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Direct measurement of acetylcholine release in detrusor smooth muscles isolated from rabbits

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Abstract In the present study, we measured acetylcholine (ACh) released from rabbit detrusor smooth muscle strips induced by electrical field stimulation (EFS) using high-performance liquid chromatography coupled with microdialysis procedure. There were frequency- and duration-dependent increases in contractile response and ACh release. There was a significant, but not simple correlation between EFS-induced contraction and ACh release. Atropine caused a decrease and increase in the contractile response and ACh release, respectively. Pretreatment with propranolol increased ACh release, but pretreatment with phentolamine had no significant effect. These results demonstrate that this method is applicable to direct measurement of ACh release by EFS, and that neurotransmitters other than ACh may relate to EFS-induced contraction. In addition, it is suggested that there are prejunctional inhibitory muscarinic receptors and beta-adrenoceptors, which contribute to ACh release induced by EFS in the rabbit detrusor smooth muscles.

Key words Acetylcholine · Detrusor smooth muscle · Microdialysis · High performance liquid chromatography · Non-adrenergic non-cholinergic neurotransmitters · Prejunctional receptor

Introduction

The parasympathetic nervous system plays an important role in the function of the lower urinary tract [10, 17]. A major neurotransmitter for physiological bladder contraction is acetylcholine (ACh) released from prejunctional parasympathetic nerve endings. In the isolated detrusor smooth muscle, ACh released from parasympathetic nerve has been mainly determined by gas chromatography [7], radioimmunoassay [23, 24], and radioisotope-labeled ACh [2, 9, 10, 27, 28, 30, 32]. However, it has been reported that these methods have significant problems in accuracy, reproductibility and simplicity. Recently, the utility of high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) coupled with a microdialysis procedure has been reported for the determination of ACh in brain [11, 21, 31, 35]. HPLC with ECD has high selectivity for ACh and choline measurement in biological samples due to the column separation and use of an enzyme-immobilized column [13]. Furthermore, microdialysis is a recently introduced and rapidly accepted sampling technique, which is useful in a wide variety of sampling situations [18, 19]. However, there is little information yet available on the measurement of ACh released from isolated urinary tract smooth muscle using this method. Therefore, in the present study, we have attempted to measure ACh release induced by electrical field stimulation (EFS) in rabbit detrusor smooth muscles using an HPLC-ECD system coupled with microdialysis. In addition, we investigated the presence of prejunctional muscarinic and adrenergic receptors, which contribute to ACh release in cholinergic nerve endings.

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Materials and methods

Tissues

Four-month-old female New Zealand white rabbits, weighing 2.0–2.5 kg, were anesthetized with 30 mg/kg sodium pentobarbital and killed by exsanguination. The bladder was removed and 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. The average weight of the muscle strips was 0.12 ± 0.01 g ($n = 56$).

Microdialysis

The microdialysis methods are summarized in Fig. 1. The dialysis probe (O-P-100-10, Eicom, Kyoto, Japan) had a 0.22 mm × 10 mm

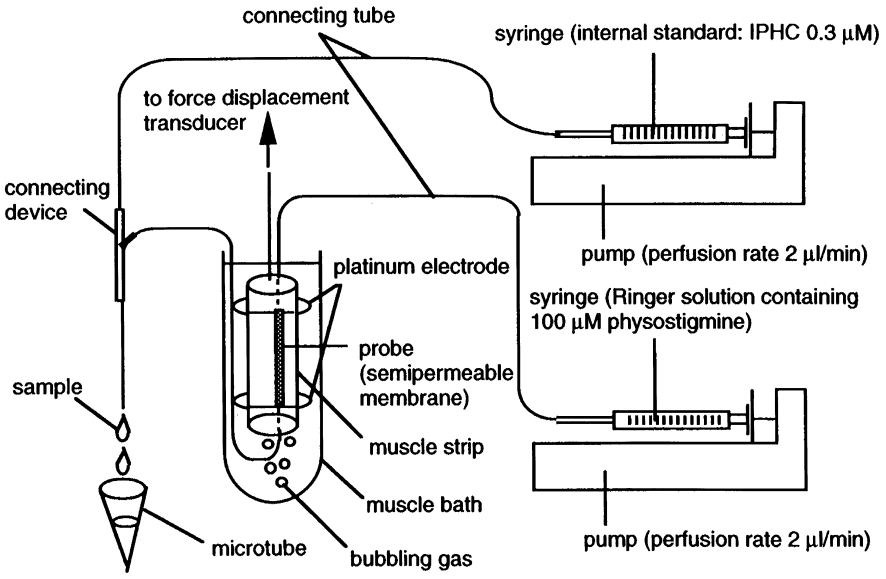

Fig. 1 Experimental set up of the microdialysis procedure

dialysis membrane of regenerated cellulose with a molecular cutoff of 50 kDa and platinum strings (0.02 mm OD) were mounted in the dialysis membrane for protection of the membrane. The probe was inserted through the muscle strip and the inlet cannula of the probe was connected to a microinfusion syringe pump (EP-60, Eicom). Ringer solution (NaCl, 147 mM; KCl, 4 mM; $CaCl_2$, 2.3 mM; pH adjusted to 7.4 with NaOH) containing 100 μM physostigmine sulfate was continuously perfused at a rate of 2 μl/min. In the present experiment, the internal standard, isopropylhomocholine (IPHC), 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/m. The strip was suspended in a 20-ml bath filled with modified Krebs-Henseleit (K-H) solution (NaCl, 117.7 mM; KCl, 4.69 mM; $CaCl_2$, 2.16 mM; $MgSO_4$, 1.20 mM; $NaHCO_3$, 24.39 mM; KH_2PO_4, 1.2 mM; glucose, 9.99 mM; 37°C at pH 7.4, gassed with 95% O_2–5% CO_2). Each muscle preparation was connected to a force displacement transducer (TB-611T; Nihon Kohden, Tokyo, Japan) and isometric forces were recorded and monitored on electronic pen recorder (R-02 A; 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 starting the experiment. Sampling was started 10 min before stimulation and dialysate was collected in a microtube every 10 min. Ten microliters of the dialysate of each sample were injected into the ACh determination system.

Electrical field stimulation

EFS 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 100, 200, 500, and 800 μs duration, respectively, and at a stimulation frequency of 2, 5, 10, 20, 30 and 40 Hz on each duration. Trains of pulses lasted for 2 s and an interval between stimulations of 2 min was observed. With every frequency, five muscle contractions were induced by shocks with an interval between them of 20 min.

Acetylcholine determination

The ACh determination by a combination of HPLC, enzyme reaction and ECD was performed as previously described with only slight modifications [11, 21, 31, 35]. In brief, a solution that consisted of 0.1 M Na_2HPO_4, pH 8.5, 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. Ten microliters of the samples collected were immediately injected into the column of the HPLC assay system by syringe loading sample injector (Model 7725; Eicom). After separation by a styrene polymer column (AC-GEL, Eicom), ACh was converted to hydrogen peroxide by a post-column enzyme reactor (AC-ENZYMEPAK, Eicom) with immobilized acetylcholinesterase (AChE) and choline oxidase. The separation column and post-column reactor were controlled isothermally at 33°C. The hydrogen peroxide was detected with an ECD system (ECD-300, Eicom) equipped with a platinum electrode. The electrode potential was set to +450 mV against an Ag/AgCl reference electrode. The amount of ACh was calculated by reference to the peak area of the standard ACh solution by a chromatogram recorder (Chromatocorder 21, System Instruments, Tokyo, Japan). ACh release during EFS was expressed in terms of amount of ACh released during EFS/weight of the strip (pmol/g).

Effect of various drugs

Two series of EFS (supramaximum voltage, 500 μs duration, 20 Hz frequency, and 2 s train) were performed at 1 h intervals. Atropine (10^{-8}–10^{-6} M), phentolamine (10^{-6} M) and propranolol (10^{-6} M) were treated 15 min before the second stimulation period, and ratios of the second/first stimulation in ACh release and contractile response were calculated.

Drugs

The following pharmacological agents were used: acetylcholine chloride, choline chloride, atropine sulfate, phentolamine hydrochloride, DL-propranolol hydrochloride and tetrodotoxin (Sigma Chemical, Tokyo, Japan); isopropylhomocholine (Eicom); sodium 1-decanesulfonate (Tokyo Kasei Kogyo, Tokyo, Japan); tetramethylammonium chloride (Nacalai Tesque, Kyoto, Japan); physostigmine sulfate (Aldrich Chemical Tokyo, Japan). All drugs were dissolved in distilled water.

Data analysis

Statistical analyses between groups were performed using analysis of variance (ANOVA) and the multiple comparison test (Fisher's test). The correlation between ACh release and contraction of the bladder muscle strips was performed using Pearson's correlation coefficient. P values of 0.05 or less were regarded as statistically significant.

Results

Chromatography

Figure 2 shows typical chromatograms for spontaneous release (before stimulation), stimulation (800 μs duration and 40 Hz frequency) and stimulation after pretreatment with 10^{-6} M of tetrodotoxin. The IPHC concentration in each sample was 1.5 pmol as an internal standard. In each chromatogram, both ACh and choline peaks were recognized, and the respective substance was well separated. In almost all samples, choline, the precursor and metabolite of ACh, was outside the limits of determination. The detection limits of ACh were about 0.02 pmol/injection. The sensitivity of the assay did not vary significantly day to day. Assays were repeated on samples frozen for at least 3 months. There was not detectable loss of activity in the period (data not shown).

Effect of EFS on contraction of muscle strip and acetylcholine release

The maximum contractile responses induced by 80 mM KCl and EFS (800 μs duration and 40 Hz frequency) in the rabbit detrusor muscle strip with microdialysis probe were 3.89 ± 0.40 g ($n = 6$) and 3.67 ± 0.38 g ($n = 6$), respectively, which were not significantly different from values in the strips without probe. There was no significant difference between KCl and EFS-induced contractions. We evaluated the contractility of the muscle strip induced by EFS before and after perfusion with 100 μM physostigmine. A tendency for a prolonged contractile response in the presence of physostigmine was observed; however, frequency response curves before and after perfusion with 100 μM physostigmine were similar. Figure 3A shows the frequency-response curve in each duration. EFS of rabbit detrusor strips caused a frequency-dependent contraction. The maximum contractile response was observed at about 20 Hz. There was no significant difference in frequency-response curves between durations of 500 and 800 μs. After pretreatment with 10^{-6} M of tetrodotoxin in the bath, EFS no longer elicited a contractile response in each strip.

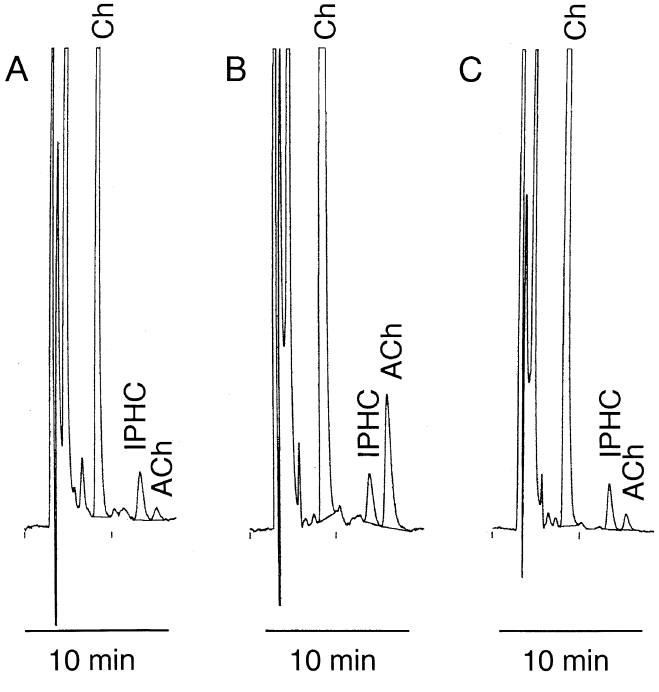

Fig. 2 Chromatograms of ACh, choline and IPHC in the dialysate. (A) Dialysate in the spontaneous (before stimulation) period. (B) Dialysate in the stimulation period (at 40 Hz frequency on 800 μs duration). (C) Dialysate in the stimulation period after pretreatment with 10^{-6} M of tetrodotoxin in the bath

The value for spontaneous ACh released from muscle strips before stimulation was 2.72 ± 0.23 pmol/g ($n = 32$). EFS produced frequency-dependent ACh release in each duration (Fig. 3B). There were duration-dependent increases in EFS-induced contraction and ACh release. There were some differences between frequency-response curve of contraction (Fig. 3A) and ACh release (Fig. 3B). In the contractile response, the maximum contractions were observed at 20 Hz in almost all durations, while ACh releases reached the maximum at 40 Hz in all durations. After pretreatment with 10^{-6} M of tetrodotoxin in the bath, the ACh release induced by EFS was significantly suppressed to the spontaneous level, but still remained (Fig. 4).

Figure 5 is a scattergram of the contractile responses and ACh releases induced by EFS in all frequencies and durations in rabbit detrusor smooth muscle strips ($n = 192$). There was a significant correlation between contractile response and ACh release ($Y = -2.894 + 5.034x - 0.881x^2$, $r = 0.740$, $P < 0.0001$, $n = 192$).

Effect of various drugs on contraction of muscle strip and acetylcholine release

The EFS-induced contractile response and ACh release in control experiments ($n = 8$) in the absence of drugs were 4.15 ± 0.42 g, 8.62 ± 0.69 pmol/g in the first stimulation period and 4.30 ± 0.41 g, 8.27 ± 0.69 pmol/g in the second stimulation period, respectively. There were no significant differences in contractile response and ACh release between the first and second stimulation period. Second/first ratios in contractile response and ACh release were 104.5 ± 2.1% (Table 1) and 95.8 ± 3.0% (Table 2), respectively, which served as controls.

Treatments with atropine (10^{-8}–10^{-6} M), phentolamine (10^{-6} M) and propranolol (10^{-6} M) did not affect the spontaneous ACh release. The presence of atropine (10^{-8}–10^{-6} M) caused a dose-dependent increase in ACh release, and 10^{-6} M of atropine significantly increased ACh release (Fig. 6B). On the other hand, atropine caused a dose-dependent inhibition of the contractile

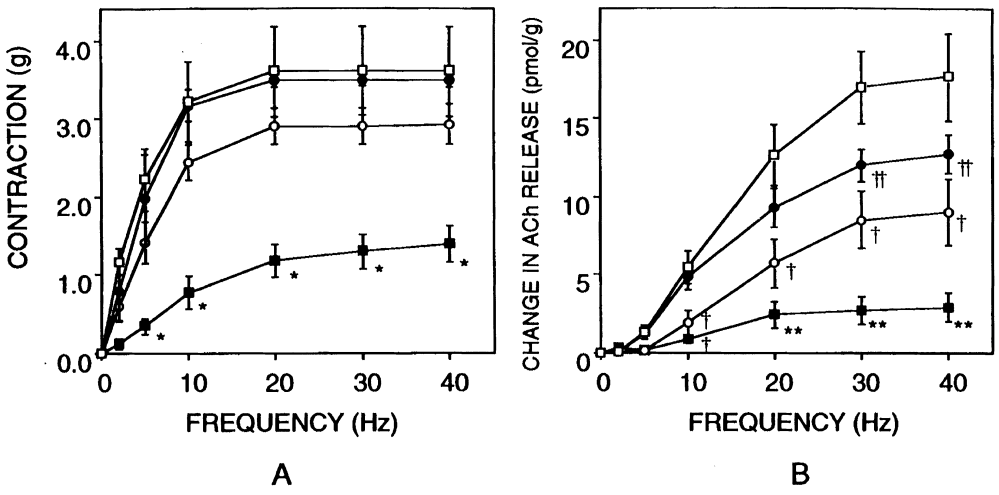

Fig. 3 (A) Frequency response curves on various durations in rabbit detrusor smooth muscles. (B) Change in ACh release induced by electrical field stimulation (EFS) from spontaneous release on various durations in rabbit detrusor smooth muscles. (*filledsquares*: 100 μs, $n = 8$; *open circiles*: 200 μs, $n = 8$; *filled circles*: 500 μs, $n = 8$; *open squares*: 800 μs, $n = 8$). Each point represent the mean ± SE; if not shown, SE bars fall within the size of the symbols used. *,**Significantly different from comparable values for 200, 500 and 800 μsec duration ($P < 0.05$). † Significantly different from comparable values for 500 and 800 μs duration ($P < 0.05$). †† Significantly different from comparable values for 800 μs duration ($P < 0.05$)

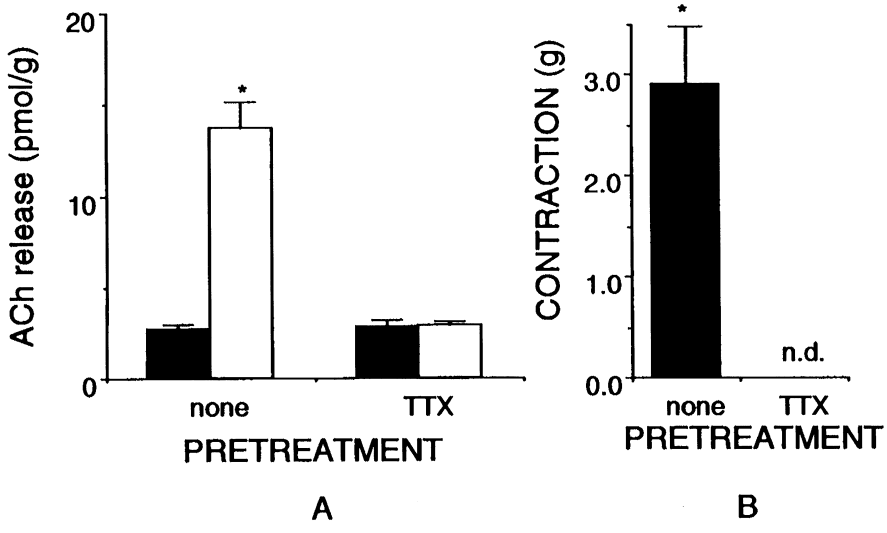

Fig. 4 The effect of pretreatment with 10^{-6} M of tetrodotoxin on ACh release (A, closed histogram: spontaneous release of ACh; open histogram: ACh release induced by EFS) and contractile response (B) in rabbit detrusor smooth muscle (n.d.: not detected). *Significantly different from comparable values for TTX pretreatment ($P < 0.01$). EFS (supramaximum voltage, 500 μs duration, 40 Hz and 2 s train) was delivered five times with an interval between stimulations of 2 min

response (Fig. 6A). Phentolamine (10^{-6} M) did not cause any significant changes in both ACh release and the contractile response, while propranolol (10^{-6} M) caused a significant increase in ACh release, but did not cause a change in the contractile response (Tables 1, 2).

Discussion

This communication is the first report to evaluate an HPLC-ECD technique coupled with microdialysis for measurement of ACh release in urinary tract smooth muscle. There have been several reports regarding the determination of ACh released from the detrusor smooth muscle strips with radioisotope-labeled ACh in some species [2, 9, 10, 27, 28, 30, 32]. However, this method has significant limitations. One of the most important limitations is that all neural compartments containing releasable ACh are not labeled with tritium equally. Therefore, changes in the amount of radiolabel released may not accurately reflect changes in the release of all ACh [4]. HPLC-ECD is extremely rapid and relatively simple, permitting the processing of multiple samples within minutes, and has excellent reproductibility, specificity and sensitivity [22]. Also, one of the advantages of HPLC-ECD is thought to be simultaneous measurement of choline as well. Toide and Arima reported that the simultaneous measurement of ACh and choline could evaluate the dynamic phenomena of ACh release and choline uptake at cholinergic nerve endings in rat brain. Recently there have been reports suggesting that HPLC-ECD may be sufficiently sensitive to detect ACh release from nerve endings in tracheal smooth muscle [4, 8, 34], and ileal longitudinal smooth muscle [26]. Thus, we applied HPLC-ECD coupled with microdialysis for the measurement of ACh release from nerve endings in rabbit detrusor smooth muscle in the present study. The detection limit of ACh in our study

was 0.02 pmol/injection, which was almost same level as that reported in the recent studies [14, 21].

Microdialysis has developed in the past two decades, and has been used mainly for the quantitation of endogenous compounds such as amino acids, amines and hormones [3, 5, 18–20]. In a recent study, we applied this procedure to measure nitric oxide induced by EFS in rabbit urethral smooth muscle strips [29]. Several investigators have used this procedure in vivo, especially in brain, for collecting samples in the measurement of ACh [11, 21, 31, 35]. In order to detect ACh, there are necessarily three basic approaches: perfusion with a dialysate containing high levels of acetylcholinesterase inhibitor (AChEI), perfusion with a dialysate containing elevated Ca^{2+} levels, and the use of microdialysis probes with an enlarged membrane surface area [14]. Recently, several publications have addressed the validity of including AChEI in the perfusion medium [11, 14, 31]. It was suggested that the perfusion levels of AChEI should be minimized if physiologically relevant data is to be obtained. However, since the activity of AchE on the synaptic membrane of cholinergic neurons is very high, released ACh is rapidly hydrolyzed. Perfusion without AChEI in the perfusate failed to demonstrate any detectable ACh in the dialysate. Quantitation of ACh was possible only when AChEI was added to the perfusion solution. In the present study, we used perfusate containing a relatively high concentration of physostigmine sulfate (100 μM). Damsma et al. [11] have reported that because of the limited dialysis efficiency, the physostigmine concentration in the surrounding fluid will be at least one order of magnitude lower than in the perfusion fluid [11], and the concentration does not have significant effects on smooth muscle contractions. In the present experimental conditions, a tendency for a pro-

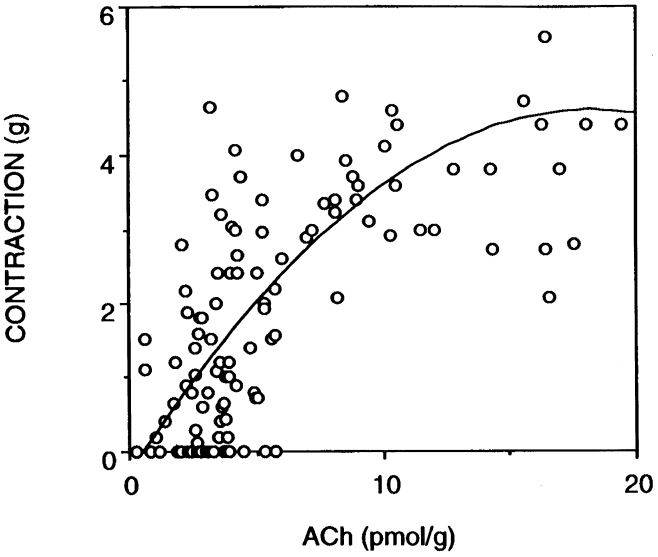

Fig. 5 The correlations between ACh release and contractile response induced by EFS in rabbit detrusor smooth muscle strips. EFS (supramaximum voltage and 2 s train) was delivered at all frequencies (2, 5, 10, 20, 30 and 40 Hz) on all durations (100, 200, 500 and 800 μs)

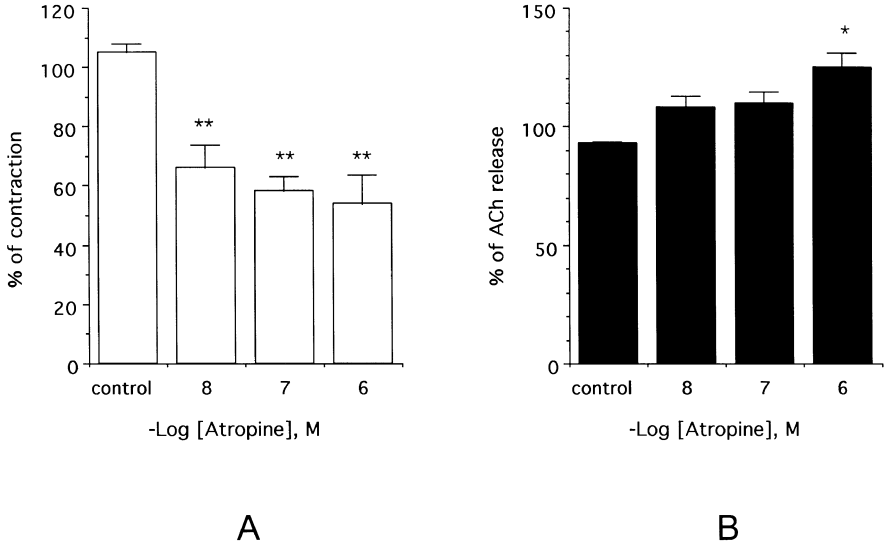

Fig. 6 The effect of pretreatment with atropine on ACh release (A) and contractile response (B) in rabbit detrusor smooth muscle ($n = 6$). *,**Significantly different from comparable values for control experiment (*$P < 0.05$, **$P < 0.01$)

Table 1 Effect of drugs on contractile response in rabbit detrusor smooth muscle strips. The concentration of each drug was 10^{-6} M in the final bath concentration

Drug	*n*	First stimulation (g)	Second stimulation (g)	Second/first ratio (%)
None (control)	8	4.15 ± 0.42	4.30 ± 0.41	104.5 ± 2.1
Atropine	6	4.53 ± 0.49	2.43 ± 0.36*	53.7 ± 6.3*
Phentolamine	6	3.93 ± 0.72	4.08 ± 0.75	104.1 ± 4.6
Propranolol	6	4.07 ± 0.55	4.30 ± 0.61	105.4 ± 2.2

* Significantly different from comparable values for control experiment (*$P < 0.01$)

Table 2 Effect of drugs on ACh release in rabbit detrusor smooth muscle strips. The concentration of each drug was 10^{-6} M in the final bath concentration

Drug	*n*	First stimulation (pmol/g)	Second stimulation (pmol/g)	Second/first ratio (%)
None (control)	8	8.62 ± 0.69	8.27 ± 0.69	95.8 ± 3.0
Atropine	6	7.60 ± 1.75	9.08 ± 2.29	120.5 ± 9,0*
Phentolamine	6	8.21 ± 0.82	8.80 ± 0.94	107.3 ± 7.6
Propranolol	6	8.80 ± 1.49	10.51 ± 2.18	117.5 ± 9.6*

* Significantly different from comparable values for control experiment (*$P < 0.05$)

longed contractile response in the presence of physostigmine was observed; however, there was no significant change between frequency-response curves before and after perfusion with 100 μM physostigmine. As a high Ca^{2+} level in dialysate may affect the contractility of detrusor smooth muscle, we chose the perfusate containing 2.3 mM Ca^{2+}. The concentration is not significantly different from the Ca^{2+} concentration of extracellular fluid (2.5 mM). In addition, Ringer solution was widely used as the perfusate of microdialysis experiments in several tissues [1, 21, 31]. The microdialysis probe used in the present study has adequate membrane surface area, and recent studies [18, 19] have confirmed its usefulness. Compared with the previous studies that did not use the microdialysis method [4, 8, 34], we were able to collect samples containing a high concentration of ACh. Thus, a small amount of the sample volume (10 μl) was enough for detection of ACh in the present study.

In the present study, the contractile response induced by KCl and EFS did not significantly change during microdialysis probe insertion, suggesting that insertion of the probe into the muscle strips may not influence to the muscle contractility. The EFS caused a frequency-dependent contraction in the rabbit detrusor strips and the maximum contractile response reached at about 20 Hz. In addition, tetrodotoxin (10^{-6} M) completely suppressed the EFS-induced contraction and ACh release, suggesting that EFS evokes pure neurogenic contraction. On the other hand, the spontaneous release of ACh after pretreatment with tetrodotoxin still remained. These findings imply that the spontaneous release of ACh was not neurogenic. Greaney et al. [14] suggested that ACh release could be mediated by injuries, which may be caused by insertion of the microdialysis probe or muscle stretching.

The amount of ACh release and the muscle contraction induced by EFS did not show a simple correlation. Although we could not explain this precisely, it was speculated that the contractility of muscle strip reached the maximum at about 20 Hz, or ACh released by 20 Hz EFS activated all ACh receptors contributing to contraction. In addition, the data may imply that neurotransmitters other than ACh contribute to contraction induced by EFS in rabbit bladder. In fact, ACh is not the sole neurotransmitter related to bladder smooth muscle contraction, and it has been reported that there is an atropine-resistant part of contraction induced by EFS in animal and human bladders [12, 25, 33]. Several authors suggested that a major portion of the atropine-resistant contractions was mediated by non-adrenergic non-cholinergic (NANC) neurotransmitters, including adenosine triphosphate (ATP), prostaglandins, or vasoactive intestinal polypeptide [6, 15, 16]. It has been frequently reported that ATP is a possible NANC neurotransmitter in bladder contraction. In the present study, there were some differences between frequency response curves of contraction and ACh releases. In the contractile response, the maximum contractions were observed at 20 Hz in almost all durations, while ACh releases reached the maximum at 40 Hz in all durations. This fact may imply that NANC neurotransmitters contribute more to the contraction induced by low frequency stimulation than by high frequency stimulation. Downie and Dean [12] suspected that the NANC component contributing to contraction induced by EFS is greater at low frequencies than at high frequencies, which is consistent with the present data.

Recently, it has been reported that prejunctional muscarinic and adrenergic receptors contributed to the neuronal release of ACh induced by EFS in detrusor smooth muscle strips in some species [2, 9, 10, 28, 30]. In the present study, we evaluated the effects of atropine, phentolamine and propranolol on ACh release in rabbit detrusor smooth muscle. Although treatment with atropine inhibited the contractile response, it increased ACh release. The data suggest that the muscarinic receptors are located pre- and postjunctionally, and that prejunctional muscarinic receptors have relatively inhibitory actions on ACh release in rabbit detrusor smooth muscle. Recently, Tobin and Sjögren [30] reported that there were muscarinic prejunctional facilitatory (M_1) and inhibitory (M_2) receptors for ACh release in rabbit detrusor smooth muscle, using radioisotope-labeled ACh. In the present study, although phentolamine had no effects on ACh release, propranolol caused an increase in ACh release. The results suggest that there are prejunctional inhibitory beta-adrenoceptors for ACh release in rabbit detrusor smooth muscles. However, propranolol did not cause any significant change in the contractile response. It was speculated that the contractility of muscle strip reached the maximum, and that increased ACh release by pretreatment with propranolol did not affect to the contractile response. The effects of prejunctional adrenoceptors on ACh release were only studied in the guinea pig urinary bladder [2]. The report evaluated the effects of several adrenergic agonists and antagonists on ^{3}H-ACh release, and concluded that ^{3}H-ACh release

was under slight control of prejunctional alpha-adrenoceptors but not regulated by beta adrenoceptors. It is suggested that the difference from the present study may be due to differences in experimental animals and conditions, however further study will be needed to clarify the role of prejunctional adrenoceptors on ACh release.

In conclusion, the present study demonstrates that this method is applicable to the direct measurement of ACh released by EFS, and suggests that a neurotransmitter other than ACh may contribute to EFS-induced contraction in rabbit detrusor smooth muscle. In addition, the presence of prejunctional inhibitory muscarinic receptors and beta-adrenoceptors for ACh release are suggested.

References

1. Ajima A, Kato T (1987) Brain dialysis: detection of acetylcholine in the striatum of unrestrained and unanesthetized rats. Neurosci Lett 81:129
2. Alberts P (1995) Classification of the presynaptic muscarinic receptor subtype that regulates ^{3}H-acetylcholine secretion in the guinea pig urinary bladder in vitro. J Pharmacol Exp Ther 274:458
3. Awdeh ZL (1976) Dialysis of micro samples. Anal Biochem 71:601
4. Baker DG, Don HF, Brown JK (1992) Direct measurement of acetylcholine release in guinea pig trachea. Am J Physiol 263:L142
5. Brand PH, Stansbury R (1979) Improved microdialysis technique. Anal Biochem 94:109
6. Burnstock G, Dumsday B, Smythe A (1972) Atropine resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. Br J Pharmacol 44:451
7. Choo LK, Mitchelson F (1980) The effect of troxypyrrolidinium, a choline uptake inhibitor, on the excitatory innervation of the rat urinary bladder. Eur J Pharmacol 61:293
8. Colasurdo GN, Loader JN, Graves JP, Larsen GL (1995) Modulation of acetylcholine release in rabbit airways in vitro. Am J Physiol 268:L432
9. D'Agostino G, Barbieri A, Grana E, Subissi A (1993) Characterization of prejunctional muscarinic autoreceptors in the rat urinary bladder. Life Sci 52:580
10. D'Agostino G, Kilbinger H, Chiari MC, Grana E (1986) Presynaptic inhibitory muscarinic receptors modulating [^{3}H] acetylcholine release in the rat urinary bladder. J Pharmacol Exp Ther 239:522
11. Damsma G, Westerink BHC, De Vries JB, Van Den Berg CG, Horn AS (1987) Measurement of acetylcholine release in freely moving rats by means of automated intracerebral dialysis. J Neurochem 48:1523
12. Downie JW, Dean DM (1977) The contribution of cholinergic postganglionic neurotransmission to contractions of rabbit detrusor. J Pharmacol Exp Ther 203:417
13. Fujimori K, Yamamoto K (1987) Determination of acetylcholine and choline in perchlorate extracts of brain tissue using liquid chromatography-electrochemistry with an immobilized-enzyme reactor. J Chromatogr 414:167
14. Greaney MD, Marshall DL, Baily BA, Acworth IN (1993) Improved method for the routine analysis of acetylcholine release in vivo: quantitation in the presence and absence of esterase inhibitor. J Chromatogr 622:125
15. Gu J, Restrick JM, Blank MA, Huang WM, Polak JM, Bloom SR, Mundy AR (1983) Vasoactive intestinal polypeptide in the normal and unstable bladder. Br J Urol 55:645
16. Johns A, Paton DM (1976) Evidence for a role of prostaglandins in atropine-resistant transmission in the mammalian urinary bladder. Prostaglandins 11:595
17. Khanna OP, Barbieri EJ, Altamura M, McMichael R (1981) Vesicourethral smooth muscle: function and relation to structure. Urology 18:211
18. Kurata N, Inagaki M, Iwase M, Nishimura Y, Kiuchi Y, Yamazaki Y, Kobayashi S, Oguchi K, Uchida E, Yasuhara H (1995) Pharmacokinetic study of trimethadione and its metabolite in blood, liver and brain by microdialysis in conscious, unrestrained rats. Molec Pathol Pharmacol 89:45
19. Kurata N, Inagaki M, Kobayashi S, Nishimura Y, Oguchi K, Yasuhara H (1993) Antypyrine concentrations in liver and blood monitored by microdialysis of unrestrained conscious rats, Chem Pathol Pharmacol 79:363
20. Lau CK, Fujitaki JM (1981) An improved method of microdialysis, Anal Biochem 110:144
21. Mizuno T, Endo Y, Arita J, Kimura F (1991) Acetylcholine release in the rat hippocampus as measured by the microdialysis method correlates with motor activity and exhibits a diurnal variation. Neuroscience 44:607
22. Potter PE, Meek JL, Neff NH (1983) Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J Neurochem 41:188
23. Shinkai M, Takayanagi I, Kato T (1991) Facilitatory effect of tachykinins and guanethidine on the acetylcholine output stimulated by nicotine from guinea-pig bladder. Br J Pharmacol 103:1191
24. Shinkai M, Takayanagi I, Kato T (1993) Tachykinin receptors of the NK2 type involved in the acetylcholine release by nicotine in guinea pig bladder. Br J Pharmacol 108:759
25. Sibley GNA (1984) A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J Physiol 354:431
26. Soejima O, Katsuragi T, Furukawa T (1993) Opposite modulation by muscarinic M_1 and M_3 receptors of acetylcholine release from guinea pig ileum as measured directly. Eur J Pharmacol 249:1
27. Somogyi GT, De Groat WC (1993) Modulation of release of [^{3}H] acetylcholine in the major pelvic ganglion of the rat. Am J Physiol 264:R1084
28. Somogyi GT, Tanowitz M, De Groat WC (1994) M1 muscarinic receptor-mediated facilitation of acetylcholine release in the rat urinary bladder. J Physiol 480:81
29. Takahashi W, Yoshida M, Wada Y, Goto S, Inadome A, Yono M, Ueda (1997) Effect of estrogen on nitric oxide-induced relaxation of the rabbit urethra. Eur J Pharmacol 339:165
30. Tobin G, Sjögren C (1995) In vivo and in vitro effect of muscarinic receptor antagonists on contractions and release of [^{3}H] acetylcholine in the rabbit urinary bladder. Eur J Pharmacol 281:1
31. Toide K, Arima T (1989) Effects of cholinergic drugs on extracellular levels of acetylcholine and choline in rat cortex, hippocampus and striatum studied by brain dialysis. Eur J Pharmacol 173:133
32. Tran LV, Somogyi GT, De Groat WC (1994) Inhibitory effect of neuropeptide Y on adrenergic and cholinergic transmission in rat urinary bladder and urethra. Am J Physiol 266:R1411
33. Wada Y, Yoshida M, Kitani K, Kikukawa H, Ichinose A, Takahashi W, Gotoh S, Inadome A, Machida J, Ueda S (1995) Comparison of the effect of various anticholinergic drugs on human isolated urinary bladder. Arch Int Pharmacodyn 330:76
34. Wang Z, Robinson NE, Yu M (1993) ACh release from horse airway cholinergic nerves: effect of stimulation intensity and muscle preload. Am J Physiol 264:L269
35. Watanabe H, Shimizu H, Matsumoto K (1990) Acetylcholine release detected by trans-striatal dialysis in freely moving rats correlates with spontaneous motor activity. Life Sci 47:829