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The activation of P_1- and P_2-purinoceptors in the guinea-pig left atrium by diadenosine polyphosphates

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1 The effects of P^1, P^2-di(adenosine) pyrophosphate (AP_2A), P^1,P^3-di(adenosine) triphosphate (AP_3A), P^1,P^4-di(adenosine) tetraphosphate (AP_4A), P^1,P^5-di(adenosine) pentaphosphate (AP_5A), ATP, α,β-methylene ADP and 2-chloroadenosine (2-ClAd) were examined in the guinea-pig driven left atrium.

2 All these purine compounds except α,β-methylene ADP produced a negative inotropic response with a rank order of potency of: 2-ClAd$>>AP_2A \geqslant ATP \geqslant AP_4A = AP_3A = AP_5A$. The EC_{50} value for 2-ClAd was approximately 1 μM, while those for the remaining compounds were in the range 10 μM – 100 μM, α,β-Methylene ADP (10 – 300 μM), a selective P_{2Y}-purinoceptor agonist, produced a small positive inotropism.

3 The P_1-purinoceptor antagonist, 8-*para*-sulphophenyltheophylline (8-*p*SPT, 20 μM) caused a rightward shift in the concentration-response curves for 2-ClAd, ATP and AP_2A, but converted the responses of AP_3A, AP_4A, and AP_5A into positive inotropisms.

4 The non-selective P_2-purinoceptor antagonist, suramin (300 μM), had no significant effect on the concentration-response curves for 2-ClAd, ATP or AP_2A, but significantly antagonized inhibitory responses to AP_3A, AP_4A and AP_5A, and excitatory responses to α,β-methylene ADP.

5 In the presence of 8-*p*SPT (20 μM), suramin (300 μM) abolished the positive inotropic responses evoked by the dinucleotides.

6 ATP was degraded far more rapidly than any of the dinucleotides, and AP_3A was the least stable of the diadenosine compounds. The relative order of stability was $AP_2A > AP_4A = AP_5A > AP_3A >>$ ATP. Suramin (300 μM) reduced the rate of degradation of ATP and AP_3A by approximately 30%. Suramin had no significant effect on the degradation of AP_2A, AP_4A or AP_5A.

7 It is concluded that the diadenosine polyphosphates cause negative inotropic responses via P_1-purinoceptors and a hitherto undefined suramin-sensitive P_2-purinoceptor, and that they appear to have positive inotropic effects mediated via another suramin-sensitive P_2-purinoceptor.

Keywords: ATP; diadenosine polyphosphates; guinea-pig heart; purinoceptors

Introduction

Adenosine and its phosphorylated derivatives, adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP) and adenosine 5'-triphosphate (ATP) are known to cause negative responses in the guinea-pig left atrium. The effects of the nucleotides are mediated via P_1-purinoceptors since they are antagonised by theophylline or 8-phenyltheophylline following degradation of the nucleotide to adenosine (Burnstock & Meghji, 1981; Moody *et al.*, 1984). This P_1-purinoceptor has been subclassified as A_1 (Evans & Schenden, 1982; Collis, 1983), and is antagonized by the A_1-selective antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (Borea *et al.*, 1989; Mantelli *et al.*, 1993). It has recently been shown that when P_1-purinoceptors are blocked a positive inotropic response to ATP can be revealed, which is mediated via P_{2Y}-purinoceptors (Mantelli *et al.*, 1993).

Recently there has also been an increasing interest in the cardiovascular effects of diadenosine polyphosphates (Gardiner *et al.*, 1994; Kengatharan *et al.*, 1994; Schluter *et al.*, 1994; Ralevic *et al.*, 1995). These compounds consist of two adenosine moieties connected via 5'-ribose linkages to each end of a polyphosphate chain. Naturally occurring diadenosine polyphosphates have a chain length varying from three to six phosphate groups (Hoyle, 1990; Pintor *et al.*, 1992b; Schluter *et al.*, 1994), and the synthetic compound P^1,P^2-di(adenosine) pyrophosphate (AP_2A) completes an homologous series. They are found in platelets, and are released upon degranulation (Flodgaard & Klenow, 1982; Luthje & Ogilvie, 1983; Schluter *et al.*, 1994), and they can act either on endothelial cells or vascular smooth muscle cells, thereby exerting a control over vascular tone. P^1,P^3-di(adenosine) triphosphate (AP_3A) mediates endothelium-dependent vasodilatation in the rat mesenteric artery (Ralevic *et al.*, 1995), and endothelium-independent vasodilatation in the rabbit mesenteric artery (Busse *et al.*, 1988). P^1,P^4-di(adenosine) tetraphosphate (AP_4A), P^1,P^5-di(adenosine) pentaphosphate (AP_5A) and P^1,P^6-di(adenosine) hexaphosphate (AP_6A) can mediate vasoconstriction in rat and rabbit mesenteric vessels by acting directly on the vascular smooth muscle (Busse *et al.*, 1988; Ralevic *et al.*, 1995), but AP_4A may also induce endothelium-dependent vasodilatation (Busse *et al.*, 1988). Furthermore, AP_3A and AP_4A can both increase hepatic portal vein pressure in perfused rat liver (Busshardt *et al.*, 1989), yet can cause coronary vasodilatation in the rabbit heart (Pohl *et al.*, 1991). *In vivo* AP_3A and AP_4A cause dilatation in rat mesenteric and hindquarter vascular beds, while AP_3A to AP_6A all cause renal vasoconstriction (Gardiner *et al.*, 1994). Also *in vivo*, AP_4A induces a fall in total peripheral resistance and slows the heart rate in dogs (Kikuta *et al.*, 1994), while AP_5A slows the heart rate in rats (Kengatharan *et al.*, 1994).

The aim of the present study was to examine the effects of a series of diadenosine polyphosphates (AP_2A to AP_5A) on the guinea-pig driven left atrium. In particular we were interested in addressing the question of whether distinct receptors for any of the diadenosine polyphosphates are present in this tissue

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that are distinct from receptors in the existing P_1/P_2-purinoceptor scheme. In addition, the ability of this cardiac tissue to degrade the diadenosine compounds was studied in order to determine whether or not their breakdown could contribute to their pharmacological profile. Finally, the characterization of the structure-activity relationships of the adenine dinucleotides could be valuable in providing information about ligand-receptor interactions.

Methods

Organ-bath experiments

Male Dunkin-Hartley guinea-pigs (320–440 g) were killed by a blow to the head and bleeding from the neck. The thorax was opened and the heart was removed and placed in Krebs solution at room temperature. The left atrium was dissected free, and was then bisected through its apex, resulting in two hemiatria. Two silk ligatures were attached to each preparation, one was tied to a rigid support placed in a 5 ml sidearm-gassed organ bath (LIN-1 tissue bath station, Linton Instrumentation, Norfolk) containing Krebs solution maintained at 30°C, and the other to an isometric force transducer (FSG-01, Experimetria, Hungary). The preparation was suspended vertically between two Pt ring electrodes, 2.5 mm diameter, 10 mm apart. The hemiatria were paced at 2.5 Hz, by a biphasic square-wave pulse (overall duration 5 ms) delivered at twice the threshold voltage (usually in the range 1.6–2.2 V) by an Experimetria ST-02 stimulator. Data were recorded by an MP100WSW data acquisition system and Acquire software (Biopac). The Krebs solution had the following composition (mM): NaCl 133, KCl 4.7, $NaHCO_3$ 16.3, NaH_2PO_4 1.35, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 7.8, and was gassed with 95% O_2/5% CO_2 giving a pH of 7.3–7.4. The Krebs solution also contained atropine (0.3 μM) and guanethidine (2 μM), to prevent interactions involving acetylcholine or sympathetic transmission. The preparations were given an initial load of 0.50–0.75 g and were allowed to equilibrate for 1 h before commencing experimentation. When the P_1-purinoceptor antagonist, 8-*para*-sulphophenyltheophylline (8-*p*SPT) (Fredholm & Sandberg, 1983), was used it was added to the organ bath and given an initial equilibration period of 30–45 min. The P_2-purinoceptor antagonist, suramin (Hoyle *et al.*, 1990), was also added directly to the organ bath as required, and because of its slow equilibration (Leff *et al.*, 1990), was given an initial period of at least 1 h.

Concentration-response relationships were constructed by adding single doses of nucleotide to the organ bath, and washing out after a maximum level of response had been observed. After the wash-out the subsequent dose was applied when the preparation had re-equilibrated (approximately 10 min). Doses of nucleotides were added in a random order in the range 0.1 μM to 1 mM. Some of the dinucleotides were limited to a maximum concentration of 100 or 300 μM, because of their cost. 2-Chloroadenosine (2-ClAd), which in contrast to the nucleotides, yields fairly stable steady-state responses, was added cumulatively (10 nM to 30 μM or more). Only one nucleotide was tested on a given preparation, before and after incubation with an antagonist. Time-controls were performed, and second curves constructed in the absence of an antagonist did not significantly differ from the first curves.

Degradation assay

The assay was carried out at 30±0.5°C in a buffer of the following composition (mM): NaCl 135, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, glucose 10 and HEPES 10. The pH was 7.4. The left atrium was obtained as described above and cut into small pieces (approximately 2–3 mg) which were placed in 24-well cell culture dishes, and pre-washed with buffer for 15–20 min. The pre-wash buffer was then changed for 250 μl buffer containing ATP (100 μM) or one of the adenine dinucleotides (100 μl). The tissues were continuously agitated during the subsequent 30 min incubation. When suramin (300 μM) was used it was added 30 min before ATP or a dinucleotide was added. At the end of the incubation period the buffer was removed, placed into a polythene phial, and frozen in liquid nitrogen. After being frozen the samples were stored at −20°C until they were assayed by reverse-phase high performance liquid chromatography (h.p.l.c.) with ultraviolet detection. The h.p.l.c. assay for ATP and adenine dinucleotides was based on that described previously (Pintor *et al.*, 1991; 1992a). The chromatography system comprised a Beckman 114M solvent delivery module coupled to an SA6500 UV/VIS absorbance detector (Severn Analytical). The column (Spherisorb ODS2, 25 cm length, 0.46 cm internal diameter, Highchrom) was equilibrated overnight with a mobile phase containing 0.2 M KH_2PO_4 and 3% (v/v) methanol, pH 6.0. The separation of nucleotides was carried out at a flow rate of 1.5 ml min^{-1}, with the spectrophotometer set at 260 nm. Concentrations of nucleotides were calculated by comparing peak heights of samples with those of known standards. The results are expressed as mean±s.e.mean of *n* experiments performed in duplicate.

Drugs used

Atropine sulphate was obtained from Phoenix Pharmaceuticals Ltd (Ireland). Guanethidine sulphate (Ismelin) was obtained from Boehringer Ingelheim. Suramin (Germanin) was a generous gift from Bayer. 8-*p*SPT was obtained from Research Biochemicals International (MA 01760, U.S.A.). All other compounds were obtained from Sigma Chemical Co.

Statistical analysis

Concentration-response curves were compared by two-way analysis of variance (ANOVA) with repeated measures. Means of groups were compared by one-way ANOVA or paired

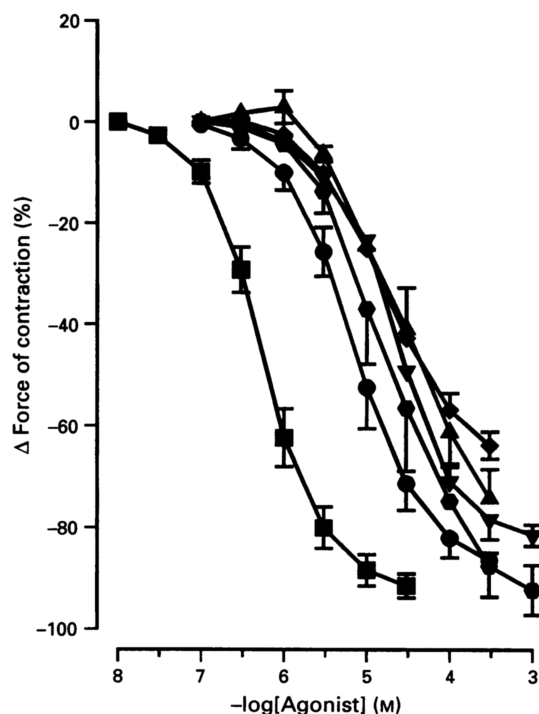

Figure 1 Guinea-pig driven left atrium: concentration-response relationships for 2-chloroadenosine (■, *n*=11), ATP (⬢, *n*=10), P^1,P^2-di(adenosine) pyrophosphate (●, *n*=8), P^1,P^3-di(adenosine) triphosphate (▲, *n*=8), P^1,P^4-di(adenosine) tetraphosphate (▼, *n*=10) and P^1,P^5-di(adenosine) pentaphosphate (◆, *n*=9). Ordinate scale shows change in force of contraction expressed as a percentage relative to force of contraction before administration of agonist. Points show mean±s.e. mean, unless occluded by symbol.

Student's t test. The term $p[A]_{50}$ is used to describe the -log[agonist] that produced a 50% decrease in force of contraction of the atrial beat. Data are given as mean ± s.e.mean (n), where n is the number of replicates, and also the number of animals. Significance was established at 5%. All figures were prepared by use of Fig P version 2.1a (Biosoft, Cambridge), and data analysis was facilitated by Fig P and Microsoft Excel 5.0 (Microsoft Corporation).

Results

The mean force of contraction of the hemiatrial preparations, paced at 2.5 Hz by twice threshold voltage, in the absence of applied agents, was 367.0 ± 23.31 mg (56 preparations from 28 animals).

In these tissues 2-ClAd, AP_2A, AP_3A, AP_4A, AP_5A and ATP all caused a concentration-dependent negative inotropic effect (Figure 1). The rank order of potency based on the $p[A]_{50}$ values was: 2-ClAd, 6.1 ± 0.11 (11) >> AP_2A, 4.9 ± 0.13 (8) ≥ ATP, 4.6 ± 0.17 (10) ≥ AP_4A, 4.3 ± 0.08 (10) = AP_3A, 4.2 ± 0.15 (8) = AP_5A, 4.1 ± 0.09 (9). 2-ClAd was at least ten times more potent than any compound (statistical significances: $P<0.001-P<0.0001$). AP_2A was significantly the most potent of the dinucleotides ($P<0.01-P<0.001$), but there was no statistically significant difference between ATP, AP_3A, AP_4A and AP_5A. α,β-Methylene ADP (10–300 μM) produced a small positive inotropism that had a maximum level of 5.8 ± 1.66% (4) at 100 μM.

When applied to the organ bath, 8-*p*SPT (20 μM) evoked a transient positive inotropic response that took a few minutes to develop fully, and which gradually subsided and plateaued during the remainder of the equilibration period. At the end of the equilibration the basal force of contraction had increased from 374.8 ± 37.54 mg to 497.0 ± 51.00 mg, with a mean increase in force of contraction of 38.8 ± 11.50% ($n=16$, $P<0.001$, paired t test). 8-*p*SPT (20 μM) caused a parallel rightward displacement of the concentration-response curves for 2-ClAd and ATP (Figure 2). In the presence of 8-*p*SPT (20 μM) the $p[A]_{50}$ values for 2-ClAd and ATP were 4.9 ± 0.13 (7) and 3.7 ± 0.10 (6), respectively and the estimated pA_2 values for 8-*p*SPT against 2-ClAd and ATP were 6.0 ± 0.14 (7) and 5.4 ± 0.18 (6) respectively: these pA_2 values are significantly different ($P<0.05$). In the presence of 8-*p*SPT (20 μM) the inhibitory responses to AP_3A, AP_4A and AP_5A were severely attenuated and most often were converted to positive inotropisms (Figure 2 and as exemplified by AP_4A in Figure 3): these concentration-response relationships did not differ significantly from one another ($P>0.20$). The curve for AP_2A was shifted rightwards, and the maximum level of response obtained prevented evaluation of a $p[A]_{50}$ value (Figure 2), and no positive inotropisms were observed.

Addition of suramin (300 μM) to the organ bath either had no effect, or gave a transient negative or positive inotropic response, either of which was slow in onset, and which declined during the equilibration period. At the end of the equilibration period the force of contraction of the hemiatrium was not significantly different from that prior to the application, being 356.2 ± 36.15 mg and 406.7 ± 52.60 mg before and after application of suramin, respectively. This represents a mean increase of 17.4 ± 15.04% ($n=12$, not significant, paired t test). Suramin (300 μM) had no significant effect on the concentration-response relationships for either 2-ClAd ($P=1.00$), ATP ($P=0.68$) or AP_2A ($P=0.15$) (Figure 4) and did not significantly alter their $p[A]_{50}$ values (Table 1). However, it shifted the concentration-response relationships for AP_3A, AP_4A and AP_5A rightwards with significant decreases in their $p[A]_{50}$ values (Table 1), but these shifts did not appear to be parallel (Figure 4). Suramin (300 μM) abolished the positive inotropic responses evoked by α,β-methylene ADP (10–300 μM). When suramin (300 μM) was applied in the presence of 8-*p*SPT

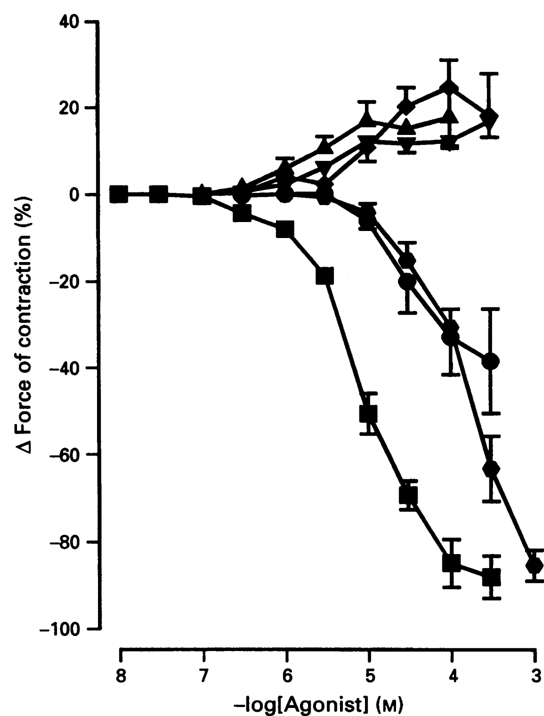

Figure 2 Guinea-pig driven left atrium: concentration-response relationships for 2-chloroadenosine (■, $n=7$), ATP (⬣, $n=6$), P^1,P^2-di(adenosine) pyrophosphate (●, $n=4$), P^1,P^3-di(adenosine) triphosphate (▲, $n=4$), P^1,P^4-di(adenosine) tetraphosphate (▼, $n=6$) and P^1,P^5-di(adenosine) pentaphosphate (◆, $n=5$) in the presence of 8-*para*-sulphophenyltheophylline (20 μM). Ordinate scale shows change in force of contraction expressed as a percentage relative to force of contraction before administration of agonist. Points show mean ± s.e. mean, unless occluded by symbol.

(20 μM) the positive inotropic responses evoked by AP_3A, AP_4A and AP_5A were severely attenuated, or even abolished (Figure 5 and as exemplified by AP_4A in Figure 3).

When suramin (300 μM) was included in the Krebs solution from the beginning of the experiment, 8-*p*SPT (20 μM) again produced a rightward shift of the concentration-response relationship for ATP (Figure 6), with an apparent pA_2 of 5.4 ± 0.14 (4). When AP_4A was tested under the same conditions, 8-*p*SPT severely attenuated the negative inotropic responses at all concentrations (Figure 6).

Degradation of nucleotides by atrial tissue

The results of the degradation study are given in Table 2. ATP was the most unstable compound, and was significantly more labile than any of the dinucleotides ($P<0.0001$). Amongst the dinucleotides there was approximately two to six times more loss of AP_3A during the incubation period than there was of AP_2A, AP_4A or AP_5A ($P=0.01-P<0.0005$). The rank order of stability of the compounds was: $AP_2A > AP_4A = AP_5A > AP_3A >> $ ATP.

Suramin (300 μM) inhibited the rate of degradation of ATP by 31.3% and reduced the rate of degradation of AP_3A by 36.7%. Suramin did not significantly affect the rate of degradation of any of the other dinucleotides (Table 2).

Discussion

The results show that the diadenosine polyphosphates, AP_2A, AP_3A, AP_4A and AP_5A are all capable of reducing the force of contraction of the guinea-pig driven left atrium. The inhibitory response was probably mediated by a P_1-purinoceptor since it was attenuated, abolished or even reversed into an excitation by 8-*p*SPT. Both 2-ClAd and ATP were also antagonized by 8-

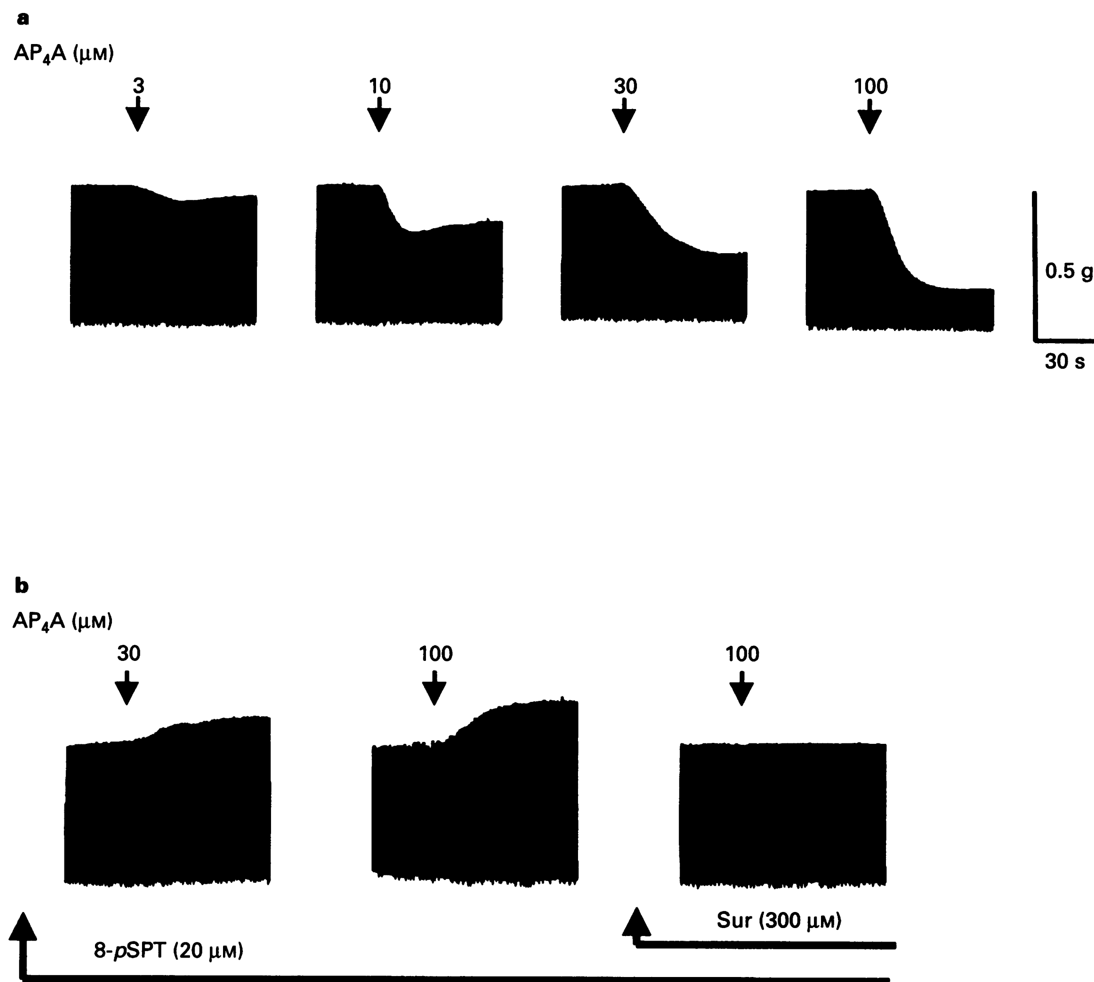

Figure 3 Effects of 8-*para*-sulphophenyltheophylline (8-*p*SPT) and suramin (Sur) on responses to P^1,P^4-di(adenosine) tetraphosphate (AP_4A) in the guinea-pig driven left atrium. (a) Control responses to AP_4A, applied as indicated by the arrows. (b) Responses to AP_4A in the presence of 8-*p*SPT and then in the additional presence of suramin. Preincubation times for 8-*p*SPT and suramin were at last 45 min and 60 min, respectively.

*p*SPT, again suggesting that these compounds were acting via P_1-purinoceptors, however the significantly greater pA_2 value when 2-ClAd rather than ATP was used as the agonist indicates that the responses evoked by 2-ClAd and ATP do not involve identical populations of receptors; the negative inotropic responses to ATP were likely to be compromised by P_2-purinoceptor-mediated positive inotropy and therefore simple competition might not be predicted. The blockade by suramin of excitatory responses to AP_3A, AP_4A and AP_5A unmasked by 8-*p*SPT, implies that the positive inotropic responses were mediated by P_2-purinoceptors.

If indeed there are two populations of receptors that the dinucleotides can activate, i.e. an inhibitory P_1- and an excitatory P_2-purinoceptor, then it might be expected that suramin, in blocking the positive inotropic response mediated via P_2-purinoceptors would thereby potentiate the inhibition, and shift the concentration-response curve for the dinucleotides to the left. However, this was not the case, and surprisingly suramin antagonized negative inotropic responses evoked by the dinucleotides. Since suramin did not affect the concentration-response relationship for 2-ClAd an interaction with P_1-purinoceptors can be ruled out. Also, since suramin did not significantly affect the degradation of AP_2A, AP_4A or AP_5A, an inhibition of degradation resulting in decreased inotropic responses can also be ruled out for these compounds. Thus it seems that there is a third population of receptors present. These receptors are activated by the dinucleotides, are suramin-sensitive, and mediate negative inotropy.

If the negative inotropic responses evoked by the nucleotides were mediated solely via a P_1-purinoceptor that is activated following degradation of the nucleotide, and consequent production of adenosine, then one might expect ATP to be substantially more potent than any of the dinucleotides since it was the least stable compound, and certainly not expect it to be less potent than AP_2A, which was the most stable compound. If this production of adenosine, which is the actual P_1-purinoceptor agonist in this preparation (Moody *et al.*, 1984), were the sole determinant of the negative inotropic response then it would be expected that there would be a distinct agonist potency order amongst ATP and the dinucleotides, but there was not. Activation of the P_2-purinoceptor by the agonist itself mediating positive inotropy will also affect apparent potency.

Suramin inhibited the breakdown of ATP but had no effect on the negative inotropic responses to ATP. Suramin inhibited the rate of degradation of ATP by 31.3%, which is equivalent to increasing the concentration of ATP at the most by 0.12 of a log unit, which in turn was equivalent to approximately half a standard error of the mean $p[A]_{50}$ value. It is therefore likely that the level of inhibition of ectoATPase by suramin is simply not great enough to be reflected in the concentration-response relationships. For AP_3A, which was degraded by the tissue at a far slower rate than ATP, the inhibition of hydrolysis by suramin would be even more difficult to determine from the concentration-response curves.

It has been shown that when P_1-purinoceptors are blocked ATP can produce a positive inotropic response mediated by P_{2Y}-purinoceptors (Mantelli *et al.*, 1993). However, in the present study, which was carried out under similar conditions,

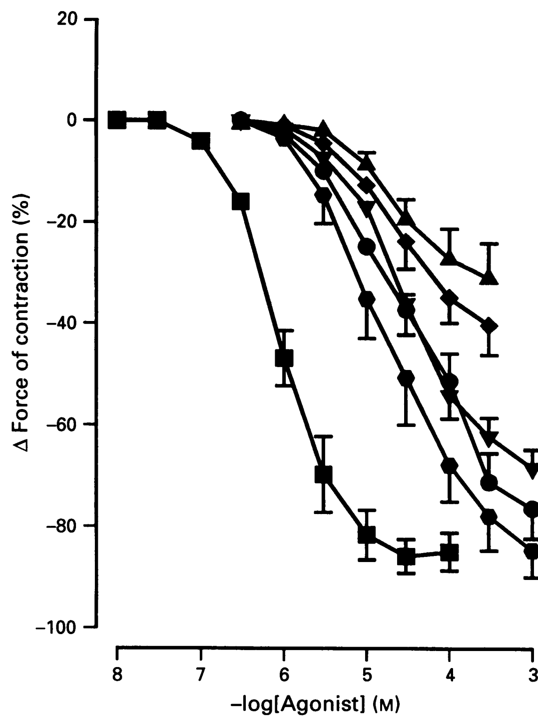

Figure 4 Guinea-pig driven left atrium: concentration-response relationships for 2-chloroadenosine (■, $n=4$), ATP (●, $n=4$), P^1,P^2-di(adenosine) pyrophosphate (●, $n=4$), P^1,P^3-di(adenosine) triphosphate (▲, $n=4$), P^1,P^4-di(adenosine) tetraphosphate (▼, $n=4$) and P^1,P^5-di(adenosine) pentaphosphate (◆, $n=4$) in the presence of suramin (300 μM). Ordinate scale shows change in force of contraction expressed as a percentage relative to force of contraction prior to administration of agonist. Points show mean ± s.e. mean, unless occluded by symbol.

Table 1 Potencies ($p[A]_{50}$ values) of 2-chloroadenosine, ATP and adenine dinucleotides at inducing negative inotropy in the guinea-pig driven left atrium in the absence and presence of suramin (300 μM)

Agonist	*Control*	*+ Suramin*
2-Chloroadenosine	5.8 ± 0.10 (4)	5.7 ± 0.10 (4)
ATP	4.5 ± 0.24 (4)	4.4 ± 0.20 (4)
AP_2A	4.6 ± 0.13 (4)	4.4 ± 0.15 (4)
AP_3A	4.3 ± 0.21 (4)	<3.0**
AP_4A	4.5 ± 0.17 (4)	4.0 ± 0.05 (4)*
AP_5A	4.1 ± 0.16 (4)	3.5 ± 0.21 (4)*

*$P<0.05$, paired t test. **The $p[A]_{50}$ value could not be evaluated in the presence of suramin.

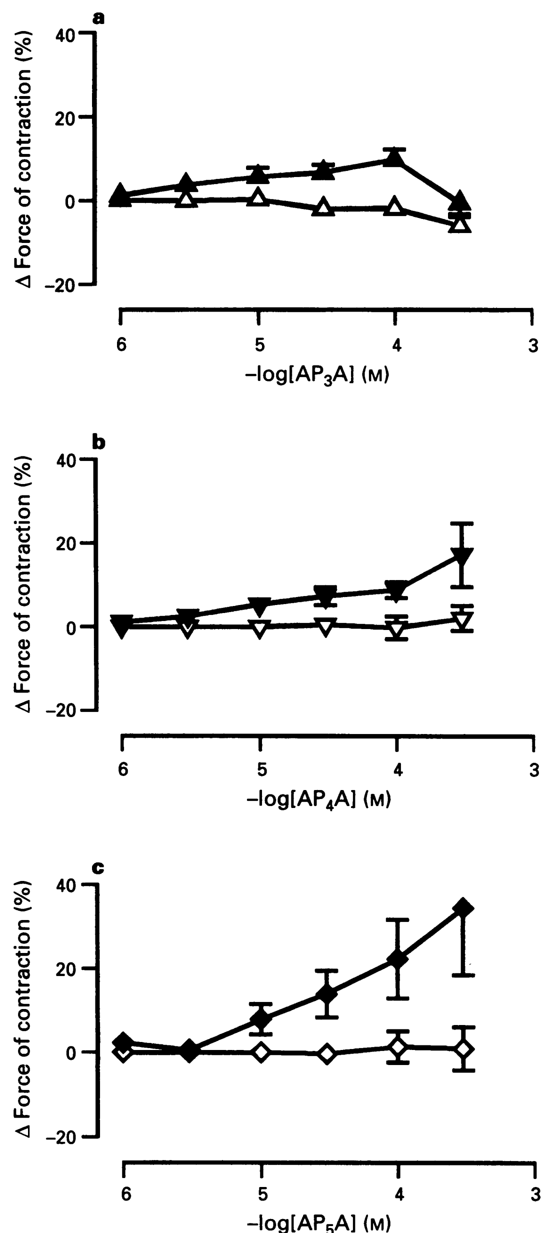

Figure 5 Guinea-pig driven left atrium: effects of suramin on concentration-response relationships for (a) P^1,P^3-di(adenosine) triphosphate (AP_3A, $n=4$), (b) P^1,P^4-di(adenosine) tetraphosphate (AP_4A, $n=4$) and (c) P^1,P^5-di(adenosine) pentaphosphate (AP_5A, $n=4$) in the presence of 8-*para*-sulphophenyltheophylline (20 μM). Closed and open symbols show responses in absence and presence of suramin (300 μM), respectively. Points show mean ± s.e. mean, unless occluded by symbol.

no positive inotropic response to ATP was seen. Perhaps this is due to a different strain of guinea-pig being used. The receptor that mediated the positive inotropic response of the dinucleotides does have some similarities with P_{2Y}-purinoceptors and few similarities with P_{2X}-purinoceptors, but does not convincingly fall into either category. Firstly, α,β-methylene ADP produced small positive inotropic responses. This compound is a very weak agonist of P_{2X}-purinoceptors in the guinea-pig vas deferens, being inactive up to 100 μM (Fedan *et al.*, 1982) and it does not activate P_{2X}-purinoceptors in the rat mesenteric arterial bed (Hoyle & Ralevic, unpublished observations), but it is a potent agonist of the P_{2Y}-purinoceptor in the guinea-pig taenia coli (Satchell & Maguire, 1975): if the receptors present in the heart were P_{2Y} then perhaps larger responses would be expected. Secondly, at P_{2X}-purinoceptors in the rat mesenteric arterial bed, guinea-pig urinary bladder and vas deferens there is a distinct agonist potency order amongst the dinucleotides, with AP_5A being the most potent, and approximately ten to 1000 times more potent than AP_4A or AP_3A, respectively (Ralevic *et al.*, 1995; Hoyle *et al.*, 1995a,b). In the driven left atrium there was no significant difference in the potency of the three largest dinucleotides, implying that this receptor is not likely to be P_{2X}. In the P_{2Y}-system of rat mesenteric artery endothelial cells, both AP_4A and AP_5A are inactive (Ralevic *et al.*, 1995), but in the guinea-pig taenia coli and human colon circular muscle where all the dinucleotides are active, there is little difference amongst their potencies (Hoyle & Burnstock, 1992; Hoyle *et al.*, 1995b). Thus the lack of a discernible potency order would be more indicative of activation of a P_{2Y}- rather than a P_{2X}-purinoceptor. Intriguingly, AP_2A is inactive in the three P_{2X}-systems mentioned above (Ralevic *et al.*, 1995; Hoyle *et al.*, 1995a,b), but it is active in the three P_{2Y}-systems

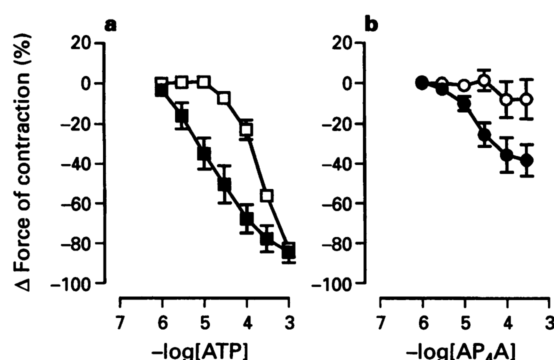

Figure 6 Guinea-pig driven left atrium: effects of 8-*para*-sulphophenyltheophylline (8-*p*SPT) on concentration-response relationships for (a) ATP and (b) P^1,P^4-di(adenosine) tetraphosphate (AP_4A) in the presence of suramin (300 μM). Closed and open symbols show responses in absence and presence of 8-*p*SPT (20 μM), respectively. Points show mean ± s.e. mean, unless occluded by symbol.

Table 2 Degradation of ATP and adenine dinucleotides by guinea-pig left atrium in the absence and presence of suramin (300 μM)

Substrate	*Degradation (pmol mg⁻¹ tissue min⁻¹)* *Control*	*+ Suramin*
ATP	179.7 ± 14.3	123.5 ± 14.7**
AP_2A	4.8 ± 1.60	7.2 ± 1.10
AP_3A	29.5 ± 3.25	18.7 ± 3.20*
AP_4A	12.1 ± 2.54	12.0 ± 1.25
AP_5A	14.2 ± 1.60	12.3 ± 1.05

The initial substrate concentration was 100 μM (25 nmol in 250 μl). All values are mean ± s.e.mean, $n=6$, except for AP_4A ($n=5$). Each assay was performed in duplicate. $^*P<0.05$, $^{**}P<0.01$ Student's *t* tests, relative to control values.

(Hoyle & Burnstock, 1992; Ralevic *et al.*, 1995; Hoyle *et al.*, 1995b). In the left atrium AP_2A did not exert a positive inotropic response, which argues against the presence of an excitatory P_{2Y}-purinoceptor in this tissue.

A receptor that specifically binds adenine dinucleotides rather than ATP has been isolated from Swiss CD1 mice cardiac muscle membranes (Walker *et al.*, 1993b; Hilderman *et al.*, 1994). Unfortunately, no functional study has been carried out to determine what action this receptor mediates. This receptor, isolated from membrane fractions, has a molecular weight of 30 kDa, and is derived from a protein whose post-translational processing, involving a serine protease, yields precursors of 67, 55 and 42 kDa (Walker & Hilderman, 1993; Walker *et al.*, 1993b; Hilderman *et al.*, 1994). However, in intact cardiac myocytes the 42 kDa form of the receptor predominates, and it has been suggested that the conversion of the 42 kDa to the 30 kDa form is an artefact (Walker *et al.*, 1993a). This compares with molecular sizes of 41–45 kD for various $P2X_1$-, $P2Y_1$- and $P2Y_2$- ($=P_{2U}$) purinoceptors (Lustig *et al.*, 1993; Webb *et al.*, 1993; Valera *et al.*, 1994). A P_{2U}-receptor has been cloned from the human epithelial CF/T43 cell line (Parr *et al.*, 1994), and is expressed in human astrocytoma 1321N1 cells, after transfection with its cDNA (Lazarowski *et al.*, 1995). At this receptor AP_4A is an agonist, being five times less potent than UTP and three times less potent than ATP; AP_3A, AP_5A and AP_2A are increasingly orders of magnitude less potent than AP_4A (Lazarowski *et al.*, 1995). Thus the dinucleotides could be acting on a P_{2U}-like receptor in the guinea-pig heart. However, in the rat mesenteric vascular bed pressor responses mediated via the P_{2U}-receptor are not antagonized by the purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (Windscheif *et al.*, 1994) but the pressor responses to AP_4A and AP_5A are almost abolished by PPADS (Ralevic *et al.*, 1995), showing that at least, these dinucleotides do not significantly activate P_{2U}-receptors.

A question that has to be considered is whether or not there is a physiological role for diadenosine polyphosphates in the regulation of cardiac contractility, and if so what could be their source? The dinucleotides AP_3A, AP_4A, AP_5A and AP_6A are found in platelets and are released during aggregation (Flodgaard & Klenow, 1982; Luthje & Ogilvie, 1983; Schluter *et al.*, 1994); the latter three are also found in adrenal medullary chromaffin granules, where they are stored in and released from secretory granules (Rodriguez del Castillo *et al.*, 1988; Pintor *et al.*, 1991; 1992b); possibilities that remain to be investigated are whether or not they are contained in a releasable form in endocardial cells or in terminals of neurones innervating the heart.

In conclusion, diadenosine polyphosphates activate two types of receptor that mediate negative inotropic responses in the guinea-pig left atrium: one is the P_1-purinoceptor and the other is an ill-defined suramin-sensitive P_2-purinoceptor. The naturally occurring dinucleotides also activate a receptor that mediates a positive inotropic response. While this receptor has some characteristics of a P_{2Y}-purinoceptor it cannot be categorically defined as such.

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