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Some pharmacological and biochemical interactions of the enantiomers of adenylyl 5′-(β, γ-methylene)-diphosphonate with the guinea-pig urinary bladder

N.J. Cusack & S.M.O. Hourani

Department of Pharmacology, University of London King's College, Strand, London WC2R 2LS

1 Adenosine 5′-triphosphate (ATP) and adenylyl 5′-(β,γ methylene)-diphosphonate (AMP-PCP) both contracted the guinea-pig urinary bladder, but the response to AMP-PCP was much greater. We synthesized the enantiomer of AMP-PCP, L-adenylyl 5′-(β,γ-methylene)-diphosphonate (L-AMP-PCP), and tested it on the guinea-pig bladder.

2 L-AMP-PCP contracted the guinea-pig bladder, and was more potent than AMP-PCP and much more potent than ATP. The potential breakdown product of L-AMP-PCP, L-adenosine, unlike adenosine (the breakdown product of AMP-PCP), did not inhibit contractions of the guinea-pig bladder.

3 ATP and its enantiomer L-adenosine 5′-triphosphate (L–ATP) were rapidly degraded by the muscle, and AMP-PCP was also degraded, but more slowly. L-AMP-PCP, however, was completely resistant to degradation.

4 L-AMP-PCP would appear to be a useful ATP analogue, as it is potent and resistant to degradation, and its potential breakdown product, L-adenosine, is inactive.

Introduction

The pharmacological actions of adenosine 5′-triphosphate (ATP) on many smooth muscle systems may be complicated by the breakdown of ATP to adenosine, which is less active than, or in some cases has the opposite action to ATP (Burnstock & Brown, 1981; Maguire & Satchell, 1981). This complication has been partly overcome by the use of analogues which are thought to be more resistant to enzymatic degradation by the tissue, such as adenylyl 5′-(β,γ-methylene)-diphosphonate (AMP-PCP), in which one of the ester oxygen linkages between the phosphate groups has been replaced by a methylene linkage (Maguire & Satchell, 1979).

In the guinea-pig bladder ATP causes contraction, but is not a very potent agonist (Ambache & Zar, 1970; Ambache *et al.*, 1977), and this has been attributed to its rapid degradation to adenosine, which inhibits contraction of the guinea-pig bladder (Burnstock *et al.*, 1972; Lukacsko & Krell, 1981). ATP and adenosine act on separate purinoceptors, which have been called P_2 and P_1 respectively (Burnstock, 1978). P_1 receptors in other tissues are stereospecific, the unnatural enantiomer of adenosine, L-adenosine, being completely inactive (Cusack *et al.*, 1979; Cusack & Planker, 1979; Brown *et al.*, 1982). P_2-receptors, on the other hand, display only limited stereoselectivity (Cusack & Planker, 1979), and in the guinea-pig bladder the unnatural enantiomer of ATP, L-adenosine 5′-triphosphate (L-ATP), is almost equipotent with ATP (Burnstock *et al.*, 1983).

The enantiomer of AMP-PCP, L-adenylyl 5′-(β,γ-methylene)-diphosphonate (L-AMP-PCP), might prove to be a very useful P_2-receptor agonist in the bladder, as it seems likely to combine high potency with resistance to degradation, and its potential breakdown product, L-adenosine, is probably inactive. We therefore synthesized L-AMP-PCP, and investigated the pharmacological effects of AMP-PCP and L-AMP-PCP on the guinea-pig isolated urinary bladder, and compared the degradation of these two analogues by the bladder with the degradation of ATP and L-ATP.

Methods

Pharmacological studies

Male albino guinea-pigs (300 to 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 attached by thread to a rigid support. The preparations were superfused with modified Krebs solution of the following ionic composition (mM): NaCl 120, KCl 5.9, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, NaH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.5, containing atropine (1 μM) and guanethidine (3.4 μM). The Krebs solution was gassed with 95% O_2 and 5% CO_2 and warmed to 35°C, and flow rate was 1 ml min^{-1}. The detrusor strips were initially mounted under 1 g tension, and mechanical activity was recorded isometrically with a Grass FT10C transducer and displayed on a Grass 79C polygraph. The preparations were equilibrated for 60 min before addition of drugs or nerve stimulation.

Non-adrenergic, non-cholinergic nerves were stimulated electrically via platinum ring electrodes by means of a Grass SD9 stimulator at a voltage of 40 V with a pulse duration of 0.3 ms and a range of frequencies from 0.2 to 50 Hz, applied in a decreasing order throughout the experiment. Drugs were added to the Krebs solution and passed through the superfusion apparatus and drug application or nerve stimulation was maintained until the tension of the detrusor strips had reached a maximum and declined. 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 are expressed as a percentage of the mean of three consecutive contractions induced by nerve stimulation at 50 Hz at the beginning of each experiment.

To investigate the effect of adenosine or L-adenosine on the responses of the detrusor strips to nerve stimulation, the preparations were superfused with adenosine or with L-adenosine at a concentration of 100 μM for 90 s before stimulating non-adrenergic, non-cholinergic nerves. For each frequency of stimulation, a control response was obtained, followed after washout by a response in the presence of adenosine and then, after another washout, by a response in the presence of L-adenosine.

Degradation studies

Guinea-pig detrusor strips prepared as described above were equilibrated for at least 60 min at 35°C in Krebs solution containing atropine and guanethidine, the solution being replaced every 10 min. Each strip was then placed in a small tube with Krebs solution (600 μl) at 35°C, containing ATP, L-ATP, AMP-PCP or L-AMP-PCP (100 μM). At various time intervals, aliquots (70 μl) were withdrawn and frozen for later analysis by high performance liquid chromatography (h.p.l.c.). As a control for non-enzymatic breakdown, ATP (100 μM) was incubated in the Krebs solution for 60 min at 35°C without a muscle strip. As a control for the possible release of nucleotides or enzymes, one muscle strip was incubated at 35°C for 60 min in Krebs solution alone, and an aliquot (70 μl) was then taken for analysis by h.p.l.c. The muscle strip was removed and ATP was added at a final concentration of 100 μM, and the incubation was continued for a further 60 min, after which another aliquot (70 μl) was taken for analysis.

Degradation of nucleotides was followed by ion-exchange h.p.l.c. as described by Cusack *et al.* (1983), using a Whatman Partisil 10-SAX column eluted with a linear gradient (0.01 to 1 M KH_2PO_4, 2 ml min^{-1}, over 15 min). Samples (60 μl) of the aliquots were directly applied to the column after a brief centrifugation at 12000 *g* in an Eppendorf centrifuge. The separated nucleosides and nucleotides were detected by ultraviolet absorption at 259 nm, and their concentrations were estimated by measuring the areas under the corresponding peaks displayed on a chart recorder, and expressed as a percentage of the area of the peak for the starting material. These areas were linearly related to concentration up to 100 μM, and the lower limit of detection was 2 μM.

Drugs

Adenosine, ATP, AMP-PCP and atropine sulphate were obtained from Sigma, London. Guanethidine monosulphate was obtained from Ciba Laboratories, Horsham. L-Adenosine (9-β-L-ribofuranosyladenine) was synthesized by the method of Holý & Sorm (1969). L-ATP was synthesized by phosphorylation of L-adenosine by the method of Holý & Sorm (1971), and L-AMP-PCP was synthesized from L-adenosine as described by Cusack *et al.* (1983).

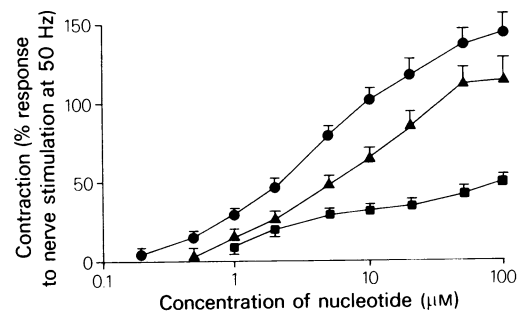

Figure 1 Contraction of the guinea-pig bladder by ATP (■), AMP-PCP (▲) or L-AMP-PCP (●). Each point is the mean of at least 10 determinations using bladder strips from at least 5 different guinea-pigs. Vertical bars show the standard errors of the mean.

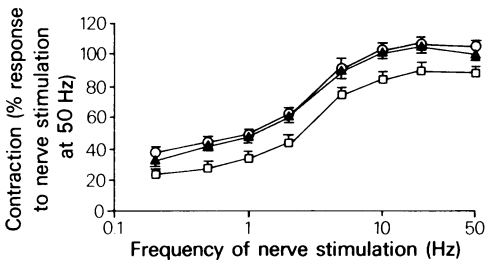

Figure 2 Contraction of the guinea-pig bladder by non-cholinergic, non-adrenergic nerve stimulation in the absence (▲) or presence of adenosine (100 μM) (□) or L-adenosine (100 μM) (○). Each point is the mean of at least 10 determinations using bladder strips from at least 5 different guinea-pigs. Vertical bars show the standard errors of the mean.

Results

Pharmacological studies

AMP-PCP and L-AMP-PCP, like ATP, both caused contraction of the guinea-pig bladder, but were more potent than ATP and achieved a greater maximal contraction (Figure 1). L-AMP-PCP was more potent than AMP-PCP, and achieved a slightly greater maximal contraction.

Adenosine (100 μM) inhibited the nerve-mediated contraction of the guinea-pig bladder, but L-adenosine (100 μM) was completely inactive (Figure 2). The inhibition by adenosine, although small, was consistent in that adenosine always reduced the response to each frequency of nerve stimulation in every tissue preparation, and, unlike L-adenosine, inhibited the spontaneous contractions of the muscle.

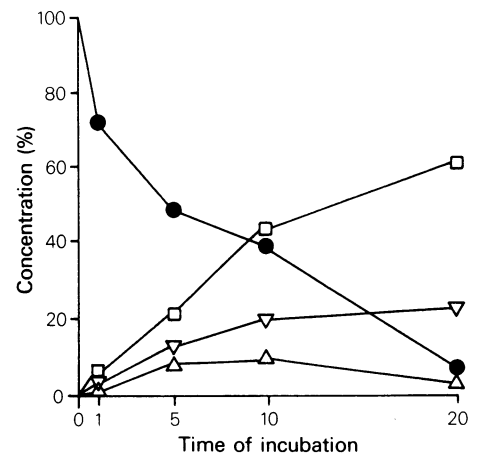

Figure 3 Degradation of ATP (100 μM) (●) to ADP (△), AMP (▽) and adenosine (□) by the guinea-pig bladder. Concentrations were estimated from the areas under the corresponding peaks obtained after h.p.l.c., and expressed as a percentage of the area of the ATP peak before incubation.

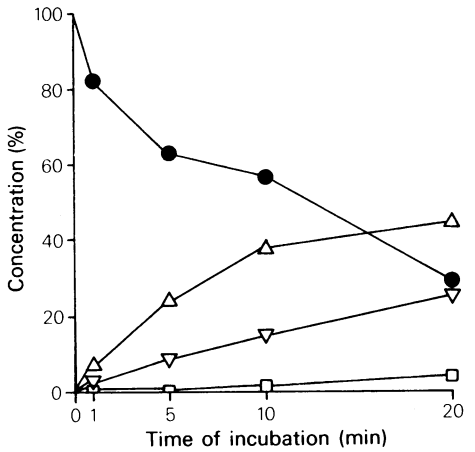

Figure 4 Degradation of L-ATP (100 μM) (●) to L-ADP (△), L-AMP (▽) and L-adenosine (□). Concentrations were estimated from the areas under the corresponding peaks obtained after h.p.l.c., and expressed as a percentage of the area of the L-ATP peak before incubation.

Degradation studies

Degradation of ATP (100 μM) was rapid, being detectable after 1 min and almost complete after 20 min. The main breakdown product was adenosine, although the intermediates, adenosine 5′-diphosphate (ADP) and adenosine 5′-monophosphate (AMP) were also detected (Figure 3). L-ATP (100 μM) was degraded less rapidly than ATP, and although breakdown was detected after 1 min, after 20 min about 30% of the L-ATP remained. The main breakdown products were L-adenosine 5′-diphosphate (L-ADP) and L-adenosine 5′-monophosphate (L-AMP), although L-adenosine was detectable after 20 min (Figure 4).

AMP-PCP (100 μM) was degraded much more slowly than ATP, and even after 60 min, approximately 40% remained. The main degradation product was adenosine, and no AMP was detected (Figure 5). L-AMP-PCP (100 μM) was completely resistant to degradation, and no breakdown products could be detected even after 60 min (Figure 5).

In the case of ATP and of AMP-PCP, somewhat less adenosine was detected after the longer incubation times than would have been expected from the corresponding breakdown of the starting materials (Figures 3 and 5), and this is presumably due to some uptake of adenosine by the muscle (Maguire & Satchell, 1981).

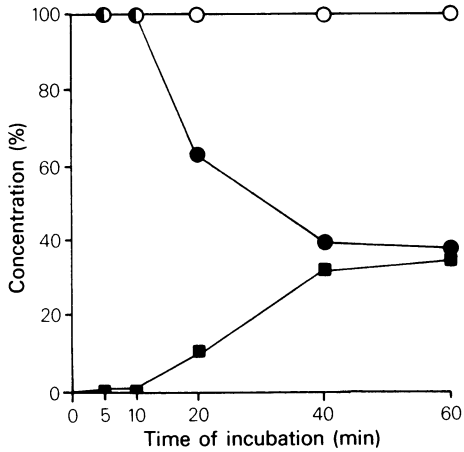

Figure 5 Degradation of AMP-PCP (100 μM) (●) to adenosine (□), and resistance to degradation of L-AMP-PCP (100 μM) (○). Concentrations were estimated from the areas under the corresponding peaks obtained after h.p.l.c., and expressed as a percentage of the area under the peak of the starting material.

ATP (100 μM) incubated with Krebs solution alone for 60 min was completely unchanged, and there was no detectable release of nucleotides or nucleosides by the muscle after 60 min incubation. ATP (100 μM) added to Krebs solution which had been incubated with muscle for 60 min was unchanged after a further 60 min incubation in the absence of the muscle, indicating that no enzymes which could degrade ATP had been released.

Discussion

These results show that L-AMP-PCP contracted the guinea-pig bladder, and was more potent than AMP-PCP and much more potent than ATP. The lack of stereoselectivity of this P_2-receptor is in agreement with the results of Burnstock *et al.* (1983), who found that ATP and L-ATP were almost equipotent at inducing contractions of the guinea-pig bladder.

Considerable indirect evidence has accumulated to suggest that ATP, and to a lesser extent AMP-PCP, are broken down to adenosine by smooth muscle preparations, fast enough to affect pharmacological responses (Maguire & Satchell, 1981). To our knowledge, however, no direct measurement of this breakdown has been reported, although there is a detailed study of the breakdown of ATP and several of its analogues, including L-ATP, AMP-PCP and L-AMP-PCP, by pig aortic endothelial cells in culture (Cusack *et al.*, 1983).

Our results show that detectable breakdown of ATP and of L-ATP occurred within 1 min, and that AMP-PCP was also degraded, but more slowly. The greater potency of AMP-PCP than of ATP could therefore indeed be due to its comparative resistance to degradation, as previously suggested (Brown *et al.*, 1979; Lukacsko & Krell, 1982). Degradation of L-AMP-PCP, on the other hand, was not detected even after 60 min incubation, and this greater resistance to degradation could explain its higher potency relative to AMP-PCP. The inactivity of L-adenosine as an inhibitor of contraction shows that the P_1-receptor of the guinea-pig bladder, like the P_1-receptor on other tissues (Cusack *et al.*, 1979; Cusack & Planker, 1979; Brown *et al.*, 1982), was stereospecific. Even if there was some local degradation of L-AMP-PCP, undetectable in our experiments, the breakdown products would therefore be inactive at this adenosine receptor.

L-AMP-PCP, like AMP-PCP (Lukascko & Krell, 1982), causes desensitization of the guinea-pig bladder to ATP (Hourani, 1984), which is evidence that these analogues act at the P_2-receptor, although in the absence of a specific, competitive ATP antagonist final proof is lacking. However, the high potency of L-AMP-PCP on the guinea-pig bladder, combined with its resistance to degradation and the lack of activity of any breakdown products on the inhibitory P_1-receptor, would appear to make this compound an attractive new ATP analogue for the study of P_2-receptors in this tissue.

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