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Adenosine- and α,β-methylene ATP-induced differential inhibition of cholinergic and non-cholinergic neurogenic responses in rat urinary bladder

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1 The effects of adenosine and α,β-methylene adenosine triphosphate (α,β-Me ATP) on single pulse-induced neurogenic responses and contractions caused by exogenously applied acetylcholine (ACh) and adenosine triphosphate (ATP) were examined in rat urinary bladder.

2 Application of single pulse stimulation (1 ms; 80 V) evoked a biphasic contractile response (abolished by tetrodotoxin, 0.5×10^{-7} M) consisting of a fast (time to peak: 1.02 ± 0.07 s) and a slow component (time to peak: 4.92 ± 1.6 s). The selective inhibition of the slow component by atropine (3×10^{-6} M) suggests the participation of both cholinergic and non-cholinergic neurotransmitters.

3 α,β-Me ATP (5×10^{-6} M) abolished ATP (10^{-4} M)-induced contractions without altering those to ACh (10^{-6} M). Further, the selective inhibition of the fast component of the neurogenic response by α,β-Me ATP is suggestive of the contribution of endogenous ATP to the non-cholinergic component.

4 Adenosine (10^{-8} M to 10^{-4} M) caused dose-related differential inhibition of the fast (IC_{50}, $1.04 \pm 0.25 \times 10^{-5}$ M) and slow (IC_{50}, $2.18 \pm 0.69 \times 10^{-6}$ M) components, thereby further supporting two modes of neurotransmission in bladder.

5 Theophylline (10^{-4} M) antagonized the inhibitory effects of adenosine on the non-cholinergic component, thereby implicating the participation of P_1-purinoceptors in neuromodulation. In contrast, theophylline at this concentration enhanced the adenosine-induced inhibition of the cholinergic component.

6 The magnitude of ATP (10^{-4} M)- and ACh (10^{-8} M)-induced contractions were almost identical to those of the fast and slow components of the neurogenic response, respectively. Comparable reduction of ATP ($30.2 \pm 3.4\%$) and ACh (100%) contractions to those of fast ($44.2 \pm 6.5\%$) and slow ($88.2 \pm 5.5\%$) components suggests the involvement of a postjunctional mechanism in adenosine-induced differential inhibition of neurogenic responses.

7 The lack of effect of erythro-6-amino-9-(2-hydroxy-3-nonyl) adenosine hydrochloride (10^{-6} M) and dipyridamole (10^{-6} M) suggests that endogenous adenosine plays little part in modulation of single pulse-induced neurogenic response.

8 The results of the present study suggest that fast and slow components of neurogenic response are mediated through ATP and ACh, respectively, possibly co-released from the same neurone in the rat bladder.

Introduction

There is considerable evidence implicating acetylcholine (ACh) and adenosine triphosphate (ATP) as the excitatory neurotransmitters mediating cholinergic and non-cholinergic neurogenic responses, respectively in mammalian urinary bladder (Brown *et al.*, 1979; Kasakov & Burnstock, 1983; Hoyle & Burnstock, 1985; Fujii, 1988; Bhat *et al.*, 1989). Further, the role of ATP as a neurotransmitter of the 'purinergic' nerves has been emphasized in guinea-pig urinary bladder by the demonstration of endogenous ATP release on transmural nerve stimulation as well as by the presence of ATP containing nerves shown by fluorescence histochemistry (Burnstock *et al.*, 1978). Purines, particularly adenosine, are known to modulate the release of classical neurotransmitters like ACh, noradrenaline and dopamine in both central and peripheral nervous systems (Snyder, 1985). In view of the release of more than one neurotransmitter (as yet unknown whether from the same or different nerves) on electric field stimulation of intramural nerves in urinary bladder, the neuromodulatory effect of adenosine in respect of both cholinergic and non-cholinergic neurotransmission needs to be examined. Single pulse stimulation parameters that separate the cholinergic and non-cholinergic contractile components distinctly (Bhat *et al.*, 1989) have been used to examine the neuromodulatory role of adenosine in rat urinary bladder. Further, adenosine deaminase inhibitor (erythro-6-amino-9-(2-hydroxy-3-nonyl)adenosine hydrochloride, EHNA) and adenosine uptake inhibitor (dipyridamole) have been employed to elucidate the role of endogenous adenosine in autonomic neurotransmission, if any, in this tissue.

α,β-Methylene ATP (α,β-Me ATP), widely used to desensitize P_2-purinoceptors, has been known to abolish or reduce electrical and contractile responses evoked by nerve stimulation in urinary bladder of several mammalian species (Kasakov & Burnstock, 1983; Fujii, 1988). It is therefore, clearly essential to examine the effects of α,β-Me ATP on both fast and slow components of single pulse-induced neurogenic response so as to establish the contribution of endogenous ATP to the non-cholinergic response in rat bladder.

Methods

Male albino rats, obtained from the Laboratory Animal Resource Section of this Institute, were used in the present study. Animals were stunned with a blow to the head and exsanguinated. The urinary bladder was dissected out and strips of 3×10 mm were cut. The smooth muscle strips were suspended in Tyrode solution of the following composition (mM): NaCl 138, KCl 5.9, $NaHCO_3$ 6.0, glucose 5.5, $NaH_2PO_4 2H_2O$ 0.5, $MgCl_2 6H_2O$ 0.5 and $CaCl_2 2H_2O$ 1.9. The solution was aerated with atmospheric air and maintained at 37 ± 0.5°C (pH 7.4). The tissues were equilibrated for a period of 1 h under a resting tension of 0.5 g.

The responses of the tissue were recorded isometrically with a force displacement transducer connected to a polygraph (M/s Medicare, India). The bladder strip was stimulated with

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a single square wave pulse of 1 ms duration at 80 V, every 15 min. The stimulation caused a biphasic contraction of the urinary bladder, consisting of an early fast phase and a late slow phase, which was abolished by tetrodotoxin (0.5×10^{-7} M), thereby suggesting its neural origin. Atropine (3×10^{-6} M), however, selectively abolished the slow component while the fast component was not altered, thus suggesting both cholinergic and non-cholinergic neurotransmission in the urinary bladder.

In order to achieve P_2-purinoceptor desensitization, the procedure described by Fujii (1988) was followed with slight modification. Briefly, the tissues were superfused with α,β-Me ATP (5×10^{-6} M) for 20 min and successive superfusions were carried out for 30–40 s at 3–4 min intervals.

To examine the effect on single pulse-induced biphasic neurogenic responses, adenosine (10^{-8} M – 3×10^{-4} M) was added cumulatively in the presence of dipyridamole (10^{-6} M) and EHNA (10^{-6} M), agents known to inhibit the inactivation of adenosine. Responses to individual doses (10^{-8} M – 3×10^{-4} M) of adenosine were elicited on some tissues and the concentration-responses relationship obtained with this protocol was the same as that obtained with cumulative doses. In some experiments, the effect of a combination of dipyridamole and EHNA was studied with a view to examining the role of endogenous adenosine, if any, in modifying the neurogenic responses elicited on single pulse field stimulation. Theophylline (10^{-4} M), a P_1-purinoceptor antagonist, was used to study the specificity of adenosine-purinoceptor interaction. Concentration-response relationship with ATP (10^{-6} M – 3×10^{-4} M) and ACh (10^{-9} M – 3×10^{-3} M), added in single doses, was established, maintaining a time interval of 25 min and 5 min, respectively between each addition. Doses of ATP (10^{-4} M) and ACh (10^{-8} M) that induced responses which matched those of fast (non-cholinergic) and slow (cholinergic) components of neurogenic response were selected to evaluate the modulatory effect of adenosine (10^{-5} M) at post-junctional site.

The following drugs were used: acetylcholine chloride (Sigma), adenosine (Sigma), adenosine triphosphate disodium salt (Sigma), α,β-methylene ATP (Sigma), atropine sulphate (Sigma), dipyridamole (Gift: German Remedies), erythro-6-amino-9-(2-hydroxy-3-nonyl) adenosine hydrochloride hydrate (EHNA; gift: Wellcome Research Laboratories), tetrodotoxin (Sigma) and theophylline (Sigma).

Results are presented as mean ± s.e.mean. Student's t test was used for the test of significance. The IC_{50} values were evaluated for each preparation by linear regression analysis.

Results

Rat urinary bladder smooth muscle, normally exhibiting autorhythmicity, when stimulated with single square wave pulses (1 ms pulse duration at 80 V) at an interval of 15 min, produced a biphasic contractile response consisting of a fast component (time to peak: 1.02 ± 0.07 s; $n = 8$) and a slow component (time to peak: 4.92 ± 1.6 s; $n = 8$), the former being always greater in magnitude than the latter. The mean absolute tensions of the fast and slow components were 0.90 ± 0.06 g ($n = 16$) and 0.50 ± 0.05 g ($n = 16$), respectively. At times in tissues exhibiting greater autorhythmicity, a third late contractile component was observed in the descending limb of the second slow phase of contraction. This component, however, was not evident in tissues exhibiting either low or no spontaneity.

Figure 1 depicts the effects of α,β-Me ATP on the responses of rat bladder to ACh, ATP and single pulse stimulation. Contractions caused by α,β-Me ATP (5×10^{-6} M) were partially persistent (about 25% of the initial response) even after five successive superfusions with this agent. However, in the presence of α,β-Me ATP, ATP (10^{-4} M)-induced contractions were abolished while ACh (10^{-6} M)-induced contractions were little affected. Further, α,β-Me ATP caused inhibition of the fast component by 38 ± 3.7% ($n = 4$) while the slow component was not altered. In the tissues pretreated with α,β-Me ATP, atropine (10^{-6} M) selectively abolished the slow component of the neurogenic response without significantly affecting the fast component.

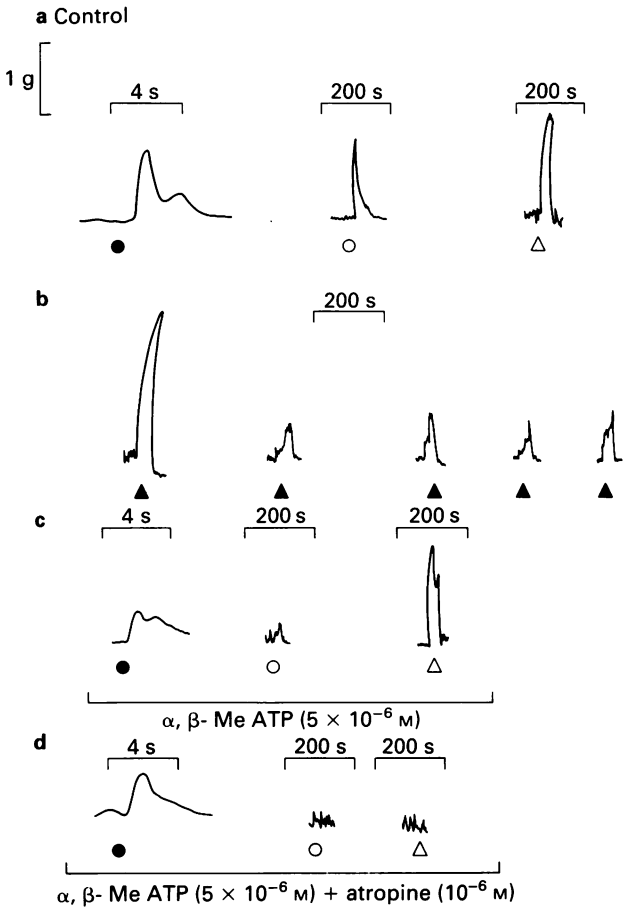

Figure 1 Effects of α,β-methylene ATP (α,β-Me ATP) on the responses to single pulse stimulation (● 1 ms, 80 V) adenosine triphosphate (ATP, ○, 10^{-4} M) and acetylcholine (ACh, △, 10^{-6} M) in rat urinary bladder: (a) control responses; (b) successive superfusions with α,β-Me ATP (5×10^{-6} M); (c) contractile responses in the presence of α,β-Me ATP (5×10^{-6} M) and (d) in the presence of α,β-Me ATP (5×10^{-6} M) and atropine (10^{-6} M).

Incubation of the tissue for 15 min with dipyridamole (10^{-6} M) and EHNA (10^{-6} M) did not significantly modify single pulse-induced biphasic contractions (Figure 2). On the other hand, adenosine (10^{-8} M–3×10^{-4} M) in the presence of dipyridamole and EHNA caused dose-related inhibition of neurogenic responses; the slow component being relatively more susceptible than the fast component (Figures 3, 4 and Table 1). In the tissues treated with dipyridamole and EHNA, theophylline (10^{-4} M) did not affect the fast component of the

Table 1 Inhibitory effect of adenosine (10^{-8}–10^{-4} M) on the fast and slow components of the contractile response to single pulse field-stimulation (1 ms; 80 V) in rat urinary bladder (pretreated with EHNA 10^{-6} M and dipyridamole 10^{-6} M) and its modulation by theophylline (10^{-4} M)

	IC_{50} (M)	
	Fast component	*Slow component*
Adenosine	$1.04 \pm 0.25 \times 10^{-5}$	$2.18 \pm 0.69 \times 10^{-6}$*
Adenosine + theophylline	$2.45 \pm 0.29 \times 10^{-5}$*	$1.32 \pm 0.19 \times 10^{-7}$*

$n = 6$–8; values are mean ± s.e.mean.
*$P < 0.05$.

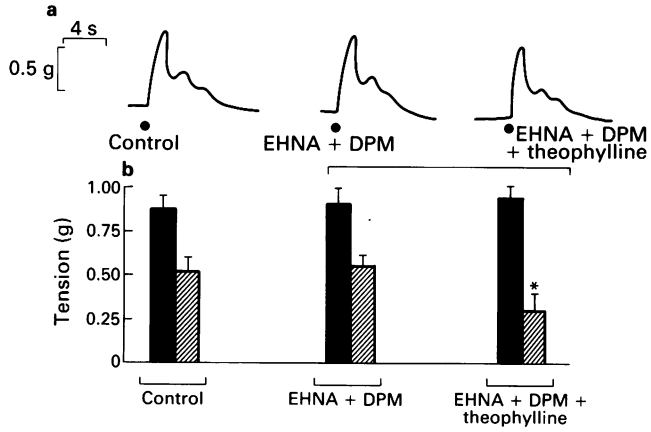

Figure 2 (a) Isometric recordings of rat urinary bladder illustrating the biphasic contractile responses to single pulse stimulation (1 ms; 80 V) and the effect of EHNA (10^{-6} M), dipyridamole (DPM: 10^{-6} M) and theophylline (10^{-4} M) on these responses and (b) mean absolute tension (g) of fast (solid column) and slow (hatched column) components of single pulse stimulus and the effect of erythro-6-amino-9-(2-hydroxy-3-nonyl) adenosine hydrochloride (EHNA, 10^{-6} M), DPM (10^{-6} M), and theophylline (10^{-4} M) on the same. Vertical bars indicate the s.e.mean (*$P < 0.05$; $n = 6$–8).

neurogenic response, while the slow component was significantly ($P < 0.05$) inhibited by about 25% (Figure 2). Further, theophylline antagonized the inhibitory effect of adenosine on the fast component of the neurogenic response (Figure 4a). This is also evident from the significant ($P < 0.05$) rise in the IC_{50} value of adenosine in the presence of theophylline as compared to controls (Table 1). In contrast, adenosine-induced dose-related inhibition of the slow component was markedly potentiated by theophylline (Figure 4b).

ATP (10^{-6} M–3 × 10^{-4} M) and ACh (10^{-9} M–3 × 10^{-3} M) elicited concentration-related contractile responses in rat urinary bladder (Figure 5a). ATP (10^{-4} M) and ACh (10^{-8} M) generated absolute tensions of 1.2 ± 0.12 g and 0.37 ± 0.05 g, respectively which were comparable to those of fast (0.95 ± 0.06 g) and slow (0.42 ± 0.05 g) components of the neurogenic responses. ATP-induced contraction was fast in time course and transient in nature as compared to that of ACh. The mean time to peak values of ATP and ACh was 7.7 ± 0.4 s and 39.3 ± 1.4 s, respectively. Adenosine (10^{-5} M) inhibited ATP- and ACh-induced contractions by about 30 and 100%, respectively as compared to the inhibition of fast (44.2 ± 6.5%; $n = 8$) and slow (88.2 ± 5.5%; $n = 8$) components of the neurogenic response.

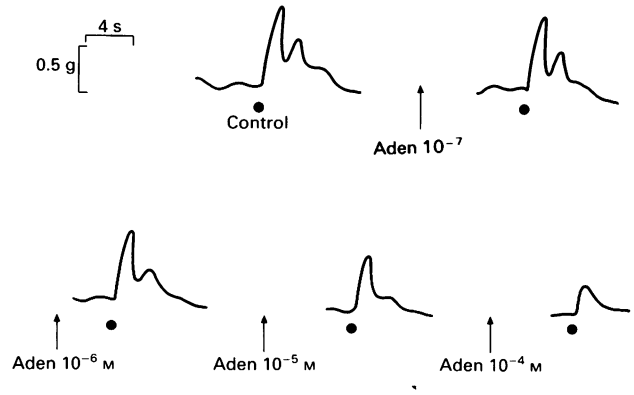

Figure 3 Effect of cumulative concentrations of adenosine (Aden, 10^{-7} M to 10^{-4} M) on the fast and slow components of contractions of single pulse stimulation in rat bladder exposed to erythro-6-amino-9-(2-hydroxy-3-nonyl) adenosine hydrochloride (EHNA, 10^{-6} M) and dipyridamole (10^{-6} M); $n = 8$.

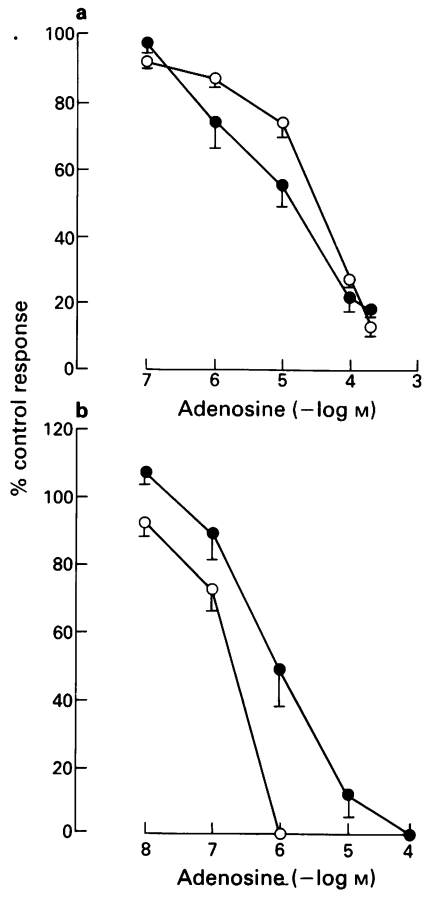

Figure 4 Effect of adenosine (10^{-8} M to 3 × 10^{-4} M) added cumulatively on the fast (a) and slow (b) components of contractions induced by single pulse stimulation in the presence (●) and absence (○) of theophylline (10^{-4} M).

Discussion

The absolute susceptibility of the biphasic contractile response elicited on single pulse stimulation to TTX and the selective inhibition of the slow component by atropine are in conformity with our earlier suggestion that the fast and slow components are mediated by a non-cholinergic neurotransmitter (possibly ATP) and a cholinergic neurotransmitter ACh, respectively (Bhat *et al.*, 1989). In order to substantiate further the role of ATP as a neurotransmitter in rat bladder, desensitization of P_2-purinoceptors by α,β-Me ATP was attempted. Persistence of residual responses to α,β-Me ATP following successive superfusions indicate that this agent partially desensitized P_2-purinoceptors in rat bladder. Similarly, depolarization by α,β-Me ATP has been reported to be partially persistent in the guinea-pig and rabbit mesenteric arteries (Ishikawa, 1985) and in rabbit ear artery (Miyahara & Suzuki, 1987). Nevertheless, in the presence of α,β-Me ATP, ATP-induced contractions were abolished without any effect on those of ACh. Similarly, while the fast component of the neurogenic response was partially inhibited by α,β-Me ATP, the slow component mediated by endogenous ACh was not altered. The selective inhibition of the non-cholinergic component of the nerve-mediated contraction could result from the desensitization of P_2-purinoceptors by α,β-Me ATP and this strongly suggests that the non-cholinergic fast component to single pulse stimulation is partly mediated by ATP. Although the role of α,β-Me ATP in inhibiting neurotransmitter release cannot be excluded in the present study, this possibility may be of little significance in view of previous reports where α,β-Me ATP has been shown not to affect neurotransmitter release in other smooth muscle preparations (rabbit

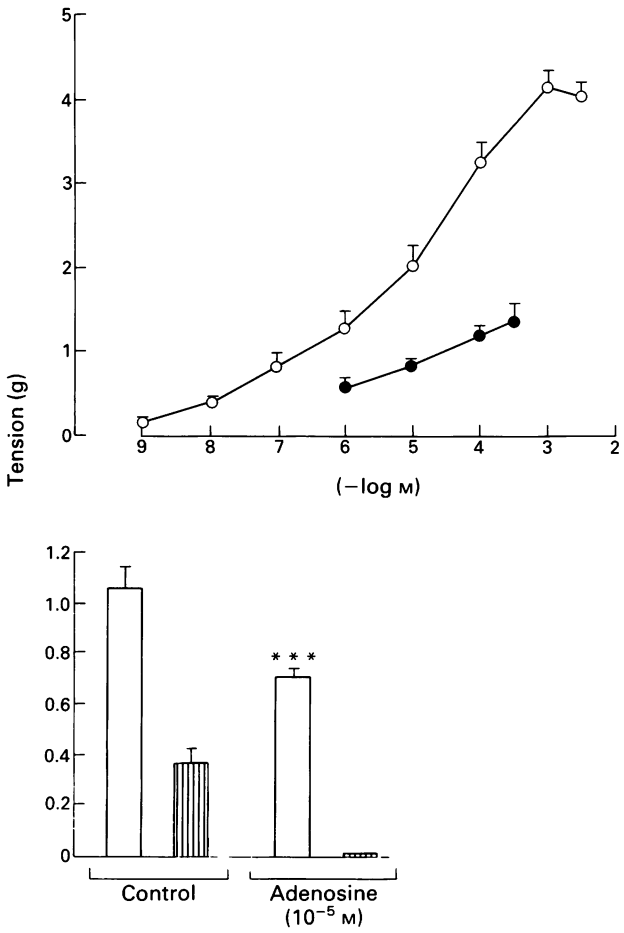

Figure 5 (a) Concentration-response curves to acetylcholine (ACh, ○) and adenosine triphosphate (ATP, ●) in rat urinary bladder and (b) effect of adenosine on the absolute tension of ATP (10^{-4} M) (open columns) and ACh (10^{-8} M) (lined columns). Vertical bars indicate the s.e.mean (***$P < 0.001$; $n = 6$).

mesenteric artery: Ishikawa, 1985; Kugelgen & Starke, 1985; guinea-pig vas deferens: Allcorn *et al.*, 1986). As observed in the present study the resistance of a significant level of the fast component to α,β-Me ATP could possibly be due to partial desensitization of P_2-purinoceptors in rat bladder. Alternatively the contribution of some neurotransmitter other than ATP to the fast component cannot be ruled out.

Since ATP and ACh are the possible neurotransmitters in rat urinary bladder, responses which matched those of fast and slow components of neurogenic responses were elicited by exogenously added ATP (10^{-4} M) and ACh (10^{-8} M), respectively. The effect of adenosine on these two agonist-induced contractile responses was evaluated with a view to (1) elucidating the postjunctional actions and (2) to examining the involvement of presynaptic/postjunctional mechanisms in adenosine-induced varied inhibition of the neurogenic responses. In rat urinary bladder, the contractions produced by ATP were fast in time course (time to peak: 7.7 ± 0.4 s) as compared to those of ACh (time to peak: 39.3 ± 1.4 s) at an equimolar concentration (10^{-4} M). This observation is in agreement with the electrophysiological observations made on the guinea-pig bladder (Fujii, 1988). Further, the characteristics of contractions to exogenously applied ATP and ACh mimic the fast and slow components of contraction originating from single pulse stimulation, in as far as the time course of action is concerned.

The abolition of the ACh response and a significant inhibition of the ATP response by adenosine is in conformity with its postjunctional inhibitory action in bladder muscle (Husted *et al.*, 1983). A similar mechanism might be involved in adenosine-induced differential inhibition of neurogenic responses as is evident from a 44% reduction of the fast component (compared to 30% with exogenous ATP) and an 88% inhibition of the slow component (compared to 100% with exogenous ACh), respectively. However, an inhibitory effect of adenosine on presynaptic release of neurotransmitter(s) in rat bladder cannot be ruled out. Assuming that ATP and ACh come from the same nerve, a prejunctional action of adenosine which might reduce the release of ATP and ACh in parallel would not result in a parallel decrease in the amplitude of neurogenic responses. Thus the results obtained with adenosine together with those of α,β-Me ATP suggest that possibly ATP and ACh are co-released from a single neurone in rat bladder and is consistent with the hypothesis of 'co-transmission' as reported earlier in several other tissues (rabbit ear artery: Saville & Burnstock, 1988; dog mesenteric artery: Machaly *et al.*, 1988; vas deferens: Sneddon *et al.*, 1984). However, the involvement of separate populations of nerves releasing ACh and ATP independently cannot be ruled out.

The specificity of antagonism by theophylline of adenosine-induced inhibition of the fast component is consistent with its P_1-purinoceptor antagonism. However, the potentiation of the inhibitory effect of adenosine on the slow component by theophylline is difficult to explain in terms of its purinoceptor antagonism. The inhibitory effect on the slow component and the potentiation of the adenosine effect by theophylline could possibly be related to its phosphodiesterase inhibitory effect as observed in other smooth muscles (Gustafsson *et al.*, 1981).

In several tissues including rat bladder, the adenosine uptake inhibitor, dipyridamole, and adenosine deaminase inhibitor, EHNA, are known to inhibit the inactivation of endogenous adenosine (Fredholm *et al.*, 1978; Dahlen & Hedqvist, 1980). However, in the present study EHNA and dipyridamole did not modify the single pulse-induced neurogenic response. This is in contrast to the inhibitory effect of dipyridamole on field-stimulated rat urinary bladder linked to accumulation of endogenous adenosine (Dahlen & Hedqvist, 1980). The discrepancy observed could probably be due to higher frequency of stimulation (e.g. 5 Hz) used in earlier studies as compared to single pulse field stimulation in the present study. Although the lack of inhibitory effect of EHNA and dipyridamole on single pulse-induced response suggests that endogenous adenosine plays little part, we cannot exclude its role altogether in neuromodulation. This is because, in the absence of a sufficient concentration of spontaneously released adenosine, any accumulation of breakdown products of purines during single pulse stimulation of intramural nerves might not have any appreciable effect before the peak contractions were reached.

In conclusion, it is suggested that (1) the varied susceptibility of cholinergic and non-cholinergic neurogenic responses in rat bladder to adenosine may involve postjunctional mechanisms, (2) the excitatory neurotransmitters possibly, ATP and ACh mediate the fast and slow components, respectively and (3) endogenous adenosine appears to play little part in the modulation of the neurogenic response elicited by single-pulse field stimulation.

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