Abrogation of thrombin-induced increase in pulmonary microvascular permeability in PAR-1 knockout mice

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Vogel, Stephen M., Xiaopei Gao, Dolly Mehta, Richard D. Ye, Theresa A. John, Patricia Andrade-Gordon, Chinnaswamy Tiruppathi, and Asrar B. Malik. Abrogation of thrombin-induced increase in pulmonary microvascular permeability in PAR-1 knockout mice. Physiol Genomics 4: 137–145, 2000.—We investigated the function of proteinase-activated receptor-1 (PAR-1) in the regulation of pulmonary microvascular permeability in response to thrombin challenge using PAR-1 knockout mice (∼−/−). Lungs were isolated and perfused with albumin (5 g/100 ml)-Krebs solution at constant flow (2 ml/min). Lung wet weight and pulmonary artery pressure (Ppa) were continuously monitored. We determined the capillary filtration coefficient (Kc) and 125I-labeled albumin (BSA) permeability-surface area product (PS) to assess changes in pulmonary microvascular permeability to liquid and albumin, respectively. Normal and PAR-1-null lung preparations received in the perfusate: 1) thrombin or 2) selective PAR-1 agonist peptide (FTLLRN-PNDK-NH2). In control PAR-1 (+/+) mouse lungs, 125I-albumin PS and Kc were significantly increased over baseline (by ∼7- and 1.5-fold, respectively) within 20 min of α-thrombin (100 nM) challenge. PAR-1 agonist peptide (5 μM) gave similar results, whereas control peptide (5 μM; FTLLRN-PNDK-NH2) was ineffective. At relatively high concentrations, thrombin (500 nM) or PAR-1 agonist peptide (10 μM) also induced increases in Ppa and lung wet weight. All effects of thrombin (100 or 500 nM) or PAR-1 agonist peptide (5 or 10 μM) were prevented in PAR-1-null lung preparations. Baseline measures of microvessel permeability and Ppa in the PAR-1-null preparations were indistinguishable from those in normal lungs. Moreover, PAR-1-null preparations gave normal vasoconstrictor response to thromboxane analog, U-46619 (100 nM). The results indicate that the PAR-1 receptor is critical in mediating the permeability-increasing and vasoconstrictor effects of thrombin in pulmonary microvessels.

proteinase-activated receptor; cultured endothelial cell monolayers; tethered ligand

PROTEINASE-ACTIVATED RECEPTORS (PARs) are activated by the proteolytic cleavage of their extracellular NH2-terminal domain (2, 4, 27). The new NH2 terminus acts as a “tethered ligand” capable of activating these receptors. Four members of this family have been cloned, three of which are activated by thrombin (PAR-1, PAR-3, and PAR-4), whereas the fourth (PAR-2) is activated by trypsin or mast cell tryptase (2).

Thrombin has important physiological functions 1) hemostasis and facilitation of wound healing, 2) pro-inflammatory action with stimulation of transvascular leukocyte migration, and 3) regulation of microvascular permeability with formation of interendothelial gaps (7, 18). Thrombin has thus been postulated to be a mediator involved in the pathogenesis of acute lung injury (10). In the intact pulmonary microcirculation, the permeability-enhancing effects of thrombin in vivo have been considered an indirect consequence of agonist-stimulated leukocyte extravasation and activation (15). The question of a direct effect of thrombin on endothelial permeability in vivo remains open (12, 28). Thrombin is known to increase permeability to liquid in cultured endothelial cell monolayers (in the absence of leukocytes) (17, 19). The increase in endothelial permeability to albumin correlates with reduced monolayer transendothelial electrical resistance in these cells, implying that thrombin can act through formation of interendothelial gaps (26). Such gaps, which have been directly observed by immunohistochemical methods, are attributed to thrombin-induced cadherin junction disassembly (14, 22). Thrombin induces myosin light chain (MLC) phosphorylation (9), an event that normally precedes actin-myosin interaction and mechanical contraction of the endothelial cell monolayer.

In the present study, we have developed a perfused mouse lung preparation to examine the direct in vivo effects of thrombin on lung microvascular permeability to protein and fluid. Because endothelial cells express multiple thrombin receptors (21, 23, 24), we specifically tested the role of PAR-1 in the regulation of endothelial permeability using PAR-1 knockout mice. Our results show that PAR-1 is requisite for the pulmonary vasoconstrictor and microvessel permeability-increasing properties of thrombin in the intact pulmonary microcirculation.
METHODS

PAR-1 Knockout Mouse Model

PAR-1-null genotype. The gene for the thrombin receptor PAR-1 was disrupted in mice by the method of homologous recombination. The detailed methodology used to create the PAR-1 knockout mouse model, as well as evidence for the physical and functional absence of PAR-1 in these mice, is provided in Darrow et al. (6).

RT-PCR analysis. For confirmation of the genotype, total RNA was made from freshly isolated mouse lung tissue, using Trizol reagent (Life Technologies, Grand Island, NY). Total RNA (2 μg) was reverse-transcribed by using SuperScript Preamplification Kit ( Gibco BRL; Life Technologies). One microliter of the RT product was amplified utilizing the following primers: TR4, a forward primer with the sequence 5'-GGCAGCCTTGGACAATGGGGC-3', (covers the first 7 bp of the mouse PAR-1 coding sequence and 14 bp of the 5' untranslated sequence preceding the initiation codon); and TR8, a reverse primer with the sequence 5'-GGTCA-GATATCCGGAGCTCC-3', derived from nucleotides 285 to 306 of the mouse PAR-1 coding sequence. The TR4 sequence has been removed from the PAR-1 genomic DNA as a result of targeted deletion (6). The resulting PCR product has an expected size of 320 bp. As a positive control, a pair of PCR primers was used for the amplification of a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene fragment with an expected size of 452 bp. PCR was performed using Taq polymerase in a total volume of 50 μl, with the following conditions: annealing at 55°C for 40 s; extension at 72°C for 60 s, and denaturing at 94°C for 40 s. After 30 cycles, 15 μl of the PCR products for the PAR-1 DNA fragment, as well as 4 μl of the PCR products for the housekeeping gene fragment, were analyzed on a 1.1% agarose gel by electrophoresis. The PCR products were visualized by ethidium bromide staining.

Experimental Procedures

Perfused mouse lung preparation. According to approved protocol of the University of Illinois Animal Care Committee, male CD-1 (n = 60) and PAR-1 knockout mice (n = 48) weighing 30–35 g were placed in an anesthesia chamber and anesthetized with 3% halothane in room air at a flow rate of 2 l/min. After induction, anesthesia was continued by means of a suture around the pulmonary artery that included the pulmonary artery via the pulmonic valve and secured by 0.025 EDTA, pH 7.4), supplemented with 5 g/100 ml of BSA (fraction V, 99% pure and endotoxin-free; Sigma-Aldrich, St. Louis, MO). Pulmonary arterial pressure was monitored throughout the experiment using a Gould pressure transducer (model P23ID; Gould Instruments, Dayton, OH). Lung wet weight was electronically nullled when the tissue was mounted, and subsequent weight changes due to gain or loss of fluid from the lung were recorded. Lung weight and arterial pressure recordings were displayed on a computer video monitor with the aid of amplifiers (model CP122; Astro-Med), an analog-to-digital converter (DAS 1800ST board; Keithley Metrabyte, Solon, OH), and commercial software for acquisition and logging of data (NoteBook Pro for Windows; Labtech, Andover, MA). All lung preparations underwent a 20-min equilibration perfusion. Lungs that were not isogravimetric at the end of the equilibration period were discarded.

Infusion of drugs. α-Thrombin was obtained from Enzyme Research Laboratories (South Bend, IN). PAR-1 agonist peptide (TFLLRNPNDK-NH2) and inactive control peptide (PTLLRNPNDK-NH2) were synthesized as described (5). Each drug was dissolved in albumin-Krebs solution at 10 times its final perfusate concentration and delivered through a side-arm in the pulmonary arterial cannula by infusion (200 μl/min, from a 10-ml syringe, with the aid of a syringe pump (model SP100i; World Precision Instruments, Sarasota, FL). Virtually immediate drug application or removal was effected by turning on or off the pump.

Pulmonary capillary filtration coefficient determination. Capillary filtration coefficient (Kfc) was measured to determine pulmonary microvascular permeability to liquid, as described (8). Briefly, after the standard 20-min equilibration perfusion, outflow pressure was rapidly elevated by 10 cmH2O for 2 min. The lung wet weight changed in a ramp-like fashion, reflecting net fluid extravasation. At the end of each experiment, lungs were dissected free of nonpulmonary tissue, and lung dry weight was determined. Kfc (ml/min-1·cmH2O·g dry wt-1) was calculated from the slope of the recorded weight change normalized to the pressure change and to lung dry weight. Normalization of the weight gain by change in outflow pressure can produce slightly higher estimates of Kfc than normalization by change in capillary pressure, as estimated with double-occlusion method. Our procedure was applied consistently before and after administration of test compounds, permitting reliable detection of drug-induced changes of Kfc. The wet-to-dry weight ratio in 6 freshly isolated (nonperfused) mouse lungs was 6.04 ± 0.4.

Pulmonary microvesSEL 125I-albumin permeability-surface area product measurement. BSA was labeled with 125I (New England Nuclear, Boston, MA) using the chloramine T method (1). Free 125I was separated from 125I-labeled BSA with a Sephadex G25 column, and contributed less than 0.3% of total radioactivity as determined by trichloroacetic acid (TCA) precipitation. Albumin-Krebs solution containing 125I-BSA (~2 × 106 counts/ml) was infused at 200 μl/min into the pulmonary artery for 3 min. A sample of the venous effluent was saved for later determination of the perfusate tracer...
PAR-1 activation increases lung vascular permeability

Experimental Protocols

Effects of PAR-1 agonist peptide and α-thrombin on pulmonary microvascular liquid permeability. We evaluated the change in $K_p$ produced by PAR-1 agonist peptide or α-thrombin in lung preparations obtained from normal or PAR-1-null mice. In one series of experiments, after the 20-min equilibration period, PAR-1 agonist peptide (perfusion concentration, 5.0 or 10.0 μM) was supplied for a 20-min period followed by a 5-min rinse with albumin-Krebs solution containing no additive. $K_p$ was measured at various times before, during, and after perfusion with PAR-1 peptide. Some preparations received, in place of agonist peptide, an inactive peptide (5 μM), as a control procedure. Each group contained $n = 4$ lungs. In a second series of experiments, 0.1 or 0.5 μM α-thrombin was provided to the pulmonary circulation for 20 min followed by a 5-min rinse. $K_p$ was measured at various times during and after administration of α-thrombin. Each group contained $n = 4$ lungs.

Effects of PAR-1 peptide or α-thrombin on $^{125}$I-albumin PS product. Isogravimetric lung preparations from normal or PAR-1 knockout mice were treated with selective PAR-1 peptide (5 μM) or α-thrombin (0.1 μM) for a period of 20 min. Then, the $^{125}$I-albumin PS product was determined. For baseline measurements $^{125}$I-albumin PS, lungs received no addition but were treated identically in all other respects to experimental lung preparations. Each group contained $n = 4$ lungs.

Effects of PAR-1 peptide or α-thrombin on MLC phosphorylation. To determine the role of PAR-1 in mediating thrombin-induced changes in MLC phosphorylation, we investigated the effects of selective PAR-1 peptide and thrombin in normal and PAR-1 knockout mice. After the 20-min equilibration perfusion, isogravimetric preparations derived from normal or PAR-1 (−/−) mice received in perfusing liquid either 5.0 μM PAR-1 agonist peptide or 0.1 μM α-thrombin. The lungs were then frozen rapidly using liquid nitrogen, after which they were immersed in acetone containing 10% (wt/vol) TCA and 10 mM dithiothreitol (DTT) (acetone/TCA/DTT) cooled to −80°C with crushed dry ice. Lungs were thawed in acetone/TCA/DTT at room temperature and then washed with acetone/DTT. MLCs were extracted for 60 min in 8 M urea, 20 mM Tris, 22 mM glycine, and 10 mM DTT. Proteins were separated by glycerol-urea polyacrylamide gel electrophoresis and blotted to nitrocellulose membranes. MLCs were specifically labeled with polyclonal rabbit anti-MLC20 antibody followed by horseradish peroxidase anti-rabbit IgG (Amersham Pharmacia Biotech, Piscataway, NJ) for visualization of unphosphorylated and phosphorylated bands of MLCs by chemiluminescence. The protein content of MLCs was verified in normal and PAR-1-null mice by

Fig. 1. Detection of proteinase-activated receptor-1 (PAR-1) transcript in mouse lung. RT-PCR was used for the detection of PAR-1 transcript in wild-type (lanes 1 and 2) and knockout (lanes 3 and 4) mice. The 320-bp PAR-1-specific fragment (P) and the 452-bp GAPDH housekeeping gene fragment (G) are marked by arrows. Shown here is an inverted color image of ethidium bromide-stained gel; result is representative of 3 experiments with identical results. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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RT-PCR. Using PAR-1-specific primers (see Methods), we amplified a gene product containing 320 bp in normal mice. That product, representing the deleted fragment of exon 1 in the mouse PAR-1 gene, was not present in PAR-1-null mice (Fig. 1, lane 3 vs. lane 1). Figure 1 also shows, as a procedural control, that our RT-PCR analysis could detect the 452-bp GAPDH housekeeping gene fragment in lung tissue obtained from normal or PAR-1-null mice; these data indicate that PAR-1 (+/−) animals did not express wild-type PAR-1 gene in lung tissue. Hence, we used these mice for the functional studies described below.

Pulmonary Vasoconstrictor Response

α-Thrombin. In normal mouse lung preparations, α-thrombin (0.5 μM) increased Ppa by 57% of baseline value within 10 min from beginning of drug infusion (Fig. 2A). As lung preparations were perfused at constant flow rate (see Methods), the observed increase in Ppa represented a rise in vascular resistance, due to the vasoconstrictor action of thrombin. α-Thrombin, at a lower concentration (0.1 μM), did not significantly affect Ppa. No statistically significant effect on Ppa was associated with α-thrombin challenge (0.5 μM) in PAR-1-null lungs (Fig. 2B), indicating that PAR-1 mediated the vasoconstrictor effect of thrombin.

PAR-1 agonist peptide. The selective PAR-1 agonist peptide (10 μM; TFLLRNPNDK-NH₂) caused a rapid (within 5 min) increase of Ppa by 111% of baseline in normal lung preparations (Fig. 3A). At 5 μM, the PAR-1 agonist peptide had no significant effect on Ppa (Fig. 3A). PAR-1 agonist peptide (10 μM) was totally ineffective in producing changes in Ppa in PAR-1-null lung preparations (Fig. 3B). Control PAR-1 peptide (10 μM; FTLLRNPNDK-NH₂), which is identical to active peptide except for the transposition of the initial pair of carboxy-terminal residues, was also without effect in either normal or PAR-1-null lung preparations (data not shown).

Thromboxane analog U-46619. To test the vascular reactivity in PAR-1-null lung preparations, we analyzed the pulmonary vasoconstrictor action of the thromboxane analog, U-46619. Infusion of U-46619 (100 nM) produced rapid (within 2 min) and pronounced (2-fold) increases in Ppa in both normal (Fig. 4A) and PAR-1-null lung preparations (Fig. 4B). Removal of U-46619 from perfusing liquid rapidly (within 5 min) reversed this vasoconstrictor action. Subsequent addition of PAR-1 agonist peptide (10 μM) produced a typical rise of Ppa in normal (Fig. 4A), but not in PAR-1-null lung preparations (Fig. 4B). Lung preparations from PAR-1-null animals thus retained normal vascular reactivity to other agonists.
Pulmonary Edema Formation

α-Thrombin. Following the 20-min equilibration period, untreated perfused mouse lung preparations characteristically gave stable baseline values for wet weight for at least 60 min (Fig. 5). Thrombin challenge (0.1 or 0.5 μM) for 60 min did not produce a significant change of lung wet from baseline level either in normal or PAR-1-null lung preparations (Fig. 5A).

PAR-1 agonist peptide. In PAR normal lung preparations, PAR-1 agonist peptide (5 μM) caused a delayed increase in lung wet weight. The increase in wet weight was significant at 40–60 min following the beginning of peptide infusion (Fig. 5B). A higher concentration of the peptide (10 μM) gave a more marked increase in wet weight (Fig. 5B). The effects of PAR-1 agonist peptide were abrogated in the PAR-1-null lung preparations (Fig. 5B).

Pulmonary Capillary Filtration Coefficient

α-Thrombin. At a concentration of 0.1 μM, α-thrombin induced a significant increase in $K_{fc}$ in PAR normal mouse lung preparations. This effect was time dependent, as $K_{fc}$ was increased by 50% and 100% of baseline value after 7 and 20 min of thrombin infusion, respectively (Fig. 6A). A higher concentration of thrombin (0.5 μM) produced a three-fold increase in $K_{fc}$ above the baseline value, measured at 20 min (Fig. 6A). The increased $K_{fc}$ was not reversible for either concentration of thrombin employed (Fig. 6A). In PAR-1-null lung preparations, thrombin was ineffective in modifying $K_{fc}$ at either of the tested concentrations (Fig. 6B).

PAR-1 agonist peptide. The PAR-1 agonist peptide (TFLLRNPNKD-NH$_2$) mimicked the effects of thrombin on $K_{fc}$. Results for normal mouse lung preparations are shown in Fig. 7A. Over a 15-min period, addition of the PAR-1 agonist peptide, at concentrations of 5 and 10 μM, produced increases in $K_{fc}$ of 2- and 4-fold of baseline value, respectively. The peptide-induced $K_{fc}$ increase was reversed after a 5-min washout period (Fig. 7A). The control peptide (FTLLRNPNKD-NH$_2$), at a concentration of 10 μM, was ineffective in normal lung preparations (Fig. 7A), thus indicating the specificity of the active PAR-1 peptide. In PAR-1-null lung preparations, PAR-1 agonist peptide (TFLLRNPNKD-NH$_2$) caused a delay in increase in lung wet weight. The increase in wet weight was significant at 40–60 min following the beginning of peptide infusion (Fig. 7B). A higher concentration of the peptide (10 μM) gave a more marked increase in wet weight (Fig. 7B). The effects of PAR-1 agonist peptide were abrogated in the PAR-1-null lung preparations (Fig. 7B).
preparations, the permeability-enhancing effects of PAR-1 agonist peptide, at 5 or 10 μM, were ablated (Fig. 7C).

Pulmonary Vessel Wall $^{125}$I-Albumin Permeability-Surface Area Product

$\alpha$-Thrombin. In PAR normal mouse lung preparations, $\alpha$-thrombin (0.1 μM, 20 min) produced marked effects on protein permeability, indicated by a sevenfold rise in $^{125}$I-albumin PS product (Fig. 8A). This effect of thrombin was absent in PAR-1-null lung preparations (Fig. 8A), thus demonstrating the essential role of PAR-1 in thrombin-induced protein permeability.

PAR-1 agonist peptide. The PAR-1 agonist peptide (5 μM) mimicked the effects of thrombin on protein permeability. $^{125}$I-albumin PS product, measured 20 min after the beginning of peptide infusion, was increased significantly (i.e., 8-fold above baseline value) in normal mouse lung preparations, but not in PAR-1-null lung preparations (Fig. 8B). In the normal lung preparations, the control peptide (5 μM) produced no significant change in $^{125}$I-albumin PS product from baseline values (Fig. 8B), thereby indicating the specificity of the PAR-1 agonist peptide.

MLC Phosphorylation

We evaluated the effect of thrombin on extent of MLC phosphorylation in mouse lung tissue pretreated with thrombin or PAR-1 agonist peptide for periods of 7 or 20 min. Western immunoblots show MLC and phosphorylated MLC (Fig. 9A). In lung tissue obtained from normal mice, thrombin (0.1 μM) induced a clear increase in MLC phosphorylation. The PAR-1 agonist peptide (5 μM) mimicked this thrombin effect (Fig. 9A). Induction of MLC phosphorylation by thrombin or agonist peptide did not occur in lung tissue taken from preparations.
PAR-1-null mice, indicating the essential role of PAR-1 in mediating the thrombin action.

**Thrombin.** Figure 9B summarizes the data for MLC phosphorylation, based on densitometric analysis of the Western immunoblots. In PAR normal lung tissue, thrombin (0.1 μM) induced a threefold increase of MLC phosphorylation above the basal level after 7 min; no further increase was observed after 20 min. The effect of thrombin was prevented in lung tissue from PAR-1-null animals.

**PAR-1 agonist peptide.** PAR-1 agonist peptide, at a concentration of 5 μM, produced a similar threefold increase of MLC phosphorylation above the basal level within the first 7 min of agonist exposure (Fig. 9C). This increase of MLC phosphorylation persisted when agonist exposure was increased to 20 min. In lung tissue obtained from PAR-1-null mice, the PAR-1 agonist peptide was ineffective in augmenting MLC phosphorylation. Thus PAR-1 agonist peptide induces MLC phosphorylation primarily through activation of PAR-1.

**DISCUSSION**

We developed an isolated-perfused mouse lung preparation in which we quantified liquid permeability ($K_{fc}$) as well as vessel permeability to albumin ($^{125}$I-albumin PS product) to address the in vivo role of PAR-1 in mediating the leakiness of pulmonary microvessels. The perfused mouse lung preparation exhibited stable baseline measurements of lung wet weight and perfusion pressure for at least 1.5 h and retained reactivity to vasoconstrictors, such as U-46619. In normal lungs, we showed that thrombin increased lung microvascular permeability to both liquid and protein. Since the lungs were buffer perfused and are absent of leukocytes (Vogel et al., unpublished observation), the findings imply that thrombin acted independently of leukocytes in increasing endothelial permeability. Furthermore, these results are consistent with observations in cultured endothelial cells demonstrating that thrombin directly increased endothelial monolayer permeability to albumin (16, 19).
In our intact mouse lung preparation, lung edema (i.e., increase in lung wet weight) followed the PAR-1 agonist peptide-induced increases in $K_{fc}$ and $125\text{I}$-albumin PS product after a 15–20 min lag. Edema formation was less pronounced with thrombin, despite the fact that thrombin (0.5 μM) and PAR-1 agonist peptide (10 μM) produced equivalent increases in $K_{fc}$. Because thrombin can act on other PARs besides PAR-1, it is possible that thrombin may have initiated compensatory responses that limited the edema formation. With sufficiently low concentration of either thrombin or PAR-1 agonist peptide, the increase in wet weight was dissociated from any change in $P_{pa}$. Hence, we attribute edema formation following PAR-1 activation to a rise in vascular permeability. At relatively high concentrations of PAR-1 agonist, a clearcut pulmonary vasoconstriction was observed both prior to and during the development of edema. The latter finding is strikingly similar to the effects of higher thrombin concentration in perfused rat lung preparations (28), in which increased postcapillary vascular resistance preceded the rise in lung wet weight.

We used PAR-1-deficient mice to explore the in vivo function of PAR-1 in mediating the thrombin-induced increase in pulmonary microvessel endothelial permeability. As shown in Figs. 6–8, basal measures of both liquid and protein permeability did not change significantly from baseline in PAR-1-null lung preparations. The stability of the isogravimetric state in PAR-1-null mouse lung preparations was also similar to the normal preparations. Moreover, baseline values for the perfusion pressure were identical in lung preparations from normal and PAR-1 knockout mice. However, normal responses to thrombin (i.e., increases in lung weight, albumin PS, $K_{fc}$, and vascular resistance) were not evident in the PAR-1-deficient lung preparations. In contrast, these preparations gave typical vasoconstrictor responses to the thromboxane analog U-46619, indicating that PAR-1 knockout mice retained normal pulmonary vasoactivity to other vasoactive mediators. In other experiments, the overexpression of PAR-1 in mice resulted in exaggerated increases in intracellular calcium and in MLC phosphorylation by the calcium-sensitive MLC kinase (9, 11). These changes preceded contraction of endothelial cells, resulting from interaction of the phosphorylated form of MLC with actin. In the intact lung, MLC phosphorylation could also play a role in endothelial and vascular smooth muscle contraction that underlies the observed vasoconstrictor and permeability-increasing effects of thrombin. In the present study, we showed that activators of PAR-1 induced the phosphorylation of MLC in normal but not in PAR-1-deficient lung preparations. Thus the results implicate PAR-1 activation in signaling MLC phosphorylation in vivo and in the mechanism of thrombin-induced increases in pulmonary microvessel permeability and vasomotor tone.

In summary, we have used the perfused mouse lung preparation to address the in vivo effects of thrombin on lung microvascular permeability to protein and fluid and vascular resistance. We specifically tested the role of PAR-1 in the regulation of endothelial permeability using PAR-1 knockout mice. The results showed that PAR-1 is requisite for the pulmonary vasoconstrictor and microvascular permeability-increasing properties of thrombin in the intact pulmonary microcirculation. Thus PAR-1 functions as the principle regulator of the pulmonary microvascular actions of thrombin in vivo.

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REFERENCES


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