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DISTRIBUTION OF P_1- AND P_2-PURINOCEPTORS IN THE GUINEA-PIG AND FROG HEART

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1 The effects of adenyl compounds were examined on the guinea-pig and frog heart in terms of the P_1/P_2-purinoceptor hypothesis.

2 The effects of two slowly degradable adenosine 5′-triphosphate (ATP) analogues; β,γ-methylene adenosine 5′-triphosphate (APPCP) and α,β-methylene adenosine 5′-triphosphate (APCPP) were also examined.

3 Adenosine, adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), ATP and APPCP produced inhibitory effects in guinea-pig atria. These inhibitory effects were antagonized competitively by theophylline and potentiated by dipyridamole. APCPP did not produce a similar inhibitory response.

4 Guinea-pig ventricles were insensitive to adenyl compounds.

5 ATP and ADP produced initial excitatory effects in frog atria which were followed by inhibitory effects. Adenosine and AMP produced inhibitory effects alone whereas APCPP produced excitatory effects only. The inhibitory effects were antagonized competitively by theophylline and potentiated by dipyridamole.

6 ATP, ADP, APPCP and APCPP evoked excitatory responses in frog ventricles. These responses were not affected by theophylline or dipyridamole. Adenosine and AMP were inactive on frog ventricles.

7 It is concluded that only P_1-receptors are present in guinea-pig atria; that both P_1- and P_2-receptors are present in frog atria; and that only P_2-receptors are present in frog ventricles. No evidence was found for the presence of either P_1- or P_2-purinoceptors in guinea-pig ventricles.

Introduction

The heart is sensitive to adenyl compounds (see Drury & Szent-Györgyi, 1929; Green & Stoner, 1950; Burnstock, 1976; 1980); these compounds have negative inotropic and chronotropic effects on the heart of both mammals (Hollander & Webb, 1957; James, 1965; Hopkins, 1973a; Fujita, Ishida, Izumi, Moritoki, Ohara & Takei, 1980) and frogs (Versprille & Van Duyn, 1966; Hartzell, 1979). In addition, an initial positive inotropic and chronotropic effect of adenosine 5′-triphosphate (ATP) has been reported in the frog heart (Versprille, 1963; Flitney, Lamb & Singh, 1977; Goto, Yatani & Tsuda, 1977).

It was recently proposed (Burnstock, 1978) that receptors for purine nucleotides and nucleosides could be classified into two types: P_1-purinoceptors, which are most sensitive to adenosine, are competitively blocked by methylxanthines, and occupation of which leads to changes in cyclic adenosine 3′,5′-monophosphate (cyclic AMP) accumulation; and P_2-purinoceptors which are most sensitive to ATP, and which are blocked (although not competitively) by quinidine, 2-substituted imidazolines, 2,2′-pyridylisatogen and apamin.

The aim of the present study was to examine the responses of the guinea-pig and frog heart to adenosine, adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), ATP and two slowly degradable ATP analogues (α,β-methylene ATP and β,γ-methylene ATP) in terms of the P_1/P_2-purinoceptor hypothesis.

Methods

Guinea-pigs (350–600 g) of either sex were killed by a sharp blow to the back of the neck. The hearts were excised and the left atria dissected free in cold bicarbonate-buffered physiological salt solution (Blinks, 1966) which was continually gassed with a mixture of 95% O_2 and 5% CO_2. The left atrium was mounted on a punctate electrode (Blinks, 1965) and then transferred to a 10 ml bath maintained at 32.5°C. An initial load of 0.5 g was applied to the

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preparation. The punctate electrode was used as a cathode (with a distant anode) to deliver electrical stimuli to the muscle (2.5 Hz, 5 ms duration) at twice threshold voltage. The right ventricle was cut into two strips which were then suspended in 10 ml organ baths. A resting tension of 1 g was applied and kept constant by readjustment during the equilibration period. The ventricle strips were electrically stimulated (2.5 Hz, 5 ms) at twice the threshold voltage. The mechanical activity was recorded isometrically by means of a Grass FT 03C force transducer and a Grass model 79D polygraph.

Frogs (*Rana pipiens*) were stunned by a blow to the back of the head, decapitated and pithed. The hearts were removed and placed in modified oxygenated Ringer solution (Gambhir & Tripathi, 1978). The spontaneously beating atria were mounted in a 10 ml bath at room temperature (17–20°C). Strips of ventricle were mounted in 10 ml baths and were stimulated electrically (0.5 Hz, 5 ms duration). The force of contraction developed by the frog atria and ventricular strips was recorded as described above.

The preparations were allowed to equilibrate for 60 min before the use of drugs. The bathing solution was changed every 15 min during the equilibration period. Responses were measured as percentages of basal levels. Statistical significance was evaluated by the t test for paired or unpaired samples and P values of 0.05 or less were considered to be significant. Regression lines were drawn from pooled observations between the 20–80% range for each of the dose-response curves. The gradients for control dose-response curves were compared with those for theophylline and dipyridamole-treated curves for divergence from parallelism ($P < 0.05$ was considered to be significant).

Drugs

Adenosine, adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP), β,γ-methylene adenosine 5′-triphosphate (APPCP), α,β-methylene adenosine 5′-triphosphate (APCPP), theophylline, indomethacin, adenosine deaminase (suspension in 3.2 M $(NH_4)_2SO_4$ solution) and 5′-nucleotidase were obtained from Sigma. Dipyridamole was obtained from Boehringer Ingelheim. All drugs were dissolved in distilled water except for indomethacin which was made up in 0.2 M sodium carbonate solution.

Results

Guinea-pig atria

Adenosine, AMP, ADP, ATP and APPCP (5–150 μM) reduced the force of contraction of guinea-pig left atria in a dose-dependent manner. The order of potency of these compounds in producing negative inotropic effects was adenosine > AMP > ADP = ATP. APPCP was slightly less potent than ATP at low concentrations (5–15 μM); however, at higher concentrations (65–650 μM) it was more potent than ATP and ADP (Figures 1 and 2). All five compounds required about 20 s to produce their maximum effect after a latency of 3–4 s.

APCPP (5–150 μM) was inactive within the time course of action of the other compounds studied ($n = 8$, see Figure 1a). However, after a time lag of about 17 s either a positive inotropic effect or a negative inotropic effect followed by a positive effect was observed.

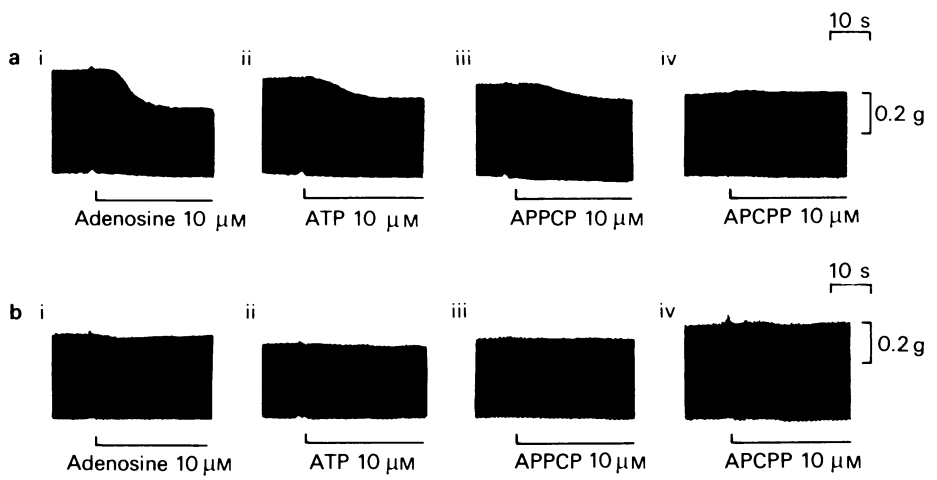

Figure 1 Responses of guinea-pig atria to: (i) adenosine; (ii) ATP; (iii) β,γ-methylene ATP (APPCP); (iv) α,β-methylene ATP (APCPP), in the absence (a) and presence (b) of theophylline (100 μM).

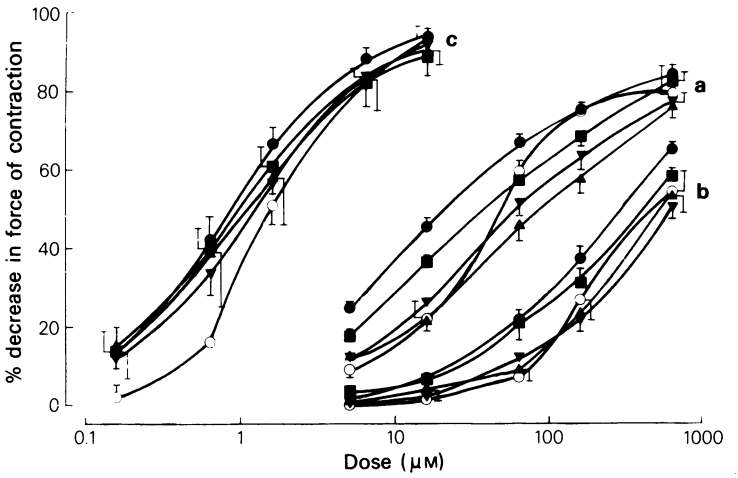

Figure 2 Log dose-response curves of electrically-driven guinea-pig atria to the negative inotropic effects of (●) adenosine; (■) AMP; (▲) ADP; (▼) ATP and (○) β,γ-methylene ATP (APPCP); (a) control; (b) in the presence of theophylline (100 μM) and (c) in the presence of dipyridamole (0.5 μM). Each point is the mean of at least 3 observations. Vertical bars show s.e.mean.

After incubation with theophylline (100 μM) for 30 min the dose-response curves to adenosine, AMP, ADP, ATP and APPCP were shifted ten fold to the right (Figure 2). The regression lines for adenosine, AMP, ADP, ATP and APPCP had gradients of 32.1, 30.8, 40.6, 42.7 and 60.2 respectively in the case of control curves and 43.9, 38.7, 45.3, 40.6 and 47.9 in the case of theophylline-treated curves. The slopes of the linear portions (in the 20–80% range) of the dose-response curves did not diverge significantly from parallel in the presence of theophylline indicating a competitive antagonism. The effect of theophylline was found to be reversible upon washout.

Incubation with dipyridamole (0.5 μM) for 30 min potentiated the negative inotropic effects of adenosine, AMP, ADP, ATP and APPCP, making the five compounds about equipotent (Figure 2). The regression lines for adenosine, AMP, ADP, ATP and APPCP had gradients of 46.2, 42.4, 42.7, 44.5 and 65.5 in the presence of dipyridamole. These gradients were not significantly different from those of controls.

In the presence of adenosine deaminase (2 u/ml) and 5′-nucleotidase (2 u/ml) the duration of action and size of the responses produced to adenosine and AMP were significantly reduced. However, adenosine deaminase and 5′-nucleotidase did not alter the effects produced by ATP and ADP.

Guinea-pig ventricles

Guinea-pig ventricular strips were found to be insensitive to adenosine, AMP, ADP and ATP (5–300 μM) in most of the preparations tested. However, in 2 out of the 11 preparations tested, an inhibitory response was produced by each compound when used in high concentrations (100–300 μM). These responses were not affected by dipyridamole (0.5 μM) or theophylline (100 μM).

Frog atria

Both ATP and ADP initially produced positive inotropic and chronotropic effects on the frog atria which were followed by negative inotropic and chronotropic responses. ATP and ADP were equipotent in producing these effects. However, adenosine and AMP produced only negative inotropic and chronotropic effects without the initial positive effects. APCPP produced positive inotropic and chronotropic effects only and took seven times as long as ATP and ADP to reach a maximum. Examples of the effects of adenosine, ATP and APCPP on frog atria are shown in Figure 3. Adenosine, AMP, ADP and ATP were not significantly different from each other in reducing the force of contraction of frog atria but ATP and ADP took 1.3 times as long as adenosine and AMP to reach a maximum. The negative inotropic and chronotropic effects of adenosine, AMP and the secondary inhibitory responses to ATP and ADP were antagonized by theophylline (100 μM), the dose-response curves being shifted ten fold to the right (Figure 4). Incubation with dipyridamole (0.5 μM) for 30 min potentiated the negative inotropic and chronotropic effects of adenosine,

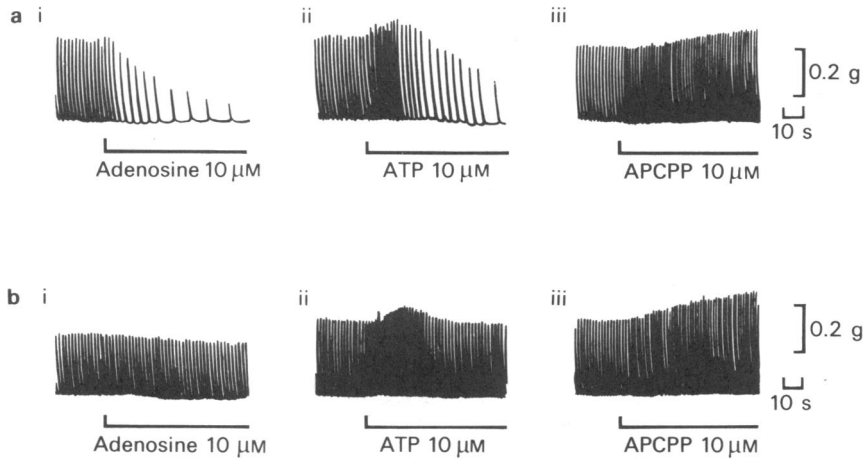

Figure 3 Responses of frog atria to: (i) adenosine; (ii) ATP; (iii) α,β-methylene ATP (APCPP); in the absence (a) and presence (b) of theophylline (100 μM).

AMP, ADP and ATP (Figure 4). The regression lines for adenosine, AMP, ADP and ATP had gradients of 44.9, 38.8, 48.9 and 42.2 respectively in the case of control curves; 59.8, 66.1, 54.8 and 48.6 in the presence of theophylline and 47.1, 40.1, 63.5 and 53.5 in the presence of dipyridamole. The gradients of the linear portions (in the 20–80% range) of the dose-response curves did not diverge significantly from parallel in the presence of theophylline and dipyridamole.

Frog ventricles

ATP, ADP, APPCP and APCPP (1–150 μM) produced positive inotropic effects (Figure 5). Adenosine and AMP in doses as high as 300 μM were inactive on most of the frog ventricular strips tested. However, in 3 out of 24 preparations, high concentrations of adenosine and AMP (100–300 μM) produced excitatory responses and in 2 other preparations produced inhibitory responses. Typical examples of these effects of adenosine, ATP and APCPP are illustrated in Figure 6. The responses to ATP and

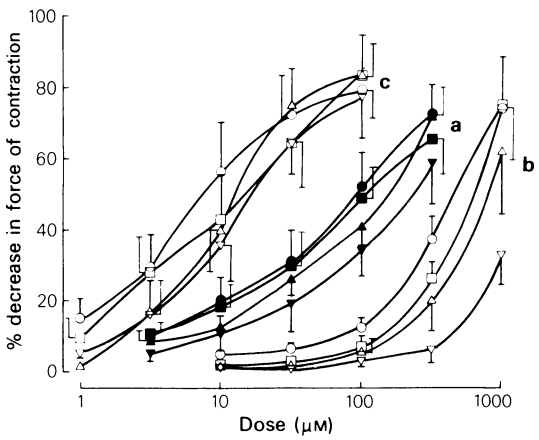

Figure 4 Log dose-response curves of spontaneously beating frog atria to the negative inotropic effects of (●) adenosine; (■) AMP; (▲) ADP and (▼) ATP alone, (a); and (○) adenosine; (□) AMP; (△), ADP and (▽) ATP in the presence of, (b) theophylline (100 μM) and (c) dipyridamole (0.5 μM). Each point is the mean of at least 5 observations; vertical lines show s.e.mean.

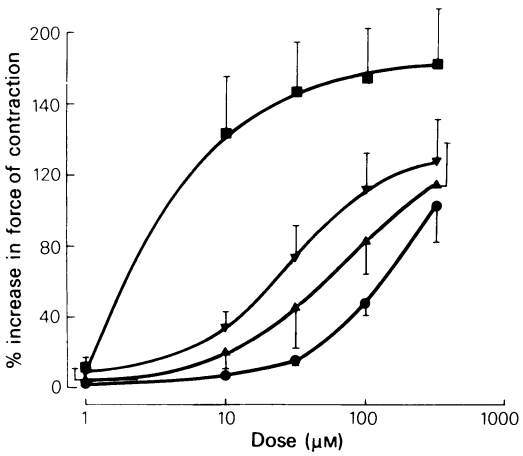

Figure 5 Log dose-response curves of electrically-driven frog ventricular strips to the positive inotropic effects of (▼) ATP; (▲) ADP; (●) β,γ-methylene ATP (APPCP) and (■) α,β-methylene ATP (APCPP). Each point is the mean of at least 6 observations. Vertical bars show s.e.mean.

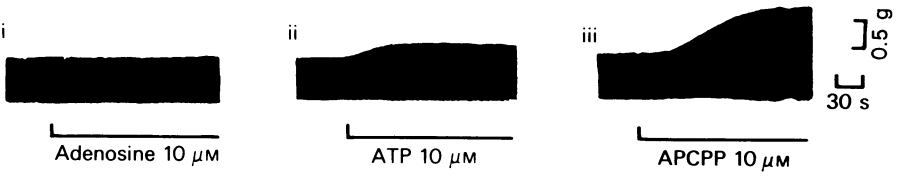

Figure 6 Responses of frog ventricular strips to: (i) adenosine; (ii) ATP and (iii) α,β-methylene ATP (APCPP).

ADP were not significantly altered by either theophylline (100 μM), dipyridamole (0.5 μM) or indomethacin (20 μM). The responses produced by APCPP took about four times as long as ATP and ADP to reach a maximum. The dose-response curve for APCPP was steeper than those for ATP, ADP and APPCP and the maximum response of the former analogue was much higher than that of ATP (Figure 5).

Discussion

This study has demonstrated different responses to adenosine and ATP in the guinea-pig and frog heart. The effects of theophylline and dipyridamole on these responses are consistent with the P_1/P_2-purinoceptor hypothesis. In guinea-pig atria, adenosine was the most potent, the order of potency being adenosine > AMP > ATP = ADP. This suggests that the effects of these compounds are due to the involvement of the postulated P_1-purinoceptor; a view substantiated by the finding that the actions of adenosine, AMP, ADP, ATP and APPCP were competitively antagonized by theophylline. It is not clear why APPCP has a distinctive sigmoid shaped dose-response curve. APCPP, which is resistant to degradation to adenosine (Satchell & Maguire, 1975; Maguire & Satchell, 1979) was inactive within the time course of action of ATP, supporting the view that ATP is metabolized to adenosine and AMP before acting on the P_1-receptor. The mode of action of APCPP in producing variable excitation or inhibition and excitation after a long latency is not known. The actions of adenosine, AMP, ADP, ATP and APPCP were potentiated by dipyridamole, which blocks adenosine uptake (Stafford, 1966; Hopkins, 1973b; Kalsner, 1975). The rapid onset of the response to ATP (Hollander & Webb, 1957; Fujita *et al.*, 1980) and the ineffectiveness of adenosine deaminase and 5′-nucleotidase on the response to ATP (confirming the results of Rubio, Belardinelli, Thompson & Berne, 1979) suggest that ATP may also have some action which is independent of its metabolism to AMP and adenosine. Nevertheless, since the action of ATP was antagonized by theophylline, there does not appear to be any evidence for the presence of a P_2-receptor in guinea-pig atria.

Guinea-pig right ventricular strips did not respond to ATP, ADP, AMP or adenosine in most preparations. Inhibitory responses were seen only at high concentrations in two preparations and, since they were not affected by dipyridamole or theophylline, it is unlikely that these effects were specific. Apart from the work of Szentmiklósi, Németh, Szegi, Papp & Szekeres (1980), these results are consistent with previous reports. Adenosine does not modify the action potentials or the contractile properties of the ventricle of guinea-pig (Lammerant & Becsei, 1973; Schrader, Rubio & Berne, 1975; Schrader, Gerlach & Baumann, 1979). P_1-purinoceptors may be present in the ventricle of other species. Adenosine increased the left ventricular contractile force in Langendorff rabbit hearts, secondary to the release of myocardial noradrenaline (Buckley, 1970a, b) and it induced a small negative inotropic effect followed by a marked positive inotropic effect on the dog ventricle (Chiba & Himori, 1975).

In frog atria, the responses to adenosine and AMP differed from those to ADP and ATP. ATP and ADP had a biphasic effect, consisting of an initial positive inotropic and chronotropic phase followed by a negative inotropic and chronotropic phase. Adenosine and AMP, in comparison, had only a negative inotropic and chronotropic effect. Since theophylline competitively blocked the inhibitory effects of adenosine, AMP and the secondary effects of ATP and ADP, it seems likely that these effects were mediated through P_1-purinoceptors. Furthermore, these inhibitory effects were potentiated by blocking adenosine uptake with dipyridamole. The excitatory actions of ATP, ADP and APCPP were unaffected by pretreatment with theophylline and therefore are not mediated via P_1-receptors, but rather by P_2-receptors which are more sensitive to ATP than to adenosine. APCPP produced an excitatory effect only; this is consistent with the view that ATP is degraded to AMP and adenosine before producing the inhibitory phase. The longer time taken for the second phase of the responses to ATP and ADP to reach a maximum compared with that taken by adenosine and AMP also supports this idea. Alternatively, APCPP could have a lower affinity than ATP

for the P_1-receptor; however, it would be necessary to block ATP breakdown in order to see which explanation is more likely. The results of this study are consistent with those of Goto, Yatani & Tsuda (1977) on frog atria. Versprille (1963) working on frog hearts, obtained an excitatory effect followed by an inhibitory effect of ATP, as in the present study, but this biphase response was followed by a third more pronounced excitatory phase that took 1–2 min to reach maximum and lasted for as long as 30 min. The mode of action of the second excitatory phase is not clear. Hartzell (1979) found that ATP as well as adenosine and AMP caused hyperpolarization in the frog sinus venosus, an effect which was competitively blocked by methylxanthines, but no initial excitatory response was reported either to ATP or adenyl imidodiphosphate, a degradation-resistant analogue. This discrepancy could be due to the fact that Hartzell (1979) used the sinus venosus only, whereas whole atria were used in the present study.

In frog ventricle strips ATP, ADP, APPCP and APCPP produced positive inotropic effects but adenosine and AMP were inactive even at high concentrations. ATP and ADP were equipotent in producing excitatory effects which were not antagonized by theophylline or potentiated by dipyridamole and therefore were not mediated via P_1-receptors. These results agree with those of Versprille (1965), who found ATP excitatory but AMP inactive on frog ventricles, and suggest that a P_2-receptor may be involved. Prostaglandins PGE_1 and PGE_2 produce excitatory effects on frog ventricles (Flitney & Singh, 1978). In addition, prostaglandins are responsible for the 'rebound contractions' that follow the inhibitory response of the guinea-pig taenia coli to ATP (Burnstock, Cocks, Paddle & Staszewska-Barczak, 1975). Since, indomethacin failed to antagonize the excitatory actions of ATP and ADP in the present study, stimulation of prostaglandin synthesis is an unlikely explanation for these excitatory responses.

The steeper dose-response curve and the greater maximal response of APCPP compared to ATP seen in frog ventricles have been shown to be characteristics of nucleotide analogues in which the polyphosphate chain is altered (Satchell & Maguire, 1975). The greater maximum effect may be due to the resistance of APCPP to degradation; the finding that APCPP took four times as long as ATP to produce a maximum effect is consistent with the work of Satchell & Maguire (1975) on guinea-pig taenia coli. In contrast with the present results, Flitney, Lamb & Singh (1977) found that ATP and ADP evoked an immediate initial positive inotropic effect on frog ventricular strips followed by a period of inhibition and then a period of longer lasting potentiation. AMP was shown to produce a qualitatively similar response to ATP and ADP at high doses (1000 μM) but adenosine produced a small inhibitory effect. In a later study, Flitney, Lamb & Singh (1978) suggested that changes in intracellular cyclic nucleotides mediated the actions of ATP. Singh & Flitney (1980) found that adenosine depressed contractility of the isolated ventricle of the frog. This effect was found to be accompanied by changes in intracellular cyclic nucleotide levels.

Thus, only P_1-receptors are present in guinea-pig atria, both P_1 and P_2 in frog atria and only P_2 in frog ventricles. No evidence was found for the presence of P_1- and P_2-receptors in guinea-pig ventricles. The distribution of purinoceptors could be the physiological consequences of different temperature control mechanisms between warm blooded and cold blooded animals. Additionally, in the frog atrium, the coronary vessels are not developed and the myocardial cells are nourished by intra-cavity blood; in this situation the regulatory mechanism of adenosine in the myocardium may be different (Goto, Yatani & Tsuda, 1978). However, it still remains to be seen whether these receptors have a physiological role and whether they mediate the actions of adenine nucleotides and nucleosides released from either neural or non-neural sites. Meanwhile, because of the marked differences in the distribution of P_1- and P_2-purinoceptors in the heart, these tissues may be useful in ligand binding studies for examining the chemistry of purine receptors.

References

BLINKS, J.R. (1965). Convenient apparatus for recording contractions of isolated heart muscle. *J. appl. Physiol.*, **20,** 755–757.

BLINKS, J.R. (1966). Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. *J. Pharmac. exp. Ther.*, **151,** 221–235.

BUCKLEY, N.M. (1970a). Cardiac effects of nucleosides after propranolol treatment of isolated hearts. *Am. J. Physiol.*, **218,** 1399–1405.

BUCKLEY, N.M. (1970b). Role of endogenous norepinephrine in cardiac inotropic effects of nucleosides. *Archs. int. Pharmacodyn. Ther.*, **188,** 92–104.

BURNSTOCK, G. (1976). Purinergic receptors. *J. theor. Biol.*, **62,** 491–503.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach.* ed. Bolis, L. & Straub, R.W. pp. 107–118. New York: Raven Press.

BURNSTOCK, G. (1980). Purinergic receptors in the heart. *Circulation Res.*, **46,** Suppl. 1, 175–182.

BURNSTOCK, G., COCKS, T., PADDLE, B. & STASZEWSKA-BARCZAK, J. (1975). Evidence that prostaglandin is responsible for the 'rebound contraction' following stimulation of non-adrenergic, non-cholinergic ('purinergic') inhibitory nerves. *Eur. J. Pharmac.*, **31,** 360–362.

CHIBA, S. & HIMORI, N. (1975). Different inotropic responses to adenosine on the atrial and ventricular muscle of the dog heart. *Jap. J. Pharmac.*, **25,** 489–491.

DRURY, A.N. & SZENT-GYÖRGYI, Â. (1929). The physiological activity of adenine compounds with special reference to their action upon the mammalian heart. *J. Physiol.*, **68,** 213–237.

FLITNEY, F.W., LAMB, J.F. & SINGH, J. (1977). Effects of ATP on the hypodynamic frog ventricle. *J. Physiol.*, **273,** 50–52P.

FLITNEY, F.W., LAMB, J.F. & SINGH, J. (1978). Effects of exogenous ATP on contractile force and intracellular cyclic nucleotide levels in the hypodynamic frog ventricle. *J. Physiol.*, **277,** 69–70P.

FLITNEY, F.W. & SINGH, J. (1978). Release of prostaglandins from the superfused frog ventricle during the development of the hypodynamic state. *J. Physiol.*, **285,** 18–19P.

FUJITA, S., ISHIDA, Y., IZUMI, K., MORITOKI, H., OHARA, M. & TAKEI, M. (1980). Potentiation by dilazep of the negative inotropic effect of adenosine on guinea-pig atria. *Br. J. Pharmac.*, **68,** 343–349.

GAMBHIR, S.S. & TRIPATHI, R.M. (1978). Dually innervated frog auricles for neuro-effector transmission studies. *Pharmac. res. Comm.*, **10,** 137–143.

GOTO, M., YATANI, A. & TSUDA, Y. (1977). An analysis of the action of ATP and related compounds on membrane current and tension components in bullfrog atrial muscle. *Jap. J. Physiol.*, **27,** 81–94.

GOTO, M., YATANI, A. & TSUDA, Y. (1978). Stabilizing effects of adenosine on the membrane currents and tension components of the bullfrog atrium. *Jap. J. Physiol.*, **28,** 611–625.

GREEN, H.N. & STONER, H.B. (1950). *Biological Actions of the Adenine Nucleotides.* London: Lewis.

HARTZELL, H.C. (1979). Adenosine receptors in frog sinus venosus: slow inhibitory potentials produced by adenine compounds and acetylcholine. *J. Physiol.*, **293,** 23–49.

HOLLANDER, P.B. & WEBB, J.L. (1957). Effects of adenine nucleotides on the contractility and membrane potentials of rat atrium. *Circulation Res.*, **5,** 349–353.

HOPKINS, S.V. (1973a). The action of ATP in the guinea-pig heart. *Biochem. Pharmac.*, **22,** 335–339.

HOPKINS, S.V. (1973b). The potentiation of the action of adenosine on the guinea-pig heart. *Biochem. Pharmac.*, **22,** 341–348.

JAMES, T.N. (1965). The chronotropic action of ATP and related compounds studied by direct perfusion of the sinus node. *J. Pharmac. exp. Ther.*, **149,** 233–247.

KALSNER, S. (1975). Adenosine and dipyridamole: action and interactions on isolated coronary artery strips of cattle. *Br. J. Pharmac.*, **55,** 439–445.

LAMMERANT, J. & BECSEI, I. (1973). Left ventricular contractility and developed tension in the intact dog submitted to an intracoronary infusion of adenosine. *J. Physiol.*, **229,** 41–49.

MAGUIRE, M.H. & SATCHELL, D.G. (1979). The contribution of adenosine to the inhibitory actions of adenine nucleotides on the guinea-pig taenia coli: studies with phosphate modified adenine nucleotide analogues and dipyridamole. *J. Pharmac. exp. Ther.*, **211,** 626–631.

RUBIO, R., BELARDINELLI, L., THOMPSON, C.I. & BERNE, R.M. (1979). Cardiac adenosine: electrophysiologic effects, possible significance in cell function, and mechanisms controlling its release. In *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides.* Ed. Baer, H.P. & Drummond, G.I. pp. 167–182. New York: Raven Press.

SATCHELL, D.G. & MAGUIRE, M.H. (1975). Inhibitory effects of adenine nucleotide analogues on the isolated guinea-pig taenia coli. *J. Pharmac. exp. Ther.*, **195,** 540–548.

SCHRADER, J., GERLACH, E. & BAUMANN, G. (1979). Sites and mode of action of adenosine in the heart. II. Ventricular myocardium. In *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides.* Ed. Baer, H.P. & Drummond, G.I. pp. 137–144. New York: Raven Press.

SCHRADER, J., RUBIO, R. & BERNE, R.M. (1975). Inhibition of slow action potentials of guinea-pig atria muscle by adenosine: A possible effect on Ca^{2+} influx. *J. mol. cell. Cardiol.*, **7,** 427–433.

SINGH, J. & FLITNEY, F.W. (1980). Adenosine depresses contractility and stimulates 3′,5′ cyclic nucleotide metabolism in the isolated frog ventricle. *J. mol. cell. Cardiol.*, **12,** 285–297.

STAFFORD, A. (1966). Potentiation of adenosine and the adenine nucleotides by dipyridamole. *Br. J. Pharmac. Chemother.*, **28,** 218–227.

SZENTMIKLÓSI, A.J., NÉMETH, M., SZEGI, J., PAPP, J. GY & SZEKERES, L. (1980). Effect of adenosine on sinoatrial and ventricular automaticity of the guinea-pig. *Naunyn-Schmiedebergs Arch. Pharmac.*, **311,** 147–149.

VERSPRILLE, A. (1963). The influence of uridine nucleotides upon the isolated frog heart. *Pflügers Arch.*, **277,** 285–292.

VERSPRILLE, A. (1965). Differences between the chronotropic effect of ATP on the isolated frog heart and the isolated frog ventricle. *Pflügers Arch.*, **283,** 82–88.

VERSPRILLE, A. & VAN DUYN, C.D. (1966). The negative chronotropic effect of adenine derivatives and acetylcholine on frog and rat hearts. *Pflügers Arch.*, **292,** 288–296.

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