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Purinergic $2X_1$ Receptors Mediate Endothelial Dependent Vasodilation to ATP

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ABSTRACT

ATP is an important endogenous mediator in the cardiovascular system. It induces endothelium dependent vasodilation, but the precise receptor pathway activated in this response is currently under debate. We have used traditional bioassay techniques to show that ATP-induced vasodilation in mesenteric vessels is endothelium-dependent. Furthermore, ATP-induced vasodilation was inhibited by both suramin and 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP (TNP-ATP), consistent with a $P2X_1$-, $P2X_2$-, or $P2X_3$-mediated event and was not potentiated by ivermectin, indicating that these responses were not $P2X_4$ receptor-mediated. ATP did not induce vasodilation in vessels from $P2X_1^{-/-}$ mice, confirming an absolute requirement for this receptor. Finally, in pure cell populations of mouse mesenteric artery endothelial cells, we show that $P2X_1$ mRNA is specifically expressed. However, in line with observations in the brain, the $P2X_1$ present in endothelial cells does not seem to be recognized by conventional antibodies. Together, these results show that ATP-induced vasodilation is mediated by $P2X_1$ receptor activation on mesenteric arterial endothelial cells. These observations establish a critical role for $P2X_1$ receptors in the ATP vasodilator pathway.

Purinergic control of vascular tone is complex, because ATP released in response to vascular insult has dual effects. ATP acts on smooth muscle cells to cause contraction and on endothelial cells to cause vasodilation. ATP and other purines act on purinergic receptors (P2), which are either membrane G-coupled proteins (P2Y) or ion channels (P2X). ATP induces vasoconstriction via activation of $P2X_1$ (Vial and Evans, 2002) receptors, whereas vasodilator responses induced by ATP are commonly thought to be mediated by P2Y receptors (Carter et al., 1988; Ralevic and Burnstock, 1988). Most recently, in the aorta, $P2Y_2$ receptors have been implicated in the dilator effects of ATP (Guns et al., 2005, 2006). However, the distribution of P2Y receptors is heterogeneous; the location of receptors is highly dependant on vessel size. For example, in the mesenteric circulation, $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors are more active in the larger vessels (Carter et al., 1988; Gitterman and Evans, 2000). Furthermore, in addition to P2Y receptors, evidence now suggests that functional $P2X_4$ receptors are present on human endothelial cells (Yamamoto et al., 2000) and their activation by sheer stress or ATP mediates vasodilation in the mouse (Yamamoto et al., 2006).

Likewise, we have recently published pharmacological evidence suggesting that ATP induces vasodilation via the activation of P2X receptors on mesenteric arteries (Stanford et al., 2001; Harrington and Mitchell, 2004). However, opinion is divided over whether $P2X_1$ receptors are actually located on the endothelium and smooth muscle (Hansen et al., 1999) or solely on smooth muscle (Vial and Evans, 2002) of blood vessels. This apparent anomaly may be explained by the lack of availability of anti-$P2X_1$ antibodies, which recognize all forms of $P2X_1$ (L. S. Harrington and J. A. Mitchell, unpublished observations; Ashour et al., 2006). In the current study, we have used mesenteric vessels from wild-type ($P2X_1^{+/+}$) and $P2X_1^{-/-}$ mice (Mulryan et

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ABBREVIATIONS: PSS, physiological salt solution; U46619, 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$; SNP, sodium nitroprusside; TNP-ATP, 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP; PBS, phosphate-buffered saline; SNP, sodium nitroprusside; ANOVA, analysis of variance; rtPCR, reverse transcription-polymerase chain reaction.

al., 2000; Vial and Evans, 2002) to determine the role of this receptor in the dilator effects of ATP and selective P2X pharmacological tools. The presence of $P2X_1$ mRNA in primary isolates of mesenteric endothelial cells was confirmed using molecular techniques.

Materials and Methods

Male Black 6 C57 ($P2X_1^{+/+}$, wild-type) or $P2X_1$ receptor-deficient ($P2X_1^{-/-}$) mice (Mulryan et al., 2000) 18 to 24 weeks old were euthanized by lethal exposure to CO_2. The mice were maintained and killed in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the United States National Institutes of Health.

The entire mesenteric bed was removed using ligatures, and placed into physiological salt solution (PSS): 119 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 0.027 mM EDTA, and 5.5 mM glucose. The mesentery was pinned flat on a dissecting dish containing PSS to allow first-order arteries to be cleaned of fat and connective tissue; these arteries were stored in fresh PSS solution at room temperature until use.

Isometric Myograph Recordings. Using tungsten wire, 2-mm segments of artery were mounted in a four channel Mulvany-Halpern myograph (model 610M; Danish Myo Technology, Aarhus, Denmark). The vessels were equilibrated to 37°C, and the solution was bubbled with 95% O_2 and 5% CO_2 for 30 min. The tension of the vessel was normalized, and changes in arterial tone were recorded via a PowerLab/800 recording unit (ADI Instruments Pty Ltd., Sydney, Australia) and analyzed using Chart 4.0 acquisition system (ADI Instruments). In this study, the first-order mesenteric arteries had a mean normalized internal diameter of 198.5 μm.

To assess the viability of the vessels, they were challenged twice with high-potassium solution: 123.7 mM KCl, 2.5 mM $CaCl_2$, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 0.027 mM EDTA, and 5.5 mM glucose.

A cumulative dose response to the thromboxane mimetic 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619) was performed (10^{-9} to 3×10^{-7} M). Vessels were contracted to an approximate EC_{80} of U46619 and the effects of accumulative additions of either ATP or ADP were determined (3×10^{-6} to 10^{-4} M). If no response was noted, addition of the next concentration of agonist was given after 2 to 3 min. The optimum tension that was gained using an EC_{80} of U46619 gave a tension that was similar between animals; 8.24 ± 0.879 mN in wild-type and 7.73 ± 0.878 mN in $P2X_1^{-/-}$ mice.

After a 30-min wash-out period, the arteries were precontracted again with EC_{80} U46619, and dose responses to acetylcholine and sodium nitroprusside (SNP) performed. Arteries that relaxed >70% in response to acetylcholine were considered to have an intact endothelium. Vessels occasionally failed this test and were disregarded. Time controls were performed by which arteries were precontracted with U46619, and any loss of tone measured at similar time points as agonists given in parallel experiments. Some experiments were performed in which the endothelial layer was removed by a human hair, leading to a loss of acetylcholine-induced vasodilatory response.

In another group of preparations, single concentration effects of 10^{-4}M ATP were studied in mesenteric arteries from both $P2X_1^{+/+}$ and $P2X_1^{-/-}$ mice. Arteries were incubated with 10^{-5} M ivermectin, 3×10^{-5}M suramin, or 3×10^{-6} M TNP-ATP for 30 min before precontraction with EC_{80} U46619. A single concentration (10^{-4} M) of ATP was added once the U46619-induced contraction had reached a plateau, and the effects were recorded for 10 min.

Magnetic Bead Separation of Endothelial Cells. Whole mesenteric beds were removed from wild-type mice and chopped into small pieces using a scalpel blade. Tissue was digested with 1 mg/ml collagenase type I (Invitrogen, Paisley, UK) in phosphate-buffered saline (PBS) for 30 min at 37°C. The digest was taken up in a syringe, passed five times through a 19-gauge needle, and sieved through a 70-μm pore size cell strainer (Falcon; BD Discovery Labware, Bedford, MA).

Cells were incubated with the primary antibody, rat anti-mouse intracellular adhesion molecule II (3 μg/ml; BD PharMingen, San Diego, CA) in PBS for 30 min at 4°C, and then rinsed once in PBS. Endothelial cells were purified by positive selection using magnetic Dynabeads coated with 10 μl (4×10^6) polyclonal sheep anti-rat IgG antibodies (Dynal Biotech, Bromborough, Wirral, UK), incubated for 5 min at 4°C. Intracellular adhesion molecule II positive cells were selected by placing the flask on a flat magnet and leaving for 5 min. Contaminating cells were removed by aspiration, taking care not to disturb bead-bound cells. Flasks were rinsed and placed back on the magnet for 5 min a couple of times, until only positive cells remained.

$P2X_1$ Analysis by rtPCR. Total RNA was extracted from brain and purified mesenteric endothelial cells (pooled samples from five mice) from wild-type mice and bladder from $P2X_1^{-/-}$ mice by means of TRIzol (Invitrogen). First-strand cDNA synthesis using reverse transcriptase (Promega, Southampton, UK) were independently primed with oligo-dT. Specific primers were designed using Primer3 and Blastn programs to amply $P2X_1$, von Willebrand Factor (endothelial cell-specific), and smooth muscle cell heavy-chain (SMHC) myosin. The primer sequences were as follows: $P2X_1$, 5′-ACTGGGAGTGTGACCTGGAC; 3′-CCAGAGCCGATGGTAGTCAT; von Willebrand factor, 5′-CAGCATCTCTGTGGTCCTGA; 3′-GATGTTGTTGTGGCAAGTGG; SMHC myosin, 5′-GGGACTTGAGTGAGGAGCTG; 3′-TTTGAACCTTTTCGCTTGCT. The PCR products were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and visualized with imaging software under UV light.

Immunofluorescence Staining. Stretched mesenteric arteries were fixed with 4% paraformaldehyde for 30 min at room temperature and stored in phosphate-buffered saline (PBS) 1% Triton X-100. To stain for $P2X_1$ receptors, the fixed arteries were blocked with 5% bovine serum albumen PBS with 1% Triton X-100 and then briefly washed. Arteries were incubated with $P2X_1$ primary antibody (rabbit polyclonal, 1:200) from Alomone (Jerusalem, Israel) overnight at 4°C. After three 20-min washes, arteries were incubated with Texas Red conjugated secondary antibody (goat anti-rabbit; 1:500) for 2 h at room temperature. To stain for nuclei, the fixed arteries were incubated in 20 nM 4′,6-diamidino-2-phenylindole dihydrochloride for 5 min, and then washed three times for 20 min each at room temperature. Vessels were mounted onto slides with aqueous fluorescent mounting medium and set with nail varnish, before images were obtained by oil immersion confocal microscopy (magnification, 650×).

Data and Statistical Analysis. Contractile or relaxant responses were calculated as a percentage of the original U46619 induced tone, and responses were recorded once plateau was achieved. Data are given as the mean ± S.E.M. for experiments (one animal per experiment).

Drugs. All drugs were purchased from Sigma Chemical Co. (Dorset, UK). Drugs were prepared each day were prepared as a high-concentration 'stock' solution and were stored at −20°C until used. All drugs were dissolved in aqueous solutions, except for TNP-ATP, which was dissolved in DMSO.

Results

ATP-Mediated Dilation Is Absent in $P2X_1^{-/-}$ Mice and Requires an Intact Endothelium in Tissue from Wild-Type ($P2X_1^{+/+}$) Mice. When added in cumulative concentrations (3×10^{-6} to 10^{-4} M) to precontracted mesenteric vessels from $P2X_1^{+/+}$ mice, ATP induced vasoconstriction followed by vasodilation (Fig. 1A). By contrast, ATP induced neither vasodilationn nor vasoconstriction in vessels from $P2X_1^{-/-}$ mice (Fig. 1B).

The effects of ATP were not due to time-dependent loss of tone, because U46619-treated vessels maintained elevated

force over the time course of the experiment similarly in tissues from $P2X_1^{-/-}$ and $P2X_1^{+/+}$ mice (Fig. 1C).

ADP (3×10^{-6} M) also induced vasodilation in mesenteric vessels from wild-type $P2X_1^{+/+}$ mice. At concentrations of 10^{-5} M and above, ADP induced constrictor responses in mesenteric vessels from the wild-type mice (Fig. 2A). In mesenteric vessels from $P2X_1^{-/-}$ mice, the vasodilator effects of ADP were enhanced and the constrictor responses abolished (Fig. 2B). Neither ATP nor ADP induced vasodilation in mesenteric vessels from which the endothelium had been removed (Fig. 3).

$P2X_1$ Receptor Located in Purified Mesenteric Endothelial Cells. Pure cell populations of mouse mesenteric artery endothelial cells were prepared using specific antibody-associated magnetic beads (Fig. 4A), and their purity was validated using rtPCR. Endothelial cells expressed the endothelial cell marker von Willebrand factor (Fig. 4B) but not the smooth muscle cell marker heavy chain myosin (Fig. 4C). Mouse mesenteric artery endothelial cells expressed $P2X_1$ mRNA (Fig. 4D). However, validated commercial antibodies to $P2X_1$ protein purchased from Alomone (Ashour et al., 2006) were found not to recognize the form in endothelial cells (Fig. 4, E and F). Similar observations have been made of $P2X_1$ in parts of the central nervous system (Watano et al., 2004; Ashour et al., 2006).

Lack of Functional $P2X_4$ Receptors in Mesenteric Arteries from Wild-Type Mice. ATP, a potent agonist of P2X receptors, induced vasodilator responses in mesenteric arteries from wild-type and not $P2X_1^{-/-}$ mice (Fig. 5). Suramin (3×10^{-5} M), which inhibits all purinergic receptors except for $P2X_4$, prevented ATP-induced vasodilator responses in tissues from wild-type mice (Fig. 5C). The selective P2X inhibitor TNP-ATP (3×10^{-6} M) inhibited the ATP-mediated response in vessels from wild-type mice (Fig. 5VD). Ivermectin (10^{-5} M) potentiates $P2X_4$ receptor-mediated responses and had no effect on ATP-mediated dilation in wild-type or $P2X_1^{-/-}$ mice (Fig. 5, E and F).

Pharmacological Characterization of Responses of Mesenteric Arteries from $P2X_1^{+/+}$ and $P2X_1^{-/-}$ Mice to U46619, Acetylcholine, SNP, and KCl. U46619 was more potent at contracting mesenteric vessels from $P2X_1^{-/-}$ than vessels from wild-type control mice (Fig. 6A). However, vessels from $P2X_1^{-/-}$ and control mice relaxed similarly in response to the endothelium-dependent vasodilator acetylcholine (Fig. 6 B) or the endothelium-independent vasodilator SNP (Fig. 6C). Contractile responses induced by high potas-

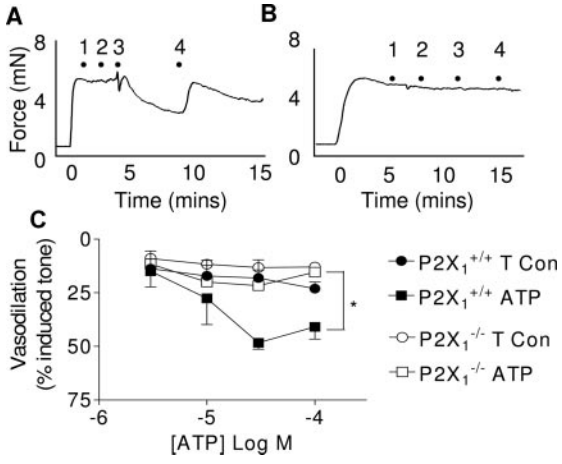

Fig. 1. Effects of ATP on mesenteric arterial tone in vessels from $P2X_1^{-/-}$ and wild type mice. Arteries precontracted with an EC_{80} of U46619 were exposed to increasing concentrations of ATP. Bullet points represent addition of 1) 3×10^{-6}M; 2) 10^{-5}M; 3) 3×10^{-5}M; 4) 10^{-4}M ATP to $P2X_1^{+/+}$ (wild type) (A) and $P2X_1^{-/-}$ mesenteric arteries (B). C, pooled data of vasodilator responses to 3×10^{-6}M to 10^{-4}M ATP in $P2X_1^{-/-}$ compared with $P2X_1^{+/+}$ mouse mesenteric arteries. Data from time controls (T Con) are included, where the arteries from $P2X_1^{+/+}$ and $P2X_1^{-/-}$ mice were exposed solely to the contractile agent. The data are represented as percentage of tone induced by U46619. Each point represents the mean ± S.E.M. for four arteries. Statistical significance between ATP responses in $P2X_1^{+/+}$ and $P2X_1^{-/-}$ arteries was established using two-way ANOVA, where * indicates $p < 0.05$.

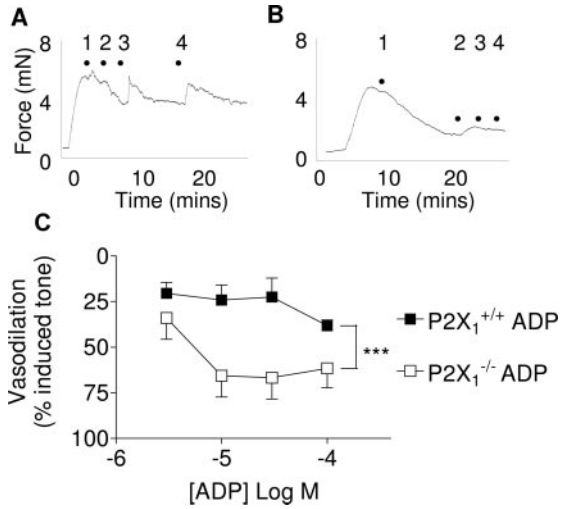

Fig. 2. Effects of ADP on mesenteric arterial tone in vessels from $P2X_1^{-/-}$ and wild-type ($P2X_1^{+/+}$) mice. Arteries precontracted with an EC_{80} of U46619 were exposed to increasing concentrations of ADP. Representative traces are shown for the effects of ADP [●: 1) 3×10^{-6}M; 2) 10^{-5}M; 3) 3×10^{-5}M; 4) 10^{-4} M] in vessels from $P2X_1^{+/+}$ (A) and $P2X_1^{-/-}$ (B) mice. C, pooled data of vasodilatory responses to 3×10^{-6}M to 10^{-4}M ADP in $P2X_1^{-/-}$ compared with $P2X_1^{+/+}$ mouse mesenteric arteries. The data are represented as percentage of tone induced by U46619. Each point represents the mean ± S.E.M. for three to four arteries. Statistical significance was established using two-way ANOVA, where *** indicates $p < 0.001$.

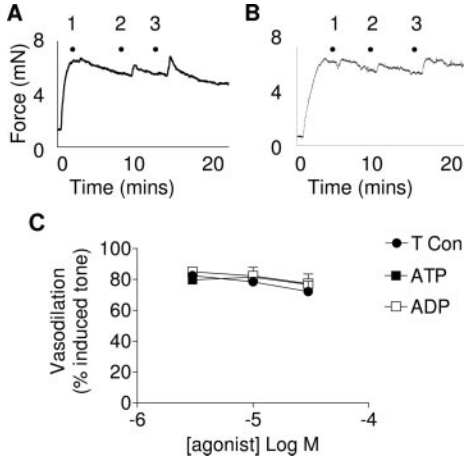

Fig. 3. Representative traces showing the effects of purines [1) 3×10^{-6}M; 2) 10^{-5}M; 3) 3×10^{-5} M] in endothelium-denuded mesenteric vessels from wild-type ($P2X_1^{+/+}$) mice. Arteries precontracted with an EC_{80} of U46619 were exposed to increasing concentrations of ATP (A) or ADP (A). C, pooled data of vasodilator response to ATP or ADP compared with time control (T Con) responses in vessels contracted with U46619 and not exposed to purine agonists. Responses are calculated as percentage of U46619-induced tone. Each point represents the mean ± S.E.M. for four arteries; there was no significant (ns) difference by two-way ANOVA.

sium did not differ between vessels from wild-type or $P2X_1^{-/-}$ mice (Fig. 6D), and mesenteric arteries did not assume spontaneous tone at any point.

Discussion

ATP induces both constrictor and dilator responses in vessels. P2X receptors mediate the contractile responses to ATP (Vial and Evans, 2002), whereas debate surrounds the nature of the receptor(s) that mediate vasodilator responses to ATP. Here we have used genetically modified mice and selective pharmacological tools to show that $P2X_1$ receptors mediate the dilator responses to ATP in mouse mesenteric arteries. We have also used molecular techniques to show that $P2X_1$ mRNA is expressed in endothelial cells from mesenteric arteries.

We have previously shown that ATP induces an atypical vascular response (Stanford et al., 2001). At low doses, ATP induces a transient dilation mediated by the activation of P2Y receptors and the consequent corelease of NO and prostacyclin. At higher doses, we showed that the dilator response induced by ATP consisted of two discernible phases: the transient phase mentioned above followed by a sustained phase, which we showed to be mediated independently of NO and prostacyclin but consistent with the release of endothelial derived hyperpolarizing factor (Stanford et al., 2001; Harrington and Mitchell, 2004). The emergence of the second and sustained phase of endothelial dependent dilation induced by ATP coincided with the emergence of the typical $P2X_1$-mediated vasoconstrictor response.

In the current study, we show that pure populations of endothelial cells isolated from mouse mesenteric artery express $P2X_1$ mRNA. Furthermore, we showed, using pharmacological tools and genetically modified mice, that ATP-induced vasodilator responses are mediated by $P2X_1$ in mouse mesenteric arteries. However, we found that currently used antibodies to $P2X_1$ did not seem to recognize $P2X_1$ in endothelial cells. Similar results have been published for a form of $P2X_1$ in the central nervous system (Ashour et al., 2006). It is not yet clear why certain anatomical tissues seem to express and function via $P2X_1$ without immunogenic reactivity. However, $P2X_1$ in endothelial cells and/or the central nervous tissues could be a spliced variant or present in a conforma-

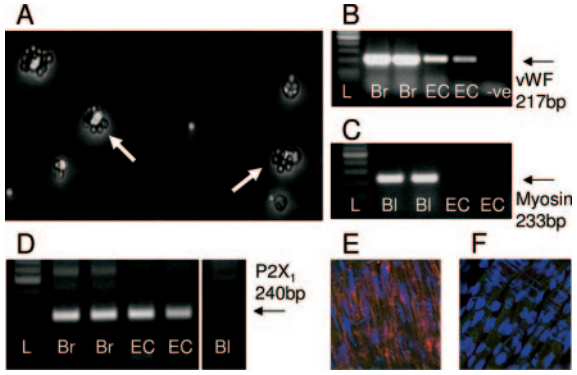

Fig. 4. A, light microscope picture of purified mesenteric endothelial cells from wild-type mice; white arrows indicate individual cells coated in magnetic Dynabeads; magnification, 200×. rtPCR detection using gene-specific primers for von Willebrand factor (B), smooth muscle heavy chain myosin (C), and $P2X_1$ (D). The estimated PCR product sizes are indicated. Lanes: L, 100-bp ladder; Br, brain; EC, mesenteric artery endothelial cell; Bl, $P2X_1^{-/-}$ mouse bladder; −ve; negative control. Confocal images taken of whole mesenteric arteries from wild-type mice, stained with $P2X_1$ antibody (red). Nuclei are shown in blue (4′,6-diamidino-2-phenylindole dihydrochloride). Pictures were taken transversely through the whole artery using the *z*-axis, showing smooth muscle cells (E) and endothelial cells (F); magnification, 650×; oil immersion.

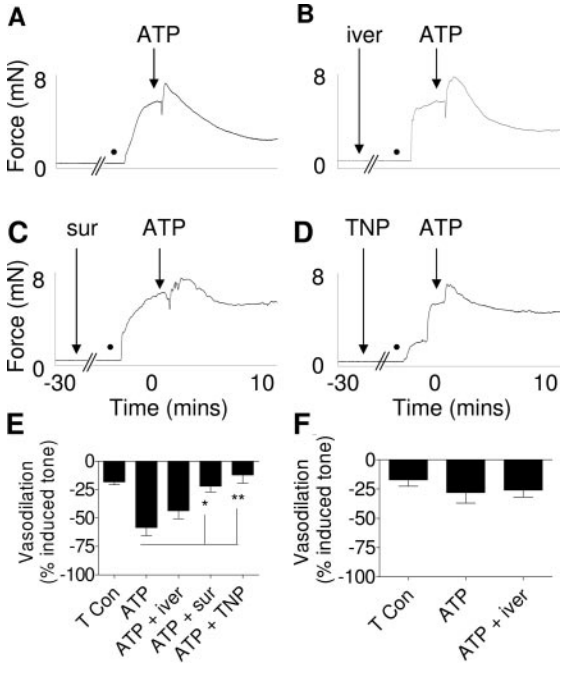

Fig. 5. The effects of ivermectin, suramin, and TNP-ATP on ATP-evoked vasodilation in wild-type ($P2X_1^{+/+}$) mice. A, ATP-mediated response; all other arteries were incubated for 30 min with 10^{-5}M ivermectin (iver) (B), 3×10^{-5}M suramin (sur) (C), 3×10^{-6}M TNP-ATP (TNP) (D). Arteries were precontracted with an EC_{80} U46619 (●) and exposed to 10^{-4}M ATP for 10 min. Data are represented as the maximum vasodilation measured as a percentage of the precontractile tone in wild-type (E) and $P2X_1^{-/-}$ (F) mice, and shown as mean ± S.E.M.; $n = 4–6$. Statistical significance was established using one-way ANOVA; ∗ indicates $p < 0.05$, and ∗∗ indicates $p < 0.01$.

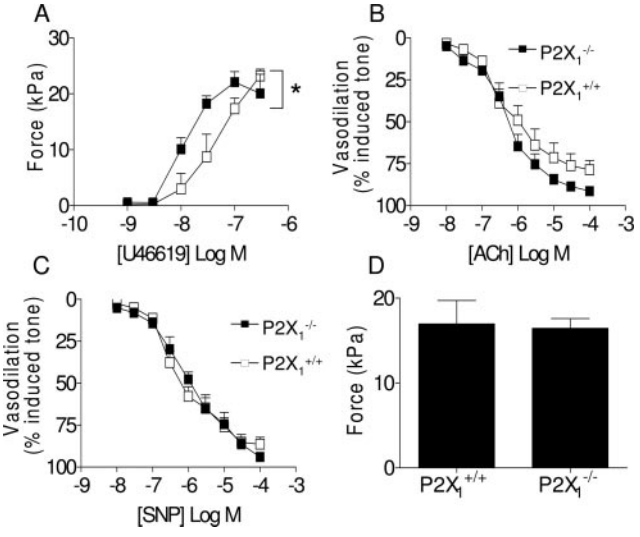

Fig. 6. Comparisons of vasoactive responses in $P2X_1^{-/-}$ compared with $P2X_1^{+/+}$ mouse mesenteric arteries. A, contraction in concentration response to U46619, force is represented as active effective pressure (AEP) measured in kilopascals. Concentration response curve to acetylcholine (B) and sodium nitroprusside (SNP) (C) in arteries precontracted to an EC_{80} U46619 (10^{-7} M)-induced tone. Vasodilation is represented as percentage of U46619-induced tone. D, contraction in response to high-potassium solution (124 mM). Each point represents four to five animals. Statistical significance was established using two-way ANOVA, where ∗ indicates $p < 0.05$.

tional state restricting access of antibodies to the epitope, as suggested by Ashour et al. (2006). We should also consider the theoretical possibility that, despite endothelial cells expressing $P2X_1$ mRNA and mediating vasodilation to ATP in a $P2X_1$-dependent manner, vascular smooth muscle mediates the initial sensing, sending a signal to the endothelium and then back again.

In addition to the data presented in the current study and in a recent report by Yamamoto et al. (2006) support the view that P2X receptors can be present on endothelial cells and mediate endothelium-dependent vasodilation. Yamamoto et al. (2006) showed that endothelial cells from the pulmonary circulation express $P2X_4$ mRNA but not mRNA for $P2X_1$ or other forms of P2X receptors (Yamamoto et al., 2006). $P2X_4$ receptors can be distinguished pharmacologically by their insensitivity to suramin, an otherwise nonselective purinergic antagonist (Buell et al., 1996; Nicke et al., 2005), low sensitivity to antagonism by TNP-ATP (Virginio et al., 1998; Nicke et al., 2005), and potentiation by ivermectin (Khakh et al., 1999). We found that ATP-induced dilator response in mesenteric arteries from wild type was abolished by TNP-ATP and suramin and not potentiated by ivermectin. These new data show that $P2X_4$ receptors are not functional on mesenteric arteries in mice used in this study. In the current study, we also show that ATP was inactive in mesenteric arteries from $P2X_1^{-/-}$ mice.

ADP is thought to mediate endothelium-dependent vasodilation via activation of $P2Y_1$ receptors (Nicholas et al., 1996; Guns et al., 2005). In rat mesenteric arteries, the vasodilator effects of ADP, but not ATP, are abolished by the $P2Y_1$ receptor antagonist MRS2179 (Buvinic et al., 2002; Guns et al., 2006), suggesting that in this tissue, ATP does not activate $P2Y_1$ receptors. In the current study, we show that ADP contracted and dilated vessels. The dilator effects were endothelium-dependent and independent of $P2X_1$, consistent with the notion that they are P2Y-mediated. The constrictor effects were mediated by $P2X_1$—indicative of contamination with ATP. The vasodilator effects of ADP were enhanced in $P2X_1^{-/-}$, probably because of the loss of the functional antagonism-induced $P2X_1$-mediated constrictor response. Some commercial preparations are known to be contaminated with ATP (Mahaut-Smith et al., 2000), which would seem to be the case with drugs used in this study.

Our observations are likely to have physiological relevance, perhaps at the site of inflammation or thrombosis or after ischemia reperfusion injury, where extracellular levels of ATP are elevated beyond the 10^{-5}M range (Carty et al., 1981; Smolenski et al., 2001; Gourine et al., 2005). Our observations are not due to some unrelated phenotype distortion, because the vessels from $P2X_1^{-/-}$ animals relax appropriately when stimulated with acetylcholine, which acts via the endothelium, or SNP, which acts directly on the smooth muscle. We also show that the vasodilator effects of ATP are mediated by the endothelium.

In conclusion, we have shown definitively that activation of $P2X_1$ receptors, most likely located on endothelial cells, mediates ATP-induced vasodilator responses in mesenteric vessels. These observations are likely to have important biological relevance at the site of inflammation or vascular insult where extracellular ATP levels are elevated.

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