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Extracellular ATP facilitates flow-induced vasodilatation in rat small mesenteric arteries

Cuiling Liu,[1] Simon Mather,[2] Yu Huang,[1] Christopher J. Garland,[2] and Xiaoqiang Yao[1]

[1]*Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China; and* [2]*Department of Pharmacology and Pharmacy, University of Bath, Bath BA2 7AY, United Kingdom*

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Liu, Cuiling, Simon Mather, Yu Huang, Christopher J. Garland, and Xiaoqiang Yao. Extracellular ATP facilitates flow-induced vasodilatation in rat small mesenteric arteries. *Am J Physiol Heart Circ Physiol* 286: H1688–H1695, 2004. First published January 8, 2004; 10.1152/ajpheart.00576.2003.—ATP can be released from endothelial cells, and this release is increased by intraluminal flow in blood vessels. In the present study, the effect of extracellular ATP (1 μM) on flow-induced vasodilatation was investigated in isolated and pressurized rat small mesenteric arteries. In the absence of extracellular ATP, only 46% of arteries developed dilatation in response to flow, and this response was both transient and unstable. In marked contrast, with ATP present, all vessels developed a prolonged and stable dilatation in response to flow. Even in the vessels that failed to respond to flow in the absence of ATP, dilatation could be stimulated once ATP was present. The ability of ATP to facilitate flow-induced vasodilatation was mimicked by UTP (1 μM), a P2Y agonist, or 3′-*O*-(4-benzoyl)benzoyl ATP (BzATP; 10 μM), an agonist for $P2X_1$, $P2X_7$, and $P2Y_{11}$ purinoceptors. The involvement of $P2X_7$ purinoceptors was further supported by the inhibitory effect of KN-62 (1 μM), a $P2X_7$ antagonist, on the action of BzATP. $P2X_1$ and $P2X_3$ purinoceptors were not involved because their receptor agonist α,β-methylene ATP had no effect. The facilitating effect of ATP on flow dilatation was also attenuated by the combined application of reactive blue 2 (100 μM), a P2Y antagonist, and suramin (100 μM), a nonselective P2X and P2Y antagonist. Furthermore, flow-induced dilatation obtained in the presence of ATP was reproducible. In contrast, in the additional presence of the ectonucleotidase inhibitor ARL-67156 (10 μM), although the first dilatation was normal, the responses to the second and later exposures to flow were greatly attenuated. The nonhydrolyzable ATP analogs adenosine-5′-(3-thiotriphosphate)trilithium salt (1 μM) and adenosine 5′-(β,γ-imido) triphosphate tetralithium salt hydrate (10 μM) had similar effects to those of ARL-67156. These data suggest that ATP acts through both P2X and P2Y purinoceptors to facilitate flow-induced vasodilatation and that ectonucleotidases prevent this effect by degrading ATP on the endothelial cell surface.

pressure myograph; P2X and P2Y purinoceptors; Ca^{2+} influx; convection and diffusion

THE HEMODYNAMICS OF BLOOD FLOW play an important role in the control of vascular tone (5, 10). Shear stress generated by blood flow causes dilatation in healthy blood vessels in vivo (3, 19). However, in isolated vascular segments, the responses to flow may vary depending on the status of the vessels such as the extent of the initial vascular tone and the presence of reactive oxygen species (3). In general, flow elicits vascular dilatation when the initial vascular tone of the vessels falls within a physiological range. At low and high vascular tone, flow actually causes vascular constriction (3). The mechanism of flow-induced dilatation has been the subject of intensive investigation, and it is known that flow-induced dilatation is predominantly endothelium dependent (3, 21, 26) and can be mediated by nitric oxide (NO) and/or prostacyclin (PGI_2) and/or endothelium-derived hyperpolarizing factors (EDHF) (5, 10). Both Ca^{2+}-dependent (9, 13, 26) and Ca^{2+}-independent (2, 11, 14, 29) flow-induced dilatation have been documented.

ATP is a vasoactive agonist that stimulates endothelial cells to release relaxing factors, thereafter influencing the tone of the underlying smooth muscle. The effect of ATP occurs via the activation of P2X and P2Y purinoceptors (18, 32). P2X receptors are ATP-gated Ca^{2+}-permeable channels, where ATP binding directly causes Ca^{2+} influx. P2Y receptors are G protein-coupled receptors and are linked to the stimulation of phospholipase C and synthesis of inositol (1,4,5)-trisphosphate [$Ins(1,4,5)P_3$], which then triggers Ca^{2+} release from intracellular stores. This release of intracellular Ca^{2+} depletes the store contents, which subsequently activates store-operated Ca^{2+} influx (30). An overall increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) level stimulates the production and release of several endothelium-derived vasodilators including NO, PGI_2, and EDHF (7, 15).

In endothelial cells in culture, interactions between flow-induced and ATP-induced changes in $[Ca^{2+}]_i$ have been reported. For example, the flow condition exaggerates ATP-induced $[Ca^{2+}]_i$ responses (27, 28), whereas extracellular ATP augments the flow-induced rise in endothelial $[Ca^{2+}]_i$ (12, 22, 37) and enhances NO production induced by flow (20, 23). However, no data are available concerning the interaction between ATP and flow-induced vascular responses at the tissue level, so it is not known whether ATP could modulate flow-induced dilatation in arteries.

In the present study, we investigated the possibility that extracellular ATP can influence flow-induced vascular dilatation in rat mesenteric arteries using a pressure myograph. Our studies demonstrated that including ATP in the superfusion solution greatly increased flow-induced vasodilatation, and the effect was mediated by both P2X and P2Y purinoceptors.

MATERIALS AND METHODS

Pressure myograph. Animals were supplied by the Laboratory Animal Service Center of the Chinese University of Hong Kong (Hong Kong, China). We followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996). Male

Address for reprint requests and other correspondence: X. Yao, Dept. of Physiology, Faculty of Medicine, The Chinese Univ. of Hong Kong, Hong Kong, China (E-mail: yao2068@cuhk.edu.hk).

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Sprague-Dawley rats (~250–280 g) were killed by inhalation of CO_2. The ileum and associated mesentery were removed and immersed in Krebs-Henseleit solution bubbled with a 95% O_2-5% CO_2 mixture. A third- or fourth-order mesenteric artery (~2–3 mm long) was carefully dissected free of the surrounding adipose tissue and mounted in a pressure myograph (Danish MyoTechnology) filled with oxygenated Tyrode solution at room temperature. The proximal part of the artery was cannulated with a glass micropipette (tip diameter ~125 μm) and secured with two fine nylon sutures. The artery was flushed with Tyrode solution containing 1% BSA to clear any intraluminal blood at a lumen pressure of <20 mmHg. The distal end of the artery was then cannulated, and both cannulation pipettes were connected to independent reservoirs set at the same height and solution level to ensure no flow. Both reservoirs were filled with Tyrode solution containing 1% BSA. The intraluminal pressure was then set to 50 mmHg by raising both reservoirs at the same time under no-flow conditions, and the artery was equilibrated for 30 min at 37°C. Tyrode solution in the myograph chamber was continuously superfused around the pressurized artery at a flow rate of 2–3 ml/min, after passing through an external reservoir that was bubbled with pure O_2. After equilibration, the vessel was pressured to 80 mmHg, and a longitudinal force was also applied to stretch the vessel until it appeared straight and then by an extra 10%. Pressure was decreased back to 50 mmHg, and the vessel was incubated for another 10 min before an experimental maneuver. A charge-coupled device (video camera module) camera attached to a light inverted microscope was used to monitor the arteries, and data were recorded to disk. External diameters of the vessels and luminal pressure were measured continuously using MyoView (version 1.1 P, 2000, Photonics Engineering).

Experimental protocols. Extraluminal application (bath administration) of phenylephrine and subsequent dilatation to acetylcholine (1 μM) were used to assess arterial viability. Arteries in which acetylcholine failed to completely reverse the constriction were discarded at this stage. Viable arteries were washed and incubated in Tyrode solution with 1% BSA in luminal solution for ~1 h and then preconstricted with phenylephrine (concentration varied to achieve similar constriction in different arteries, 0.1–4 μM) to achieve an approximate 60–80% reduction in vessel diameter. Once a stable diameter was obtained, flow (shear stress 3.5–10.7 dyn/cm^2) was initiated by establishing a pressure gradient (5–6 mmHg) by moving the two reservoirs an equal distance (5 cm) but in opposite vertical directions at the same time. This ensured that the change in flow did not cause a simultaneous change in transmural pressure. In some experiments, intraluminal flow rate was changed by varying the vertical distance between two solution reservoirs. The mean intraluminal pressure was maintained at 50 mmHg throughout the flow protocol. Changes in the luminal solution were achieved by switching a three-way valve to access another reservoir to perfuse the artery for 15–20 min. Changes in the superfusion (bath) solution involved perfusing the chamber with a new solution for 40–50 min. At the end of each experiment, the viability of the endothelium was again assessed by exposure to 1 μM acetylcholine. Any artery that did not develop >80% relaxation was discarded and the data were excluded. With inhibitors, vessels were preincubated with 1 μM 4-[(2*S*)-2-[(5-isoquinolinylsulfonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl]phenyl isoquinolinesulfonic acid ester (KN-62), 100 μM reactive blue 2, or/and 100 μM suramin for at least 40 min before exposure to the purinoceptor agonist 3′-*O*-(4-benzoyl)benzoyl ATP (BzATP; 10 μM) or 1 μM ATP.

Suppliers, drugs, and solutions. The pressure myograph was from Danish MyoTechnology; and the microscope was a Zeiss Axiovert 25 (inverted) microscope. Phenylephrine hydrochloride and suramin were obtained from RBI. Acetylcholine, ATP, 6-*N*,*N*-diethyl-D-β,γ-dibromomethylene ATP (ARL-67156), adenosine-5′-(3-thiotriphosphate)trilithium salt (ATP-γ-S), BzATP, UTP, adenosine 5′-(β,γ-imido)triphosphate tetralithium salt hydrate (AMP-PNP), α,β-methylene ATP, and BSA (fraction V) were purchased from Sigma. Reactive blue 2 and KN-62 were from Tocris. All chemicals were dissolved in H_2O.

The Krebs-Henseleit solution contained (in mM) 119 NaCl, 25 $NaHCO_3$, 1 $MgCl_2$, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, and 11 D-glucose. The Tyrode solution contained (in mM) 117 NaCl, 1 $MgCl_2$, 4.7 KCl, 1.2 KH_2PO_4, 1.6 $CaCl_2$, 10 HEPES, 30 D-mannitol, and 11 D-glucose; pH 7.4. pH was adjusted with 10 M NaOH.

Data analysis. Vasodilatation to flow was calculated as a percentage by following equation

$$\%\text{Dilatation} = 100 \times (D_f - D_{phe}/D_i - D_{phe})$$

where D represents the vessel external diameter; D_f is the maximum diameter during flow, D_{phe} is the diameter after phenylephrine constriction and before flow, and D_i is the initial diameter before phenylephrine constriction. Statistical evaluation of data was made using a paired or unpaired Student's *t*-test. Significance was assumed at $P < 0.05$. In all experiments, n is the number of mesenteric arteries from different rats.

RESULTS

Effect of ATP on flow-induced dilatation. Isolated small mesenteric arteries with a vessel external diameter between 300 and 450 μm at 50 mmHg were preconstricted with phenylephrine, and fluid flow was established to induce dilatation. Because flow-induced dilatation might be influenced by the extent of the initial vascular tone, different vessels were first preconstricted to a similar level (60–80% reduction in vessel diameter) by titrating the concentration of applied phenylephrine (0.1–4 μM). Fifty-four percent (53/99) of the vessels did not develop dilatation to flow at all, although they were able to dilate in response to acetylcholine (1 μM) (Fig. 1*A*). The rest [46% (46/99)] of the arteries showed dilatation to flow challenge (Fig. 1*B*). The flow-induced dilatation was transient, typically only lasting for <5 min in total (Fig. 1*B*). An additional flow challenge failed to elicit any dilatation (Fig. 1*B*). These vessels were washed with and stabilized in Tyrode solution for 1 h and then reconstricted with phenylephrine. Again, flow was still unable to elicit dilatation in these vessels (Fig. 1*B*).

In marked contrast, vessels that were initially bathed in an ATP (1 μM)-containing medium both intraluminally and extraluminally always developed marked dilatation to flow (100%, $n = 19$). Dilatation consisted of an initial transient peak of relaxation that was followed by a plateau phase, which continued as long as the flow was on (>10 min). Moreover, in the presence of ATP, the vessels were able to dilate repeatedly in response to flow challenges (Fig. 1*C*), and they responded to flow after wash and reconstriction with phenylephrine (Fig. 1*D*).

In vessels that did not develop dilatation to flow at all (53/99), the application of 1 μM ATP, through both the intraluminal and extraluminal solutions, enabled the vessels to respond with dilatation to flow (Fig. 2*A*; $n = 21$). As mentioned before, some vessels displayed initial flow dilatation in the absence of exogenously applied ATP but then failed to respond to a subsequent flow challenge, even after wash and stabilization in Tyrode solution for 1 h. However, the application of ATP also enabled these vessels to respond to flow (Fig. 2*B*; $n = 14$). We examined the effect of a ATP concentration dose on flow dilatation in the vessels that failed to dilate in the absence of exogenously applied ATP. In these experiments, a series of different concentrations of

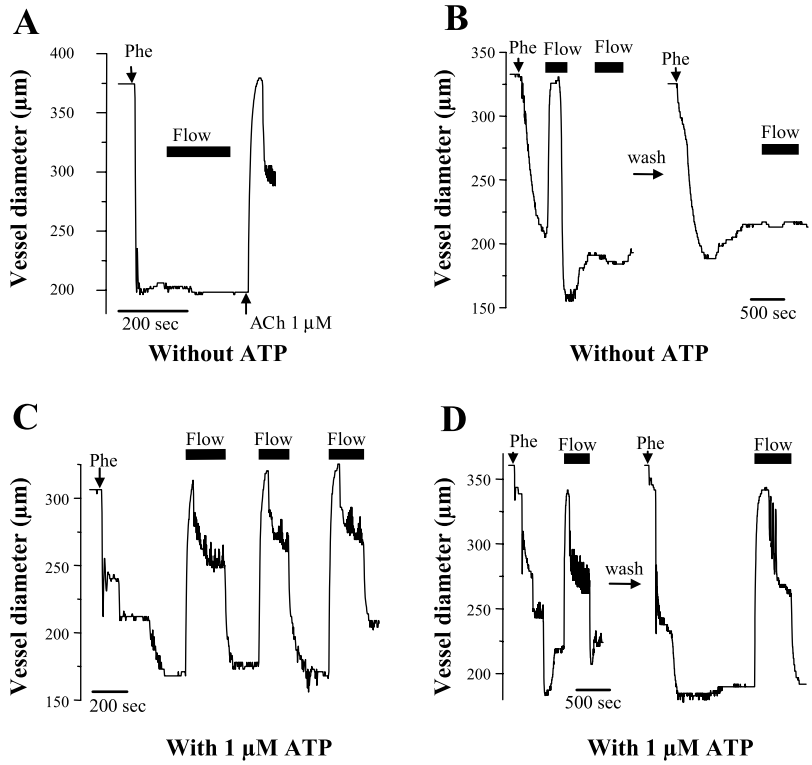

Fig. 1. Differential vascular responses to flow in the absence or presence of extracellular ATP. *A* and *B*: representative traces in the absence of ATP. *C* and *D*: representative traces in the presence of 1 μM ATP. The vessels were preconstricted with phenylephrine hydrochloride (Phe; 0.1–4 μM). In the time periods indicated by the solid bars on the top of the traces, intraluminal flow was applied with Tyrode solution containing 1% BSA (*A* and *C*). After the initial round of experiments, some vessels (*B* and *D*) were washed by and maintained in Tyrode solution for 1 h, followed by reconstriction with Phe and a second round of flow experiments. Acetylcholine (ACh; 1 μM) was added to demonstrate that the vessels were healthy. Each trace is representative of 6–14 experiments.

ATP from 30 nM to 3 μM were included in both intraluminal and extraluminal solutions. ATP increased the peak amplitudes of flow dilatation in a dose-dependent manner (Fig. 2, *C* and *D*). Furthermore, at a high ATP concentration of 1 or 3 μM, flow dilatation was more stable, and the peak amplitudes of dilatory responses to the first and second flow challenges were similar (Figs. 1*C* and 2*C*). In contrast, at a low ATP concentration of 30 or 100 nM, the dilatory responses to the first flow challenge were larger than the responses to the second flow challenge (Fig. 2*C*).

Taken together, these data clearly demonstrate that extracellular ATP is a crucial factor facilitating the flow-induced

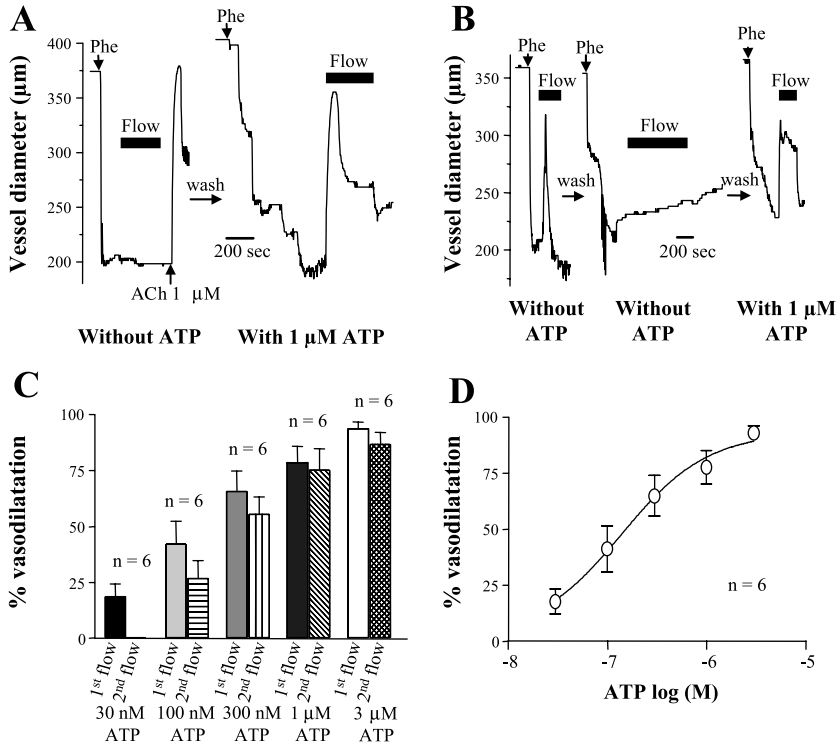

Fig. 2. Effect of ATP to enable nonresponding arteries to develop flow-induced dilatation. *A*: representative trace showing the switching effect of ATP in vessels that did not have initial flow dilatation. *B*: switching effect of ATP in vessels that were unable to respond to additional flow challenge. Vessels were preconstricted with Phe, and flow was applied in the time periods indicated by the solid bars on the top of the traces. After the initial round of experiments, the vessels were washed by and maintained in Tyrode solution for 1 h, followed by reconstriction with Phe and a second round of flow experiments. *C* and *D*: summary of the concentration-dependent effect of ATP on the peak amplitude of dilatory responses to the first (*C* and *D*) and second flow challenges (*C*) in vessels that did not have initial flow dilatation ($n = 6$). A representative trace for these experiments (*C* and *D*) can be found in Fig. 1*C* (1 μM ATP). Values are means ± SE.

dilatation in rat small mesenteric arteries. It enabled nonresponding arteries to respond to flow with dilatation.

Effect of UTP and BzATP on flow-induced dilatation. A number of purinoceptor agonists were used to investigate the specific type(s) of purinoceptor involved in the action of ATP. The results show that the P2Y purinoceptor agonist UTP (35) and the $P2X_7$ purinoceptor agonist BzATP (32) could mimic the action of ATP. If both intraluminal and extraluminal solutions contained UTP (1 μM) or BzATP (10 μM), all vessels displayed flow dilatation ($n = 5$ for each data set), and the magnitudes of flow dilatation were similar to those in ATP-containing solution (Fig. 3*C*). Furthermore, similar to the effect of ATP, both UTP and BzATP could enable the arteries to dilate in response to flow (Fig. 3, *A* and *B*). In the presence of either agent, flow dilatation was prolonged, and the vessels were able to dilate to repetitive flow challenges (Fig. 3, *A* and *B*).

BzATP is not specific to the $P2X_7$ receptor. It also activates $P2X_1$ receptors (18), and, in addition, it stimulates $P2Y_{11}$ receptors, causing Ins(1,4,5)P_3 production and intracellular Ca^{2+} release (8). KN-62, a potent noncompetitive antagonist of $P2X_7$ receptors (6), was therefore used to confirm the involvement of $P2X_7$ receptors. KN-62 (1 μM) reduced the effect of BzATP on flow dilatation by 21% (Fig. 3, *D* and *E*; $n = 8$), and this inhibitory effect was reversed after wash (Fig. 3*D*). These experiments support the notion that the effect of BzATP was at least partly due to its action on $P2X_7$ receptors. The residual effect of BzATP could be due to incomplete inhibition of KN-62 on $P2X_7$ receptors or the involvement of $P2Y_{11}$ receptors. The participation of $P2X_1$ receptors was unlikely because α,β-methylene ATP (5 μM), an agonist that acts mostly on $P2X_1$ and $P2X_3$ receptors, was found to be ineffective ($n = 5$).

Two additional purinoceptor antagonists, reactive blue 2 and suramin, were tested. Neither the P2Y antagonist reactive blue 2 (100 μM) nor the nonselective P2X and P2Y antagonist suramin (100 μM) individually had any effect on flow dilatation. However, the combined application of these agents significantly decreased the maximal dilatation to flow (Fig. 4, *A* and *B*). The inhibitory effect of reactive blue 2 plus suramin was reversible. After washout for ~50 min, the flow-induced dilatation recovered.

Effect of ATP-γ-S, AMP-PNP, and ARL-67156 on flow-induced dilatation. Previous studies have already demonstrated that ATP can augment flow-induced Ca^{2+} influx into vascular endothelial cells (12, 22, 37). An increased Ca^{2+} influx should stimulate the activity of NO synthase (NOS) and phospholipase

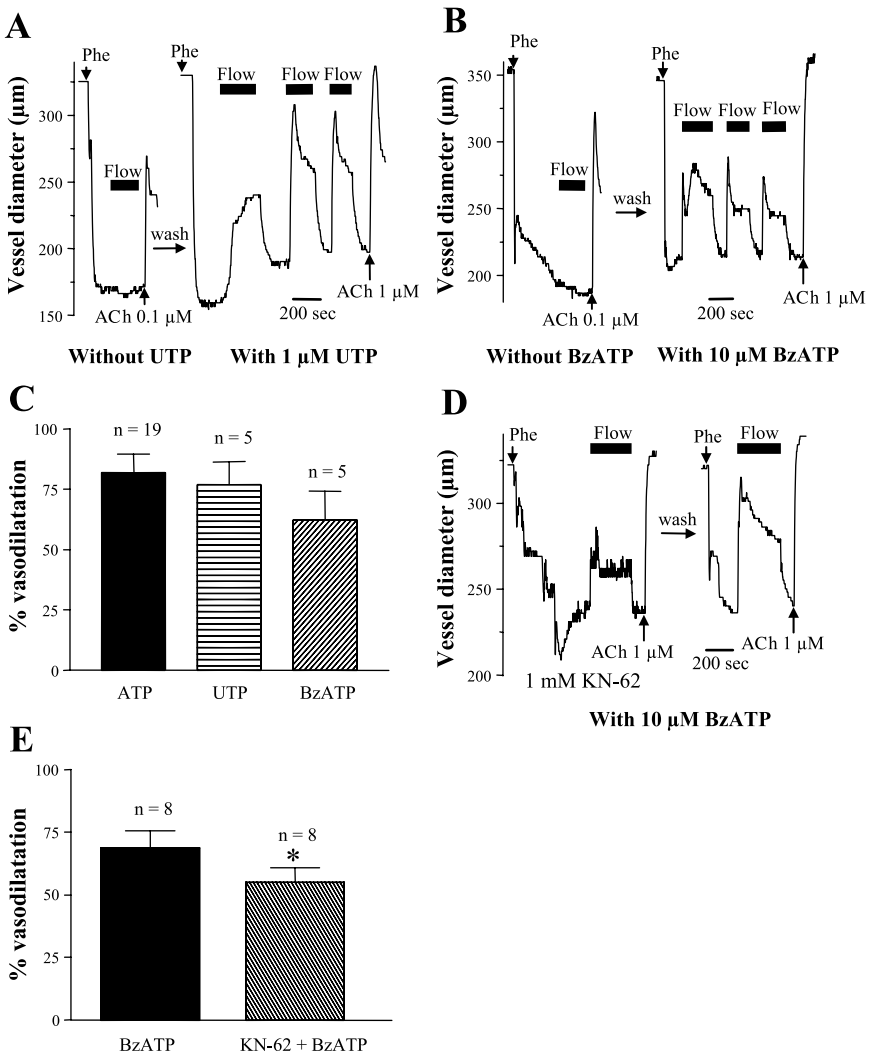

Fig. 3. Effect of UTP and 3′-*O*-(4-benzoyl)benzoyl ATP (BzATP) to enable nonresponding arteries to develop flow-induced dilatation. *A* and *B*: representative traces. The conditions were the same as in Fig. 2*A* except that 1 μM UTP (*A*) or 10 μM BzATP (*B*) was used to replace ATP. *C*: comparison of the peak flow vasodilatation with 1 μM ATP ($n = 19$), 1 μM UTP ($n = 5$), and 10 μM BzATP ($n = 5$). *D*: representative trace showing the effect of 1 μM KN-62 on dilatation in the presence of 10 μM BzATP. *E*: comparison of the peak flow responses with 10 μM BzATP and 1 μM KN-62 plus 10 μM BzATP ($n = 8$ for each group). Values are means ± SE. *$P < 0.05$.

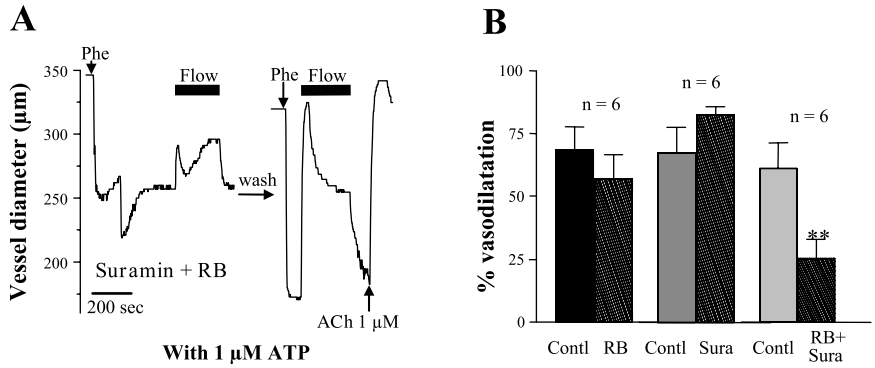

Fig. 4. Effect of reactive blue 2 (RB) and suramin (Sura) on flow-induced vasodilatation. *A*: representative traces showing the effect of 100 μM RB plus 100 μM Sura on dilatation in the presence of 1 μM ATP. The conditions were the same as in Fig. 1*D* except that the vessels were treated with RB and Sura. *B*: summary of responses in the presence of 100 μM RB alone, 100 μM Sura alone, and 100 μM RB plus 100 μM Sura ($n = 6$ for each group). Contl, control. Values are means ± SE. $^{**}P < 0.01$.

A_2 and activate K^+ channels, leading to the production and release of vascular relaxing substances (NO, PGI_2, and EDHF). This scheme suggests that the effect of ATP on flow-induced vasodilatation may involve the interaction of ATP with purinoceptors on the endothelial cell surface and that ectonucleotidase may influence the flow dilatation by hydrolyzing ATP. Two nonhydrolyzable ATP analogs, ATP-γ-S and AMP-PNP, were used to test this hypothesis. As with ATP, when either ATP-γ-S (1 μM) or AMP-PNP (10 μM) was included in both the intraluminal and extraluminal solutions, all arteries dilated in response to flow ($n = 5$). However, in the presence of 1 μM ATP, repetitive exposure of the arteries to flow caused reproducible dilatation of a similar magnitude (Figs. 1*C* and 5, *A* and *B*), whereas in the presence of ATP-γ-S or AMP-PNP, dilatation decreased to repetitive flow challenges. The peak dilatation obtained in response to the first flow challenge was the largest, but the responses to the second and later flow challenges decreased in magnitude for both ATP-γ-S (Fig. 5, *C* and *D*) and AMP-PNP (Fig. 5, *E* and *F*). Similar results were obtained in vessels that were bathed in 1 μM ATP together with 10 μM ARL-67156, an ectonucleotidase inhibitor (36), applied in the intraluminal solution (Fig. 5, *G* and *H*). These results are consistent with the notion that, in the absence of agonist degradation, more agonists are present on cell surface; therefore, additional ATP delivery by flow should cause less change in agonist concentration in the unstirred surface layer, resulting in a decreased flow-induced Ca^{2+} influx and a reduced flow dilatation.

Effect of flow rate on vasodilatation. If the facilitating effect of ATP on flow-induced dilatation is related to ATP delivery to the cell surface by flow and ATP degradation by cell surface ectonucleotidase, an increase in flow rate is expected to increase ATP delivery, thereby enhancing the dilatory response. To test this hypothesis, the intraluminal flow rate in the vessels was varied by changing the pressure difference between the two ends of the vessels while maintaining the transmural pressure constant. On the basis of Darcy's law, the flow rate is directly proportional to the pressure difference (1). Only those vessels that failed to dilate in the absence of exogenously applied ATP were used for the experiments. In the presence of 30 nM to 1 μM ATP, the vessels showed an increased dilatory response to flow as the pressure difference (and thus the flow rate) increased. The relationship between the flow dilatation and pressure difference (and thus flow rate) was proportional ($n = 6$; Fig. 6). These results agree with the hypothesis that flow may enhance dilatation by increasing ATP delivery to endothelial cell surface.

DISCUSSION

The major new finding of this study is that extracellular ATP can dramatically facilitate flow-induced dilatation in rat small mesenteric arteries. In the absence of extracellular ATP, only 46% of isolated mesenteric arteries dilated in response to a flow stimulus. The application of extracellular ATP (1 μM) enabled the nonresponding vessels to respond to flow. In the presence of ATP, all arteries developed dilatation to flow, and this response was more stable and repeatable than that in vessels without ATP present.

Several purinoceptor agonists and antagonists were used to determine the type(s) of purinoceptor(s) involved. It was found that the action of ATP could be mimicked by UTP, a P2Y agonist, and BzATP, a $P2X_1$, $P2X_7$, and $P2Y_{11}$ agonist. In the presence of either agent, all vessels developed marked dilatation to flow. Both agents could enable the nonresponding arteries to respond to flow with dilatation. These results suggest that both P2X and P2Y purinoceptors mediated the action of ATP. Within the P2X subgroup, the involvement of $P2X_7$ purinoceptors could be pinpointed based on the effectiveness of BzATP and the inhibitory role of KN-62 on BzATP action. $P2X_1$ and $P2X_3$ purinoceptors were not involved, because α,β-methylene ATP, an agonist that acts mostly on $P2X_1$ and $P2X_3$ purinoceptors, was ineffective. Because KN-62 could only reduced the action of BzATP by ~21%, it is likely that $P2Y_{11}$ purinoceptors, another target for BzATP, are also involved. We also tested the effect of reactive blue 2, a P2Y antagonist, and suramin, a nonselective P2X and P2Y antagonist. Neither reactive blue 2 nor suramin alone affected the flow-induced dilatation, but the combined administration of both agents significantly attenuated the dilatation. Because suramin cannot inhibit several P2Y subtypes such as $P2Y_4$ and $P2Y_6$ purinoceptors (32), a combined administration of suramin and reactive blue 2 is expected to inhibit additional subtypes of P2Y and P2X purinoceptors than suramin does alone. Taken together, these data support the involvement of both P2X and P2Y purinoceptors in mediating the action of ATP. As to the specific receptor subtypes, $P2X_7$ and $P2Y_{11}$ purinoceptors were likely to be involved, whereas $P2X_1$ and $P2X_3$ purinoceptors did not participate. Caution is needed in interpreting the data generated from the use of reactive blue 2 and suramin because these agents may disturb other cellular pathways in addition to their actions on P2 purinoceptors. However, these data are consistent with the hypothesis of dual participation by P2X and P2Y purinoceptors, which is mainly derived from the use of UTP, BzATP, and KN-62.

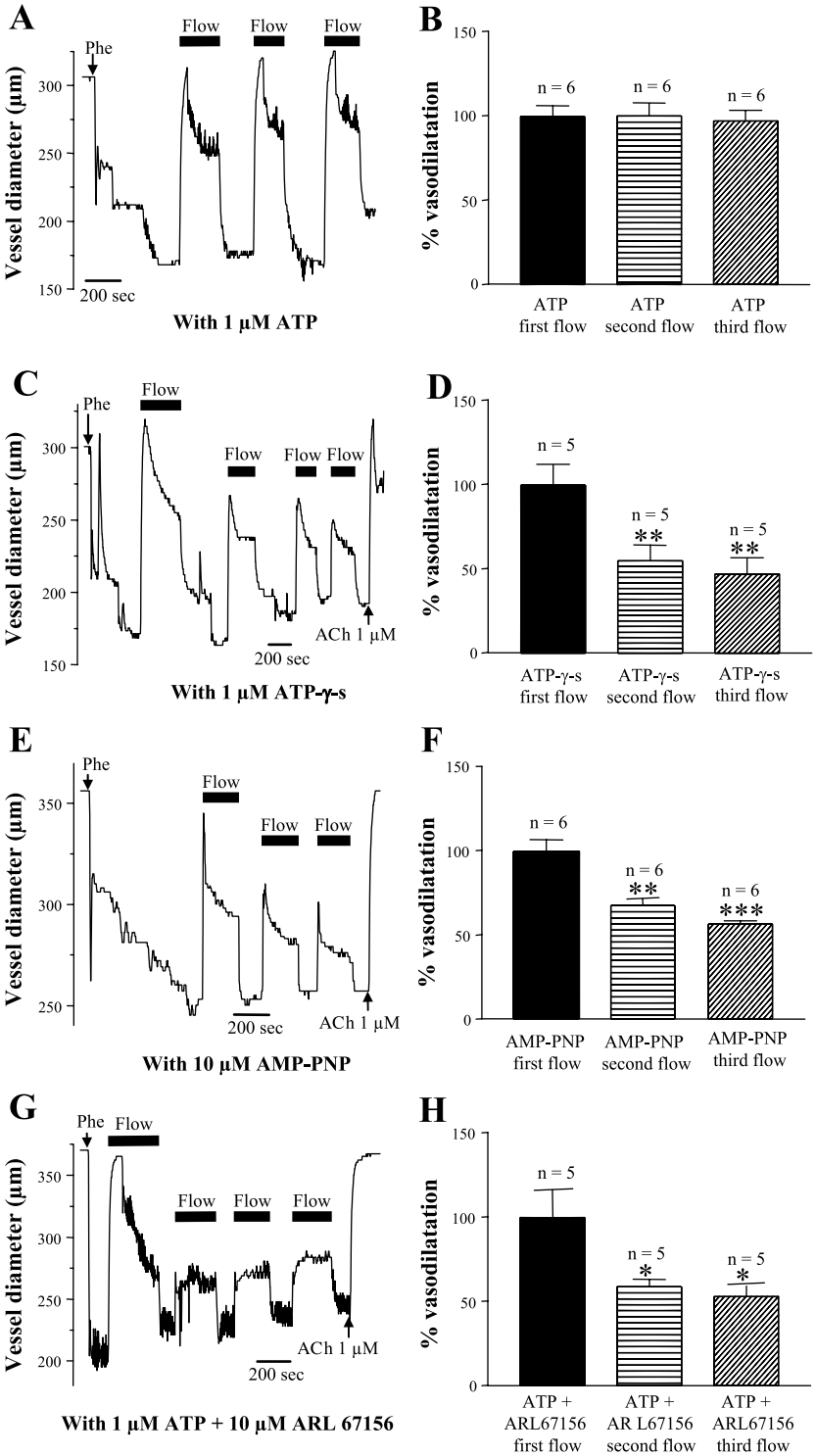

Fig. 5. Comparison of vasodilatation to flow in arteries in the presence of ATP, adenosine-5′-(3-thiotriphosphate)trilithium salt (ATP-γ-S), adenosine 5′-(β,γ-imido)triphosphate tetralithium salt hydrate (AMP-PNP), or ARL-67156 (only in the luminal solution) plus ATP. *A*, *C*, *E*, and *G*: representative traces showing the flow responses in the presence of 1 μM ATP (*A*), 1 μM ATP-γ-S (*C*), 10 μM AMP-PNP (*E*), or 10 μM ARL-67156 plus 1 μM ATP (*G*). *B*, *D*, *F*, and *H*: summary of the flow responses to repeated flow challenges in the presence of 1 μM ATP (*B*), 1 μM ATP-γ-S (*D*), 10 μM AMP-PNP (*F*), and 10 μM ARL-67156 plus 1 μM ATP (*H*). Values are means ± SE; n = 5–6 for each group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

We also explored the possible mechanism through which ATP enhanced flow-induced dilatation. Previous studies have already demonstrated that ATP can augment flow-induced Ca^{2+} influx into endothelial cells (12, 22, 37). A rise in $[Ca^{2+}]_i$ is expected to stimulate the activity of NOS and phospholipase A_2 and activate K^+ channels, leading to the production and release of vascular relaxing substances (NO, PGI_2, and EDHF) from the endothelium (7, 15). Concerning the mechanism of how ATP can enhance flow-induced increases in $[Ca^{2+}]_i$, a convection and diffusion model has been proposed (10, 12, 27). It is hypothesized that the processes of convection and diffusion regulate ATP delivery to the purinoceptors on the endothelial cell membrane. The amount of receptor binding then represents a balance between the delivery of ATP and its degradation by ectonucleotidases on the endothelial cell surface. Increases

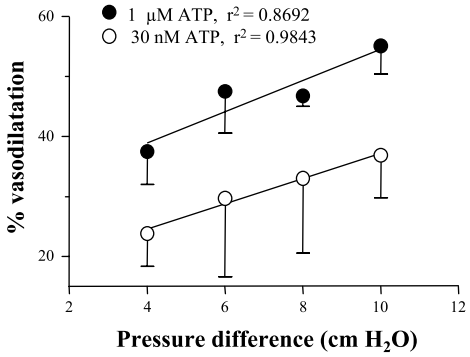

Fig. 6. Effect of flow rate on the peak amplitudes of flow dilatation. The vessels were preconstricted with Phe. The intraluminal flow rate in the vessels was varied by changing the pressure difference between the two ends of the vessels. The pressure difference was directly proportional to flow rate, and therefore it was used to represent the flow rate. Values are means ± SE; n = 6 for each ATP concentration.

in flow would be predicted to augment the delivery of ATP to the unstirred layer of endothelial cell surface and shift the balance in favor of receptor binding, causing an increased $[Ca^{2+}]_i$ response (10, 12, 27). If the effect of ATP on flow dilatation is mediated by the action of ATP on $[Ca^{2+}]_i$, it is likely that flow-induced dilatation may follow the principle of the convection and diffusion model. Several lines of evidence suggest that this may indeed be the case. First, in the presence of ATP, the amplitude of each dilatation in response to repeated exposure to flow was similar. In contrast, when the flow was applied in the presence of the nonhydrolyzable analogs ATP-γ-S or AMP-PNP, the maximal dilatation to the first flow challenge was the largest, but the responses to the second and later flow challenges decreased in magnitude (Fig. 5). These results agree with the notion that a nonhydrolyzable analog could not be effectively cleared from the endothelial cell surface by ectonucleotidases, and thus repeated application of flow might cause less change in agonist concentration in the unstirred surface layer. Second, inhibition of ectonucleotidases by the intraluminal administration of ARL-67156 also reduced the amplitude of flow responses to the second and later flow as ATP-γ-S and AMP-PNP did (Fig. 5, *G* and *H*), consistent with the concept that, as the amount of ATP cleared by ectonucleotidases decreases, additional ATP delivery by flow would cause less change in surface ATP concentration. Third, the magnitude of flow dilatation increased in proportional to the flow rate (Fig. 6). Furthermore, flow dilatation increased as the concentration of exogenously applied ATP increased (Figs. 2 and 6). These data also agree with the convection and diffusion model because the model predicts that an increase in flow rate should increase ATP delivery, thereby enhancing dilatory responses.

It is obvious that the convection and diffusion model alone could not fully explain the flow-induced dilatation. As mentioned, in the absence of agonist degradation, the amplitudes of flow dilatation in response to the second and later flow challenges were smaller than the one to the first challenge (Fig. 5, *C–H*). Nevertheless, the vessels still contracted each time when flow was turned off and again dilated when flow was subsequently reestablished under these conditions (Fig. 5, *C–H*). This contradicts with the convection and diffusion model, which predicts that the vessels should not develop additional dilatations to the second and later challenges in the absence of agonist degradation. Therefore, additional mechanisms, which could not be accounted for by the convention and diffusion model, are also involved in the flow vasodilatation. In fact, studies have well demonstrated that shear stress may induce vasodilatation via a Ca^{2+}-independent pathway (5). In this pathway, flow stress directly activates phosphatidylinositol 3-kinase (PI3K), which subsequently stimulates protein kinase B (Akt), causing an increased activity of endothelial NOS (eNOS) (5). Therefore, it appears that flow may induce vasodilatation through two separate mechanisms. One is Ca^{2+} dependent, and the other is Ca^{2+} independent. The convection and diffusion model cannot be used to explain the Ca^{2+}-independent pathway, because this pathway is directly activated by flow. Interestingly, the Ca^{2+}-independent component was also affected by ATP. Without exogenously applied ATP, the whole flow responses including the Ca^{2+}-dependent one (the one that can be accounted for by the convection and diffusion model) and the Ca^{2+}-independent one (the one that cannot be explained by the convection and diffusion model) all disappeared. The effect of ATP on Ca^{2+}-independent flow dilatation might be related to the fact that purinoceptor agonists themselves could stimulate the PI3K-Akt-eNOS pathway (17, 33), thereby potentiating the Ca^{2+}-independent dilatory response to flow.

The finding that ATP stimulates flow-induced dilatation has profound physiological implications. It has been reported that ATP is released from the endothelium (23, 25, 31, 34), and the release of ATP increases under conditions of intraluminal flow (4, 25). Our data suggest that the released ATP may influence flow-induced vascular dilatation. Consistent with this finding, a previous study has estimated that the amount of ATP released from the endothelium may be ~300 nM (23). This value is close to the concentration of ATP that can elicit a rise in $[Ca^{2+}]_i$ (23). ATP may not be the only Ca^{2+}-mobilizing agonist that may enhance the flow-induced vascular dilatation. Previous studies have demonstrated that endothelin-1 and angiotensin II can potentiate flow-induced vasodilatory responses (16, 24). It is likely that flow can also evoke the release of these Ca^{2+}-mobilizing agonists from endothelial cells and that these agonists in turn may serve to enhance the flow-induced vasodilatation.

In conclusion, the presence of extracellular ATP can dramatically increase the ability of mesenteric small resistance arteries to respond to flow challenge by dilating. In the presence of exogenous extracellular ATP, the flow-induced vasodilatation becomes more stable and reproducible. Both P2X and P2Y purinoceptors appear to mediate the action of ATP, whereas ectonucleotidases may influence the effect of ATP by causing ATP degradation on the endothelial cell surface.

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