Responses of the aorta of the garter snake (*Thamnophis sirtalis parietalis*) to purines

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1 Isolated aortic rings from the garter snake (*Thamnophis sirtalis parietalis*) were investigated in order to identify and classify responses to adenosine and adenosine 5'-triphosphate (ATP) and their analogues as part of a comparative study of vertebrate purinoceptors.

2 Adenosine, D-5'-(N-ethylcarboxamide) adenosine (NECA), R- and S-N⁶-(2-phenylisopropyl) adenosine (R- and S-PIA) and 2-chloroadenosine (2-CA) all concentration-dependently relaxed aorta precontracted with noradrenaline (NA). The order of potency was: NECA > R-PIA = 2-CA > adenosine > S-PIA. Individual pD₂ values for the analogues were: NECA 7.12 ± 0.13 (9), R-PIA 5.93 ± 0.25 (7), 2-CA 5.64 ± 0.40 (5), adenosine 5.04 ± 0.10 (13) and S-PIA 4.26 ± 0.10 (7). The order of potency has characteristics of both A₁ and A₂ receptors and cannot satisfactorily be classified according to the P₁-(adenosine) purinoceptor subtypes established in mammalian preparations.

3 ATP, α,β-methylene ATP (α,β-MeATP), 2-methylthio ATP (2MeSATP), β,γ-methylene ATP (β,γ-MeATP) and uridine 5'-triphosphate (UTP) all concentration-dependently constricted the isolated aorta. The order of potency was α,β-MeATP = 2MeSATP > ATP > β,γ-MeATP > UTP. Only ATP, α,β-MeATP and 2MeSATP consistently produced a maximum response; pD₂ values were: ATP 3.98 ± 0.07 (10), α,β-MeATP 5.86 ± 0.15 (12) and 2MeSATP 6.06 ± 0.23 (9). In vessels precontracted with NA neither ATP nor 2MeSATP caused relaxation in the presence or absence of the endothelium.

4 Suramin (0.1 mM) inhibited vasoconstriction to ATP, α,β-MeATP, 2MeSATP and β,γ-MeATP; however, since contractions to ATP and analogues did not reach a maximum response in the presence of this and other antagonists, pD₂ values could not be calculated.

5 Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 30 μM), a P_{2X}-purinoceptor antagonist, antagonized constrictions to α,β-MeATP only. Reactive blue 2 (RB2; 30 μM), a P_{2Y}-purinoceptor antagonist, inhibited vasoconstrictions to 2MeSATP only.

6 Indomethacin (30 μM) inhibited vasoconstriction in response to ATP and 2MeSATP, but not α,β-MeATP, suggesting that the presence of an unaltered phosphate chain on the ATP analogue was necessary to stimulate the production of a prostanoid.

7 Repeated administration of α,β-MeATP (3 μM) caused desensitization of the receptor responsible for the constriction due to α,β-MeATP whereas the responses to ATP and 2MeSATP were unaltered.

8 In summary, both P₁-purinoceptors mediating vasodilatation and P₂-purinoceptors mediating vasoconstriction are present on the garter snake aorta. However, in contrast to mammalian vessels, both P_{2X} and P_{2Y} subtypes mediate vasoconstriction. There was no evidence for vasodilatation to ATP or analogues. Stimulation of the P₂-purinoceptor by ATP and 2MeSATP caused the synthesis of a prostanoid. In addition, the possibility of a receptor activated by ATP, separate from P_{2X} and P_{2Y} subtypes is discussed since contractions to ATP proved to be insensitive to both PPADS and RB2. A comparison is made of purinoceptors in the garter snake aorta with those in other vertebrate vessels.

Keywords: Garter snake aorta; adenosine; ATP; P_{2X}-purinoceptor; P_{2Y}-purinoceptor; vasodilatation; vasoconstriction

Introduction

Since the initial observation of Drury & Szent Györgyi (1929) that adenosine compounds have potent inhibitory actions on the mammalian heart, extensive investigations have shown that both adenine nucleosides and nucleotides have important physiological actions concerning the local control of the vasculature (Su, 1985; Burnstock & Kennedy, 1986; Burnstock, 1990b; Olsson & Pearson, 1990).

Two distinct receptor classes have been recognised for adenosine and adenosine 5'-triphosphate (ATP) (Burnstock, 1978, 1990a); P₁- and P₂-purinoceptors. P₁-purinoceptors are identified as being preferentially activated by adenosine and adenosine 5'-monophosphate (AMP) and are blocked by methylxanthines (see Kennedy, 1990); this receptor has been further subdivided into A₁- and A₂-receptors and an A₃ subtype has been proposed using analogue potency orders

(Van Calker *et al.*, 1979; Londos *et al.*, 1980; Ribeiro & Sebastiao, 1986; Stone, 1991); the P₁-purinoceptor has been subdivided further (see Stone, 1989; Hoyle, 1992). P₂-purinoceptors are described as being preferentially activated by ATP and adenosine 5'-diphosphate (ADP); these were initially divided into P_{2X} and P_{2Y} subgroups, based on potency orders of ATP analogues (Burnstock & Kennedy, 1985); additional subtypes of purinoceptors have been proposed, for example P_{2Z}, P_{2T}, and P_{2U} subclasses (Gordon, 1986; Hoyle & Burnstock, 1991a; Stone, 1991; Hoyle, 1992; O'Connor, 1992). Further subclasses have been put forward including P_{2R}, P_{2S} and P₃, but lack widespread support (see Hoyle & Burnstock, 1991a,b; Stone, 1991).

Both P₁- and P₂-purinoceptor agonists have widespread actions on the cardiovascular system. Typically, adenosine dilates blood vessels by activating vascular smooth muscle A₂-receptors (Berne *et al.*, 1983; Burnstock & Kennedy, 1986; Collis, 1989), although endothelial adenosine receptors have

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been identified (Vials & Burnstock, 1993). Activation of A₁-purinoceptors generally mediates prejunctional inhibition of neurotransmitter release (Verhaeghe *et al.*, 1977).

In the cardiovascular system ATP stimulates both P_{2X}- and P_{2Y}-purinoceptors (Ralevic & Burnstock, 1991). P_{2X}-purinoceptors are found predominantly on vascular smooth muscle cells and mediate vasoconstriction (Burnstock & Kennedy, 1986; Burnstock, 1990a,b). Activation of endothelial P_{2Y}-purinoceptors results in vasodilatation, via endothelium-derived relaxing factor (EDRF) known to be nitric oxide (NO) (De Mey & Vanhoutte, 1981; Houston *et al.*, 1987; Pearson & Gordon, 1989; Boeynaems & Pearson, 1990) and prostacyclin (Moncada & Vane, 1979), however, some vascular smooth muscle P_{2Y}-purinoceptors have been demonstrated such as in the rabbit portal vein (Mathieson & Burnstock, 1985) and hepatic artery (Brizzolara & Burnstock, 1991).

Whereas there is extensive documentation of the presence and activity of both P₁- and P₂-purinoceptors on mammalian vascular preparations, information from lower vertebrate groups is limited. P₁-purinoceptors have been identified in the coronary artery of the trout (Small *et al.*, 1990), gill vasculature of the cichlid (Okafor & Oduleye, 1986) and dogfish atria and aorta (Meghji & Burnstock, 1984a; Evans, 1992). In the coronary artery of the skate, adenosine constricted vascular rings whereas ADP and ATP caused vasodilatation at high concentrations (Farrell & Davie, 1991a) indicating different purinoceptor subtypes. ATP is excitatory in the atria of the frog (Hoyle & Burnstock, 1986), axolotl and toad (Meghji & Burnstock, 1983a,b). There is very little documentary evidence of the effect of purine compounds on the vasculature of reptiles or birds, although dilatation in response to ATP has been observed in the perfused foot vasculature of ducks and chickens (McGregor, 1979). The present investigation was carried out to examine the effect of adenosine and ATP on the isolated dorsal aorta of the garter snake.

Methods

General Procedures

Red-backed garter snakes (*Thamnophis sirtalis parietalis*) of either sex supplied by Blades Biological, Cowden, Edenbridge, Kent, were anaesthetized with sagatal (50 mg kg⁻¹, i.p.) and decapitated. The brain was destroyed with a metal seeker. The dorsal aorta was rapidly removed and placed in a physiological saline solution. Ring segments of approximately 5–6 mm were dissected free and carefully mounted so as not to damage the endothelial layer in 10 ml organ baths containing continuously gassed (95% O₂/5% CO₂) saline of the following composition (mM): NaCl 144.17, KCl 4.95, Na₂H₂PO₄ 1.20, NaHCO₃ 29.60, MgSO₄ 1.21, CaCl₂ 2.75 and glucose 5.50 (Yamanouye *et al.*, 1992). Experiments were carried out at room temperature, 23 ± 1.0°C. For some experiments the endothelium was removed by rubbing the intima with a tungsten wire. The efficiency of this technique was tested by the ability of the vessel to relax to acetylcholine (ACh) when precontracted with noradrenaline (NA; data not shown).

Segments were mounted horizontally in the organ bath by inserting 2 tungsten wires through the lumen of the vessel and attached to a rigid support and a Grass force displacement transducer FT03C. Mechanical activity was displayed on a Grass ink-writing oscillograph. An initial load of 1 g was applied and the tissues were allowed to equilibrate for 1 h.

Concentration-response relationships

Prior to the experiment, the integrity of the endothelial layer was ascertained by the response to ACh on the NA (EC₅₀ concentration) precontracted aorta. Vasodilatation to ACh

indicated that the endothelium was intact, whereas the absence or only a very limited degree of vasodilatation indicated that the endothelium had been disrupted during preparation.

Concentration-response curves were constructed for NA to obtain an EC₅₀ value for each segment, which was used to constrict the vessel in order to observe relaxations to adenosine and analogues D-5'-(N-ethylcarboxamide) adenosine (NECA), R- and S-N⁶-(2-phenylisopropyl) adenosine (R- and S-PIA) and 2-chloroadenosine (2-CA).

Concentration-response curves were constructed for ATP and analogues α,β -methylene ATP (α,β -MeATP), β,γ -methylene ATP (β,γ -MeATP), 2-methylthio ATP (2MeSATP) and uridine triphosphate (UTP) on vessels at basal tone.

In order to characterize the purinoceptors, the concentration-response curves for adenosine and analogues were repeated in the presence of the P₁-purinoceptor antagonists 8-*p*-sulphophenyl-theophylline (8-PSPT) and caffeine; those of ATP, α,β -MeATP, β,γ -MeATP and 2MeSATP were repeated in the presence of the P₂-purinoceptor antagonist suramin, the P_{2X}-purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2', 4'-disulphonic acid (PPADS), the P_{2Y}-purinoceptor antagonist reactive blue 2 (RB2), and finally the prostanoicid inhibitor, indomethacin. All antagonists were equilibrated for 20 min.

Drugs used

NA bitartrate, adenosine, NECA, R-PIA, S-PIA, 2CA, ATP, α,β -MeATP, β,γ -MeATP, UTP, indomethacin, RB2 and ascorbic acid were supplied by Sigma Chemical Co; 2MeSATP, cyclopentyladenosine (CPA), CGS21680 (2-[*p*-(2-carboxyethyl) phenethyl amino]-5'-N-ethylcarboxamido adenosine) and 8-PSPT by Research Biochemicals Inc.; suramin was the kind gift of I.C.I. and PPADS the kind gift of Dr G. Lambrecht, Johann Wolfgang Goethe University, Frankfurt, Germany. NA was dissolved in 100 μ M ascorbic acid; indomethacin in 0.2 M NaCO₃ solution and NECA, R- and S-PIA, CPA and CGS21680 in dimethyl sulphoxide (DMSO) to produce a stock solution, with subsequent dilutions in distilled water. All other drugs were dissolved in distilled water.

Statistical analysis

Vasodilator responses are expressed as mean % relaxation of the NA-induced contraction $\pm$ standard error (s.e.) (*n*) and vasoconstrictor responses are expressed as a percentage of the maximum response, or as a mean maximum tension developed in g. Significance was tested by analysis of variance followed by Tukey's test, or a paired Student's *t*-test. A probability of *P* < 0.05 was taken as significant.

Results

Vasodilator responses to adenosine and analogues

On NA-precontracted (EC₅₀ concentration) vessels, adenosine and analogues NECA, R- and S-PIA and 2-CA caused concentration-dependent, endothelium-independent relaxations, the maximum relaxation being between 90–100%. Individual pD₂ values were: NECA 7.12 $\pm$ 0.13 (9), R-PIA 5.93 $\pm$ 0.25 (7), 2-CA 5.64 $\pm$ 0.40 (5), adenosine 5.04 $\pm$ 0.10 (13) and S-PIA 4.26 $\pm$ 0.10 (7). Analysis of variance showed the order of potency for the compounds to be: NECA > R-PIA = 2-CA > adenosine > S-PIA (Figure 1).

Adenosine antagonists were examined for their ability to inhibit the dilatation to adenosine, however, 8-PSPT (up to 10 μ M) and caffeine (up to 0.1 mM), inhibited constriction of the aorta to NA at all concentrations of antagonist tested and could not be used. The more selective adenosine agonists

CPA and CGS 21680, selective for A₁- and A₂-receptors respectively, were without activity on this preparation.

Neither ATP (up to 10 mM) nor 2MeSATP (up to 30 μ M) caused relaxation of precontracted vessels in either the absence or presence of the endothelium; instead, a further constriction was observed to the agonists.

Vasoconstrictor responses to ATP and analogues

At basal tone, ATP, α,β -MeATP, β,γ -MeATP, 2MeSATP and UTP all concentration-dependently and endothelium-independently constricted the isolated aortic rings. ATP, α,β -MeATP and 2MeSATP reached a maximum contraction, pD₂ values being: ATP 3.98 ± 0.07 (10), α,β -MeATP 5.86 ± 0.15 (12) and 2MeSATP 6.06 ± 0.23 (9). There was no significant difference between the pD₂ values for α,β -MeATP and 2MeSATP, but both these compounds were more potent than ATP; $P < 0.05$ (Figure 2).

Since concentration-response curves to β,γ -MeATP and UTP did not consistently reach a maximum response, the 5 agonists were also compared by their absolute tensions developed. Analysis of variance and Tukey's test revealed the order of potency to be: α,β -MeATP = 2MeSATP > ATP = β,γ -MeATP = UTP (data not shown).

Desensitization to α,β -MeATP

Repeated applications of α,β -MeATP (3 μ M) caused diminished contractions until there was no response to an additional dose of α,β -MeATP. In the presence of α,β -MeATP the responses to ATP and 2MeSATP were unchanged.

Effect of antagonists on constrictor responses to ATP and analogues

Suramin (0.1 mM) significantly inhibited the constrictor responses to ATP, α,β -MeATP, β,γ -MeATP and 2MeSATP. As maximum responses to the ATP analogues were not always reached in the presence of suramin and the other antagonists,

pD₂ values were not calculated. Student's *t*-test revealed that suramin significantly inhibited responses to all the ATP analogues; $P < 0.05$ (Figures 3a–3d).

In the presence of PPADS (30 μ M) the constrictor response to α,β -MeATP was significantly ($P < 0.05$) inhibited (Figure 4), while having no effect on the ability of the other agonists to constrict the aorta (not shown).

RB2 (30 μ M) significantly ($P < 0.05$) inhibited constrictor responses to 2MeSATP only, without having an effect on the responses of the other agonists (Figure 5).

Contractile responses to ATP and 2MeSATP were significantly ($P < 0.05$) inhibited by indomethacin (30 μ M) (Figure 6a and 6b) whereas the responses to the other agonists were unaffected.

Discussion

The results from this study reveal that the dorsal aorta of the garter snake possesses both P₁- and P₂-purinoceptors that are antagonistic in action and appear to be independent of the presence of an intact endothelium, the P₁-purinoceptor mediating vasodilatation and P₂-purinoceptors mediating vasoconstriction.

Identification of the P₁-purinoceptor subtype mediating vasodilatation of the aorta, founded on potency order alone, can be somewhat misleading. An agonist profile of R-PIA > adenosine > NECA describes an A₁-purinoceptor and the reverse an A₂-purinoceptor, according to the original definition by Van Calcar *et al.* (1979). In addition, a further criterion has been suggested to aid the identification of P₁-purinoceptors, the stereoselectivity for the PIA isomers (Stone, 1989). A potency ratio of the R/S isomers of PIA, with R-PIA approximately 100 times more potent than S-PIA indicates an A₁-purinoceptor whereas a ratio of 10 or less is indicative of an A₂-receptor. However, the potency ratios of R- and S-PIA have proved extremely variable.

Based on these two sets of criteria, the results from this

Figure 1 Cumulative concentration-response relationships for adenosine ($\blacktriangle$, $n = 13$), NECA ($\blacklozenge$, $n = 9$), R-PIA ($\blacksquare$, $n = 7$), S-PIA ($\bullet$, $n = 7$) and 2-CA ($\blacktriangledown$, $n = 5$) on noradrenaline (EC₅₀ concentration) precontracted garter snake aorta. All symbols represent mean % relaxation $\pm$ s.e. (unless masked by the symbol). For abbreviations, see text.

Figure 2 Concentration-response relationships for ATP ($\bullet$, $n = 10$), α,β -MeATP ($\blacktriangle$, $n = 12$) and 2MeSATP ($\blacksquare$, $n = 9$) on basal tone of the dorsal aorta of the garter snake. All symbols show mean % contraction $\pm$ s.e. (unless masked by the symbol). For abbreviations, see text.

study can be interpreted in a variety of ways, and may be indicative of both an A₁- and an A₂-receptor. The order of potency for NECA and R-PIA suggest that the receptor is of the A₂ subtype, since NECA was significantly more potent than the PIA isomers (Van Calker *et al.*, 1979; Londos *et al.*, 1980). If the stereoselectivity of the isomers for PIA is considered alone, then the opposite interpretation can be concluded; i.e. the receptor might be considered of the A₁ subtype since R-PIA is approximately 100 times more potent than S-PIA (Ribeiro & Sebastiao, 1986; Stone, 1989). The

identification of this receptor is confounded further if one takes into consideration the proposed existence of the A₃-receptor believed to exist on nerve endings and the heart and not coupled to adenylyl cyclase. The A₃-receptor has been described as having an agonist profile of cyclohexyl adenosine (CHA), R-PIA and NECA being the most potent, irrespective of their order, and more potent than 2-CA (Ribeiro & Sebastiao, 1985). The adenosine receptor of the snake aorta does not appear to fall into either P₁-purinoceptor subtype, thus it could also be argued that the receptor

Figure 3 Effect of suramin (0.1 mM) on the concentration-response relationships for ATP, α,β -MeATP and β,γ -MeATP, on the dorsal aorta of the garter snake. (a) Concentration-response relationship for ATP in the absence (●, $n = 5$) and presence (○, $n = 5$) of suramin (0.1 mM). (b) Concentration-response relationship for α,β -MeATP in the absence (▲, $n = 6$) and presence (△, $n = 6$) of suramin (0.1 mM). (c) Concentration-response relationship for 2MeSATP in the absence (■, $n = 6$) and presence (□, $n = 6$) of suramin (0.1 mM). (d) Concentration-response relationship for β,γ -MeATP in the absence (◆, $n = 5$) and presence (◇, $n = 5$) of suramin (0.1 mM). All symbols show mean % contraction $\pm$ s.e. (unless masked by the symbol). *Indicates significance, $P < 0.05$. For abbreviations, see text.

from this study is of the A₃ type, proposed for those receptors that cannot be classified according to classical criteria.

Figure 4 Concentration-response relationship for α,β -MeATP in the absence ($\blacktriangle$, $n=6$) and presence ($\triangle$, $n=6$) of PPADS ($30\ \mu\text{M}$), on the dorsal aorta of the garter snake. All symbols show mean % contraction $\pm$ s.e. (unless masked by the symbol). *Indicates significance, $P<0.05$. For abbreviations, see text.

Figure 5 Concentration-response relationship for 2MeSATP in the absence ($\blacksquare$, $n=6$) and presence ($\square$, $n=6$) of RB2 ($30\ \mu\text{M}$), on the dorsal aorta of the garter snake. All symbols show mean % contraction $\pm$ s.e. (unless masked by the symbol). *Indicates significance, $P<0.05$. For abbreviations, see text.

The adenosine antagonists examined in this study inhibited the constrictor activity of NA by an unknown mechanism, classification of the receptor by the use of selective antagonists was therefore denied and as such the receptor can only be classified by the use of the somewhat misleading agonist potency order. Lack of activity of selective agonists and antagonists is not uncommon when studying more primitive organisms. For instance the adenosine antagonist

Figure 6 Effect of indomethacin ($30\ \mu\text{M}$) on the concentration-response relationships for ATP and 2MeSATP, on the dorsal aorta of the garter snake. (a) Concentration-response relationship for ATP in the absence ($\bullet$, $n=5$) and presence ($\circ$, $n=5$) of indomethacin ($30\ \mu\text{M}$). (b) Concentration-response relationship for 2MeSATP in the absence ($\blacksquare$, $n=5$) and presence ($\square$, $n=5$) of indomethacin ($30\ \mu\text{M}$). All symbols show mean % contraction $\pm$ s.e. (unless masked by the symbol). *Indicates significance, $P<0.05$. For abbreviations, see text.

1,3-dipropyl-8-cyclopentylxanthine (DPCPX) was found to be ineffective at blocking adenosine in the stomach and intestine of the stickleback (Knight & Burnstock, 1993).

There are many instances in the literature where subtypes of adenosine receptors have been identified although the potency order does not agree with the classical definition. For instance, the receptor inhibiting nerve evoked contractions of the rat vas deferens has an agonist profile of NECA > R-PIA > 2-CA, the R/S ratio being 100 and the receptor is classified by the authors as an A₁ subtype on this basis, even though NECA is the more potent (Paton & Webster, 1984). Similarly, in bovine coronary arteries, the inhibitory adenosine receptor is classified as an A₂-receptor, the potency order being NECA > R-PIA > 2-CA although the R/S ratio is much greater than 10, being in fact 32 (Mustafa & Askar, 1985). Clearly, identification of adenosine receptor subtypes on agonist potency alone, particularly when the order does not agree exactly with those described for classical A₁, A₂ or A₃ subtypes is inappropriate. Considering the ambiguity of the interpretation of the potency order of the adenosine analogues, it may be appropriate to conclude that the receptor identified in this study is unique in nature possessing characteristics of all 3 adenosine receptors when defined by the order of potency alone.

This study has revealed the presence of P₂-purinoceptors on the aorta that are also independent of an intact endothelium. The order of potency: α,β -MeATP = 2MeSATP > ATP > β,γ -MeATP > UTP, would suggest an undifferentiated P₂-purinoceptor. The possibility of the receptor being specific for UTP, a P_{2U} subtype (O'Connor, 1992) is discounted since UTP was significantly less potent than all the ATP analogues.

Selective blockade of the ATP analogues indicates the presence of both P_{2X}- and P_{2Y}-purinoceptors. All of the ATP analogues were inhibited by suramin (0.1 mM) which blocks both P_{2X}- and P_{2Y}-purinoceptors (Dunn & Blakeley, 1988; Hoyle *et al.*, 1990). Thus, all the ATP analogues mediated vasoconstriction via P₂-purinoceptors.

The recently developed compound PPADS has shown specificity for the P_{2X}-purinoceptor on the vas deferens of the rabbit and guinea-pig (Lambrecht *et al.*, 1992; McLaren *et al.*, 1994), rabbit bladder (Ziganshin *et al.*, 1993) and ear and saphenous artery (Ziganshin *et al.*, 1994). PPADS (30 μ M) was found to inhibit selectively contractions in response to α,β -MeATP while having no effect on 2MeSATP. Interestingly, PPADS was ineffective in blocking the response to ATP itself. In addition, the presence of a P_{2X}-purinoceptor is confirmed since the repeated administration of α,β -MeATP resulted in desensitization of the response; ATP may or may not be acting at this receptor, since its continued activity after desensitization with α,β -MeATP or blockade with PPADS could indicate a lack of activity at this receptor, or an action on a separate receptor.

The activity of RB2 indicates that there is also a P_{2Y}-purinoceptor present on the aorta of the snake. RB2 (30 μ M) selectively attenuated responses to 2MeSATP only, without an effect on α,β -MeATP or ATP. Contractile responses to 2MeSATP are also inhibited by indomethacin, indicating that 2MeSATP stimulated the production of a prostanoid; similarly ATP contractions were also sensitive to indomethacin, indicating that it too was activating the production of a prostanoid, although whether via an action on a separate, unidentified purinoceptor subtype is still open to conjecture.

The presence of a dual population of receptor subtypes producing the same response is somewhat unusual; however, examples can be found: A₁- and A₂-purinoceptors have been identified in the rat duodenum, both causing relaxation (Nicholls *et al.*, 1992). Commonly, vascular responses to α,β -MeATP and 2MeSATP are antagonistic in nature; α,β -

MeATP mediating vasoconstriction and 2MeSATP mediating vasodilatation; however there was no evidence of vasodilatation to purine compounds.

While this study has not revealed the mechanism of action for the response to α,β -MeATP, it has revealed that stimulation of the P_{2Y}-purinoceptor results in the formation of a prostanoid, since ATP and 2MeSATP were inhibited by indomethacin. This is consistent with a number of mammalian vascular systems (Needleman *et al.*, 1974; Boeynaems & Galand, 1983) where prostaglandin synthesis is initiated by ATP at the endothelial level, mediating vasodilatation (see Moncada, 1982). However, in this preparation, the prostaglandin is not endothelial in origin, since vasoconstriction to 2MeSATP occurred even when the endothelium was not intact (as revealed by the lack of vasodilatation to ACh). In a number of vascular systems, some members of the prostaglandin family can initiate vasoconstriction, for example, the rabbit aorta constricts to prostaglandin E₁ (PGE₁) and PGE_{F1 α} (Khairallah *et al.*, 1967; Strong & Bohr, 1967). The nature of the agonist also appears to be important, since prostaglandin formation occurred only in response to an agonist possessing an unaltered phosphate chain of the purine molecule (Brown & Burnstock, 1981); α,β -MeATP and β,γ -MeATP did not stimulate prostaglandin formation.

There is evidence that both adenosine and ATP play a part in vascular control of lower vertebrate animals. Adenosine has been shown to dilate the perfused gills of the hagfish (Axelsson *et al.*, 1990) and have effects upon the heart of the trout, frog and toad (Meghji & Burnstock, 1983b; 1984b; Hoyle & Burnstock, 1986; Lazou & Beis, 1987). Both an A₁- and an A₂-receptor have been identified in the aorta of the dogfish (Evans, 1992). ATP has been shown to have activity in a variety of different vessels from lower vertebrates, such as the systemic vasculature and coronary artery of the rainbow trout (Wood, 1977; Small *et al.*, 1990) and steelhead trout (Small & Farrell, 1990), mako shark coronary artery (Farrell & Davie, 1991b) and gill vascular bed (Colin & Leray, 1979; Colin *et al.*, 1979). However, a full characterization of these responses was not performed and as such the subtype, if any, of the purinoceptor is unknown. The dual activity of ATP, vasodilatation and vasoconstriction, on the coeliac mesenteric artery and anterior cardinal vein of the trout, suggested to Olson & Villa (1991) the possibility of a dual population of ATP receptors that were antagonistic in nature.

In summary, the results from the present study have shown the presence of both P₁- and P₂-purinoceptors on the smooth muscle of the garter snake aorta. The P₁-purinoceptor, mediating vasodilatation, exhibiting the characteristics of an A₁-, an A₂- and the less accepted A₃-receptor such that its classification is limited to that of a unique or unclassified adenosine receptor. The presence of P_{2X}- and P_{2Y}-purinoceptors mediating vasoconstriction was revealed by selective antagonism by PPADS and RB2, and desensitization by α,β -MeATP. The P_{2Y}-purinoceptor is somewhat unusual in that it mediates vasoconstriction via the production of a prostaglandin. The possibility of a receptor specific to ATP is postulated, since the response to ATP was not affected by PPADS or RB2, but, by the action of suramin, was acting via a P₂-purinoceptor. This study has revealed that antagonistic control of vascular smooth muscle by separate receptors for both adenosine and ATP, is a primitive feature which has now been demonstrated in a variety of lower vertebrates.

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