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The possible effect of nitric oxide on relaxation and noradrenaline release in the isolated rabbit urethra

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Abstract

We evaluated the effects of N^{ω}-nitro-L-arginine (L-NNA, a nitric oxide (NO) synthase inhibitor) and carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO, a NO scavenger) on NO-mediated relaxation and noradrenaline release from adrenergic nerve endings induced by electrical field stimulation in the rabbit urethra. Electrical field stimulation caused frequency-dependent relaxation of rabbit urethral smooth muscles precontracted with phenylephrine. The relaxation responses were significantly inhibited by treatment with L-NNA or carboxy-PTIO. The inhibitory effect of carboxy-PTIO was significantly weaker than that of L-NNA. Electrical field stimulation caused significant noradrenaline release from adrenergic nerve endings in the rabbit urethra. Treatment with carboxy PTIO enhanced electrical field stimulation-induced noradrenaline release, and simultaneous application of L-NNA and carboxy-PTIO did not further enhance noradrenaline release in the rabbit urethra. As carboxy-PTIO reacts only with the free radical NO, the present results suggest that free radical NO and NO-containing compounds are involved in the L-NNA-sensitive nitrergic nerve-mediated relaxation in the rabbit urethra. At the same time free radical NO has a prejunctional action by which it may inhibit noradrenaline release from adrenergic nerves.

Keywords: Urethra; Nitric oxide (NO); Noradrenaline; Nitrergic nerve

1. Introduction

Urethral smooth muscle is innervated by autonomic nerves: adrenergic, cholinergic, and nonadrenergic, noncholinergic (NANC) nerves (Andersson, 1993; De Groat, 1995). There are reports demonstrating that nitric oxide (NO) is one of the neurotransmitters released from the nitrergic component of NANC nerves (Rand and Li, 1995a,b). Recently, attention has been focused on the role of NO in lower urinary tract function, and NO has been identified as a nitrergic neurotransmitter contributing to relaxation in various mammalian urethras in vitro (Dokita et al., 1991; Andersson et al., 1992; Hashimoto et al., 1993; Ehrén et al., 1994; Leone et al., 1994) and in vivo (Bennett et al., 1995; Kakizaki et al., 1997). However, some reports have suggested that neurotransmitters released from nitrergic nerves do not appear to be free-radical NO only, and may include NO-containing compounds in rat anococcygeus muscles and gastric fundus (Rand and Li, 1995b), cat airway (Tanaka et al., 1996) and some arteries (Simonsen et al., 1995; Yoshida et al., 1998).

The physiological and pathological effects of NO are usually examined indirectly by suppressing NO synthase with inhibitors, such as N^{ω}-monomethyl-L-arginine (L-NMMA) and N^{ω}-nitro-L-arginine (L-NNA). However, to detail the physiological significance of free radical NO in a biological system, it is essential to analyse NO-dependent responses by using a specific reagent that neutralizes free radical NO directly. Until recently, only a few substrates, including endogenous compounds, e.g., haem-containing protein, such as haemoglobin (Kosaka et al., 1992) and superoxide anions (Saran et al., 1990), were known to interact directly with free radical NO and effectively antagonize its physiological action. Recently, we found a new class of NO scavenger, i.e., a series of derivatives of 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide

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(PTIO), that showed a unique radical–radical reaction with NO, resulting in the generation of NO_2^-/NO_3^- and 2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl (PTI) (Akaike et al., 1993). Furthermore, PTIOs have potent inhibitory activity against endothelium-derived relaxing factors (EDRF) in rabbit aortic ring preparations, the inhibitory potential of PTIOs being comparable to that of NO synthase inhibitors (Akaike et al., 1993). Thus, it seems that the antagonistic action of this type of compound against NO will be useful for analysing the pathophysiological roles of NO in vivo and in vitro (Yoshida et al., 1993, 1994).

In the present study, assuming that part of the nitrergic nerve-mediated urethral relaxation relates to free radical NO and NO-containing compounds, we attempted to investigate the effects of a water-soluble carboxy derivative of PTIO (carboxy-PTIO) on relaxations induced by electrical field stimulation in rabbit urethral smooth muscles. In addition, we examined the effects of carboxy-PTIO on noradrenaline release from prejunctional adrenergic nerve endings, using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) coupled with a microdialysis procedure, since several reports demonstrated that the endogenous free radical NO has a prejunctional action in inhibiting excitatory neuroeffector transmission in several tissues (Belvisi et al., 1991; Jing et al., 1995; Tanaka et al., 1996).

2. Materials and methods

2.1. Tissue preparation

Forty female New Zealand white rabbits weighing 2.2–2.5 kg were killed by exsanguination after intravenous administration of 50 mg/kg sodium pentobarbital. The abdomen was opened and the urethra was dissected free down to the entrance of the vaginal wall and was placed into Krebs–Henseleit (K–H) solution. The urethra was opened by a longitudinal posterior incision and was cut into transverse muscle strips, approximately 2×10 mm for both relaxation and noradrenaline-release experiments.

2.2. Relaxation experiments

The relaxation experiments were performed as previously described (Yoshida et al., 1992; Takahashi et al., 1997). The muscle strips were transferred to 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 a 1-h equilibration period, the muscle strip was stretched to the length for optimal force development, and the bath solution was changed every 15 min. The resting tension at the length for optimal force development in both groups was 600–800 mg. This was determined in preliminary experiments, in which muscle strips were stretched stepwise and stimulated with 10 μM phenylephrine at each length. When the phenylephrine-induced contraction was within 10% of the previous one, the length was considered optimal for isometric force development.

In this study, we investigated the effects of L-NNA and carboxy-PTIO on cys-NO- and electrical field stimulation-induced relaxations of rabbit urethral smooth muscles precontracted with 1 μM phenylephrine. For evaluation of muscle contractility, the concentration–response curves for phenylephrine (0.01–10 μM) and 80 mM KCl-induced contractions were recorded before and after experiments. Cys-NO (0.1 μM–1 mM) was cumulatively added, and electrical field stimulation was applied through two parallel platinum electrodes (10 mm wide and 8 mm apart) fixed to both sides of the inner surface of the muscle bath, so that a current pulse would pass transversely across the muscle strips of the rabbit urethra. Electrical impulses for field stimulation of intrinsic nerves were delivered with a stimulator (SEN-3301, Nihon Kohden) and boosted by an amplifier (SEG-3104, Nihon Kohden). They delivered square-wave pulses (voltage: supramaximum, pulse duration: 2 ms, frequency: 0.5–15 Hz, train duration: 3 s at 2-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 out several times with K–H solution and were equilibrated for 60 min, precontracted with 1 μM phenylephrine and a second series of electrical field stimuli were applied. In the preliminary study, we evaluated the inhibitory potential of carboxy-PTIO and L-NNA on electrical field stimulation-induced relaxation in the rabbit urethra over a wider concentration range (1–300 μM). The inhibitory effects started at 10 μM and were saturated at 100 μM. Thus, in these experiments, L-NNA (10–100 μM) and carboxy-PTIO (10–100 μM) were added for 30 min before the second series of electrical field stimulation and then the effect of the pretreatment with each drug on electrical field stimulation-induced relaxation was evaluated. When used, L-arginine (5 mM) was added to the organ chamber 5 min after L-NNA (100 μM) or carboxy-PTIO (100 μM) was added.

2.3. Noradrenaline release experiments

In the present experiment, the microdialysis method (Shintani et al., 1994; Inadome et al., 1998) was used for collecting the samples containing noradrenaline released from urethral smooth muscle. A microdialysis probe (Kurata et al., 1993) (outer diameter: 220 μm, inner diameter: 200 μm, length: 10 mm) cellulose membrane

protected with platinum strings (outer diameter: 0.02 mm), molecular cut-off 50 kDa, A-I-8-03, Eicom, Kyoto, Japan) was inserted into the urethral strip and the inlet cannula of the probe was connected to a microsyringe pump (EP-60, Eicom). The probe was perfused with Ringer solution containing 0.05 mM ascorbic acid at a constant flow rate of 2 μl/min. As in the functional experiment, the strip with the microdialysis probe was suspended in the muscle bath. Following a 2-h equilibration period, dialysate was collected for 10 min for evaluation of basal noradrenaline release. Then, electrical field stimulation (voltage: supramaximum, pulse duration: 0.5 ms, frequency: 40 Hz, train duration: 3 s at 1 min interval for 10 min) was applied through two parallel platinum electrodes (10 mm wide and 8 mm apart) placed so that a current pulse would pass transversely across the muscle strips of the rabbit urethra, and dialysate was collected every 10 min in polyethylene tubes at room temperature. Following a 1-h equilibration period, each strip pretreated with carboxy-PTIO (100 μM), and both carboxy-PTIO (100 μM) and L-NNA (100 μM) for 30 min was stimulated under the same conditions, and the dialysate was collected. Each dialysate fraction was stored at −20°C for measurement of noradrenaline.

The noradrenaline determination using HPLC–ECD was performed as previously described with only slight modifications (Itoh et al., 1990). In brief, a solution that consist of 0.1 M phosphate buffer, pH 6.0, containing 5% methanol, 50 mg/l Na_2EDTA and 500 mg/l octanesulfonic acid, was delivered as the mobile phase at a rate of 1.0 ml/min. A volume of 10 μl of the sample collected was immediately injected into the column of the HPLC assay system by means of a syringe-loading sample injector (Model 7725, Eicom). The reversed phase separation column (Eicompak MA-5ODS, Eicom) was controlled isothermally at 25°C. Noradrenaline was detected with an ECD system (ECD-300, Eicom) equipped with a graphite electrode. The electrode potential was set to +400 mV against an Ag/AgCl reference electrode. A standard solution containing 0.1 pmol of noradrenaline was injected every working day and the amount of noradrenaline was calculated by reference to the peak area of the standard noradrenaline solution by a chromatogram recorder (Chromatocorder 21, System Instruments, Tokyo, Japan). Noradrenaline release is expressed in terms of the amount of noradrenaline in 10 μl dialysate fraction/weight of the strip (pmol/g wet weight of urethra).

2.4. Solutions and drugs

The 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 solution was made by omitting $CaCl_2$ from the K–H solution and adding 0.1 mM EGTA. Ringer solution was composed as follows (mM): NaCl, 147.0; KCl, 4.0; $CaCl_2$, 2.3 and the pH was adjusted to 7.4 with NaOH. KCl solution (80 mM) was prepared by replacing Na^+ by equimolar amounts of K^+ in the K–H solution. Cys-NO was prepared from L-cystein 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 the preliminary experiment, this vehicle had no effect on the contractility of the rabbit urethral smooth muscles.

L-Arginine hydrochloride, tetrodotoxin, EGTA, EDTA, hexamethonium chloride, L-cysteine, guanethidine monosulphate, DL-propranolol hydrochloride and indomethacin were obtained from Sigma, carboxy-PTIO was provided by Dojindo Laboratories, Kumamoto, Japan. All other chemicals were obtained from commercial sources. Concentrations are expressed as final concentrations in the organ baths. Indomethacin was dissolved in 1% Na_2CO_3 immediately before use. Unless otherwise specified, drugs were dissolved in distilled water, and 0.2 ml volumes were added to the bath.

2.5. Data analysis

In the functional experiment, the relaxation induced by Ca^{2+}-free K–H solution was taken as 100% and percent relaxation was calculated. Data are expressed as means ± S.E.M. Statistical analysis of differences between groups was performed using an 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 curves for phenylephrine and KCl (80 mM)-induced contractions were not significantly different before and after the experiment. The pretreatment with L-NNA and carboxy-PTIO did not have a significant effect on the resting tension or on the contractile response induced by 1 μM phenylephrine. In the presence of atropine (1 μM), propranolol (1 μM) and indomethacin (10 μM), exogenously applied cys-NO evoked a transient relaxation of urethral smooth muscles precontracted with 1 μM phenylephrine in a concentration-dependent manner. Although L-NNA (100 μM) did not affect the concentration-dependent relaxation induced by cys-NO, carboxy-PTIO (100 μM) significantly inhibited the relaxation (Fig. 1).

Electrical field stimulation caused frequency-dependent relaxations in rabbit urethral smooth muscles precontracted with 1 μM phenylephrine in the presence of atropine (1 μM), propranolol (1 μM) and indomethacin (10 μM). The maximum relaxation was 93.5 ± 4.4% (n = 20). The relaxation responses developed rapidly and were transient, and we did not observe longer-lasting second-phase relaxation

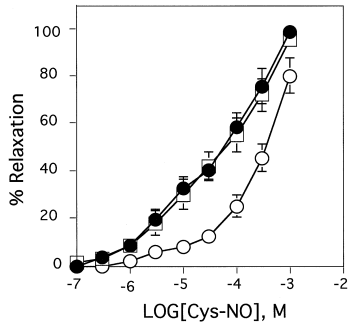

Fig. 1. Effects of carboxy-PTIO and L-NNA on the cys-NO-induced relaxation of the rabbit urethra. Cys-NO (0.1 μM–1 mM) was applied with carboxy-PTIO (100 μM) (○), L-NNA (100 μM) (□) or without drugs (●) to the strips of rabbit urethral smooth muscles under tension induced by phenylephrine (1 μM) in the presence of atropine (1 μM), propranolol (1 μM) and indomethacin (1 μM). Each point is the mean ± S.E.M. derived from seven experiments. Average absolute tension development evoked by phenylephrine (1 μM) was 1.23 ± 0.21 g ($n = 7$).

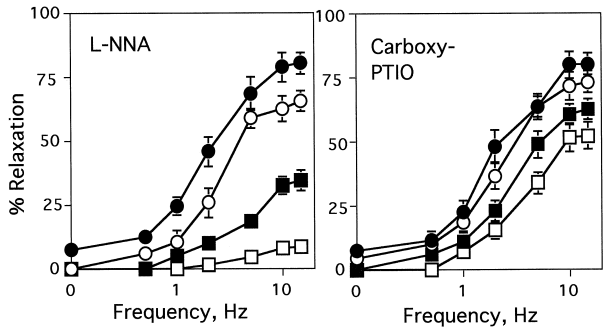

Fig. 3. Effects of L-NNA and carboxy-PTIO on frequency–response curves for electrical field stimulation-induced relaxation in the rabbit urethra. Electrical field stimulation (voltage: supramaximum, pulse duration: 2 ms, frequency: 0.5–15 Hz, train duration: 3 s at 2-min intervals) was delivered to the strips of rabbit urethral smooth muscles under tension induced by phenylephrine (1 μM) in the presence of atropine (1 μM), propranolol (1 μM) and indomethacin (1 μM). Each point is the mean ± S.E.M. derived from 10 experiments. ●: control, ○: 10 μM, ■: 30 μM, □: 100 μM of each drug. Average absolute tension development evoked by phenylephrine (1 μM) was 1.28 ± 0.26 g ($n = 20$).

responses in the present study. All relaxation responses were almost completely blocked by tetrodotoxin (1 μM), but not by hexamethonium (100 μM). The pretreatment with L-NNA (10–100 μM) and carboxy-PTIO (10–100 μM) caused concentration-dependent inhibition of the relaxation induced by electrical field stimulation. Typical tracings for both drugs are shown in Fig. 2. These inhibitory effects of L-NNA (100 μM) were overcome by L-arginine (5 mM), but those of carboxy-PTIO (100 μM) were not affected by L-arginine (5 mM). Fig. 3 shows the effects of the pretreatment with L-NNA and carboxy-PTIO on the frequency–response curves for electrical field stimulation. The inhibitory effect of carboxy-PTIO on the frequency–response curve was significantly weaker than that of L-NNA. The inhibition by carboxy-PTIO (100 μM) of the relaxation response induced by 15 Hz electrical field stimulation was 38.3 ± 4.2% ($n = 10$), which was significantly smaller than the effect of L-NNA (100 μM) (87.6 ± 5.2%; $n = 10$).

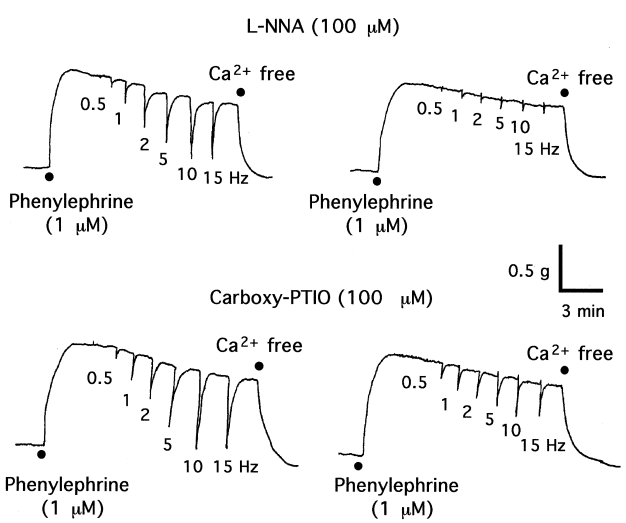

Fig. 2. Representative tracings showing the effects of L-NNA and carboxy-PTIO on electrical field stimulation-induced relaxation in the rabbit urethra. Electrical field stimulation (voltage: supramaximum, pulse duration: 2 ms, frequency: 0.5–15 Hz: train duration 3 s at 2-min intervals) was delivered to strips of rabbit urethral smooth muscle under tension induced by phenylephrine (1 μM) in the presence of atropine (1 μM), propranolol (1 μM) and indomethacin (1 μM). Electrical field stimulation caused frequency-dependent relaxation in the rabbit urethra. Pretreatment with L-NNA (100 μM) or carboxy-PTIO (100 μM) inhibited the relaxation responses. The inhibitory effect of L-NNA was significantly greater than that of carboxy-PTIO.

3.2. Noradrenaline release experiments

After insertion of the microdialysis probe, the contractile response induced by electrical field stimulation (voltage: supramaximum, pulse duration: 0.5 ms, frequency: 40 Hz, train duration: 2 s at 1-min interval) was 0.67 ± 0.08 g ($n = 20$) in rabbit urethral smooth muscles, which was similar to the contractile response before probe insertion. In the preliminary experiment, noradrenaline release induced by electrical field stimulation every 1 h was constant for 5 to 6 h. Before electrical field stimulation, the amount of noradrenaline released into the dialysate fraction (basal release) was 0.13 ± 0.02 pmol/g wet weight of urethra ($n = 20$). The amount of noradrenaline released into the dialysate fraction during electrical field stimulation was 0.37 ± 0.03 pmol/g wet weight of urethra ($n = 20$), which was significantly higher than the basal release. Pretreatment with tetrodotoxin (1 μM) or guanethidine (1 μM) significantly inhibited noradrenaline release during electrical field stimulation to 0.14 ± 0.03 pmol/g wet weight of urethra ($n = 5$), and 0.15 ± 0.02 pmol/g wet weight of urethra ($n = 5$), respectively, which is similar to the basal release. Fig. 4 shows the effects of L-NNA and

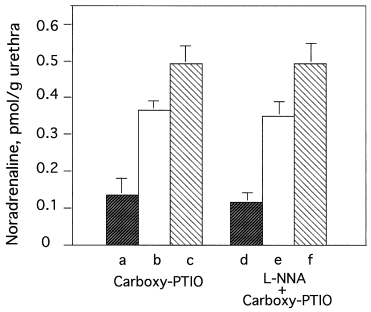

Fig. 4. Effects of L-NNA and carboxy-PTIO on electrical field stimulation-induced noradrenaline release from adrenergic nerve in the rabbit urethra. The release of noradrenaline was measured by HPLC with electrochemical detection coupled with microdialysis. A microdialysis probe was inserted into the urethral strip and was perfused with Ringer solution containing 0.05 mM ascorbic acid at a constant flow rate of 2 μl/min in the muscle bath. Electrical field stimulation (voltage: supramaximum, pulse duration: 0.5 ms, frequency: 40 Hz, train duration: 3 s at 1-min interval for 10 min) was applied to the muscle strips under tension induced by phenylephrine (1 μM) in the presence of atropine (1 μM), propranolol (1 μM) and indomethacin (1 μM), and dialysate was collected every 10 min. Each bar is the mean ± S.E.M. derived from 10 experiments. (a) Basal noradrenaline release before treatment with carboxy-PTIO (100 μM); (b) noradrenaline release during electrical field stimulation before treatment with carboxy-PTIO (100 μM); (c) noradrenaline release during electrical field stimulation after treatment with carboxy-PTIO (100 μM); (d) basal noradrenaline release before treatment with both L-NNA (100 μM) and carboxy-PTIO (100 μM); (e) noradrenaline release during electrical field stimulation before treatment with both L-NNA (100 μM) and carboxy-PTIO (100 μM); (f) noradrenaline release during electrical field stimulation after treatment with both L-NNA (100 μM) and carboxy-PTIO (100 μM).

carboxy-PTIO on noradrenaline release during electrical field stimulation of rabbit urethral smooth muscles. Pretreatment with carboxy-PTIO (100 μM) caused a significant increase in noradrenaline release (0.49 ± 0.05 pmol/g wet weight of urethra; $n = 10$). The simultaneous application of L-NNA (100 μM) and carboxy-PTIO (100 μM) did not further enhance noradrenaline release (0.49 ± 0.06 pmol/g wet weight of urethra; $n = 6$) in rabbit urethral smooth muscles. After pretreatment with carboxy-PTIO (100 μM), the contraction (0.94 ± 0.10 g; $n = 10$) induced by electrical field stimulation (voltage: supramaximum, pulse duration: 0.5 ms, frequency: 40 Hz, train duration: 2 s) was significantly greater than the control contraction (0.69 ± 0.06 g; $n = 10$). When both L-NNA and carboxy-PTIO were applied, the contractile response was 0.96 ± 0.10 g ($n = 10$).

4. Discussion

The present results demonstrate that carboxy-PTIO, one of the NO scavengers, only partially suppressed the L-NNA-sensitive NANC nerve-mediated relaxations in rabbit urethral smooth muscle preparations. These observations are in sharp contrast to those obtained with rabbit aortic ring preparations, where carboxy-PTIO (100 μM) almost completely blocked the vasorelaxation, possibly induced by EDRF/NO released from endothelial cells, elicited by stimulation with acetylcholine and adenosine triphosphate (Akaike et al., 1993). By using electron-spin resonance spectroscopy, we demonstrated that carboxy-PTIO reacted only with free radical NO in a stoichiometric manner in a neutral solution with a rate constant of 10^4 M^{-1} S^{-1}, resulting in the generation of NO_2^-/NO_3^- and carboxy-PTI. Neither carboxy-PTI nor NO_2^-/NO_3^- has an effect on vascular smooth muscle tone (Akaike et al., 1993; Yoshida et al., 1993). The inhibitory effect of carboxy-PTIO was almost similar to that of NO synthase inhibitors, such as L-NNA or L-NMMA in rabbit aortic ring preparations (Akaike et al., 1993). In view of the molecular size of carboxy-PTIO (molecular weight: 299.28), a large proportion of carboxy-PTIO used in the present experiment should reach the neuroeffector junction in rabbit urethral smooth muscles. Thus, the neuronal release of NO-containing compounds, as well as free radical NO, which would in turn release NO in the urethral tissue, could account for the observed difference between aortic ring preparations and urethral smooth muscles in the inhibitory effect of carboxy-PTIO on relaxation responses. As carboxy-PTIO has high selectivity for free radical NO, the present data indicate that both free radical NO and NO-containing compounds are involved in the L-NNA-sensitive nitrergic nerve-mediated relaxation in rabbit urethral smooth muscles.

Several reports have demonstrated that the neurotransmitter released from nitrergic nerves does not appear to be identical to free radical NO. Rand and Li (1995b) reported that the relaxation of the rat anococcygeus muscle induced by exogenous NO was inhibited by carboxy-PTIO, but the relaxation induced by nitrergic nerve stimulation was not completely reduced by carboxy-PTIO. Also, in the cat airway smooth muscle (Tanaka et al., 1996), carboxy-PTIO suppressed the amplitude of the relaxation induced by nitrergic nerve stimulation by about 50%. Simultaneous application of NO synthase inhibitor further reduced the carboxy-PTIO-resistant relaxation to 20–30% of the control value. The authors suggested that carboxy-PTIO only partially inhibited NO synthase-sensitive nitrergic nerve-mediated relaxation. Furthermore, there have been a number of reports suggesting that part of EDRF is an adduct of NO rather than free radical NO (Verdernikov et al., 1992; Mülsch, 1994; Stamler, 1994). Recently, we also suggested that the contribution of free radical NO to vascular tone may vary among different blood vessels (Yoshida et al., 1998). In that report, we showed that carboxy-PTIO almost completely inhibited acetylcholine-induced (EDRF-mediated) relaxation in rabbit aortic and femoral artery ring preparations precontracted with phenylephrine. In contrast, the maximum inhibition produced by carboxy-PTIO in renal, mesenteric, or pulmonary arteries was about 70%. The data suggested that acetylcholine-induced relaxation in

the aorta and femoral artery can be attributed solely to the release of free radical NO from endothelial cells. In contrast, in renal, mesenteric, and pulmonary arteries, acetylcholine-induced relaxations are mediated not only by free radical NO but also by NO-containing compounds, such as nitrosothiols (Stamler, 1994). These data are consistent with the present data, and may further support the finding that the neuronal release of NO-containing compounds as well as free radical NO contributes to the relaxation responses induced by electrical field stimulation in rabbit urethral smooth muscles.

In the present study, we used HPLC–ECD detection coupled with microdialysis for measurement of noradrenaline released from the rabbit urethra. The HPLC–ECD system is extremely rapid, relatively simple, permitting the processing of multiple samples within minutes, and has excellent reproducibility, specificity and sensitivity (Salzman and Sellers, 1982). In addition, the microdialysis method has developed in the past two decades, and several investigators have used this method to collect samples for the measurement of endogenous noradrenaline in various tissues (Itoh et al., 1990; Kiss et al., 1995; Drijfhout et al., 1996). Using microdialysis, we recently measured NO_2^-/NO_3^- (Takahashi et al., 1997) and acetylcholine (Inadome et al., 1998) release induced by electrical field stimulation in rabbit urethral and bladder smooth muscles, respectively. However, little information is as yet available about the measurement of noradrenaline release by this method in the urinary tract. This is the first report to use the HPLC–ECD detection coupled with microdialysis for measurement of noradrenaline release in urinary tract smooth muscles.

In the present study, the basal release of noradrenaline could be measured in the absence of electrical field stimulation. The basal release of noradrenaline after pretreatment with tetrodotoxin or guanethidine still remained. Greaney et al. (1993) suggested that neurotransmitter release could be elicited by injury. In the present experiments insertion of the microdialysis probe or muscle stretching may have caused tissue damage. The noradrenaline release during electrical field stimulation was significantly increased, and pretreatment with guanethidine or tetrodotoxin significantly inhibited noradrenaline release during electrical field stimulation. The data suggested that noradrenaline release in the rabbit urethra was caused by adrenergic nerve stimulation. In the present study, carboxy-PTIO significantly enhanced noradrenaline release induced by electrical field stimulation. However, simultaneous application of carboxy-PTIO and L-NNA did not further enhance noradrenaline release. These observation imply that endogenous free radical NO, but not NO-containing compounds, has a prejunctional action in suppressing noradrenaline release from adrenergic nerve endings in the rabbit urethra.

It is generally accepted that adrenergic neuroeffector transmission is regulated by the coexistence and corelease of several transmitter substances along with noradrenaline and the negative feedback control on noradrenaline release (Stjärne, 1989). Mutoh et al. (1987) reported that the activation of prejunctional muscarinic receptors in adrenergic nerve endings in dog bladder neck depressed noradrenaline release induced by electrical field stimulation. Recently, the prejunctional inhibitory regulation by NO of excitatory neurotransmitter release has been demonstrated (Belvisi et al., 1991; Jing et al., 1995; Tanaka et al., 1996). Belvisi et al. (1991) suggested that NO released by nerve stimulation modulated cholinergic neurotransmission in the guinea-pig trachea. Jing et al. (1995) also demonstrated that endogenous and exogenous NO had a prejunctional action in inhibiting excitatory neuroeffector transmission, presumably by suppressing transmitter release from the vagus nerve in the cat airway. Furthermore, Tanaka et al. (1996) reported that carboxy-PTIO or N^{ω}-nitro-L-arginine methyl ester (L-NAME) enhanced excitatory junctional potentials induced by electrical field stimulation, and that simultaneous application of L-NAME and carboxy-PTIO did not further enhance the excitatory junction potential in cat tracheal and bronchial tissues. These findings suggest that free radical NO has a prejunctional action which inhibits excitatory neuroeffector transmission. The results are consistent with our results which suggest that free radical NO has a prejunctional inhibitory action on noradrenaline release from adrenergic nerve endings in the rabbit urethra. Thus, the present study demonstrates that free radical NO released from nitrergic nerves may directly relax rabbit urethral smooth muscle and at the same time inhibit the release of noradrenaline from adrenergic nerve ending. These observations imply that free radical NO has a double inhibitory role on rabbit urethral smooth muscle contractions. Several reports (Hakoda et al., 1991; Xie et al., 1991) have demonstrated that vasoactive intestinal polypeptide has a similar role in bronchoconstriction.

In conclusion, because carboxy-PTIO reacts only with free radical NO, the present data indicate that both free radical NO and NO-containing compounds are involved in the L-NNA-sensitive nitrergic nerve-mediated relaxation in rabbit urethral smooth muscles, and, at the same time, free radical NO may have a prejunctional inhibitory action on noradrenaline release from adrenergic nerves.

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