British Journal of Pharmacology (2000) 131, 1561–1568

www.nature.com/bjp

Properties of P2X and P2Y receptors are dependent on artery diameter in the rat mesenteric bed

[1]D.P. Gitterman & *,[1]R.J. Evans

[1]Department of Cell Physiology & Pharmacology, University of Leicester, Medical Sciences Building, University Road, Leicester, LE1 9HN

1 P2 receptor mediated contractile responses have been characterized in different diameter arteries from the rat mesenteric arterial vasculature (first, second to third and fifth to sixth order for large, medium and small arteries) using wire myograph and diamtrak video imaging.

2 α,β-methylene ATP (α,β-meATP) evoked transient concentration-dependent contractions in mesenteric arteries with EC_{50} values of 0.4, 2.5 and 107 μM for small, medium and large arteries respectively.

3 Suramin (10–100 μM) produced substantial parallel rightward shifts of the concentration-response curve to α,β-meATP in small and medium-sized arteries with pA_2 of 5.1. Responses in large vessels were unaffected by suramin.

4 Immunohistochemical analysis of arterial sections revealed no substantial differences in expression patterns of P2X receptors between different sizes of artery. $P2X_1$ receptors were expressed at high levels, $P2X_4$ and $P2X_5$ receptors were also detected on smooth muscle. The P2X receptor response is dominated by $P2X_1$ receptor in small and medium arteries but the nature of the receptor mediating the suramin insensitive α,β-meATP mediated response in large arteries is unclear.

5 The P2Y receptor agonist UTP was significantly more potent in small than in medium or large arteries (EC_{50} values: 15.0 μM small, 88.5 μM diamtrak medium 1.6 mM myography medium and 1.4 mM large). Responses in both small and medium-sized vessels were reduced by suramin (30–100 μM). The sensitivity to UTP and suramin indicates the presence of $P2Y_2$ receptors.

6 This study shows that P2 receptors do not have a homogenous phenotype throughout the mesenteric vascular bed and that the properties depend on artery size.
British Journal of Pharmacology (2000) **131,** 1561–1568

Keywords: P2X receptors; P2Y receptors; artery; suramin; immunohistochemistry

Abbreviations: iso-PPADS, iso-pyridoxalphosphate-6-azophenyl-2′-5′-disulphonate; α,β-meATP, α,β-methylene ATP

Introduction

P2 receptors are activated by extracellular nucleotides and play an important role in the control of vascular tone and blood pressure (Boarder & Hourani, 1998). They can be divided into two classes, ionotropic P2X receptors and metabotropic P2Y receptors (Abbracchio & Burnstock, 1994; Ralevic & Burnstock, 1998). P2X receptors for ATP are ligand-gated cation channels and are found on many different cell types, including vascular smooth muscle, neurones and blood cells (Buell *et al*., 1996; Collo *et al*., 1996; Surprenant *et al*., 1996; Vulchanova *et al*., 1996). ATP is co-stored and co-released with noradrenaline from sympathetic nerves and in arteries P2X receptors mediate membrane depolarization and a component of constriction associated with sympathetic nerve stimulation (Burnstock, 1997). Following P2X receptor activation calcium can enter the cell either directly through the calcium permeant P2X receptor channel (Benham & Tsien, 1987; Valera *et al*., 1994) or through voltage-gated calcium channels opened in response to P2X receptor mediated membrane depolarization (Bulloch & McGrath, 1988).

Genes encoding seven different P2X receptors ($P2X_{1-7}$) have so far been identified (Burnstock, 1997). They can form either homomeric or heteromeric channels with at least three subunits giving rise to a wide range of receptor phenotypes (Nicke *et al*., 1998; North & Surprenant, 2000). In vascular smooth muscle the α,β-methylene ATP (α,β-meATP) and L-β,γ-methylene ATP sensitive rapidly desensitizing P2X receptor phenotype corresponds most closely to that of recombinant $P2X_1$ receptors (Valera *et al*., 1995; Evans & Surprenant, 1996). This is consistent with studies showing that the $P2X_1$ receptor is the predominant P2X receptor isoform expressed in smooth muscle (Collo *et al*., 1996) and that P2X receptor mediated responses are absent in vas deferens smooth muscle from $P2X_1$-receptor-deficient knock-out mice (Mulryan *et al*., 2000). However there is some evidence to suggest that there may be a heterogenous distribution of P2X receptor isoforms and P2X receptor properties throughout the arterial vasculature (Nori *et al*., 1998; Lewis & Evans, 2000a).

Metabotropic G-protein coupled P2Y receptors can also contribute to the control of vascular tone. At least four different isoforms of the P2Y receptor have been cloned in the rat corresponding to $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors (Burnstock, 1997). $P2Y_1$ receptors are activated by ADP and ATP, and in the vasculature they are found on endothelial cells where they mediate vasodilation (Webb *et al*., 1993; Burnstock, 1997). In contrast $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors are expressed on smooth muscle, are activated by uridine nucleotides and mediate vasoconstriction (Urquilla, 1978; von Kugelgen & Starke, 1990; Hartley & Kozlowski, 1997; Erlinge *et al*., 1998; Hartley *et al*., 1998).

*Author for correspondence; E-mail RJE6@le.ac.uk

Small arteries and arterioles are known to account for over 45% of peripheral resistance to blood flow through the vasculature and therefore substantially contribute to systemic blood pressure. It seems possible that as large conduit arteries and small resistance arteries perform different functions they may also have different P2 receptor mediated properties. Indeed, variations in distribution or properties depending on arterial size have already been demonstrated for inward rectifier potassium channels (Quayle *et al*., 1996), L-type calcium channels (Bowles *et al*., 1997) and calcium-activated chloride channels (Clapp *et al*., 1996). No systematic comparison of P2 receptor-mediated contractile responses in different sizes of artery has yet been carried out. In the meseneteric circulation P2X and P2Y receptors can mediate artery constriction (Sjoblom-Widfeldt, 1990; Ralevic *et al*., 1995; Lagaud *et al*., 1996; Lewis *et al*., 1998) and vessels can be studied from the main conduit superior mesenteric artery down to small resistance arteries allowing direct comparisons of responses between different sized vessels. The aim of this study was thus to characterize the properties of P2 receptors in different calibre arteries using the rat mesenteric arcade as a model vascular bed.

Methods

Adult male Wistar rats (200–300 g) were killed by cervical dislocation or CO_2 asphyxiation followed by femoral artery exsanguination. A portion of the gut with attached mesenteric arcade was removed and mesenteric arteries of different diameters were dissected; large vessels from the superior mesenteric artery, medium-sized vessels were from second or third order branches and small vessels correspond to fifth or sixth order arteries.

Contraction studies

Large and medium artery rings (537 ± 23 μm, range 386–806 μm and 215 ± 7 μm, range 131–342 μm internal diameter, $n = 30$ and 40 arteries respectively) were mounted in a Mulvany myograph using standard procedures (Lagaud *et al*., 1996); changes in arterial tone were recorded and analysed using a MacLab data acquisition system. The lower limit of vessel that can be mounted in the myograph is dependent on the ability to insert two 40 μm tungsten wires through the lumen therefore the theoretical limit is a vessel with an internal diameter of at least 80 μm. In addition it is technically very difficult to work on small arteries in the myograph without damaging them. As a result we have studied small resistance arteries using the video imaging diamtrak method. In order to control for differences in methodology between myography and diamtrak we have collected data for medium arteries with both systems. We are therefore able to make direct comparisons between small/medium and medium/large vessels. Small arteries were dissected carefully cleaning away all connective tissue and pinned out (stretched to approximately 150% of their resting length) in a Sylgard coated organ bath (volume 2 ml). The organ bath was placed on the stage of an inverted microscope and changes in external arterial diameter were analysed using Diamtrak software as previously described (Neild, 1989). This method uses a video camera to produce a digitized image of the blood vessel, this is then analysed on line by a computer programme (Diamtrak) that measures the outside diameter (dark edge) of the artery. The outside diameter of the medium arteries was 293 ± 5 μm (range 278–321, wall thickness accounts for $\sim$40% giving an internal diameter of $\sim$180 μm), and for small arteries was 102 ± 3 μm range (69–135 μm, $n = 40$ arteries) (wall thickness accounts for $\sim$40% giving a mean internal diameter of $\sim$60 μm).

Tissues were superfused with a physiological saline solution (composition in mM): NaCl 150, KCl 2.5, HEPES 10, $CaCl_2$ 2.5, $MgCl_2$ 1, pH to 7.3 with NaOH. Experiments were conducted at 32–34°C for diamtrak and 34–36°C for myography. Drugs were made as concentrated stock solutions in MilliQ distilled water and added to the superfusate at the required final concentration, in general only one agonist was tested per artery. Reproducible responses to agonists were obtained when applications were separated by 30 min intervals. In experiments testing the effect of antagonists, suramin was pre-superfused for 30 min before being added in combination with the agonist. The procedure for the UTP cross-desensitization experiment was as follows: an application of UTP was made; this was followed by an application of α,β-meATP allowing the response to fully return to baseline tone; in the continued presence of α,β-meATP a second application of UTP was then made. The two contractile responses for UTP were then compared.

Data analysis

Data are expressed as mean $\pm$ s.e.mean throughout and n = number of animals, unless otherwise stated. Concentration-response data were fitted by the least squares method using Microcal Origin software with the following equation:

$$\text{response} = \alpha[A]^{n_H}/([A]^{n_H} + [A_{50}]^{n_H}) \quad (1)$$

where α is the asymptote, n_H is the Hill coefficient, [A] the agonist concentration and A_{50} the agonist concentration producing a half maximal response (EC_{50}), $pA_{50} = -\log EC_{50}$. Differences between means were tested using either a 2 sample or paired, two tailed t-test, as appropriate. A P value of <0.05 was considered statistically significant. pA_2 values for suramin were estimated using Schild analysis for competitive receptor antagonists. A full Schild regression was made for medium-sized arteries, while in small vessels two data points were used to estimate a pA_2.

Immunohistochemical studies

Mesenteric arteries were dissected as above and immunohistochemical analysis of P2X receptor expression was performed as described previously (Lewis & Evans, 2000). Briefly, embedded tissues were cut into 12 μm transverse sections and mounted on pre-subbed slides. Sections were fixed in paraformaldehyde, permeabilized with a 0.5% Triton-X (Sigma) solution and incubated with primary and secondary antisera. Anti-$P2X_1$, $P2X_2$, $P2X_4$ and $P2X_7$ antibodies (Alomone, Israel) were all used at a dilution of 1 : 200. Anti-$P2X_5$ and $P2X_6$ antibodies (gift from Roche Bioscience) were used at 1 : 1000 and anti-$P2X_3$ was used at 1 : 5000 (gift from Dr L. Vulchanova, University of Minnesota, U.S.A.). The secondary antibody was in each case fluorescein isothiocyanate (FITC) conjugated anti-rabbit IgG raised in donkey (Jackson Immunoresearch) used at a 1 : 100 dilution. All dilutions were made using 10% donkey serum (Jackson Immunoresearch) in phosphate buffered saline (PBS). When blocking peptides were used, the antibody was pre-incubated with its corresponding antigen peptide for 1 h at room temperature. To test for non-specific antibody

binding, control slides were incubated with secondary antisera only and non-immune donkey serum only. Tissue sections mounted in Citifluor (UKC Chem Lab, U.K.) were examined under epifluorescence and images were captured using Scionimage software. Immunohistochemical studies were conducted on at least three arteries from different animals. The level of immunoreactivity for a given P2X receptor subunit seen between animals was reproducible. The level of immunoreactivity was estimated by eye and assigned to the following categories; + + + = strong expression, + + = moderate expression, + = weak expression, ± = barely detectable expression, − = no expression.

Drugs

α,β methylene ATP, suramin, phenylephrine, UTP (Sigma, U.K.). iso-pyridoxalphosphate-6-azophenyl-2′-5′-disulphonate (iso-PPADS) (Tocris Cookson, U.K.).

Results

Sensitivity to P2X$_1$ receptor agonist, α,β-meATP

The metabolically stable ATP analogue α,β-meATP evoked concentration-dependent constrictions of rat mesenteric arteries. At higher concentrations, responses rapidly reached a peak and declined toward baseline in the continued presence of the agonist (Figure 1a–c). There was a marked difference in sensitivity to α,β-meATP based on the diameter of the vessel (Figure 1). The mean EC_{50} values for small, medium and large arteries were ~0.4, 2.5 and 107 μM (coresponding pA_{50} values were 6.4±0.1 small, 5.7±0.1 medium diamtrak 5.6±0.1 medium myography, and large 4.0±0.1; n=4-5, and Hill slopes were 1.5±0.2, 1.2±0.1 diamtrak and 1.2±0.2 myography, and 0.9±0.1, respectively). These correspond to significant differences in sensitivity to α,β-meATP between small and medium ($P<0.005$) and medium and large ($P<0.005$) vessels. The sensitivity to α,β-meATP was the same for medium arteries whether determined using diamtrak or myography techniques.

The differences in potency of α,β-meATP may have been caused by anatomical differences between large and smaller arteries, affecting access of the agonist to the receptor. To test for such a putative diffusional barrier, contractile responses to the α_1-adrenoceptor agonist phenylephrine were studied. There was no significant difference between mean EC_{50} values for phenylephrine in all three vessel sizes (Figure 2a); 1.6, 3.0 and 2.9 μM (pA_{50} = 5.9±0.1, 5.6±0.2 and 5.6±0.2, n=3–4) for small, medium and large respectively. The Hill slopes for small, medium and large were 1.6±0.4, 4.2±1.5 and 1.8±0.6, respectively. In addition 'agonist-independent' constrictions were evoked by superfusion with a solution containing a high concentration of potassium ions (KCl). There was no difference in response to KCl between medium (diamtrak or myography) and large arteries. The concentration of KCl required to evoke ~30% maximum response was however significantly lower for small arteries ($P<0.05$) (Figure 2b).

It is possible that at the very high concentrations of α,β-meATP used in large arteries, the constrictions may have been caused by small amounts of contaminating ATP activating a P2Y receptor. The effect of ATP was therefore tested at a concentration equivalent to a putative 5% contamination of the α,β-meATP, i.e. a 1 mM solution of α,β-meATP would thus contain 50 μM contaminating ATP. This concentration of ATP however produced a contraction that was only 1.8±1.9% of the response to 1 mM α,β-meATP (n=3), demonstrating that the observed responses were evoked by α,β-meATP and not due to ATP contamination.

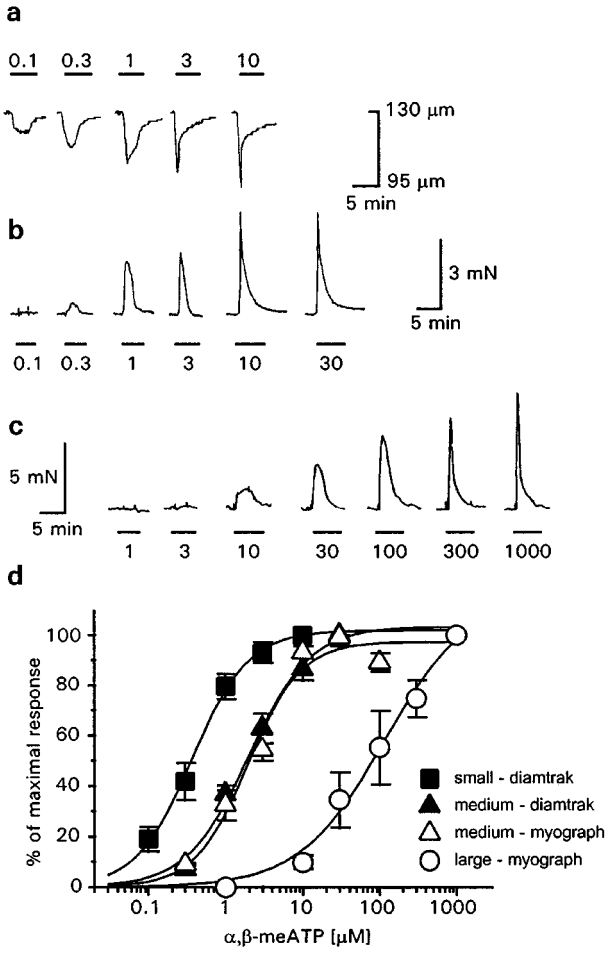

Figure 1 Characterization of contractile responses to α,β-methylene ATP. (a), (b) and (c) show contractions in small, medium and large arteries respectively; periods of application are indicated by the bar. The transient nature of contractile responses can be clearly seen in all three vessel sizes. (d) Concentration-response curves showing different α,β-meATP sensitivity depending on vessel size. Data are mean responses±s.e.mean (n=4–5) and are expressed as per cent of the maximal response to α,β-meATP in each tissue.

Effect of removal of extracellular calcium on constrictions evoked by α,βmeATP

To test whether the requirement for calcium influx from the extracellular space for smooth muscle contraction was the same in all sizes of artery, calcium was removed from the extracellular solution. Under these conditions contractile responses to 10 μM α,β-meATP were abolished in small and medium-sized arteries and constrictions to 300 μM α,β-meATP were abolished in large arteries (n=3–4).

Sensitivity of α,β-meATP mediated responses to the P2 receptor antagonists

Due to the lack of subtype-selective agonists for different P2X receptors, one way of distinguishing between isoforms is to determine antagonist sensitivity. In the rat, suramin is a potent antagonist at the $P2X_{1,2,3}$ and $P2X_5$ receptor isoforms

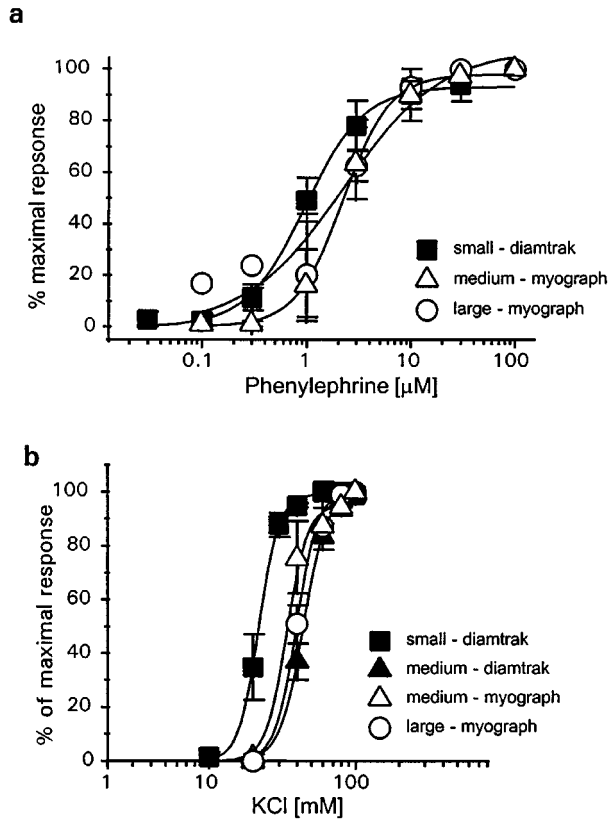

Figure 2 Small, medium and large arteries all display similar sensitivity to phenylephrine, and KCl is more potent on small arteries. The three concentration response curves to phenylephrine show that the EC_{50} values are all approximately 2 μM (a). Contractile responses to a solution containing a high concentration of KCl, small arteries were more sensitive (b). Data are mean responses$\pm$s.e.mean ($n=3-4$) and are expressed as per cent of the maximal response in each tissue.

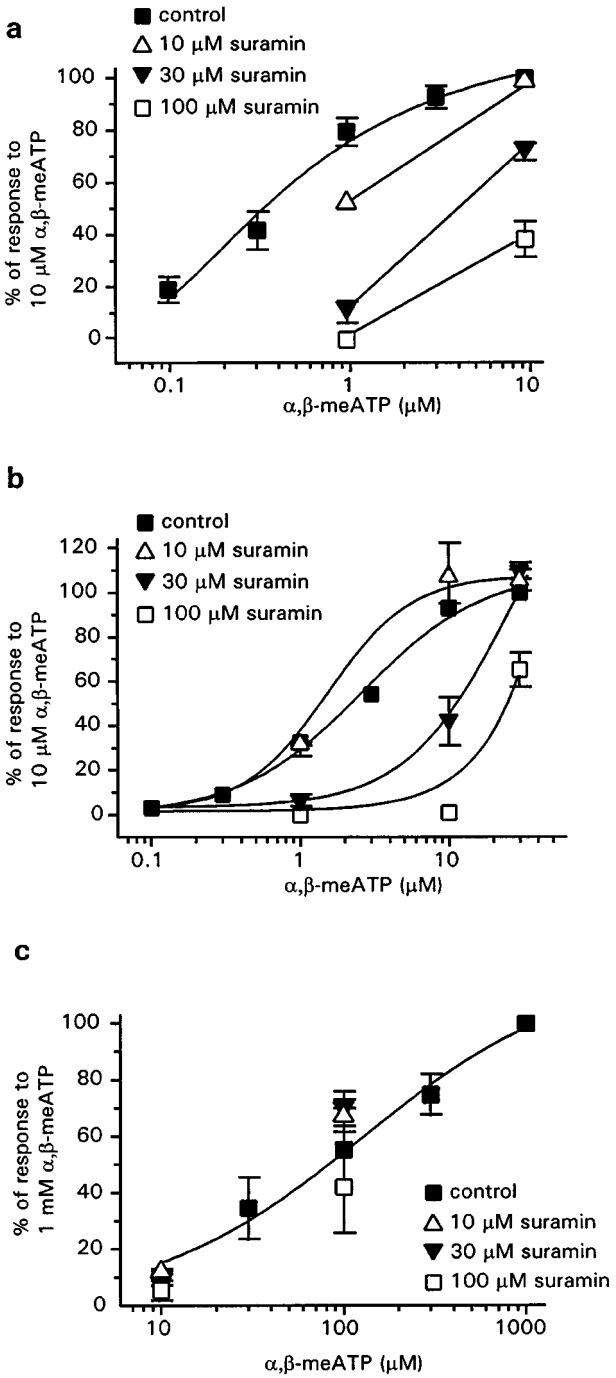

Figure 3 Suramin sensitivity of contractile responses to α,β-meATP in different-sized mesenteric arteries. Concentration response curves for responses to α,β-meATP alone and in the presence of suramin. A clear right-ward shift of the curve can be seen in small (a) and medium-sized (b) arteries but not in large (c). Data are mean responses$\pm$s.e.mean ($n=3-6$) and are expressed as per cent of the response to 10 μM α,β-meATP in small and medium arteries and per cent of the response to 1 mM α,β-meATP in large arteries.

and a weak antagonist at $P2X_4$ and $P2X_6$ receptors. Suramin had no effect on the resting tone of the arteries but produced substantial rightward shifts of the concentration response curve for α,β-meATP in the small and medium arteries (Figure 3a,b). These data were used to estimate a pA_2 value using Schild analysis. The values obtained for small and medium-sized arteries were 5.14 and 5.08, respectively. In contrast, suramin had no effect on responses to α,β-meATP in large arteries (Figure 3c), suggesting that the agonist is not acting at suramin sensitive P2X receptors in this tissue. Iso-PPADS is an effective antagonist at P2X receptors in medium mesenteric arteries (Lagaud *et al.*, 1996; Lewis & Evans, 2000b). In large arteries the P2 receptor antagonist iso-PPADS (30 μM) inhibited responses to α,β-meATP (300 μM) by 41.8$\pm$14.2%, $n=3$).

Immunohistochemical detection of P2X receptor subunits

The expression of defined P2X receptor isoforms was determined using receptor specific antibodies (Table 1) and extends a previous study characterizing P2X receptor expression in medium mesenteric arteries (Lewis & Evans, 2000b). There is substantial expression of $P2X_1$ receptor subunits in all vessel sizes (Figure 4). The $P2X_4$ receptor is expressed in the smooth muscle of medium and large arteries and weakly in small vessels, $P2X_5$ receptor immunoreactivity was weak/barely detectable in all arteries. $P2X_7$ is weakly expressed in the outer non-smooth muscle layers of medium-sized arteries and in the smooth muscle layer of large arteries. $P2X_{2,3}$ and $P2X_6$ receptor immunoreactivity was below the limit of detection in arterial tissue.

Characterization of contractile responses to UTP

Our results from experiments with α,β-meATP suggested there is heterogeneity in P2X receptor expression dependent on the size of the artery. In order to test whether such variation was also found for P2Y receptors, concentration-

Table 1 Immunohistochemistry on sections of small, medium and large mesenteric arteries.

	Small	*Medium*	*Large*
$P2X_1$	+++	+++	++
$P2X_2$	−	−	−
$P2X_3$	−	−	−
$P2X_4$	±	+/++	+
$P2X_5$	±	±	±
$P2X_6$	−	−	−
$P2X_7$	±	+	+

+++ = strong expression, ++ = moderate expression, + = weak expression, ± = barely detectable expression, − = no expression.

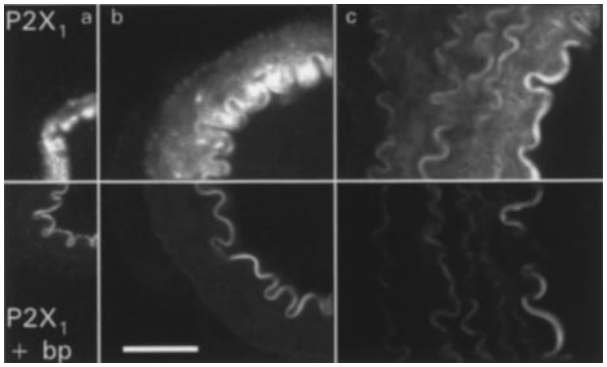

Figure 4 Immunohistochemical visualization of $P2X_1$ receptors in rings of small (a), medium (b) and large (c) mesenteric arterial rings. $P2X_1$ receptor-specific immunoreactivity is associated with the smooth muscle layer of all three sizes of artery (top panels). Specific immunoreactivity is blocked by pre-incubation of the antibody with its specific antigen blocking peptide, leaving only the autofluorescence of the elastic laminae (bottom panels). Calibration bar 50 μm.

response relationships for UTP were constructed. UTP evoked sustained contractions of mesenteric arteries (Figure 5). The EC_{50} values for UTP were 15 μM, 88 μM, 1.6 mM and 1.4 mM ($pA_{50} = 5.0 \pm 0.2$, 4.1 ± 0.1, 2.9 ± 0.1 and 2.9 ± 0.2, $n = 4-6$) for small, medium (diamtrak), medium (myography) and large arteries, respectively (Figure 4). The slopes of concentration responses curves were 1.1 ± 0.3, 2.2 ± 0.3, 0.9 ± 0.1 and 1.3 ± 0.2, respectively. In diamtrak studies on medium arteries UTP was significantly more potent at small compared to medium arteries ($P < 0.02$). In dimatrak studies the potency of UTP was significantly higher than that estimated in myograph studies ($P < 0.005$).

To ensure that observed responses were actually being mediated by P2Y and not P2X receptors, a cross-desensitization experiment was conducted. Responses to UTP prior to and following desensitization of the P2X receptor with α,β-meATP were compared. The second response to UTP expressed as a percentage of the first was 99.7 ± 8.0, 126.3 ± 5.2 and 229.0 ± 10.7% for small, medium and large arteries respectively ($n = 3-6$) (Figure 5a,b). This confirms that UTP is acting at a different receptor to α,β-meATP. The potentiation of UTP responses in medium and large arteries following α,β-meATP treatment may reflect a sensitization of the responses to subsequent agonist stimulation.

In order to determine which P2Y receptor subtype UTP was acting at, the effect of suramin was tested. In the rat, $P2Y_2$ receptors are sensitive to suramin while $P2Y_4$ receptors are not. In medium sized arteries 30 μM (Figure 5c) and 100 μM suramin reduced responses to 1 mM UTP by 48.8 ± 2.5 and 65.5 ± 6.2% respectively ($n = 4$). In small

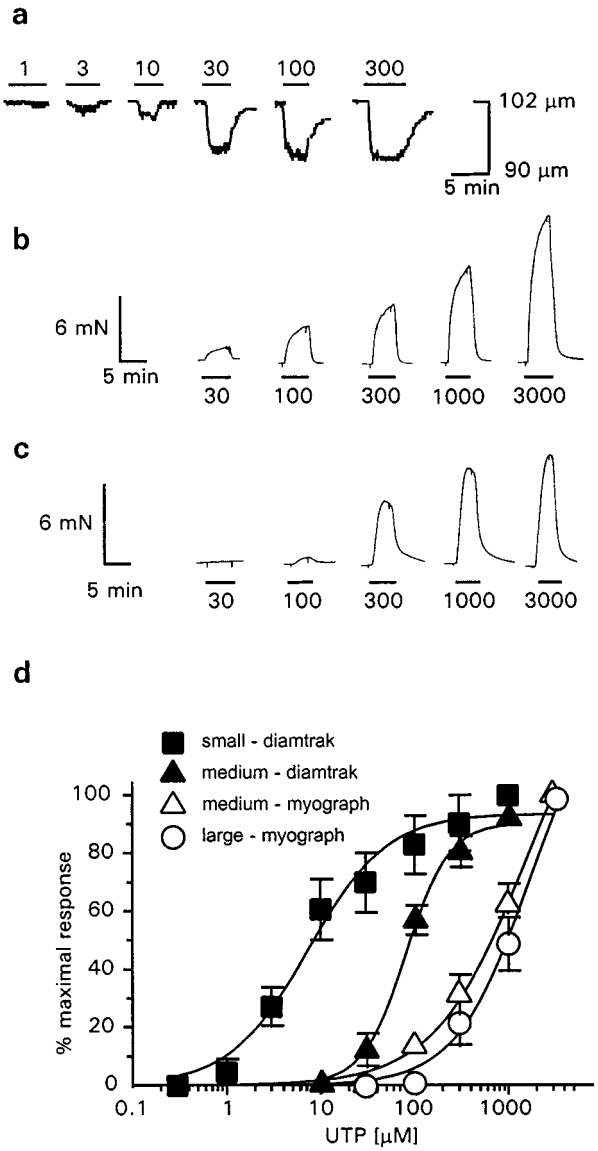

Figure 5 Characterization of contractile responses to UTP. Typical responses in small (a), medium (b) and large (c) arteries, showing sustained constrictions to UTP; periods of application are indicated by the bar. Concentration-response curves for UTP in the three sizes of mesenteric artery show substantial differences in sensitivity of the arteries depending on their size or method of recording (d). Data points are mean responses ± s.e.mean ($n = 4-6$) and are expressed as a per cent of the maximal response in small arteries and as per cent of the response to 3 mM UTP in medium-sized and large arteries.

vessels, contractions to 30 μM UTP were reduced by 64.3 ± 9.1% (Figure 5d) and 96.3 ± 3.7% respectively ($n = 5$).

Discussion

In this study we have shown that the properties of P2 receptors in rat mesenteric arteries depend on the diameter of the vessel. For P2X receptors the differences in receptor function seem to result from the differences in contractile sensitivity and expression of different receptor subtypes. Small and medium vessels share a similar α,β-meATP and suramin sensitive phenotype while the large vessels show a relatively α,β-meATP insensitive and suramin insensitive phenotype. In contrast P2Y receptors in the medium and large vessels share similar properties while UTP is more potent in smaller diameter arteries.

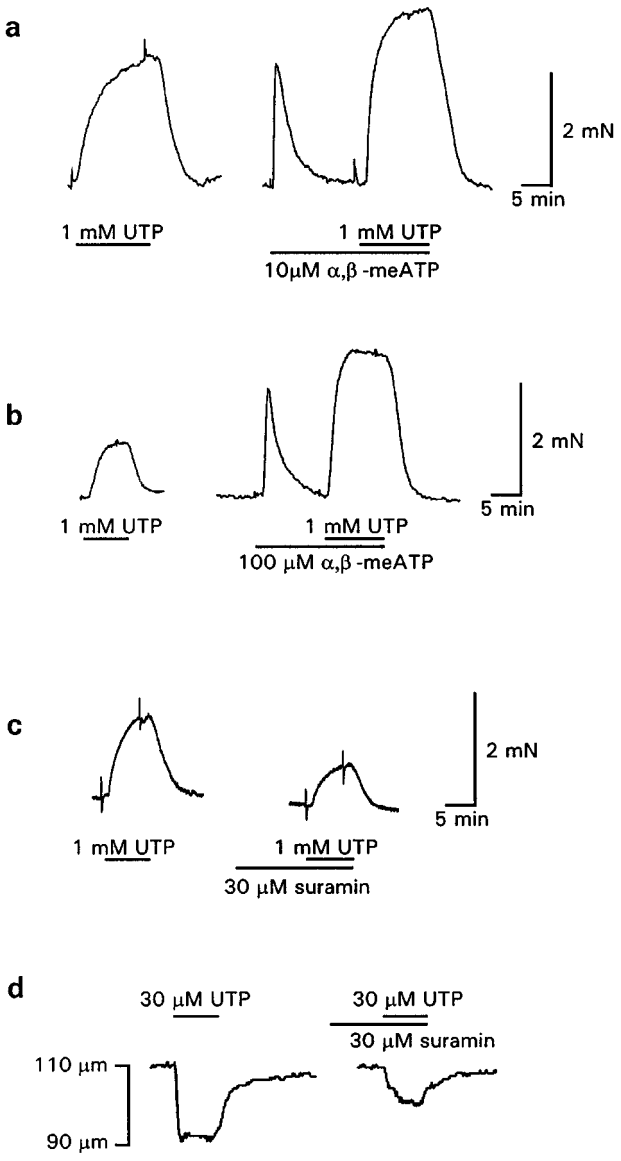

Figure 6 Contractions to UTP are mediated through receptors other than $P2X_1$ and are suramin sensitive. Cross-desensitization experiments in medium-sized (a) and large (b) arteries. Responses to UTP are no smaller after the $P2X_1$ receptor has been desensitized with α,β-meATP. Suramin (30 μM) reduced responses to UTP by approximately 50% in both medium-sized (c) and small (d) arteries.

The metabolically stable ATP analogue α,β-meATP produced concentration-dependent contractile responses in all arteries. Ca^{2+} influx was essential for vasoconstriction and indicates that the responses are mediated by P2X receptor ion channels and not by metabotropic P2Y receptors. There was however a significant difference in the potency of the agonist depending on the diameter of the vessel. Small and medium-sized arteries had EC_{50} values of 0.4 and 2.5 μM respectively. The small difference in sensitivity to α,β-meATP can be accounted for by an increased calcium sensitivity of the muscle in small arteries as indicated by the increased sensitivity of small arteries to KCl. For example a concentration of KCl (20 mM) failed to evoke a contraction in medium but evoked $\sim$35% of maximum response in the small arteries, 40 mM KCl evoked $\sim$40% contraction in medium vessels and $\sim$95% in small arteries. If we compare the shifts in the amplitude of the response for α,β-meATP evoked responses we see a similar pattern e.g. 0.3 μM α,β-meATP evoked $\sim$5% max response in medium compared to $\sim$40% in small and 1 μM α,β-meATP evoked $\sim$37% in medium compared to $\sim$80% in large. So the effective change in per cent response between small and medium for a given concentration of KCl or α,β-meATP are the same and are likely to result from a change in calcium sensitivity of the contractile machinery. The lack of increased potency of phenylephrine in small compared to medium arteries may indicate differences in receptor expression and/or coupling. The EC_{50} values for α,β-meATP are similar to values obtained in other arteries and smooth muscle preparations (von Kugelgen *et al*., 1990; Galligan *et al*., 1995; Lagaud *et al*., 1996).

Contractions of small and medium arteries were suramin sensitive and the estimates of pA_2 values for small and medium arteries are identical and agree well with those previously quoted for vas deferens and submucosal arterioles (von Kugelgen *et al*., 1990; Khakh *et al*., 1994; Galligan *et al*., 1995). These properties bear the hallmark of recombinant $P2X_1$ receptors (Valera *et al*., 1994) and are consistent with the immunohistochemical studies showing that the $P2X_1$ receptor is expressed at high levels in the smooth muscle layer. We have recently shown that the $P2X_1$ receptor is essential for P2X receptor mediated smooth muscle contraction in the vas deferens (Mulryan *et al*., 2000), and it therefore appears that responses in small and medium-sized mesenteric arteries are dominated by the $P2X_1$ receptor phenotype. The role of $P2X_4$ and $P2X_5$ receptor subunits expressed on mesenteric arteries however remains unclear.

Two key features distinguished P2X receptor mediated responses in large arteries from smaller vessels: (1) the sensitivity of large arteries to α,β-meATP was approximately 25–100 fold lower and (2) the sensitivity to the P2 receptor antagonist suramin. A diffusional barrier limiting agonist access to large vessels is unlikely to account for these differences as the sensitivity to KCl, phenylephrine and UTP was the same for medium and large vessels in the myograph. Ionic currents recorded from recombinant $P2X_1$ receptors have shown that maximal stimulation of the $P2X_1$ receptor is achieved at a concentration of 10–30 μM α,β-meATP (Evans *et al*., 1995). This concentration of agonist however produces contractions in large vessels that are equivalent to only 10% of the maximal response. There are a number of caveats to classifying receptors based on agonist sensitivities and the use of antagonists is often more conclusive. Suramin was an effective antagonist at small/medium arteries (pA_2 5.1) but had no effect on α,β-meATP evoked responses in large arteries. Taken together these results provide compelling evidence that the contractile P2X phenotype in large mesenteric arteries is not $P2X_1$-like and that a different P2X receptor mediates the α,β-meATP evoked contraction.

Comparing the pharmacological properties of large arteries with those of the recombinant receptors reveals a mixture of phenotypes: the rapidly desensitizing response to agonists is characteristic of $P2X_1$ and $P2X_3$ receptor isoforms, the relative insensitivity to α,β-meATP is seen in the $P2X_{2,4,5,6\text{ and }7}$ receptors and the lack of effect of suramin is shared by the $P2X_4$ and $P2X_6$ receptor isoforms. The fact that no single receptor can account for the phenotype of the large mesenteric artery P2X receptor raises the possibility of a novel heteromultimer of P2X receptor subunits. More than one type of isoform forming functional heteromeric receptors has been reported on several occasions (Lewis *et al*., 1995; Le *et al*., 1998; Haines *et al*., 1999). However immunohistochemical analysis revealed no major differences in patterns of P2X receptor isoform expression between the three sizes of

vessel and may suggest that a novel additional subunit is expressed in large arteries.

In addition to testing agonists at P2X receptors, we also characterized constrictions to UTP in the mesenteric circulation. This pyrimindine has been shown to have a contractile effect in numerous arterial preparations (von Kugelgen & Starke, 1990; Ralevic & Burnstock, 1991; Hartley *et al*., 1998) that is mediated by either $P2Y_2$ or $P2Y_4$ receptors present on the surface of smooth muscle cells. In the rat, $P2Y_2$ receptors are suramin sensitive, while $P2Y_4$ receptors are suramin insensitive (Boarder & Hourani, 1998). In myograph studies the potency of UTP was the same for medium and large arteries and was essentially the same as that reported recently (Malmsjo *et al*., 2000). The sensitivity to UTP was increased $\sim$20 fold for medium arteries when contractions were studied using the diamtrak technique. In contrast there was no change in the sensitivity between diamtrak or myograph studies for responses to KCl or α,β-meATP. It has previously been shown in rabbit mesenteric arteries that the sensitivity of contractions to other G-protein coupled receptors is dependent on the degree of vessel tone/pressurization or depolarization with potassium (Dunn *et al*., 1994). It is known that stretching vessels also results in membrane depolarization. Recent work has shown that oscillations in calcium release from intracellular stores following P2Y receptor activation can be potentiated by membrane depolarization (Mason *et al*., 2000). Thus one possible explanation for these findings is that in mesenteric arteries stretch induced depolarization associated with pinning out vessels for diamtrak analysis accounts for the increase in sensitivity to UTP in diamtrak studies. However as KCl and α,β-meATP evoked contractions are dependent on calcium influx they are not affected by vessel stretching.

In mesenteric arteries UTP responses were suramin sensitive which indicates the involvement of $P2Y_2$ receptors similar to those in pulmonary arteries (Hartley *et al*., 1998). In small mesenteric arteries UTP had an EC_{50} value of 15.0 μM, no evidence of other vessels with such a high sensitivity to UTP has been reported previously. This increased potency may reflect the increased sensitivity seen for KCl evoked contractions in small arteries. It could also be due to an increased P2Y receptor reserve, a differential degree of nucelotidase metabolism of UTP or the existence of a novel P2Y receptor. However, it is clear, that under physiological conditions UTP released from damaged cells or platelets (Ralevic & Burnstock, 1998) can act as a potent vasoconstrictor at small arteries.

In summary we have demonstrated that arteries do not have a homogenous P2X receptor contractile phenotype and that the agonist sensitivity of P2 receptors in the rat mesenteric circulation is dependent on the diameter of the artery and the degree of vessel tone. These features may relate to the different physiological/functional role(s) played by different calibre arteries.

This work was supported by the MRC and the Wellcome Trust. We would also like to thank Dr L. Vulchanova and Roche Bioscience for P2X receptor antibodies.

References

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

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

BOARDER, M.P. & HOURANI, S.M.O. (1998). The regulation of vascular function by P2 receptors: multiple sites and multiple receptors. *Tr. Pharmacol. Sci.,* **19,** 99–107.

BOWLES, D.K., HU, Q., LAUGHLIN, M.H. & STUREK, M. (1997). Heterogeneity of L-type calcium current density in coronary smooth muscle. *Am. J. Physiol.,* **273,** H2083–H2089.

BUELL, G., MICHEL, A., LEWIS, C., COLLO, G., HUMPHREY, P.P.A. & SURPRENANT, A. (1996). $P2X_1$ receptor activation in HL60 cells. *Blood,* **7,** 2659–2664.

BULLOCH, J.M. & MCGRATH, J.C. (1988). Selective blockade by nifedipine of 'purinergic' rather than adrenergic nerve-mediated vasopressor responses in the pithed rat. *Br. J. Pharmacol.,* **95,** 695–700.

BURNSTOCK, G. (1997). The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacol.,* **36,** 1127–1139.

CLAPP, L.H., TURNER, J.L. & KOZLOWSKI, R.Z. (1996). Ca^{2+} activated Cl- currents inpulmonary arterial myocytes. *Am. J. Physiol.,* **270,** H5177–H1584.

COLLO, G., NORTH, R.A., KAWASHIMA, E., MERLO-PICH, E., NEIDHART, S., SURPRENANT, A. & BUELL, G. (1996). Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J. Neurosci.,* **16,** 2495–2507.

DUNN, W.R., WELLMAN, G.C. & BEVAN, J.A. (1994). Enhanced resistance artery sensitivity to agonists under isobaric compared with isometric conditions. *Am. J. Physiol.,* **266,** H147–H155.

ERLINGE, D., HOU, M., WEBB, T.E., BARNARD, E. & MOLLER, S. (1998). Phenotype changes of the vascular smooth muscle cell regulate P2 receptor expression as measured by quantitative RT–PCR. *Biochem. Biophys. Res. Comm.,* **248,** 864–870.

EVANS, R.J., LEWIS, C., BUELL, G., VALERA, S., NORTH, R.A. & SURPRENANT, A. (1995). Pharmacological characterization of heterologously expressed ATP-gated cation channels (P2X purinoceptors). *Mol. Pharmacol.,* **48,** 178–183.

EVANS, R.J. & SURPRENANT, A. (1996). P2X receptors in autonomic and sensory neurons. *Semin. Neurosci.,* **8,** 217–223.

GALLIGAN, J.J., HERRING, A. & HARPSTEAD, T. (1995). Pharmacoligical characterization of purinoceptor-mediated constriction of submucosal arterioles in guinea pig ileum. *J. Pharm. Exp. Ther.,* **274,** 1425–1430.

HAINES, W.R., TORRES, G.E., VOIGT, M.M. & EGAN, T.M. (1999). Properties of the novel ATP-gated ionotropic receptor composed of the $P2X_1$ and $P2X_5$ isoforms. *Mol. Pharmacol.,* **56,** 720–727.

HARTLEY, S.A., KATO, K., SALTER, K.J. & KOZLOWSKI, R.Z. (1998). Functional evidence for a novel suramin-insensitive pyrimidine receptor in rat small pulmonary arteries. *Circ. Res.,* **83,** 940–946.

HARTLEY, S.A. & KOZLOWSKI, R.Z. (1997). Electrophysiological consequences of purinergic receptor stimulation in isolated rat pulmonary arterial myocytes. *Circ. Res.,* **80,** 170–178.

KHAKH, B.S., MICHEL, A. & HUMPHREY, P.P.A. (1994). Estimates of antagonist affinities at P2X purinoceptors in rat vas deferens. *Eur. J. Pharmacol.,* **263,** 301–309.

LAGAUD, G.J.L., 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.

LE, K.-T., BABINSKI, K. & SEGUELA, P. (1998). Central $P2X_4$ and $P2X_6$ channel subunits coassemble into a novel heteromeric ATP receptor. *J. Neurosci.,* **18,** 7152–7159.

LEWIS, C., NEIDHART, S., HOLY, C., NORTH, R.A., BUELL, G. & SURPRENANT, A. (1995). Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. *Nature,* **377,** 432–435.

LEWIS, C., SURPRENANT, A. & EVANS, R.J. (1998). 2′,3′-O-(2,4,6-trinitrophenyl) adenosine 5′-triphosphate (TNP-AT) – a nanomolar affinity antagonist at rat mesenteric artery P2X receptor ion channels. *Br. J. Pharmacol.,* **124,** 1463–1466.

LEWIS, C.J. & EVANS, R.J. (2000a). Comparison of P2X receptors in rat mesenteric basilar and septal (coronary) arteries. *J. Auton, Nerv. Sys.,* **81,** 69–74.

LEWIS, C .J. & EVANS, R.J. (2000b). Lack of run-down of smooth muscle P2X receptor currents recorded with the amphotericin permeabilised patch technique; physiological and pharmacological characterisation of the properties of mesenteric artery P2X receptor ion channels. *Br. J. Pharmacol.* **131,** 1659–1666.

MALMSJO, M., ADNER, M., HARDEN, T.K., PENDERGAST, W., EDVINSSON, L. & ERLINGE, D. (2000). The stable pyrimidines UDPβS and UTPγS discriminate between the P2 receptors that mediate vascular contraction and relaxation of the rat mesenteric artery. *Br. J. Pharmacol.,* **131,** 51–56.

MASON, M.J., HUSSAIN, J.F. & MAHAUT-SMITH, M.P. (2000). A novel role for membrane potential in the modulation of intracellular Ca^{2+} oscillations in rat megakaryocytes. *J. Physiol.,* **524,** 437–446.

MULRYAN, K., GITTERMAN, D.P., LEWIS, C.J., VIAL, C., LECKIE, B.J., COBB, A.L., BROWN, J.E., CONLEY, E.C., BUELL, G., PRITCHARD, C.A. & EVANS, R.J. (2000). Reduced vas deferens contraction and male infertility in mice lacking $P2X_1$ receptors. *Nature,* **403,** 86–89.

NEILD, T.O. (1989). Measurement of arteriole diameter changes by analysis of television images. *Blood Vessels,* **26,** 48–52.

NICKE, A., BAUMERT, H., RETTINGER, J., EICHELE, A., LAMBRECHT, G., MUTSCHLER, E. & SCHMALZING, G. (1998). $P2X_1$ and $P2X_3$ receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO J.,* **17,** 3016–3028.

NORI, S.L., FUMAGALLI, L., BO, X., BOGDANOV, Y. & BURNSTOCK, G. (1998). Coexpression of mRNAs for $P2X_1$, $P2X_2$ and $P2X_4$ receptors in rat vascular smooth muscle: an in situ hybridization and RT–PCR study. *J. Vasc. Res.,* **35,** 179–185.

NORTH, R.A. & SURPRENANT, A. (2000). Pharmacology of cloned P2X receptors. *Ann. Rev. Pharmacol. Toxicol.,* **40,** 563–580.

QUAYLE, J.M., DART, C. & STANDEN, N.B. (1996). The properties and distribution of inward rectifier potassium currents in pig coronary arterial smooth muscle. *J. Physiol.,* **494,** 715–726.

RALEVIC, V. & BURNSTOCK, G. (1991). Effects of purines and pyrimidines on the rat mesenteric arterial bed. *Circ. Res.,* **69,** 1583–1590.

RALEVIC, V. & BURNSTOCK, G. (1998). Receptors for purines and pyrimidines. *Pharmacol. Rev.,* **50,** 413–492.

RALEVIC, V., HOYLE, C.H.V. & BURNSTOCK, G. (1995). Pivotal role of phosphate chain length in vasoconstrictor versus vasodilator actions of adenine dinucleotides in rat mesenteric arteries. *J. Physiol.,* **483,** 703–713.

SJOBLOM-WIDFELDT, N. (1990). Neuro-muscular transmission in blood vessels: phasic and tonic components. An in-vitro study of mesenteric arteries of the rat. *Acta Physiol. Scandanavica,* **138** (supplement 587).

SURPRENANT, A., RASSENDREN, F., KAWASHIMA, E., NORTH, R.A. & BUELL, G. (1996). The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor $P2X_7$. *Science,* **272,** 735–738.

URQUILLA, P.R. (1978). Prolonged contraction of isolated human and canine cerebral arteries induced by uridine 5′-triphsophate. *Stroke* **9,** 133–136.

VALERA, S., HUSSY, N., EVANS, R.J., ADAMI, N., NORTH, R.A., SURPRENANT, A. & BUELL, G. (1994). A new class of ligand-gated ion channel defined by P2X receptor for extraxcellular ATP. *Nature,* **371,** 516–519.

VALERA, S., TALABOT, F., EVANS, R.J., GOS, A., ANTONARAKIS, S.E., MORRIS, M.A. & BUELL, G. (1995). Characterization and chromosomal localization of a human P(2X) receptor from the urinary bladder. *Receptors and Channels,* **3,** 283–289.

VON KUGELGEN, I., BULTMANN, R. & STARKE, K. (1990). Interactions of adenine nucleotides, UTP and suramin in mouse vas deferens; suramin-sensitive and suramin-insensitive components in the contractile effect of ATP. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **342,** 198–205.

VON KUGELGEN, I. & STARKE, K. (1990). Evidence for two separate vasoconstriction-mediating nucleotide receptors, both distinct from the P2X-receptor, in rabbit basilar artery: a receptor for pyrimidine nucleotides and a receptor for purine nucleotides. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **341,** 538–546.

VULCHANOVA, L., ARVIDSSON, U., RIEDL, M., BUELL, G., SURPRENANT, A., NORTH, R.A. & ELDE, R.P. (1996). Differential distribution of two ATP-gated ion channels (P2X receptors) determined by immunohistochemistry. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 8063–8067.

WEBB, T.E., SIMON, J., KRISHEK, B.J., BATESON, A.N., SMART, T.G., KING, B.G., BURNSTOCK, G. & BARNARD, E.A. (1993). Cloning and functional expression of a brain G-protein-coupled ATP receptor. *FEBS Lett.,* **324,** 219–225.

(Received September 5, 2000
Revised October 4, 2000
Accepted October 6, 2000)