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Prejunctional α-adrenoceptors regulate nitrergic neurotransmission in the rabbit urethra

Hiroshi Seshita, Masaki Yoshida*, Wataru Takahashi, Akito Inadome, Makoto Yono, Yutaka Miyamoto, Shigetaka Murakami, Shoichi Ueda

Department of Urology, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto 860-8556, Japan

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Abstract

We evaluated the effects of prejunctional α-adrenoceptors on nitric oxide (NO)-mediated urethral relaxation in rabbits using a muscle bath technique and high-performance liquid chromatography coupled with a microdialysis procedure. The amount of NO_2^-/NO_3^- released during electrical field stimulation was measured by an NO_2^-/NO_3^- analyzer based on the Griess method. Pretreatment with phenylephrine (0.01 μM) and yohimbine (0.1–10 μM) significantly reduced the relaxation responses induced by electrical field stimulation. In contrast, pretreatment with clonidine (0.01 μM) and prazosin (0.01–1 μM) enhanced the relaxation responses. Cys-NO-induced relaxations of rabbit urethral smooth muscle were not affected by pretreatment with α-adrenoceptor agonists and antagonists. The amount of NO_2^-/NO_3^- released by electrical field stimulation increased after pretreatment with clonidine (0.01 μM) and prazosin (0.01–1 μM), but decreased after pretreatment with phenylephrine (0.01 μM) and yohimbine (0.1–10 μM). The results suggest that the release of NO from nitrergic nerves in the rabbit urethra is reduced and increased by stimulation of prejunctional α_1- and α_2-adrenoceptors, respectively.

Keywords: Urethra; Nitric oxide (NO); Adrenoceptor; Nitrergic nerve; (Rabbit)

1. Introduction

Urethral smooth muscle is innervated by autonomic nerves: adrenergic, cholinergic and non-adrenergic, non-cholinergic (NANC) nerves. There are reports demonstrating that nitric oxide (NO) is one of the neurotransmitters released from the nitrergic component of NANC nerves, and it has been confirmed that NO released from nitrergic nerves relaxes urethral smooth muscles in various mammalian urethra in vitro (Dokita et al., 1991; Andersson et al., 1991; Hashimoto et al., 1993; García-Pascual et al., 1996; Werkström et al., 1995) and in vivo (Persson et al., 1992; Bridgewater et al., 1993; Bennett et al., 1995; Kakizaki et al., 1997).

It has been generally accepted that autonomic neurotransmission is regulated by the co-existence and co-release of several transmitter substances (Mutoh et al., 1987; Saenz de Tejada et al., 1989; Rand and Li, 1995). Recently, we demonstrated that NO released from nitrergic nerves inhibited noradrenaline release from adrenergic nerves in the rabbit urethra (Yoshida et al., 1998). Some studies have shown that the release of NO from vasodilator nerves can be inhibited by stimulation of prejunctional muscarinic receptors or α-adrenoceptors in several vascular vessels (Ayajiki et al., 1993; Bryan et al., 1995; Segarra et al., 1998). Furthermore, Simonsen et al. (1997) reported that the release of a NANC neurotransmitter, which is primarily thought to be NO, was inhibited by stimulation of prejunctional α_2-adrenoceptors in horse penile resistance arteries. However, there is little information available about the prejunctional modulation of nitrergic neurotransmission in urethral smooth muscles.

The present study was therefore designed to investigate the prejunctional α-adrenoceptor regulation of nitrergic

* Corresponding author. Tel.: +81-96-373-5240; fax: +81-96-373-5242.

E-mail address: masaki@kaiju.medic.kumamoto-u.ac.jp (M. Yoshida).

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nerve-mediated relaxation in rabbit urethral smooth muscles.

2. Materials and methods

2.1. Tissue preparation and relaxation experiments

Female rabbits weighing 2.5–3.0 kg were anesthetized with 30 mg/kg sodium pentobarbital and killed by exsanguination. The urethra was dissected free down to the entrance of the vaginal wall and was placed in modified Krebs–Henseleit (K–H) solution. The urethra was cut into transverse muscle strips, approximately 2×10 mm. The strips were suspended in 20-ml organ baths filled with K–H solution at 37°C bubbled with 95% O_2 and 5% CO_2, resulting in a pH of 7.4, and were attached to two L-shaped metal specimen holders by tying both ends of the preparations with silk ligatures. One end of each strip was connected to a force-displacement transducer (TB-611T, Nihon Kohden) and isometric forces were recorded and monitored on a pen-writing recorder (R-02A, RIKADENKI). During an hour equilibration period, the muscle strip was stretched to the length of optimal force development, and the bath solution was changed every 15 min. The resting tension at the length of optimal force development was 600–800 mg, according to the previous report (Takahashi et al, 1997).

For evaluation of muscle contractility, the concentration–response curves for phenylephrine or 80 mM KCl-induced contractions were first constructed. Electrical field stimulation was applied to the strips precontracted with 0.1 μM endothelin-1 through two parallel platinum electrodes (10-mm wide and 8-mm apart). Electrical impulses for field stimulation of intrinsic nerves were delivered with a stimulator (SEN-3301, Nihon Kohden) and boosted by the amplifier (SEG-3104, Nihon Kohden). They delivered square-wave pulses (voltage, supramaximum; pulse duration, 2 ms; frequency, 0.5–15 Hz; and train duration, 3 s at 1-min intervals). After the experiment, K–H solution was changed to Ca^{2+}-free K–H solution to obtain 100% relaxation. Subsequently, strips were washed several times with K–H solution and were equilibrated for 60 min and then precontracted by endothelin-1 in the presence of the atropine (1 μM) and indomethacin (10 μM) to block muscarinic receptors and prostaglandins release, respectively.

The next series of electrical stimulation was applied and adrenoceptor agonists (phenylephrine and clonidine) or antagonists (prazosin and yohimbine) were added for 30 min before electrical stimulation. In the experiment using adrenoceptor agonists, guanethidine (10 μM) was also added to K–H solution to block intrinsic adrenergic neurotransmission. To evaluate the action of α-adrenoceptor agonists and antagonists on postjunctional receptors sites in NO-mediated relaxation in the rabbit urethra, the relaxation responses induced by an NO donor (cys-NO) in the rabbit urethral smooth muscles precontracted with endothelin-1 were measured in the presence and absence of phenylephrine (0.01 μM), clonidine (0.01 μM), prazosin (1 μM) and yohimbine (10 μM). In this study, cys-NO (0.1 μM–1 mM) was cumulatively added.

2.2. Measurement of NO release

The NO release from urethral smooth muscle was measured using the microdialysis method as previously reported (Ohta et al., 1994; Takahashi et al., 1997). In brief, a microdialysis probe (Kurata et al., 1993) (outer diameter, 220 μm; inner diameter, 200 μm; length, 10 mm; cellulose membrane, molecular cut-off, 50 kDa, A-I-8-03, Eicom, Kyoto, Japan) was inserted into the urethral strip. The probe has inlet and outlet cannulas with 28 gauge sharp needles on both ends, which help to insert the probe into the rabbit urethral strip easily. The probe was perfused with Ringer solution at a constant flow rate of 2 μl/min using a microsyringe pump (EP-60, Eicom). As in the relaxation experiments, the strip with microdialysis probe was set up, and the muscle strip was stretched to the length of optimal force development. Under this condition, the length of muscle strip was about 15–18 mm, and the microdialysis probe was positioned in the central part of the strip. Following a 2-h equilibration period, dialysate was collected for 10 min (20 μl) for evaluation of basal NO release. Then, electrical field stimulation (supramaximum voltage; pulse duration, 2 ms; frequency, 7 Hz; train duration, 3 s at 1-min intervals for 10 min) was applied to strips precontracted with endothelin-1, and dialysate during electrical stimulation was collected in polyethylene tubes at room temperature.

To quantitate the NO_2^-/NO_3^- in the dialysate fraction, 10-μl dialysate was directly infused into an automated NO_2^-/NO_3^- analyzing system by means of a syringe loading sample injector (Model 7725; Eicom). The system was based on the Griess reaction. The levels of NO_2^-/NO_3^- in the standard solution and the samples were determined as a mixture of NO_2^-/NO_3^-. NO_2^-/NO_3^- release is expressed in terms of the amount of NO_2^-/NO_3^- in 10 μl dialysate fraction/weight of the strip (pmol/g wet weight of urethra). The effects of pretreatment with α-adrenoceptor agonists (phenylephrine 0.01 μM and clonidine 0.01 μM) and antagonists (prazosin 0.01–1 μM and yohimbine 0.1–10 μM) on the electrical field stimulation-induced NO_2^-/NO_3^- release from rabbit urethral smooth muscles were evaluated.

2.3. Solutions and drugs

Ringer solution was composed as follows (mM): NaCl, 147; KCl, 4.0; $CaCl_2$, 2.3, and the pH was adjusted to 7.4. K–H solution was composed as follows (mM): NaCl, 117.7; KCl, 4.69; $CaCl_2$, 2.16; $MgSO_4$, 1.20; $NaHCO_3$, 24.39; KH_2PO_4, 1.20 and glucose, 9.99. Ca^{2+}-free solu-

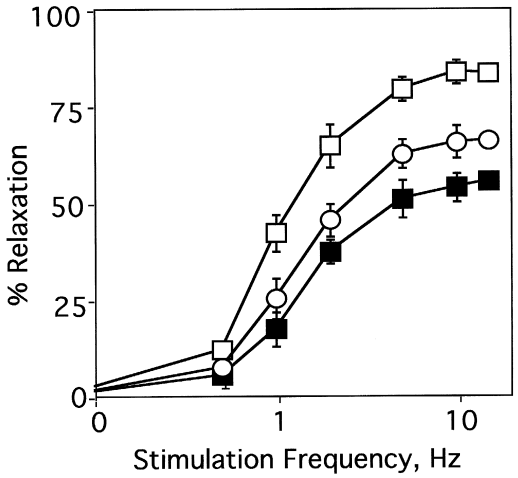

Fig. 1. Effects of α-adrenoceptor agonists on electrical field stimulation-induced relaxation of the female rabbit urethra. Muscle strips were precontracted with 0.1 μM endothelin-1. Electrical field stimulation (voltage, supramaximum; pulse duration, 2 ms; frequency, 0.5–15 Hz; train duration, 3 s at 1-min intervals) was applied to the muscle strips in the presence of atropine (1 μM), guanethidine (10 μM) and indomethacin (10 μM). (○): control, (■): 0.01 μM phenylephrine, (□): 0.01 μM clonidine. Each point shows mean ± S.E.M. ($n = 7$); if not shown, S.E. bars fall within the size of the symbols.

tion was made by omitting $CaCl_2$ from K–H solution and adding 0.1 mM EGTA. KCl solution (80 mM) was prepared by replacing Na^+ by equimolar amounts of K^+ in K–H solution. Cys-NO was prepared from L-cysteine and sodium nitrite as described by Thornbury et al. (1991). The vehicle, in which cys-NO was dissolved, contained 1 M HCl, methanol and concentrated sulphuric acid. In a preliminary experiment, this vehicle had no effect on the contractility of rabbit urethral smooth muscles.

The following drugs were used. L-phenylephrine hydrochloride, prazosin hydrochloride, clonidine hydrochloride, yohimbine hydrochloride, guanethidine monosulphate, atropine sulfate, tetrodotoxin, hexamethonium chloride, L-cysteine, L-arginine, N^{ω}-nitro-L-arginine (L-NNA) and EGTA, which were obtained from Sigma. Indomethacin was obtained from Nacalai tesque, Japan. All other chemicals were obtained from commercial sources. Concentrations are expressed as final bath concentrations. Indomethacin was dissolved in 1% w/v sodium bicarbonate; all other drugs were dissolved in distilled water, and 0.2 ml volumes were added to the bath.

2.4. Data analysis

For relaxation experiments, the relaxation induced by Ca^{2+}-free K–H solution was taken as 100% and the percent relaxation was calculated. The data are expressed as means ± S.E.M. Statistical analysis of differences between groups was performed using analysis of variance (ANOVA) and Fisher's multiple comparison test. P values of 0.05 or less were taken as statistically significant.

3. Results

3.1. Relaxation experiments

The concentration response curve for phenylephrine and the contractile response induced by 80 mM KCl were not significantly different before and after the experiment. Endothelin-1 (0.1 nM–3 μM) caused concentration-dependent contractions in the rabbit urethral strip. The maximum contractile response was 1.54 ± 0.23 g ($n = 16$) and the ED_{50} value was 34.0 ± 2.5 nM ($n = 16$). Endothelin-1 (0.1 μM) produced a contractile response of 1.05 ± 0.27 g ($n = 16$), which was not affected by treatment with phenylephrine (0.01 μM), clonidine (0.01 μM), prazosin (0.01–1 μM), and yohimbine (0.1–10 μM). Electrical field stimulation (supramaximum voltage, 2 ms pulse duration, 0.5–15 Hz frequency and 3-s train) caused frequency-dependent relaxations of urethral strips precontracted with 0.1 μM endothelin-1. All relaxation responses were almost completely blocked by tetrodotoxin (1 μM), but not by hexamethonium (100 μM). Pretreatment with L-NNA (100 μM) caused inhibition of the relaxation, which was overcome by L-arginine (5 mM). In the presence of guanethidine (10 μM), indomethacin (10 μM) and atropine (1 μM), pretreatment with phenylephrine (0.01 μM) and clonidine (0.01 μM) significantly decreased and increased the relaxation response, respectively (Fig. 1). The maximum relaxation before and after treatment with phenylephrine and clonidine is shown in Table 1. There

Table 1
Effects of various α-adrenoceptor agonists and antagonists on electrical field stimulation-induced maximum relaxation of female rabbit urethra

	Maximum relaxation (%)			
	0.01 μM phenylephrine ($n = 7$)	0.01 μM clonidine ($n = 7$)	1 μM prazosin ($n = 7$)	10 μM yohimbine ($n = 7$)
Before	65.7 ± 1.48	64.8 ± 1.44	65.8 ± 2.26	65.5 ± 2.62
After	55.3 ± 2.28[a]	83.1 ± 0.90[a]	87.5 ± 1.42[a]	45.2 ± 1.20[a]

Relaxations are expressed as percentages of the tension induced by 0.1 μM endothelin-1 and data are means ± S.E.M.; n = number of experiments.Electrical field stimulation (voltage, supramaximum; pulse duration, 2 ms; frequency, 15 Hz; train duration, 3 s at 1-min intervals) was delivered.

[a] Significantly different from the comparable values before pretreatment with α-adrenoceptor agonists and antagonists.

were significant differences in the maximum relaxation before and after treatment with phenylephrine or clonidine.

The effects of α-adrenoceptor antagonists on the relaxation response induced by electrical field stimulation are shown in Fig. 2. Pretreatment with prazosin (0.01–1 μM) increased the relaxation responses induced by electrical field stimulation in a concentration-dependent manner (Fig. 2A), while treatment with yohimbine (0.1–10 μM) decreased the relaxation responses (Fig. 2B). The maximum relaxations induced by electrical field stimulation before and after treatment with prazosin (1 μM) and yohimbine (10 μM) are also shown in Table 1. There was a significant increase and decrease in relaxant responses after treatment with prazosin and yohimbine, respectively. In

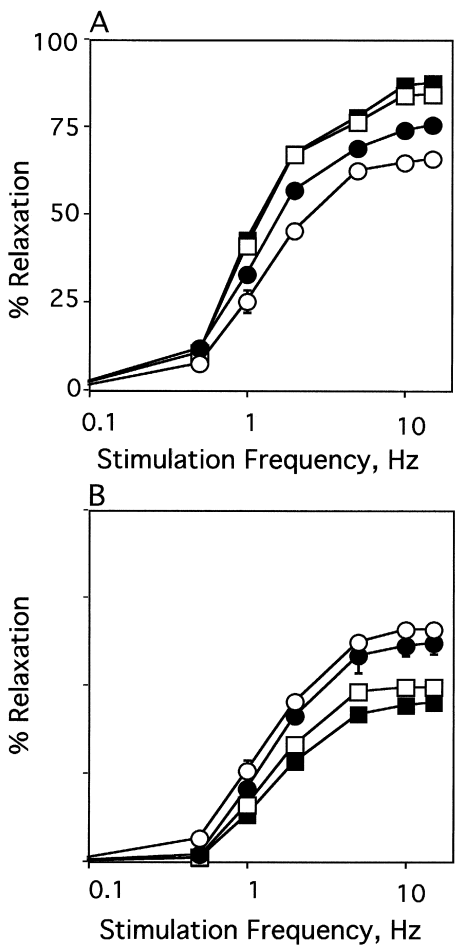

Fig. 2. (A) Effects of prazosin on field stimulation-induced relaxation of the female rabbit urethra. (○): control, (●): 0.01 μM prazosin, (□): 0.1 μM prazosin, and (■): 1 μM prazosin. (B) Effects of yohimbine on field stimulation-induced relaxation in the female rabbit urethra. (○): control, (●): 0.1 μM yohimbine, (□):1 μM yohimbine and (■):10 μM yohimbine. Electrical field stimulation (voltage, supramaximum; pulse duration, 2 ms; frequency, 0.5–15 Hz; train duration, 3 s at 1-min intervals) was applied to the muscle strips under tension induced by 0.1 μM endothelin-1 in the presence of atropine (1 μM) and indomethacin (10 μM). Each point shows mean ± S.E.M. ($n = 7$); if not shown, S.E. bars fall within the size of the symbols.

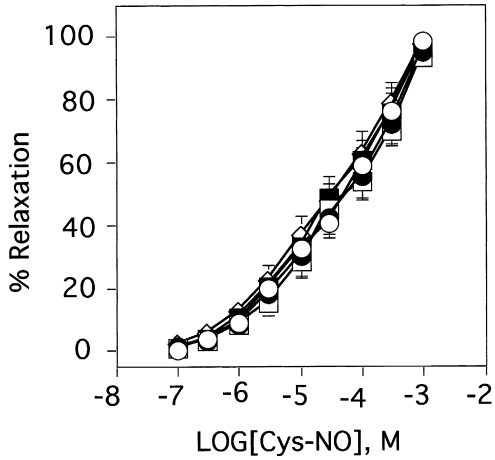

Fig. 3. Effects of pretreatment with α-adrenoceptor agonists and antagonists on the cys-NO-induced relaxation of the female rabbit urethra. (○): control, (●): 0.01 μM phenylephrine, (□): 0.01 μM clonidine, (■): 1 μM prazosin and (◇): 10 μM yohimbine. Cys-NO (0.1 μM–1 mM) was applied to the muscle strips under tension induced by 0.1 μM endothelin-1 in the presence of atropine (1 μM) and indomethacin (10 μM). Each point shows mean ± S.E.M. ($n = 7$); if not shown, S.E. bars fall within the size of the symbols.

the presence of atropine (1 μM) and indomethacin (10 μM), exogenously applied cys-NO evoked a transient relaxation of urethral smooth muscles precontracted with endothelin-1 in a concentration-dependent manner. Pretreatment with phenylephrine (0.01 μM), clonidine (0.01 μM), prazosin (1 μM) and yohimbine (10 μM) did not have significant effects on the cys-NO-induced relaxations in the rabbit urethra (Fig. 3).

3.2. Measurement of NO release

The concentration–response curves for phenylephrine and the contractile response induced by 80 mM KCl were not significantly different between urethral smooth muscles with and without the microdialysis probe. After insertion of the microdialysis probe, the contractile response induced by endothelin-1 (0.1 μM) was 1.00 ± 0.15 g, which was similar to the contractile response before probe insertion. Furthermore, insertion of the microdialysis probe did not have a significant effect on the relaxation response induced by electrical field stimulation in the rabbit urethral smooth muscles. In a preliminary experiment, NO_2^-/NO_3^- release induced by electrical field stimulation every hour was constant for 6 to 7 h. To evaluate the effects of electrical field stimulation with platinum electrodes on the content of NO_2^-/NO_3^- in the buffer solution, we measured the NO_2^-/NO_3^- content in K–H solution before and after electrical field stimulation (supramaximum voltage; pulse duration, 2 ms; frequency, 7 Hz; train duration, 3 s at 1-min intervals for 10 min). After electrical field stimulation, the content of NO_2^-/NO_3^- in the K–H solution was

0.43 ± 0.24 pmol/10 μl buffer ($n = 5$), which was not significantly different from the content before electrical stimulation (0.35 ± 0.21 pmol/10 μl buffer; $n = 5$). The amount of NO_2^-/NO_3^- released from urethral smooth muscle strips into the dialysate fraction before electrical field stimulation (basal release) was 11.46 ± 0.51 pmol/g urethra ($n = 10$). The amount of NO_2^-/NO_3^- released into the dialysate fraction during electrical field stimulation of urethral smooth muscle strips was 28.93 ± 3.01 pmol/g urethra ($n = 10$), which was significantly higher than the basal release. Pretreatment with tetrodotoxin (1 μM) significantly inhibited NO_2^-/NO_3^- release during electrical field stimulation to 10.43 ± 1.96 pmol/g urethra ($n = 10$), which was similar to the basal release. The effect of L-NNA (100 μM) on the content of NO_2^-/NO_3^- in K–H solution was also evaluated, because L-NNA contains nitrates. Without urethral smooth muscle strips, the NO_2^-/NO_3^- content of K–H solution with 100 μM L-NNA in the muscle bath was 2.94 ± 0.31 pmol/10 μl buffer ($n = 6$), which was significantly higher than that of K–H solution without L-NNA (0.31 ± 0.18 pmol/10 μl buffer; $n = 6$). However, in the microdialysis procedure, 100 μM L-NNA in the K–H solution did not increase basal NO_2^-/NO_3^- release (6.67 ± 0.73 pmol/g urethra: $n = 6$), and decreased the electrical field stimulation-induced NO_2^-/NO_3^- release from rabbit urethral smooth muscles to 5.75 ± 0.64 pmol/g urethra ($n = 6$).

The effects of α-adrenoceptor agonists on NO_2^-/NO_3^- release in rabbit urethral smooth muscles induced by electrical field stimulation are shown in Table 2. Pretreatment with phenylephrine (0.01 μM) and clonidine (0.01 μM) significantly decreased and increased NO_2^-/NO_3^- release from the rabbit urethral smooth muscles. Fig. 4 shows the effects of treatment with prazosin (A) and yohimbine (B) on NO_2^-/NO_3^- release induced by electrical field stimulation. The amount of NO_2^-/NO_3^- released by electrical field stimulation increased and decreased after prazosin (0.01–1 μM) and yohimbine (0.1–10 μM) pretreatment in a concentration-dependent manner, respectively. After 0.1 and 1 μM prazosin pretreatment, NO_2^-/NO_3^- release induced by electrical field stimulation was 36.07 ± 2.64 and

Table 2
Effects of α-adrenoceptor agonists on NO_2^-/NO_3^- release induced by electrical field stimulation of the female rabbit urethra

	NO_2^-/NO_3^- production (pmol/g weight of urethra)	
	0.01 μM phenylephrine ($n = 7$)	0.01 μM clonidine ($n = 7$)
Before	29.6 ± 3.15	30.8 ± 2.44
After	20.5 ± 2.28[a]	40.1 ± 2.90[a]

The data are means ± S.E.M.; n = number of experiments. The release of NO_2^-/NO_3^- was measured by high-performance liquid chromatography coupled with microdialysis method. Electrical field stimulation (voltage, supramaximum; pulse duration, 2 ms; frequency, 7 Hz; train duration, 3 s at 1-min intervals for 10 min) was delivered.

[a]Significantly different from the comparable values before pretreatment with α-adrenoceptor agonists.

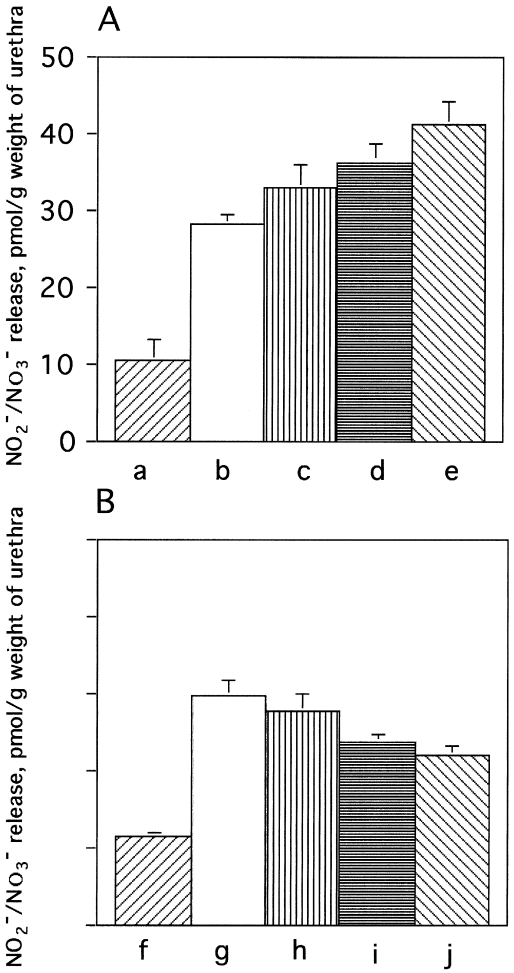

Fig. 4. Effects of pretreatment with prazosin (A) and yohimbine (B) on NO_2^-/NO_3^- during electrical field stimulation of the female rabbit urethra. The release of NO_2^-/NO_3^- was measured by high-performance liquid chromatography with electrochemical detection coupled with microdialysis. A microdialysis probe was inserted into the urethral strip and was perfused with Ringer solution at a constant flow rate of 2 μM/min in the muscle bath. Electrical field stimulation (voltage, supramaximum; pulse duration, 2 ms; frequency, 7 Hz; train duration, 3 s at 1-min intervals for 10 min) was applied to the muscle strips under tension induced by endothelin-1 in the presence of atropine (1 μM) and indomethacin (10 μM), and dialysate was collected every 10 min. Each point shows mean ± S.E.M. ($n = 7$). (A) (a) Basal NO_2^-/NO_3^- production before electrical field stimulation; (b) NO_2^-/NO_3^- production during electrical field stimulation; (c) NO_2^-/NO_3^- production during electrical field stimulation after treatment with 0.01 μM prazosin; (d) NO_2^-/NO_3^- production during electrical field stimulation after treatment with 0.1 μM prazosin; (e) NO_2^-/NO_3^- production during electrical field stimulation after treatment with 1 μM prazosin. (B) (f) Basal NO_2^-/NO_3^- production before electrical field stimulation; (g) NO_2^-/NO_3^- production during electrical field stimulation. (h) NO_2^-/NO_3^- production during electrical field stimulation after treatment with 0.1 μM yohimbine; (i) NO_2^-/NO_3^- production during electrical field stimulation after treatment with 1 μM yohimbine; (j) NO_2^-/NO_3^- production during electrical field stimulation after treatment with 10 μM yohimbine.

41.25 ± 2.78 pmol/g urethra, respectively, which was significantly higher than the value before treatment (28.13 ± 1.35 pmol/g urethra). In contrast, 1 and 10 μM yohimbine treatment caused a significant decrease in NO_2^-/NO_3^- release (23.68 ± 1.02 and 21.75 ± 1.35 pmol/g urethra, respectively) compared to the value before treatment (29.73 ± 1.96 pmol/g urethra).

4. Discussion

There are many reports showing that NO contributes to urethral relaxation in experimental animals (Dokita et al., 1991; Andersson et al., 1991; Werkström et al., 1995; Bennett et al., 1995; García-Pascual et al., 1996) and humans (Andersson et al., 1992; Ehrén et al., 1994; Dixon and Jen, 1995). In the present experiment, electrical field stimulation-induced urethral relaxations were completely inhibited by pretreatment with L-NNA, an inhibitor of NO synthase, and the inhibition was overcome by the addition of L-arginine, a precursor of NO. These results are consistent with previous reports.

With regard to measurement of NO release in biological models, Ohta et al. (1994) reported an assay system for consecutive measurement of NO production in the rat brain that was combined with microdialysis methods. In this method, NO production is measured, based on the Griess reaction, as the amount of its stable metabolites, NO_2^- and NO_3^-. This method has the advantage of consecutive measurement, high precision, good reproducibility, technical simplicity and relatively short resolution time (Ohta et al., 1994; Iversen et al., 1997). It is not technically difficult to insert a microdialysis probe into the urethral smooth muscle strip, which is 10-mm long, because there are 28 gauge sharp needles in both ends of the probe, which help to insert the probe easily. After setting of the muscle strip in the muscle bath, we stretched the muscle strip to obtain optimal force development. In this condition, the length of the muscle strip was about 15–18 mm, and the microdialysis probe was located in the central part of the muscle strip. Furthermore, the effects of the microdialysis probe on the contractile responses to endothelin-1, KCl, and phenylephrine, and on the electrical field stimulation-induced relaxation response were negligible in the present experiment. Wiklund et al. (1993) reported that electrical stimulation with platinum electrodes changed the content of nitrite in the buffer solution. However, in the present experiment, after electrical stimulation without a muscle strip, the amount of NO_2^-/NO_3^- in the K–H solution of the muscle bath did not significantly change. The difference may be due to different experimental conditions and procedures. It is also possible that L-NNA affects the content of nitrite in the buffer solution in the muscle bath, because L-NNA may contain a high amount of nitrite. The content of NO_2^-/NO_3^- in K–H solution containing 100 μM L-NNA was significantly higher than the NO_2^-/NO_3^- content of the K–H solution without L-NNA. However, in the microdialysis procedure, 100 μM L-NNA in K–H solution did not cause a significant increase in the basal NO_2^-/NO_3^- release from urethral smooth muscle strips. Thus, we could measure NO release induced by electrical field stimulation in rabbit urethral smooth muscles using this method. In the present experiment, electrical field stimulation caused NO release from isolated urethral strips, and this release was significantly inhibited by pretreatment with tetrodotoxin and L-NNA. These findings are consistent with our recent report (Takahashi et al., 1997), suggesting that electrical field stimulation evokes neurogenic responses, and that the L-arginine/NO pathway contributes to the relaxation induced by nitrergic nerve stimulation in the rabbit urethra.

In the present experiment, we evaluated the effects of α-adrenoceptor agonists and antagonists on the relaxation responses and NO release induced by electrical field stimulation in precontracted urethral strips. Usually, precontraction of urethral strips is elicited with an α_1-adrenoceptor agonist, phenylephrine (Dokita et al., 1991; Ehrén et al., 1994; Takahashi et al., 1997; Yoshida et al., 1998). However, we used endothelin-1 for precontraction of rabbit urethral strips in the present experiment, because it is possible that pretreatment with α-adrenoceptor agonists and antagonists would have affected the contractile response induced by phenylephrine. There are several reports showing that endothelin-1 has significant contractile response in rabbit urethral smooth muscles, which is related to the formation of inositol phosphates (García-Pascual et al., 1993). Pretreatment with α-adrenoceptor agonists (phenylephrine and clonidine) and antagonists (prazosin and yohimbine) did not have a significant effect on endothelin-1-induced contractile responses at the concentrations used in this experiment.

In the present study, pretreatment with α_1-adrenoceptor agonist reduced nitrergic nerve-mediated relaxation and NO release, while pretreatment with α_2-adrenoceptor agonist increased the relaxation and NO release. Pretreatment with α_1- and α_2-adrenoceptor antagonists enhanced and reduced the relaxation response and NO release induced by nitrergic nerve stimulation in the rabbit urethral smooth muscles in a concentration-dependent manner, respectively. The relaxation responses induced by exogenously administered NO donor were not affected by pretreatment with α_1- and α_2-adrenoceptor agonists and antagonists, suggesting that postjunctional α_1- and α_2-adrenoceptors in rabbit urethral smooth muscles did not contribute to NO-mediated relaxation responses in the present experiment. These results demonstrate that α_1- and α_2-adrenoceptors exist prejunctionally on nitrergic nerve endings in the rabbit urethra, and imply that nitrergic neurotransmission is reduced by the stimulation of α_1-adrenoceptors and is increased by the stimulation of α_2-adrenoceptors. This is the first report to clarify the prejunctional α-adrenoceptor-mediated regulation of NO release from nitrergic nerve in the rabbit urethra.

There are several reports demonstrating that autonomic neurotransmission in the urinary tract is regulated by the coexistence and co-release of several transmitter substances, and that negative feedback mechanisms contribute to the release of autonomic neurotransmitters (Mutoh et al., 1987; Holmquist et al., 1994; Tanaka et al., 1996; Inadome et al., 1998; Yoshida et al., 1998). In regard to the regulation of NO release from nitrergic nerve, several studies have shown that the release of NO from vasodilator nerves can be inhibited by stimulation of prejunctional muscarinic receptors or α_2-adrenoceptors (Lefebvre and Smits, 1992; Boeckxstaens et al., 1993; De man et al., 1994). Recently, Simonsen et al. (1997) demonstrated that the release of the NANC neurotransmitter, which is primarily thought to be NO, was inhibited by stimulation of prejunctional α_2-adrenoceptors in horse penile resistant arteries. Furthermore, Boeckxstaens et al. (1993) reported that stimulation of prejunctional α_2-adrenoceptors inhibited the nitrergic nerve-mediated relaxation induced by electrical field stimulation in the canine isolated ileocolonic junction. In addition, De Man et al. (1994) suggested that the prejunctional α_2-adrenoceptor-mediated modulation of NO-related substance release might involve neural K^+ channels. In the present study, the stimulation of prejunctional α_2-adrenoceptors enhanced the nitrergic nerve-mediated responses in the rabbit urethra. Although species and/or tissue-related differences in the prejunctional regulation of α-adrenoceptor subtypes for NO release from nitrergic nerve ending may be involved (Deplanne et al., 1998), the reasons for the different results in this and other reports is not clear from our experiments. Further studies are needed in this regard.

There are a few reports about the prejunctional regulation of α_1-adrenoceptor-mediated regulation of NO release from nitrergic nerves. Simonsen et al. (1997) reported that nitrergic neurotransmission was not affected by prejunctional α_1-adrenoceptors in horse penile resistance arteries. However, the present study demonstrated that stimulation of prejunctional α_1-adrenoceptors reduced the nitrergic nerve-mediated responses in the rabbit urethra. These data may provide an explanation for the modulation of urethral tone. The urethral smooth muscles are innervated by two main neurons, adrenergic and nitrergic nerves (Yoshida et al., 1998). Noradrenaline released from adrenergic nerves causes urethral contraction mainly through postjunctional α_1-adrenoceptors on urethral smooth muscle. At the same time, noradrenaline regulates NO release through α-adrenoceptors at prejunctional sites on nitrergic nerve endings. The dual effects of noradrenaline on pre- and postjunctional adrenoceptors may contribute to the coordination of adrenergic and nitrergic neural communication. However, the exact physiological explanation still remains to be clarified.

In conclusion, the present study demonstrates that the release of NO from nitrergic nerves is regulated by prejunctional α-adrenoceptors in the rabbit urethra. The stimulation of α_1-adrenoceptors reduces electrical field stimulation-induced NO release, and the stimulation of α_2-adrenoceptors increases NO release from nitrergic nerves.

References

Andersson, K.-E., García-Pascual, A., Forman, A., Tøttrup, 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., García-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.

Ayajiki, K., Okamura, T., Toda, N., 1993. Nitric oxide mediates and acetylcholine modulates, neurally induced relaxation of bovine cerebral arteries. Neuroscience 54, 819–825.

Bennett, B.C., Kruse, M.N., Roppolo, J.R., Flood, H.D., Fraser, M.O., De Groat, W.C., 1995. Neural control of urethral outlet activity in vivo: role of nitric oxide. J. Urol. 153, 2004–2009.

Boeckxstaens, G.E., De Man, J.G., Pelckmans, P.A., Herman, A.G., Van Maercke, Y.M., 1993. α_2-Adrenoceptor-mediated modulation of the nitrergic innervation of the canine isolated ileocolonic junction. Br. J. Pharmacol. 109, 1079–1084.

Bridgewater, M., Macneil, H.F., Brading, A.F., 1993. Regulation of tone in pig urethral smooth muscle. J. Urol. 150, 223–228.

Bryan, J.R.R.M., Steenberg, M.L., Eichler, M.Y., Johnson, T.D., Swafford, M.W.G., Suresh, M.S., 1995. Permissive role of NO in α_2-adrenoceptor-mediated dilations in rat cerebral arteries. Am. J. Physiol. 269, H1171–H1174.

De Man, J.G., Boeckxstaens, G.E., Herman, A.G., Pelckmans, P.A., 1994. Effect of potassium channel blockade and α_2-adrenoceptor activation on the release of nitric oxide from non-adrenergic non-cholinergic nerves. Br. J. Pharmacol. 112, 341–345.

Deplanne, V., Palea, S., Angel, I., 1998. The adrenergic, cholinergic and NANC nerve-mediated contractions of the female rabbit bladder neck and proximal, medial and distal urethra. Br. J. Pharmacol. 123, 1517–1524.

Dixon, J.S., Jen, P.Y., 1995. Development of nerves containing nitric oxide synthase in the human male urogenital organs. Br. J. Urol. 76, 719–725.

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.

Ehrén, I., Iversen, H., Jansson, O., Adolfsson, J., Wiklund, N.P., 1994. Localization of nitric oxide synthase activity in the human lower urinary tract and its correlation with neuroeffector responses. Urology 44, 683–687.

García-Pascual, A., Persson, K., Holmquist, F., Andersson, K.-E., 1993. Endthelin-1-induced phosphoinositude hydrolysis and contraction in isolated rabbit detrusor and urethral smooth muscle. Gen. Pharmacol. 24, 131–138.

García-Pascual, A., Costa, G., Labadia, A., Persson, K., Triguero, D., 1996. Characterization of nitric oxide synthase activity in sheep urinary tract: functional implications. Br. J. Pharmacol. 118, 905–914.

Hashimoto, S., Kigoshi, S., Muramatu, I., 1993. Nitric oxide-dependent and -independent neurogenic relaxation of isolated dog urethra. Eur. J. Pharmacol. 231, 209–214.

Holmquist, F., Persson, K., Bødker, A., Andersson, K.-E., 1994. Some pre- and postjunctional effects of castration in rabbit isolated corpus cavernosum and urethra. J. Urol. 152, 1011–1016.

Inadome, A., Yoshida, M., Takahashi, W., Yono, M., Seshita, H., Miyamoto, Y., Kawano, T., Ueda, S., 1998. Prejunctional muscarinic receptors modulating acetylcholine release in rabbit detrusor smooth muscles. Urol. Int. 61, 135–141.

Iversen, H.H., Celsing, F., Leone, A.M., Gustafsson, L.E., Wiklund, P., 1997. Nerve-induced release of nitric oxide in the rabbit gastrointestinal tract as measured by in vivo microdialysis. Br. J. Pharmacol. 120, 702–706.

Kakizaki, H., Fraser, M.O., De Groat, W.C., 1997. Reflex pathways controlling urethral striated and smooth muscle function in the male rat. Am. J. Physiol. 272, R1647–R1656.

Kurata, N., Inagaki, M., Kobayashi, S., Nishimura, Y., Oguchi, K., Yasuhara, H., 1993. Antipyrine concentrations in liver and blood monitored by micro-dialysis of unrestrained conscious rats. Chem. Pathol. Pharmacol. 79, 363–369.

Lefebvre, R.A., Smits, G.J.M., 1992. Modulation of non-adrenergic non-cholinergic inhibitory neurotransmission in rat gastric fundus by the α_2-adrenoceptor agonist, UK-14304. Br. J. Pharmacol. 107, 256–261.

Mutoh, S., Ueda, S., Fukumoto, Y., Machida, J., Ikegami, K., 1987. Effect of adrenergic and cholinergic drugs on the noradrenergic transmission in bladder neck smooth muscle. J. Urol. 138, 212–215.

Ohta, K., Araki, N., Shibata, M., Hamada, J., Komatsumoto, S., Shimazu, K., Fukuuchi, Y., 1994. A novel in vivo assay system for consecutive measurement of brain nitric oxide production combined with the microdialysis technique. Neurosci. Lett. 176, 165–168.

Persson, K., Igawa, Y., Mattiasson, A., Andersson, K.-E., 1992. Effect of inhibition of the L-arginine/nitric oxide pathway in the rat lower urinary tract in vivo and in vitro. Br. J. Pharmacol. 107, 178–184.

Rand, M.J., Li, C.G., 1995. Nitric oxide as a neurotransmitter in peripheral nerves: nature of transmitter and mechanism of transmission. Annu. Rev. Physiol. 57, 659–682.

Segarra, G., Medina, P., Domenech, C., Martínez León, J.B., Vila, J.M., Aldasoro, M., Lluch, S., 1998. Neurogenic contraction and relaxation of human penile deep dorsal vein. Br. J. Pharmacol. 124, 788–794.

Simonsen, U., Prieto, D., Hernandez, M., Sáenz de Tejada, I., García-Sacristán, A., 1997. Prejunctional α_2-adrenoceptors inhibit nitrergic neuro-transmission in horse penile resistance arteries. J. Urol. 157, 2356–2360.

Saenz de Tejada, I., Kim, N., Lagan, I., Krane, R.J., Goldstein, I., 1989. Regulation of aderenergic activity in penile corpus cavernosum. J. Urol. 142, 1117–1121.

Takahashi, Y., Yoshida, M., Wada, Y., Goto, S., Inadome, A., Yono, M., Ueda, S., 1997. Effect of estrogen on nitric oxide-induced relaxation of the rabbit urethra. Eur. J. Pharmacol. 339, 165–171.

Tanaka, H., Jing, L., Takahashi, S., Ito, Y., 1996. The possible role of nitric oxide in relaxations and excitatory neuroeffector transmission in the cat airway. J. Physiol. (London) 493, 785–791.

Thornbury, K., Ward, S.M., Dalziel, H.H., Carl, A., Westfall, D.P., Sanders, K.M., 1991. Nitric oxide and nitrosocysteine mimic nonadrenergic noncholinergic hyperpolarization in canine proximal colon. Am. J. Physiol. 261, G553–G557.

Werkström, V., Persson, K., Ny, L., Bridgewater, M., Brading, A.F., Andersson, K.-E., 1995. Factors involved in the relaxation of female pig urethra evoked by electrical field stimulation. Br. J. Pharmacol. 116, 1599–1604.

Wiklund, C.U., Wiklund, N.P., Gustafsson, L.E., 1993. Modulation of neuroeffector transmission by endogenous nitric oxide: a role for acetylcholine receptor activated nitric oxide formation, as indicated by measurements of nitric oxide/nitrate release. Eur. J. Pharmacol. 240, 235–242.

Yoshida, M., Akaike, T., Inadome, A., Takahashi, W., Seshita, H., Yono, M., Goto, S., Maeda, H., Ueda, S., 1998. The possible effect of nitric oxide on relaxation and noradrenaline release in the isolated rabbit urethra. Eur. J. Pharmacol. 357, 213–219.