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Effects of phosphorothioate analogues of ATP, ADP and AMP on guinea-pig taenia coli and urinary bladder

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1 Phosphorothioate analogues of ATP, ADP and AMP were tested on the guinea-pig taenia coli and urinary bladder.

2 The **Rp** diastereoisomers of the phosphorothioate analogues, ATP-α-S and ADP-α-S were respectively 7 and 3 times more effective than the **Sp** diastereoisomers in causing relaxation of the taenia coli. No stereoselectivity was observed for the diastereoisomers of ATP-β-S.

3 In guinea-pig bladder, no stereoselectivity was observed for any of the phosphorothioate analogues.

4 These results show that P_2-purinoceptors mediating inhibitory responses in the guinea-pig taenia coli show marked stereoselectivity, while P_2-purinoceptors mediating excitatory responses in the guinea-pig bladder show little stereoselectivity.

Introduction

Adenine nucleotides have diverse actions on smooth muscle, causing relaxation of the mammalian gut and contraction of the mammalian urinary bladder; these effects mimic the response to nerve stimulation in the presence of cholinoceptor and adrenoceptor antagonists (for reviews, see Burnstock, 1979; 1981).

Some 2-substituted analogues of adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP) and adenosine 5′-monophosphate (AMP) are considerably more potent than their parent nucleotides at inducing relaxation of the guinea-pig taenia coli (Satchell & Maguire, 1975; Cusack & Planker, 1979), but the 2-substituted analogues are not more effective than ATP at contracting the guinea-pig bladder (Burnstock *et al.*, 1983). Studies with pairs of enantiomers of 2-substituted analogues of ATP, ADP and AMP showed that while considerable stereoselectivity exists for the relaxant effects in the taenia coli (Cusack & Planker, 1979; Burnstock *et al.*, 1983), virtually no stereoselectivity exists for contraction of the guinea-pig bladder (Burnstock *et al.*, 1983). Since it appears that the phosphate rather than the adenine or ribose moiety of the nucleotides is the more important requirement for activity, and to investigate further the selectivity of relaxation and of contraction, we tested a series of phosphorothioate analogues of ATP, ADP and AMP having phosphate oxygens replaced by sulphur, some of which exist as pairs of diastereoisomers.

Methods

Guinea-pig taenia coli

Guinea-pigs of either sex (250–400 g) were stunned and exsanguinated and the abdomen opened. The longitudinal muscle of the caecum (taenia coli), together with the underlying Auerbach's plexus were dissected free and kept moist with modified Krebs solution of the following ionic composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.3, $NaHCO_3$ 16.3, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 7.7. Strips of taenia coli approximately 1.5 cm in length were attached by thread to a rigid support and transferred to overflow organ baths (volume 10 ml), where they were continually gassed by 95% O_2, 5% CO_2 and maintained at 36.5 ± 0.5°C. Guanethidine (3.4 μM) was present throughout. The preparations were initially placed under a resting tension of 1 g and allowed to equilibrate for 1 h. Mechanical activity was recorded under isometric conditions with a Dynamometer UFI force transducer and displayed on a Grass polygraph. The tone of the preparations

was standardised by the addition of carbachol (50 nM routinely during an 8 min cycle, but in the range 50–60 nM as the preparations aged) to allow quantification of the magnitude of the inhibitory responses (Brown & Burnstock, 1981). Concentration-response curves were obtained for ATP, ADP or AMP together with a maximum of two appropriate ATP, ADP or AMP analogues. Drug-induced relaxations were expressed as percentage inhibitions of the carbachol contractions.

Guinea-pig bladder

Mucosal-free strips of the detrusor of the bladder were prepared by the method of Ambache & Zar (1970). The preparations were suspended in modified Krebs solution (as described above) containing guanethidine (3.4 μM) and atropine (1 μM) in 2 ml organ baths. The Krebs solution was maintained at 36.5 ± 0.5°C and bubbled with a 95% O_2, 5% CO_2 gas mixture. The preparations were placed initially under 0.5 g resting tension and allowed to equilibrate for 40 to 60 min. The mechanical activity was recorded isometrically with a Dynamometer UF1 force transducer and displayed on a Grass polygraph. Electrical field stimulation was achieved by passing square-wave pulses (4 Hz, 0.3 ms duration and supramaximal voltage) to platimum ring electrodes (separated by 10 mm) from a Grass SD9 stimulator. The duration of a period of electrical stimulation was sufficient to allow the neurogenic excitatory responses to decay to one third of the maximal amplitude attained (usually 7–10 s). Since it is known that ATP and other spasmogens produce a prolonged increase in the sensitivity of the bladder smooth muscle membrane to excitatory stimuli, an interval of 15 min separated additions of drugs. Drug-induced contractions were expressed as a percentage of the mean of two successive responses to field stimulation elicited prior to addition of the drug, since these responses would reflect any increased excitability of the muscle.

Drugs

Guanethedine monosulphate was obtained from Ciba-Geigy, U.K., adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), atropine sulphate and carbamyl chlorine chloride (carbachol) were purchased from Sigma, London. Adenosine 5′-O-(3-thiotriphosphate) (ATP-γ-S), adenosine 5′-O-(2-thiodiphosphate)(ADP-β-S) and adenosine 5′-O-thiomonophosphate (AMPS) were purchased from Boehringer Mannheim. The **Rp** diastereoisomer of adenosine 5′-O-(2-thiotriphosphate) (ATP-β-S) was synthesized enzymically from ADP-β-S by phosphorylation with the combination acetate kinase/acetyl phosphate, and the contaminating **Sp** diastereoisomer was removed by treatment with myosin. The **Sp** diastereoisomer of ATP-β-S was synthesized enzymically from ADP-β-S by phosphorylation with the combination pyruvate kinase/phosphoenol pyruvate and contaminating **Rp** diastereoisomer was removed by treatment with hexokinase (Eckstein & Goody, 1976; Jaffe & Cohn, 1978). Adenosine 5′-O-(1-thiotriphosphate) (ATP-α-S) was synthesized chemically by pyrophosphorylation of AMPS, and the **Rp** and **Sp** diastereoisomers obtained, separated by isocratic (0.01 M KH_2PO_4, 2 ml min^{-1}) high performance liquid chromatography on a reverse phase column (μ Bondapak C18, Waters Associates) (Eckstein & Goody, 1976; Cusack & Hourani, 1982). Adenosine 5′-O-(2-thiodiphosphate) ADP-α-S was synthesized chemically by phosphorylation of AMPS and the **Rp** and **Sp** diastereoisomers obtained separated by isocratic (0.05 M $NH_4H_2PO_4$, 2 ml min^{-1}) high performance liquid chromatography on the reverse phase column (Eckstein & Goody, 1976; Cusack & Hourani, 1981). All nucleotide analogues were purified by ion exchange chromatography and examined by high performance liquid chromatography before use, and stock solutions were assayed by ultraviolet spectroscopy.

Statistical methods

Results given are expressed as the mean ± standard error of the mean (s.e.mean). EC_{50} values were calculated according to the method of Waud (1975).

Results

Guinea-pig taenia coli

ATP, ADP, AMP and each of the phosphorothioate analogues relaxed the carbachol-contracted taenia coli in a concentration-dependent manner and reached the same maximum as their reference nucleotides (Figure 1.) ATP-γ-S, and ATP-β-S were equipotent with ATP (Figure 1a, b). Both **Rp** ATP-α-S (pEC_{50} 7.25 ± 0.09) and **Sp** ATP-α-S (pEC_{50} 6.37 ± 0.08) diastereoisomers were more potent than ATP (pEC_{50} 5.53 ± 0.05) (Figure 1c). Similarly **Rp** ADP-α-S (pEC_{50} 7.23 ± 0.13) and **Sp** ADP-α-S (pEC_{50} 6.69 ± 0.08) were more potent than ADP (pEC_{50} 6.28 ± 0.36) (Figure 1e). ADP-β-S was more effective than ADP only at higher concentrations (Figure 1d), and similarly, AMPS was more effective than AMP at higher concentrations (Figure 1f).

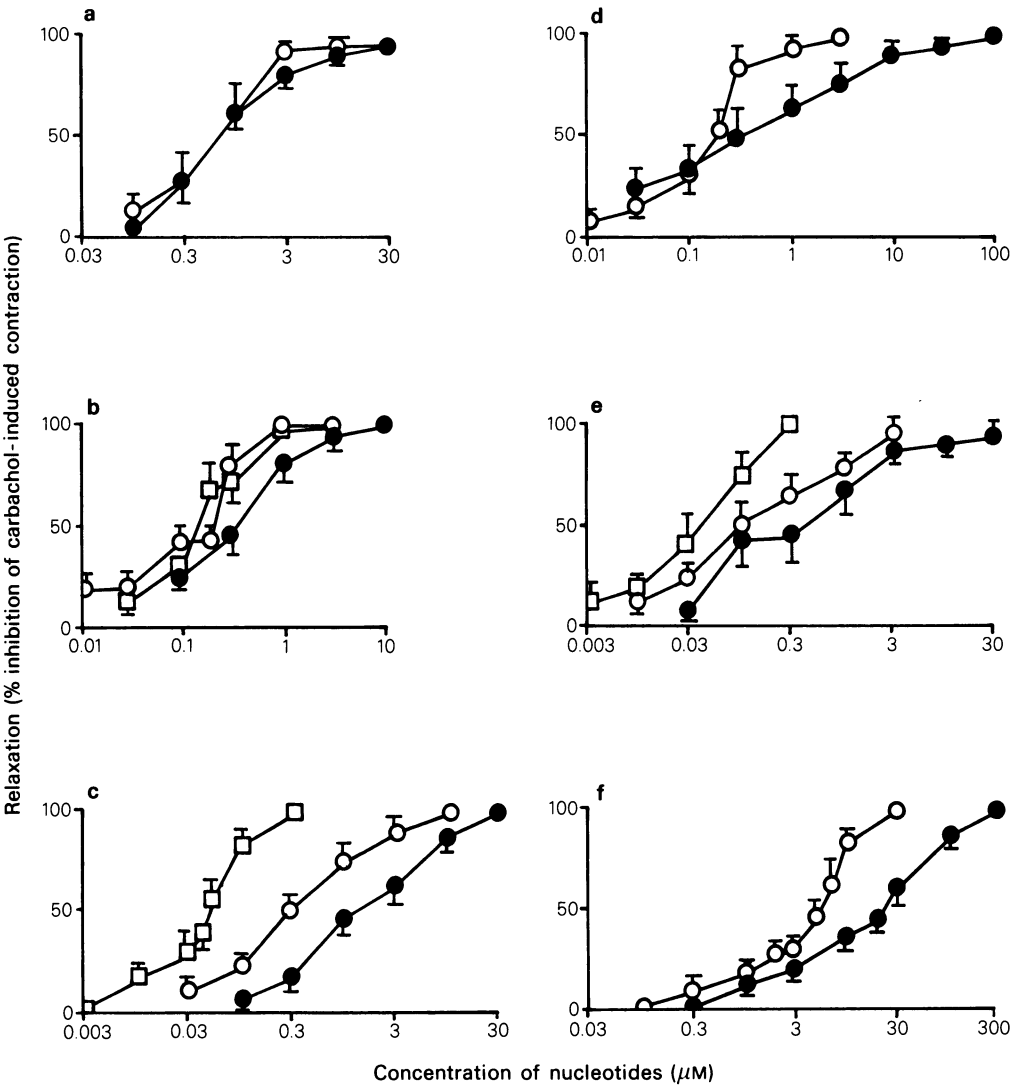

Figure 1 Log concentration-response curves of the guinea-pig taenia coli to the inhibitory effects of phosphorothioate analogues of adenine nucleotides: (a) ATP (●) and ATP-γ-S (○); (b) ATP (●), **Rp** ATP-β-S (□) and **Sp** ATP-β-S (○); (c) ATP (●), **Rp** ATP-α-S (□) and **Sp** ATP-α-S (○); (d) ADP (●) and ADP-β-S (○); (e) ADP (●), **Rp** ADP-α-S (□) and **Sp** ADP-α-S (○); (f) AMP (●) and AMPS (○). Each point is the mean of 8 observations on at least 8 different animals, and vertical bars show s.e.mean.

Guinea-pig bladder

ATP, ADP and each of the phosphorothioate analogues contracted the bladder in a concentration-dependent manner (Figure 2). ATP-γ-S, and both of the diastereoisomers of ATP-β-S were more effective, at every concentration, than ATP in contracting the bladder (Figure 2a, b). Both diastereoisomers of ATP-α-S were equipotent with ATP (Figure 2c). ADP-β-S was equipotent with ADP (Figure 2d), and both diastereoisomers of ADP-α-S were less potent than ADP (Figure 2e). AMP, even at 300 μM concentration, did not contract the bladder, but AMPS was almost as effective as ATP and achieved 75% of the neurogenic response at 300 μM concentration (Figure 2f).

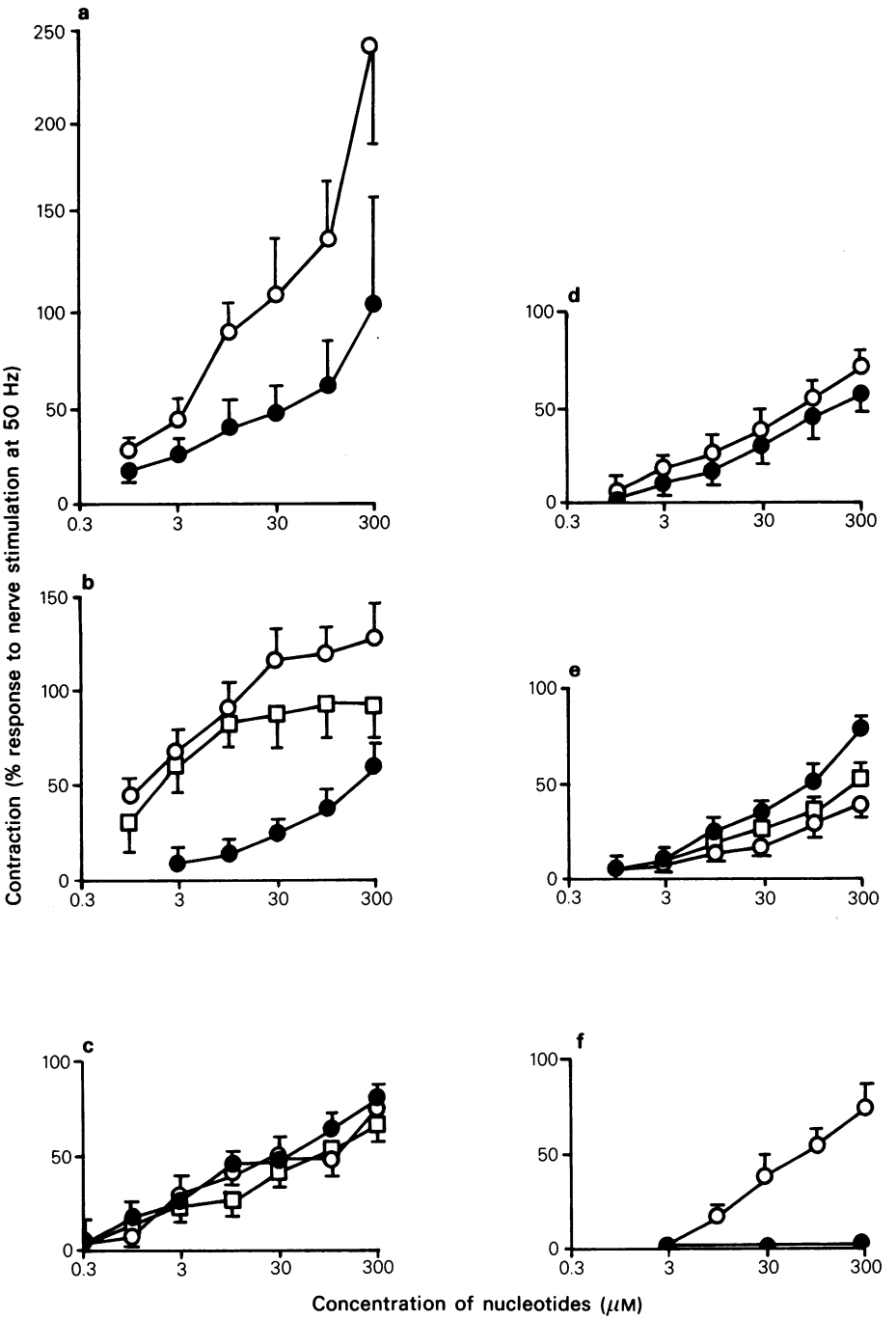

Figure 2 Log concentration-response curves of the guinea-pig urinary bladder to the excitatory effects of phosphorothioate analogues of adenine nucleotides: (a) ATP (●) and ATP-β-S (○); (b) ATP (●), Rp ATP-β-S (□) and Sp ATP-β-S (○); (c) ATP (●), Rp ATP-α-S (□) and Sp ATP-α-S (○); (d) ADP (●) and ADP-β-S (○); (e) ADP (●), Rp ADP-α-S (□) and Sp ADP-α-S (○); (f) AMP (●) and AMPS (○). Each point is the mean of 8 observations on at least 8 different animals, and vertical bars indicate s.e.mean.

Discussion

These results show that ATP-γ-S, ATP-β-S, ATP-α-S, ADP-β-S, ADP-α-S and AMPS all induced relaxation of the carbachol-contracted guinea-pig taenia coli and contraction of the guinea-pig urinary bladder in a manner similar to that of ATP (Ambache & Zar, 1970; Burnstock *et al.*, 1972; Satchell & Burnstock, 1975).

Concentration-response curves showed that all the phosphorothioate analogues were at least as potent as the parent nucleotides ATP, ADP or AMP in relaxing the guinea-pig taenia coli. No stereoselectivity was observed for the diastereoisomers of ATP-β-S, but for ATP-α-S and ADP-α-S stereoselectivity for the **Rp** diastereoisomer was found. **Rp** ATP-α-S was approximately 7 times more potent than **Sp** ATP-α-S, and **Rp** ADP-α-S was approximately 3 times more potent than **Sp** ADP-α-S at their pEC_{50} values.

Concentration-response curves for contraction of the guinea-pig bladder by ATP-γ-S, the diastereoisomers of ATP-α-S, ADP, ADP-β-S and the diastereoisomers of ADP-α-S were similar to that of ATP and all failed to reach a maximum even at a concentration of 300 μM. The diastereoisomers of ATP-β-S were much more potent than ATP and did reach maximum values. AMP did not contract the bladder but AMPS was almost as effective as ATP, and would appear to act on P_2-purinoceptors in this tissue.

The P_2-purinoceptor on the taenia coli can display a marked stereoselectivity for enantiomers of adenine nucleotides in which the modification resides in the ribose component (Cusack & Planker, 1979; Burnstock *et al.*, 1983), and in the present study stereoselectivity extends to some of the phosphorothioates in which the modification is in the phosphate.

Stereoselectivity to diastereoisomers of phosphoriothioates is also found in the human platelet ADP receptor where **Sp** ADP-α-S is 5 times more potent than **Rp** ATP-α-S in inducing platelet aggregation, and where **Sp** ATP-α-S is 5 times more potent than **Rp** ATP-α-S in inhibiting competitively ADP-induced aggregation (Cusack & Hourani, 1981; 1982).

In contrast, the bladder response failed to show stereoselectivity either for the enantiomers of ATP (Burnstock *et al.*, 1983) or, in the present study, for any of the diastereoisomers of the phosphorothioates.

The stereoselectivity for **Rp** diastereoisomers of ATP in taenia coli, but not the bladder, demonstrated in the present study, together with our earlier report for 2-methylthio-ATP in taenia coli, but not bladder (Burnstock *et al.*, 1983) suggest that further studies might be undertaken to explore whether or not there are subtypes of P_2-purinoceptors. Other differences have also been reported between the taenia coli and bladder; for example, apamin, a potassium channel blocker (Banks *et al.*, 1979) inhibits ATP actions in the taenia but not in the bladder (Shuba & Vladimirova, 1980), and, in addition, ultraviolet light mimics the response of the taenia but not of the bladder, to ATP (Burnstock & Wong, 1978).

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