British Journal of Pharmacology (1999) 127, 1597–1602
http://www.stockton-press.co.uk/bjp

Evidence that the ATP-induced increase in vasomotion of guinea-pig mesenteric lymphatics involves an endothelium-dependent release of thromboxane A_2

[1]Jinnan Gao, [1]Jun Zhao, [1]Sharyn E. Rayner & *,[1]Dirk F. Van Helden

[1]The Neuroscience Group, Discipline of Human Physiology, Faculty of Medicine & Health Sciences, The University of Newcastle, Callaghan, NSW, 2308, Australia

1 Experiments were made to investigate mechanisms by which adenosine 5′-trisphosphate (ATP) enhanced vasomotion in mesenteric lymphatic vessels isolated from young guinea-pigs.

2 ATP (10^{-8}–10^{-3} M) caused a concentration-dependent increase of perfusion-induced vasomotion with the endothelium mediating a fundamental role at low ATP concentrations (10^{-8}–10^{-6} M).

3 The response to 10^{-6} M ATP showed tachyphylaxis when applied at intervals of 10 min but not at intervals of 20 or 30 min.

4 Suramin (10^{-4} M) or reactive blue 2 (3×10^{-5} M) but not PPADS (3×10^{-5} M) abolished the excitatory response to 10^{-6} M ATP confirming an involvement of P_2 purinoceptors.

5 The excitatory response to 10^{-6} M ATP was abolished by treatment with either pertussis toxin (100 ng ml^{-1}), antiflammin-1 (10^{-9} M), indomethacin (3×10^{-6} M) or SQ29548 (3×10^{-7} M), inhibitors of specific G proteins, phospholipase A_2, cyclo-oxygenase and thromboxane A_2 receptors respectively.

6 ATP simultaneously induced a suramin-sensitive inhibitory response, which was normally masked by the excitatory response. ATP-induced inhibition was mediated by endothelium-derived nitric oxide (EDNO) as the response was abolished by N^G-nitro-L-arginine (L-NOARG; 10^{-4} M), an inhibitor of nitric oxide synthase.

7 We conclude that ATP modulates lymphatic vasomotion by endothelium-dependent and endothelium-independent mechanisms. One of these is a dominant excitation caused through endothelial P_2 purinoceptors which because of an involvement of a pertussis toxin sensitive G-protein may be of the P_{2Y} receptor subtype. Their stimulation increases synthesis of phospholipase A_2 and production of thromboxane A_2, an arachidonic acid metabolite which acts as an endothelium-derived excitatory factor.

Keywords: Lymphatic vessels; vasomotion; endothelium; ATP; phospholipase A_2; arachidonic acid, thromboxane A_2, P_{2Y} purinoceptors

Abbreviations: ACh, acetylcholine; ATP, adenosine 5′-trisphosphate; EDNO, endothelium-derived nitric oxide; L-NOARG, N^G-nitro-L-arginine; SNP, sodium nitroprusside; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid; RB2, reactive blue 2

Introduction

Many collecting lymphatic vessels have smooth muscle in their walls which can phasically constrict, a phenomenon often referred to as lymphatic 'vasomotion' (Johnson, 1980; Auckland & Reed, 1993). These vessels have frequently occurring unidirectional valves which form vessels into multiple chambers termed lymphangions, each capable of acting as an independent 'primitive heart' to propel lymph (Mislin, 1983). Luminal perfusion is a key factor for initiating vasomotion (Florey, 1927; Smith, 1949), with such activity modulated by many factors including hormones/neurotransmitters such as noradrenaline (McHale & Roddie, 1983) and endothelium released substances such as endothelium-derived nitric oxide (EDNO; Yokoyama & Ohhashi, 1993; von der Weid *et al*., 1996). These substances modulate vasomotion by acting on the intrinsic pacemaker mechanisms (Van Helden, 1993; Van Helden *et al*., 1996).

Adenosine 5′-trisphosphate (ATP), a sympathetic co-transmitter for both blood (Burnstock, 1995) and lymphatic vessels (Hollywood & McHale, 1994), modulates vascular tone and lymphatic vasomotion. The mechanisms underlying ATP-induced responses on blood vessels are quite diverse varying between different tissues and species. However, the pathways whereby ATP modulates lymphatic vasomotion are not known.

ATP generally produces its effects by acting on P_2 purinoceptors (Burnstock, 1990) which can be classified into two major superfamilies: P_{2X} purinoceptors, which are ligand-gated ion channel receptors and P_{2Y} purinoceptors, which are G protein-coupled receptors (Abbracchio & Burnstock, 1994; Fredholm *et al*., 1994; Barnard *et al*., 1994). Both P_{2X} and P_{2Y} receptors can be further classified into up to seven subtypes (Surprenant *et al*., 1996). Most studies on blood vessels have shown that ATP modulates vasoconstriction mainly by a direct action on P_{2X} or P_{2Y} purinoceptors on the smooth muscle (Benham & Tsien 1987; Abbracchio & Burnstock, 1994; Harden *et al*., 1995; Lagaud, 1996; McLaren *et al*., 1998). However, there have been reports which indicate that ATP can cause endothelium-dependent vasoconstriction through release of thromboxane A_2 (Shirahase *et al*., 1990; 1991; Dominiczak *et al*., 1991). By contrast, vasodilation is mainly mediated by

*Author for correspondence.

P_{2Y} purinoceptors localised on the endothelium (De Mey & Vanhoutte, 1981; Martin *et al*., 1985; Palmer *et al*., 1987; Pearson & Carter, 1990; Gordon, 1990).

Our present experiments indicate that ATP increases vasomotion of guinea-pig mesenteric lymphatics in both an endothelium-dependent and endothelium-independent manner. The principle findings reported here are that ATP can stimulate the release of an endothelium-derived excitatory factor, thromboxane A_2 which diffuses onto the smooth muscle to increase perfusion-induced vasomotion.

Methods

Tissue preparation

Young guinea-pigs (<10 days) of either sex were killed by overexposure to the inhalation anaesthetic halothane (5–10% in air) followed by decapitation. The small intestine and attached mesentery were rapidly removed and placed in a physiological saline solution of the following composition (in mM): $CaCl_2$ 2.5, KCl 5, $MgCl_2$ 2, NaCl 120, $NaHCO_3$ 25, $NaHPO_4$ 1 and glucose 10. The pH was maintained at 7.2 by constant bubbling with a 95% O_2 and 5% CO_2 gas mixture. Lymphatic vessels (diameter < 300 μm) with associated arteries, veins and mesothelium were dissected out and pinned flatly onto a Sylgard-coated (Dow Corning) base of a small organ bath (volume 1.0 ml) and superfused at a rate of 6 ml min^{-1} with the physiological saline heated to 34–36°C. A fine glass cannula connected to an infusion pump was loosely inserted into the distal end of the lymphatic vessel with perfusion rates of about 2.5 μl min^{-1} used to induce spontaneous constrictions. Possible blockage of the cannula through precipitation of calcium was prevented by using a low-calcium physiological saline solution ($CaCl_2$ 1.2 mM). Tissues were normally used within 1–4 h of isolation (except for pertussis toxin experiments) and used fresh or stored from 2–8 h at 4°C in physiological saline until use.

Recording technique

The activities of lymphatic vessels were monitored by a video camera attached to an inverted microscope and analysed visually or videoscopically with vessel edges tracked at 25 Hz by a computer-based algorithm (Beresford-Smith *et al*., 1993).

Removal of endothelium

The endothelium of lymphatics was removed *in vitro* by repeatedly (5–6 times) passing brief (5–10 s) streams of air through the lumen of the vessels at a rate of about 3 μl min^{-1}. The success of lysis was confirmed by applying acetylcholine (ACh, 100 μM) followed by sodium nitroprusside (SNP 100 μM). A negative response to ACh and a positive response to SNP was used as confirmation of successful lysis. The use of SNP was necessary as it has been shown that some 40% of guinea-pig mesenteric lymphatic vessels do not respond in any way to either ACh or SNP (von der Weid *et al*., 1996). Lysis based on this testing procedure proved successful in about 50% of treated vessels.

Experimental protocols

Experiments were normally performed using a 15 min control period, a 5 min test period followed by a 30 min washout period. Results were analysed by recording the vasomotion, measured as lymphangion constriction rate for 4 min at the end of the initial control period, 1 min after application of the agonist and 4 min at the end of washout period. The protocol was in part repeated when a pharmacological inhibitor was used but now in the presence of the inhibitor which was applied for a total of 20 min (15 min before and 5 min during application of ATP). There followed a 30 min washout period before ATP was again used. Except where noted, analysis has been based on comparisons of the constriction rate of individual lymphangions to that of the relevant control immediately preceding the response, both averaged over a 4 min period.

Chemicals

Chemicals used were acetylcholine (ACh), adenosine 5′-trisphosphate (ATP), N^G-nitro-L-arginine (L-NOARG), indomethacin and sodium nitroprusside (SNP) from Sigma-Aldrich, Sydney, Australia and suramin from Biomol Pty Ltd, Sydney, Australia, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and reactive blue 2 from Tocris Cookson Ltd; and SQ29548 ([1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino) carbonyl] hydrazino] methyl] - 7 - oxabicyclo [2.2.1]hept-2-yl]-5-heptanoic acid), antiflammin-1 (Met-Gln-Met-Lys-Val-Leu-Asp-Ser) and pertussis toxin from RBI (Australian Laboratory Services, Sydney, Australia). Stock solutions were ACh, ATP, PPADS, reactive blue 2, SNP (10^{-1} M), antiflammin-1 (10^{-6} M), pertussis toxin (0.1 μg μl^{-1}) all in distilled water, L-NOARG (10^{-1} M) in 0.1 M HCl, indomethacin (3×10^{-3} M) and SQ29548 (3×10^{-4} M) in ethanol, with all stock solutions stored at −20°C. The concentration of ethanol was always less than 0.1% which itself was found to have no significant effect on lymphangion constriction rate.

Statistical analysis

The data are expressed as the mean ± standard error of the mean (s.e. mean) with statistical comparisons made using a two tailed paired Student's *t*-test. Differences were considered significant (*) and highly significant (**) when the *P* values was less than 0.05 and 0.01 respectively with n indicating the number of lymphangions used.

Results

Effect of ATP on vasomotion

Perfused lymphatic vessels exhibited spontaneous constrictions (mean 14 ± 2 constrictions min^{-1}; $n = 228$ lymphangions). Application of different concentrations of ATP produced a concentration-dependent increase in lymphangion constriction rate with a maximum response of 200 ± 10% ($n = 9$, $P < 0.01$) at a concentration of 10^{-3} M (Figure 1). The endothelium was involved in this response, as removal of the endothelium altered the sensitivity to ATP with ATP concentrations higher than 10^{-6} M (e.g. 10^{-5} M) compared to 10^{-8} M, now required to give responses significantly greater than control (Figure 1). Therefore, subsequent studies investigating endothelial mechanisms were made using 10^{-6} M ATP, a concentration which did not produce any significant effects on endothelium-denuded tissues but significantly increased lymphangion constriction rate by 126 ± 6% ($n = 12$; $P < 0.05$). Repetitive application of 10^{-6} M ATP caused significant tachyphylaxis when applied at a short interval (10 min; 61 ± 4%; $P < 0.01$;

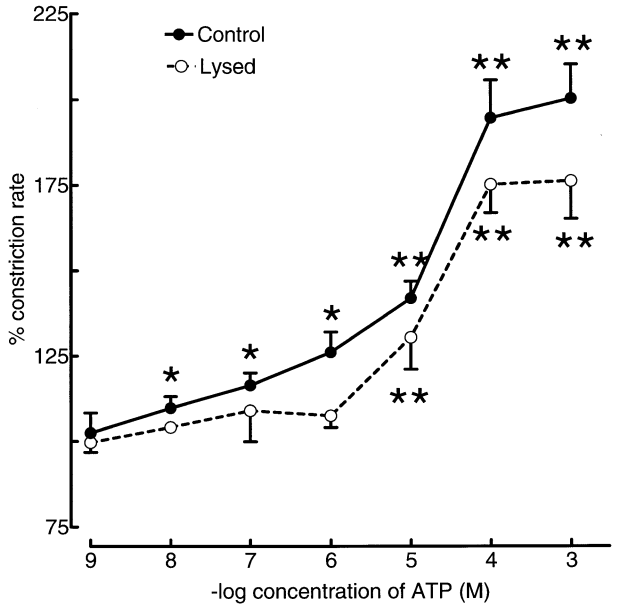

Figure 1 The effects of ATP at concentrations in the range 10^{-9}–10^{-3} M on lymphangion constriction rate in vessels with or without an endothelium. Data were normalized with respect to the corresponding control lymphangion constriction rate with $n=9-12$ lymphangions for all points. Vertical lines denote s.e.mean. $^{*}P<0.05$, $^{**}P<0.01$.

$n=6$) but had no significant effect at the longer intervals (20 min, $n=6$; 30 min; $n=6$) tested.

ATP receptor subtypes

Bath application of 10^{-4} M suramin, a non-specific P_2 purinoceptor antagonist (Dunn & Blakeley, 1988; Hoyle *et al.*, 1990) had no significant effects on perfusion-induced constriction rate but completely abolished the 10^{-6} M ATP-induced increase in this response (Figure 2a). This effect of suramin was reversible as after 30 min wash, the ATP-induced response returned to values not significantly different from the control response before application of suramin. PPADS (3×10^{-5} M), a P_2-receptor antagonist able to discriminate between some native and recombinant P_{2X}- and P_{2Y}-receptor subtypes (Humphrey *et al.*, 1995; see also Ziyal *et al.*, 1997), did not significantly affect either perfusion-induced constriction rate or this activity after modulation by ATP (Figure 2b). Reactive blue 2 (3×10^{-5} M), a P_{2Y} purinoceptor inhibitor (Manzini *et al.*, 1986; Burnstock & Warland, 1987), but which also can inhibit some P_{2X} receptors (Humphrey *et al.*, 1995), did not itself affect perfusion-induced constriction rate (Figure 2c). However, it was not possible to assess its effectiveness as an antagonist to ATP in this experiment as application of 10^{-6} M ATP in the presence of reactive blue 2 markedly inhibited the basal perfusion-induced constriction rate (Figure 2c). This was probably through a competing effect, at least in

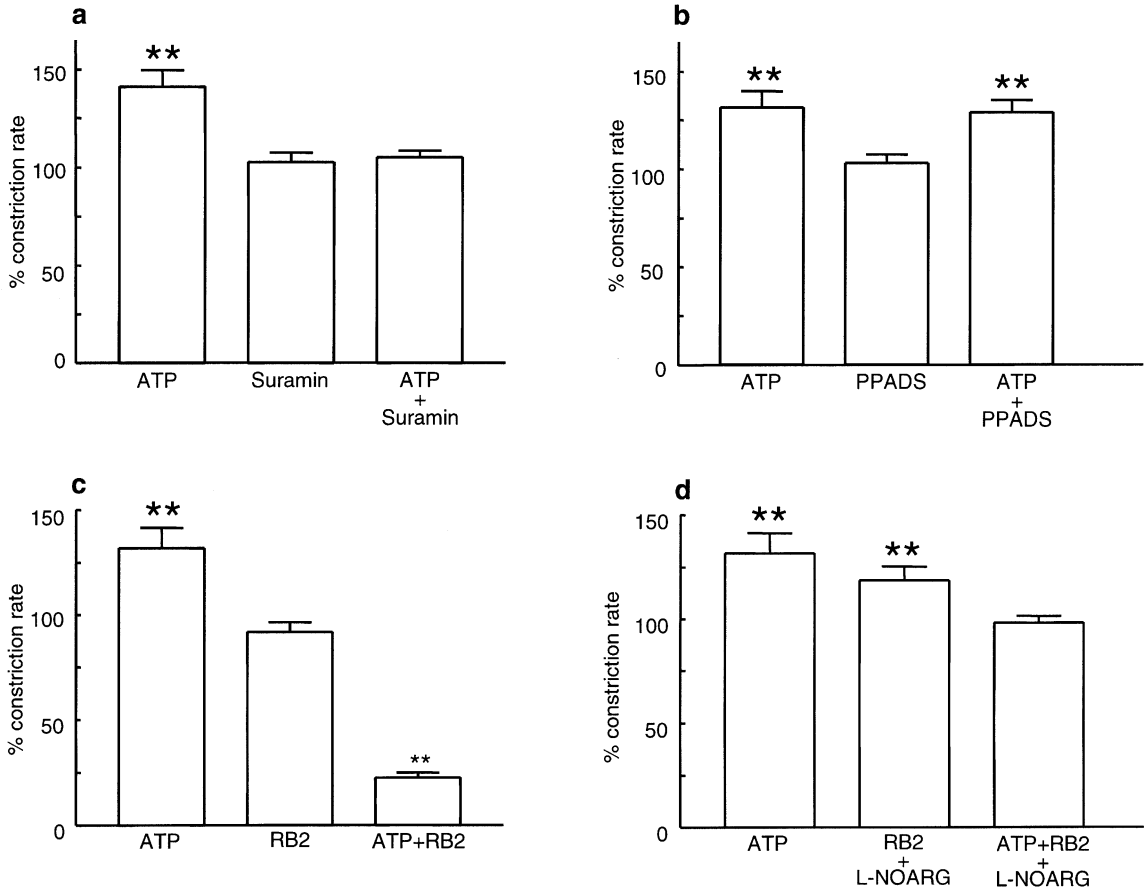

Figure 2 The effects of antagonists of P_2 purinoceptors on lymphangion constriction rate before and during application of 10^{-6} M ATP. The data presented are for suramin (10^{-4} M) (a); PPADS (3×10^{-5} M) (b), reactive blue 2 (RB2, 3×10^{-5} M) (c) and RB2 together with L-NOARG (10^{-4} M) an inhibitor of NO synthase (d). Data were normalized with respect to the corresponding lymphangion constriction rate just before application of either ATP or ATP plus inhibitor with each measurement based on data from $n=6-12$ lymphangions. Vertical lines denote s.e.mean. $^{**}P<0.01$.

part caused by an ATP-induced release of EDNO as perfusion-induced constrictions persisted at control levels upon addition of ATP in the presence of both reactive blue 2 and L-NOARG (10^{-4} M), an inhibitor of nitric oxide synthase (Figure 2d). Taken together, these findings indicate that the excitatory and inhibitory response were both mediated by P_2 purinoceptors but these receptor types were different as while both were blocked by suramin, only the excitatory response was blocked by reactive blue 2.

The data also indicate another phenomenon, namely that there was significant endogenous release of EDNO in control perfused vessels. This follows as there was a significant increase in constriction rate upon addition of L-NOARG to either the control solution (Figure 3) or to the control solution with reactive blue 2 (Figure 2d) before application of ATP. Importantly, addition of ATP in the presence of L-NOARG caused a significant increase in constriction rate ($P<0.01$; Figure 3), further indicating that the excitatory purinoceptor-induced response occurred independent of the inhibitory mechanism. Subsequent experiments were made in the presence of 10^{-4} M L-NOARG.

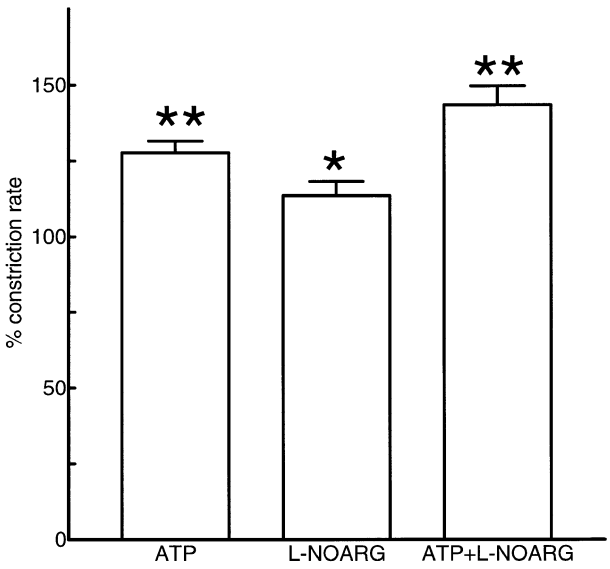

Figure 3 The effects of L-NOARG an inhibitor of NO synthase on lymphangion constriction rate before and during application of 10^{-6} M ATP. Data were normalized with respect to the corresponding lymphangion constriction rate just before application of either ATP or ATP plus L-NOARG with each measurement based on data from $n=9$ lymphangions. Vertical lines denote s.e.mean. $^{*}P<0.05$, $^{**}P<0.01$.

Basis for P_2 purinoceptor-mediated excitation

An endothelium-derived excitatory factor which has been reported to increase pacemaking in these lymphatics is

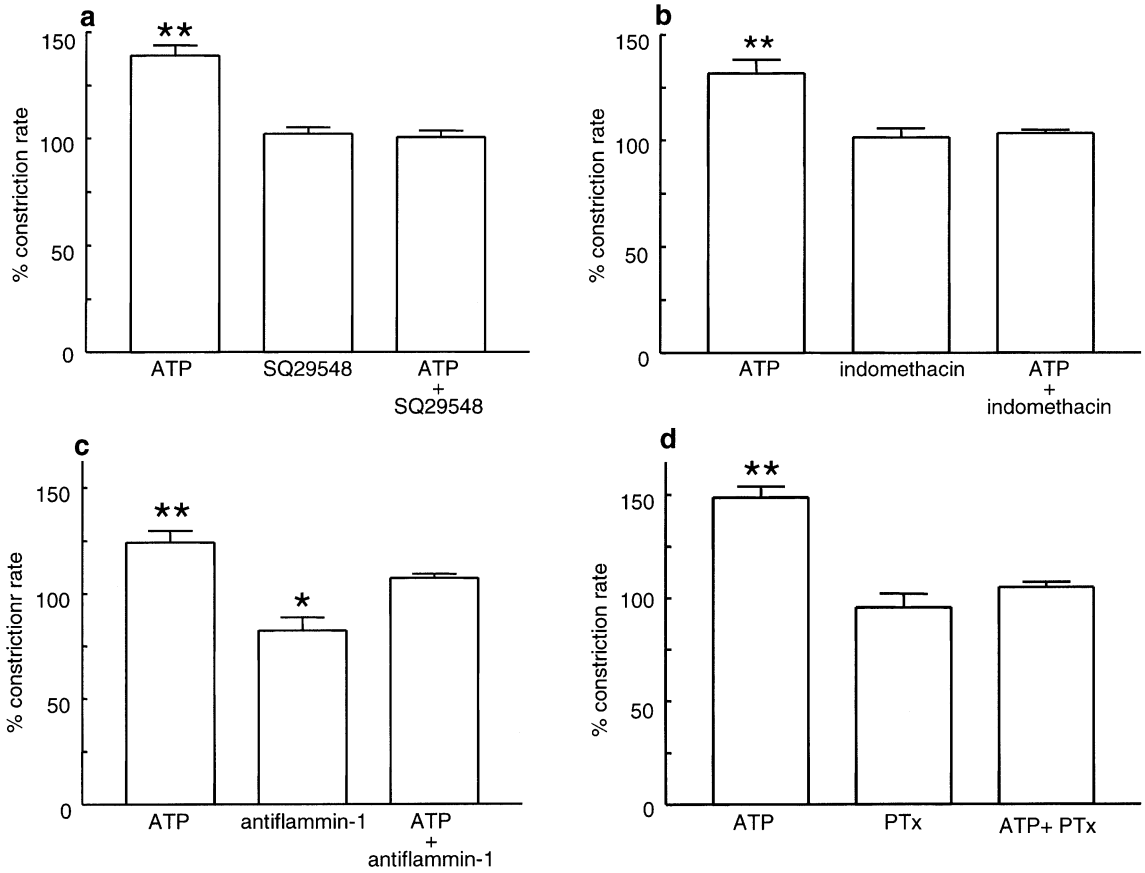

Figure 4 The effects of various inhibitors on lymphangion constriction rate before and during application of 10^{-6} M ATP. The data presented are for SQ29548 (3×10^{-7} M) an inhibitor thromboxane A_2 receptors (a), indomethacin (3×10^{-6} M) an inhibitor of cyclo-oxygenase (b), antiflammin-1 (10^{-9} M) an inhibitor of phospholipase A_2 (c) and pertussis toxin (PTx, 100 ng ml^{-1}) an inhibitor of specific G proteins (d). Data were normalized with respect to the corresponding lymphangion constriction rate just before application of either ATP or ATP plus inhibitor with each measurement based on data from $n=6-12$ lymphangions. All experiments were performed in the presence of 10^{-4} M L-NOARG. Vertical lines denote s.e.mean. $^{*}P<0.05$, $^{**}P<0.01$.

thromboxane A_2 which results through substance P acting to increase phospholipase A_2 and the production of arachidonic acid (Rayner & Van Helden, 1997). Therefore, we undertook experiments to determine if ATP activated this pathway.

The first experiments undertaken were to determine whether thromboxane A_2, a metabolite of arachidonic acid, was involved in the excitatory response. This was examined using SQ29548 a thromboxane A_2 receptor antagonist (see Monshizadegan *et al*., 1992; Hunt *et al*., 1992). SQ29548 at 3×10^{-7} M completely inhibited the response to 10^{-6} M ATP (Figure 4a), this implicating thromboxane A_2 as the endothelium-derived excitatory factor.

Thromboxane A_2 is a metabolite of arachidonic acid formed by the cyclo-oxygenase pathway. Therefore, blockade of this pathway should also prevent the generation of thromboxane A_2 and resultant increase in vasomotion. This premise was tested using the cyclo-oxygenase inhibitor indomethacin which applied at 3×10^{-6} M abolished the ATP-induced enhancement in constriction rate (Figure 4b).

The possibility that receptor stimulation acted by increasing phospholipase A_2, the activation of which might generate arachidonic acid and hence metabolites such as thromboxane A_2 (see Hershey *et al*., 1991) was examined. This was done by application of the phospholipase A_2 inhibitor antiflammin-1 (Lloret & Moreno, 1992) applied at 10^{-9} M, a concentration which does not have non-specific actions such as blocking thromboxane A_2 receptors (Rayner & Van Helden, 1997). Antiflammin-1 abolished the response to 10^{-6} M ATP (Figure 4c) indicating that phospholipase A_2 is activated by P_2 receptor stimulation.

Coupling between receptors and enzyme activation is likely to be mediated by a G protein, some of which are inhibited by pertussis toxin. Therefore, the response to ATP was compared before and after 4 h exposure to pertussis toxin at 100 ng ml^{-1}. Pertussis toxin abolished the ATP-induced enhancement of constriction rate (Figure 4d), an action which was not due to an action on G proteins mediating the thromboxane A_2 receptor-induced activation of the smooth muscle as these are insensitive to pertussis toxin (Rayner & Van Helden, 1997). Taken together, these findings indicate that ATP-induced endothelium-dependent excitation results through ATP acting on endothelial P_2 purinoceptors to increase synthesis of phospholipase A_2 by a pertussis toxin-sensitive G protein and the production of thromboxane A_2.

Discussion

It is known that ATP increases lymphatic vasomotion, a finding reported for sheep mesenteric lymphatics where data was presented indicating that ATP is a functional co-transmitter in sympathetic nerves (Hollywood & McHale, 1994). However, the mechanisms underlying this response have not been determined. Our findings indicate that ATP is also excitatory when applied to guinea-pig mesenteric lymphatics. ATP activates at least three lymphatic mechanisms, an excitatory and a competing inhibitory mechanism which are both endothelium-dependent and a third excitatory mechanism which is endothelium-independent. The present report has considered the endothelium-dependent mechanisms.

ATP was found to act primarily and possibly exclusively through the endothelium at concentrations below 10^{-5} M. Our results indicate that ATP acts on the endothelium to produce competing excitatory and inhibitory actions, the excitatory response, namely the enhancement in lymphatic vasomotion, being dominant. The nature of the receptors and intracellular mechanisms underlying the two responses were different. The excitatory response was most likely mediated by P_{2Y} receptors as the pharmacology confirmed a role for P_2 purinoceptors with P_{2Y} receptors known to be G-protein coupled (Introduction). Reactive blue 2, blocked the excitatory response but not the inhibitory response. Under these conditions, the inhibitory response became completely dominant, ATP now causing marked inhibition of all perfusion-induced contractile activity. Classification of the receptors underlying the ATP-induced inhibition was not made but should comply with the findings that the receptors were blocked by suramin but not by PPADS or reactive blue 2. Importantly, ATP-induced stimulation of these inhibitory receptors induced release of EDNO, the inhibition being abolished by an inhibitor of NO synthase.

Consequent experiments performed in the presence of a NO synthase inhibitor indicated that P_{2Y} purinoceptors mediated the ATP-induced excitatory response. Pharmacological analysis of the intracellular mechanisms activated by the P_{2Y} receptors indicated that stimulation of these receptors caused excitation by producing thromboxane A_2 from the endothelium. This endothelium-derived excitatory factor was likely to arise through activation of phospholipase A_2 by a pertussis toxin-sensitive G-protein, resultant production of arachidonic acid and breakdown to the metabolite thromboxane A_2 through the cyclo-oxygenase pathway. This mechanism is analogous to that activated by substance P receptors in this same tissue. The primary difference is that substance P appears to invoke only this mechanism (Rayner & Van Helden, 1997) whereas ATP also activates an endothelium-mediated inhibitory response and acts directly on the smooth muscle at higher ATP concentrations.

The financial support of the National Health and Medical Research Council of Australia is gratefully acknowledged.

References

ABBRACCHIO, M.P. & BURNSTOCK, G. (1994). Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacol. Ther.*, **64,** 445–475.

AUCKLAND, K. & REED, R.K. (1993). Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol. Rev.*, **73,** 1–78.

BARNARD, A.E., BURNSTOCK, G. & WEBB, E.T. (1994). G protein-coupled receptors for ATP and other nucleotide: a new receptor family. *Trends Pharmacol. Sci.*, **15,** 67–70.

BENHAM, C.D. & TSIEN, R.W. (1987). A novel receptor-operated, Ca^{2+}-permeable channels activated by ATP in smooth muscle. *Nature,* **328,** 275–278.

BERESFORD-SMITH, B., NESBITT, K.V. & VAN HELDEN, D.F. (1993). Edge detection at multiple locations using a 'radar' tracking algorithm as exemplified in isolated guinea-pig lymphatic vessels. *J. Neurosci. Methods,* **49,** 69–79.

BURNSTOCK, G. & WARLAND, J.J.I. (1987). P_2-purinoceptors of two subtypes in the rabbit mesenteric artery: reactive blue 2 selectively inhibits responses mediated via the P_{2Y}- but not the P_{2X}-receptor. *Br. J. Pharmacol.*, **90,** 383–391.

BURNSTOCK, G. (1990). Overview. Purinergic mechanisms. *Anal. New York Acad. Sci.*, **603,** 1–17.

BURNSTOCK, G. (1995). Noradrenaline and ATP: co-transmitters and neuromodulators. *J. Physiol. & Pharmacol.*, **46,** 365–384.

DE MEY, J.G. & VANHOUTTE, P.M. (1981). Role of the intima in the cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol.,* **316,** 347–355.

DOMINICZAK, A.F., QUILLEY, J. & BOHR, D.F. (1991). Contraction and relaxation of rat aorta in response to ATP. *Am. J. Physiol.,* **261,** H243–H251.

DUNN, P.M. & BLAKELEY, A.G.H. (1988). Suramin: a reversible P2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol.,* **93,** 243–245.

FLOREY, H. (1927). Observations on the contractility of lacteals:II. *J. Physiol.,* **63,** 1–18.

FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.,* **46,** 143–156.

GORDON, J.L. (1990). The effects of ATP on endothelium. *Anal. New York Acad. Sci.,* **603,** 46–51.

HARDEN, T.K., BOYER, J.L. & NICHOLAS, R.A. (1995). P2-purinergic receptors: subtype-associated signalling responses and structure. *Ann. Rev. Pharmacol. Toxicol.,* **35,** 541–579.

HERSHEY, A.D., POLENZANI, L., WOODWARD, R.M., MILEDI, R. & KRAUSE, J.E. (1991). Molecular and genetic characterization, functional expression, and mRNA expression patterns of a rat substance P receptor. *Anal. New York Acad. Sci.,* **623,** 3–77.

HOLLYWOOD, M.A. & MCHALE, N.G. (1994). Mediation of excitatory neurotransmission by the release of ATP and noradrenaline in sheep mesenteric lymphatic vessels. *J. Physiol.,* **481,** 415–423.

HOYLE, C.H.V., KNIGHT, G.E. & BURNSTOCK, G. (1990). Suramin antagonizes responses to P2-purinoreceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli. *Br. J. Pharmacol.,* **99,** 617–621.

HUMPHREY, P.P.A., BUELL, G., KENNEDY, I., KHAKH, B.S., MICHEL, A.D., SURPRENANT, A. & TREZISE, D.J. (1995). New insights on P_{2X} receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **352,** 585–596.

HUNT, J.A., MERRITT, J.E., MACDERMOT, J. & KEEN, M. (1992). Characterization of the thromboxane receptor mediating prostacyclin release from cultured endothelial cells. *Biochem. Pharmacol.,* **43,** 1747–1752.

JOHNSON, P.C. (1980). The myogenic response. In *Handbook of Physiology: The Cardiovascular System*. eds. Geiger, S.R., Bohr, D.F., Somlyo, A.P. & Sparks, H.V., Vol. II. pp. 400–442. Bethesda, Maryland: American Physiological Society.

LAGAUD, G.J., STOCLET, J.C. & ANDRIANTSITOHAINA, R. (1996). Calcium handling and purinoceptor subtypes involved in ATP-induced contraction in rat small mesenteric arteries. *J. Physiol.,* **492,** 689–703.

LLORET, S. & MORENO, J.J. (1992). In vitro and in vivo effects of the anti-inflammatory peptides, antiflammins. *Biochem. Pharmacol.,* **44,** 1437–1441.

MANZINI, S., HOYLE, C.H.V. & BURNSTOCK, G. (1986). An electrophysiological analysis of the effect of reactive blue 2, a putative P2-purinoceptor antagonist, on inhibitory junction potentials of rat caecum. *Eur. J. Pharmacol.,* **127,** 197–204.

MARTIN, W., CUSACK, N.J., CARLETON, J.S. & GORDON, J.L. (1985). Specificity of P2-purinoceptor that mediates endothelium-dependent relaxation of the pig aorta. *Eur. J. Pharmacol.,* **108,** 295–299.

MCHALE, N.G. & RODDIE, I.C. (1983). The effect of intravenous adrenaline and noradrenaline infusion of peripheral lymph flow in the sheep. *J. Physiol.,* **341,** 517–526.

MCLAREN, G.J., BURKE, K.S., BUCHANAN, K.J., SNEDDON, P. & KENNEDY, C. (1998). Evidence that ATP acts at two sites to evoke contraction in the rat isolated tail artery. *Br. J. Pharmacol.,* **124,** 5–12.

MISLIN, H. (1983). The lymphangion. In *Lymphangiology*. ed. Foldi, M. & Casley-Smith, J.R. pp. 165–175. Stuttgart, New York: F.K. Schattauer Verlag.

MONSHIZADEGAN, H., HEDBERG, A. & WEBB, M.L. (1992). Characterization of binding of a specific antagonist, [^{3}H]-SQ29548, to a soluble thromboxane A_2/prostaglandin H_2 (TP) receptor in human platelet membranes. *Life Sci.,* **51,** 431–437.

PALMER, R.M. FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature,* **327,** 524–526.

PEARSON, J.D. & CARTER, T.D. (1990). Effects of extracellular ATP on the release of vasoactive mediators from endothelium. *Anal. New York Acad. Sci.,* **603,** 267–273.

RAYNER, S.E. & VAN HELDEN, D.F. (1997). Evidence that the substance P-induced enhancement of pacemaking in lymphatics of the guinea-pig mesentery occurs through endothelial release of thromboxane A_2. *Br. J. Pharmacol.,* **121,** 1589–1596.

SHIRAHASE, H., USUI, H., SHIMAJI, H., KURAHASHI, K. & FUJIWARA, M. (1990). Endothelium-dependent contraction induced by platelet-derived substances in canine basilar arteries. *J. Pharmacol. Exp. Ther.,* **255,** 182–186.

SHIRAHASE, H., USUI, H., SHIMAJI, H., KURAHASHI, K. & FUJIWARA, M. (1991). Endothelium-independent and endothelium-dependent contractions mediated by P2X- and P2Y-purinoceptors in canine basilar arteries. *J. Pharmacol. Exp. Ther.,* **256,** 683–688.

SMITH, R.O. (1949). Lymphatic contractility: A possible mechanism of lymphatic vessels for the transport of lymph. *J. Exp. Med.,* **90,** 497–509.

SURPRENANT, A., RASSENDREN, F., KAWASHIMA, E., NORTH, R.A. & BUELL, G. (1996). The cytolytic P_{2Z} receptor for extracellular ATP identified as a P_{2X} receptor ($P2X_7$). *Science,* **272,** 735–738.

VAN HELDEN, D.F. (1993). Pacemaker potentials in lymphatic smooth muscle of the guinea-pig mesentery. *J. Physiol.,* **471,** 465–479.

VAN HELDEN, D.F., VON DER WEID, P.-Y. & CROWE, M.J. (1996). Intracellular Ca^{2+} release: a basis for electrical pacemaking in lymphatic smooth muscle. In *Smooth Muscle Excitation*. eds. Bolton, T.B. & Tomita, T., London.

VON DER WEID, P.-Y., CROWE, M.J. & VAN HELDEN, D.F. (1996). Endothelium-dependent modulation of pacemaking in lymphatic vessels of the guinea-pig mesentery. *J. Physiol.,* **493,** 563–575.

YOKOYAMA, S. & OHHASHI, T. (1993). Effects of acetylcholine on spontaneous contractions in isolated bovine mesenteric lymphatics. *Am. J. Physiol.,* **264,** H1460–H1464.

ZIYAL, R., ZIGANSHIN, A.U., NICKEL, P., ARDANUY, U., MUTSCHLER, E., LAMBRECHT, G. & BURNSTOCK, G. (1997). Vasoconstrictor responses via P2X-receptors are selectively antagonized by NF023 in rabbit isolated aorta and saphenous artery. *Br. J. Pharmacol.,* **120,** 954–960.

(Received February 23, 1999
Revised April 30, 1999
Accepted May 7, 1999)