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Enhancement of sympathetic purinergic neurotransmission in the guinea-pig isolated vas deferens by the novel ecto-ATPase inhibitor ARL 67156

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1 Field stimulation of the sympathetic nerves of the guinea-pig isolated vas deferens with trains of pulses for 20 s at 1-8 Hz produced characteristic biphasic contractions. The effect of the novel ecto-ATPase inhibitor, 6-N,N-diethyl-D-β,γ-dibromomethyleneATP (ARL 67156, formerly known as FPL 67156), on the magnitude of the initial, predominantly purinergic peak of this response was studied in order to determine the influence of enzymatic degradation of adenosine 5'-triphosphate (ATP) on its action as a neurotransmitter.

2 The peak magnitude of the response to nerve stimulation was significantly increased in a concentration-dependent manner by ARL 67156 (5-100 μM) and the size of the neurogenic response at 4 Hz was approximately doubled in the presence of ARL 67156 (100 μM).

3 ARL 67156 (100 μM) has a rapid onset of action. The enhancing effect on neurogenic contractions was maximal after 10 min, was well maintained for at least 30 min and was rapidly reversed, with responses returning to control levels 10 min after washout.

4 The neurogenic contraction in the presence of prazosin (0.1 μM) was purely purinergic, as it was abolished by the P_2-purinoceptor antagonist, PPADS (100 μM). ARL 67156 (100 μM) produced a similar degree of enhancement of neurogenic responses in the absence and presence of prazosin, supporting the view that the enhancing effects of ARL 67156 on neurogenic contractions result from potentiation of the action of ATP.

5 Exogenous ATP and α,β-methyleneATP produced rapid transient contractions. Responses to ATP were increased in magnitude and duration in the presence of ARL 67156 (100 μM), whereas those to the stable analogue, α,β-methyleneATP were not significantly affected.

6 Contractions to exogenous noradrenaline (10 μM) and KCl (40 mM) were significantly enhanced by ARL 67156 (100 μM), but this potentiation was abolished by PPADS (100 μM). Therefore, this effect of the ecto-ATPase inhibitor may be due to a build up of endogenous ATP, increasing the sensitivity of the smooth muscle to other agonists.

7 It is concluded that ARL 67156 potentiates the action of ATP, and that when ATP acts as a neurotransmitter its postjunctional actions are greatly attenuated by enzymatic degradation.

Keywords: ATP; ecto-ATPase; ARL 67156; purinergic transmission; vas deferens

Introduction

Since the initial proposal of the purinergic nerve hypothesis (Burnstock, 1972), considerable evidence has accumulated to support the idea that adenosine 5'-triphosphate (ATP) is an important neurotransmitter in a wide variety of tissues, often acting as a cotransmitter with noradrenaline (NA) or acetylcholine (see Burnstock, 1990). For example, when ATP and NA are released as cotransmitters from sympathetic nerves in the guinea-pig vas deferens, ATP mediates excitatory junction potentials (e.j.p.s), which in turn initiate action potentials and the predominantly purinergic, phasic component of the neurogenic contraction (Sneddon *et al.*, 1982; Sneddon & Westfall, 1984). NA does not contribute to e.j.p.s, but produces the smaller, predominantly noradrenergic, tonic phase of the contraction.

The purinergic nerve hypothesis envisaged that the postjunctional actions of ATP would be curtailed by its sequential degradation to adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate, adenosine and inosine. The enzymes responsible for this process have since been characterized and the most prominent for conversion of ATP to ADP is ecto-ATPase (for review seen Ziganshin *et al.*, 1994). Consistent with a role in modulation of neurotransmission, ecto-ATPase is present in a wide variety of tissues, including the guinea-pig vas deferens (Harris, 1972; Bailey & Hourani, 1994).

Many types of agents have been tested as inhibitors of ecto-ATPase, but none is sufficiently selective to be a useful tool for investigating the role of ecto-ATPases in purinergic neurotransmission. However, a recently developed analogue of ATP, 6-N,N-diethyl-D-β,γ-dibromomethylene ATP (ARL 67156, formerly known as FPL 67156), may be a major breakthrough in this field. ARL 67156 inhibits ecto-ATPase with a pIC_{50} of 4.62 in human blood cells (Crack *et al.*, 1995) and 5.1 in the rat vas deferens (Khakh *et al.*, 1995). It enhances contractions of the rabbit ear artery evoked by exogenous ATP, but not responses to the stable analogue α,β-methyleneATP (α,β-meATP, Crack *et al.*, 1995). This suggests that its enhancing effect on contractions is due to inhibition of ATP breakdown and not to non-selective effects on the smooth muscle, since if this were the case then it would be expected that responses to α,β-meATP would also have been enhanced. ARL 67156 is also a weak P_{2X}-purinoceptor antagonist, with a pA_2 of 3.3 (Crack *et al.*, 1995), which implies that if used at 10-100 μM, then effective inhibition of ecto-ATPase should be achieved, with relatively little receptor antagonism.

We have now used ARL 67156 to investigate the role of

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ecto-ATPase in modulating purinergic neurotransmission in the guinea-pig isolated vas deferens. A preliminary account of these results has been published (Sneddon *et al.*, 1995).

Methods

Albino male guinea-pigs (250–400 g) were killed by asphyxiation with CO_2 and subsequent cervical dislocation. The vasa deferentia were removed, cleaned of connective tissue and mounted in 2 ml horizontal baths. The tissues were allowed to equilibrate under a resting tension of 1 g at 35°C for 1 h in a physiological salt solution of the following composition (mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11; and bubbled with 95% O_2, 5% CO_2. Tension was recorded with Scaime transducers connected via Universal amplifiers (Gould) to a pen recorder (Gould 2107).

Sympathetic nerves were stimulated by electrical field stimulation for 20 s at 10 min intervals with a pulse width of 0.5 ms and supramaximal voltage, using a Grass S44 stimulator and Grass SIU5F stimulus isolation unit. When studying the effects of ARL 67156 or prazosin on neurogenic contractions, three reproducible control responses were obtained before addition of the drug. When examining the effects of ARL 67156 on responses to exogenous agonists, three reproducible control responses to ATP (100 μM), NA (10 μM) or KCl (40 mM) were obtained at 20 min intervals or 30 min intervals for α,β-meATP (0.5 μM). ARL 67156 (100 μM) was then added 10 min before the fourth application of agonist. Similarly, when using pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) to study the interaction between ARL 67156 and exogenous NA or KCl, three reproducible control responses to NA or KCl were obtained, then PPADS (100 μM) was added for 40 min, before further agonist responses were evoked. In the continued presence of PPADS, ARL 67156 (100 μM) was then added and 10 min later a further response to NA or KCl obtained.

Statistics

Values in the text refer to mean ± s.e.mean. Statistical significance of the results was tested by Student's paired *t* test. Differences were considered significant when $P < 0.05$.

Drugs

Adenosine 5′-triphosphate (disodium salt), α,β-methyleneATP (lithium salt), prazosin (all Sigma), ARL 67156, provided by Astra plc, and PPADS, a gift from Dr G. Lambrecht, University of Frankfurt, were dissolved in distilled water and kept as 10^{-1} M stock solutions. Potassium chloride (Sigma) was kept as a 2 M stock. (-)-Noradrenaline bitarate (Sigma) was dissolved in acid saline and kept as a 10^{-1} M stock solution.

Results

Field stimulation of the sympathetic nerves of the guinea-pig vas deferens with trains of pulses for 20 s at 1–8 Hz produced characteristic biphasic contractions, as illustrated in Figures 1, 3 and 4. The initial, transient peak reached a maximum within 5 s, then declined to a lower level, which was usually well maintained throughout the stimulation period. In all cases the results described below refer to the peak magnitude of the contraction. The magnitude of the responses increased with stimulation frequency. Preliminary experiments showed that stimulation at 16 and 32 Hz produced slightly larger responses than at 8 Hz, but these were not included in subsequent experiments since the time required for recovery after such stimulation was too long.

Effects of ARL 67156 on neurogenic contractions

The time course of the effect of ARL 67156 on neurogenic contractions was investigated (Figure 1). ARL 67156 (100 μM) increased the amplitude of neurogenic contractions with a rapid onset of action. The effect was maximal after 10 min and was well maintained for at least 30 min, with responses returning close to control levels 10 min after washout.

Concentration-effect relationship for ARL 67156

The concentration-effect relationship for ARL 67156 (1–100 μM) was studied by quantifying the enhancement of the peak contraction evoked by nerve stimulation at 4 Hz. Responses were significantly increased in a concentration-dependent manner by ARL 67156, from 5 to 100 μM (Figure 2). Higher concentrations were not used since only a limited amount of the compound was available, and it has previously been shown that ARL 67156 has a pA_2 of 3.3 as an antagonist at P_{2X}-purinoceptors (Crack *et al.*, 1995). Therefore, 100 μM ARL 67156 was used in all subsequent experiments.

Contractions of the guinea-pig vas deferens to nerve stimulation at 1, 2, 4 and 8 Hz were all significantly increased by 100 μM ARL 67156, as shown in Figure 3. The mean magnitudes of responses at 1, 2, 4 and 8 Hz were increased from

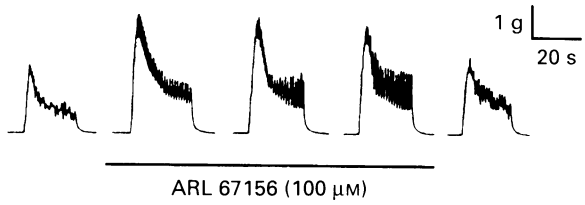

Figure 1 The time course of the effect of ARL 67156 on neurogenic contractions evoked by trains of pulses for 20 s at 2 Hz. The trace shows contractions recorded at 10 min intervals. ARL 67156 (100 μM) was added immediately after the end of the control response (first panel), left in contact with the tissue for 30 min (second, third and fourth panels), then washed out and stimulation repeated (fifth panel).

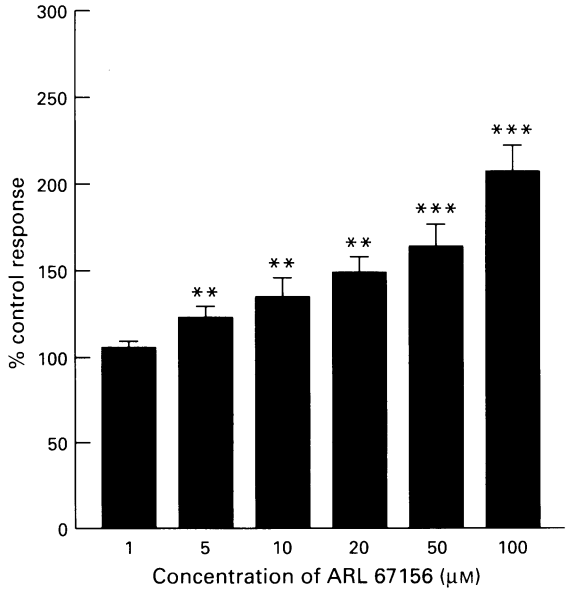

Figure 2 The concentration-effect relationship of ARL 67156 on neurogenic contractions evoked by trains of pulses for 20 s at 4 Hz. Initially, three reproducible control responses were obtained, then ARL 67156 was added at increasing concentrations (1, 5, 10, 20, 50 and 100 μM) 10 min before each stimulation. The graph shows the mean data ± s.e.mean ($n = 6$). $^{**}P < 0.01$; $^{***}P < 0.001$.

0.33± 0.07 g to 0.69±0.14 g; from 0.55±0.11 g to 0.88± 0.12 g; from 1.32±0.20 g to 2.08±0.23 g and from 2.89± 0.22 g to 4.00±0.45 g ($n=7$) respectively. The greatest percentage increase was seen at 1 Hz and this decreased with increasing frequency of stimulation.

In order to investigate the effect of ARL 67156 on the purinergic component of the neurogenic response, the α_1-adrenoceptor antagonist, prazosin, was used to inhibit the noradrenergic component (Figure 4). Prazosin (0.1 μM) abolished the response to exogenous Na (10 μM, not shown) and slightly reduced the magnitude of the phasic component of the neurogenic contraction at 4 Hz from 1.60±0.08 g to 1.26±0.12 g ($n=8$, $P<0.05$). The response to nerve stimulation in the presence of prazosin was significantly increased by subsequent addition of ARL 67156 (100 μM) from 1.26±0.12 g to 2.23±0.17 g ($n=8$, $P<0.05$). This degree of enhancement was not significantly different from that seen in the absence of prazosin. The final section of Figure 4 confirms that the contractile response in the presence of prazosin was purely purinergic, since it was abolished by the P_2-purinoceptor antagonist, PPADS (100 μM).

Effects of ARL 67156 on exogenously applied P_{2X}-purinoceptor agonists

In order to characterize the site of action of ARL 67156, its effect on the responses to submaximal concentrations of exogenous ATP (100 μM) and α,β-meATP (0.5 μM) was tested. Both ATP and α,β-meATP produced rapid, transient contractions, which reached a peak in about 5 s and then subsided rapidly, even in the continued presence of the agonist (Figure 5a). The response to exogenous ATP was increased in the presence of ARL 67156 (100 μM), and although the peak of the response was still transient, the level of tension remained elevated for longer in the presence of the ecto-ATPase inhibitor. Neither the magnitude nor the time course of the response to α,β-meATP was affected by ARL 67156 (100 μM). Figure 5b shows the effect of ARL 67156 on the responses to ATP and to α,β-meATP expressed as a percentage of control. The mean magnitude of the responses to ATP was increased from 2.65±0.30 g to 4.22±0.42 g ($n=11$) by ARL 67156, whereas the response to the stable analogue, α,β-meATP was not significantly affected (control 1.51±0.13 g to 1.46±0.10 g, $n=6$).

Effects of ARL 67156 on the responses to other contractile agents

The effect of ARL 67156 on the responses to sub-maximal concentrations of exogenous NA and KCl was also tested and the results are shown in Figure 6. In the presence of ARL 67156 (100 μM) contractions to NA (10 μM) and KCl (40 mM) were significantly enhanced over control responses, from 1.16±0.25 g to 3.41±0.44 g ($n=13$) and from 3.08±0.30 g to 4.20±0.34 g ($n=9$) respectively. This could suggest that ARL 67156 is not selective for ecto-ATPase, but causes a general increase in smooth muscle sensitivity. However, an alternative

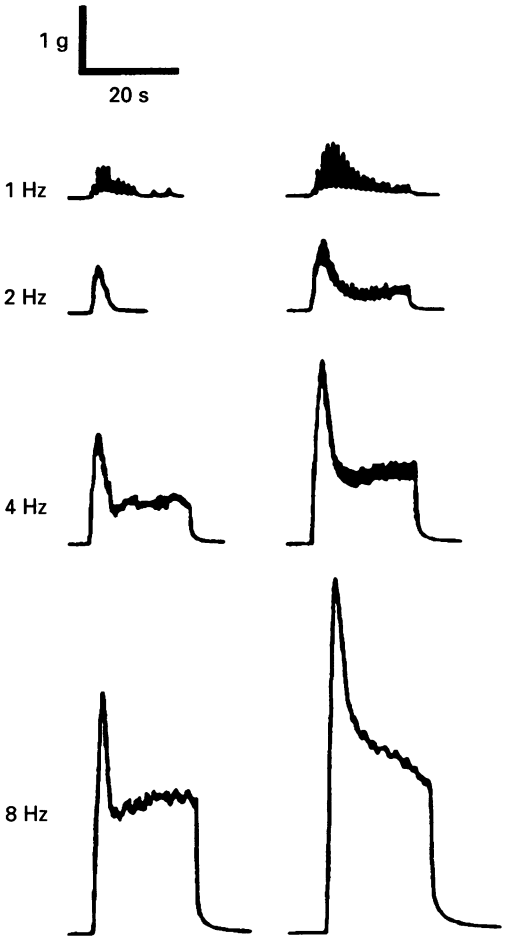

Figure 3 The effect of ARL 67156 on neurogenic contractions evoked at 1, 2, 4 and 8 Hz. The traces on the left show control responses, and those on the right show responses after 10 min in the presence of ARL 67156 (100 μM). All responses are from the same muscle strip.

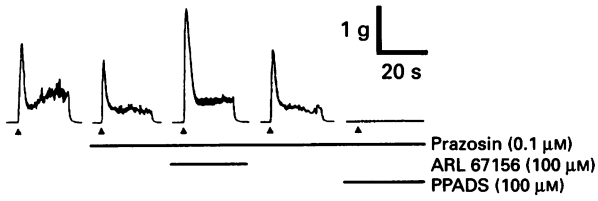

Figure 4 The effect of ARL 67156 on neurogenic contractions at 4 Hz for 20 s in the presence of prazosin; (▲) indicates the start of the 20 s period of stimulation. After the control response (first panel) prazosin (0.1 μM) was added, and was present for the remainder of the experiment. After 20 min in prazosin, nerve stimulation was repeated (second panel). ARL 67156 (100 μM) was added and another neurogenic contraction obtained 10 min later (third panel). ARL 67156 was washed out and 10 min later the potentiation was rapidly reversed (fourth panel). PPADS (100 μM) was added 20 min before the final stimulation (fifth panel).

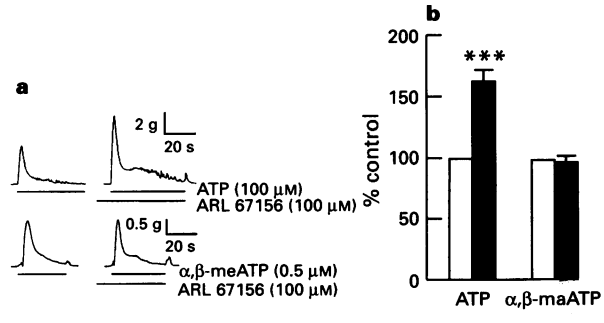

Figure 5 The effect of ARL 67156 on contractions to ATP and α,β-meATP. Section (a) shows typical responses to ATP (100 μM) and α,β-meATP (0.5 μM) before and 10 min after the addition of ARL 67156 (100 μM). The graph in (b) shows the mean magnitude of responses to ATP (100 μM, $n=11$) and α,β-meATP (0.5 μM, $n=6$) after addition of ARL 67156 (100 μM, solid columns) expressed as a percentage of control responses (open columns). (The statistical significance level shown on the graph was determined by analysis of the raw data expressed in grams, ***$P<0.001$).

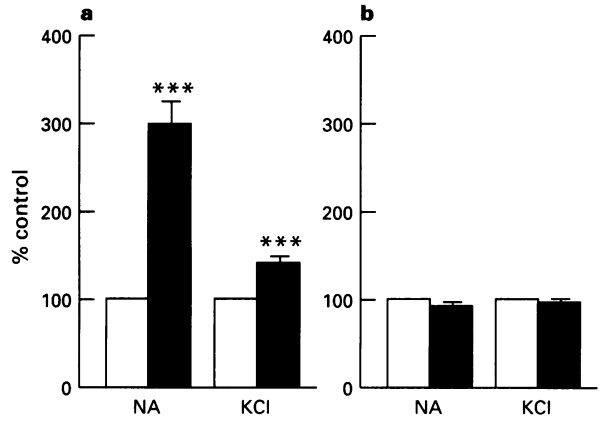

Figure 6 The effect of ARL 67156 on contractions to NA and KCl. In each graph the response to NA (10 μM) and KCl (40 μM) in the presence of ARL 67156 (solid column) is expressed as a percentage of the control response (open column). Responses were obtained either in the absence (a) or in the presence (b) of PPADS (100 μM). (The statistical significance level shown on the graph was determined by analysis of the raw data expressed in grams. ***$P<0.001$).

explanation is that the observed increase in the magnitude of the responses to NA and KCl was due to a build-up of ATP in the prolonged presence of the ecto-ATPase inhibitor, sensitizing the smooth muscle via P_2-purinoceptors. In order to test this possibility, the effect of ARL 67156 on the response to NA and KCl was examined in the presence of the P_2-purinoceptor antagonist, PPADS. PPADS (100 μM) also significantly enhanced contractions to NA and KCl from control values of 1.16 ± 0.25 g to 2.39 ± 0.22 g ($n=7$) and from 4.66 ± 0.41 g to 5.26 ± 0.32 g ($n=6$) respectively. At this time, contractions to exogenous NA and KCl were no longer enhanced by ARL 67156 (100 μM), going from control values of 2.39 ± 0.22 g to 2.17 ± 0.17 g ($n=7$) and from 5.26 ± 0.32 g to 5.19 ± 0.34 g ($n=6$) respectively.

Discussion

The results of this study show that ARL 67156 produces a substantial increase in the magnitude of the purinergic component of neurogenic contractions of the guinea-pig vas deferens. Since contractions evoked by exogenous ATP were enhanced in a similar fashion, this suggests that the postjunctional actions of the purinergic neurotransmitter are normally greatly attenuated by rapid hydrolysis by ecto-ATPase.

In the guinea-pig vas deferens, ATP is released as a co-transmitter with NA. Our initial experiments showed that ARL 67156 enhanced the phasic, predominantly purinergic, component of the neurogenic response, more than the tonic, predominantly noradrenergic, component. In the presence of prazosin, contractions to NA were abolished and the remaining neurogenic response was entirely purinergic, as it was abolished by the P_2-purinoceptor antagonist, PPADS. ARL 67156 produced a similar degree of potentiation of this purely purinergic contraction as it had in the absence of prazosin, confirming that the enhancing effect of ARL 67156 on neurogenic responses is due to its ability to enhance the action of ATP as a neurotransmitter.

The ability of ARL 67156 to potentiate neurogenic contractions of the guinea-pig vas deferens was greatest at low frequencies of stimulation. The mechanism underlying this effect is unclear, but may reflect the fact that as greater amounts of ATP are released at the higher frequencies, so proportionally less ATP may be broken down and thus ARL 67156 will appear to have less of an effect. Alternatively, the inverse relationship may be due to less prejunctional inhibition by adenosine. By inhibiting extracellular breakdown of ATP, ARL 67156 also inhibits the production of adenosine, the major breakdown product of ATP in this tissue (Bailey & Hourani, 1994). Adenosine can act at prejunctional P_1-purinoceptors to depress sympathetic neurotransmission in the guinea-pig vas deferens (Sneddon *et al.*, 1984; Driessen *et al.*, 1994), an effect which is greatest at low frequencies of stimulation. Thus, inhibition of adenosine production may be expected to cause greater potentiation of contractions evoked at low frequencies of stimulation, as was seen here. However, in a previous study, the P_1-purinoceptor antagonist, 8-phenyltheophylline, had no effect on neurogenic contractions in this tissue (Sneddon *et al.*, 1984), and so the involvement of endogenous adenosine in the potentiating action of ARL 67156 is uncertain. The mechanism underlying the frequency-dependence of the action of ARL 67156 is currently under study.

In the present experiments ARL 67156 was effective at 5–100 μM, which is very similar to the concentration-range over which it inhibits ecto-ATPase activity in human blood cells (Crack *et al.*, 1995) and rat vas deferens (Khakh *et al.*, 1995). In human blood cells the highest concentration used (1000 μM) inhibited enzyme activity by about 95%, with an IC_{50} of 24 μM (Crack *et al.*, 1995). Such a high concentration could not be used in the present study as limited amounts of the compound were available. Also, ARL 67156 is a weak antagonist at P_{2X}-purinoceptors, ($pA_2=3.3$, Crack *et al.*, 1995), so at concentrations above 100 μM, measurement of the enhancing effect of ARL 67156 on purinergic responses would be inaccurate, as receptor antagonism would significantly inhibit the postjunctional action of ATP. At the highest concentration used here in the guinea-pig vas deferens, 100 μM, ARL 67156 inhibited ecto-ATPase activity in human blood cells by approximately 70%. Thus, if it were possible to produce complete ecto-ATPase inhibition without antagonizing P_{2X}-purinoceptors, it is likely that sympathetic purinergic responses in the guinea-pig vas deferens could be increased by even more than was seen here.

In the present study, ATP and α,β-meATP both evoked rapid, transient contractions, with a similar time course to the purinergic component of the neurogenic response. However, ARL 67156 only potentiated responses to ATP, consistent with it acting to inhibit ecto-ATPase. Cunnane & Manchanda (1988) previously depressed ecto-ATPase activity in the guinea-pig vas deferens by cooling the tissue from 35°C to 25°C. This greatly decreased the rate of decay of purinergic e.j.ps and of the depolarization evoked by exogenous ATP, but had no effect on the depolarization elicited by α,β-meATP. Again this supports the conclusion that the actions of ATP are greatly attenuated by ecto-ATPase. This has important consequences for the characterization of P_2-purinoceptors. In the absence of selective antagonists, P_2-purinoceptors have been classified largely on the basis of rank order of agonist potency (Burnstock & Kennedy, 1985; Gordon, 1986; O'Connor *et al.*, 1991). In many tissues, including the guinea-pig vas deferens, α,β-meATP appears to be 100–1000 times more potent than ATP at the P_{2X}-purinoceptor. However, now that the influence of agonist breakdown by ecto-ATPase has become clear, it is recognised that ATP is in fact more potent than α,β-meATP and the pharmacological properties of the P_{2X}-purinoceptor have been redefined (see Kennedy & Leff, 1995).

The enhancing effects of ARL 67156 on contractions evoked by nerve stimulation or exogenous agonists developed rapidly and were readily reversed upon washout of the drug, consistent with an extracellular site of action. ARL 67156 is a charged molecule and a structural analogue of ATP and so would be unlikely to gain rapid access to an intracellular site. Ecto-ATPases have been purified from a range of tissues (see Ziganshin *et al.*, 1994) and in some cases cDNA sequences isolated. The ecto-ATPases were found to be identical to previously identified cell adhesion molecules (Lin & Guidotti, 1989; Culic *et al.*, 1992; Dzhandzhugazyan & Bock, 1993; Edlund *et al.*, 1993; McCuaig *et al.*, 1993; Najjar *et al.*, 1993).

These glycoproteins are largely extracellular and the region which encodes ecto-ATPase activity is found in the extracellular portion. The identity of the ecto-ATPase/cell adhesion molecule present in the guinea-pig vas deferens is not known.

In these experiments the responses of the guinea-pig vas deferens to exogenous NA and KCl were significantly enhanced by ARL 67156. This was unexpected, since it was not obvious how NA, KCl and ATP could be potentiated by ARL 67156, while α,β-meATP was unaffected. Exogenous NA has been shown to induce the release of ATP in this tissue (Katsuragi *et al.*, 1991; Vizi *et al.*, 1992) and so it may be that the potentiation of NA by ARL 67156 was due to inhibition of breakdown of the released ATP. However, this is unlikely as α,β-meATP also evokes release of ATP (Katsuragi *et al.*, 1991), but was not potentiated by ARL 67156 here. Furthermore, contractions evoked by ATP reach a peak within 5 s and those by NA within 20 s (this study, not shown; Sneddon & Westfall, 1984), whereas NA-induced release of ATP is much slower, taking 2–6 min to reach a peak (Katsuragi *et al.*, 1991; Vizi *et al.*, 1992).

A more likely explanation for the potentiation of NA and KCl by ARL 67156 is that in the prolonged presence of the ecto-ATPase inhibitor there was an accumulation of endogenous ATP in the extracellular space, which could act to potentiate contractions to NA and KCl. Indeed, ATP and NA have previously been shown to interact synergistically in the guinea-pig vas deferens (Holck & Marks, 1978; Sakai *et al.*, 1979; Kazic & Milosavljevic, 1980). Furthermore, as KCl acts in part by releasing neuronal ATP and NA, then ARL 67156 could potentiate KCl-induced contractions (a) by inhibiting the breakdown of the released ATP and (b) by potentiating synergism between ATP and NA. Since ARL 67156 did not potentiate NA or KCl in the presence of PPADS, this suggested that the potentiation seen in the absence of PPADS was not due to a general, non-selective sensitization of the smooth muscle, but rather was due to a specific mechanism involving P_2-purinoceptors and some form of receptor cross-talk. However, this interpretation is complicated by the fact that PPADS also potentiated contractions to NA and KCl. The mechanism underlying this effect is unclear, but it should be noted that PPADS can also inhibit ecto-ATPase activity (Khakh *et al.*, 1995) and depolarizes smooth muscle cells in the vas deferens via a mechanism unrelated to P_2-purinoceptors (McLaren *et al.*, 1994).

The subtype of P_2-purinoceptor involved in the synergism is not clear. At the concentration used in this study, 100 μM, PPADS is certainly an antagonist at P_{2X}-purinoceptors in the guinea-pig vas deferens (McLaren *et al.*, 1994). However, at 100 μM, PPADS is also an antagonist of at least one subtype of P_{2Y}-purinoceptor, as it has a pA_2 of 5.9 at the P_{2Y}-purinoceptor present in turkey erythrocytes (Boyer *et al.*, 1994). The mechanism by which ATP potentiated NA is also unclear. Recently, pathways have been characterized by which agonists acting at one type of receptor can sensitize smooth muscle to agonists acting through other receptors (Somlyo & Somlyo, 1994; Walsh, 1994), but further studies are required to determine if ATP can activate these mechanisms.

In conclusion, the results of these experiments support the hypothesis that when ATP acts as a neurotransmitter in the guinea-pig vas deferens, its postjunctional actions are greatly reduced by the enzymatic degradation. Thus, ecto-ATPase appears to have a physiological role in modulating purinergic neurotransmission in this tissue. Since ecto-ATPase is present throughout the body, ARL 67156 may also enhance responses to neuronally-released ATP in other tissues where ATP acts as a neurotransmitter, such as the urinary bladder, various parts of the gastroinestinal tract, arteries and the central nervous system.

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