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ORIGINAL ARTICLE

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The role of cholinesterases in rat urinary bladder contractility

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Abstract This study examines the effects of inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) on acetylcholine (ACh)-induced contraction in rat urinary bladder smooth muscle. Neostigmine, a non-selective ChE inhibitor, caused concentration-dependent contractions in rat urinary bladder strips, whereas tetraisopropylpyrophosphoramide (iso-OMPA; a BuChE inhibitor) failed to affect the resting tone of the preparations. Neostigmine (1 μM) markedly augmented the contractile responses to ACh. Although iso-OMPA (10 μM) also potentiated ACh-induced contraction, the effect was less than that evoked by neostigmine. The activities of AChE in rat urinary bladder strips were significantly ($P < 0.05$) higher than those of BuChE. These results indicated that AChE, rather than BuChE, plays an important role in controlling ACh-induced contractions of rat urinary bladder.

Keywords Acetylcholine · Acetylcholinesterase · Butyrylcholinesterase · Urinary bladder

Introduction

The parasympathetic nervous system plays a critical role in regulating urinary bladder function [10]. Activation of the parasympathetic nerve causes the release of acetylcholine (ACh), which in turn leads to a muscarinic receptor-mediated contraction that empties the bladder. Cholinesterase (ChE) inhibitors have been shown to potentiate the contractile responses to exogenous ACh or to electrical field stimulation of urinary bladder preparations [7, 10, 11, 12, 13]. This implies that ChE activity plays an important role in controlling ACh-induced contraction of the urinary bladder. Although two different ChEs have been described in many tissues, namely, acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BuChE; EC 3.1.1.8), no reports have distinguished between the relative contributions of the different ChEs involved in the contractions to muscarinic receptor agonists in the urinary bladder. In airway smooth muscles and pulmonary blood vessels, not only AChE but also BuChE play an important role in controlling ACh-induced responses [1, 2, 3, 4, 9, 14]. Because urinary bladder functions, like respiratory functions, are regulated by the parasympathetic nervous system, we hypothesized that both AChE and BuChE might control ACh-induced contraction of the bladder.

To examine this hypothesis, we evaluated the effects of neostigmine (a non-selective ChE inhibitor) and tetraisopropylpyrophosphoramide (iso-OMPA, a BuChE inhibitor) on contractile responses to ACh in the rat urinary bladder. We also determined the activities of AChE and BuChE.

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Materials and methods

Preparation of rat urinary bladder strips

Male Wistar rats weighting 250–290 g were maintained on standard rat chow and tap water at libitum with 12:12 h light:dark cycles in a quiet environment. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The urinary bladder was removed and placed in ice-cold Krebs bicarbonate buffer (KRB, composition in mM: NaCl, 119; KCl, 4.8; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25 and glucose, 10.0) (pH 7.4). Four longitudinal strips of approximately 2 mm×10 mm were isolated from the bladder body. These were used for the measurement of mechanical responses and of ChE activity.

Measurement of mechanical activity

One end of each strip was attached to an isometric force displacement transducer (model TB-611T, Nihon Kohden, Tokyo, Japan) by a cotton thread, and the other end was tied to a stainless steel holder. Tension was digitized at a sampling rate of 2 Hz with the use of a 12-bit analog-to-digital converter [model

AD12–8(PM), Contec, Osaka, Japan] interfaced with a dedicated laboratory computer system (PC9821 Nr150, NEC, Tokyo, Japan). Strips were mounted in 20-ml jacketed organ baths filled with KRB gassed with 95% O_2-5% CO_2 at 37°C. The preparations were placed under initial load (1 g) and the resting tension was adjusted every 15 min. The tissues were allowed to equilibrate for 90 min and the bath fluid was changed every 15 min with fresh KRB solution.

After this, all preparations were contracted with ACh (100 μM). The tissues were washed with fresh KRB solution and allowed to return to their resting tone. When the resting tone was established, two different protocols were followed. In the first protocol, each ChE inhibitor (neostigmine or iso-OMPA) was added to the bath fluid at 10 min intervals in a cumulative fashion, beginning with the lowest concentration. In the second protocol, the preparations were incubated for 10 min in KRB solution containing either neostigmine (1 μM) or iso-OMPA (10 μM), and subsequently a concentration-response curve to ACh was determined.

ChE activity in intact tissues

Each tissue was equilibrated for 60 min in an organ chamber that was warmed to 37°C and filled with 5 ml KRB. The buffer was continuously bubbled with a mixture of 95% O_2-5% CO_2 and was changed every 10 min. After equilibration, the activities of AChE and BuChE were determined at 37°C by Ellman et al.'s method [6] with a slight modification. Briefly, acetylthiocholine (0.8 mM) or butyrylthiocholine (0.8 mM) was added to the organ chamber containing an isolated bladder strip and incubated for 20 min. The entire volume of fluid in each chamber was removed to determine ChE activity by measuring the concentration of the hydrolysis product, thiocholine. An aliquot (100 μl) of 10 mM 5,5′-dithio-bis(2-nitrobenzoic acid) was added to a 900-μl sample of tissue chamber fluid to produce thionitrobenzoate anion. Absorbance of the adduct was measured spectrophotometrically at 412 nm. To correct for nonspecific hydrolysis of the substrate, aliquots of each ChE preparation were incubated under conditions of complete inhibition of either AChE [with 100 μM 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one-dibromide (BW284c51)] or BuChE (with 100 μM iso-OMPA) and the changes in the absorbance under these conditions were subtracted from those obtained under the control conditions. The enzyme activity in urinary bladder strips was expressed as nanomoles of substrate hydrolyzed/h/mg tissue.

Data analysis and statistics

Data were expressed as the mean ± SEM. Isolated bladder strips contract spontaneously with irregular frequency and varying amplitude under the experimental conditions adopted in the present study, therefore, the average tension during a steady-state period (3 min) for each concentration was used to assess contractility. Contractile responses to neostigmine or ACh were expressed as percentages of the reference contraction induced by ACh (100 μM). The maximal contractile responses (E_{max}) and the half-maximum effective concentration values (EC_{50}) were calculated using the GraphPad Prism program (GraphPad Software, San Diego, Calif., USA). The pD_2 values were calculated as the negative logarithm of EC_{50}. Data were analyzed using Student's *t*-test. A *P* value less than 0.05 was considered significant.

Drugs

The following drugs were used: acetylcholine chloride, acetylthiocholine iodide, atropine sulfate, *S*-butyrylthiocholine, BW284c51, 5,5′-dithio-bis(2-nitrobenzoic acid), iso-OMPA, neostigmine bromide (Sigma Chemical, St. Louis, Mo., USA). Stock solutions were diluted appropriately using KRB.

Results

Effects of neostigmine and iso-OMPA

Figure 1 shows effects of neostigmine and iso-OMPA on the resting tension of rat urinary bladder preparations. Neostigmine concentration-dependently elevated the resting tension. E_{max} values were 88.7 ± 8.8% ($n = 4$) of the responses to ACh (100 μM) and pD_2 values were 5.49 ± 0.04 ($n = 4$). The tensions induced by ACh (100 μM) were 2.0 ± 0.1 g ($n = 27$). The neostigmine responses were completely reversed by atropine (1 μM) (data not shown). In contrast, iso-OMPA did not affect the resting tension ($n = 3$).

Figures 2 and 3 show the effects of neostigmine and iso-OMPA on ACh-induced contractions in rat urinary bladder preparations. As illustrated in panel A, the responses to ACh were biphasic with a rapid phasic contraction followed by a sustained contraction. Pretreatment of the preparations with neostigmine (1 μM) markedly augmented the ACh-induced contractions (Fig. 2, $n = 5$). As shown in Table 1, neostigmine significantly ($P < 0.05$) affected both E_{max} and pD_2 for ACh. Iso-OMPA also significantly ($P < 0.05$) potentiated ACh-induced responses, however, the potentiation by iso-OMPA (10 μM) was much smaller (Fig. 3 and Table 1) ($n = 5$).

ChE activity in intact tissues

Figure 4 shows the activities of AChE and BuChE in rat urinary bladder preparations. The activity of AChE was significantly ($P < 0.05$) higher than that of BuChE ($n = 4$).

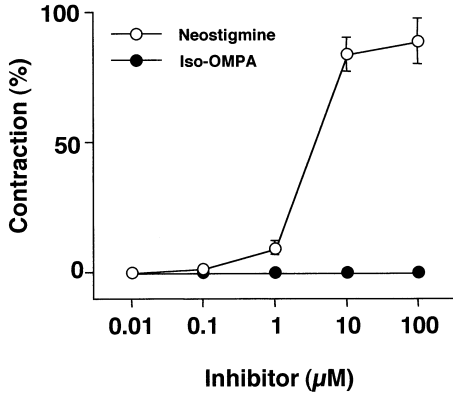


Fig. 1 The effects of neostigmine and tetraisopropylpyrophosphoramide (iso-OMPA) on the resting tension of the rat urinary bladder preparations. Contractile responses are expressed as percentages of the reference contraction induced by acetylcholine (100 μM). Each *point with a vertical bar* represents the mean ± SEM from three or four separate preparations

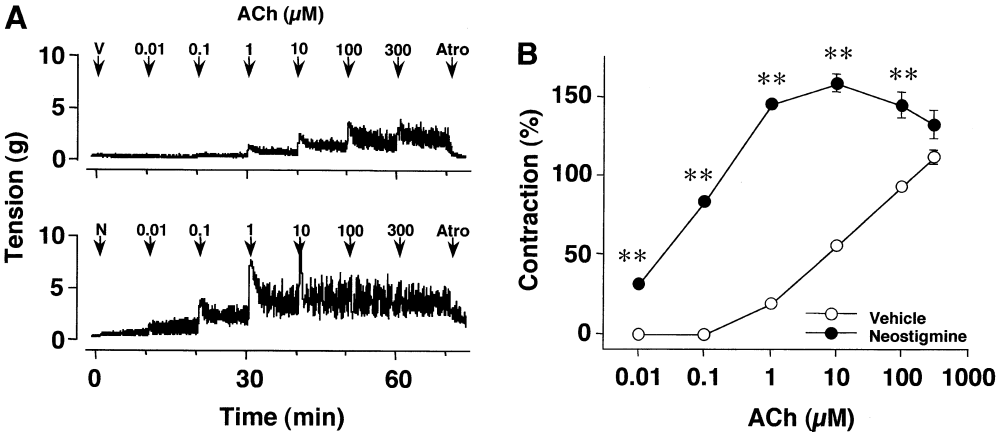


Fig. 2 The effect of neostigmine on acetylcholine (ACh)-induced contraction in rat urinary bladder preparations. **A** Typical tracings of tension developed with ACh in the absence (*V*) and presence (*N*) of neostigmine (1 μM). Increasing concentrations of ACh caused a concentration-dependent increase in tension. The increased tension was reversed by exposure to 1 μM atropine (*Atro*). **B** Neostigmine significantly augmented the ACh-induced contraction. Contractile responses are expressed as percentages of the reference contraction induced by ACh (100 μM). Each *point with a vertical bar* represents the mean ± SEM of five separate preparations. $^{**}P < 0.01$ vs corresponding control values

Discussion

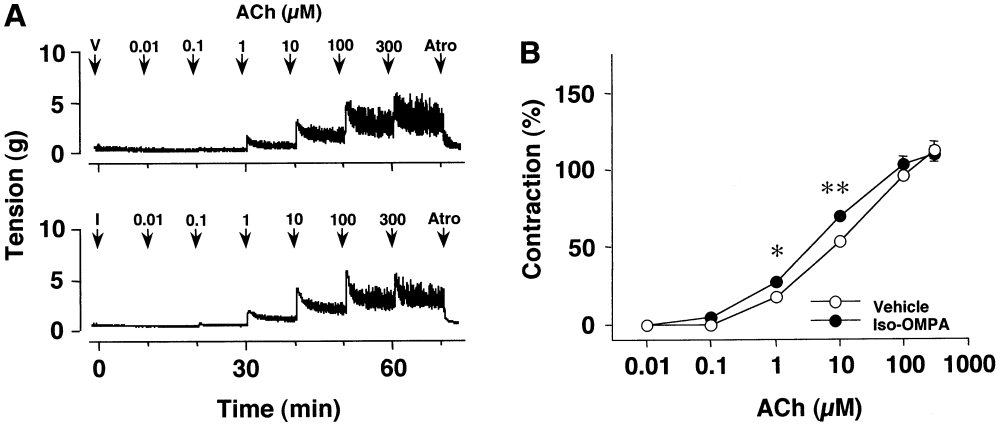
In the present study, we detected activities of both AChE and BuChE in isolated rat urinary bladder. Although these enzymes might contribute to the regulation of the ACh-induced contraction of bladder preparations, the efficacy of iso-OMPA (a BuChE inhibitor) was significantly lower than that of neostigmine (a non-selective ChE inhibitor).

BW284c51, a selective AChE inhibitor, exhibits an anti-muscarinic effect [3]. Therefore, we examined the actions of neostigmine and iso-OMPA to assess the relative contributions of AChE and BuChE involved in the regulation of resting tension and ACh-induced contractions of the rat urinary bladder. Neostigmine elevated the resting tension in a concentration-dependent manner. On the other hand, iso-OMPA (10 μM), which produced complete inhibition of BuChE and 13% inhibition of AChE at this concentration [3], did not change the resting tension. The contractile responses to neostigmine may not only be due to the prevention of endogenous ACh degradation but also to the stimulation of release of endogenous ACh from nerve terminals [8], because 100 μM iso-OMPA, which inhibits AChE, failed to affect the resting tension.

Table 1 Effect of neostigmine (1 μM) and iso-OMPA (10 μM) on the contraction produced by acetylcholine in rat urinary bladder strips. Emax are expressed as the percentage of the reference contraction induced by acetylcholine (100 μM). The values represent the mean ± SEM from four to five separate preparations. $^{**}P < 0.01$, $^{*}P < 0.05$ vs corresponding control absence values

	Emax		pD_2values	
	Absence	Presence	Absence	Presence
Neostigmine	109.4 ± 4.8	148.0 ± 6.5**	5.06 ± 0.10	7.06 ± 0.06**
Iso-OMPA	111.7 ± 5.8	109.2 ± 6.0	4.99 ± 0.11	5.32 ± 0.08*

Fig. 3 The effect of iso-OMPA on ACh-induced contraction in the rat urinary bladder preparations. **A** Typical tracings of tension developed with ACh in the absence (*V*) and presence (*I*) of iso-OMPA (10 μM). Increasing concentrations of ACh caused a concentration-dependent increase in tension. The increased tension was reversed by exposure to 1 μM atropine (*Atro*). **B**, Iso-OMPA significantly augmented the ACh-induced contraction. Contractile responses were expressed as percentages of the reference contraction induced by ACh (100 μM). Each point with a vertical bar represents the mean ± SEM of five separate preparations. $^{*}P < 0.05$, $^{**}P < 0.01$, vs corresponding control values



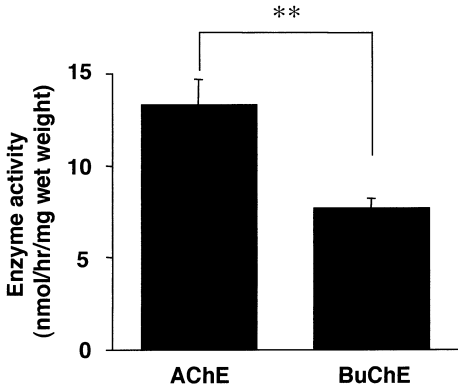


Fig. 4 Activities of AChE and butyrylcholinesterase (BuChE) in intact urinary bladder preparations. Enzyme activities in intact tissues were determined spectrophotometrically from tissue-bath fluid using a modification of Ellman et al.'s method [6]. Activity was obtained by normalization to tissue wet weight. Each *column with a vertical bar* represents the mean ± SEM from four separate preparations. $^{**}P < 0.01$

Both neostigmine (1 μM) and iso-OMPA (10 μM) caused a significant leftward shift of contractile responses to ACh. However, the effect of neostigmine (1 μM) on the ACh response was more pronounced than that of iso-OMPA (10 μM). Although neostigmine could inhibit both AChE and BuChE, most of the observed potentiation by neostigmine seems to be attributable to inhibition of AChE, because iso-OMPA had only a small effect. Furthermore, the enhancement of ACh-induced contraction by iso-OMPA may be partly attributed to its inhibitory effect on AChE activity. Thus, AChE plays a critical role in controlling ACh-induced contraction of rat urinary bladder. The present study also indicates that AChE activity is higher than BuChE activity in the rat urinary bladder. This result supports the findings that AChE, rather than BuChE, plays an important role in the regulation of resting tension and ACh-induced contraction.

Electrical field stimulation of bladder muscle produces contraction of the bladder. This was potentiated by inhibition of ChEs. Field stimulation, however, may release neurotransmitters other than ACh [5]. Therefore, we evaluated the effects of ChE inhibitors on the contraction elicited by exogenous ACh, but not by electrical field stimulation, to assess the relative role of ChEs in controlling of ACh-induced response. In airway smooth muscle and pulmonary blood vessels, it has been proposed that ACh hydrolysis may be controlled by a dual enzymatic process [4, 9]. That is to say, AChE is mainly responsible for the local neuronal synaptic regulation of ACh and BuChE protects against an increased release of ACh or against an extra-neuronal appearance of ACh. In the rat urinary bladder, even contractile responses to exogenous ACh were markedly potentiated by AChE inhibition, rather than BuChE inhibition, therefore AChE must play an important role in controlling the cholinergic-mediated response to electrical field stimulation in the urinary bladder.

In conclusion, the present study indicates the presence of AChE and BuChE activities in the rat urinary bladder. Furthermore, the results suggest that ACh hydrolysis in the rat urinary bladder could be mainly regulated by AChE, although both AChE and BuChE are present.

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