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Blockade of adenosine receptors unmasks a stimulatory effect of ATP on cardiac contractility

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1 The effects of ATP, α,β-methylene ATP and β,γ-methylene ATP on the contractile tension of guinea-pig isolated left atria were evaluated.

2 ATP (1– 100 μM) produced a concentration-dependent negative inotropic effect; this response was converted to a positive inotropic effect in the presence of the antagonist of adenosine A_1 receptors, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 0.1 μM), and in the presence of 8-phenyltheophylline (10 μM), an antagonist of A_1 and A_2 receptors.

3 The positive inotropic effect of ATP was antagonized by the P_2 receptor antagonist, suramin (500 μM). Reactive blue 2 (30– 500 μM), a putative P_{2y} receptor antagonist, concentration-dependently reduced and finally abolished the effect of ATP.

4 In the presence of 8-phenyltheophylline, the stable analogues of ATP, α,β-methylene ATP and β,γ-methylene ATP (1– 30 μM), produced a concentration-dependent increase in atrial contractility of a lesser degree than that induced by ATP.

5 The results suggest that when inhibitory adenosine receptors are blocked, ATP produces a positive inotropic effect, probably mediated by P_{2y} receptor stimulation.

Keywords: ATP; heart; positive inotropism; P_2-purinoceptors

Introduction

Adenine nucleotides exert well-known depressant effects in the mammalian heart, consisting of a negative chronotropic and inotropic response, an anti-adrenoceptor effect and an inhibition of adrenergic neurotransmission (for two reviews see Burnstock, 1980; Pelleg *et al.*, 1990). These effects are mediated by stimulation of adenosine receptors, since they are antagonized by the non-selective adenosine receptor antagonist, 8-phenyltheophylline. According to some reports, adenosine and ATP, at high concentrations, induce a positive inotropic effect in ventricular preparations (Legssyer *et al.*, 1988; Dorigo *et al.*, 1988; Scamps *et al.*, 1990; Szentmiklosi *et al.*, 1990). In chick ventricular myocytes pretreated with either 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), a selective A_1 receptor antagonist, or the G_i ribosylating agent, pertussis toxin, adenosine induces a stimulation of adenylate cyclase activity and an increase in contractile amplitude (Xu *et al.*, 1992). On the other hand, ATP produces a small positive inotropic effect in rat papillary muscle, but a negative inotropic effect in rat atria (Scamps *et al.*, 1990). The inhibitory response observed in rat atria is converted to a positive effect in preparations obtained from pertussis toxin-pretreated animals (Scamps *et al.*, 1990). A biphasic, negative and positive effect for ATP in spontaneously beating guinea-pig atria has also been reported by Dorigo *et al.* (1988); the negative phase is abolished by 8-phenyltheophylline, thus suggesting that adenosine receptors are not involved in this positive inotropic effect. In the present study we have evaluated the influence of ATP on cardiac contractility in guinea-pig left atria, and have attempted to characterize the receptors involved in the observed effects.

Methods

Male guinea-pigs (250–400 g) were killed by a blow on the head. The hearts were rapidly removed and left atria were isolated and vertically mounted in an organ bath 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 and glucose 10. The solution was oxygenated with 95% O_2 and 5% CO_2, kept at 30°C and had a pH of 7.4. The preparations were electrically driven at 1 Hz by point stimulation with square wave pulses of 0.5 ms duration and supra-threshold voltage (5 V). Resting tension was adjusted at 0.5–0.7 g and developed tension was recorded by an isometric transducer on a pen recorder (Battaglia Rangoni KV 135). Atria were allowed to equilibrate for 1 h before drug administration. ATP and analogues were added to the bath solution in a cumulative way at intervals of 3–4 min. Antagonists were present in Tyrode solution for the duration of the experiment, or added to the bath at least 15 min before ATP administration. Since ATP caused desensitization, only one concentration-response curve for ATP was determined in a single preparation. Therefore concentration-response curves in the absence and in the presence of antagonists were carried out in different preparations. The effect induced by ATP and analogues was evaluated as the difference between the basal contractile tension and the tension after drug administration (net increase in mg).

Drugs

Adenosine 5'-triphosphate (ATP), lithium salt of α,β-methylene ATP, sodium salt of β,γ-methylene ATP, reactive blue 2 and prazosin hydrochloride were all obtained from Sigma Chemical Co., St. Louis, U.S.A.; suramin (Bayer, Milan, Italy), 8-phenyltheophylline (Calbiochem, San Diego, U.S.A.), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; Research Biochemical Inc., Natick, MA, U.S.A.), cimetidine hydrochloride (Smith, Kline Beecham, Welwyn Garden City, U.K.); 2-hydroxy-5-{2-[hydroxy-3-(4-[(1-methyl-4-trifluoromethyl) 1H-imidazol-2-yl-]-phenoxy)-propyl]aminoethoxyl}-benzamide (CGP 20712A) was kindly supplied by Ciba Geigy, Basel, Switzerland.

Drugs were prepared fresh daily in distilled water. A stock solution of 8-phenyltheophylline was made up in 80% ethanol containing 0.2 mM NaOH; further dilutions were made in distilled water.

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Statistics

The values reported are means ± s.e.mean. Statistical significance was calculated with Student's *t* test for unpaired data. A P value <0.05 was considered significant.

Results

Guinea-pig left atria, electrically stimulated at 1 Hz, developed a basal contractile tension of 530.3 ± 36.3 mg ($n = 30$). Concentrations of ATP ranging from 1 to 100 μM were tested. The nucleotide produced a fast developing negative inotropic effect at concentrations higher than 3 μM (Figure 1). The maximum negative inotropic effect, obtained with 100 μM ATP, consisted of a reduction of cardiac contractility of 44.6 ± 1.4% ($n = 4$; Table 1). The selective A_1 receptor antagonist, DPCPX (0.1 μM), did not affect the atrial contractile tension for a period of 40 min (Figure 1). However, in the presence of the antagonist, the negative inotropic effect of ATP was converted to a concentration-dependent positive effect. Records obtained from a typical experiment showing that the A_1 antagonist induced a reversal of the ATP effect are shown in Figure 1. The maximum positive inotropic effect induced by 30 μM ATP in the presence of DPCPX, consisted of an increase in contractile tension of 32.0 ± 7% ($n = 6$). The positive inotropic response to ATP was not modified by pretreatment of the preparations with 1 μM CGP 20712A, 1 μM prazosin, or 1 μM cimetidine, selective antagonists of β_1-adrenoceptors, α_1-adrenoceptors and H_2-receptors, respectively (data not shown in figure). In order to test whether the positive inotropic effect detected in the presence of the A_1 receptor antagonist was mediated by stimulation of A_2 adenosine receptors, further experiments were performed in the presence of 10 μM 8-phenyltheophylline, a non-selective adenosine receptor antagonist. A concentration-dependent positive inotropic response to ATP was also detected in the presence of the antagonist; the maximum effect was observed with a concentration of 30 μM ATP, and consisted of a net increase in contractile tension of about 120 mg ($n = 24$; Figure 2). A further increase in the ATP concentration to 100 μM did not induce another increase in contractile tension; moreover, in some experiments, the increase in ATP concentration pro-

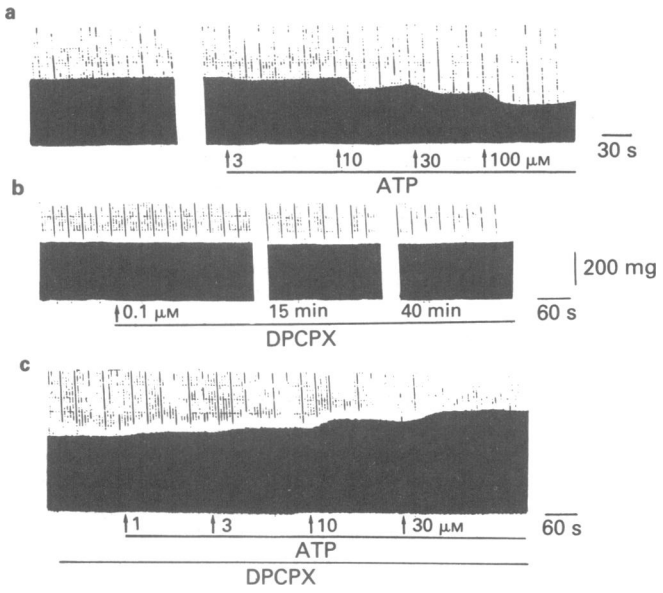

Figure 1 (a) Negative inotropic effect of ATP in guinea-pig left atria, electrically stimulated at 1 Hz; (b) lack of effect of 0.1 μM 1, 3-dipropyl-8-cyclopentylxanthine (DPCPX) on the contractile tension during an observation period of 40 min; (c) positive inotropic effect of ATP in the presence of 0.1 μM DPCPX.

Table 1 Negative inotropic effect induced by ATP in guinea-pig left atria electrically stimulated at 1 Hz

ATP concentration (μM)	*% reduction of contractility*
1	0 ± 0
3	4.2 ± 1.4
10	15.2 ± 2.3
30	27.7 ± 1.7
100	44.6 ± 1.4

Values are mean ± s.e.means of 4 experiments.

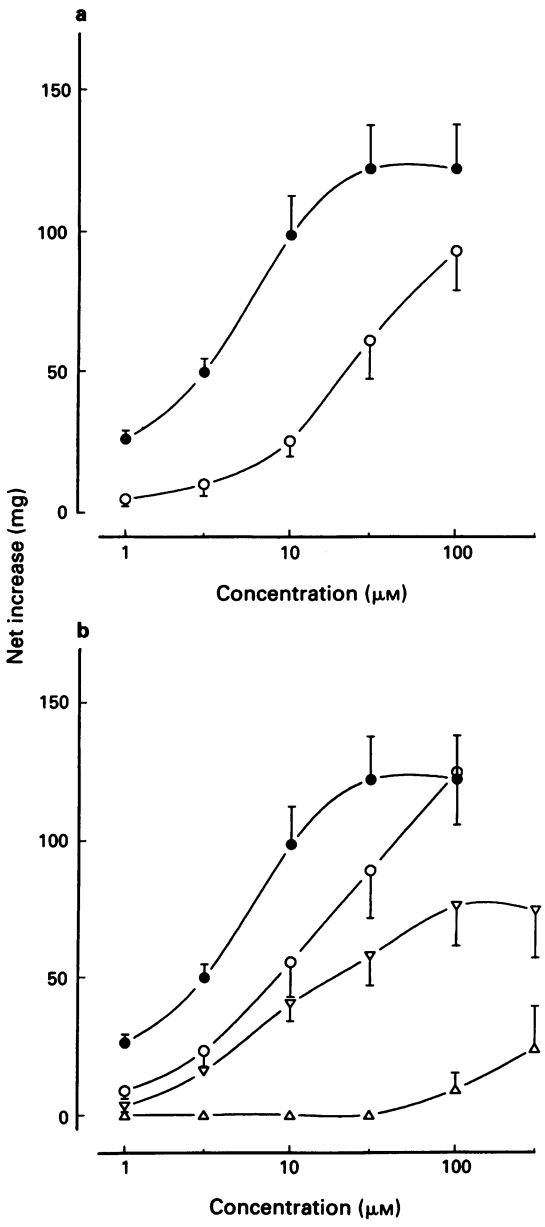

Figure 2 Concentration-response curves for the effect of ATP in the absence and in the presence of antagonists on force of contraction of guinea-pig isolated left atria preincubated with 10 μM 8-phenyltheophylline and electrically stimulated at 1 Hz. (a) (●) ATP alone ($n = 24$); (○) ATP in the presence of 500 μM suramin ($n = 10$). (b) (●) ATP alone ($n = 24$); (○) ATP in the presence of 30 μM reactive blue 2 ($n = 10$); (▽) ATP in the presence of 100 μM reactive blue 2 ($n = 6$); (△) ATP in the presence of 500 μM reactive blue 2 ($n = 6$). The effects are expressed as net increase in mg over the baseline value. Symbols indicate means ± s.e.mean.

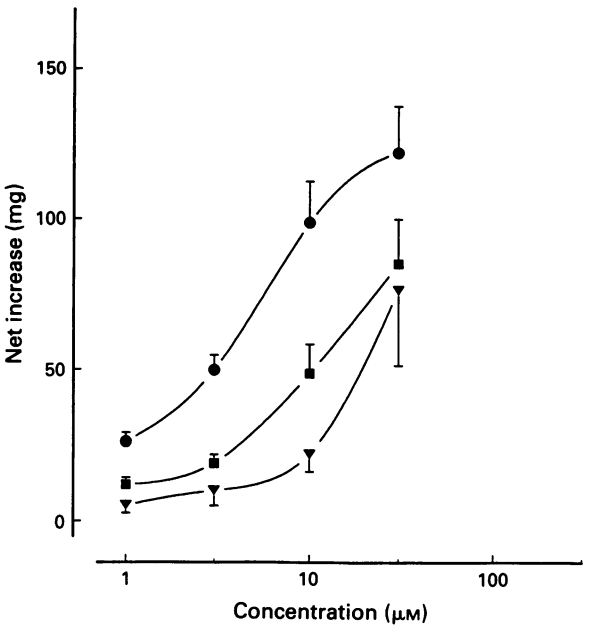

Figure 3 Concentration-response curves for the effect of ATP (●, $n = 24$), α,β-methylene ATP (■, $n = 8$) and β,γ-methylene ATP (▼, $n = 6$) on force of contraction of guinea-pig isolated left atria preincubated with 10 μM 8-phenyltheophylline and electrically stimulated at 1 Hz. The effects are expressed as net increase in mg over the baseline value. Symbols indicate means ± s.e.mean.

duced a slight reduction of tension, probably because of the displacement of the competitive antagonist 8-phenyltheophylline from A_1 receptors. The concentration-response curve for ATP was shifted to the right in a statistically significant manner by 500 μM suramin, an antagonist of P_2 purinoceptors ($n = 10$; Figure 2a). Moreover, reactive blue 2 (30–500 μM), a dye which has been reported active as a P_{2y} antagonist, significantly reduced the effect of ATP in a concentration-dependent manner. The shift of the curve observed in the presence of reactive blue 2 suggested a non-competitive type of antagonism since, in the presence of the dye, 100 μM ATP was no longer able to produce its maximum positive inotropic effect, and the ATP response was completely abolished by 500 μM reactive blue (Figure 2b). The effect of reactive blue 2 was not due to a nonspecific effect on muscular cells since the highest concentration of the antagonist (500 μM) did not affect the ability of the preparations to develop an inotropic effect in response to increasing concentrations of either calcium or noradrenaline (not shown in figure).

In addition, the stable analogues of ATP, α,β-methylene ATP and β,γ-methylene ATP, produced a positive inotropic effect in the concentration range 1–30 μM, in the presence of 8-phenyltheophylline. The curves showing the effects of the analogues, in comparison with that of ATP, are shown in Figure 3. The analogues were less potent than ATP, α,β-methylene ATP being more active than β,γ-methylene ATP.

Discussion

The results of the present study demonstrate that the usual negative inotropic effect of ATP can be converted to a positive inotropic response by blockade of adenosine receptors. The receptors involved in the cardiodepressant effects of adenine nucleotides have been identified as adenosine receptors of the A_1 subtype, which are linked to different intracellular pathways. The negative inotropic and chronotropic effects, which are predominant in atrial tissue, are due to an increase in K^+ current (Belardinelli & Isemberg, 1983; Bruckner *et al.*, 1985); the antiadrenoceptor effect, which is better observed in ventricular preparations, is due to an inhibition of β-receptor stimulation-induced cyclic AMP accumulation (Dobson, 1983). Both these kinds of A_1 receptor-mediated effects are linked to pertussis toxin-sensitive G proteins (Endoh *et al.*, 1983). After inactivation of G proteins by pertussis toxin, or blockade of A_1 receptors, both adenosine (Bohm *et al.*, 1988; Xu *et al.*, 1992) and ATP (Scamps *et al.*, 1990) can induce a positive inotropic effect on cardiac preparations. The cardiac stimulant response to adenosine and ATP had been tentatively attributed to different mechanisms: a stimulation of cyclic AMP by adenosine in the presence of DPCPX has been reported in chick ventricular myocytes (Xu *et al.*, 1992). It has also been demonstrated that ATP is able to increase calcium current in rat ventricular preparations (Bjornsson *et al.*, 1989; De Young & Scarpa, 1989; Scamps *et al.*, 1990) and to produce inositoltrisphosphate formation in the rat ventricle (Legssyer *et al.*, 1988). The above mentioned effects are not antagonized by adenosine receptor antagonists, isobutylmethylxanthine (IBMX) or 8-phenyltheophylline, thus suggesting that specific ATP receptors, (P_2 receptors) are involved in the excitatory response of ATP.

In agreement with the previously reported observations, a positive inotropic response to ATP was observed in the present study, after blockade of inhibitory adenosine receptors. The increase in cardiac tension produced by ATP was not modified by pretreatment with CGP 20712A, prazosin or cimetidine, selective antagonists of β_1-adrenoceptors, α_2-adrenoceptors and H_2-receptors, respectively. These findings clearly indicate that the cardiac stimulant response to ATP was not attributable to noradrenaline or histamine release. These results also demonstrate that adenosine A_2 receptors, which have been reported able to stimulate cyclic AMP production in several preparations (Londos *et al.*, 1980; Paton, 1984), were not involved in the positive inotropic response to ATP. Furthermore, the increase in cardiac contractility produced by ATP was antagonized by drugs reported as antagonists of P_2 receptors, such as suramin and reactive blue 2 (Kennedy, 1990), indicating that the effect of the nucleotide was mediated by stimulation of specific ATP receptors. A positive inotropic effect, even if to a lesser degree than ATP, was also produced by the two more stable analogues, α,β-methylene ATP and β,γ-methylene ATP. ATP receptors have been recently classified into P_{2x} and P_{2y} subtypes, on the basis of agonist potency order and of the ability to produce different functional responses in vascular preparations, i.e. a vasoconstrictor and a vasodilator effect, respectively (Burnstock & Kennedy, 1985; Kennedy, 1990). The order of potency of the agonists tested in the present study, (ATP > α,β-methylene ATP > β,γ-methylene ATP), is indicative of a P_{2y} receptor subtype. The concentration-positive inotropic response curve for ATP was shifted to the right by suramin, an antagonist of ATP receptors which is not able to discriminate between P_{2x} and P_{2y} receptors (Kennedy, 1990; Blakeley *et al.*, 1991). Moreover, the concentration-response curve to the agonist was also shifted in a concentration-dependent fashion by reactive blue 2, an anthraquinone-sulphonic acid derivative which has been reported as able to antagonize selectively the effects mediated by P_{2y} receptor stimulation (Burnstock & Warland, 1987; Taylor *et al.*, 1989). According to previous reports, the stimulation of P_{2y} receptors by ATP induces a rapid and transient accumulation of inositol 1,4,5-trisphosphate in a variety of cultured cells, such as endothelial cells, hepatocytes, leukocytes and erythrocytes. This effect is associated with a rapid increase in cytoplasmic calcium concentration (Boeynaems & Pearson, 1990). An increase in calcium concentration by ATP has also been reported in ventricular rat myocytes (De Young & Scarpa, 1989), associated with enhanced contractility (Danziger *et al.*, 1988). Moreover it has been demonstrated that ATP and other analogues increase the intracellular calcium concentration in rat myocytes with an order of potency of the agonists indicative of the P_{2y} receptor subtype (Bjornsson *et al.*, 1989).

The concentration-range of ATP able to increase the intracellular calcium level in this last study is comparable to the concentrations found able to increase the contractility in atrial preparations. Thus, the hypothesis can be advanced that the ATP-induced positive inotropic effect observed in the present study was mediated by stimulation of specific P_2 receptors, presumably belonging to the P_{2y} subtype, and that, according to previous observations, this effect was mediated by an increase in intracellular calcium.

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