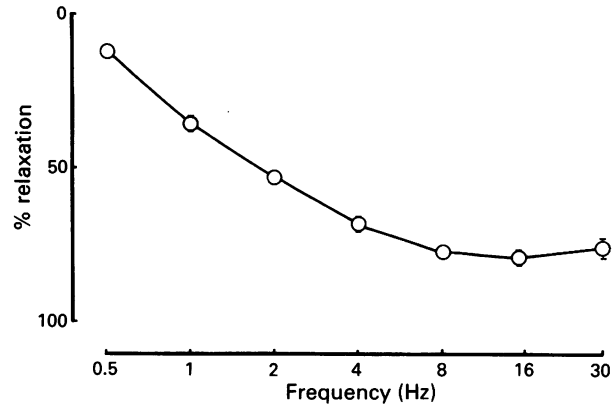

Figure 3 Frequency-response relations for electrically-induced relaxations in rat isolated urethral preparations contracted by noradrenaline (10^{-5}–3×10^{-5} M, $n = 23$, N = 15). Each point is expressed as percentage relaxation of the agonist-induced tension, and represents mean with s.e.mean (vertical bars, where bigger than the symbol).

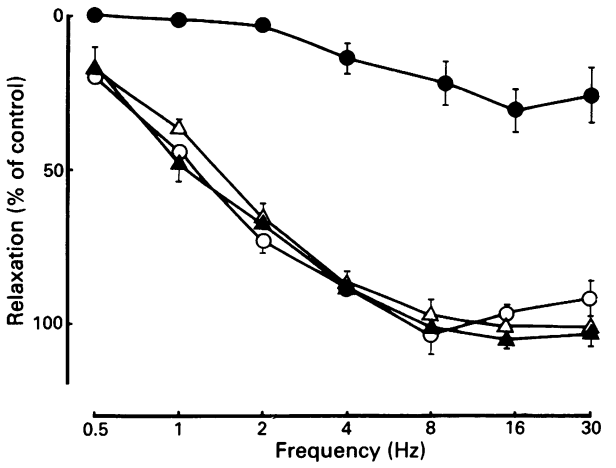

Figure 4 Frequency-response relations for electrically-induced relaxations in rat isolated urethral preparations contracted by noradrenaline (10^{-5}–3×10^{-5} M). Responses were recorded in controls (○) or after pretreatment with L-N^G-nitro-arginine methyl ester (L-NAME) 10^{-4} M (●), D-NAME 10^{-4} M (▲) or L-NAME 10^{-4} M + L-arginine 10^{-3} M (△). Each point is expressed as percentage of the maximal response before treatment, and represents mean ($n = 4$–8); s.e.mean shown by vertical bars.

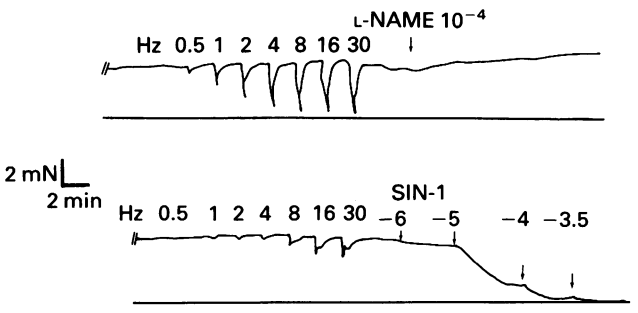

Figure 5 Original tracing showing the effect of pretreatment with L-N^G-nitro-arginine methyl ester (L-NAME, 10^{-4} M) for 15 min on relaxation induced by electrical stimulation in rat isolated urethra. Precontraction above baseline (shown as a solid line) was induced by noradrenaline (3×10^{-5} M). 3-Morpholino-sydnonimin (SIN-1, expressed as log molar concentrations) was applied at the end of the experiment.

SNP, SIN-1 and NO caused concentration-related relaxations of the longitudinal urethral preparations (Figure 6). The maximal relaxation produced by SNP, SIN-1 and NO was 99 ± 1% ($n = 6$), 100 ± 0% ($n = 6$), and 93 ± 4% ($n = 6$) of the NA-induced tension, respectively. Preincubation with L-NAME did not affect the relaxant response to the drugs.

Detrusor The detrusor preparations developed spontaneous, myogenic activity during the equilibration period. SIN-1 (10^{-4} and 3×10^{-4} M) reduced the amplitude of the spontaneous contractile activity in 5 out of 10 strips. L-NAME (10^{-6}–10^{-4} M) had no effect on spontaneous contractions. Detrusor strips precontracted by carbachol (10^{-6}–10^{-5} M; $n = 10$, N = 7) or K^+ (35 mM; $n = 7$, N = 5) showed contractions in response to electrical stimulation (Figure 7). If α,β-methylene ATP (10^{-5} M) and/or atropine (10^{-6} M) were included (in an attempt to suppress the effects of released excitatory transmitters) electrical stimulation of the detrusor still did not cause relaxation. The response to electrical stimulation in the detrusor was not dependent on the direction which the muscle strips were taken, as in the urethra, since it was shown in separate experiments ($n = 4$) that strips taken longitudinally did not behave differently from the standard preparations.

SNP, SIN-1 and NO produced small relaxations of carbachol-contracted detrusor preparations (Figure 8). The maximal relaxation evoked by SNP, SIN-1 and NO averaged 14 ± 5% ($n = 7$), 41 ± 7% ($n = 7$) and 36 ± 5% ($n = 6$) of the carbachol-induced tension, respectively (Figure 8). Isoprenaline (10^{-5} M) was added to some detrusor strips ($n = 6$) to

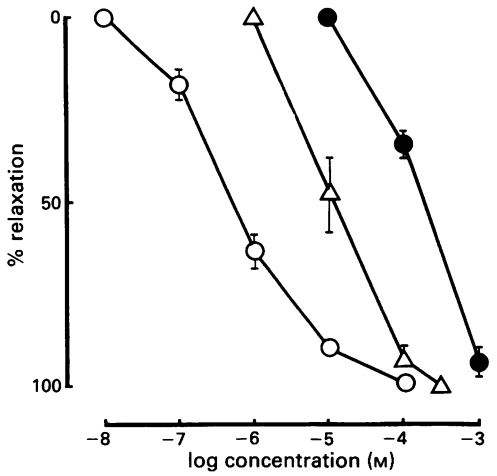

Figure 6 Concentration-response curves obtained by addition of NO (present in acidified solution of $NaNO_2$) (●), 3-morpholino-sydnonimin (△), and sodium nitroprusside (○) to rat isolated urethral preparations contracted by noradrenaline (NA) 10^{-5}–3×10^{-5} M). Each point is expressed as percentage relaxation of the NA-induced tension, and represents mean ($n = 6$) with s.e.mean shown by vertical bars.

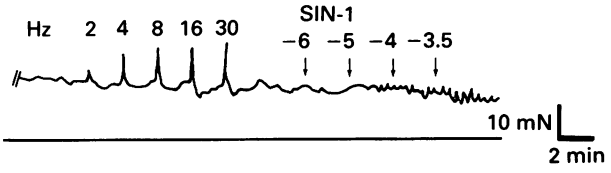

Figure 7 Original tracing showing the response to electrical stimulation in rat isolated detrusor preparations contracted above baseline (shown as a solid line) by 10^{-5} M carbachol. 3-Morpholino-sydnonimin (SIN-1) (expressed as log molar concentrations) was applied at the end of the experiment.

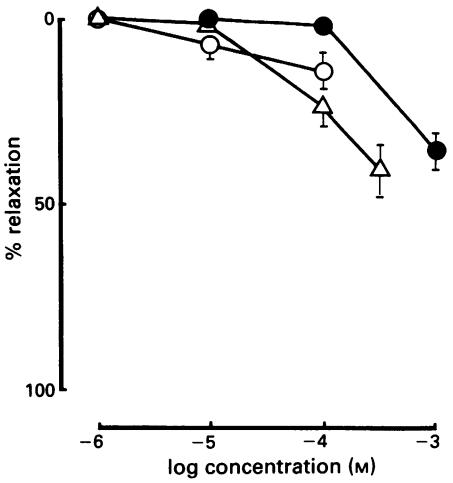

Figure 8 Concentration-response curves obtained by addition of NO (present in acidified solution of $NaNO_2$) (●), 3-morpholino-sydnonimin (Δ), and sodium nitroprusside (○) to rat isolated detrusor preparations contracted by carbachol (10^{-5} M). Each point is expressed as percentage relaxation of the carbachol-induced tension, and represents mean ($n = 6-7$) with s.e.mean shown by vertical bars.

confirm that the smooth muscle was able to relax. The relaxant response produced by isoprenaline was 56 ± 3% of the carbachol-induced tension.

Contractile responses produced by electrical field stimulation (20 Hz) of the detrusor strips were markedly depressed by TTX (10^{-6} M), but about 30–40% of the response persisted after treatment with atropine (10^{-6} M). The electrically-induced contractions were unaffected by addition of L-NAME (10^{-6} M–10^{-4} M; $n = 9$) or L-Arg (10^{-3} M; $n = 8$).

Discussion

The present study shows that inhibition of NO formation from L-Arg in the lower urinary tract of conscious rats stimulates detrusor activity. The regular micturition pattern observed prior to administration of L-NAME was changed into an irregular pattern after injection of the drug, and there was a marked bladder hyperactivity and impairment of bladder capacity. The effects of L-NAME were antagonized by L-Arg, and could not be reproduced by D-NAME, supporting that the effects are mediated by the L-Arg/NO system. It may be speculated that this system has an inhibitory function during bladder filling by acting on the detrusor, as suggested by James *et al.* (1991), or that it inhibits the micturition reflex by an effect on the trigone or the urethra (Persson *et al.*, 1991b; Persson & Andersson, 1992).

In agreement with previous findings in other species, including man (Andersson *et al.*, 1983; 1991; 1992; Klarskov *et al.*, 1983; Dokita *et al.*, 1991; Garcia-Pascual *et al.*, 1991; Persson *et al.*, 1991a; Persson & Andersson, 1992), we observed that the rat isolated urethra exhibits a NANC-nerve mediated relaxation, which can be inhibited by L-NAME. The effects of L-NAME were prevented by L-Arg and could not be reproduced by D-NAME, suggesting that as *in vivo*, the L-Arg/NO system was involved. Interestingly, there was a difference between longitudinal and circular preparations; all the longitudinal, but only 25% of the circular preparations showing relaxation when stimulated electrically. Functional differences between circular and longitudinal orientation of urethral smooth muscle have previously been demonstrated (Persson & Andersson, 1976; Hassouna *et al.*, 1983; Mattiasson *et al.*, 1990). However, the different degrees of relaxation seen in the present study may not be due solely to the orientation of the muscle fibres. Another study of the female rat urethra (Andersson *et al.*, 1990) has shown that the electrically-induced responses of the circularly oriented urethral muscle mainly reflects activation of striated rather than smooth muscle components. The importance of this difference in NANC-mediated relaxation for the function of the rat urethra is, however, not known.

Previous studies have shown that in the rabbit and sheep urethra, the relaxant response is more pronounced at high than at low tension levels (Andersson *et al.*, 1983; Garcia-Pascual *et al.*, 1991), and that spontaneous release of NO seems to occur only at high levels of tension (Andersson *et al.*, 1992). Also in this study, L-NAME increased the tension when applied to precontracted rat urethral strips, but not at baseline level. This suggests that regulation of urethral tension via the L-Arg/NO pathway dominates at high urethral pressures.

Bladder neck relaxation is thought to occur via increased activity in inhibitory nerves (de Groat & Kawatani, 1985). If these inhibitory nerves release NO, direct smooth muscle relaxation should be expected in response to NO and NO-related substances in the bladder outlet region. In fact, such a relaxant effect was evident in the rat urethra after application of NO, SIN-1 and SNP. L-NAME did not affect the relaxant response of the added drugs, i.e. it does not inhibit the smooth muscle effects of NO. It therefore seems reasonable to assume that the functional bladder changes recorded after L-NAME administration during cystometry reflects inhibition of NO synthase, and that NO is an important factor for bladder outlet smooth muscle relaxation. The present results furthermore lend support to the view that unstable detrusor contractions may be initiated from the bladder outlet region (Hindmarsh, 1983, Low *et al.*, 1989, Andersson, 1990). The fact that L-NAME administration causes bladder hyperactivity may be attributed to its ability to inhibit NO-formation in the smooth muscle of the detrusor and urethra. Vasoconstriction in the outlet region can be expected, and the amount of blood in the urethral venous plexus may induce changes in urethral pressure (Rud *et al.*, 1980). Thus, it cannot be excluded that changes in the blood supply may contribute to the observed increase in reflex activity.

In contrast to the findings in the urethra, electrical stimulation of the precontracted rat detrusor in the presence of atropine and after desensitization with α,β-methylene ATP, did not produce relaxation, but further contraction. Such a response has been observed also in pig detrusor (Persson & Andersson, 1992). However, if the pig detrusor preparations were almost maximally contracted, it was possible to induce minor relaxations of both neuronal and non-neuronal nature, which could be inhibited by L-NOARG (Persson & Andersson, 1992). This was not found in the rat detrusor. On the other hand, the experimental conditions in the rat detrusor, with its pronounced excitatory NANC-component, makes relaxation studies complicated. James *et al.* (1991) found that in K^+ (20 mM) contracted preparations of the human detrusor, electrical stimulation evoked relaxations sensitive to L-NOARG, but insensitive to TTX, and they suggested this to be an important factor for bladder relaxation during the filling phase.

The mechanisms behind detrusor relaxation during filling are not fully understood. In the cat, Edvardsen (1968) found an increased sympathetic activity during this phase. Supporting the importance of this finding, Maggi *et al.* (1985) provided evidence for a sympathetic inhibition of the reflex activation of the rat detrusor in response to physiological filling. On the other hand, no influence on the bladder function, at rest or during voiding, could be demonstrated in human patients with various lesions of the sympathetic nervous system (Nordling, 1983). Klevmark (1977) found no support for an inhibitory reflex via the sympathetic nervous system and suggested that accommodation of the bladder to increasing amount of fluid depends almost completely upon the viscoelastic properties of the detrusor muscle. Theoretically, an increased activity of NO-releasing inhibitory nerves to the detrusor, could be one factor keeping the bladder

relaxed during bladder filling. The findings *in vitro* that SNP, SIN-1, and NO were only moderately effective in relaxing the detrusor compared to their effects on the urethra, and that the drugs *in vivo* decreased bladder capacity, micturition volume, and threshold pressure do not favour this view. The increased activity in the micturition reflex seen after administration of SNP and SIN-1, could be ascribed to a drop in urethral pressure, provided that these drugs *in vivo* cause relaxation of urethral smooth muscle as they do *in vitro*. SIN-1 also increased the frequency and amplitude of spontaneous contractions. These effects may seem paradoxical, but can be explained if the drug increases the release of excitatory transmitters from nerves. In fact, such an effect of SIN-1 has been demonstrated in penile erectile tissue (Holmquist *et al.*, unpublished). Thus, NO-mediated relaxation of the detrusor smooth muscle does not seem to be of main functional importance in the rat bladder.

Taken together, the present results suggest that the L-Arg/NO pathway is of functional importance in the bladder outlet region; its role in the detrusor is still unsettled. They also suggest that the site of action of L-NAME for inducing bladder hyperactivity in the rat is the outlet region rather than the detrusor muscle.

The authors thank Mrs Magdalena Dawiskiba for skillful technical assistance. This study was supported by the Swedish Medical Research Council (grant no 6837), the Medical Faculty, University of Lund, and the Sasakawa Health Science Foundation.

References

ANDERSSON, K.-E. (1990). Autonomic neurotransmission and the unstable bladder. *Neurourol. Urodyn.*, **9**, 555–557.

ANDERSSON, K.-E., GARCIA-PASCUAL, A., FORMAN, A. & TØTTRUPT, A. (1991). Non-adrenergic, non-cholinergic nerve-mediated relaxation of rabbit urethra is caused by nitric oxide. *Acta Physiol. Scand.*, **141**, 133–134.

ANDERSSON, K.-E., GARCIA-PASCUAL, A., PERSSON, K., FORMAN, A. & TØTTRUP, A. (1992). Electrically-induced, nerve-mediated relaxation of rabbit urethra involves nitric oxide. *J. Urol.*, **147**, 253–259.

ANDERSSON, K.-E., MATTIASSON, A. & SJÖGREN, C. (1983). Electrically induced relaxation of the noradrenaline contracted isolated urethra from rabbit and man. *J. Urol.*, **129**, 210–214.

ANDERSSON, P.O., MALMGREN, A. & UVELIUS, B. (1990). Functional responses of different muscle types of the female rat urethra in vitro. *Acta Physiol. Scand.*, **140**, 365–372.

ASMUSSEN, M. & ULMSTEN, U. (1976). Simultaneous urethrocystometry with a new technique. *Scand. J. Urol. Nephrol.*, **10**, 7–11.

COOLSAET, B. (1985). Bladder compliance and detrusor activity during the collection phase. *Neurourol. Urodyn.*, **4**, 263–273.

DE GROAT, W.C. & KAWATANI, M. (1985). Neural control of the urinary bladder: possible relationship between peptidergic inhibitory mechanisms and detrusor instability. *Neurourol. Urodyn.*, **4**, 285–300.

DOKITA, S., MORGAN, W.R., WHEELER, M.A., YOSHIDA, M., LATIFPOUR, J. & WEISS, R.M. (1991). N^G-nitro-L-arginine inhibits non-adrenergic, non-cholinergic relaxation in rabbit urethral smooth muscle. *Life Sci.*, **48**, 2429–2436.

EDVARDSEN, P. (1968). Nervous control of urinary bladder in cats. III. Effects of autonomic blocking agents in the intact animal. *Acta Physiol. Scand.*, **72**, 183–193.

FURCHGOTT, R.F., KHAN, M.T., JOTHIANANDAN, D. & KHAN, A.S. (1988). Evidence that the endothelium-derived relaxing factor of rabbit aorta is nitric oxide. In *Vascular Neuroeffector Mechanisms*. ed. Bevan, J.A., Majewski, H., Maxwell, R.A. & Story, D.F. pp. 77–84. Oxford: IRL Press.

GARCIA-PASCUAL, A., COSTA, G., GARCIA-SACRISTAN, A. & ANDERSSON, K.-E. (1991). Relaxation of sheep urethral muscle induced by electrical stimulation of nerves: involvement of nitric oxide. *Acta Physiol. Scand.*, **141**, 531–539.

HASSOUNA, M., ABDEL-HAKIM, A., ABDEL-RAHMAN, M., GALEANO, C. & ELHILALI, M.M. (1983). Response of the urethral smooth muscle to pharmacological agents. 1. Cholinergic and adrenergic agonists and antagonists. *J. Urol.*, **129**, 1262–1264.

HINDMARSH, J.R., GOSLING, P.T. & DEANE, A.M. (1983). Bladder instability. Is the primary defect in the urethra? *Br. J. Urol.*, **55**, 648–651.

JAMES, M.J., BIRMINGHAM, A.T. & BATES, C.P. (1991). Relaxation of human isolated detrusor strips in response to electrical field stimulation: a possible role for nitric oxide in the human bladder. *J. Urol.*, **145**, 307A, abstract 380.

KLARSKOV, P., GERSTENBERG, T.C., RAMIREZ, D. & HALD, T. (1983). Non-cholinergic, non-adrenergic nerve mediated relaxation of trigone, bladder neck and urethral smooth muscle in vitro. *J. Urol.*, **129**, 848–850.

KLEVMARK, B. (1977). Motility of the urinary bladder in cats during filling at physiological rates. II. Effects of extrinsic bladder denervation on intramural tension and on intravesical pressure patterns. *Acta Physiol. Scand.*, **101**, 176–184.

KULSENG-HANSSEN, S. (1987). Urethral pressure variations in women with neurological symptoms: II. Relationship to urethral smooth muscle. *Neurourol. Urodyn.*, **6**, 79–85.

LOW, J.A. (1977). Urethral behaviour during the involuntary detrusor contraction. *Am. J. Obstet. Gynecol.*, **128**, 32–42.

LOW, J.A., ARMSTRONG, J.B. & MAUGER, G.M. (1989). The unstable urethra in the female. *Obstet. Gynecol.*, **74**, 69–74.

MAGGI, C.A., SANTICIOLI, P. & MELI, A. (1985). Sympathetic inhibition of reflex activation of bladder motility during filling at a physiological-like rate in urethane anaesthetized rats. *Neurourol. Urodyn.*, **4**, 37–45.

MALMGREN, A., SJÖGREN, C., UVELIUS, B., MATTIASSON, A., ANDERSSON, K.-E. & ANDERSSON, P.-O. (1987). Cystometrical evaluation of bladder instability in rats with infravesical outflow obstruction. *J. Urol.*, **137**, 1291–1294.

MATTIASSON, A., ANDERSSON, K.-E., ANDERSSON, P.-O., LARSSON, B., SJÖGREN, C. & UVELIUS, B. (1990). Nerve-mediated functions in the circular and longitudinal muscle layers of the proximal female rabbit urethra. *J. Urol.*, **143**, 155–160.

MCGUIRE, E.J. (1978). Reflex urethral instability. *Br. J. Urol.*, **40**, 200–204.

NORDLING, J. (1983). Influence of the sympathetic nervous system on lower urinary tract in man. *Neurourol. Urodyn.*, **2**, 3–26.

PERSSON, C.G.A. & ANDERSSON, K.-E. (1976). Adrenoceptor and cholinoceptor mediated effects in the isolated urethra of cat and guinea-pig. *Clin. Exp. Pharmacol Physiol.*, **3**, 415–426.

PERSSON, K. & ANDERSSON, K.-E. (1992). Nitric oxide and relaxation of pig lower urinary tract. *Br. J. Pharmacol.*, **106**, 416–422.

PERSSON, K., GARCIA-PASCUAL, A., FORMAN, A., TØTTRUP, A. & ANDERSSON, K.-E. (1991a). Nitric oxide and non-adrenergic, non-cholinergic nerve-mediated relaxation of isolated pig and rabbit urethral and pig trigonal smooth muscle. In *Proceedings Biology of Nitric Oxide*, London, Oct 1–3.

PERSSON, K., IGAWA, Y., MATTIASSON, A. & ANDERSSON, K.-E. (1991b). Inhibition of the L-arginine/nitric oxide pathway causes bladder hyperactivity in the rat. *Acta Physiol. Scand.*, **144**, 107–108.

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.

RUD, T., ULMSTEN, U. & ANDERSSON, K.-E. (1978). Initiation of voiding in healthy women and those with stress incontinence. *Acta Obstet. Gynecol. Scand.*, **57**, 457–462.

RUD, T., ANDERSSON, K.-E., ASMUSSEN, M., HUNTING, A. & ULMSTEN, U. (1980). Factors maintaining the intraurethral pressure in women. *Invest. Urol.*, **17**, 343–347.

SPEAKMAN, M.J., WALMSLEY, D. & BRADING, A.F. (1988). An in vitro pharmacological study of the human trigone – a site of non-adrenergic, non-cholinergic neurotransmission. *Br. J. Urol.*, **61**, 304–309.

TANAGHO, E.A. & MILLER, E.R. (1970). Inhibition of voiding. *Br. J. Urol.*, **42**, 175–183.

TANG, P.C. & RUCH, T.C. (1955). Non-neurogenic basis of bladder tonus. *Am. J. Physiol.*, **181**, 249–257.

ULMSTEN, U., ANDERSSON, K.-E. & PERSSON, C.G.A. (1977). Diagnostic and therapeutic aspects of urge urinary incontinence in women. *Urol. Int.*, **32**, 88–96.

ULMSTEN, U., HENRIKSSON, L. & IOSIF, S. (1982). The unstable female urethra. *Am. J. Obstet. Gynecol.*, **144**, 93–97.

(Received March 11, 1992
Revised May 7, 1992
Accepted May 12, 1992)