Functional Evidence for a Novel Suramin-Insensitive Pyrimidine Receptor in Rat Small Pulmonary Arteries

S. Anthony Hartley, Kenichi Kato, Katie J. Salter, Roland Z. Kozlowski

Abstract—Uridine nucleotides are known to cause constriction of pulmonary arterial smooth muscle. However, the P2 receptor subtypes underlying the contractile effects of these nucleotides in the pulmonary circulation have not been determined. We have used myography and the patch-clamp recording technique to compare the effects of UTP and UDP in isolated small pulmonary arteries (diameter 100 to 400 μm) and their constituent smooth muscle cells. In endothelium-denuded arteries, both UTP and UDP (0.01 to 3 mmol/L) induced concentration-dependent increases in tension that were independent of P2X receptor stimulation. The UDP-mediated increase in tension was significantly less sensitive to the nonselective P2 receptor blocker suramin than the UTP-mediated increase in tension. In single isolated arterial myocytes, voltage-clamped at −50 mV (close to the resting membrane potential of these cells), application of both UTP and UDP evoked periodic oscillations of inward current primarily because of a Ca2+-activated Cl− current (I_{Cl,Ca}). Oscillations of I_{Cl,Ca} evoked by UTP were reversibly inhibited by suramin, although those evoked by UDP were insensitive to the antagonist. In addition to confirming the presence of classical P2Y2 receptors, these results also provide functional evidence for the existence of a novel UDP receptor in pulmonary arterial myocytes, which may contribute to pyrimidine-evoked vasoconstriction. This notion is supported by molecular evidence that demonstrates the presence of P2Y6 receptor transcripts in rat pulmonary arterial smooth muscle. (Circ Res. 1998;83:940-946.)

Key Words: artery, pulmonary □ nucleotide, uridine □ channel, Cl−

adenine and uracil nucleotides such as ATP and UTP are potent modulators of cardiovascular function. These nucleotides can be released either intracellularly from perivascular nerves or extraluminally from a range of sources, such as aggregating platelets and endothelial cells.1–3 These nucleotides interact with various P2 receptors to produce a range of physiological responses. P2 receptors located on the endothelium mediate vasodilatation, and receptors located on the smooth muscle mediate vasoconstriction.4 Consequently, the effects of such nucleotides are dependent on their site of action. Direct evidence of the regulated release of uridine nucleotides is limited,5 but the vasomotor activity of UTP in human cerebral arteries6 in addition to activation of other cell functions,7 has led to the suggestion that extracellular uridine nucleotides are also important signaling molecules that mediate their effects through P2 receptor stimulation. These receptors have been divided into 2 broad groups: P2X receptors, which are intrinsic ion channels, and P2Y receptors, which are G protein–coupled receptors. The P2X receptor family comprises subtypes P2X1–6. The P2Y family includes the receptors P2Y1 and P2Y2 (formerly known as P2Y and P2U, respectively), the P2T receptor (yet to be cloned), and 5 further cloned subtypes (P2Y3–7).8

Since the initial observation in cerebral vessels, UTP has been shown to produce constriction of a number of arterial vessels including guinea pig coronary artery,9 rabbit ear artery,10 rat tail and femoral arteries,11 and rat pulmonary artery.12 The first evidence that a novel class of uridine nucleotide-responsive receptors may exist came with the observation that stimulation of G protein–coupled P2X (P2Y2) receptors (originally cloned by Lustig et al13) by either ATP or UTP promoted an increase in the intracellular Ca2+ concentration ([Ca2+]i). Evidence for selective “pyrimidinergic” receptors was provided by Lazarowski et al,14 who reported the existence of uridine nucleotide–specific receptors on C6-2B rat glioma cells. Since the identification of this receptor type, 2 further nucleotide sequences encoding 2 different G protein–coupled receptors that are selectively activated by uridine nucleotides have been described,15,16 indicating the possible existence of a family of pyrimidinergic receptors. Studies that use expression cloning17 indicate that these 3 receptors can be clearly delineated into (1) a UDP-prefering, uridine nucleotide–specific receptor (P2Y6), (2) a UDP-prefering receptor (P2Y4), and (3) a P2Y2 receptor activated by both UTP and ATP. Although mRNA for both P2Y2 receptors and P2Y6 receptors is present in vascular smooth muscle cells,15 existing functional studies have demonstrated only the presence of P2Y2 receptors. Studies in isolated pulmonary arterial smooth muscle cells of both the rat and the rabbit have shown the existence of P2Y2 receptors.
blocked by the nonselective P2 receptor antagonist suramin. However, in the pulmonary vascular bed, both UTP- and UDP-induced vasoconstriction is thought to be insensitive to suramin, indicating the existence of a suramin-insensitive receptor activated by uridine nucleotides. Consistent with these observations, the existence of a UTP-specific receptor has been shown in guinea pig endothelial cells and canine coronary arteries. In the present study, we have investigated the actions of UTP and UDP in whole arteries and single-isolated myocytes of the rat small pulmonary artery. Our experiments have been designed to determine whether (1) a novel suramin-insensitive receptor activated by uridine nucleotides exists, and (2) whether it plays a functional role in mediating contraction.

Materials and Methods

Arterial Preparation and Mounting

Male albino Wistar rats (200 to 250 g body weight) were killed by an overdose of IP Euthatol (pentobarbitone sodium B.P.), and cervically dislocated. Small pulmonary arterial vessels, 100 to 400 μm in diameter, were removed from the lungs and placed in physiological salt solution (PSS) of the following composition (mmol/L): NaCl 150, KCl 5.4, MgCl₂ 1.2, CaCl₂ 1.2, HEPES 5, and glucose 10 (pH 7.4 with NaOH). These vessels, ~1 to 2 mm in length, were denuded of endothelium by gentle rubbing with thin surgical thread and mounted in a Mulvaney myograph (volume 10 mL), which was perfused at a rate of 2 mL/min with solution A maintained at 37°C. Two tungsten wires (30 μm diameter) were inserted through the lumen, and mechanical activity was recorded isometrically by a force transducer connected to 1 of the 2 tungsten wires; the other wire was attached to a support carried by a micromanipulator. After subjecting the muscle to an initial tension of 25 mm Hg (3.3 Kpa), the muscle was equilibrated for 25 min (Tₒ). To verify effective endothelium removal, the sensitivity of vessels to acetylcholine after preconstriction with phenylephrine was determined. All vessels denuded of endothelium were not relaxed by acetylcholine (Figure 1). Concentration-response curves to UTP (0.01 to 3 mmol/L) and UDP (0.01 to 3 mmol/L) were constructed noncumulatively, which allowed the tension to return to baseline between additions.

Single Cell Isolation and Electrophysiological Experiments

Pulmonary arterial smooth muscle cells were isolated through the use of a dispersion procedure, previously described by Albarwani et al., and modified to include an incubation with collagenase (type VIII, 1.5 mg/mL), protease (type I, 0.1 mg/mL), and elastase (type II-A, 0.3 mg/mL) for 4 minutes at 37°C after pretreatment with papain. Cells were stored at 4°C and remained viable for ~10 hours.

Membrane current from pulmonary arterial myocytes was recorded with the use of the perforated patch configuration of the patch-clamp recording technique at room temperature (20°C to 25°C). The cells were bathed in PSS, and the pipette was filled with solution consisting of the following (mmol/L): 125 KCl, 4 MgCl₂, 10 HEPES, 0.02 EGTA, and pH 7.3 with KOH. The viability of each cell preparation was assessed by qualitatively visualizing the ability of isolated cells to contract in response to UTP and UDP. In general, cells isolated by the use of our methods could be maintained in the perforated patch configuration for up to 1 hour. However, the experiments presented here rarely lasted for >15 minutes. To examine the effects of the uridine nucleotides on membrane current, cells were voltage-clamped at ~50 mV, unless otherwise stated. Electodes (3 to 6 MΩ) were pulled from borosilicate glass capillaries (Clark Electromedical) with a vertical puller (Narishige Ltd). Ionic currents were detected with the use of an Axopatch 200A amplifier (Axon Instruments). Series resistance and capacity compensation facilities were used when necessary. Data were filtered at 2 kHz with the use of a Digidata 1200 interface (Axon Instruments) and recorded either on-line with a personal computer or off-line on a modified DAT recorder (Sony DTC-100ES). Data were analyzed with the use of pClamp software (version 6.1; Axon Instruments Inc).

Molecular Studies

Total RNA was isolated from endothelium-denuded rat pulmonary arteries and aortas using TRIZOL reagent (Gibco Life Technologies) according to the recommendations of the manufacturer. cDNA was synthesized from 1 μg total RNA, in the presence of (100 ng) random hexamer primer (Perkin Elmer), 1 mmol/L dNTPs (Promega), 20 U of RNasin ribonuclease inhibitor (Promega), and 50 U of Moloney murine leukemia virus reverse transcriptase (Perkin Elmer) according to the recommendations of the manufacturer. Polymerase chain reaction (PCR) was conducted through the use of oligonucleotide primers designed from the published rat P2Y₆ sequence, which has been described previously by Webb et al.;
sensed primer 5'-GGAGACCTTGCGCTGCGTGGTA-3', and antisense primer 5'-TACCCAGCGCATCACGCGCGCGCCG-3'. PCR was performed in a buffer containing (in mmol/L) 50 KCl, 10 Tris-HCl (pH 8.3), 1.5 MgCl₂, and 0.01% (wt/vol) gelatin (Perkin Elmer), with the use of 10% of the cDNA synthesis reaction, 80 ng of each primer, 200 μmol/L of each dNTP, and 1.25 U of Taq DNA polymerase (Promega) in a total volume of 50 μL. The reaction conditions were as follows: 30 seconds at 94°C, 55°C, and 72°C, respectively, for 35 cycles followed by 1 cycle at 72°C for 10 minutes. Both negative control reactions (in which no cDNA was included in the PCR reaction mix) and positive control reactions (with primers designed to amplify GAPDH and with cDNA synthesized from total RNA isolated from rat aorta, which is known to express the P2Y₆ receptor) were performed. A mock cDNA synthesis reaction, in which no reverse transcriptase was added, was also performed as a control for genomic DNA contamination of the RNA sample and for DNA contamination in the PCR reaction.

PCR products were separated on a 1.2% (wt/vol) agarose gel by electrophoresis, and the bands excised. After extraction of the DNA using the QIAquick gel extraction kit (QIAGEN), the PCR product was cloned into the pCRII dual vector Invitrogen (INV) and introduced into INV<sup>α</sup> competent cells. Plasmid DNA was prepared using QIAfilter plasmid midipreps (QIAGEN), and the double-stranded plasmid inserts were sequenced in both directions with the use of the Sequenase 2.0 kit (Amersham International) to confirm their identity.

Specialty Chemicals
Amphotericin B, collagenase (type VIII), dithiothreitol, elastase (type IIA), EGTA, HEPES, niflumic acid, papain (papaya latex), protease (type I), UDP (sodium salt), and UTP (sodium salt) were all purchased from Sigma (Poole). Suramin (hexadsodium salt) was obtained from ICN Biochemicals Ltd.

Data Analysis
To compare the ability of a range of nucleotides to activate oscillatory inward currents, membrane current elicited over a 1-minute period was digitized at 100 Hz and integrated (pClamp software; version 6.2), as previously described, giving a value in nA · ms. Contractions were normalized and expressed as N/mm². Data are presented throughout as mean±SEM. When presented graphically, the SEM is represented by the associated error bars. Statistical significance was assessed using a Student t test. P values ≤0.05 were considered significant.

Results
Contractile Effects of UTP and UDP
UTP (0.01 to 3 mmol/L) and UDP (0.1 to 3 mmol/L) produced reproducible concentration-dependent contractions of endothelium-denuded rat small pulmonary arteries. The endothelium was removed to ensure that effects of the nucleotides could be attributed to their actions on the smooth muscle cells and to obviate any relaxation mediated through P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors present on vascular endothelial cells. Contractions in response to UTP and UDP developed slowly and were maintained during administration of the nucleotide. Typical traces showing the changes in contraction produced by the addition of the nucleotides UTP (0.01 to 3 mmol/L) and UDP (0.01 to 3 mmol/L) are shown in Figure 2A and 2B. The concentration-response curves to UTP and UDP are shown in Figure 2C. In the presence of α,β-methylene ATP (100 μmol/L), a selective P2X receptor agonist that causes desensitization of these receptors, the magnitude of the contractile response induced by either UTP or UDP was unchanged, which indicates that P2X receptor stimulation is not involved in the contractile responses to either uridine nucleotide (not shown).

Effects of Suramin on the Contractile Actions of UTP and UDP
In a number of vascular and neuronal preparations, suramin has been a nonselective antagonist at P2 receptors. To investigate the action of suramin on UTP- and UDP-induced contractions, pulmonary arteries were preincubated with the antagonist for 30 minutes before the addition of the nucleotides (used at 1 mmol/L). The antagonist was present in the bath solution throughout the experiment. As illustrated in Figure 3A, suramin (100 μmol/L) significantly reduced the contractile response of UTP to 57±7% (n=8) of control, which was obtained in the absence of the antagonist. The UDP-induced contraction was only slightly (24±5%, n=8), but significantly, inhibited on application of 100 μmol/L suramin (Figure 3B). A significant difference was found between the inhibitory effects of suramin on the UTP- and UDP-induced contractions. The UDP-induced contraction was significantly less resistant to suramin than the UTP-induced contraction (Figure 3C). This difference indicated the possible existence of a novel UDP-activated receptor relatively insensitive to suramin. To verify this notion and to
between small pulmonary arteries have a resting membrane potential showed in an earlier study that myocytes isolated from rat (10 m) the contractile responses of 1 mmol/L UTP and 1 mmol/L UDP, respectively. C, Bar graph showing the mean±SEM inhibition by 100 μmol/L suramin on the UTP- and UDP-induced contraction of isolated arteries. The number of arteries used for each nucleotide is shown in parentheses. *Significant (P≤0.05) difference between the suramin-sensitivity of the UTP- and UDP-induced contractions.

minimize the potential influence of nucleoside diphosphokinase or ectonucleotidase enzymes known to reside on the extracellular surface of most cells,7 the effects of UTP and UDP were investigated with the use of single rat pulmonary arterial myocytes.

Electrophysiological Effects of UTP and UDP
We48 have shown that extracellular application of UTP (10 μmol/L) to myocytes isolated from rat small pulmonary arteries evokes periodic oscillations of a Ca2+-activated Cl− current (I_{Cl,Ca}) via the activation of P2Y2 receptors. We showed in an earlier study that myocytes isolated from rat small pulmonary arteries have a resting membrane potential between −44 mV and −53 mV (−49.3±1.1 mV, n=15). We have found consistently that the extracellular addition of UTP (10 μmol/L) to cells maintained in the perforated patch configuration at a holding potential of −50 mV (close to the resting membrane potential of these cells) evoked periodic oscillations of inward current in 16 of 21 cells. Under identical conditions, extracellular application of UDP (10 μmol/L) evoked periodic oscillations of inward current in 24 of 36 cells. These oscillations were reversibly inhibited (n=5) by niflumic acid (50 μmol/L, a blocker of Cl− channels),28 which confirms that they were caused by activation of I_{Cl,Ca} (data not shown). The magnitude of I_{Cl,Ca} measured over a 1-minute period, as discussed in Materials and Methods, was not significantly different between the 2 nucleotides: 420±113 nA·ms (n=11) for UTP and 412±64 nA·ms (n=20) for UDP. Higher concentrations of UDP (100 μmol/L and 500 μmol/L) evoked currents of similar magnitude: 429±55 nA·ms (in 4 of 4 cells) and 437±34 nA·ms (in 3 of 3 cells), respectively. This suggests activation of I_{Cl,Ca} by UTP and UDP is “all or nothing,” and if activated, the current magnitude may be a maximal response. Because there is significant cross-contamination in commercially available UTP and UDP, we examined the effects of the 2 nucleotides at a concentration of 0.5 μmol/L. This concentration represents the maximum (5%) likely to be found as a contaminant within commercially available UTP or UDP when used at 10 μmol/L. Neither nucleotide had any effect at this concentration (data not shown), which obviated the possibility that the responses presented were the consequences of cross-contamination.

Effects of Antagonists on the Electrophysiological Actions of UTP and UDP
Application of suramin (100 μmol/L throughout, unless otherwise stated) totally inhibited oscillations of I_{Cl,Ca} evoked by 10 μmol/L UTP (Figure 4A, n=5). In contrast, application of suramin had no significant inhibitory effect on activation of I_{Cl,Ca} evoked by 10 μmol/L UDP (Figure 4B, n=6). Furthermore, pretreatment of cells with suramin for 3 minutes had no effect on the action of 10 μmol/L UDP (in the continued presence of suramin). The nucleotide still evoked oscillations of I_{Cl,Ca} of similar magnitude to those evoked in the absence of the antagonist (n=4, data not shown). Additional experiments using a range of concentrations of suramin (0.01 to 1 mmol/L) further highlighted the differential sensitivity of the UDP- and UTP-activated oscillations of I_{Cl,Ca} to the antagonist. The results are expressed quantitatively in Figure 4C. Unlike suramin, application of the known P2Y receptor blocker Cibacron Blue (10 μmol/L), a concentration reported to be selective for P2Y receptors (Burnstock and Warland),29 significantly inhibited oscillations induced by both UTP and UDP (Figure 5A, 5B, and 5C). This finding further supports that UTP and UDP are acting via P2Y receptors.

Molecular Studies
To determine whether P2Y6 receptor transcripts are expressed in rat pulmonary arteries, reverse transcription (RT)-PCR experiments were performed. In these experiments, rat aortas, which have already been shown to express P2Y6 receptors,15 were used as a positive control. RT-PCR, which used oligonucleotide primers designed to amplify a 450-bp region of the P2Y6 receptor, yielded a single PCR product in experiments through the use of total RNA isolated from rat pulmonary arteries and aortas (Figure 6). Cloning and sequencing of these products confirmed that they represented amplification of the selected region of the published P2Y6 receptor sequence (Accession No. D63665).24

Discussion
The present study compares the contractile and electrophysiological effects of UTP and UDP in rat small pulmonary...
arterial muscle. Our report provides for the first time functional evidence of the existence of UDP receptors in arterial myocytes pharmacologically distinct from P2Y2 receptors.

We agree with previous studies performed in the pulmonary vascular bed of the rat, which indicated that the nucleotides UTP and UDP (0.01 to 3 mmol/L) caused concentration-dependent increases in the tension of isolated small pulmonary arteries of the rat. There are several reports indicating the presence of P2Y2 receptors in pulmonary arterial smooth muscle cells that are equally sensitive to the purine ATP and the pyrimidine UTP. The existence of such receptors could explain the ability of UTP to cause contraction of pulmonary arteries independently of P2X receptor stimulation. However, because UDP has been shown unequivocally not to be an agonist at the P2Y2 receptor, UDP could only be mediating its contractile effects in this tissue through either (1) the in situ conversion of UDP to UTP

Figure 4. Effect of suramin on the UTP- and UDP-evoked periodic oscillations of \( I_{\text{Cl, Ca}} \) in single isolated arterial myocytes. A, Representative record demonstrating the inhibitory effect of extracellular suramin (100 \( \mu \text{mol/L} \)) on UTP-evoked oscillations of \( I_{\text{Cl, Ca}} \). B, Representative record demonstrating the insensitivity of UDP-evoked oscillations of \( I_{\text{Cl, Ca}} \) to extracellular suramin (100 \( \mu \text{mol/L} \)). C, Bar graph showing the mean±SEM inhibition by suramin (0.01 \( \text{mmol/L} \)) of \( I_{\text{Cl, Ca}} \) activated by UTP and UDP (both 10 \( \mu \text{mol/L} \)). The number of cells is shown in parentheses. *Significant (\( P<0.05 \)) inhibition by suramin.

Figure 5. Effect of Cibacron Blue on the UTP- and UDP-evoked periodic oscillations of \( I_{\text{Cl, Ca}} \) in single isolated arterial myocytes. A, Representative record demonstrating the inhibitory effect of extracellular Cibacron Blue (10 \( \mu \text{mol/L} \)) on UTP-evoked oscillations of \( I_{\text{Cl, Ca}} \). B, Representative record demonstrating the insensitivity of UDP-evoked oscillations of \( I_{\text{Cl, Ca}} \) to extracellular Cibacron Blue (10 \( \mu \text{mol/L} \)). C, Bar graph showing the mean±SEM inhibition by Cibacron Blue of \( I_{\text{Cl, Ca}} \) activated by (1) UTP and (2) UDP (both 10 \( \mu \text{mol/L} \)). The number of cells is shown in parentheses. *Significant (\( P<0.05 \)) inhibition by Cibacron Blue.

Figure 6. Agarose gel electrophoresis of PCR amplification products from rat pulmonary artery and aorta using oligonucleotide primers for P2Y2 receptor and GAPDH. Both sets of primers yield a single band (450 and 561 bp, respectively) in reactions that contained RNA and reverse transcriptase. No bands were observed in any of the negative control lanes (2, 4, 5, 7, 9, and 10).
through the activity of an ectonucleoside diphosphokinase and subsequent activation of P2Y$_2$ receptors, or (2) the activation of an additional pyrimidine receptor that is exclusively sensitive to UDP. The increases in tension induced by both UTP and UDP were inhibited by suramin. However, the UDP-mediated contraction was significantly less sensitive to the antagonist than was the UTP-mediated contraction. This marked difference in the sensitivity to suramin obviates the possibility that the 2 nucleotides are acting via the same P2Y$_2$ receptor.

Our original observations were extended in this study when we showed that UDP, like UTP, can result in activation of I$_{Ca,L}$. However, in contrast to UTP, the oscillations in I$_{Ca,L}$ evoked by UDP were totally insensitive to suramin (100 µmol/L) and were only significantly inhibited when suramin was used at concentrations $>100$ µmol/L: a concentration known to have nonspecific inhibitory effects on other ion channels and cell functions. Furthermore, as suramin selectively inhibited activation of I$_{Ca,L}$ by UTP but not UDP, calcium release from intracellular stores is probably not affected. Thus, under conditions under which the influence of ectonucleotidases is negligible (isolated single cells) and no significant conversion of UDP to UTP can occur, UDP can still elicit effects (activation of I$_{Ca,L}$) that are insensitive to suramin. Because of this pharmacological difference and the fact that UDP, unlike UTP, does not activate P2Y$_2$ receptors, a novel UDP-selective receptor probably exists in pulmonary arterial smooth muscle.

Because UDP is known to be inactive at both the P2Y$_2$ and P2Y$_4$ receptors, the most likely receptor candidate for the effect of UDP in pulmonary arterial smooth muscle cells is the P2Y$_4$ receptor. When expressed in C6 rat glioma cells, the P2Y$_4$ receptor was relatively resistant to blockade by suramin, with receptor-mediated increases in IP$_3$ being reduced by only $\approx 20\%$. This lack of potency is consistent with our findings with suramin (1) in isolated vessels in which UDP-mediated increases in tension were only reduced by $\approx 25\%$, and (2) in single cells in which UDP-mediated activation of I$_{Ca,L}$ was only reduced by $\approx 15\%$. P2Y$_4$ receptors (and their encoding mRNA) are expressed in the rat aortas in which they have been suggested to perform a potential physiological role in vascular function. The P2Y$_4$ receptor transcript is also found in cultured rat aortic smooth muscle cells and may be expressed in the smooth muscle layer of the vascular wall where it may be involved in the regulation of vascular tone. Our molecular data are consistent with these results through the expression of identical mRNA in pulmonary arterial smooth muscle. Like P2Y$_2$ and P2Y$_4$ receptors, P2Y$_4$ receptors are coupled to phospholipase C, and their stimulation mediates an increase in the [Ca$^{2+}$]$_i$, which in vascular smooth muscle may elicit activation of Ca$^{2+}$-sensitive membrane currents (such as I$_{Ca,L}$) and, therefore, induce contraction.

The existence of P2Y$_4$ receptors in pulmonary arterial smooth muscle explains the observed suramin insensitivity of the UDP-induced vasoconstriction of the pulmonary vascular bed of the rat. The 2 chief reasons for this conclusion are as follows. First, the P2Y$_4$ receptor is reportedly UDP-selective but is weakly activated by UTP. Consequently, in multicellular tissue preparations that require higher concentrations of nucleotides to elicit a given response than single cells, vasoconstriction evoked by UTP may be mediated at least in part through stimulation of suramin-resistant P2Y$_4$ receptors. This could also explain the apparent insensitivity of UTP- and UDP-induced contraction of canine coronary artery. Secondly, a much greater ectonucleotidase activity exists in multicellular preparations that require higher concentrations of purine nucleotides may be released from cells under a variety of pathological conditions such as inflammation, trauma, and hypoxia. With the existence of constriction-mediating pyrimidine receptors on vascular smooth muscle, the release of uridine nucleotides under such conditions would be of extreme importance in the pulmonary circulation. In the pulmonary circulation, their release may play a role in hypoxic pulmonary vasoconstriction or in certain forms of pulmonary hypertension when there is loss or trauma to the endothelium.

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**References**


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