Pannexin1 Regulates α1-Adrenergic Receptor-Mediated Vasoconstriction


Rationale: The coordination of vascular smooth muscle cell constriction plays an important role in vascular function, such as regulation of blood pressure; however, the mechanism responsible for vascular smooth muscle cell communication is not clear in the resistance vasculature. Pannexins (Panx) are purine-releasing channels permeable to the vasoconstrictor ATP and thus may play a role in the coordination of vascular smooth muscle cell constriction.

Objective: We investigated the role of pannexins in phenylephrine- and KCl-mediated constriction of resistance arteries.

Methods and Results: Western blot, immunohistochemistry, and immunogold labeling coupled to scanning and transmission electron microscopy revealed the presence of Panx1 but not Panx2 or Panx3 in thoracodorsal resistance arteries. Functionally, the contractile response of pressurized thoracodorsal resistance arteries to phenylephrine was decreased significantly by multiple Panx inhibitors (mefloquine, probenecid, and 10⁶Panx1), ectonucleotidase (apyrase), and purinergic receptor inhibitors (suramin and reactive blue-2). Electroporation of thoracodorsal resistance arteries with either Panx1-green fluorescent protein or Panx1 small interfering RNA showed enhanced and decreased constriction, respectively, in response to phenylephrine. Lastly, the Panx inhibitors did not alter constriction in response to KCl. This result is consistent with coimmunoprecipitation experiments from thoracodorsal resistance arteries, which suggested an association between Panx1 and α1D-adrenergic receptor.

Conclusions: Our data demonstrate for the first time a key role for Panx1 in resistance arteries by contributing to the coordination of vascular smooth muscle cell constriction and possibly to the regulation of blood pressure. (Circ Res. 2011;109:00-00.)

Key Words: pannexins • phenylephrine • adrenergic receptor • smooth muscle cells • vasoconstriction

In resistance arteries, the coordination of vascular smooth muscle cell (VSMC) constriction helps to regulate blood flow and peripheral resistance and thus overall blood pressure. Although it has been hypothesized that gap junctions link VSMCs in resistance arteries to provide this coordination, the gap junctional coupling of VSMCs is controversial, and the presence of gap junctions and the gap junction proteins (connexins) is difficult to observe. Thus, VSMCs may use other mechanisms to communicate and coordinate their responses.

Pannexins (Panx) are tetra-spanning membrane proteins that mediate paracrine intercellular communication via release of purines such as ATP or UTP. The physiological function of Panx remains poorly documented in the vasculature, where there is currently no indication of Panx expression or function. We thus hypothesized that pannexins may coordinate VSMC constriction through the release of purines and activation of purinergic receptors.

Methods

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

Mice

Male mice (12 to 16 weeks) were used according to the University of Virginia Animal Care and Use Committee guidelines.
Electron Microscopy
Thoracodorsal resistance arteries (TDAs) were immunolabeled with Panx1 antibody and 10-nm gold beads and imaged with a JEOL 6400 scanning electron microscope.

Vessel Transfection
The VSMCs of TDAs were transfected with either a plasmid that contained Panx1-green fluorescent protein or Panx1 small interfering RNA and were cultured for 16 to 18 hours.

Coimmunoprecipitation
Panx1 or α1D-adrenergic receptor antibody was conjugated to Dynabeads, incubated with TDA lysates, and run on an SDS-PAGE gel.

Statistical Analysis
One-way or 2-way ANOVA, followed by a Bonferroni test, was used for comparisons between treatments. *P*<0.05 was significant.

Results
Panx1 Is Expressed in VSMCs of TDAs
Using transmission electron microscopy, we could not observe gap junctions between VSMCs in TDAs but could clearly identify tight junctions between endothelial cells (ECs; Figure 1A). The absence of membrane apposition was confirmed in other resistant arteries, whereas gap junctions could be observed between VSMCs in mouse aorta (Online Figure I). Therefore, we tested for the presence of the 3 Panx isoforms in TDA. Using Western blot (Figure 1B) and immunolabeling, we could only identify Panx1, both in ECs and VSMCs and between VSMCs (Figures 1C–E). In mouse aorta, we could not detect any Panx isoform in the VSMCs (Online Figure I). Immuno-scanning electron microscopy on TDAs with an extracellular loop Panx1 antibody revealed the presence of the protein on the VSMC plasma membrane (Figure 1F).

Phenylephrine-Induced Constriction Is Mediated by Panx
The data above clearly demonstrate that Panx1 is expressed in VSMCs; however, its functional role in the resistance arteries is unknown. We therefore constructed dose-response curves using the α1-adrenergic receptor agonist phenylephrine in the presence of 3 Panx inhibitors: mefloquine (Figure 2A), 10Panx1 peptide (Figure 2B), and probenecid (Figure 2C). Panx inhibitors all significantly reduced phenylephrine-induced vasoconstriction.

Figure 1. Panx1 is expressed in the membrane of VSMCs in TDA. A, Representative transmission electron microscopy image of TDA demonstrates close apposition (arrow) between ECs, whereas VSMCs are separated by a large intercellular space (arrowheads). B, Duplicate Western blots of TDA lysates for the 3 Panx isoforms (Panx1, Panx2, and Panx3) and Panx1 antibody incubated with cognate peptide. C, Immunofluorescence of a transverse section of TDA labeled for Panx1 (red); nuclei are stained with DAPI (blue). D, Immunofluorescence of a lateral view of VSMCs of a TDA labeled for Panx1 (red); arrow indicates direction of blood flow. E, Immuno-transmission electron microscopy labeling of TDA for Panx1; enlargement of red box is shown on right. F, Representative scanning electron microscopy of a TDA (left); enlarged images of VSMCs (right) from immuno-scanning electron microscopy labeled for Panx1 (gold beads were pseudocolored in pink). Samples were labeled with antibodies against the extracellular loop of Panx1 (EL-Panx1) or the C-terminal of Panx1 (CT-Panx1). VSMCs without labeling or primary antibodies are also shown. Scale bar, 1 μm in A and E, 5 μm in D, and 10 μm in C and F. Asterisks, vessel lumen.
(Figures 2A–C; Online Figures II and III) but did not affect ATP-induced vasoconstriction (Online Figure IV).

The Panx channels release purines, which induce constriction when applied to TDA (Online Figure IV). We thus tested the effect of apyrase, an ectonucleotidase that degrades purines. Both 1 and 10 U/mL significantly reduced TDA constriction in response to phenylephrine (Figure 2D; Online Figures II and III). Purines such as ATP and UTP are known to bind purinergic receptors present on VSMCs; therefore, we treated the vessel with suramin, a purinergic receptor inhibitor (Figure 2E; Online Figures II and III), and reactive blue-2 (Figure 2F; Online Figures II and III), which inhibits the P2Y subfamily of purinergic receptors. Both antagonists significantly inhibited phenylephrine-induced constriction. To test whether phenylephrine-induced constriction was due to Panx1 lo-

Figure 2. Panx is involved in phenylephrine constriction of TDA. The effect of cumulative concentrations of phenylephrine on internal diameter of pressurized TDA was significantly decreased after treatment with Panx inhibitors mefloquine (A; 10 μmol/L), 100Panx1 peptide (B; 200 μmol/L), and probenecid (C; 2 mmol/L and 500 μmol/L). D, Effect of apyrase (1 and 10 U/mL). We also inhibited purinergic receptors with suramin (E; 100 and 300 μmol/L) and P2Y receptors with reactive blue-2 (F; 75 μmol/L). n Indicates number of vessels; value in parentheses is the number of mice. *P<0.05.

Figure 3. Modulation of Panx1 expression in VSMCs modifies phenylephrine-induced constriction of TDA. The VSMCs of TDAs were transfected with either a plasmid containing Panx1-green fluorescent protein (Panx1-GFP) or Panx1 small interfering RNA (siRNA). A. The effect of cumulative concentrations of phenylephrine was investigated in each condition. B, Immunofluorescence of TDA transfected with Panx1-GFP (top), untransfected TDA (middle), and TDA transfected with Panx1 siRNA (bottom) labeled for Panx1. Scale bar, 5 μm. Asterisks in A indicate P<0.05 and in B, vessel lumen. In B, arrows point to internal elastic lamina, arrowheads point to EC.
calized on ECs, we perfused probenecid, apyrase, and reactive blue-2 in the lumen of TDAs and found no changes in phenylephrine responses, a result consistent with experiments on endothelium-denuded TDAs in which probenecid inhibited phenylephrine-induced constriction to the same extent as observed on intact TDAs (Online Figure V). Lastly, cultured VSMCs from coronary resistance arteries (expressing both α1D-adrenergic receptor and Panx1) had significant increases in ATP release after phenylephrine stimulation that was inhibited by 10Panx1, whereas cultured aortic VSMCs (expressing only α1D-adrenergic receptor) and normal rat kidney cells (which do not express α1D-adrenergic receptor or Panx1) did not have any increase in ATP release after phenylephrine stimulation (Online Figure VI).

Modulation of Panx1 Expression Modifies Phenylephrine-Induced Constriction of TDAs

TDAs were transfected either with Panx1-green fluorescent protein or with Panx1 small interfering RNA (Online Figure VII). When Panx1 was overexpressed, the response of TDAs to phenylephrine was increased by approximately 30%, whereas underexpression of Panx1 induced a 45% decrease in constriction in response to phenylephrine (Figure 3A; Online Figure VIII). Transfection of TDAs with control small interfering RNA did not affect phenylephrine-induced constriction (Online Figure VII). Immunolabeling of transfected TDAs revealed that Panx1 expression was modified only in VSMCs and not in ECs (Figure 3B), which was confirmed with an anti-green

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Figure 4. Panx1 is associated with α1D-adrenergic receptor and is not involved in KCl constriction. A. Previously used inhibitors (mefloquine, 10 μmol/L; 10Panx1, 200 μmol/L; probenecid, 2 mmol/L; apyrase, 10 U/mL; suramin, 300 μmol/L; or reactive blue-2, 75 μmol/L) had no effect on 40 mmol/L KCl-induced constriction. However, the L-type Ca<sup>2+</sup> channel inhibitor diltiazem (10 μmol/L) significantly inhibited KCl-induced constriction. *P<0.05. RB-2 indicates reactive blue-2. B. Immunolabeling of transverse sections of TDA labeled for Panx1 (red) and α1D-adrenergic receptor (green). Nuclei are stained in blue; asterisks indicate lumen. Arrows point to colocalized labeling for Panx1 and α1D-adrenergic receptor. Scale bar, 10 μm. C. Immunogold labeling of TDA for Panx1 (arrows) and α1D-adrenergic receptors (arrowheads). Scale bar, 1 μm. IEL indicates internal elastic lamina. D. Interaction between Panx1 with α1D-adrenergic receptors was revealed when lysates from TDAs were immunoprecipitated (IP) either with α1D-adrenergic receptor (α1D; top left) or Panx1 (bottom left) and blotted (Western blot [WB]) with Panx1 or α1D-adrenergic receptor antibodies, respectively. Panx1 and α1D-adrenergic receptor pull down were verified by blotting with Panx1 or α1D-adrenergic receptor antibodies (middle), and the absence of IgG was also controlled (right). E. Summary of phenylephrine (PE)-induced vasoconstriction. Application of phenylephrine stimulates α1D-adrenergic receptor (α1D; 1), followed by activation of Panx1 channel (2), which leads to the opening of the Panx1 channel and the release of purines in the extracellular space (3). Purines bind to P2Y receptors (4), which reinforces the α1D-adrenergic receptor constriction (5).
fluorescent protein antibody (Online Figure VII). Endothelial and smooth muscle function were not altered by transfection (Online Figure IX).

Panx1 Is Associated With α1-Adrenergic Receptor and Is Not Involved in KCl Constriction

We investigated whether Panx inhibitors could alter a receptor-independent stimulus such as KCl. We tested the 3 different Panx inhibitors and the purinergic inhibitors and found that none of them affected TDA constriction in response to KCl (Figure 4A). We thus hypothesized that a specific interaction may occur between Panx1 and α1D-adrenergic receptor, the primary adrenergic receptor responsible for VSMC constriction (Online Figure X). Immunolabeling of TDAs with Panx1 and α1D-adrenergic receptor antibodies revealed colocalization of the 2 proteins (Figures 4B and 4C). The association between Panx1 and α1D-adrenergic receptor was confirmed by coimmunoprecipitation from TDA lysates (Figure 4D).

Discussion

In the microcirculation, coordination of VSMC contraction is essential for the regulation of blood flow distribution and peripheral vascular resistance. Gap junctions are likely responsible for VSMC coordination in conduit arteries; however, their role in VSMC coordination in resistance arteries remains unclear. Our transmission electron microscopy results indicate that VSMCs of TDAs are unlikely to be coupled exclusively via gap junctions, because the VSMCs are separated by relatively large intercellular spaces. The close apposition of EC plasma membranes, characteristic of gap junctions, indicates that the intercellular space observed between VSMCs was not an artifact of fixation. Our demonstration of Panx1 expression could suggest a mechanism that allows VSMCs to coordinate their responses independent of gap junctions.

Pannexin channels release purines in response to different stimuli. In the vasculature, the concentration of purines is finely regulated by ectonucleotidase at the surface of VSMCs and contributes to local regulation of vascular tone. Our findings suggest that Panx1, purines, and purinergic receptors are involved in TDA constriction in response to phenylephrine. However, we also found staining for Panx1 in ECs, which are known to release ATP, although our data show that the inhibition of phenylephrine responses was not due to Panx1 in ECs. Nevertheless, Panx1 expression in ECs suggests that Panx1 could be involved in EC functions such as vasodilation or inflammatory cell adhesion.

The present data imply that Panx1 is specifically involved in phenylephrine-induced, but not KCl-induced constriction. This suggests that Panx1 is not activated solely by the rise of intracellular calcium concentrations or mechanical stretch. Reports from cell culture studies have suggested that purine release through Panx1 may occur after activation of Gα11-coupled α1-adrenergic receptor but not after stimulation of Gs-coupled α2-adrenergic receptors or Gq-coupled β-adrenergic receptors. Thus, Panx1 could also be involved in TDA constriction in response to other Gα11-coupled receptor agonists, such as serotonin, endothelin-1, or angiotensin II. However, our evidence indicates that Panx1 and α1D-adrenergic receptor are closely associated at the protein level, which suggests that Panx1 and the α1-adrenergic receptor may be part of a signaling microdomain.

The present data provide the first demonstration of Panx expression in the vasculature and of its function in the control of vasoconstriction. Our results suggest that the release of purines through Panx1 channels on VSMCs is triggered by phenylephrine stimulation and participates in control of vascular tone through purinergic receptors (Figure 4D). Thus, Panx1 could contribute to the coordination of VSMC constriction and the regulation of blood pressure via catecholamines released by sympathetic nerves.

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Disclosures

None.

References


**Novelty and Significance**

**What Is Known?**
- Intercellular communication between smooth muscle cells (SMCs) within the vascular wall is essential for the control of vasoreactivity, but the mechanism for this remains unclear.
- Pannexin channels participate in intercellular communication through the release of purines such as ATP, a potent vasoconstrictor.

**What New Information Does This Article Contribute?**
- Pannexin1 (Panx1) is present in the SMCs of resistant arteries and plays a role in phenylephrine-induced vasoconstriction.
- Panx1 and the α1D-adrenergic receptor are part of the same protein complex.
- When phenylephrine binds to the α1D-adrenergic receptor, Panx1 opens to release purines that can act on purinergic receptors present on SMCs to enhance phenylephrine-induced vasoconstriction.

Intercellular communication between SMCs serves to regulate the vasoconstriction of resistance arteries, a fundamental process for the control of blood flow and peripheral resistance; however, the exact mechanisms of SMC communication remain unclear. Here, we focused on Panx1, a protein known to participate in the release of ATP that has not been described in the vasculature. Our data indicate that Panx1 releases ATP during contraction of resistance arteries in response to phenylephrine, thereby controlling the intensity of vasoconstriction. These data are the first to describe the presence of, and a role for, Panx1 in the vascular wall, providing a novel mechanism for SMC communication.
Pannexin1 Regulates α1-Adrenergic Receptor–Mediated Vasoconstriction

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**Supplementary methods**

**Western blot**
Thoracodorsal arteries (TDA) from 4 mice were homogenized on ice in lysis buffer containing Tris-HCl 50 mmol/L, NaCl 150 mmol/L, EDTA 5 mmol/L, sodium deoxycholate 1%, Triton X-100 0.5%, Na3VO4 500 µmol/L, AEBSF 10 µmol/L, NaF 10 mmol/L, in PBS and pH adjusted to 7.4. Protein lysates were sonicated and subjected to electrophoresis on 4-12 % Bis-Tris gels (Invitrogen), transferred to nitrocellulose, and visualized and analyzed using Li-Cor Odyssey Imager as previously described1.

**Immunofluorescence**
Mice were deeply anesthetized with pentobarbital (40 mg/kg) and perfused transcardially with 5 ml of heparinized phosphate-buffered saline (PBS) followed by 5 ml of 4 % paraformaldehyde in PBS. TDA were isolated, placed in 4 % paraformaldehyde for 1 hour and then placed in 70 % ethanol for further paraffin embedding. Paraffin was removed and sections were subjected to immunohistochemistry as previously described2. For double immunolabelling experiments, sections were first incubated with Panx1 antibody and the secondary antibody coupled to Alexa Fluor 543. Sections were then incubated with the blocking solution for 6 hours and with α1D- adrenoreceptor antibody followed by incubation with the secondary antibody coupled to Alexa Fluor 647. Imaged for the double immunostaining were captured with a Zeiss LSM 700.

**Electron microscopy**

**Ultrastructure electron microscopy**
Mice were deeply anesthetized with pentobarbital (40 mg/kg) and perfused transcardially with 5 ml of heparinized phosphate-buffered saline (PBS) followed by 5 ml with 4% paraformaldehyde and 2% glutaraldehyde in PBS. Thoracodorsal arteries were isolated and ultrastructure TEM images were obtained as previously described1.

**Immunolabeling on TEM sections**
Mice were deeply anesthetized with pentobarbital (40 mg/kg) and perfused transcardially with 5 ml of heparinized phosphate-buffered saline (PBS) followed by 5 ml with 4% paraformaldehyde and 0.5% glutaraldehyde in PBS. Thoracodorsal arteries were isolated and processed for ultrathin sections and immunogold labeling as previously described3. For double immunolabelling experiments, sections were first incubated with Panx1 antibody and the secondary antibody coupled to 25 nm gold beads. Sections were then incubated with the blocking solution for 6 hours and with α1D- adrenoreceptor antibody followed by incubation with the secondary antibody coupled to 10 nm beads.

**Immunolabeling coupled to SEM**
Mice were deeply anesthetized with pentobarbital (40 mg/kg) and perfused transcardially with 5 ml of heparinized phosphate-buffered saline (PBS) followed by 5 ml of 4% paraformaldehyde and 0.5% glutaraldehyde in PBS. Thoracodorsal arteries were isolated and placed for 30 minutes in 4% paraformaldehyde and 0.5% glutaraldehyde at 4°C. After a quick wash in PBS, adventitia was removed in 2 steps: first the vessels were treated with 1 mg/mL collagenase type VIII (Sigma) supplemented with 55 ug/mL CaCl2 in PBS at 37°C for 4 hours on a shaker; second, after a quick wash in PBS, vessels were placed in 30% KOH in PBS and heated at 60°C for 4 minutes. Vessels were then subjected to immunolabeling following the same protocol used for immunolabeling on TEM sections except that samples were not permeabilized. Vessels were fixed with 1% osmium tetraoxide for 1 hour, and after a quick wash in PBS, vessels were successively dehydrated in 40%, 60%, 80% and 100% ethanol for 10 minutes each following a final dehydration using the critical point dryer. Finally, vessels were coated with gold and imaged using a JEOL 6400 scanning electron microscope.

**Vessel Cannulation:**
Mice were sacrificed with an overdose of pentobarbital (60-90 mg/kg) injected intraperitonealy. First order thoracodorsal arteries (TDA) were isolated (with an internal diameter of approximately 200-250 µm) and placed in Krebs-HEPES buffer 4. Arteries were cannulated at both ends with glass micropipettes.
and secured with 10-0 nylon suture in a pressure myograph (Danish MyoTechnology). The chamber was superfused with Krebs-Hepes solution, gassed with air, and maintained at 37°C while the vessel was perfused with Krebs-HEPES supplemented with 1% BSA. Vessels in the denuded (“-E”) groups were perfused with air for 5-10 minutes. Vessels were maintained in a no-flow state and held at a constant transmural pressure of 80 mmHg equivalent to the transmural pressure of these vessels in vivo 5, 6. Vessels were allowed to equilibrate for 30 minutes before stimulation either with cumulative concentrations of phenylephrine (10^{-8} to 10^{-4} mol/L), ATP (10^{-8} to 10^{-3} mol/L) or a single dose of KCl (40 mmol/L). When indicated, drugs were applied to the vessel during the equilibration period and the initial diameter was determined at the end of this equilibration period. Integrity of endothelial function was assessed at the end of each agonist-induced constriction using 10 µmol/L acetylcholine. To determine whether the smooth muscle function was fully intact in the endothelium denuded groups (“-E”) compared to the intact groups (“E”), vessels were superfused with 40 mM KCl solution for 20 minutes.

**Vessel transfection**

Mice were sacrificed with an overdose of pentobarbital (60-90 mg/kg) injected intraperitonealy. Thoracodorsal arteries were isolated and placed in RPMI media supplemented with penicillin (2mM)/streptomycin (50 U/mL) (Gibco) and CaCl₂ (2mmol/L). The TDA were then transferred to a cuvette containing 100 µl of Nucleofector solution (Lonza) complemented either with 5 µg of a plasmid containing a Panx1-GFP construct previously described 7, 10 nmol/L of siRNA targeting Panx1 (Ambion) or 10 nmol/L of control siRNA (Thermoscientific). The cuvette was placed in the Nucleofector cuvette holder and subjected to electroporation (program A033). Arteries were then placed in RPMI supplemented with penicillin (2mM)/streptomycin (50 U/mL) (Gibco), CaCl₂ (2mmol/L) and BSA 1% in an incubator for 14 to 18 hours. After incubation, TDA were cannulated and pressurized at 40 mmHg for 15 minutes and then at 80 mmHg for 15 minutes before performing dose response to PE.

**Co-immunoprecipitation**

Rabbit IgG/IgM Dynabeads (Invitrogen) were washed three times with 200 µL of Blocking Buffer (0.5 % BSA, 0.2 % Fish Skin Gelatin in PBS; pH was adjusted to 7.4) to block non-specific binding sites. During each wash, beads were gently mixed by rotation for five minutes. Beads were then pulled down with a magnet for one minute and the supernatant was removed and replaced with fresh blocking buffer. Dynabeads were incubated with either rabbit anti-α₁D-adrenoreceptor antibody (Alomone, 1:30) or rabbit anti-Panx1 CT-395 antibody (1:50) overnight at 4°C. Antibodies were conjugated to Dynabeads by washing twice with conjugation buffer (Sodium phosphate 20 mmol/L, NaCl 150 mmol/L, pH adjusted to 7.4) followed by incubation with 5 mmol/L BS³ (Thermo Scientific) in conjugation buffer and a final wash with Quenching Buffer (1mol/L Tris-HCl, pH adjusted to 7.4). Beads were then washed three times with blocking buffer and stored at 4°C until use. Protein extracts from TDA were prepared as described above in the western blot section. Protein extracts from TDA were incubated with antibody-conjugated Dynabeads for 2 hours at 4°C on tube rotator. The beads were then pulled down with a magnet and washed twice with lysis buffer for 15 minutes followed by a final wash with PBS at 4°C. Beads were then pulled down with a magnet, resuspended in Laemmli buffer and incubated for 10 minutes at room temperature to remove bound proteins. Samples were subjected to SDS-PAGE, transferred to nitrocellulose and labeled with either rabbit anti-α₁D-adrenoreceptor antibody (1:250) or Rabbit anti-Pannexin 1 CT-395 antibody (1:250).

**ATP assay**

Primary mouse aortic smooth muscle cells and human coronary smooth muscle cells (Lonza) were grown in DMEM F12 supplemented with 10%. Normal rat kidney cells (NRK) were cultured in DMEM high glucose supplemented with 10% FBS. Cells were placed in DMEM supplemented with 1 % BSA one hour before the experiment to prevent further ATP degradation by FBS. Cells were treated for 5 minutes with the pannexin inhibitors 10Panx1 (300 µM) prior to stimulation with 10^{-4} M PE. The media were collected 15 minutes after 10^{-4} PE and 50 µL of the media was mixed with 50 µL of CellTiter-Glo reagent.
(Promega) in an opaque-walled 96 well plate according to the manufacturer’s instructions. Luminescence was measured with a 1450 Microbeta TriLux luminometer (Perkin Elmer).

**Statistics**
All results are expressed as mean ± SEM. Statistics were performed using Origin Pro 6.0 software. All experiments were analyzed using 1-way ANOVA or 2-way ANOVA followed by Bonferroni’s post test for differences between treatments where indicated. \( P \) value less than 0.05 was considered significant.

**Antibodies**
Antibodies directed against the C-tail of Panx1 (mPanx1 CT-395), Panx2 (mPanx2 CT-494) and Panx3 (mPanx3 CT-379) have been previously described. Additionally, an antibody targeting the extracellular loop of Panx1 (mPanx1 EL2-247) was used for immuno-SEM experiments.

**Drugs**
Probenecid, diltiazem, reactive blue-2 (RB-2) and apyrase were purchased from Sigma. Mefloquine and suramin were respectively purchased from Bioblocks and Fisher. Panx1 was produced by GenScript.


Supplementary Figure Legends

Supplementary figure I: SMC from large vessels are coupled with gap junctions as opposed to SMC from resistance vessels. Representative TEM of transverse sections of three resistant arteries: artery from corporal tissue (A), cremaster artery (B) and coronary artery (C); and of a non-resistant vascular bed: the mouse aorta (D). These images demonstrate close apposition with the presence of gap junctions (arrows) between SMC only in the aorta whereas SMC are separated by a large intercellular space (arrowheads) in resistant arteries. Immunolabelling for Panx1 (E), Panx2 (F) and Panx3 (G) on transverse sections of aorta. Pannexin staining is shown in red, nuclei are stained in blue and the elastic lamina autofluorescence appears in green. Scale Bar is 1µm in A through D and each box in E, F and G is 100 µm x 100 µm. “*” indicates vessel lumen, IEL=Internal Elastic Lamina, RBC=Red Blood Cells.

Supplementary figure II: Original recordings of vasoreactivity experiments on cannulated TDA. Representative traces illustrating the development of tone (A) and the contractile response to cumulative concentration of PE (B) in TDA in absence of pharmacological agent (control) or in presence of Mefloquine 10 µmol/L, 10µmol/L, Probenecid 2 mmol/L, Apyrase 10 U/mL, Suramin 300 µmol/L, Reactive Blue-2 100 µmol/L.

Supplementary figure III: cumulative concentration response curves to PE up to 10^{-2} mol/L. Effect of Mefloquine 20 µmol/L, 10µmol/L, Probenecid 500 µmol/L, Apyrase 10 U/mL, Suramin 300 µmol/L, Reactive Blue-2 100 µmol/L on dose response to PE from 10^{-8} mol/L to 10^{-2} mol/L demonstrate that all the pharmacological agents induce an upward shift in the dose response curves.

Supplementary figure IV: Effect of pannexin inhibitors on ATP induced constriction. The effect of Mefloquine 10 µmol/L, 10µmol/L, Probenecid 2 mmol/L on cumulative concentrations of ATP (from 10^{-8} to 3.10^{-7}) shows that pannexin inhibitors do not alter ATP vasoconstriction of TDA.

Supplementary Figure V: Endothelial Panx1 is not involved in the response to PE. When applied only intraluminaly, RB-2 (A, 75µmol/L), probenecid (B, 2mmol/L), and apyrase (C, 1 U/mL) do not modify PE induced constriction. Furthermore, denudation of endothelium does not affect the inhibitory effect of 2 mmol/L Probenecid on PE response (D).

Supplementary figure VI: Effect of PE on ATP release in coronary SMC, aortic SMC and NRK cells. Immunofluorescence of coronary SMC (A), aortic SMC (B) and NRK cells (C) demonstrates the presence of both Panx1 and α1D-adrenoreceptor in cultured resistance artery SMC (coronary SMC) whereas only α1D-adrenoreceptor is present in aortic SMC and neither Panx1, nor α1D-adrenoreceptor is expressed in NRK cells. Each box is 50 µm x 50 µm. (D) The release of ATP was measured after stimulation of each cell type with PE (10^{-4} M) and revealed a release of ATP after PE stimulation only in cultured resistance artery SMC whereas aortic SMC and NRK cells did not release ATP after PE stimulation.

Supplementary figure VII characterization of transfection of Panx1-GFP and Panx1 siRNA in TDA. (A) Immunofluorescence of transverse sections of non transfected TDA (left panel) or transfected (right panel) with the Panx1-GFP plasmid (5 µg). Immunolabeling of sections for GFP revealed the presence of GFP (red) in VSMC of vessels transfected with the Panx1-GFP plasmid whereas the staining is absent from the non transfected artery. Enlarged images are shown in bottom panel. IEL = Internal Elastic Lamina. Scale Bar: 10 µm. (B) Western blot of protein lysates of TDA transfected with Panx1-GFP (5 µg), Panx1-siRNA (10 nmol/L), control siRNA (10 nmol/L) and non transfected TDA (upper panel). Quantification of the Panx1 band
normalized to the GAPDH band demonstrate that the amount of Panx1 protein is decreased when TDA are transfected with Panx1 siRNA while the amount of endogenous Panx1 is unchanged when TDA are transfected with Panx1-GFP or control siRNA or in control electroporated TDA (lower panel). (C) Effect of transfection of TDA with control siRNA (10 nmol/L) on PE response.

Supplementary figure VIII: Original recordings of vasoreactivity experiments on transfected TDA. Representative traces illustrating the development of tone (A) and the contractile response to cumulative concentration of PE (B) in TDA transfected either with Panx1 siRNA (10 nmol/L), control siRNA (10 nmol/L) or Panx1-GFP (5 µg).

Supplementary figure IX: The integrity of VSMC and EC functions is preserved after transfection of TDA. (A) Effect of acetylcholine (10 µmol/L) on control TDA preconstricted with 100 mol/L PE was compared to TDA transfected with the Panx1-GFP plasmid (5 µg). (B) Relaxation to 10 µmol/L acetylcholine was compared on control TDA and TDA transfected with Panx1 siRNA (10 nmol/L) preconstricted with 40 mmol/L KCl. (C) KCl responses were compared between control TDA and TDA transfected with Panx1 siRNA (10 nmol/L).

Supplementary figure X: α1D-adrenoreceptor is present in SMC and mediates the constriction to PE in TDA. (A) Western Blot of heart lysates using α1D-adrenoreceptor antibody pre-incubated or not with control peptide to assess for antibody specificity. (B) Immunofluorescence of transverse sections of TDA for α1D-adrenoreceptor (red) demonstrates the presence of α1D-adrenoreceptor only in SMC but not in EC. Scale Bar is 10 µm. (C) effect of α1A- adrenoreceptor inhibitor (5-methylurapidil, 10 nmol/L), α1B- adrenoreceptor inhibitor (L-765.314, 60 nmol/L) and α1D- adrenoreceptor (BMY 7378, 10 nmol/L) on PE response shows that only α1D- adrenoreceptor mediates PE constriction in TDA.
Suppl Fig VIII