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Desensitization of the guinea-pig urinary bladder by the enantiomers of adenylyl 5′-(β,γ-methylene)-diphosphonate and by substance P

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1 Adenosine 5′-triphosphate (ATP), substance P (SP) and non-cholinergic nerve stimulation contracted the guinea-pig urinary bladder. SP and two poorly-degradable analogues of ATP, the enantiomers of adenylyl 5′-(β,γ-methylene)-diphosphonate (AMP-PCP and L-AMP-PCP), were used to desensitize the guinea-pig bladder.

2 Desensitization of the bladder by AMP-PCP (50 μM) or by L-AMP-PCP (50 μM) abolished the responses to ATP, and inhibited the responses to non-cholinergic nerve stimulation and to SP. The responses to histamine were unaffected.

3 Desensitization by SP (1 μM) inhibited the responses to SP itself, but not the responses to ATP, L-AMP-PCP or non-cholinergic nerve stimulation.

4 These results suggest that SP may act partly by releasing ATP, and support the suggestion that ATP rather than SP is the non-cholinergic stimulatory transmitter.

Introduction

In the guinea-pig urinary bladder adenosine 5′-triphosphate (ATP) causes contraction and adenosine inhibits contraction, and ATP has been suggested as the transmitter released from non-cholinergic stimulatory nerves (Burnstock *et al.*, 1972; 1978), although this suggestion has been challenged (Ambache *et al.*, 1977). In the absence of a competitive, specific ATP antagonist, desensitization has been used to investigate this problem. The use of ATP itself as the desensitizing ligand has led to conflicting results (Ambache & Zar, 1970; Ambache *et al.*, 1977; Dean & Downie, 1978), which have been attributed to the synthesis of prostaglandins induced by ATP (Burnstock *et al.*, 1978) and to the breakdown of ATP to adenosine, which is inhibitory (Lukacsko & Krell, 1981; 1982).

Analogues of ATP in which one of the ester oxygen linkages in the triphosphate chain has been replaced by a methylene linkage are less easily degradable than ATP, and apparently do not cause the synthesis of prostaglandins (Brown & Burnstock, 1981). Recently Kasakov & Burnstock (1983) used adenosine 5′-(α,β-methylene)-triphosphonate to desensitize the guinea-pig bladder, and found a greatly decreased response to ATP and to nerve stimulation, but only a slightly diminished response to histamine and to acetylcholine. Since adenosine 5′-(α,β-methylene)-triphosphonate is potentially capable of being broken down to adenosine, a better desensitizing ligand would be a poorly degraded analogue of L-adenosine 5′-triphosphate (L-ATP), such as L-adenylyl 5′-(β,γ-methylene)-diphosphonate (L-AMP-PCP). This is even more potent at contracting the guinea-pig bladder than its enantiomer adenylyl 5′-(β,γ-methylene)-diphosphonate (AMP-PCP), and is broken down even more slowly, if at all, its potential breakdown product, L-adenosine, being completely inactive as an inhibitor of the guinea-pig bladder (Cusack & Hourani, 1984).

The peptide substance P (SP) also causes contraction of the guinea-pig bladder, and SP-like immunoreactivity has been found throughout the bladder (Alm *et al.*, 1978). SP is probably not the stimulatory transmitter, however, because SP analogues which act as antagonists in other tissues do not block the response to nerve stimulation, although interpretation of these results is complicated by the finding that these analogues act as partial agonists in the guinea-pig bladder, rather than as pure antagonists (Husted *et al.*, 1981; Leander *et al.*, 1981).

SP and the enantiomers of AMP-PCP were used to desensitize the guinea-pig bladder to investigate the possibility that ATP or SP (or both) may be the neurotransmitter released from stimulatory nerves in

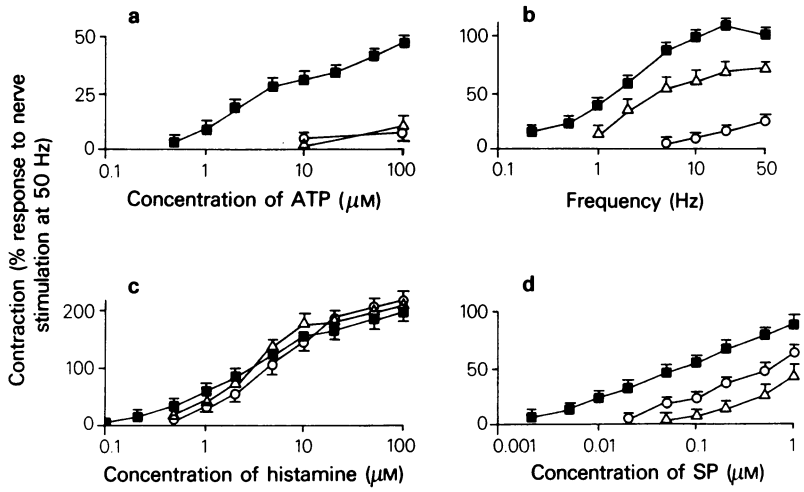

Figure 1 Contraction of the guinea-pig bladder by (a) ATP, (b) nerve stimulation, (c) histamine or (d) substance P (SP) before (■) or after desensitization with AMP-PCP (50 μM) (△) or L-AMP-PCP (50 μM) (○). Each point is the mean of at least 10 determinations using bladder strips from at least 5 different guinea-pigs. Vertical bars show the standard errors.

the guinea-pig bladder, and to see whether there is any interaction between the effects of these two putative transmitters.

Methods

Male albino guinea-pigs (300–500 g) were stunned by a blow to the head and exsanguinated. Mucosa-free detrusor strips (10 × 2 mm) were prepared from the urinary bladder and set up as described in the preceding paper (Cusack & Hourani, 1984). Stimulation of non-adrenergic, non-cholinergic nerves was also carried out as described in that paper. Drugs were added to the Krebs solution, and passed through the superfusion apparatus which had no glass components, the warming coil being a length of fine bore stainless steel tubing. Drug application or nerve stimulation was maintained until the tension of the detrusor strips had reached a peak and declined, or in the case of SP and histamine (which have a very prolonged action) had achieved a plateau.

Concentration-response curves or frequency-response curves were obtained non-cumulatively, with at least 10 min washout between drug applications or nerve stimulations, and up to 30 min after high concentrations of agonists. All contractions were expressed as a percentage of the mean of three consecutive contractions induced by nerve stimulation at 50 Hz at the beginning of each experiment.

Desensitization of the detrusor strips was achieved by superfusion with SP (1 μM) for 2 min or with AMP-PCP (50 μM) or L-AMP-PCP (50 μM) for 30 s, followed by a 3 min washout to allow the muscle to relax before challenging with nerve stimulation or with an agonist. This treatment abolished or greatly reduced the response to a second challenge with the desensitizing drug, and in the case of the enantiomers of AMP-PCP completely inhibited the spontaneous contractions of the muscle. The desensitization was reversible in each case, full recovery of the response to the desensitizing agonist being achieved after 30 min washout.

Drugs

ATP, AMP-PCP, SP and atropine sulphate were obtained from Sigma, London. Guanethidine monosulphate was obtained from Ciba Laboratories, Horsham. L-AMP-PCP was a generous gift from Dr N.J. Cusack, and had been synthesized as described by Cusack *et al.* (1983).

SP was dissolved in 0.01 M acetic acid at an initial concentration of 500 μM, and stored frozen in aliquots. Each day a new aliquot was thawed out and a range of dilutions was made in 0.01 M acetic acid, which were further diluted 100 times in Krebs solution just before use. Solutions of SP were not allowed to come into contact with any glass, to which SP binds avidly, and were mixed gently to avoid oxidation (see Stewart, 1983).

Results

Desensitization of the guinea-pig bladder with the enantiomers of AMP-PCP (50 μM) completely blocked the responses to ATP (Figure 1a) and inhibited the responses to non-cholinergic nerve stimulation, L-AMP-PCP being more inhibitory than AMP-PCP (Figure 1b). Responses to histamine were unaffected (Figure 1c), but responses to SP were inhibited, AMP-PCP being more inhibitory than L-AMP-PCP (Figure 1d). Representative polygraph tracings demonstrating the effect of L-AMP-PCP are shown in Figure 2.

Desensitization of the guinea-pig bladder with SP (1 μM), which inhibited the responses to SP itself (Figure 3a), had no effect on the responses to L-AMP-PCP (Figure 3b) and potentiated the responses to ATP (Figure 3c) and to non-cholinergic nerve stimulation (Figure 3d).

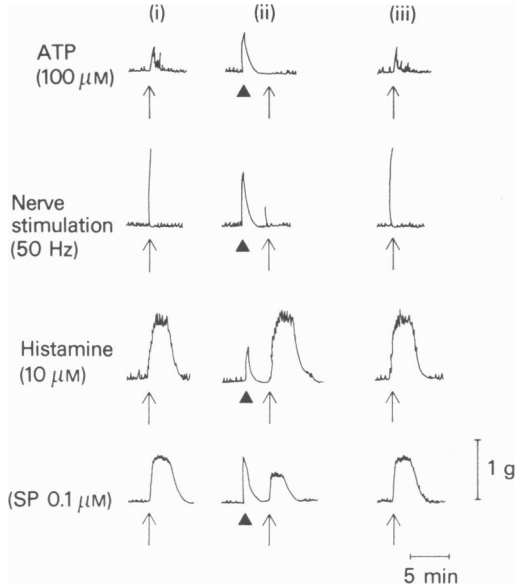

Figure 2 Desensitization of the guinea-pig bladder by L-AMP-PCP. The tissue was challenged with ATP, electrical stimulation, histamine or substance P (SP) at the times indicated by the arrows, either (i) before or (ii) 3 min after or (iii) 30 min after superfusing with L-AMP-PCP (50 μM) (▲) for 30 s. An upward deflection indicates a contraction.

Discussion

These results show that both enantiomers of AMP-PCP desensitized the guinea-pig bladder to ATP, and that this desensitization was not a non-specific inhibition because the responses to histamine were unaffected. The responses to non-cholinergic nerve stimulation were also inhibited, which is evidence in favour of the hypothesis that ATP might be the transmitter released from non-cholinergic nerves (Burnstock *et al.*, 1978). L-AMP-PCP was more effective at inhibiting the responses to nerve stimulation than was AMP-PCP, probably because it is a more potent agonist (Cusack & Hourani, 1984).

That the guinea-pig bladder can be specifically

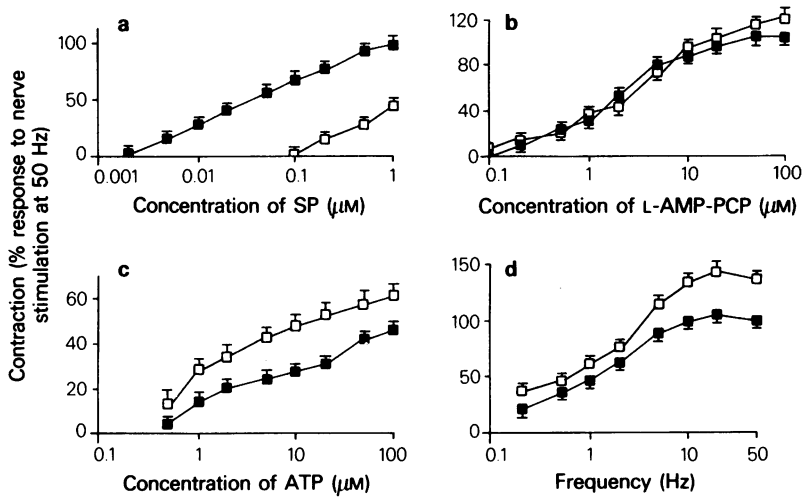

Figure 3 Contraction of the guinea-pig bladder by (a) substance P (SP), (b) L-AMP-PCP, (c) ATP or (d) nerve stimulation, before (■) or after (□) desensitization with SP (1 μM). Each point is the mean of at least 10 determinations using bladder strips from at least 5 different guinea-pigs. Vertical bars show the standard errors.

desensitized by a poorly-degradable ATP analogue agrees with the results of Kasakov & Burnstock (1983), who showed that adenosine 5'-(α,β-methylene)-triphosphonate could block the responses to ATP but not to histamine or to acetylcholine. These authors used only one concentration of histamine, but the full concentration-response curves presented here show that histamine was unaffected over a wide range of concentrations.

Unlike the responses to histamine, the responses to SP were inhibited by desensitization by the enantiomers of AMP-PCP. As L-AMP-PCP could only give rise to the inactive L-adenosine (Cusack & Hourani, 1984), this inhibition cannot be due merely to the formation of adenosine, although this could explain why AMP-PCP is slightly more inhibitory than L-AMP-PCP. The cross-desensitization must therefore imply some interaction between ATP and SP.

Desensitization of the guinea-pig bladder by SP did not inhibit the responses to ATP, L-AMP-PCP or nerve stimulation. This lack of effect on the responses to nerve stimulation does not support the suggestion that SP is the neurotransmitter released from non-cholinergic nerves, and is in agreement with the results reported by Husted *et al.* (1981) and Leander *et al.* (1981), who found that analogues of SP with some inhibitory properties had no effect on the responses to nerve stimulation in the bladder.

That desensitization of ATP receptors resulted in a decreased response to SP but that the reverse is not true, suggests that ATP must be in some way involved in the response of the guinea-pig bladder to SP. One possibility is that SP induces the release of ATP, in a similar way to its action in the guinea-pig ileum, where SP is thought to release acetylcholine (Chahl, 1982). The responses of the guinea-pig bladder to nerve stimulation and to ATP, but not to the poorly-degraded L-AMP-PCP, were potentiated by desensitization with SP, possibly because ATP released by SP slows down the breakdown of ATP subsequently added exogenously or released from non-cholinergic nerves.

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