Br. J. Pharmacol. (1988), **94**, 699–706

Comparative study of the actions of AP_5A and α,β-methylene ATP on nonadrenergic, noncholinergic neurogenic excitation in the guinea-pig vas deferens

[1]Ian MacKenzie, Karen A. Kirkpatrick & Geoffrey Burnstock

Department of Anatomy and Developmental Biology and Centre for Neuroscience, University College London, Gower Street, London WC1E 6BT

1 The agonistic and antagonistic effects of two nucleotide analogues, P^1,P^5-di-(adenosine-5′) pentaphosphate (AP_5A) and α,β-methylene ATP (α,β-Me ATP), have been compared in the guinea-pig isolated vas deferens.

2 In organ bath studies, both AP_5A and α,β-Me ATP were approximately 100 times more potent than ATP in producing phasic contractions of the vas deferens smooth muscle. Repeated additions of either agonist (1–10 μM) produced desensitization to a subsequent addition of the test substance. AP_5A and α,β-Me ATP were approximately equipotent in the production of desensitization.

3 After desensitization had been produced in the vas deferens by AP_5A or α,β-Me ATP, excitatory responses elicited by ATP (100–150 μM) and nonadrenergic field stimulation (2–20 Hz) were blocked, whereas those elicited by carbachol (1–10 μM) were augmented.

4 Intracellular electrical recordings demonstrated that AP_5A and α,β-Me ATP produced similar effects on membrane activity of the vas deferens. Concentration-dependent depolarizations alone were produced by both substances until the voltage threshold for action potential discharge was attained; thereafter, action potential discharges were superimposed on the depolarization and an accompanying phasic contraction was recorded. Upon restoration of the membrane potential to its control value (5–10 min after addition of either AP_5A or α,β-Me ATP), excitatory junction potentials (e.j.ps) elicited by field stimulation (up to 3 Hz) and spontaneous e.j.ps were reduced by AP_5A (>0.1 μM) in a concentration-dependent manner (as previously described for α,β-Me ATP).

5 The antagonistic effects of AP_5A on mechanical responses elicited by field stimulation were more quickly reversed on washout of AP_5A than were the effects of α,β-Me ATP, suggesting some dissimilarity in their mechanism of action at the receptor level.

6 The antagonistic effects of AP_5A on the nonadrenergic contractile responses of the vas deferens were not produced by the structurally related P^1,P^4-di-(adenosine-5′) tetraphosphate (AP_4A) even with cumulative concentrations up to 200 μM.

7 Desensitization of P_2-purinoceptors can be produced by some nucleotide analogues such as AP_5A and α,β-Me ATP, whose activity may arise partly because of their structural conformation and stability.

Introduction

The guinea-pig vas deferens receives an excitatory sympathetic innervation; the biphasic mechanical response elicited by nerve stimulation consists of a rapid phasic contraction which exhibits pharmacological properties characteristic of a nonadrenergic mediator (Ambache & Zar, 1971) and a slower tonic contraction whch typifies a noradrenergic response (Swedin, 1971; McGrath, 1978).

Although there have been several hypotheses advanced to reconcile the apparently anomalous properties of the primary phasic contraction within the framework of a purely noradrenergic system which mediates sympathetic excitation (Swedin, 1971; see Hirst & Neild, 1980), the weight of direct

[1] Author for correspondence at: Department of Pharmacology, St George's Hospital Medical School, London SW17 0RE.

experimental evidence at present lies with those experiments which utilize receptor-blocking agents as pharmacological tools. Two nucleotide derivatives have proven useful in recent years in the characterization of these components of sympathetic neuromuscular excitation. Arylazidoaminopropionyl ATP ($ANAPP_3$) and α,β-methylene ATP (α,β-Me ATP) are both effective agonists and, under the appropriate conditions, antagonists at the excitatory P_2-purinoceptor activated in smooth muscle by ATP. For example, the photolysed form of $ANAPP_3$ blocked both the phasic nonadrenergic component of neurogenic excitation in the vas deferens and the phasic response elicited by ATP (Fedan *et al.*, 1981); α,β-Me ATP produced desensitization to its own action and a comparable antagonism of phasic contractions elicited by nerve stimulation and ATP (Meldrum & Burnstock, 1983). Similarly, intracellular recording techniques have demonstrated that both $ANAPP_3$ (Sneddon & Westfall, 1984) and α,β-Me ATP (Sneddon & Burnstock, 1984; Stjärne & Åstrand, 1984; Allcorn *et al.*, 1986) antagonize the excitatory junction potentials (e.j.ps) which give rise to the action potentials underlying nonadrenergic phasic contraction in the vas deferens. These data have led to a concensus of opinion that ATP and noradrenaline may be released as cotransmitters from sympathetic nerves supplying the vas deferens (Fedan *et al.*, 1981; Meldrum & Burnstock, 1983; Sneddon & Westfall, 1984; Stjärne & Åstrand, 1985; Kirkpatrick & Burnstock, 1987).

In the present experiments we have used intracellular recording and organ bath techniques to study the desensitization process induced by α,β-Me ATP in the guinea-pig vas deferens. We have examined whether this antagonistic activity was confined to the substituted nucleotide and have explored some of the structural requirements of the receptor for the desensitizing agonist. We have shown that the pharmacological profile of α,β-Me ATP is shared by a diadenosine nucleotide, P^1,P^5-di-(adenosine-5') pentaphosphate (AP_5A), but not by a closely related compound, P^1,P^4-di-(adenosine-5') tetraphosphate (AP_4A).

Methods

Guinea-pigs (250–400 g) were stunned and exsanguinated. The vasa deferentia were removed and mounted in overflow organ baths containing Krebs solution of the following composition (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15.4, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.5. This solution was maintained at 36–37°C and continually gassed with a mixture of 95% O_2/5% CO_2. Preparations were mounted under an initial load of 1 g. Isometric tension was recorded (Grass FT03C) and displayed on a Grass polygraph. Drugs were added directly to the bath.

Electrical field stimulation was achieved by delivering (at either 5 min or 20 min intervals) trains of pulses (at various frequencies up to 20 Hz and width of 0.5 ms) via Pt ring electrodes and a Grass stimulator.

Electrophysiological experiments

The vasa deferentia were bisected and the prostatic end of the tissue was retained and pinned out for intracellular recording. Although we have not illustrated them in the present study, simultaneous isometric recordings of mechanical activity were also obtained from a short contiguous length of the preparation. The recording chamber was continually perfused by a solution of identical composition to that described above, which was also maintained at 36°C by means of a local heating coil and feedback thermistor. Drugs were added to the continually flowing Krebs solution.

Conventional intracellular recording techniques were used; electrical (and mechanical) recordings (WPI M707) were displayed on a storage oscilloscope and could be retained on an instrumentation tape recorder (Hewlett Packard) or chart recorder (Gould 2200S). Intracellular microelectrodes (60–70 MΩ) were filled with 3 M KCl. Electrical field stimulation (Pt plates; 0.5 ms pulse width) at variable frequency (generally up to 3 Hz, but occasionally at 10–20 Hz) used trains of pulses delivered from a Grass S44 stimulator and stimulus isolation unit.

Drugs

α,β-Methylene ATP (α,β-Me ATP), adenosine 5'-triphosphate (ATP), carbamylcholine (carbachol) and tetrodotoxin were obtained from Sigma. Other drugs and their sources include, guanethidine sulphate (Ciba-Geigy), prazosin hydrochloride (Pfizer), P^1, P^4-di-(adenosine-5') tetraphosphate (AP_4A) and P^1,P^5-di-(adenosine-5') pentaphosphate (AP_5A) (both Boehringer). Drugs were dissolved in distilled water and stored as frozen stock concentrations for a few days; care was taken with ATP, AP_5A, AP_4A and α,β-Me ATP and these were made up immediately before use since there was some indication (particularly at the lower concentrations used for the electrophysiological experiments) that some loss of activity may have arisen with the nucleotide analogues upon prolonged storage.

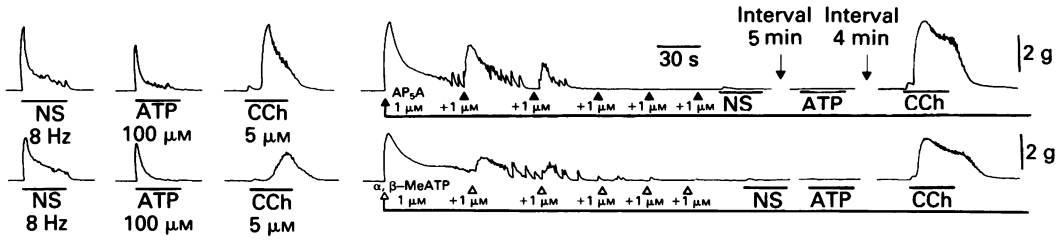

Figure 1 Contractile responses to nerve stimulation (NS, 8 Hz for 30 s), ATP (100 μM) and carbachol (CCh, 5 μM) were obtained in two preparations from the same animal. In the upper panel, AP_5A elicited an initial contraction which was progressively smaller upon subsequent additions (six additions, each at 1 μM). When the tissue was desensitized to AP_5A, responses to nerve stimulation and ATP were blocked, in contrast to that elicited by carbachol. In the lower panel, α,β-Me ATP was used as the desensitizing agonist in the contralateral vas deferens and produced a similar selective desensitization to that produced by AP_5A, when used at the same concentration. Periods of exposure to the excitatory stimuli are denoted by horizontal bars. Prazosin (5 μM) was continually present in both these experiments.

Statistical analysis

Results are expressed as the mean ± s.e.mean, with the number of determinations n given in parentheses. Statistical significance between two data samples was tested by either Student's t-test or paired t-test. A probability level of $P < 0.05$ was considered statistically significant.

Results

Organ bath experiments

In these experiments, neurogenic responses were established at variable frequency (a stimulation period of 30 s was routinely used) and supramaximal voltage; these excitatory responses were blocked by tetrodotoxin (1 μM). After supramaximal voltage was established, frequency-response relationships were examined (2–20 Hz) and the characteristic biphasic mechanical response of the intact vas deferens became apparent. At this point prazosin (5–10 μM) was routinely added for the duration of the experiments to exclude the contribution of noradrenaline to the neurogenic 'control' response.

Additionally, reproducible responses to exogenous ATP and carbachol were established (before and after prazosin). A concentration of carbachol was then chosen (1–10 μM) that produced a response of similar magnitude to that of a nerve stimulation response (at 8 Hz) which, in turn, was approximately 75% of the maximal response over the frequency range studied here (71.7 ± 1.8%, $n = 33$). A concentration of ATP was chosen (usually 100–150 μM) that elicited reproducible responses of a similar magnitude to those of the other two stimuli. After these control responses had been established, then α,β-Me ATP was tested for its capacity to induce desensitization to its own agonist (excitatory) action.

α,β-Me ATP was used routinely in the concentration range 1–10 μM. Within this concentration range α,β-Me ATP elicited a phasic contraction upon its first addition to the organ bath; this phasic excitatory response declined within a minute or so and, when the muscle reattained its former atonic state, another addition of α,β-Me ATP was made (Figure 1). The amplitude of this excitatory response was progressively attenuated as the number of subsequent additions of α,β-Me ATP was increased, until a point was reached at which addition of α,β-Me ATP elicited no further excitatory response. At this time, desensitization to the excitatory action of α,β-Me ATP was judged to be complete, since responses to neurogenic stimulation were substantially or completely blocked ($n = 14$). Responses to ATP were similarly antagonized, whereas responses to carbachol were either unaffected or even increased slightly (Table 1).

AP_5A and α,β-Me ATP (each at a concentration of 1 μM) both produced phasic excitatory responses on their initial exposure which were of similar magnitude ($AP_5A = 3.2 \pm 0.35$ g; α,β-Me ATP = 3.1 ± 0.27; $n = 10$ in each case), but larger than those produced by a much higher concentration of ATP (ATP = 2.1 ± 0.19 g; $n = 20$ for 100 μM). AP_5A also produced desensitization to its own action (1–5 μM, $n = 12$; Figure 1). A series of paired experiments followed in which we tried to quantify the ability of AP_5A and α,β-Me ATP to produce desensitization. It was found that AP_5A and α,β-Me ATP were similarly effective in the production of desensitization and antagonism of neurogenic responses (Figure 1, Table 1). However, one manner in which AP_5A and α,β-Me ATP differed in their action was in the way that neurogenic excitatory responses

Table 1 Comparison of the effects of repetitive additions of α,β-Me ATP (1 μM) or AP_5A (1 μM) on excitatory responses elicited by ATP (100 μM), carbachol (5 μM) and field stimulation (8 Hz for 30 s) in a series of paired experiments in the guinea-pig vas deferens ($n = 5$)

	Desensitizing agonist:	*α,β-Me ATP*	*AP_5A*
Successive additions at 1 μM required for desensitization		5.6 ± 0.57	5.2 ± 0.42
% change in magnitude of control response after desensitization	ATP (100 μM)	−98 ± 1.1	−98 ± 1.2
	Nerve stimulation (8 Hz)	−95 ± 0.9	−91 ± 2.5
	Carbachol (5 μM)	+24 ± 10.4	+22 ± 8.9

Prazosin (5 μM) was continually present.

recovered following desensitization. In four experiments of the type shown in Figure 1 and Table 1, neurogenic excitatory responses were more quickly and more completely restored following the washout of AP_5A (after desensitization had been produced) in comparison with parallel responses in tissues when α,β-Me ATP had been used (Figure 2).

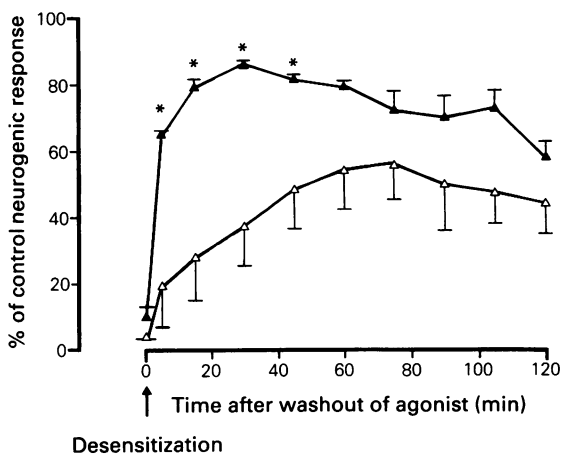

Figure 2 The rate and extent of recovery of the neurogenic contractions from desensitization were followed in seven pairs of experiments. Data shown in this illustration came from four of those experiments described in Table 1 (and shown in Figure 1). Desensitization was produced by either agonist and responses to nerve stimulation, ATP and carbachol were then tested. AP_5A (▲) or α,β-Me ATP (△) was then washed out (time = 0) and responses to nerve stimulation were tested over a 2 h period at those times indicated on the graph. They were compared to their control (pre-desensitization) amplitudes. Neurogenic responses were more quickly and more completely restored in preparations which had been exposed to AP_5A (statistically significant changes indicated by asterisks). Prazosin (5 μM) was continually present.

In a few further experiments ($n = 5$), we also studied the action of AP_4A, an analogue of AP_5A which has one less phosphate group in the polyphosphate bridge linking the two adenosine moieties. AP_4A was a less potent agonist than either AP_5A or α,β-Me ATP (AP_4A at 1 μM = 0.53 ± 0.05 g; $n = 4$) and there was no parallel desensitization induced against the neurogenic response, even with multiple (up to 20) additions of low agonist concentration (1 μM) or additions of higher agonist concentration (6–10 μM), yielding cumulative concentrations of up to 200 μM.

Electrophysiological experiments

The prostatic end of the vas deferens exhibits a prominent nonadrenergic innervation whose electrical correlate is a graded e.j.p. (Burnstock & Holman, 1961). In the present experiments the resting membrane potential averaged −65 ± 0.7 mV ($n = 54$). Spontaneous e.j.ps (usually 5–10 mV, but up to 28 mV) were superimposed on an otherwise stable membrane potential. After a satisfactory impalement had been obtained, electrical responses to field stimulation were elicited at several frequencies (routinely trains of 10 pulses at 0.2, 0.5, 0.75 and 1 Hz were studied in each cell, but these were sometimes studied at 2 or 3 Hz). E.j.ps exhibited a frequency-dependent facilitation before they attained a steady-state value, with the ultimate firing of action potentials which completely depolarized the cell membrane potential to 0 mV, or by an additional 5–15 mV.

Agonists such as ATP or α,β-Me ATP (Burnstock *et al.*, 1986) elicited electrical (and mechanical) responses which were similar to those produced by AP_5A. (Intervals of 12–20 min separated subsequent additions of agonist at a fixed concentration to ensure reproducibility; neurogenic responses were also periodically studied during recovery periods to provide an indication of any desensitization that

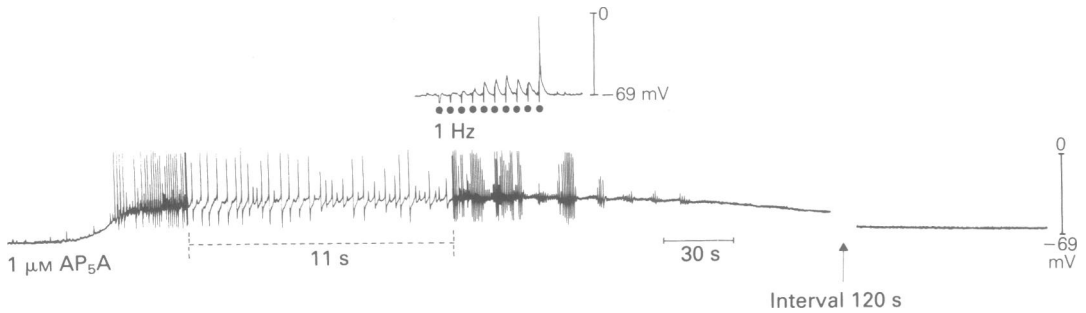

Figure 3 Electrical responses elicited by field stimulation (1 Hz, 10 pulses) and AP_5A in the vas deferens are shown here. The e.j.ps facilitated to reach the electrical threshold for the initiation of action potentials. AP_5A (1 μM) elicited an initial depolarization and then an action potential discharge. (The time course of the action potentials is shown with more clarity at the faster chart speed.) The action potentials became less frequent and then stopped before the depolarization fell off and the former (pre-drug) membrane potential was recorded again (even in the continual presence of AP_5A). E.j.ps elicited thereafter were antagonized by AP_5A in a concentration-dependent manner (Figures 4 and 5).

might have arisen.) Low concentrations of AP_5A produced a concentration-dependent membrane depolarization; higher concentrations of AP_5A, upon reaching the electrical threshold for the activation of voltage-dependent Ca^{2+} channels (Burnstock *et al.*, 1986), elicited a depolarization with a superimposed discharge of action potentials (Figure 3). These action potentials also attained 0 mV; they were accompanied by mechanical excitation.

The antagonistic effects of AP_5A (0.1–10 μM) on fully facilitated e.j.ps (taken as the mean amplitude of the 6th–10th pulses in a train) were assessed in a series of experiments where control responses to nerve stimulation were obtained over a wide frequency range (0.2–1 or 2 Hz) in a single cell, and were then examined in the presence of progressively increasing concentrations of AP_5A, once the depolarization (and any action potential discharge) had waned and the membrane potential had attained its former (pre-drug) level. E.j.p. amplitudes were significantly reduced by AP_5A (Figure 4) in a concentration-dependent manner; action potentials were not produced by those e.j.ps, since they no longer reached the electrical threshold for action potential initiation (Figure 5). As the concentration of AP_5A was increased, the frequency and amplitude of spontaneous e.j.ps was also reduced.

Discussion

A prominent component of the neurogenic excitatory response in the guinea-pig vas deferens and urinary bladder is resistant to the action of adrenoceptor and muscarinic receptor antagonists, but is antagonized by two compounds ($ANAPP_3$ and α,β-Me ATP) which are structurally related to ATP (Fedan *et al.*, 1981; Kasakov & Burnstock, 1983; Meldrum & Burnstock, 1983). These observations support the hypothesis that ATP may act as a cotransmitter (with noradrenaline) in the vas deferens (Fedan *et al.*, 1981) or as a transmitter in the

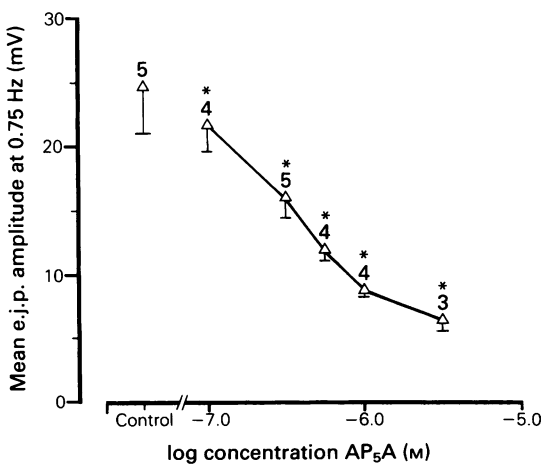

Figure 4 The concentration-dependent inhibition of e.j.p. amplitude by AP_5A is shown here. The control response is the mean amplitude of a fully-facilitated e.j.p. elicited at 0.75 Hz (mean of 6th to 10th pulse in a train) in a series of cells (number of cells indicated). AP_5A was added in progressively increasing concentrations and e.j.p. amplitudes were redetermined in the same cells once drug-induced depolarization had subsided (Figure 5). Significant antagonism of e.j.p. amplitude was observed over all the concentration-range shown here (indicated by asterisks).

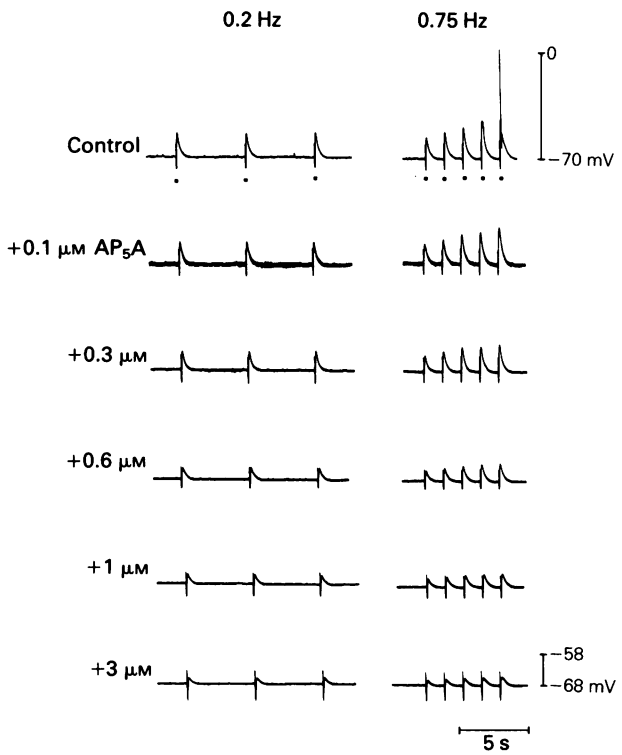

Figure 5. Control e.j.ps elicited by stimulation at various frequencies (as shown) were progressively antagonized by increasing AP_5A concentrations (up to 3 μM). The action potentials elicited by stimulation were absent once the e.j.ps fail to attain the electrical threshold for action potential initiation. E.j.ps shown here at p.2 Hz were fully-facilitated (8th to 10th pulses in a train) and underwent facilitation at 0.75 Hz (first five pulses in a train).

bladder (in addition to acetylcholine) (Burnstock *et al.*, 1978). An analogous cotransmitter role for ATP in several vascular tissues has also been postulated on the basis of similar experimental observations (Sneddon & Burnstock, 1984; Ishikawa, 1985; Kügelgen & Starke, 1985; Kennedy *et al.*, 1986; Burnstock & Warland, 1987).

Of the two 'antagonists' of the nonadrenergic or noncholinergic responses described so far, α,β-Me ATP is more potent than $ANAPP_3$ (Fedan *et al.*, 1981; Meldrum & Burnstock, 1983) and does not require prior photolysis by u.v. irradiation. In the present experiments, we have shown directly that a third nucleotide derivative, AP_5A, was approximately equipotent with α,β-Me ATP in its agonistic and antagonistic (or desensitization) effects in organ bath experiments. We have, furthermore, quantified the concentration-dependent antagonism of AP_5A on e.j.ps of the vas deferens in continuous recordings from single cells with intracellular microelectrodes. AP_5A (present work) and α,β-Me ATP (Sneddon & Burnstock, 1984) have similar antagonistic effects on spontaneous and evoked e.j.ps in the vas deferens, and a similar agonistic action on the membrane potential (Burnstock *et al.*, 1986; present work). A postsynaptic action at a restricted locus rather than a presynaptic site of action of AP_5A seems more likely, since spontaneous e.j.ps were antagonized and since contractile responses to carbachol were not reduced in parallel with those elicited by nerve stimulation and ATP. One striking difference between the action of AP_5A and α,β-Me ATP in organ bath studies was the faster (within 5 min), more complete recovery of the transmission mechanism from desensitization induced by AP_5A. This infers some mechanistic difference in the action of these two substances at their receptor (receptor, rather than receptors, since ATP is similarly antagonized by both compounds). Preliminary experiments have also shown that AP_5A antagonizes atropine-resistant neurogenic contractions and phasic contractions to ATP in detrusor muscle strips from the guinea-pig (MacKenzie, Kirkpatrick & Burnstock, unpublished observations) in a similar manner to

α,β-Me ATP, and suggests some common properties of the mechanism of the antagonism at the P_2-purinoceptor.

The structure of adenine nucleotide derivatives contributes to changes in pharmacological potency at the P_2-purinoceptor when compared to ATP in these and other tissues (Stone, 1981) which could derive from either their steric presentation at the receptor or at the active site of their metabolizing enzymes. The stereochemistry of ATP (stylistically represented by structural notation AOPOPOP, where A = the adenosine group, O = anhydride oxygen, P = phosphate group), and AP_5A (AOPOPOPOPOPOA) are possibly quite similar—the dinucleotide lacks the longer C—P bond length

and larger bond angle exhibited by α,β-Me ATP (AOPCPOP, where C is a methylene substituent) (Maguire & Satchell, 1979). At physiological pH, AP_5A and AP_4A exist in an extended (more linear) form (Kolodny *et al.*, 1979). Furthermore, if one imagines a plane of symmetry passing through the central phosphoryl group in the polyphosphate 'bridge' of AP_5A, this yields two shorter fragments which are, essentially, equivalent to the presence of two ATP molecules in close proximity with the added advantage that there are no exposed terminal phosphates (at least in the unbound form) available for enzymatic degradation. The metabolic routes for dinucleotide degradation in smooth muscle (Pearson *et al.*, 1985) may be similar for ATP and AP_5A (once the pentaphosphate has been cleaved at some point by whatever available mechanism; how this can be achieved is unclear) and may involve the production of di- and monophosphates. The length of the dinucleotide (and its products of metabolism) is certainly important—the deletion of a single phosphate moiety yields a compound (AP_4A, AOPOPOPOPOA) with little agonist and subsequent 'desensitizing' activity. The stability of α,β-Me ATP is an important determinant in its activity but its metabolites would (by virtue of its methylene bridge) differ from those of ATP and AP_5A.

Another interesting property of AP_5A and AP_4A is that they are naturally occurring. It is difficult, however, to explain how the well-documented inhibition of myosin adenylate kinase by AP_5A (Lienhard & Secemski, 1973) might contribute to its antagonistic activity in the vas deferens, when nucleotides are thought to be incapable of entering the muscle cell membrane in their unmetabolized form. Information about the storage of AP_5A is sparse and the assay techniques used can also cross-react with ATP (Momsen, 1978; Ogilvie, 1981). We are also unaware of any literature which describes the action of AP_5A in the whole animal; it might be a useful tool in experiments where rapid nucleotide degradation to adenosine (the consequence of ATP action) would be an otherwise undesirable action. Long-term binding of the nucleotides α,β-Me ATP or AP_5A to their receptors, or their long-term modification of associated receptor-operated second messenger systems might also be possible.

To date, therefore, the antagonism of excitatory nucleotide receptors requires their prior activation (and/or physical modification, e.g. photolysis and the antagonistic effects of $ANAPP_3$) by synthetic or endogenous nucleotide analogues whose structural conformation and metabolic stability are important determinants in the expression of their biological activity.

We are grateful to the M.R.C. for financial support and to Dr F.A. Cribbin for her assistance in the preparation of the manuscript.

References

ALLCORN, R.J., CUNNANE, T.C. & KIRKPATRICK, K. (1986). Actions of α,β-methylene ATP and 6-hydroxydopamine on sympathetic neurotransmission in the vas deferens of the guinea-pig, rat and mouse: support for cotransmission. *Br. J. Pharmacol.*, **89**, 647–659.

AMBACHE, N. & ZAR, M.A. (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol.*, **216**, 359–389.

BURNSTOCK, G. & HOLMAN, M.E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.*, **155**, 115–133.

BURNSTOCK, G. & WARLAND, J.J.I. (1987). P_2-purinoceptors of two subtypes in the rabbit mesenteric artery: reactive blue 2 selectively inhibits responses mediated via the P_{2Y}- but not the P_{2X}-purinoceptor. *Br. J. Pharmacol.*, **90**, 383–391.

BURNSTOCK, G., COCKS, T., CROWE, R. & KASAKOV, L. (1978). Purinergic innervation of the guinea-pig urinary bladder. *Br. J. Pharmacol.*, **63**, 125–138.

BURNSTOCK, G., MacKENZIE, I. & MANZINI, S. (1986). Regulation of voltage-dependent calcium channels in the guinea-pig vas deferens by the dihydro-pyridines nifedipine and Bay K 8644. *J. Physiol.*, **373**, 62P.

FEDAN, J.S., HOGABOOM, G.K., O'DONNELL, J.P., COLBY, J. & WESTFALL, D.P. (1981). Contribution by purines to the neurogenic response of the vas deferens of the guinea-pig. *Eur. J. Pharmacol.*, **69**, 41–53.

HIRST, G.D.S. & NEILD, T.O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature*, **283**, 767–768.

ISHIKAWA, S. (1985). Actions of ATP and α,β-methylene ATP on neuromuscular transmission and smooth muscle membrane of the rabbit and guinea-pig mesenteric arteries. *Br. J. Pharmacol.*, **86**, 777–787.

KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analog, α,β-methylene ATP, to produce desensitisation of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **86**, 291–294.

KENNEDY, C., SAVILLE, V.L. & BURNSTOCK, G. (1986). The contributions of noradrenaline and ATP to the responses of the rabbit central ear artery to sympathetic nerve stimulation depend on the parameters of stimulation. *Eur. J. Pharmacol.*, **122**, 291–300.

KIRKPATRICK, K. & BURNSTOCK, G. (1987). Sympathetic nerve-mediated release of ATP from the guinea-pig vas deferens is unaffected by reserpine. *Eur. J. Pharmacol.*, **138**, 207–214.

KOLODNY, N.H., KISTENEFF, E., REDFIELD, C. & RAPAPORT, E. (1979). Ap_4A and Ap_5A prefer folded unstacked conformations at pH 4–5, in sharp contrast with Ap_2A and Ap_3A. *FEBS Lett.*, **107**, 121–124.

KÜGELGEN, I. v. & STARKE, K. (1985). Noradrenaline and adenosine triphosphate as co-transmitters of neurogenic vasconstriction in rabbit mesenteric artery. *J. Physiol.*, **367**, 435–455.

LIENHARD, G.E. & SECEMSKI, I.I. (1973). P^1,P^5-di (adenosine-5′) pentaphosphate, a potent multisubstrate inhibitor of adenylate kinase. *J. Biol. Chem.*, **248**, 1121–1123.

McGRATH, J.C. (1978). Adrenergic and 'non-adrenergic' components in the contractile response of the vas deferens to a single indirect stimulus. *J. Physiol.*, **283**, 23–39.

MAGUIRE, M.H. & SATCHELL, D.G. (1979). Specificity of adenine nucleotide receptors: inhibition of the guinea pig taenia coli by adenine nucleotide analogs. In *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides*. ed. Baer, H.P. & Drummond, G.I. pp. 33–43. New York: Raven Press.

MELDRUM, L.A. & BURNSTOCK, G. (1983). Evidence that ATP acts as a co-transmitter with noradrenaline in sympathetic nerves supplying the guinea-pig vas deferens. *Eur. J. Pharmacol.*, **92**, 161–163.

MOMSEN, G. (1978). Firefly luciferase reacts with P^1,P^5-di(adenosine-5′-) pentaphosphate and adenosine 5′-tetraphosphate. *Biochem. Biophys. Res. Commun.*, **84**, 816–822.

OGILVIE, A. (1981). Determination of diadenosine tetraphosphate (Ap_4A) levels in subpicomole quantities by a phosphodiesterase luciferin-luciferase coupled assay: application as a specific assay for diadenosine tetraphosphatase. *Analyt. Biochem.*, **115**, 302–307.

PEARSON, J.D., COADE, S.B. & CUSACK, N.J. (1985). Characterization of ectonucleotides on vascular smooth-muscle cells. *Biochem. J.*, **230**, 503–507.

SNEDDON, P. & BURNSTOCK, G. (1984). Inhibition of excitatory junction potentials in guinea-pig vas deferens by α,β-methylene ATP: further evidence for ATP and noradrenaline as cotransmitters. *Eur. J. Pharmacol.*, **100**, 85–90.

SNEDDON, P. & WESTFALL, D.P. (1984). Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens. *J. Physiol.*, **347**, 561–580.

STJÄRNE, L. & ÅSTRAND, P. (1984). Discrete events measure single quanta of adenosine 5′-triphosphate secreted from sympathetic nerves of guinea-pig and mouse vas deferens. *Neuroscience*, **13**, 21–28.

STJÄRNE, L. & ÅSTRAND, P. (1985). Relative pre- and postjunctional roles of noradrenaline and adenosine 5′-triphosphate as neurotransmitters of the sympathetic nerves of guinea-pig and mouse vas deferens. *Neuroscience*, **14**, 929–946.

STONE, T.W. (1981). Actions of adenine dinucleotides on the vas deferens, guinea-pig taenia caeci and bladder. *Eur. J. Pharmacol.*, **75**, 93–102.

SWEDIN, G. (1971). Studies on neurotransmission mechanisms in the rat and guinea-pig vas deferens. *Acta Physiol. Scand.*, Suppl. **369**, 1–34.

(Received November 10, 1987
Revised February 2, 1988
Accepted February 29, 1988)