Br. J. Pharmac. (1980), **70**, 443–452

CONTRIBUTION OF PROSTAGLANDINS TO THE ADENOSINE TRIPHOSPHATE-INDUCED CONTRACTION OF RABBIT URINARY BLADDER

K.-E. ANDERSSON, S. HUSTED & C. SJÖGREN

Department of Clinical Pharmacology, University of Lund, Lund, Sweden and Institute of Pharmacology, University of Aarhus, Aarhus, Denmark & Research Laboratories of AB LEO, Helsingborg, Sweden

1 Adenosine 5'-triphosphate (ATP) produced an inital rapid, phasic contraction and a later, slowly developing tonic contraction in the isolated detrusor of the rabbit but mainly a rapid, phasic response in the guinea-pig bladder.

2 Electrical field stimulation elicited only a rapid, phasic contraction in both rabbit and guinea-pig bladders.

3 Prostaglandin synthesis inhibition by means of indomethacin and suprofen abolished the tonic response to ATP in the rabbit detrusor, leaving the phasic part of the contraction almost unaffected. The ATP-induced contraction in guinea-pig bladder was not influenced by indomethacin.

4 The contractile response of rabbit urinary bladder to prostaglandins $F_{2\alpha}$ and E_2 and to carbachol were not significantly influenced by indomethacin. The contractions induced by the prostaglandins were similar to the tonic response to ATP.

5 Tetrodotoxin, atropine, phentolamine, and theophylline did not alter the ATP-induced contraction. However, the calcium antagonists, nifedipine and nimodipine, abolished the phasic ATP response and greatly reduced the tonic part of the contraction.

6 Tachyphylaxis occurred on repeated addition of ATP; the response to field stimulation was progressively reduced only after indomethacin pretreatment.

7 ATP and prostaglandins may contribute to the non-adrenergic, non-cholinergic component of the excitation of rabbit and guinea-pig bladder.

Introduction

Non-cholinergic, non-adrenergic inhibitory transmission in various parts of the vasculature and of the intestinal and respiratory tracts has been attributed to adenosine 5'-triphosphate (ATP) release from 'purinergic' nerves (Burnstock, 1972). ATP release shown to occur during intramural nerve stimulation of the isolated detrusor of the rabbit suggested an involvement of this compound also in the atropine-resistant excitatory response of the mammalian urinary bladder (Burnstock, Dumsday & Smythe, 1972; Burnstock, Cocks, Kasakov & Wong 1978a; Dean & Downie 1978b), although this possibility was discounted by, e.g., Ambache & Zar (1970), Ambache, Daly, Killick & Woodley (1977), and Weetman & Turner (1977).

Isolated strips of guinea-pig urinary bladder respond to exogenous ATP with a rapid and transient contraction mimicking the response to electrical field stimulation more closely than other excitatory substances tested (Ambache & Zar, 1970; Dumsday, 1971; Burnstock *et al.*, 1978a). A significant influence of prostaglandins of the E and F series has been demonstrated on ATP-induced contractions (Burnstock *et al.*, 1978a; Dean & Downie, 1978a) and on atropine-resistant, nerve mediated responses of the rat (Choo & Mitchelson, 1977) and guinea-pig urinary bladder (Burnstock *et al.*, 1978a). These findings, and the blocking effect of indomethacin on ATP-induced contractions in rabbit urinary bladder (Dean & Downie 1978a), and on stimulation of non-cholinergic, non-adrenergic nerves of guinea-pig urinary bladder (Burnstock *et al.*, 1978a) suggest that the atropine-resistant neurotransmission in the urinary bladder may involve both ATP and prostaglandins acting in cooperation.

In the present study, the contractile effects of ATP in the rabbit and guinea-pig urinary bladder were studied. Evidence is presented, which suggests that the ATP-induced response in the rabbit detrusor consists of an initial rapid, phasic contraction mimicking the

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response to electrical stimulation of non-cholinergic, non-adrenergic nerves, and a tonic contraction mediated by prostaglandins released by ATP. Further, species differences in the response to ATP are considered.

Methods

Preparations

Adult albino rabbits weighing 1.8 to 2.5 kg, or albino guinea-pigs, 600 to 800 g, were killed by a blow on the neck and bled rapidly from the carotid arteries. The urinary bladder was excised by a transverse cut below the trigone. Visible connective tissue, fat and serosa were dissected away, and the lower part, including the trigone, was removed by a transverse cut and discarded. The remaining part of the urinary bladder was cut along a lateral surface giving a rectangular sheet of tissue about 1.2 to 1.5 cm long (unstretched) and 0.8 to 1.0 cm wide. The mucosal layer was gently removed, and strips (4–6) were then cut vertically, i.e. from the lower trigonal end of the bladder towards the fundus, each strip being approximately 2 to 3 mm × 6 to 8 mm (unstretched). A strip of bladder tissue was made by passing ligatures through its ends.

The strips of bladder tissue were then placed in an organ bath between two platinum wire electrodes (diameter 0.5 mm). The two electrodes were placed parallel to each other on both sides of the strip at a distance of 6 to 8 mm with sufficient gaps between electrodes and bladder to allow for unimpeded muscular contraction.

Electrical stimulation

The preparations were excited by transmural electrical stimulation with a Grass stimulator (model S 88). Monophasic 0.5 ms pulses of maximal current strength (225 to 250 mA) and with a frequency of 15 Hz were automatically delivered in 5 s trains unless otherwise stated every third minute. This ensured selective and supramaximal excitation of the nerves in the tissue, as checked with tetrodotoxin (TTX 0.5 μg/ml), which abolished the contractile response. Furthermore, the ganglion blocking agent, hexamethonium (300 μM), did not significantly influence the contractile response elicited by stimulation.

Recording

Isometric contractions were recorded by means of a force displacement transducer (Grass FT 03 or Statham G 10 B) connected to a pen recorder (Grass m7 or W/W Recorder 1200).

Salt solutions

Electrodes and tissues were suspended in 20 ml organ baths containing Krebs solution (Na^+-Krebs) of the following composition (mM): Na^+ 140.2, K^+ 4.6, Ca^{2+} 1.5, Mg^{2+} 1.2, Cl^- 129.0, HCO_3^- 20.0, $H_2PO_4^-$ 1.2 and glucose 11.1. The solution was maintained at 37°C, equilibrated before and during the experiment with carbogen containing 5.6% CO_2 and 94.4% v/v O_2; the pH was 7.4.

The chemicals used were of analytical grade. Fresh solutions were prepared on the day of the experiment.

Drugs

The following drugs were used: atropine sulphate monohydrate (Sigma Chemical Co), indomethacin (Dumex), suprofen (Janssen), nimodipine (1,4-dihydro-2,6-diemethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarbonic acid-isopropyl-(2-methoxyethyl)-ester; Bay a 9736; Bayer AG), nifedipine (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarbonic acid dimethyl ester; Bay a 1040; Bayer AG), hexamethonium bromide (Sigma), tetrodotoxin (TTX; Sankyo Co, Ltd.), adenosine 5'-triphosphate disodium salt (ATP, Sigma), guanethidine sulphate (Ciba), prostaglandins E_2 and $F_{2\alpha}$ (Upjohn), theophylline (DAK), phentolamine (Regitin; Ciba) and carbachol (DAK). Indomethacin was added from stock solutions containing 2.5 mM in 5 ml 99% ethanol plus 95 ml 0.1 M phosphate buffer (pH = 7.2). The prostaglandins were dissolved in 99% ethanol as stock solutions 2.5 mM and diluted with 0.9% w/v NaCl solution (saline) to the final required concentrations.

Statistical analysis

Student's *t* test (unpaired samples) was used to compare means. Compucorp model alpha 325 was used for these calculations.

Experimental

The strip was stretched to 30 mN tension for 10 min; then the tension was reduced to 20 mN throughout the experimental period. The preparations were allowed to equilibrate for 30 min before the start of the experiment. In the following control period, the strip of bladder tissue was stimulated 3 to 5 times until the change in response to stimulation was less than ±10%. The last response thus recorded was used as control.

The effect of drugs added cumulatively on the tension response evoked by field stimulation was checked in a standard manner: 2.5 min after the control response, the lowest concentration of the drug to be tested was added; the responses after 0.5, 3.5, 6.5

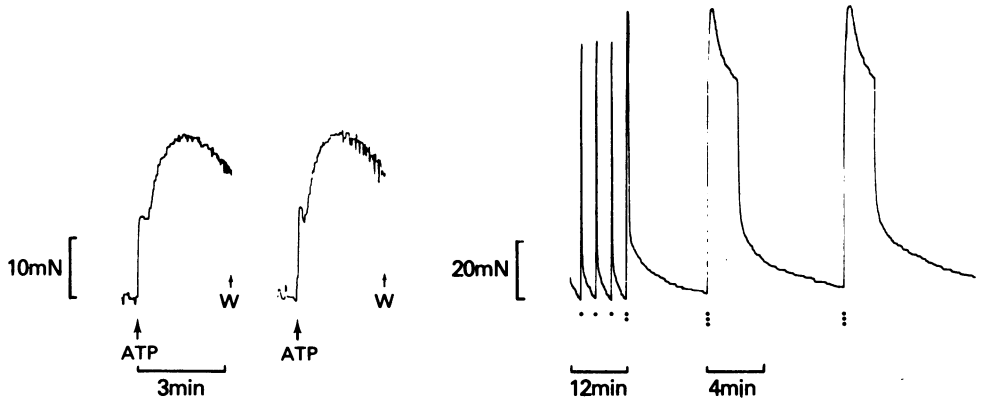

Figure 1 Contractile responses of a rabbit urinary bladder preparation to ATP 100 μM and to electrical field stimulation. W = wash-out (×3) in a 20 min period between two successive ATP additions. Train duration: (.) 5 s; (:) 30 s; (⁝) 120 s.

min etc. exposure were recorded. When a steady state response level was reached, the next concentration of drug was added. The response during the last stimulation of each period was used for calculations.

When the effect of drugs added non-cumulatively was studied, an interval of 20 min after wash-out was allowed between each drug addition. During this time, the bathing fluid was changed repeatedly. Comparisons between drugs were made when the contractile response had reached a steady state (less than 10% difference between two successive contractions). Each strip of bladder tissue was used for only one experiment.

Results

Effects of ATP and of transmural nerve stimulation

Rabbit The response of the rabbit urinary bladder to ATP 100 μM consisted of an initial rapid, phasic contraction (starting within 1 s and reaching maximum within 5 to 7 s), and a later increase in tension which was slow developing and tonic in character (starting within 15 to 20 s and being maintained for several minutes even after washing; Figure 1). Figure 1 also shows superimposed, rapid changes in tension during the tonic phase.

The time course of the contractile response to intramural nerve stimulation with increasing train length (5 to 120 s) is also shown in Figure 1. These responses consisted predominantly of rapid, phasic contractions, which were not followed by tonic contractions.

Guinea-pig The contractile responses (mainly rapid phasic contractions) of guinea-pig bladder to ATP 100 μM and electrical field stimulation are shown in Figure 2. In some experiments, a slight tonic contraction was seen after the phasic response. As in the rabbit bladder, superimposed, rapid changes in tension were seen during the tonic phase.

Effects of indomethacin on the response of rabbit detrusor to ATP and on contractions elicited by electrical field stimulation

Indomethacin 5 to 10 μM abolished the tonic response of the rabbit detrusor to ATP (Figure 3 and Table 1). This effect was reversible on wash-out and could also be obtained by suprofen 30 μM, another inhibitor of prostaglandin synthesis. The phasic response to exogenous ATP in rabbit bladder was reduced to 64 ± 14% ($n = 7$) of the control by indomethacin 5 μM (Table 1). This reduction was statistically significant. The phasic response elicited by exogenous ATP in guinea-pig bladder was not significantly influenced by indomethacin (5 μM, Figure 3). However, the slight tonic response seen in some experiments disappeared on addition of indomethacin. Indomethacin reduced the resting tension and the spontaneous activity. These effects developed in less than 30 min.

Guanethidine in an adrenergic neurone blocking concentration (3.4 μM) did not influence the response of rabbit detrusor to electrical field stimulation (Figure 4a), whereas atropine 1 μM significantly reduced the electrically evoked response, the effect being maximal within less than 5 min (Figure 4a).

The contraction induced by electrical stimulation of non-cholinergic, non-adrenergic neurones in rabbit detrusor pretreated with atropine 1 μM and guanethidine 3.4 μM was markedly reduced by indomethacin 5

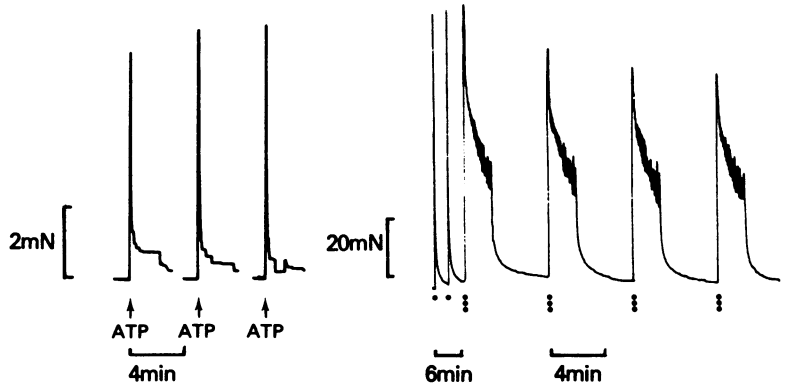

Figure 2 Contractile responses of a guinea-pig urinary bladder preparation to ATP 100 μM and to electrical field stimulation. Between each ATP addition there was a 20 minute wash-out (×3) period. Train duration: (.) 5 s; (⋮) 120 s.

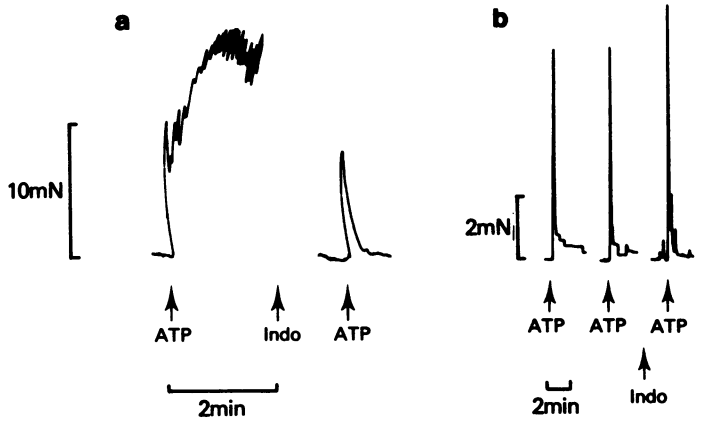

Figure 3 The effect of indomethacin 5 μM (contact time 30 min) on the rabbit urinary bladder (a) and guinea-pig urinary bladder (b) to exogenous ATP 100 μM. Between each ATP addition there was a 20 min washout (×3) period, except after indomethacin (Indo) treatment, when the interval between ATP additions was 40 min.

Table 1 Effect of various drugs on the phasic and tonic responses to exogenous ATP 100 μM in rabbit detrusor

			Before drug addition		*After drug addition*	
Drug added	*Concentration* (μM)	n	*Phasic response* mN ± s.e. mean	*Tonic response* mN ± s.e. mean	*Phasic response* % of control	*Tonic response* % of control
Indomethacin	5.0	7	13 ± 1	23 ± 3	64 ± 14*	0****
Atropine	1.0	4	16 ± 3	31 ± 6	113 ± 9^{NS}	103 ± 4^{NS}
Phentolamine	180	6	12 ± 2	23 ± 6	52 ± 8*	39 ± 9*
Nifedipine	0.15	5	9 ± 3	20 ± 5	0****	12 ± 4****
	0.3	3	9 ± 1	14 ± 8	0****	10 ± 6*
Theophylline	50	8	6 ± 2	13 ± 4	104 ± 13^{NS}	88 ± 4^{NS}

NS = not significant ($P > 0.05$); *$P < 0.05$; ****$P < 0.001$.

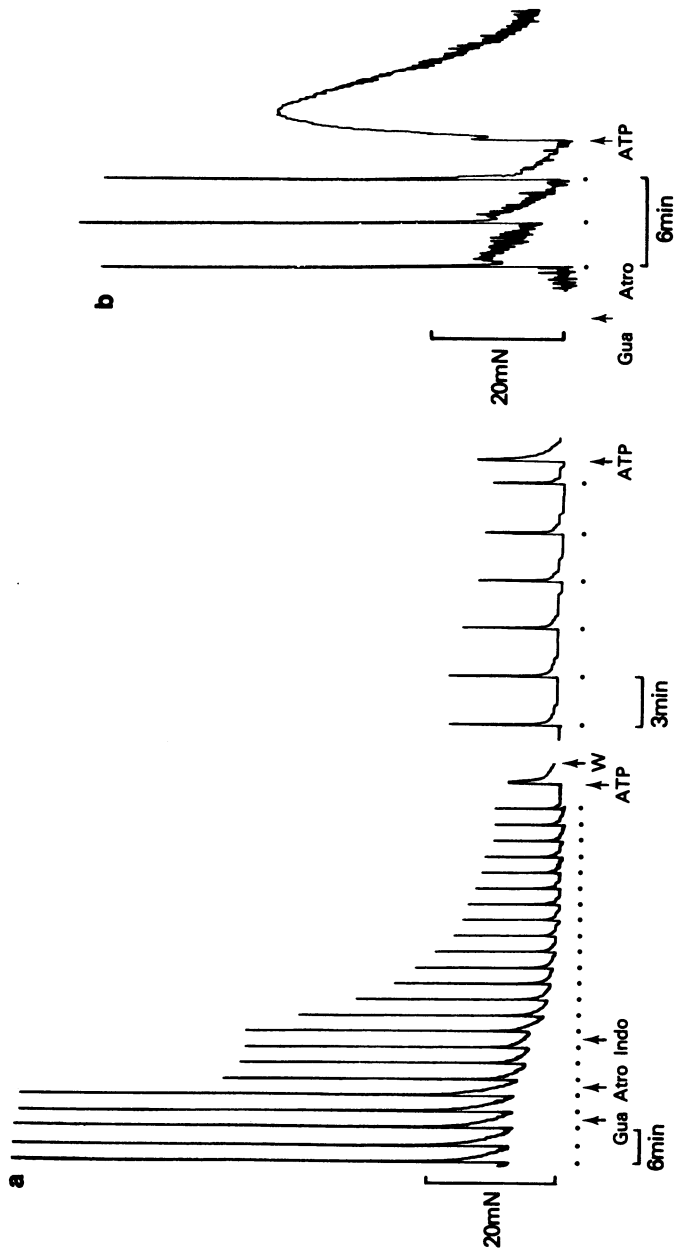

Figure 4(a) Contractile responses of the rabbit detrusor to electrical field stimulation and to ATP 100 μM in the presence of guanethidine (Gua) 3.4 μM, atropine (Atro) 1.0 μM and indomethacin (Indo) 5 μM. W, 3 washouts during a 20 min period with guanethidine, atropine and indomethacin still present. (b) Contractile responses of the rabbit detrusor to electrical field stimulation and to ATP 100 μM in the presence of guanethidine (Gua) 3.4 μM and atropine (Atro) 1.0 μM.

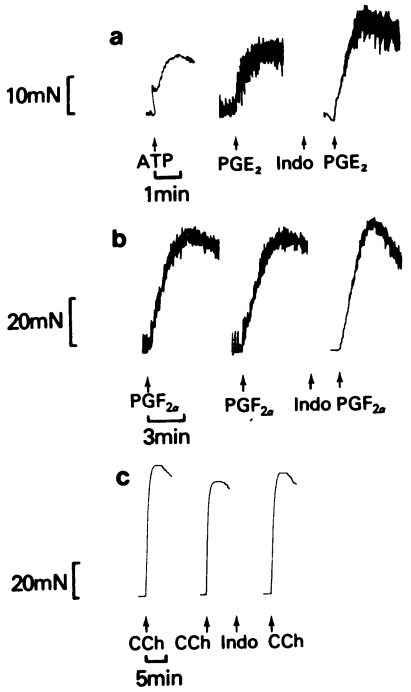

Figure 5 Contractile responses of rabbit urinary bladder to ATP 100 μM and prostaglandin E_2 (PGE_2) 0.025 μM (a), $PGF_{2\alpha}$ 0.28 μM (b), and carbachol (CCh) 1.4 μM (c). The effects of indomethacin (Indo) 5μM (contact time 30 min) are also shown. Between the additions of excitatory agents, there was a 20–40 minute wash-out (3 ×) period.

to 10 μM (Figure 4a). ATP 100 μM added to the same preparation mimicked the remaining field stimulated response. The rates of contraction were similar. However, relaxation was slower after ATP. The amplitude of the electrically induced contractions was higher when only atropine 1 μM and guanethidine 3.4 μM was present in the bath and there was a pronounced tonic response after ATP addition (Figure 4b).

Time course of contractions produced by ATP, carbachol, prostaglandin E_2 and $F_{2\alpha}$

The effects of PGE_2, $PGF_{2\alpha}$ and carbachol on rabbit isolated bladder are shown in Figure 5. The contractions produced by the prostaglandins had similarities with the tonic response elicited by ATP. The contractile activity induced by the prostaglandins developed slowly (beginning within about 15 to 20 s) and on it were superimposed rapid changes in tension. Compared with the contractions produced by carbachol, those elicited by the prostaglandins were slower to develop (Figure 5) and lasted longer. No rapid changes in tension were seen after carbachol.

Indomethacin 5 μM had no effect on the contractile responses elicited either by carbachol or by $PGF_{2\alpha}$ but it caused considerable reduction in spontaneous activity. The contractile effect of PGE_2 was slightly augmented (Figure 5).

Effects of atropine, phentolamine, tetrodotoxin, theophylline and nifedipine on ATP-induced contractions in rabbit detrusor

The action of ATP was probably not caused by initiation of nerve impulses in the preparation because it persisted in the presence of tetrodotoxin 0.25 μg/ml, a concentration that completely blocked the phasic response to electrical stimulation in this preparation.

To test if the phasic response to exogenous ATP was due to neuronal release of acetylcholine, atropine 0.03 μM or 1.0 μM was added (Table 1). Both the phasic and the tonic responses were unaltered by atropine.

The 2-substituted imidazoline, phentolamine, 18 μM had no effect on the response to exogenous ATP 100 μM. In a concentration of 180 μM, the α-adrenoceptor blocking drug increased the tone and spontaneous activity of the preparation, and significantly decreased both phasic and tonic responses to exogenous ATP (Table 1).

Theophylline 50 to 100 μM did not alter the ATP-induced contraction (Table 1).

Nifedipine 0.15 and 0.3 μM (which is thought to inhibit Ca^{2+} influx in smooth muscle cells during excitation) abolished the phasic response to ATP (Table 1 and Figure 6), and the tonic response was markedly reduced to 12 ± 4% and 10 ± 6% of the control. Increasing the nifedipine concentration to 1 and 1.5 μM did not abolish the tonic ATP response. The response to ATP was completely re-established on wash-out of the preparation (Figure 6). Nimodipine 0.25 μM, a more stable nifedipine analogue, had a similar blocking effect (Figure 6).

Tachyphylaxis to ATP in rabbit and guinea-pig bladder and the effect of indomethacin

Series of three electrically induced contractions were elicited at intervals of 6 to 12 min (Figures 7a and b); 3 to 5 min after each series, ATP 100 μM was added to the bath. A small decline in the magnitude of the electrically induced contractions both within and between successive series was often observed especially in experiments continued for more than 1 h. The contractile effect of ATP decreased successively (Figure 7a). Tachyphylaxis to ATP, and a more pronounced tachyphylaxis of contractions elicited by field stimulation, was seen in indomethacin 5 μM pretreated preparations (Figure 7b). In experiments where indomethacin was omitted, tachyphylaxis to

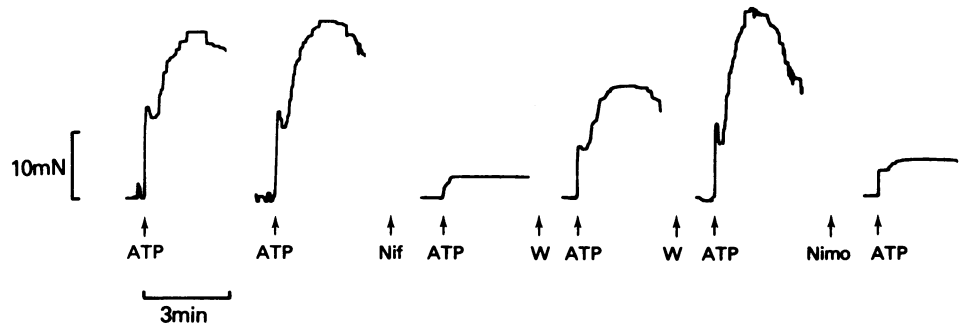

Figure 6 Effects of nifedipine (Nif) 0.3 μM and nimodipine (Nimo) 0.25 μM, 30 min contact time, on the contractile response to ATP 100 μM in rabbit urinary bladder. The nifedipine-mediated inhibition was reversible on washing (W) the preparation. Between each addition of ATP, there was a wash-out (× 3) period of 20 or 40 min.

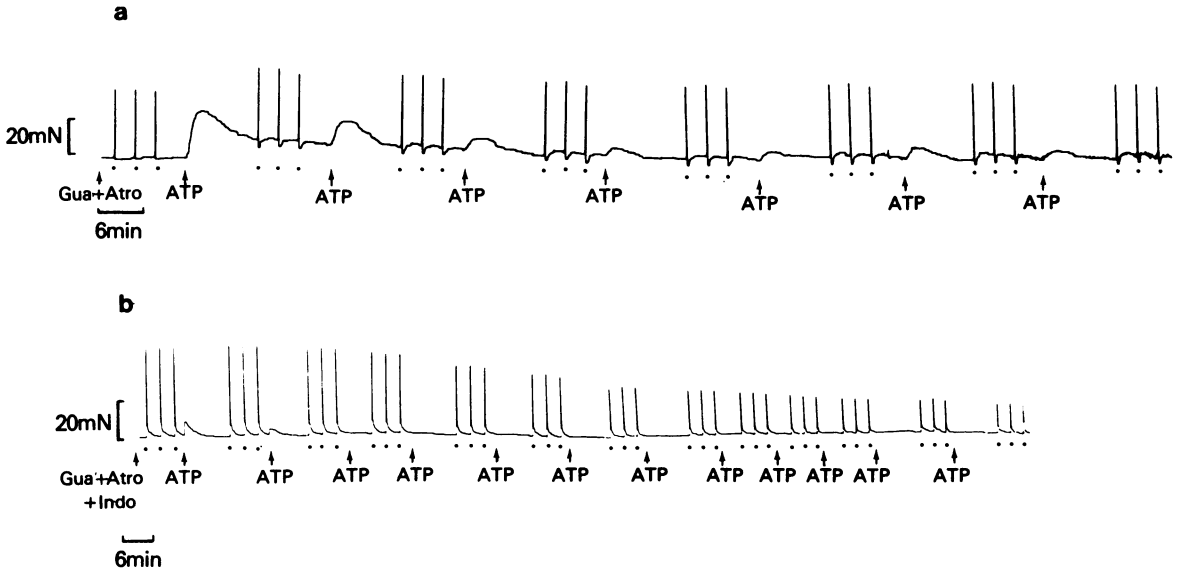

Figure 7 Effects of ATP 100 μM and electrical field stimulation on rabbit urinary bladder strips after 60 min pretreatment and in the continuing presence of (a) guanethidine (Gua) 3.4 μM and atropine (Atro) 1.0 μM, and (b) guanethidine (Gua) 3.4 μM, atropine (Atro) 1.0 μM and indomethacin (Indo) 5 μM.

ATP was followed by an increase in both tone and spontaneous activity of the preparation, whereas the response to nerve stimulation was unaffected.

Guinea-pig bladder treated in the same way as the rabbit detrusor showed similar behaviour, although no marked increase in spontaneous activity and tone was seen in experiments where indomethacin was omitted.

Discussion

Effect of indomethacin on the contractile responses to electrical nerve stimulation and to ATP

ATP effectively contracted the isolated guinea-pig and rabbit detrusors. In rabbit bladder, and to a much smaller extent in guinea-pig detrusor, the phasic ATP

response was followed by a delayed, sluggish contraction similar to those elicited by PGE_2 and $PGF_{2\alpha}$. This tonic ATP-induced response was abolished by indomethacin with only little change in the phasic response. Indomethacin in the concentrations used probably acts mainly by inhibition of prostaglandin synthesis (Andersson, Hedner & Persson 1974). Thus, the present findings suggest that the tonic ATP response is produced by prostaglandins. Kamikawa, Serizawa & Shimo (1977) showed a similar effect of indomethacin on 'rebound' contraction to exogenous ATP of longitudinal muscles of the guinea-pig digestive tract. Burnstock, Cocks, Paddle & Staszewska-Barczak (1975) found a total inhibition by indomethacin of the 'rebound' contraction following stimulation of non-adrenergic, non-cholinergic ('purinergic') inhibitory nerves in guinea-pig taenia coli. In contrast to the findings of Dean & Downie (1978a) on rabbit detrusor, Sjögren & Andersson (1979) found no inhibitory effect of indomethacin on contractions produced by ATP in guinea-pig bladder. A dual effect of ATP on rabbit detrusor was not observed by Dean & Downie (1978b). However, the marked difference in tonic ATP response between guinea-pig and rabbit detrusor might explain the observed difference concerning the effect of indomethacin.

In studies on purinergic mechanisms in the urinary bladder, guinea-pig has often been used (Ambache & Zar 1970; Burnstock *et al.*, 1978a; Burnstock, Cocks, Crowe & Kasakov 1978b; Sjögren & Andersson 1979). It has been emphasized that the bladder response to ATP closely mimics the response to non-cholinergic, non-adrenergic nerve stimulation. In the present study, a similarity was found only in indomethacin pretreated rabbit detrusor preparations.

The marked difference in contractile events on ATP addition and during non-cholinergic and non-adrenergic nerve stimulation could not be explained by the short duration of electrical stimulation, as an increase in train length from 5 to 120 s did not change the contractile pattern. However, during longer periods of nerve stimulation, an increase in spontaneous activity was constantly seen, suggesting synthesis of prostaglandins. Whether the source of ATP release during transmural nerve stimulation of isolated urinary bladder (Burnstock *et al.*, 1978b) is from hypothetical specific purinergic neurones (Burnstock 1972) or from cholinergic neurones (Silinsky & Hubbard 1973; Dowdall, Boyne & Whittaker 1974; Silinsky 1975) has not been clarified. However, both adrenergic nerves and sensory nerves (Burnstock *et al.*, 1978a) as well as extraneuronal sites have been ruled out as the source of ATP.

Whatever the source(s) of endogenous ATP, the failure of released nucleotide to stimulate synthesis of prostaglandins in amounts large enough to influence the contractile events, might be explained by the presence of enzymes in the synaptic region, which quickly metabolize both ATP and ADP (Su 1975; Westfall, Stitzel & Rowe 1978). It has been suggested that ADP, formed from degradation of ATP, stimulates prostaglandin synthesis and thus gives rise to the tonic phase of the ATP-induced contraction (Husted, Sjögren & Andersson, unpublished data).

Another explanation of the difference between the effect of exogenous and endogenously released ATP might be that exogenous ATP exerts its excitatory effect on the extrasynaptic regions of the smooth muscle fibres, as was described for isolated frog muscle (cf. Ribeiro 1978).

Actions of drugs on ATP-induced contractions

Neither the phasic nor the tonic response of rabbit detrusor to exogenous ATP was influenced by atropine, excluding the possibility of neuronal acetylcholine release as mediator of the ATP response. Similar results were obtained by Ambache & Zar (1970) on guinea-pig bladder.

In agreement with the findings of Dumsday (1971) and Ambache & Zar (1970) on guinea-pig bladder, the contractile effect of exogenous ATP was not influenced by tetrodotoxin, excluding the influence of nerve impulse generation in the preparation.

It has been argued that 2-substituted imidazoline derivatives, such as clonidine, phentolamine, and yohimbine, antagonize adenosine and adenine nucleotide effects on guinea-pig taenia coli (Tomita & Watanabe, 1973; Ambache *et al.*, 1977) and neurones in the cerebral cortex (Stone & Taylor, 1978). High concentrations of these substances increase tone and spontaneous activity in guinea-pig bladder (Burnstock *et al.*, 1978a), and guinea-pig taenia coli (Tomita & Watanabe, 1973). In agreement with observations on guinea-pig vas deferens, detrusor (Ambache *et al.*, 1977), and taenia coli (Tomita & Watanabe, 1973), phentolamine, in an α-adrenoceptor blocking concentration, did not reduce the response to exogenous ATP in rabbit detrusor. Phentolamine in a high concentration reduced both the phasic and the tonic responses and also markedly increased tone and spontaneous activity of the preparation. These unspecific effects of high concentrations of phentolamine were also emphasized by Burnstock *et al.* (1978a), Tomita & Watanabe (1973), and Ambache *et al.* (1977), but the mechanism(s) of action is still unknown.

Theophylline, in concentrations below those necessary for calcium mobilization from intracellular pools (Dönges, Heitmann, Jungbluth, Meinertz, Schmelzle & Scholz 1977) or phosphodiesterase inhibition (Daniel & Crankshaw 1974), had no effect on either the phasic or the tonic response to exogenous ATP. This is in accordance with results reported by Burnstock (1978c) and De Mey, Burnstock & Vanhoutte

(1979), who found that theophylline did not block the actions of ATP, unless it was broken down first to adenosine.

In a study on rabbit urinary bladder (Husted, Sjögren & Andersson, 1980), it was found that atropine and the inhibitors of Ca^{2+} influx in smooth muscle cells, nimodipine and nifedipine reduced the response to electrical field stimulation in a concentration-dependent way. The maximum inhibition was 40%, 69%, and 58% respectively, of the control response. When atropine was added cumulatively to nimodipine pretreated strips, the maximum inhibitory effect was raised to 100%. These results agree with those obtained in the present experiments, where the atropine-resistant part of the response to electrical nerve stimulation seemed to be dependent on Ca^{2+} influx.

Time course of contractions produced by ATP, carbachol, prostaglandin E_2 and $F_{2\alpha}$

Confirming the results of Hills (1976), and in agreement with previous observations on urinary bladder from man (Andersson, Ek & Persson 1977) and guinea-pig (Ambache & Zar 1970), a contractile effect of PGE_2 and $PGF_{2\alpha}$ was found in rabbit detrusor. Both compounds produced a delayed sluggish contraction with superimposed, rapid tension changes. This response had similarities with the tonic contraction induced by exogenous ATP. Generally, the sensitivity of the preparation to PGE_2 was higher than to $PGF_{2\alpha}$, a result in agreement with that found in human detrusor (Andersson *et al.*, 1977). The responses to PGE_2 and $PGF_{2\alpha}$ were more slowly developing and of longer duration than those evoked by carbachol, but they were all unaffected by indomethacin. Further, the spontaneous activity, which was abolished by indomethacin, was re-established when PGE_2 and $PGF_{2\alpha}$ were added.

By means of bioassay and thin-layer chromatography of the bathing fluid, Bultitude, Hills & Shuttleworth (1976) found evidence for release of prostaglandin-like (E type) material during electrically induced contraction of the rabbit urinary bladder. Needleman, Minkes & Douglas (1974) showed that ATP induced synthesis of prostaglandins. It is possible that prostaglandin synthesis and release during field stimulation is mediated by release of ATP. The prostaglandins may either contribute to the resultant contractile response (Johns & Paton 1976; 1977), or modulate the release of transmitter(s) (Wennmalm 1971), or the effectiveness of the transmitter (Choo & Mitchelson 1977; Burnstock *et al.*, 1978a).

Tachyphylaxis and the effect of indomethacin

As pointed out by Burnstock *et al.* (1978a), desensitization of the guinea-pig bladder to ATP in the presence of indomethacin was accompanied by a progressive decrease in response to transmural nerve stimulation; this was also observed in the present experiments. With no indomethacin present, the response to field stimulation was unchanged, even though tachyphylaxis to ATP had developed.

Similar results were obtained in rabbit detrusor. It thus seems plausible that the presence (release) of prostaglandins plays a role in the size of the electrically induced contractions (Burnstock *et al.*, 1978a), while tachyphylaxis to exogenous ATP is still present. This may be caused by adenosine accumulation (Hayashi, Kunimoto, Mori, Shinozuka & Yamada 1978), which might decrease the contractile response to exogenous ATP (Sjögren & Andersson 1979).

Thus, the present results suggest that ATP may play a role in bladder contractions. The involvement of prostaglandins in this respect needs further clarification.

The skilful technical assistance of Kirsten Aggerholm and the excellent secretarial work of Birgitta Dahlbo and Lena Lindstrand are gratefully acknowledged.

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(Received October 1, 1979.
Revised January 11, 1980.)