Possible role of diadenosine polyphosphates as modulators of cardiac sensory–motor neurotransmission in guinea-pigs

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1. Isolated guinea-pig atria were used to study the neuromodulatory effect of diadenosine polyphosphates (AP_nA) on cardiac capsaicin-sensitive sensory–motor neurotransmission.

2. In the presence of atropine, guanethidine and propranolol, electrical field stimulation (EFS) of the atrial preparations evoked a positive inotropic response which is known to be mediated by release of calcitonin gene-related peptide (CGRP) from sensory–motor nerves. P^1,P^2-diadenosine pyrophosphate (AP_2A), P^1,P^3-diadenosine triphosphate (AP_3A), P^1,P^4-diadenosine tetraphosphate (AP_4A), P^1,P^5-diadenosine pentaphosphate (AP_5A) and P^1,P^6-diadenosine hexaphosphate (AP_6A) inhibited in a concentration-dependent way (0·1–30 μM) cardiac responses to EFS. The inhibitory effect of AP_nA was mimicked by adenosine.

3. All the AP_nA tested had a direct negative inotropic effect, by reducing in a concentration-dependent manner the basal contractile tension. The inotropism of AP_nA was comparable to that of adenosine.

4. Both inhibition of cardiac responses to EFS and negative inotropism of AP_2A, AP_3A and AP_4A were sensitive to the antagonism by the A_1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 0·1–1 nM). The extent of antagonism of DPCPX for the AP_nA tested was comparable to that for adenosine.

5. Despite the direct negative inotropism, AP_4A tested at the highest concentration used did not affect the cardiac responses to the neurotransmitter CGRP, applied exogenously.

6. These results have demonstrated that in isolated guinea-pig atria AP_nA inhibited sensory–motor neurotransmission, without affecting cardiac responses to exogenous CGRP. The effect of AP_nA was sensitive to antagonism by DPCPX, which suggests it operates via the activation of prejunctional A_1 adenosine receptors. A postjunctional negative inotropism was also shown, mediated by myocardial A_1 adenosine receptors.

Diadenosine polyphosphates are naturally occurring molecules consisting of two adenosine moieties bridged by a chain of a variable number (from two to six) of phosphates. The different dinucleotides are therefore identified as AP_nA, where n stands for the number of phosphate groups in each compound.

Diadenosine polyphosphates were first detected in mammalian hepatocytes (Rapaport & Zamecnik, 1976) and platelets, where AP_4A and AP_3A are colocalized (Flodgaard & Klenow, 1982; Luthje & Ogilvie, 1983). AP_4A and AP_5A have later been detected in several regions of the central nervous system and their release by depolarizing agents has been shown in synaptosomal preparations (Pintor, Diaz, Torres & Miras-Portugal, 1992) and *in vivo* (Pintor, Porras, Mora & Miras-Portugal, 1993). Appreciable quantities of AP_4A and AP_5A have also been found in chromaffin granules (Rodriguez del Castillo, Torres, Delicado & Miras-Portugal, 1988) and in cholinergic vesicles from *Torpedo marmorata* (Pintor & Miras-Portugal, 1993). The discovery of AP_nA in secretory systems and the demonstration of their release in both catecholaminergic and cholinergic systems have suggested a possible role for these compounds as neurotransmitters and/or neuromodulators. This hypothesis is further supported by the findings that in several innervated tissues AP_nA can interact with the purinoceptor systems for adenosine (P_1-purinoceptors) and ATP (P_2-purinoceptors; Abbracchio & Burnstock, 1994; Baxi & Vishwanatha, 1995). The existence of a specific receptor for AP_nA has also been suggested (Pintor & Miras- Portugal, 1995). However, the information on the neuromodulatory actions of these compounds and the pharmacological characterization of their neuronal receptors has been limited largely to the central nervous system and chromaffin cells (Stone & Perkins, 1981; Castro, Torres, Miras-Portugal & Gonzales, 1990; Klishin, Lozovaya, Pintor, Miras-Portugal & Kristal, 1994).

In this study we have examined the possible modulatory action of AP_nA on the peripheral function of capsaicin-

sensitive sensory nerves in the heart. We also aimed to characterize the receptor system(s) involved in the neuromodulatory effects of AP_nA. Isolated guinea-pig atria were used as the experimental model since in this preparation it has been shown that stimulation of primary sensory fibres, sensitive to the neurotoxic action of capsaicin, leads to peripheral release of neuropeptides, namely calcitonin gene-related peptide (CGRP), substance P (SP) and related neurokinins (NKs). The interaction of CGRP with specific myocardial receptors results in a positive inotropism, while SP and NKs have little or no effect on myocardial contractility (Saito, Ishikawa, Kimura & Goto, 1987; Rubino, 1993; Rubino & Burnstock, 1996). The term 'sensory–motor neurotransmission' has been used to identify the peripheral activity of capsaicin-sensitive sensory fibres, where CGRP has been identified as the active neurotransmitter (Burnstock, 1990; Rubino & Burnstock, 1996). The effects of P^1,P^2-diadenosine pyrophosphate (AP_2A), P^1,P^3-diadenosine triphosphate (AP_3A), P^1,P^4-diadenosine tetraphosphate (AP_4A), P^1,P^5-diadenosine pentaphosphate (AP_5A) and P^1,P^6-diadenosine hexaphosphate (AP_6A) were tested on the cardiac responses to electrical field stimulation of the isolated atria and were compared with those of adenosine. The pharmacological characterization of the receptor systems involved in the effects of AP_nA was carried out by using the selective antagonist of adenosine A_1 receptors, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).

METHODS

Experimental preparation

The experimental model used was as previously described (Rubino, Amerini, Mantelli & Ledda, 1991). Male guinea-pigs (250–300 g) were killed by cervical dislocation and the atrial myocardium, containing both right and left sections, was isolated. Preparations were vertically mounted in 10 ml organ baths containing Tyrode solution of the following composition (mM): NaCl, 115; KCl, 4·7; $CaCl_2$, 1·8; $MgSO_4$, 1·2; KH_2PO_4, 1·2; $NaHCO_3$, 25; glucose, 10; oxygenated with 95% O_2 and 5% CO_2 and kept at a constant temperature of 30 °C, in order to reduce the metabolic need of the tissue. Atria were stretched until the maximum contractile tension was reached. The isometric contraction was recorded by an isometric force–displacement transducer (model FT 03C, Grass Instrument Co., Quincy, MA, USA) and a DC preamplifier on a pen recorder (model 79D, Grass).

Sensory–motor neurotransmission

The preparations were electrically driven at a constant rate (4 Hz) by punctuate electrodes connected with a pulse generator (model S9, Grass). Electrical field stimulation (EFS) was performed according to Saito *et al.* (1987). Trains of field pulses (20 Hz frequency; 100–120 V intensity; 1 ms duration for 5 s) were applied through two platinum plates, parallel to the preparations, connected with a second pulse generator (model S45, Grass). The parasympathetic component of the response to EFS was eliminated by the addition of atropine (0·3 μM) to the Tyrode solution. Furthermore, guanethidine (5 μM) and propranolol (1 μM) were present throughout the experiment in order to eliminate sympathetic neurotransmission. After a period of equilibration of at least 60 min, trains of field pulses were applied at 10 min intervals. The response to EFS was evaluated as the difference between the maximum tension developed after applying the field pulses and the basal contractile tension (milligrams). Since responses to each train remained reproducible for many consecutive tests, it was possible to obtain cumulative concentration–effect curves for the AP_nA tested. Increasing concentrations of the agonists were added to the bathing solution after a control train of field pulses. EFS was then applied again after 4 min contact with each concentration of the drugs tested. Two concentration–response curves were usually obtained in each preparation. The effect of the agonists tested was evaluated as the percentage inhibition of the control response. The antagonist DPCPX was added 30 min before the application of the control train of field pulses.

Cardiac responses to exogenous agonists

The negative inotropic effect of AP_nA and adenosine was evaluated as the percentage reduction of the basal contractile tension. Cumulative concentration–response curves for exogenous CGRP were obtained by adding increasing concentrations of the peptide, until the maximum inotropic effect was reached. In order to avoid desensitization, each preparation was used for a single dose–response curve to CGRP in the absence or in the presence of a single concentration of AP_4A.

Drugs used

Adenosine hemisulphate, AP_2A, AP_3A, AP_4A, AP_5A, AP_6A, capsaicin and CGRP were from Sigma. Atropine sulphate was from BDH (Poole, UK), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was from Cambridge Research Biochemicals (Atlantic Beach, NY, USA), propranolol (Inderal) was from ICI (Macclesfield, UK) and guanethidine sulphate (Ismelin) was from Ciba Laboratories (Horsham, UK).

Data evaluation

Data shown in the text and figures are reported as means ± S.E.M. Since maximal effects were not reached, the rank order of potency of agonists was evaluated from the −log concentration ($p[A]_{30}$) that gave 30% inhibition of sensory–motor neurotransmission and 30% reduction of basal contractile tension. The $p[A]_{30}$ values were statistically compared by using Student's t test for unpaired data and results were considered significantly different when $P < 0.05$.

RESULTS

Sensory–motor neurotransmission

Isolated guinea-pig atria developed a basal contractile tension of 234·8 ± 14·3 mg ($n = 25$). EFS elicited cardiac responses that consisted of a transient increase in contractile tension. The maximum cardiac response to EFS (136·2 ± 10·3 mg; $n = 25$) was reached in about 30 s and contractile tension declined to basal values in about 3 min (Fig. 1*A*). The cardiac response to EFS was abolished in preparations treated with capsaicin (1 μM), which itself evoked a positive inotropic response due to activation of capsaicin-sensitive sensory–motor nerves (Fig. 1*B* and *C*).

Effect of diadenosine polyphosphates on sensory–motor neurotransmission

Representative tracings showing the effects of EFS under control conditions and in the presence of increasing concentrations of AP_3A are shown in Fig. 2. AP_3A inhibited the contractile response to EFS in a concentration-

Table 1. $p[A]_{30}$ values for diadenosine polyphosphates and adenosine

	Inhibition of neurotransmission	Reduction of basal contractile tension
AP_2A	5·97 ± 0·11	5·18 ± 0·07
AP_3A	5·82 ± 0·16	4·84 ± 0·11
AP_4A	5·64 ± 0·11	4·84 ± 0·13
AP_5A	5·68 ± 0·12	4·78 ± 0·11
AP_6A	6·07 ± 0·13	5·05 ± 0·11
Adenosine	5·72 ± 0·08	4·94 ± 0·12

Values are given as means ± S.E.M. of 4–6 observations.

dependent way such that at the highest concentration (10 μM) AP_3A reduced sensory–motor neurotransmission by about 50%. It may also be noted that, in the presence of this concentration of AP_3A, basal contractile tension was reduced by about 20%. All AP_nA tested showed a concentration-dependent inhibitory effect on sensory–motor neurotransmission (Fig. 3). None of the concentration–response curves reached a plateau; higher concentrations of the agonists were not tested in order to avoid drastic effects on basal contractile tension (see next section). Therefore, the order of potency of the AP_nA was evaluated from the $p[A]_{30}$ values. No significant differences were observed among these values, which indicates a similar potency for all the AP_nA tested (Table 1). In the same concentration range of AP_nA, adenosine inhibited sensory–motor neurotransmission, having a $p[A]_{30}$ value not significantly different from those of the AP_nA (Fig. 3 and Table 1).

Effect of diadenosine polyphosphates on basal contractile tension

All the AP_nA tested had negative inotropic effects. Basal contractile tension was reduced in a concentration-dependent way, as shown in Fig. 4. No significant differences were found among the agonists tested, as revealed by the $p[A]_{30}$ values (Table 1). However, at the higher concentrations tested, AP_2A appeared to elicit a greater negative inotropic action than the other AP_nA. The maximal effect achieved at a concentration of 30 μM was 52·8 ± 4·2% ($n = 7$) reduction of basal contractile tension (Fig. 4). The negative inotropic action of AP_nA was comparable to that of adenosine (Fig. 4 and Table 1).

Antagonism by DPCPX

The selective A_1 adenosine receptor antagonist DPCPX *per se* had no effect on basal contractile tension or sensory–motor neurotransmission. However, DPCPX (0·1 and 1 nM) shifted to the right and in a parallel way the concentration–response curves constructed for the inhibitory effect of AP_3A and AP_4A on sensory–motor neurotransmission (Fig. 5*A* and *B*). The antagonism of DPCPX was concentration dependent. The $p[A]_{30}$ value for AP_3A was shifted from 5·82 ± 0·16 ($n = 5$) to 5·65 ± 0·09 ($n = 4$) and to 5·22 ± 0·12 ($n = 4$) in the presence of 0·1 and 1 nM DPCPX, respectively. The $p[A]_{30}$ values for AP_4A were 5·64 ± 0·11 ($n = 5$), 5·23 ± 0·12 ($n = 4$) and 4·96 ± 0·02 ($n = 4$) in the absence and in the presence of 0·1 and 1 nM DPCPX, respectively. Similarly, DPCPX antagonized the inhibitory effect of adenosine in a concentration-dependent manner (Fig. 5*C*). The $p[A]_{30}$ value for adenosine was reduced from 5·72 ± 0·08 ($n = 4$) to 5·51 ± 0·19 ($n = 4$) and to 5·16 ± 0·08 ($n = 4$), in the presence of 0·1 and 1 nM DPCPX, respectively. The pA_2 ($-\log K_d$) values determined for 1 nM DPCPX at the level of

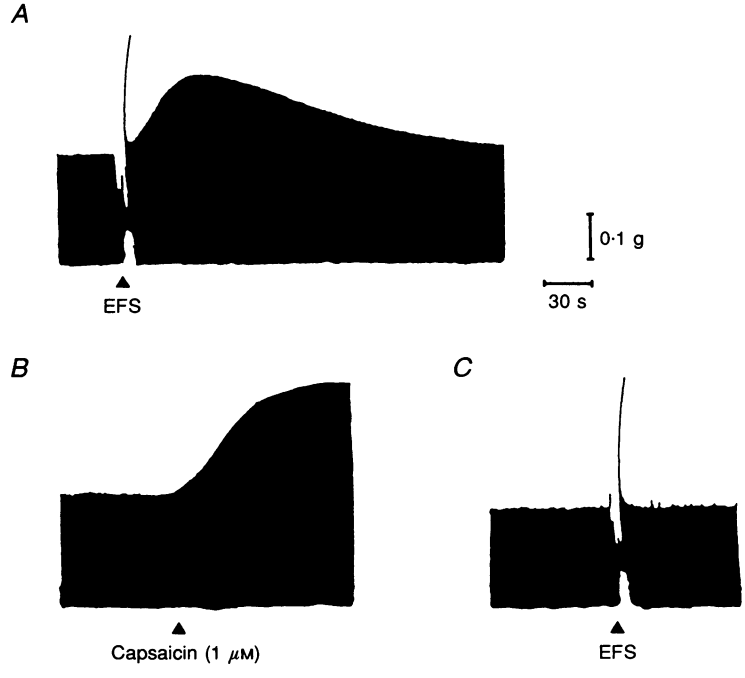

Figure 1. Desensitizing effect of capsaicin on the cardiac responses to electrical field stimulation

Representative recordings of an experiment showing cardiac responses to electrical field stimulation (EFS) before and after application of capsaicin (1 μM). Atria were driven at 4 Hz. EFS, applied in the presence of atropine (0·3 μM), guanethidine (5 μM) and propranolol (1 μM), elicited a transient increase in contractile tension, which declined to basal values in about 3 min (*A*). Capsaicin (1 μM) evoked a positive inotropic effect (*B*) due to the activation of sensory–motor nerves, after which EFS did not evoke cardiac responses any more (*C*).

30% inhibition of sensory–motor neurotransmission were 9·47 *versus* AP_3A, 9·58 *versus* AP_4A and 9·42 *versus* adenosine. The antagonism of 0·1 nM DPCPX on the inhibitory effect of AP_2A on sensory–motor neurotransmission is shown in Fig. 5*D*.

When the direct negative inotropic effect of AP_3A, AP_4A and adenosine was evaluated in the presence of 0·1 and 1 nM DPCPX, concentration–response curves were shifted to the right in a concentration-dependent way (Fig. 6*A*, *B* and *C*). DPCPX at 0·1 nM antagonized to the same extent the negative inotropic effect of AP_2A (Fig. 6*D*), which appeared to reduce the basal contractile tension more than the other AP_nA. The pA_2 values calculated for 0·1 nM DPCPX at the level of 30% reduction of basal contractile tension were 9·93 *versus* AP_2A, 10·07 *versus* AP_3A and 10·6 *versus* AP_4A.

Effect of AP_4A on the cardiac response to exogenous CGRP

In order to evaluate whether the inhibitory effect of AP_nA on sensory–motor neurotransmission was due to pre- or post-junctional mechanisms, the influence of a representative compound of the polyphosphate family was tested on the cardiac response to the neurotransmitter CGRP applied exogenously. In preparations with a basal contractile tension of 232·8 ± 21·6 mg ($n = 14$), CGRP elicited a concentration-dependent positive inotropic effect. At the highest concentration tested (30 nM), CGRP increased the contractile tension by 218·7 ± 25·5 mg ($n = 14$). In the presence of 3 and 30 μM AP_4A the concentration–response curves to CGRP were superimposable on that obtained in the absence of AP_4A (Fig. 7). CGRP at 30 nM increased the contractile tension by 228·3 ± 33·6 mg ($n = 10$) and

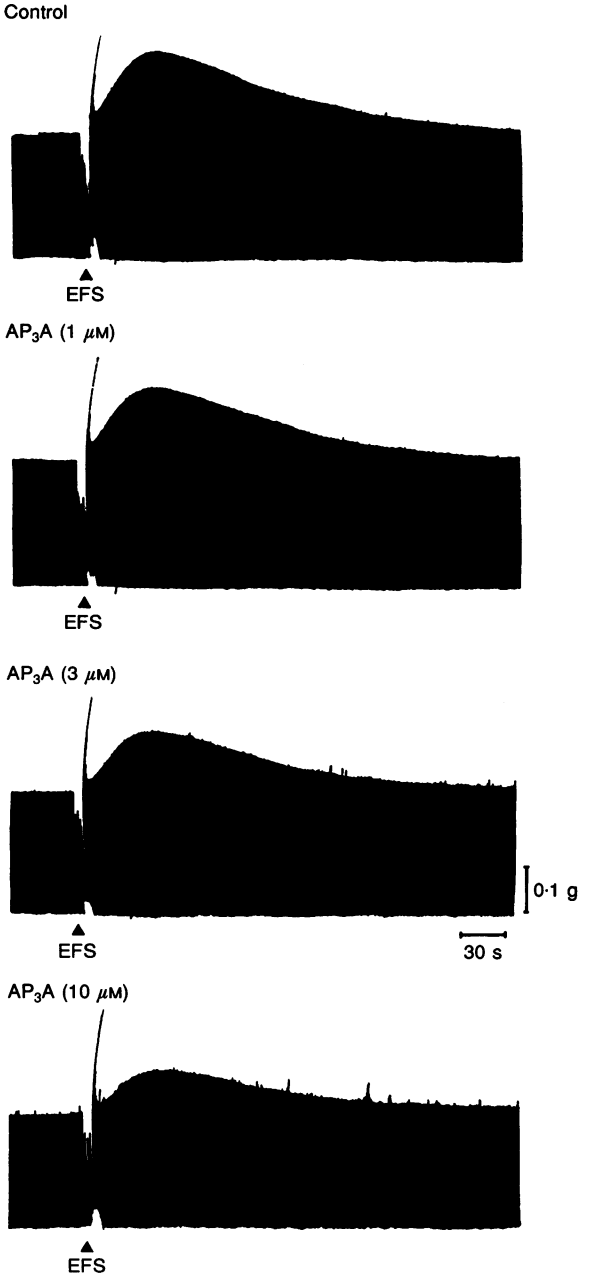

Figure 2. Inhibitory action of AP_3A on sensory–motor neurotransmission

Representative traces of an experiment showing cardiac responses to EFS (20 Hz, 1 ms, 120 V, for 5 s) in the absence (Control) and in the presence of increasing concentrations (1–10 μM) of AP_3A. Atria were driven at 4 Hz. EFS, applied in the presence of atropine (0·3 μM), guanethidine (5 μM) and propranolol (1 μM), elicited a transient increase in contractile tension which was reduced in the presence of AP_3A.

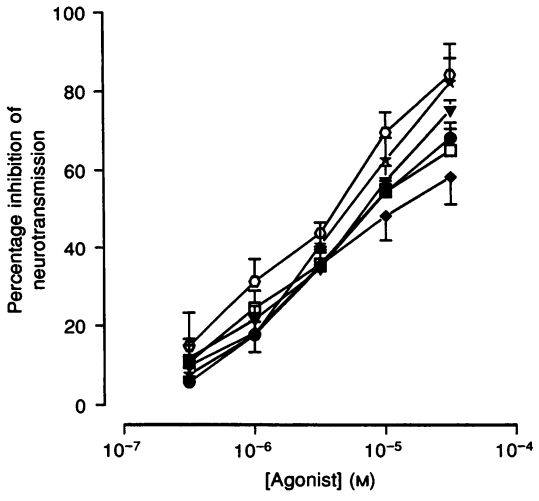

Figure 3. Concentration–response curves for AP_nA and adenosine on sensory–motor neurotransmission

Inhibitory effect of AP_2A (★), AP_3A (□), AP_4A (●), AP_5A (▼), AP_6A (○) and adenosine (◆) on sensory–motor neurotransmission in isolated guinea-pig atria, driven at 4 Hz. Points are means ± S.E.M. of 4–6 experimental observations.

273·5 ± 62·0 mg ($n = 4$) in the presence of AP_4A at 3 and 30 μM, respectively.

DISCUSSION

The results of this study demonstrate that, in isolated guinea-pig atria, AP_nA modulate capsaicin-sensitive sensory–motor neurotransmission via prejunctional A_1 adenosine receptors. A postjunctional inotropic effect of AP_nA is also shown, via activation of myocardial A_1 adenosine receptors.

Sensory–motor neurotransmission was evaluated in this study by EFS of isolated guinea-pig atria, driven at 4 Hz and in the presence of atropine, guanethidine and propranolol. Under these experimental conditions cardiac responses to EFS were abolished by capsaicin, which confirmed that we were evaluating sensory–motor neurotransmission (Saito *et al.* 1987; Rubino *et al.* 1991). All the AP_nA tested and adenosine reduced cardiac responses to EFS in the same range of concentrations and without significant differences among the $p[A]_{30}$ values. These data indicate that AP_nA and adenosine were equipotent in modulating sensory–motor neurotransmission and suggest that AP_nA were acting via a common receptor system. The selectivity of DPCPX for A_1 adenosine receptors is well established at the concentrations of antagonist used in this study (Lohse, Klotz, Linderborn-Fotinos, Reddington, Schwabe & Olsson, 1987). The extent of antagonism of DPCPX for AP_2A, AP_3A, AP_4A and adenosine was comparable, as demonstrated by the rightward shift of the concentration–response curves and by the similar pA_2 values, which supports the hypothesis that a single receptor system was involved in the inhibitory effects observed. Taken together these observations indicate that adenosine receptors of the A_1 subtype mediate the neuromodulatory action of AP_nA on cardiac sensory–motor neurotransmission.

All AP_nA studied were equipotent to adenosine in reducing the basal contractile tension, which suggests the interaction of AP_nA with the A_1 receptors that mediate the negative inotropic effect of adenosine in the atrial myocardium (Collis, 1983; Belardinelli, Linden & Berne, 1989). Studies on mouse heart cell membranes have demonstrated the existence of specific binding sites for AP_4A, where AP_5A and AP_6A have similar, while AP_3A has reduced, efficacy (Hilderman, Martin, Zimmerman & Pivorun, 1991;

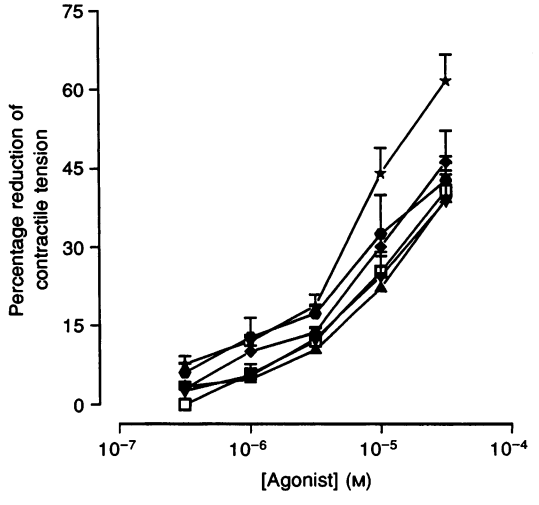

Figure 4. Concentration–response curves for AP_nA and adenosine on basal contractile tension

Negative inotropic effect of AP_2A (★), AP_3A (□), AP_4A (●), AP_5A (▲), AP_6A (⬢) and adenosine (◆) in isolated guinea-pig atria driven at 4 Hz. Points are means ± S.E.M. of 4–6 experimental observations.

Hilderman, Lilien, Zimmerman, Tate, Dimmick & Jones, 1994). However, the functional data obtained in the present study with the adenosine antagonist DPCPX do not support the hypothesis of a specific receptor system for AP_nA in the myocardium. Indeed, despite the greater inotropism of AP_2A, similar pA_2 values were calculated for DPCPX *versus* AP_2A, AP_3A and AP_4A thus indicating the functional interaction of all AP_nA tested with the same receptor population, namely the myocardial A_1 adenosine receptors.

The AP_nA may be cleaved by ectohydrolases to $AP_{(n-1)}A$ and AMP and further degraded to adenosine by ectonucleotidases (Ogilvie, Luthje, Pohl & Busse, 1989; Rodriguez-Pascual, Torres, Rotlan & Miras-Portugal, 1992). In view of the direct and neuromodulatory effects of adenosine and related nucleotides in the myocardium (Belardinelli *et al.* 1989; Rubino *et al.* 1991), it could be inferred that AP_nA exert their effects after hydrolytic cleavage to adenosine. However, in contrast to the rapid degradation of ATP by ectoenzymes, AP_nA are only slowly hydrolysed in the heart (Pohl, Ogilvie, Lamontagne & Busse, 1991) as well as in other tissues (Ogilvie *et al.* 1989; Rodriguez-Pascual *et al.* 1992), thus suggesting that AP_nA exert their actions as uncleaved compounds. Moreover, the assumption that the final degradation product of AP_nA might be the main mediator of their effects would imply a distinct potency for adenosine and AP_nA. In this study there were no differences in the concentration–response curves of AP_nA and adenosine, which argues against the hypothesis that AP_nA were acting via their metabolite adenosine.

The negative inotropic action of AP_nA might suggest that the polyphosphates reduced the cardiac responses to the CGRP that was released by EFS via a postjunctional mechanism of inhibition of sensory–motor neurotransmission. However, at the two concentrations tested AP_4A did not modify cardiac responses to exogenous CGRP, thus providing indirect evidence for a prejunctional inhibition of sensory–motor neurotransmission. It is likely

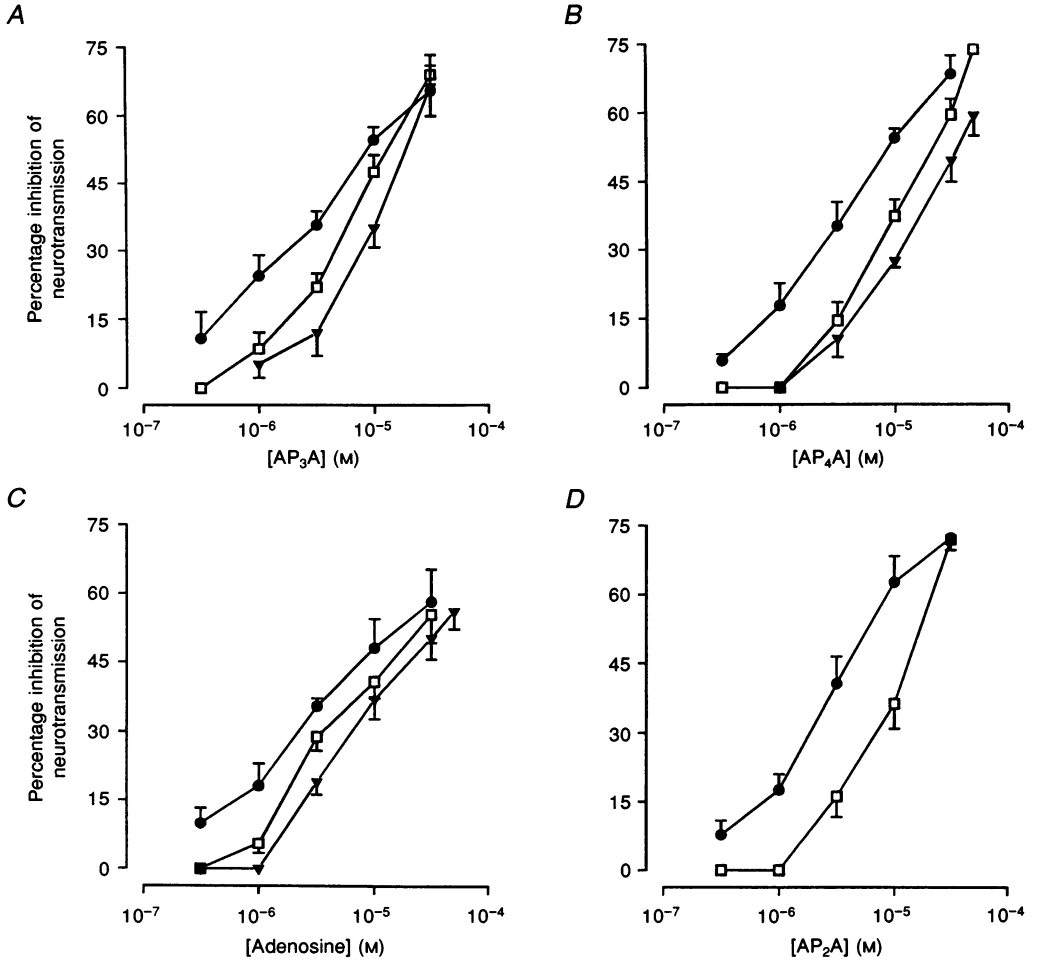

Figure 5. Antagonism of DPCPX *versus* the inhibitory effect of AP_3A, AP_4A, adenosine and AP_2A on sensory–motor neurotransmission

Concentration–response curves for AP_3A (*A*), AP_4A (*B*), adenosine (*C*) and AP_2A (*D*) in the absence (●) and in the presence of DPCPX at 0·1 (□) and 1 nM (▼). Points are means ± S.E.M. of 4–6 experimental observations.

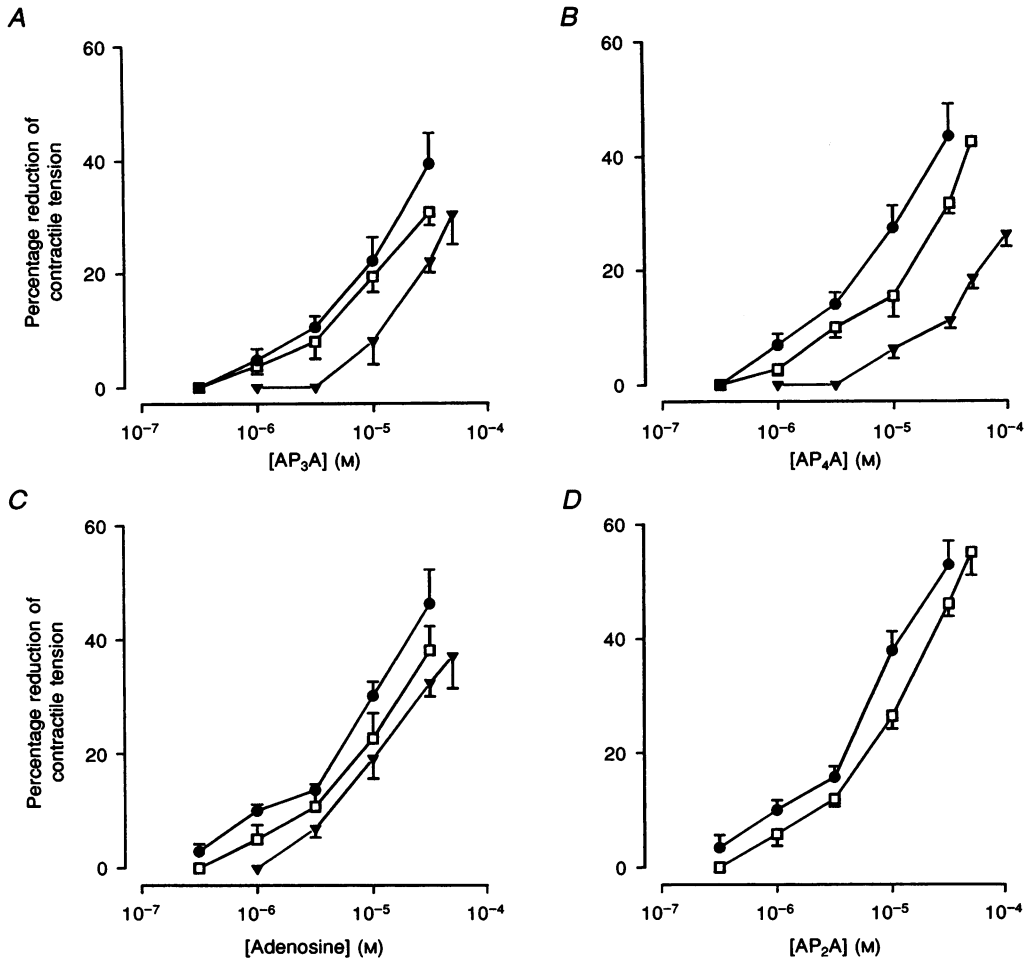

Figure 6. Antagonism of DPCPX *versus* the inotropic effect of AP_3A, AP_4A, adenosine and AP_2A

Concentration–response curves for AP_3A (*A*), AP_4A (*B*), adenosine (*C*) and AP_2A (*D*) in the absence (●) and in the presence of DPCPX at 0·1 (□) and 1 nM (▼). Points are means ± S.E.M. of 4–6 experimental observations.

that inhibition of sensory–motor neurotransmission is caused by a reduced release of neuropeptides, including the active neurotransmitter CGRP, following EFS in the presence of AP_nA. Although the contribution of the inotropic effect of AP_nA to the reduction of sensory–motor neurotransmission cannot be ruled out as a postjunctional component of the neuromodulatory action of AP_nA, pre- and postjunctional effects of polyphosphates can be distinguished on the basis

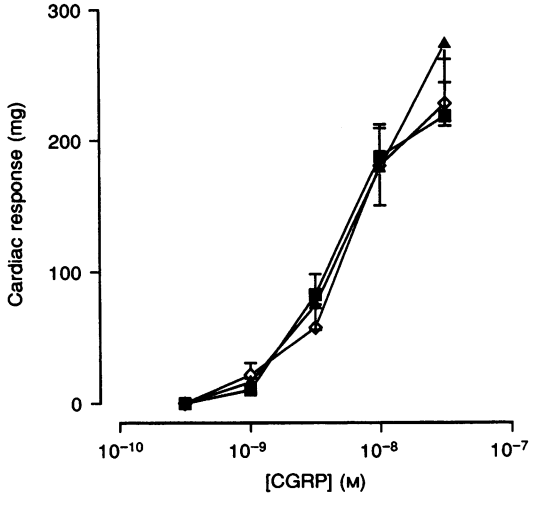

Figure 7. Effect of AP_4A on the inotropic responses to exogenous CGRP

Concentration–response curves to CGRP constructed in isolated guinea-pig atria driven at 4 Hz in the absence (■; $n = 14$) and in the presence of AP_4A at 3 (◇; $n = 10$) and 30 μM (▲; $n = 4$). Points are means ± S.E.M.

of the following observations. First, the $p[A]_{30}$ values calculated for the negative inotropic effect were about one log unit higher than those calculated for the reduction of cardiac response to EFS. Second, the inhibitory effect on sensory–motor neurotransmission induced by the highest concentration of AP_nA and adenosine used (30 μM) was greater (about 70%) than the negative inotropic effect (about 40%). Therefore, AP_nA and adenosine appeared to be more potent as neuromodulators of sensory–motor neurotransmission than as direct inotropic agents.

The findings of this investigation confirm previous observations in that adenosine modulates cardiac sensory–motor neurotransmission via prejunctional inhibitory A_1 receptors (Rubino *et al.* 1991; Rubino, 1993). Indeed, the present study provides the first evidence for a modulatory action of AP_nA on the peripheral nervous system, namely on cardiac sensory–motor neurotransmission, and indicates that prejunctional A_1 adenosine receptors are involved in this effect. These results are in line with observations showing the neuromodulatory effects of AP_nA in the central nervous system. It has been shown that AP_3A and AP_5A depress the firing of spontaneously active cortical neurons via methylxanthine-sensitive mechanisms; the involvement of adenosine receptors has therefore been suggested (Stone & Perkins, 1981). More recently, it has been demonstrated that in the rat hippocampus the adenosine antagonist cyclopentyltheophylline antagonizes the inhibitory action of AP_4A and AP_5A on synaptic transmission, thus suggesting that these compounds modulate the neuronal activity via activation of A_1 adenosine receptors (Klishin *et al.* 1994).

In summary, the results of the present study demonstrate that AP_nA reduce cardiac sensory–motor neurotransmission without affecting inotropic responses to exogenous CGRP, thus indicating a prejunctional neuromodulatory action of AP_nA. Furthermore, this study demonstrates a post-junctional negative inotropic action of AP_nA which is shared with that of adenosine. Both neuromodulatory and direct effects are mediated by A_1 adenosine receptors. The contribution of sensory–motor neurotransmission in the regulation of cardiac function has recently received great attention. Release of CGRP from sensory–motor nerves has been shown in pathophysiological conditions, such as myocardial infarction (Mair, Lechleiner, Langel, Wiedermann, Dienstl & Saria, 1990), when the biological activity of adenosine and related compounds has also been demonstrated to have physiological relevance (Belardinelli *et al.* 1989). In this light, both direct and neuromodulatory effects of the parent compound of adenosine AP_nA might be considered as an additional mechanism of regulation of cardiac function. Furthermore, the long-lasting biological activities of AP_nA compared to ATP and adenosine may offer an exciting pharmacological potential. It is noteworthy that cross-talk between sympathetic and sensory–motor innervation has been shown in the heart, where the cotransmitters of sympathetic autonomic neurotransmission noradrenaline, ATP and NPY have neuromodulatory actions on sensory–motor neurotransmission (Rubino, 1993; Rubino & Burnstock, 1996). Corelease of AP_4A with catecholamines has been shown from chromaffin cells, which are considered in many respects a representative system of neuronal secretory responses (Castillo, Moro, Delvalle, Sillero, Garcia & Sillero, 1992). Based on these observations it is tempting to speculate that AP_nA coreleased from cardiac sympathetic terminals might contribute to the cross-talk between sympathetic and sensory–motor neurotransmission in the pathophysiological regulation of cardiac function.

Abbracchio, M. & Burnstock, G. (1994). Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacology and Therapeutics* **64**, 445–475.

Baxi, M. D. & Vishwanatha, J. K. (1995). Diadenosine polyphosphates: their biological and pharmacological significance. *Journal of Pharmacological and Toxicological Methods* **33**, 121–128.

Belardinelli, L., Linden, J. & Berne, R. M. (1989). The cardiac effects of adenosine. *Progress in Cardiovascular Diseases* **32**, 73–97.

Burnstock, G. (1990). The Fifth Heymans Memorial Lecture – Ghent, February 17, 1990. Co-transmission. *Archives Internationales de Pharmacodynamie et de Thérapie* **304**, 7–33.

Castillo, C. J. F., Moro, M. A., Delvalle, M., Sillero, A., Garcia, A. G. & Sillero, M. A. G. (1992). Diadenosine tetraphosphate is co-released with ATP and catecholamines from bovine adrenal medulla. *Journal of Neurochemistry* **59**, 723–732.

Castro, E., Torres, M., Miras-Portugal, M. T. & Gonzales, M. P. (1990). Effect of diadenosine polyphosphates on catecholamine secretion on isolated chromaffin cells. *British Journal of Pharmacology* **100**, 360–364.

Collis, M. G. (1983). Evidence for A_1-adenosine receptors in the guinea-pig atrium. *British Journal of Pharmacology* **78**, 207–212.

Flodgaard, H. & Klenow, H. (1982). Abundant amounts of the diadenosine 5′,5‴-P^1,P^4-tetraphosphate are present and releasable, but metabolically inactive in human platelets. *Biochemical Journal* **208**, 737–742.

Hilderman, R. H., Lilien, J. E., Zimmerman, J. K., Tate, D. H., Dimmick, M. A. & Jones, G. B. (1994). Adenylated dinucleotide binding to the adenosine 5′,5‴-P^1,P^4-tetraphosphate mouse heart receptor. *Biochemical and Biophysical Research Communications* **29**, 749–755.

Hilderman, R. H., Martin, M., Zimmerman, J. K. & Pivorun, E. B. (1991). Identification of a unique membrane receptor for adenosine 5′,5‴-P^1,P^4-tetraphosphate. *Journal of Biological Chemistry* **266**, 6915–6918.

Klishin, A., Lozovaya, N., Pintor, J., Miras-Portugal, M. T. & Kristal, O. A. (1994). Possible functional role of diadenosine polyphosphates: negative feed-back for excitation in hippocampus. *Neuroscience* **58**, 235–236.

Lohse, M. J., Klotz, K. N., Linderborn-Fotinos, J., Reddington, M., Schwabe, U. & Olsson, R. A. (1987). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) – a selective high affinity radioligand for A_1 adenosine receptors. *Naunyn–Schmiedeberg's Archives of Pharmacology* **336**, 204–210.

LUTHJE, J. & OGILVIE, A. (1983). The presence of diadenosine 5′,5‴-P^1,P^3triphosphate (Ap_3A) in human platelets. *Biochemical and Biophysical Research Communications* **115**, 253–260.

MAIR, J., LECHLEINER, P., LANGEL, T., WIEDERMANN, C., DIENSTL, F. & SARIA, A. (1990). Plasma CGRP in acute myocardial infarction. *Lancet* **335**, 168.

OGILVIE, A., LUTHJE, J., POHL, U. & BUSSE, R. (1989). Identification and partial characterization of an adenosine (5′) tetraphospho (5′) adenosine hydrolase in intact bovine aortic endothelial cells. *Biochemical Journal* **259**, 97–103.

PINTOR, J., DIAZ, M. A., TORRES, M. & MIRAS-PORTUGAL, M. T. (1992). Presence of diadenosine polyphosphates Ap_4A and Ap_5A in rat brain synaptic terminals. Ca^{2+} dependent release evoked by 4-aminopyridine and veratridine. *Neuroscience Letters* **136**, 141–144.

PINTOR, J. & MIRAS-PORTUGAL, M. T. (1993). Diadenosine polyphosphates – Ap_nA – as new neurotransmitters. *Drug Development Research* **28**, 259–262.

PINTOR, J. & MIRAS-PORTUGAL, M. T. (1995). A novel receptor for diadenosine polyphosphate coupled to calcium increase in rat midbrain synaptosomes. *British Journal of Pharmacology* **115**, 895–902.

PINTOR, J., PORRAS A., MORA, F. & MIRAS-PORTUGAL, M. T. (1993). Amphetamine-induced release of diadenosine polyphosphates Ap_4A and Ap_5A from caudate putamen of conscious rat. *Neuroscience Letters* **150**, 13–16.

POHL, U., OGILVIE, A., LAMONTAGNE, D. & BUSSE, R. (1991). Potent effects of AP_3A and AP_4A on coronary resistence and autacoid release of intact rabbit heart. *American Journal of Physiology* **260**, H1692–1697.

RAPAPORT, E. & ZEMECNIK, P. C. (1976). Presence of diadenosine 5′, 5‴-P^1,P^4 tetraphosphate in mammalian cells in levels varying widely with the proliferative activity of the tissue: A possible positive pleiotypic activator. *Proceedings of the National Academy of Sciences of the USA* **73**, 3984–3988.

RODRIGUEZ DEL CASTILLO, A., TORRES, M., DELICADO, E. G. & MIRAS-PORTUGAL, M. T. (1988). Subcellular distribution studies of diadenosine polyphosphates Ap_4A and Ap_5A in bovine adrenal medulla: presence in chromaffin granules. *Journal of Neurochemistry* **51**, 1696–1703.

RODRIGUEZ-PASCUAL, F., TORRES, M., ROTLAN, P. & MIRAS-PORTUGAL, M. T. (1992). Extracellular hydrolysis of diadenosine polyphosphates Ap_nA by bovine chromaffin cells in culture. *Archives of Biochemistry and Biophysics* **297**, 176–183.

RUBINO, A. (1993). Non-adrenergic non-cholinergic (NANC) neural control of the atrial myocardium. *General Pharmacology* **24**, 539–545.

RUBINO, A., AMERINI, S., MANTELLI, L. & LEDDA F. (1991). Adenosine receptors involved in the inhibitory control of non-adrenergic non-cholinergic neurotransmission in guinea-pig atria belong to the A1 subtype. *Naunyn–Schmiedeberg's Archives of Pharmacology* **344**, 464–470.

RUBINO, A. & BURNSTOCK, G. (1996). Capsaicin-sensitive sensory-motor neurotransmission in the peripheral control of cardiovascular function. *Cardiovascular Research* **31**, 467–479.

SAITO, A., ISHIKAWA, T., KIMURA, S. & GOTO, K. (1987). Role of calcitonin gene-related peptide as cardiotonic neurotransmitter in guinea-pig left atria. *Journal of Pharmacology and Experimental Therapeutics* **243**, 731–736.

STONE, T. W. & PERKINS, M. N. (1981). Adenosine dinucleotides effect on rat cortical neurones. *Brain Research* **229**, 241–245.

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