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Studies on the stereoselectivity of the P_2-purinoceptor on the guinea-pig vas deferens

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1 ATP, 2-chloro-ATP, 2-methylthio-ATP and their unnatural L-enantiomers, **Rp** and **Sp** diastereoisomers of the ATP phosphorothioate analogues, ATPαS and ATPβS, were tested on the guinea-pig vas deferens.

2 The 2-substituted analogues of ATP were no more effective than ATP in causing contraction of the vas deferens. However, stereoselectivity was observed with each pair of enantiomers of ATP, 2-chloro-ATP and 2-methylthio-ATP.

3 No stereoselectivity was observed for the phosphorothioate analogues. **Rp**- and **Sp**-ATPβS were more effective than ATP at eliciting contractions of the vas deferens.

4 These results show that unlike the P_2-purinoceptor mediating excitatory responses in the guinea-pig bladder, the P_2-purinoceptor mediating contraction in the guinea-pig vas deferens displays stereoselectivity.

Introduction

Adenine nucleotides have potent pharmacological actions on smooth muscle and can induce relaxation of mammalian gut and contraction of mammalian urinary bladder and vasa deferentia (for reviews see Burnstock, 1978; 1981). In the guinea-pig vas deferens, the response to nerve stimulation is biphasic with an initial rapid twitch component followed by a slower secondary tonic component (Ambache & Zar, 1971). The second, tonic, component seems to be adrenergic as it is mimicked by application of exogenous noradrenaline and abolished by α_1-adrenoceptor antagonists and by treatment with reserpine (Ambache & Zar, 1971; Swedin, 1971). The response to exogenous ATP closely mimics the initial, phasic component and is abolished by the photoaffinity analogue of ATP, 3′-arylazidoaminopropionyl-ATP ($ANAPP_3$) (Fedan *et al.*, 1981; Sneddon *et al.*, 1982) and also after desensitization by the P_2-purinoceptor agonist α, β-methylene-ATP (Meldrum & Burnstock, 1983). Since both phases of the nerve response are substantially reduced by 6-hydroxydopamine, which selectively destroys adrenergic nerve terminals (Fedan *et al.*, 1981), this has led to the proposal that noradrenaline and ATP are released together as cotransmitters from these sympathetic nerves.

Some 2-substituted analogues of ATP such as 2-azido, 2-chloro- and 2-methylthio-ATP are considerably more potent than ATP at inducing relaxation of the guinea-pig taenia coli (Satchell & Maguire, 1982; Cusack & Planker, 1979), although they are not more effective than ATP at contracting the guinea-pig bladder (Burnstock *et al.*, 1983). A comparison of the responses induced by pairs of enantiomers of the 2-substituted analogues of ATP showed that, while considerable stereoselectivity was exhibited by the inhibitory P_2-purinoceptor mediating relaxation of the taenia coli, virtually no stereoselectivity was observed for the excitatory P_2-purinoceptor mediating contraction of the bladder (Burnstock *et al.*, 1983). Further studies using the **Rp** and **Sp** diastereoisomers of the phosphorothioate analogues of adenine nucleotides confirmed the stereoselectivity of the inhibitory P_2-purinoceptor in the intestine and the lack of stereoselectivity at the excitatory P_2-purinoceptor in the bladder (Burnstock *et al.*, 1984). In the present study we have used pairs of enantiomers of 2-substituted analogues of ATP and pairs of diastereoisomers of phosphorothioate analogues of ATP to investigate the stereoselectivity at the excitatory P_2-purinoceptor on the guinea-pig vas deferens.

Methods

Albino male guinea-pigs, 200–300 g, were killed by a blow to the head and exsanguinated. The whole vasa deferentia were removed and mounted in 2 ml organ baths with one end of the tissue fixed to a rigid support and the other attached to the transducer. The preparations were suspended in modified Krebs solution of the following ionic composition (mM); NaCl 133, KCl 4.7, $NaHCO_3$ 16.3, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 7.7. The solution was gassed with 95% O_2, 5% CO_2 and maintained at 36.5 ± 0.5°C. The preparations were initially placed under a resting tension of 1 g and allowed to equilibriate for 1 h. Contractions were measured by a force-displacement transducer (Grass FT03C) and recorded using a Grass polygraph. Owing to the availability of some of the adenine nucleotide analogues, high bath concentrations of some analogues could not be feasibly used to obtain a maximum response. In order to compare the potency of the analogues, the drug-induced contractions were expressed as a percentage of the maximal contraction of the tissue obtained to 120 mM KCl (Fedan *et al.*, 1982).

Drugs

Adenosine 5′-triphosphate (ATP) was purchased from Sigma, London. Adenosine 5′-O-(2-thiodiphosphate) (ADPβS) and adenosine 5′-O-thiomonophosphate (AMPS) were purchased from Boehringer Mannheim. 9-β-L-Ribofuranosyladenine (L-adenosine) synthesized from L-xylose (Acton *et al.*, 1964) was phosphorylated to 9-β-L-ribofuranosyladenine 5′-triphosphate (L-ATP) as described by Holý & Sorm (1971). 2-Chloro-9-β-L-ribofuranosyladenine (2-chloro-L-adenosine) was synthesized as previously described (Cusack *et al.*, 1979). 2-Methylthioadenosine and 2-methylthio-9-β-L-ribofuranosyladenine (2-methylthio-L-adenosine) were obtained by displacement of chloride from 2-chloro-adenosine (Maguire *et al.*, 1971) and 2-chloro-L-adenosine (Cusack *et al.*, 1979), respectively, with methanethiol. 2-Chloroadenosine 5′-triphosphate (2-chloro-ATP) and 2-methylthioadenosine 5′-triphosphate (2-methylthio-ATP) were synthesized by phosphorylation followed by pyrophosphorylation of 2-chloradenosine and 2-methylthioadenosine, respectively (Gough *et al.*, 1973), and 2-chloro-9-β-L-ribofuranosyladenine 5′-triphosphate (2-chloro-L-ATP) and 2-methylthio-9-β-L-ribofuranosyladenine 5′-triphosphate (2-methylthio-L-ATP) were synthesized in an identical manner from 2-chloro-L-adenosine and 2-methylthio-L-adenosine. The **Rp** diastereoisomer of adenosine 5′-O-(2-thiotriphosphate) (ATPβS) was synthesized enzymically from ADPβS by phosphorylation with the combination acetate kinase/acetyl phosphate, and any contaminating **Sp** diastereoisomer was removed by treatment with myosin. The Sp diastereoisomer of ATPβS was synthesized enzymically from ADPβS by phosphorylation with the combination pyruvate kinase/phosphoenol pyruvate and any contaminating **Rp** diastereoisomer was removed by treatment with hexokinase (Eckstein & Goody, 1976; Jaffe & Cohn, 1978). Adenosine 5′-O-(1-thiotriphosphate) (ATPαS) was synthesized chemically by pyrophosphorylation of AMPS, and the **Rp** and **Sp** diastereoisomers obtained were separated by isocratic (0.01 M KH_2PO_4, 2 ml min^{-1}) high pressure liquid chromatography on a reverse phase column (μBondapak C18, Waters Associates) (Eckstein & Goody, 1976; Cusack & Hourani, 1982). The purity of the nucleoside triphosphates was checked by high pressure liquid chromatography 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).

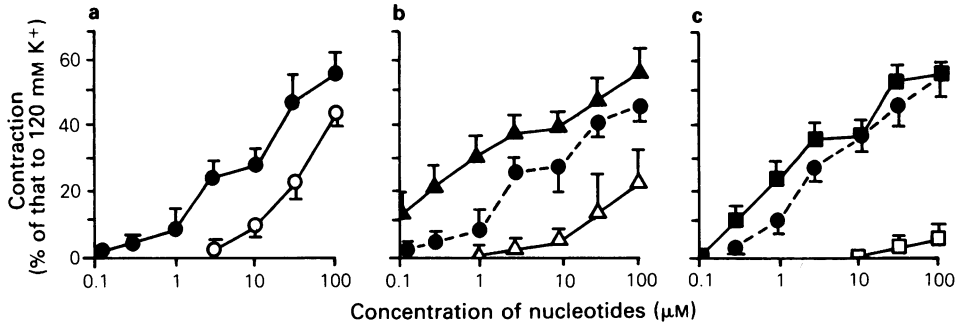

Figure 1 Concentration-response curves of the guinea-pig vas deferens to the contractions of (a) ATP (●), and L-ATP (○), (b) 2-chloro-ATP (▲), 2-chloro-L-ATP (△), and ATP (●), (c) 2-methylthio-ATP (■), 2-methylthio-L-ATP (□) and ATP (●). Each point is the mean of 8 observations on at least 8 different animals, and vertical lines indicate s.e.mean.

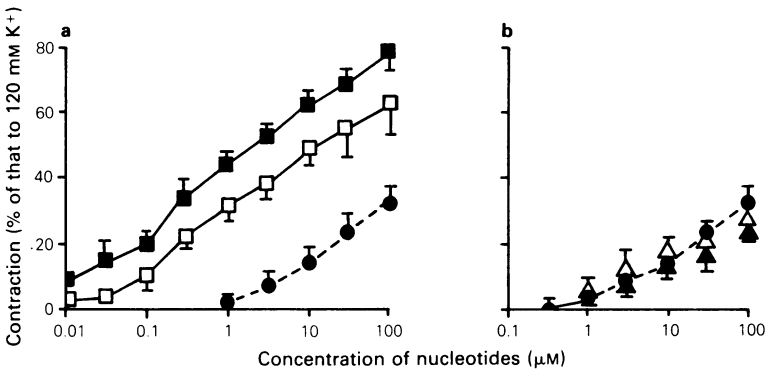

Figure 2 Concentration-response curves of the guinea-pig vas deferens to the contractions of (a) ATP (●), Rp-ATPβS (□) and Sp-ATPβS (■); (b) ATP (●), Rp-ATPαS (△) and Sp-ATPαS (▲).

Results

ATP, L-ATP, 2-chloro-ATP, 2-chloro-L-ATP, 2-methylthio-ATP, Rp-ATPαS, Sp-ATPαS, Rp-ATPβS and Sp-ATPβS all contracted the guinea-pig vas deferens in a concentration-dependent manner, but 2-methylthio-L-ATP was active only at high concentrations (Figures 1 and 2).

ATP was more effective at inducing contractions than L-ATP (Figure 1a). 2-Chloro-ATP was more effective than its L-enantiomer, at every concentration, at causing contraction of the vas deferens. In addition, 2-chloro-ATP, at lower concentrations ($< 100\,\mu M$) was more effective than ATP at contracting the vas deferens. However, at higher concentrations ($> 300\,\mu M$) the nucleotides were equipotent. The L-enantiomer of 2-chloro-ATP was less potent than ATP at every concentration (Figure 1b). 2-Methylthio-ATP was more effective than 2-methylthio-L-ATP at every concentration. 2-Methylthio-ATP was virtually equipotent with ATP over the concentration range tested (Figure 1c).

Sp- and Rp-ATPβS were more effective than ATP at every concentration, at contracting the vas deferens. In addition, the Rp diastereoisomer of ATPβS was more effective than the Sp diastereoisomer (Figure 2a). Both diastereoisomers of ATPαS were equipotent with ATP (Figure 2b).

Discussion

These results show that all of the ATP analogues can induce contraction of the guinea-pig vas deferens in a manner established for that of ATP itself (Hölck & Marks, 1978; Westfall *et al.*, 1978). These similarities, together with their structural relationship to ATP itself, suggest that these analogues all act at the P_2-purinoceptor, but in the absence of studies with reversible competitive ATP antagonists, this cannot be definitely proved. The results obtained with pairs of enantiomers of ATP show that 2-substitution gave little or no enhancement of the contractile response and this resembles their behaviour on the guinea-pig urinary bladder where 2-substitution does not lead to analogues more potent than ATP (Burnstock *et al.*, 1983). On the other hand, the considerable stereoselectivity exhibited, especially towards the enantiomer of 2-methylthio-ATP is comparable to the high degree of stereoselectivity displayed by the inhibitory P_2-purinoceptor on the guinea-pig taenia coli for this analogue (Burnstock *et al.*, 1983).

In contrast, no stereoselectivity was observed for the Rp and Sp diastereoisomers of either of the phosphorothioate analogues of ATP. In addition, while neither diastereoisomer of ATPαS was more effective than ATP at inducing contraction of the vas deferens, each diastereoisomer of ATPβS was much more effective than ATP, and this resembles the activity of these analogues on the guinea-pig bladder (Burnstock *et al.*, 1984).

Our previous studies on the stereoselectivity of P_2-purinoceptors led us to consider that a basis might exist for their subdivision (Burnstock *et al.*, 1983; 1984), but the results of the present study appear to complicate the issue, since stereoselectivity of the excitatory P_2-purinoceptor on the vas deferens seems to exhibit some of the characteristics of the inhibitory P_2-purinoceptor on the guinea-pig taenia coli. Further studies with selective inhibitors of the inhibitory and excitatory responses are required to clarify this situation.

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