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The effect of nitric oxide on acetylcholine release in the rabbit bladder

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Abstract

We evaluated the effects of nitric oxide (NO) on acetylcholine release and the contractile response induced by electrical field stimulation in rabbit bladder smooth muscles using a muscle bath and high performance liquid chromatography coupled with microdialysis. Electrical field stimulation (supramaximum voltage, pulse duration 0.5 ms, frequency 5 and 20 Hz) was applied to a smooth muscle strip isolated from rabbit bladder. With low-frequency (5 Hz) stimulation, pretreatment with N^{ω}-nitro-L-arginine (L-NNA) (100 μM) significantly increased electrical field stimulation-induced acetylcholine release and contractile response, which were reduced by the addition of L-arginine. Pretreatment with sodium nitroprusside in the absence or presence of L-NNA significantly decreased electrical field stimulation-induced acetylcholine release and contractile response. In contrast, with high frequency (20 Hz) stimulation, pretreatment with L-NNA and sodium nitroprusside had no significant effect on either contractile response or acetylcholine release. Pretreatment with sodium nitroprusside caused no significant changes in carbachol and ATP-induced contractile responses. Sodium nitroprusside and L-NNA had no significant effects on the atropine-resistant part of the contraction induced by electrical field stimulation in rabbit bladder smooth muscles. The results suggest that there is a NO-mediated mechanism inhibiting acetylcholine release from cholinergic nerve endings in rabbit bladder, which may contribute to bladder function.

Keywords: Bladder; Nitric oxide (NO); Acetylcholine

1. Introduction

The parasympathetic nervous system plays an important role in the function of the lower urinary tract (D'Agostino et al., 1986). A major neurotransmitter for physiological bladder contraction is acetylcholine released from prejunctional parasympathetic nerve endings. Nitric oxide (NO) is also known to play an important role in various pathophysiological conditions (Moncada et al., 1991). NO synthase activity has also been demonstrated in nerves supplying animal and human urogenital organs (Andersson et al., 1991; Vizzard et al., 1994; Bennett et al., 1995; Dixon and Jen, 1995; Werkström et al., 1995; Garcia-Pascual et al., 1996; Jen et al., 1996).

Regarding the role of NO in lower urinary tract function, 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). However, the role of NO in bladder smooth muscle is not clear. Persson et al. (Persson and Andersson, 1992; Persson et al., 1992) have indicated that neurogenic relaxation, involving the L-arginine/NO pathway, could be demonstrated consistently in the pig and rat urethral but not in the detrusor smooth muscles. In addition, there are reports of the presence of NO synthase in the nerves innervating the detrusor muscle and of a lack of effect of NO donors on the cGMP levels in this muscle (Smet et al., 1996; McNeill et al., 1992). It has been reported recently that NO prejunctionally inhibits acetylcholine release from cholinergic nerve endings in several smooth muscles (Sekizawa et al., 1993; Kilbinger and Wolf, 1994), However, there is little information available about the prejunctional modulation of NO for bladder smooth muscle contractions.

The present study was therefore designed to evaluate the prejunctional effects of NO on the contractile response and acetylcholine release induced by electrical field stimu-

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lation in rabbit bladder smooth muscles. This is the first report demonstrating the role of NO in acetylcholine release and the neurogenic contractile response evoked by electrical field stimulation in bladder smooth muscles.

2. Materials and methods

2.1. Tissues

Female New Zealand white rabbits weighing 2.5–3.0 kg were anesthetized with 50 mg/kg sodium pentobarbital and killed by exsanguination. The abdomen was opened and the bladder was dissected free from connective tissue and the vaginal wall. Uniform longitudinal strips of the posterior wall of the bladder dome (3×10–12 mm) were prepared.

2.2. Microdialysis procedure

Microdialysis for the smooth muscle strips was performed as previously described (Takahashi et al., 1997; Inadome et al., 1998a; Yoshida et al., 1998; Seshita et al., 2000). The dialysis probe (O-P-100-10; Eicom, Kyoto, Japan) had a 0.22×10-mm dialysis membrane of regenerated cellulose with a molecular cut-off of 50 kDa, and platinum strings (0.22-mm outer diameter) were mounted for protection of the dialysis membrane. The probe was inserted through the muscle strip, and the inlet cannula of the probe was connected to a microfusion syringe pump (EP-60, Eicom). Ringer solution containing 100 μM physostigmine sulfate was continuously perfused at a rate of 2 μl/min. In the present experiment, the internal standard, isopropylhomocholine, which was delivered by the microinfusion syringe pump, was fed into the perfusate tube distal to the semipermeable membrane at a rate of 2 μl/mim. The strips were suspended in a 20-ml organ bath filled with modified Krebs–Henseleit (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, Tokyo, Japan), and isometric forces were recorded and monitored on an electronic pen recorder (R-02A, Rikadenki Kogyo, Tokyo, Japan). Each strip was stretched until optimal stress developed (about 1.0 g resting tension) and was allowed to equilibrate for 120 min before the start of the experiment. Sampling was started 10 min before stimulation and dialysate was collected in a microtube every 10 min.

2.3. Acetylcholine determination

Acetylcholine was determined with a combination of high performance liquid chromatography (HPLC), enzyme reaction, and electrochemical detection (ECD) as previously described, with only slight modifications (Damsma et al., 1987; Toide and Arima, 1989; Mizuo et al., 1991; Watanabe et al., 1990). In brief, a solution containing 300 mg/l sodium 1-decanesulfonate and 65 mg/l tetramethylammonium chloride, was delivered as the mobile phase at a rate of 0.6 ml/min. A 10-μl portion of the samples collected was immediately injected into the column of the HPLC assay system with a syringe-loading sample injector (model 7725; Eicom). After separation on a styrene polymer column (AC-GEL, Eicom), acetylcholine was converted to hydrogen peroxide with a post-column enzyme reactor (AC-Enzymepak, Eicom) with immobilized acetylcholinesterase and choline oxidase. The separation column and post-column reactor were controlled isothermally at 33 °C. Hydrogen peroxide was detected with an ECD system (ECD-300, Eicom) equipped with a platinum electrode. The electrode potential was set at +450 mV against a Ag/AgCl reference electrode. The amount of acetylcholine was calculated by reference to the peak area of the standard acetylcholine solution by a chromatogram recorder (Chromatocorder 21; System Instruments, Tokyo). The detection limit of acetylcholine was 0.02 pmol/injection. Acetylcholine release during electrical field stimulation was expressed in terms of the amount of acetylcholine released during electrical field stimulation/wet weight of the muscle strips (pmol/g).

2.4. Functional contractile response experiments

In all experiments, indomethacin (10 μM) and guanethidine (10 μM) were added to K–H solution to exclude the effect of prostaglandins and intrinsic adrenergic neurotransmission. For evaluation of muscle contractility, contractile responses for 80 mM KCl were obtained before and after the experiments. Electrical field stimulation was generated between two parallel platinum electrodes (10 mm wide and 8 mm apart). The intramural nervous system of the strips was stimulated by rectangular pulses of 500 μs duration at 2–40 Hz. Trains of pulses for 2 s, with a 2-min interval between stimulations were used. Five muscle contractions were induced by shocks at one frequency period, and the interval between them was 20 min. The mean value of the peak tensions developed by all twitches during the stimulation period was taken as the contractile response (g).

In a preliminary study, we obtained frequency–response curves for acetylcholine release induced by electrical field stimulation (2–80 Hz). Release at 5 and 20 Hz was about 40% and 80% of the maximum acetylcholine release, respectively. At frequencies higher and lower than 40 and 5 Hz, the amount of acetylcholine release was maximum and slight, respectively. Under such conditions, we could not evaluate the changes in acetylcholine release after drug

treatments. Thus, in the present study, we chose 5 and 20 Hz as low and high stimulation frequencies, respectively.

The effects of pretreatment with N^{ω}-nitro-L-arginine (L-NNA, 100 μM) and L-arginine (100 μM) on the contractile responses and acetylcholine release induced by electrical field stimulation were evaluated. The effect of the NO-donor, sodium nitroprusside (100 μM), in the absence and presence of L-NNA on the contractile responses and acetylcholine release induced by electrical field stimulation was evaluated. Three series of electrical field stimulation (5 or 20 Hz) were performed at 1-h intervals. Drugs were added 20 min before the second and the third stimulations, respectively. The effects of drugs on electrical field stimulation-induced contractile responses were calculated from the ratios of the second/first and third/first stimulation, respectively. Electrical field stimulation (5 and 20 Hz) was also applied in the presence of atropine (1 μM). The residual atropine-resistant contraction was evaluated by desensitization of P_{2X} purinoceptors with α,β-methylene ATP (10 μM per application, 3–5 times at 10-min intervals) until the contraction caused by this agent was absent. Then, electrical field stimulation was repeated in the presence of atropine and α,β-methylene ATP. The effect of the pretreatment with L-NNA and sodium nitroprusside (100 μM) on the atropine-resistant and α,β-methylene ATP-sensitive contractions was evaluated.

Concentration–response curves were made for carbachol (0.01–100 μM) and ATP (0.001–5 mM) in rabbit bladder smooth muscles, and the effects of pretreatment with sodium nitroprusside (100 μM) on these curves were also evaluated. Sodium nitroprusside was added in the bath 20 min before the concentration–response curves for carbachol and ATP, and frequency–response curves for electrical field stimulation.

2.5.Solutions and drugs

Ringer solution was composed as follows (mM): NaCl, 147 mM; KCl, 4 mM; $CaCl_2$, 2.3 mM 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. KCl solution (80 mM) was prepared by replacing Na^+ with equimolar amounts of K^+ in K–H solution. The following drugs were used: acetylcholine chloride, choline chloride, guanethidine monosulphate, L-arginine, L-NNA, sodium nitroprusside, atropine sulfate, carbachol chloride, ATP, α,β-methylene ATP, hexamethonium chloride and tetrodotoxin (Sigma, Tokyo); isopropylhomocholine (Eicom); sodium 1-decanesulfonate (Tokyo Kasei Kogyo, Tokyo); tetramethylammonium chloride, indomethacin (Nacalai Tesque, Kyoto); physostigmine sulfate (Aldrich Chemical, Tokyo). 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. Concentrations are expressed as final bath concentrations.

2.6. Data analysis

The data are expressed as means ± S.E.M. Groups were compared 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

The contractile responses induced by 80 mM KCl were not significantly different before and after experiments. The electrical field stimulation-induced contractile responses in control experiments in the absence of drugs were 3.68 ± 0.36 g for the low frequency ($n = 12$) and 5.72 ± 0.89 g for the high frequency ($n = 8$), respectively (Table 1). The contractile responses induced by electrical field stimulation were almost completely blocked by tetrodotoxin (1 μM) but not by hexamethonium (100 μM).

Acetylcholine released spontaneously from rabbit bladder strips before electrical field stimulation was considered as basal release. Electrical field stimulation caused significant increases in acetylcholine release. Pretreatment with tetrodotoxin (1 μM) abolished the increase in acetylcholine release induced by electrical field stimulation, but did not alter spontaneous acetylcholine release. The values for basal release of acetylcholine with the low (5 Hz)- and high (20 Hz)-frequency stimulations were 2.23 ± 0.59 and 3.85 ± 1.30 pmol/g, respectively (Table 1).

With low-frequency (5 Hz) stimulation, electrical field stimulation caused a significant increase in acetylcholine release (4.22 ± 1.25 pmol/g), and pretreatment with L-NNA (100 μM) caused a significant increase in acetylcholine release (8.31 ± 2.45 pmol/g). The elevation in acetylcholine release caused by L-NNA was significantly inhibited to the control value (4.88 ± 1.55 pmol/g) by

Table 1
Contractile response and ACh release induced by EFS in the absence of drugs

EFS frequency	Contraction (g)	ACh release (pmol/g)	
		Basal	Stimulation
5 Hz ($n = 12$)	3.68 ± 0.36	2.23 ± 0.59	4.22 ± 1.25
20 Hz ($n = 8$)	5.72 ± 0.89	3.85 ± 1.30	11.88 ± 1.98

Mean values ± S.E.M. are shown. n = number of experiments. Electrical field stimulation (EFS; voltage: supramaximum, pulse duration: 500 μs, train duration: 2 s at 2-min intervals for 10 min) was delivered and the interval between stimulations was 2 min.

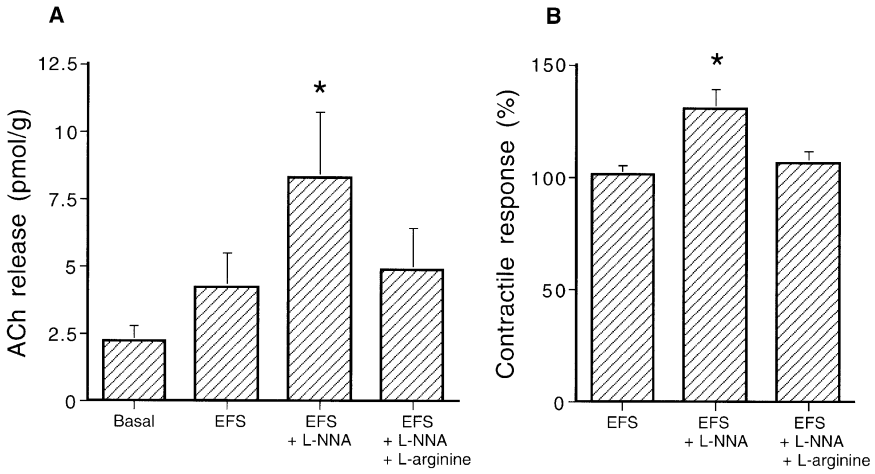

Fig. 1. The effects of pretreatment with L-NNA and addition of L-arginine on acetylcholine (ACh) release (A) and contractile responses (B) induced by electrical field stimulation (EFS) (low frequency: 5 Hz) in rabbit bladder smooth muscles. Three series of EFS were performed at 1-h intervals. L-NNA and L-arginine were added 20 min before the second and the third stimulation, respectively. ACh release during EFS was expressed in terms of amount of ACh released during EFS/wet weight of the muscle strips (pmol/g). The effects of L-NNA and L-arginine on the EFS-induced contractile responses were calculated from the ratios of the second/first and third/first stimulation, respectively. EFS (voltage: supramaximum, pulse duration: 500 μs, frequency: 5 Hz, train duration: 2 s) was delivered five times, and the interval between stimulations was 2 min. Each column represents the mean ± S.E.M. of 12 experiments. * Significantly different from the value of EFS without drugs ($P < 0.05$).

addition of L-arginine (100 μM) (Fig. 1). Instead, with the high frequency (20 Hz) stimulation, although electrical field stimulation caused a significant increase in acetylcholine release (11.88 ± 1.98 pmol/g), neither the pretreatment with L-NNA (100 μM) nor the addition of L-arginine (100 μM) had significant effects on acetylcholine release (Fig. 2).

With low-frequency (5 Hz) stimulation, pretreatment with L-NNA (100 μM) caused a significant increase in the contractile response induced by electrical field stimulation (130.8 ± 8.3%), and the addition of L-arginine returned the increase to the control value (Fig. 1). However, with high-frequency stimulation, neither the pretreatment with L-NNA nor the addition of L-arginine (100 μM) had a

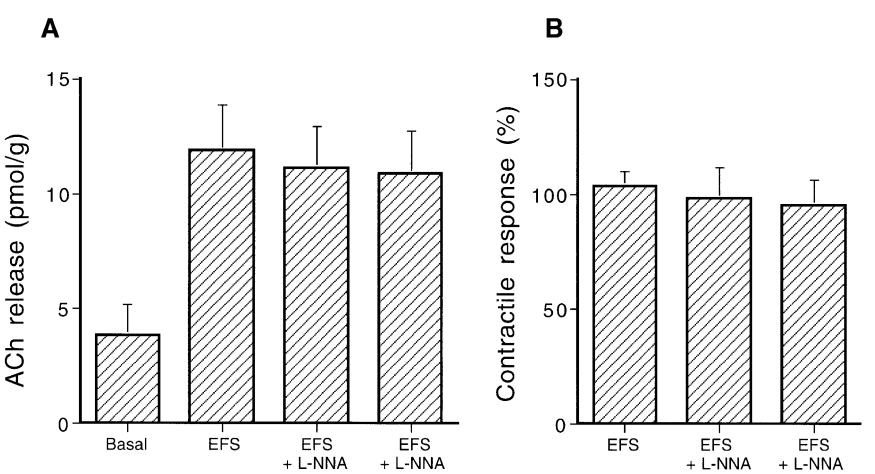

Fig. 2. The effects of pretreatment with L-NNA and addition of L-arginine on ACh release (A) and contractile response (B) induced by EFS (high frequency: 20 Hz) in rabbit bladder smooth muscles. Three series of EFS were performed at 1-h intervals. L-NNA and L-arginine were added 20 min before the second and the third stimulation, respectively. ACh release during EFS was expressed in terms of amount of ACh released during EFS/wet weight of the muscle strips (pmol/g). The effects of L-NNA and L-arginine on the EFS-induced contractile responses were calculated from the ratios of the second/first and third/first stimulation, respectively. EFS (voltage: supramaximum, pulse duration: 500 μs, frequency: 20 Hz, train duration: 2 s) was delivered five times, and the interval between stimulations was 2 min. Each column represents the mean ± S.E.M. of eight experiments.

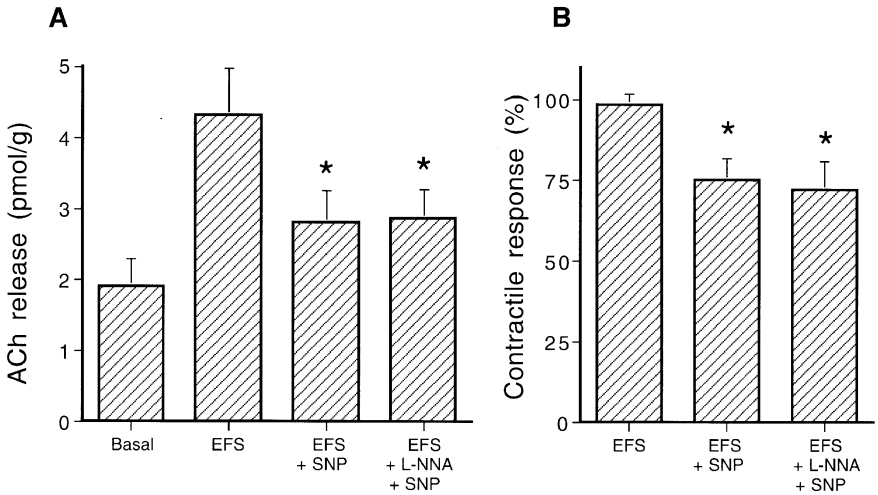

Fig. 3. The effects of pretreatment with sodium nitroprusside (SNP) on ACh release (A) and contractile responses (B) induced by EFS (low frequency: 5 Hz) in rabbit bladder smooth muscles. Three series of EFS were performed at 1-h intervals. L-NNA and/or SNP were added 20 min before the second and the third stimulation, respectively. ACh release during EFS was expressed in terms of amount of ACh released during EFS/wet weight of the muscle strips (pmol/g). The effects of SNP and L-NNA on the EFS-induced contractile responses were calculated from the ratios of the second/first and third/first stimulation, respectively. EFS (voltage: supramaximum, pulse duration: 500 μs, frequency: 5 Hz, train duration: 2 s) was delivered five times, and the interval between stimulation was 2 min. Each column represents the mean ± S.E.M. of five experiments. * Significantly different from the value of EFS without drugs ($P < 0.05$).

significant effect on the contractile response induced by electrical field stimulation (Fig. 2). Pretreatment with sodium nitroprusside (100 μM) caused a significant decrease in the acetylcholine release (2.81 ± 0.45 pmol/g) and contractile response (75.5 ± 7.0%) induced by low-frequency (5 Hz) electrical field stimulation. After preincubation with L-NNA, the addition of sodium nitroprusside caused a significant decrease in both acetylcholine release (2.87 ± 0.42 pmol/g) and contractile response (72.1 ± 9.0%) (Fig. 3). With high-frequency (20 Hz) stimulation, the addition of sodium nitroprusside (100 μM) in the absence and presence of L-NNA did not cause a significant effect on the contractile response induced by electrical field stimulation (Fig. 4).

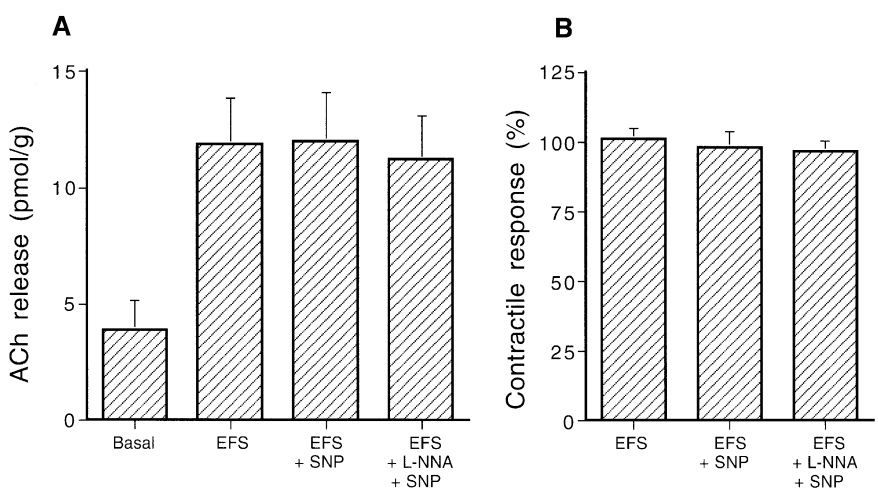

Fig. 4. The effects of pretreatment with SNP on ACh release (A) and contractile responses (B) induced by EFS (high frequency: 20 Hz) in rabbit bladder smooth muscles. Three series of EFS were performed at 1-h intervals. L-NNA and/or SNP were added 20 min before the second and the third stimulation, respectively. ACh release during EFS was expressed in terms of the amount of ACh released during EFS/wet weight of the muscle strips (pmol/g). The effects of SNP and L-NNA on the EFS-induced contractile responses were calculated from the ratios of the second/first and third/first stimulation, respectively. EFS (voltage: supramaximum, pulse duration: 500 μs, frequency: 5 Hz, train duration: 2 s) was delivered five times, and the interval between stimulations was 2 min. Each column represents the mean ± S.E.M. of five experiments. * Significantly different from the value of EFS without drugs ($P < 0.05$).

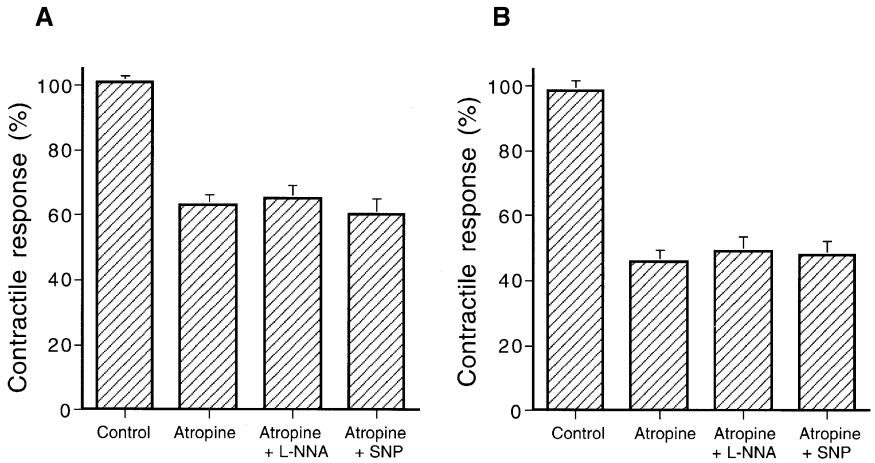

Fig. 5. The effects of treatment with L-NNA and SNP on atropine-resistant contractions induced by EFS in rabbit bladder smooth muscles. EFS was performed in the presence of atropine (1 μM). L-NNA (100 μM) and SNP (100 μM) were added to the bath 20 min before EFS. EFS (voltage: supramaximum, pulse duration: 500 μs, frequency: 5 Hz (A) and 20 Hz (B), train duration: 2 s) was delivered five times, and the interval between stimulations was 2 min. Each column represents the mean ± S.E.M. of five experiments.

Pretreatment with atropine (1 μM) decreased the electrical field stimulation (5 and 20 Hz)-induced contractile responses by 63.2 ± 3.0% ($n = 5$) and 45.8 ± 3.5% ($n = 5$) of the control contractions, respectively. The residual contractions were almost completely abolished by repeated exposure to α,β-methylene ATP. These atropine-resistant and α,β-methylene ATP-sensitive contractions were not significantly affected by the treatment with L-NNA (100 μM), and sodium nitroprusside (100 μM) (Fig. 5). Carbachol (0.01–100 μM) and ATP (0.001–10 mM) caused concentration-dependent contractile responses in rabbit bladder smooth muscles. Pretreatment with sodium nitroprusside (100 μM) did not have significant effects on the concentration–response curves for carbachol and ATP (Fig. 6).

4. Discussion

In the present study, both low (5 Hz)- and high (20 Hz)-frequency stimulation caused significant contractile

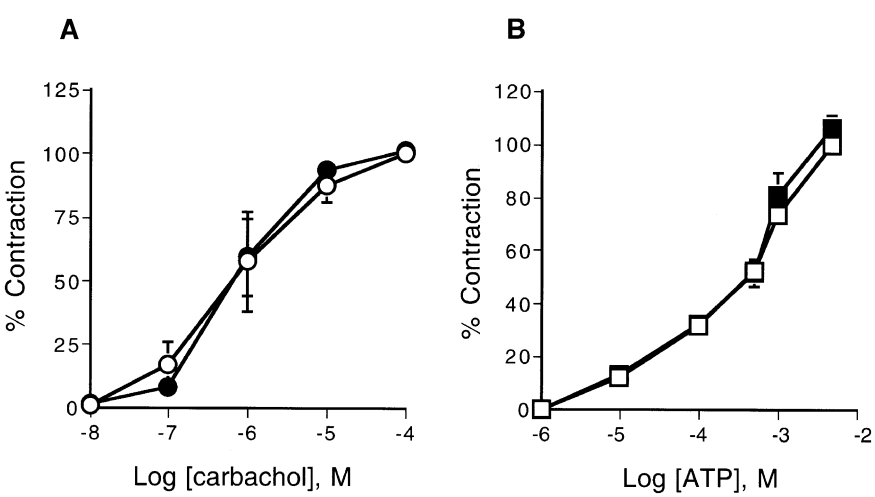

Fig. 6. The effects of pretreatment with SNP on the concentration–response curves for carbachol (A) and ATP (B) in rabbit bladder smooth muscle. For each experiment, contractile responses are expressed as percentages of the maximum contractile response in the absence of SNP. SNP (100 μM) was added 20 min before concentration–response curves were made for carbachol (0.01–100 μM) and ATP (0.01–5 mM). (○) control, (●) pretreatment with SNP, (□) control, (■) pretreatment with SNP. Each point represents the mean ± S.E.M. of five experiments; if not shown, the bars fall within the size of the symbols.

responses and increases in acetylcholine release in rabbit bladder smooth muscles. Treatment with tetrodotoxin significantly suppressed the electrical field stimulation-induced contractile responses and acetylcholine release, suggesting that electrical field stimulation evoked neurogenic contraction in this experiment. However, the basal release of acetylcholine after pretreatment with tetrodotoxin still remained. Greaney et al. (1993) suggested that a basal acetylcholine release could be mediated by injuries which may result from insertion of the microdialysis probe. These findings indicate that basal release of acetylcholine is not neurogenic, and are consistent with our previous reports (Inadome et al., 1998b).

In the present study, pretreatment with sodium nitroprusside, a NO donor, did not have any effect on carbachol-induced contractile responses. L-NNA increased the release of acetylcholine induced by electrical field stimulation at 5 Hz, and L-arginine, the precursor for the formation of NO, reduced the release of acetylcholine. Moreover, pretreatment with sodium nitroprusside in the presence or absence of L-NNA caused a significant decrease in the release of acetylcholine induced by electrical field stimulation at 5 Hz. These results suggest that NO does not have an effect on postjunctional muscarinic receptor-induced contractions in rabbit bladder smooth muscles, and that NO prejunctionally exerts an inhibitory action on acetylcholine release from cholinergic nerve endings. Wiklund et al. (1993a) and Kilbinger and Wolf (1994) reported similar results for guinea-pig ileum and mesenteric plexus. In the present contraction study, pretreatment with L-NNA enhanced the electrical field stimulation (5 Hz)-induced contractile response and addition of L-arginine suppressed the response. In contrast, pretreatment with sodium nitroprusside in the absence or presence of L-NNA reduced the electrical field stimulation (5 Hz)-induced contractile response. These findings are similar to reports demonstrating that inhibition of NO synthase enhanced the electrical field stimulation-induced cholinergic contractions of guinea pig ileum and taenia coli (Wiklund et al., 1993b; Knudsen and Tøttrup, 1992), and of stomach preparations of rat and rabbit (Lefebvre et al., 1992; Baccari et al., 1993). Thus, our data support a prejunctional modulation by endogenous NO of acetylcholine release from cholinergic nerve endings in rabbit bladder smooth muscles.

It has been reported that the effects of NO synthase inhibitors on the release of acetylcholine vary among tissues and species (Kilbinger, 1996). In the isolated trachea of man and guinea-pig (Ward et al., 1993; Brave et al., 1991), NO synthase inhibitors fail to affect acetylcholine release while, in the rat trachea (Sekizawa et al., 1993), NO synthase inhibitors increase the electrical field stimulation-induced acetylcholine release. Furthermore, it has been reported that treatment with a NO synthase inhibitor caused a decrease in the electrical field stimulation-induced acetylcholine release from the guinea-pig gastric fundus (Sotirov et al., 1999). It remains unclear whether this variability is due to differences in stimulation conditions, tissues or species, and further investigations are required in this regard.

In the present experiments, the pretreatment with L-NNA had no significant effects on the electrical field stimulation (20 Hz)-induced acetylcholine release and contractile response of rabbit bladder smooth muscles. The reason for the difference in reaction between high (20 Hz) and low (5 Hz) frequencies is not clear. In our previous study (Inadome et al., 1998b), the maximum contractile response induced by electrical field stimulation was observed at 20 Hz, while acetylcholine release reached a maximum at 40 Hz in rabbit bladder smooth muscles. In the present experiment, we did not evaluate the effects of L-NNA on the contractile responses induced by 20 Hz, which was the maximum contractile response. However, treatment with L-NNA also had no significant effect on 20 Hz-induced acetylcholine release, which was about 80% of the maximum release at 40 Hz. On the basis of these findings, it is suggested that a NO-mediated mechanism inhibiting acetylcholine release and acetylcholine-induced contractile response plays a role mainly in the low-frequency, rather than in the high-frequency stimulation. It has been shown that inhibition of NO production by NO synthase inhibitors causes bladder hyperactivity and decreased bladder capacity in the filling phase of micturition in the rat bladder (Andersson, 1993; Persson et al., 1992). Thus, the present results may suggest that NO has an important role as a neuromodulator inhibiting bladder overactivity and preserving bladder capacity by suppressing acetylcholine release.

With regard to nerve-mediated urinary bladder contractions, it is generally accepted that, in addition to the cholinergic contribution, an atropine-resistant, non-adrenergic, non-cholinergic neural component exists in most mammalian bladder smooth muscle. The exact mechanism of the atropine-resistant contraction in electrical field stimulation is not clear. Several neurotransmitters are considered to be responsible for the non-cholinergic portion of bladder smooth muscle contractions. It has been reported that ATP is the most plausible non-cholinergic neurotransmitter (Burnstock, 1996; Hoyle et al., 1989). In the present study, treatment with atropine decreased the electrical field stimulation-induced contractile responses by 40–60% of the control contractions. The combination of atropine and purinergic desensitization by α,β-methylene ATP inhibited almost all the contractile responses induced by electrical field stimulation. The results are similar to previous reports (Yokota and Yamaguchi, 1996), and suggest that ATP is a main non-cholinergic neurotransmitter in the atropine-resistant part of the contraction in rabbit bladder smooth muscles. In the present study, treatment with L-NNA (specific NO synthase inhibitor) had no significant effects on the atropine-resistant part of electrical field stimulation-induced contraction. Furthermore, sodium nitroprusside (a NO donor) did not have a significant effect

on concentration–response curves to ATP and the atropine-resistant part of the contraction induced by electrical field stimulation. These findings could suggest that NO does not affect the ATP release from nerve endings or ATP-induced contractile responses in rabbit bladder smooth muscles.

In conclusion, the present study demonstrated that a NO synthase inhibitor caused significant increases and that a NO donor caused a significant decrease in the acetylcholine release and the contractile response induced by electrical field stimulation in rabbit bladder smooth muscles. The results suggest that there is an inhibitory mechanism of acetylcholine release from cholinergic nerve endings which is modulated by endogenous NO. NO may have an important role as a neuromodulator inhibiting bladder contraction.

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