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EFFECTS OF ADENOSINE 5′-TRIPHOSPHATE (ATP) AND β-γ-METHYLENE ATP ON THE RAT URINARY BLADDER

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1 High concentrations of adenosine 5′-triphosphate (ATP, 100 to 1000 μM) were required to cause contraction of the rat urinary bladder, while adenosine and adenosine 5′-monophosphate (AMP, 1 to 50 μM) produced relaxation.

2 One hundred fold lower concentrations of β-γ-methylene ATP, which is resistant to degradation to AMP and adenosine, caused dose-dependent, phasic contractions which mimicked atropine-resistant responses to nerve stimulation.

3 Adenosine and AMP caused dose-dependent inhibition of carbachol-induced contractions; theophylline competitively antagonized this inhibition but not the contractile responses to β-γ-methylene ATP, ATP or atropine-resistant nerve stimulation.

4 These results suggest that the insensitivity of the rat bladder to ATP is due to its rapid degradation to AMP and adenosine and support the hypothesis that the bladder receives a purinergic excitatory innervation.

Introduction

Contraction of the mammalian urinary bladder to parasympathetic nerve stimulation is largely resistant to block by atropine (Langley & Anderson, 1895; Henderson, 1923; Henderson & Roepke, 1934; 1935; Ambache, 1955; Ursillo, 1961; Hukovic, Rand & Vanov, 1965; Ambache & Zar, 1970). One explanation of this phenomenon is that the bladder receives a non-cholinergic as well as a cholinergic excitatory innervation (Ambache & Zar, 1970; Dumsday, 1971; Burnstock, Dumsday & Smythe, 1972). Evidence has been presented for adenosine 5′-triphosphate (ATP) as the non-cholinergic transmitter in the guinea-pig bladder (Burnstock, *et al*, 1972; Burnstock, Cocks, Crowe & Kasakov, 1978) and rabbit bladder (Downie & Dean, 1977). However, in the rat bladder, high concentrations of ATP cause only weak contractions (Burnstock, *et al*, 1972; Clark & Mitchelson, 1974). This insensitivity of the rat bladder to ATP has recently been taken as evidence against ATP being the non-cholinergic transmitter (Clark & Michelson 1974; Ambache, Killick & Woodley, 1977) since an important criterion for establishing a neurotransmitter is that the response to nerve stimulation is closely mimicked by the response to exogenous application of the substance (Eccles, 1964).

In the present study, the sensitivity of the rat bladder to ATP has been re-examined and in particular compared with that to β-γ-methylene ATP (termed here APPCP), which is resistant to degradation.

Methods

Male Wistar rats (200 to 250 g) were stunned by a blow to the back of the head and exsanguinated. Thin strips of the detrusor of the bladder were prepared by the method of Ambache & Zar (1970) for the guinea-pig bladder. The tissues were incubated in 20 ml organ baths containing modified Krebs solution (Bülbring, 1953) at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. The muscles were initially stretched to a tension of 0.25 g and contractions recorded by a Grass FT10 isometric transducer and displayed on a Grass polygraph.

In those experiments in which the responses to field stimulation were examined, the muscles were threaded through a pair of ring platinum electrodes. Stimulation was by 0.2 ms pulses at supramaximal voltage at the frequencies given in the text; in these experiments the Krebs solution contained both atropine (1.4 μM) and guanethidine (3.4 μM) to eliminate muscarinic and adrenergic responses.

Since the bladder has low tone, relaxations to adenosine and its derivatives were demonstrated as

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a reduction in the contractile response to a test dose of carbachol (0.1 to 0.5 μM). The purine compound was added 30 s before the addition of carbachol and the contraction then measured. Responses were calculated as the percentage reduction in the carbachol-induced contraction from the control group. Log dose-percentage maximum response curves were constructed for the inhibitory agonists by calculating the mean ± s.e. mean of the effective concentrations required to give a certain percentage of maximum response. This avoids biassing the curves towards a lower slope (Waud, 1975).

The following drugs were used: adenosine (Sigma), adenosine 5′-monophosphate (AMP, Sigma), adenosine 5′-triphosphate (ATP, Sigma), atropine sulphate (Antigen Ltd), carbamylcholine chloride (carbachol, Sigma), guanethidine sulphate (Ciba), indomethacin (Sigma), β-γ-methylene adenosine triphosphate (APPCP, Sigma), theophylline (Sigma). Doses are given as final molar concentrations in the organ bath unless otherwise stated.

Results

Responses to ATP

ATP had no effect on the rat bladder muscle until concentrations of 100 μM were reached. This and higher concentrations of ATP caused weak phasic contractions which were rapid in onset, but faded during maintained contact with the tissue (Figure 1a).

In preliminary experiments, low concentrations of ATP (1 to 100 μM) were found to inhibit carbachol-induced contractions although in higher concentrations within this range (10 to 100 μM) ATP sometimes potentiated these contractions. ATP is a potent stimulant of prostaglandin synthesis in a wide range of tissues (Needleman, Minkes & Douglas, 1974; Burnstock, Cocks, Paddle & Staszewska-Barczak, 1975) and prostaglandins have been shown to potentiate the contractile response to intramural nerve stimulation in the bladder of the guinea-pig (Burnstock *et al.*, 1978) and rat (Choo & Mitchelson, 1977). Therefore to test whether the potentiating effect of ATP on carbachol-induced contractions was due to stimulation of prostaglandin synthesis, the experiments were repeated in the presence of the prostaglandin synthesis inhibitor, indomethacin. Under those conditions low concentrations of ATP (1 to 100 μM) always caused dose-related inhibition of carbachol-induced contractions (see Methods) with a threshold of about 1 μM and a maximum of 100 μM (Figure 2). This inhibition was antagonized by theophylline (50 μM) which produced a parallel shift in the dose-response curve with no change in the maximum (Figure 2). Theophylline had no effect on the contractions produced by high concentrations of ATP.

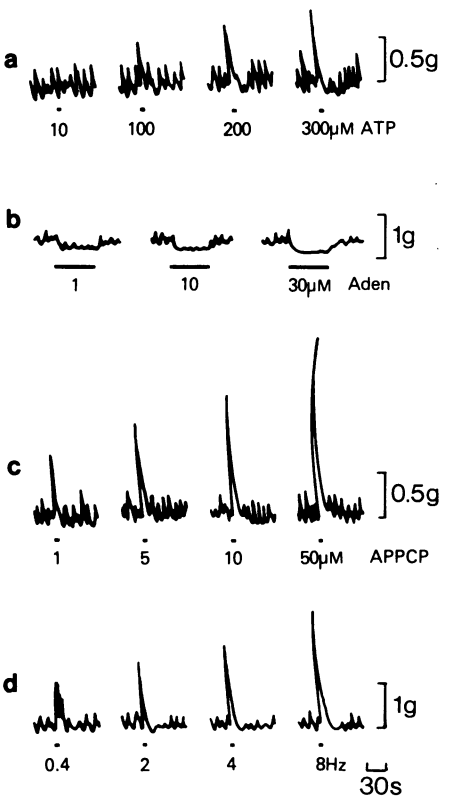

Figure 1 Responses of the rat isolated bladder to (a) ATP, (b) adenosine (Aden), (c) β-γ-methylene ATP (APPCP) and (d) intramural nerve stimulation with 0.2 ms duration pulses delivered at supramaximal voltage of 30 to 50 V for 10 s at the frequencies indicated. Guanethidine (3.4 μM) was present throughout and in (d) atropine (1.4 μM) was added to the bath 60 min before stimulation. Note that the time scale is the same in all traces.

Responses to adenosine and AMP

Adenosine and AMP caused slow relaxations of the rat bladder which once fully developed were maintained for prolonged periods but were rapidly reversed on washing. The effect of adenosine is illustrated in Figure 1b. The inhibitory effects of both adenosine and AMP were also measured as the percentage reduction of the contractions produced by a test dose of carbachol. These reductions were dose-related with a threshold of about 1 μM and a maximum of 30 μM. The inhibitory effects of adenosine and AMP were antagonized by theophylline which

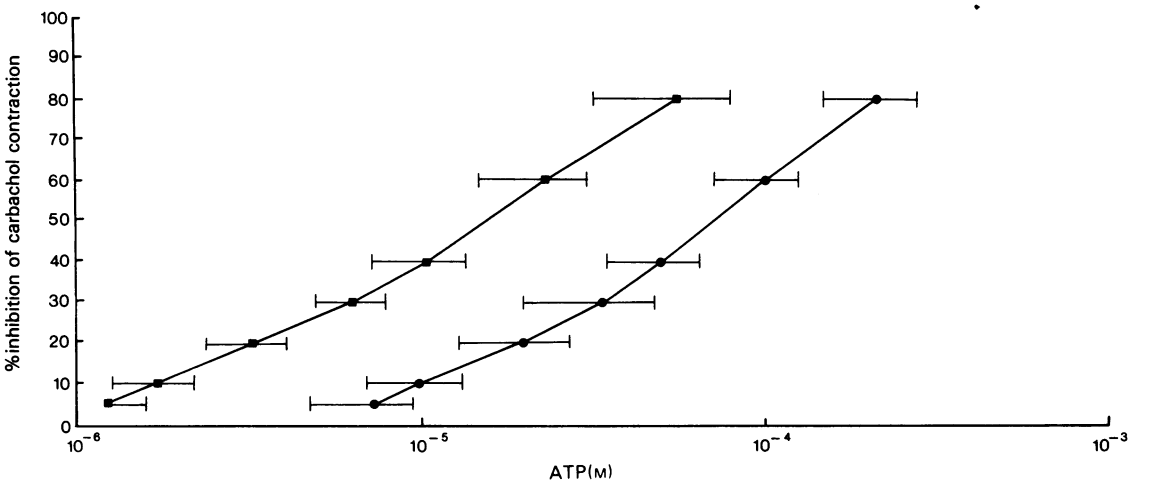

Figure 2 Log dose-response curves to low concentrations of ATP in the rat isolated bladder. Inhibitory responses to ATP were calculated as the percentage inhibition of carbachol contractions (see Methods). (■) Control; (●) after incubation for 30 min with theophylline (50 μM). Horizontal lines show s.e. means. Guanethidine (3.4 μM) and indomethacin (5 μM) were present throughout.

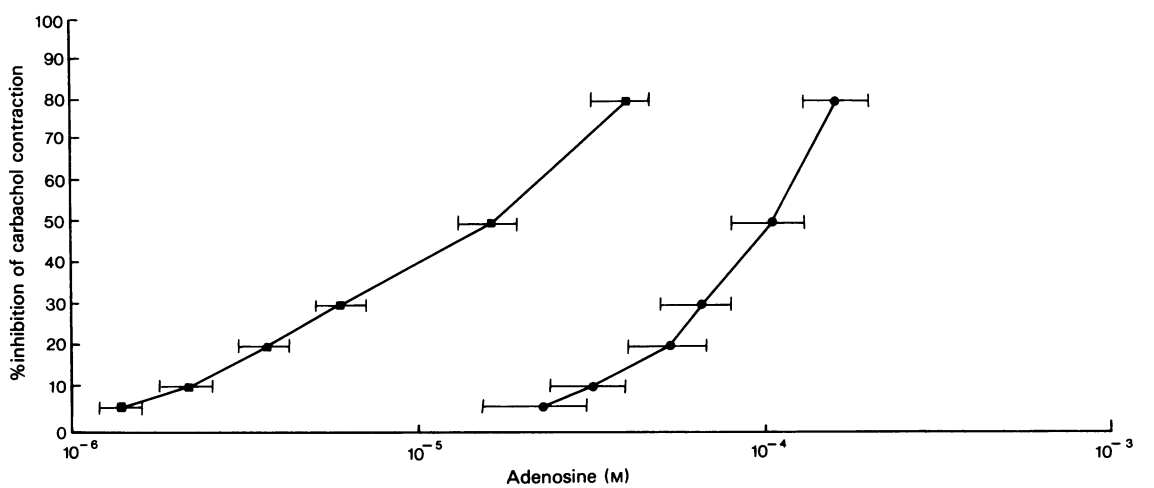

Figure 3 Log dose-response curves to adenosine in the rat isolated bladder. Inhibitory responses to adenosine were calculated as the percentage inhibition of carbachol contractions (see Methods). (■) Control; (●) after incubation for 30 min with theophylline (50 μM). Horizontal lines show s.e. means. Guanethidine (3.4 μM) was present throughout.

produced a parallel shift in a dose-response curve with no change in the maximum. The results with adenosine are illustrated in Figure 3.

Responses to β-γ-methylene ATP (APPCP)

Low concentrations of APPCP produced rapid dose-related contractions which reached maxima in 2 to 3 s, but faded during maintained contact (Figure 1c). The threshold concentration was about 1 μM with a maximum of 30 μM. In accordance with the higher sensitivity of the tissue to this drug the maximum response was significantly greater than that for ATP. Incubation with indomethacin (5 μM) for 60 min caused a reduction of 10 to 20% in the APPCP response, but theophylline did not antagonize the contractile responses to APPCP and no parallel shift in the dose-response curve was observed (Figure 4). Sub-

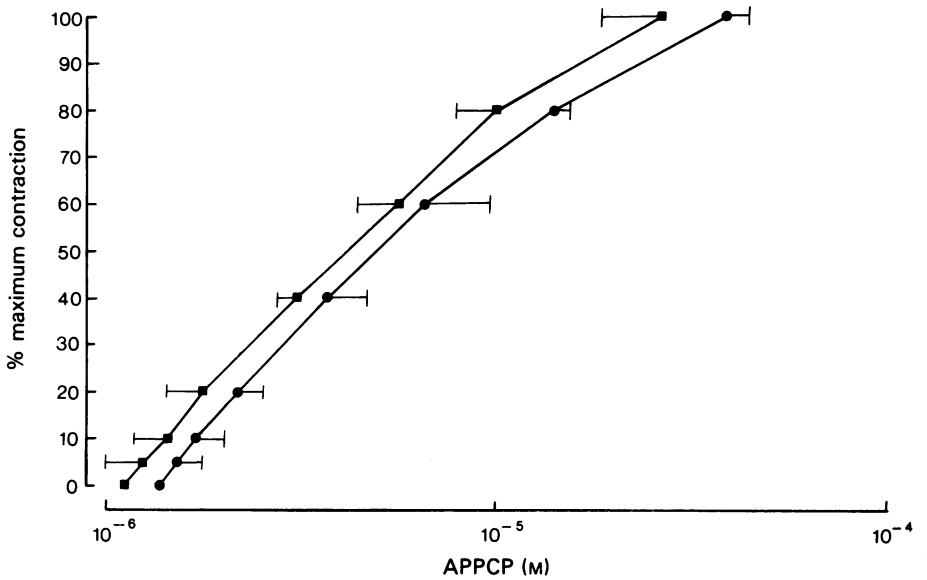

Figure 4 Log dose-response curves to β-γ-methylene ATP (APPCP) in the rat isolated bladder. (■) Control; (●) after incubation for 30 min with theophylline (50 μM). Horizontal lines show s.e. means. Guanethidine (3.4 μM) was present throughout.

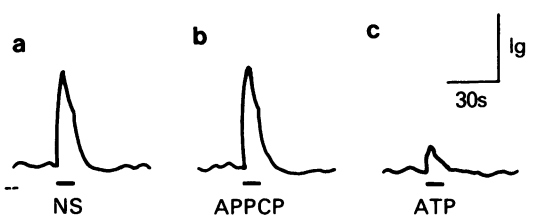

Figure 5 Comparison of the excitatory response of the rat isolated bladder to (a) intramural nerve stimulation (NS: 0.1 ms duration pulses delivered at 5 Hz and supramaximal voltage (50 V) for 10 s); (b) β-γ-methylene ATP (APPCP, 1 μM) and (c) ATP (200 μM). Atropine (1.4 μM) and guanethidine (3.4 μM) were present throughout.

threshold doses of APPCP did not affect carbachol-induced contractions.

Responses to electrical field stimulation

In the presence of both atropine (1.4 μM) and guanethidine (3.4 μM), electrical field stimulation of the bladder (0.2 ms duration pulses and supramaximal voltage for periods up to 10 s) produced rapid, phasic contractions which were similar to those in response to APPCP and high concentrations of ATP (Figure 5). The magnitude of contraction increased with increasing frequency of stimulation (0.2 to 30 Hz) (Figure 1d). These responses were unaffected by theophylline (50 μM).

Discussion

The rat urinary bladder is approximately 100 times more sensitive to APPCP than to ATP. APPCP has a methylene group (—CH_2—) inserted between the end two phosphate groups of ATP which results in resistance to degradation to AMP and adenosine. Therefore, rapid breakdown of exogenously applied ATP may be an important factor contributing to its weak agonistic action in the rat bladder.

Extracellular degradation of ATP to adenosine is possible via enzymes such as ATPases, nucleotide pyrophosphatase, alkaline phosphatases and 5'-nucleotidase. These enzymes are situated in the plasma membrane of several cell types with their active sites facing the extracellular space (Dunkley, Manery & Dryden, 1966; Woo & Manery, 1973; 1975; De Pierre & Karnovsky, 1974; Trams & Lauter, 1974; Abney, Evans & Parkhouse, 1976). Haugen & Skrede (1977) recently demonstrated high levels of several of these ectoenzymes throughout the gastrointestinal tract of both man and rat. To our knowledge there is no information available about the rate of degradation of extracellular nucleotides in smooth muscle. However,

Paddle & Burnstock (1974) found rapid degradation of ATP upon passage through the coronary vasculature.

Two consequences of extracellular breakdown of ATP need to be considered. The first is that unlike ATP which contracts the bladder, both adenosine and AMP were found to cause a competitive slow, maintained relaxation. The second consequence of ATP breakdown is the reduction of the concentration of ATP at receptor sites. It seems unlikely that the insensitivity of the rat bladder to ATP is due to physiological antagonism by the inhibitory breakdown products AMP or adenosine. Low doses of theophylline, which competitively antagonized the inhibitory responses to adenosine and AMP but not the contractile response to ATP or APPCP (see also Burnstock, 1978), did not potentiate the responses to either intramural nerve stimulation or ATP. Furthermore, the responses to both intramural nerve stimulation and ATP are rapid in onset and reach peak contraction within 2 to 3 s, whereas the relaxations to AMP and adenosine are slow in onset and reach maximum after 30 s. Therefore, it is not surprising that theophylline did not potentiate these responses since any accummulation of inhibitory breakdown products during either response would not have an appreciable effect before peak contraction was attained. Thus a more likely explanation is that the responses appear to be critically dependent on the initial high concentration of the transmitter or agonist reached at the receptor.

The ability of low concentrations of ATP to block contractions to carbachol when it is applied at least 30 s before the addition of carbachol, could be explained by breakdown of ATP to AMP and adenosine which have an inhibitory action. The finding that theophylline competitively antagonizes this effect supports this suggestion.

The possibility should be considered that ATP and APPCP act indirectly by inducing prostaglandin synthesis (Needleman *et al.*, 1974; Burnstock *et al.*, 1975). Prostaglandins have been implicated in the contractile response to non-cholinergic nerve stimulation in the bladder of the rat (Choo & Mitchelson, 1977), and rabbit and monkey (Johns & Paton, 1977). However, this is an unlikely explanation for the APPCP contraction in the rat bladder, since indomethacin treatment only inhibited it by 10 to 20%.

Although the rat bladder is insensitive to ATP, the demonstration that low concentrations of APPCP mimic the excitatory responses to non-adrenergic, non-cholinergic nerve stimulation supports the proposal that the rat urinary bladder receives a purinergic excitatory innervation.

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