expression of p75NTR in mouse pulmonary arteries and the putative role in neuronal survival, suggesting that its true role in disease pathogenesis is not fully understood.

A recent study (33) has demonstrated a novel biological role for p75NTR as a regulator of fibrin deposition after nerve injury and in a model of lung fibrosis. The expression of p75NTR in nonneuronal cells, such as endothelial cells (ECs), SMCs, and hepatic stellate cells, has also led to a link between p75NTR upregulation and pathologies such as atherosclerosis (41), melanoma formation (13), lung inflammation (30), and liver disease (27); all of these disease are associated with defects in fibrin degradation. Chronic thromboembolic pulmonary hypertension (CTEPH) is another disease whose main etiology is decreased fibrinolysis and pulmonary vasculopathy. Morris et al. (23) provided evidence to suggest that the resistance of CTEPH patient fibrin to plasmin-mediated lysis may be due to alterations in fibrinogen. However, previous studies of fibrinolysis also suggested that an upregulation of tissue-type plasminogen activator and type 1 plasminogen activator inhibitor (PAI-1) may also contribute to the deranged fibrinolysis in CTEPH patients (25). The expression of PAI-1 itself is dependent on the expression and function of p75NTR; p75NTR increases PAI-1 (33), but only after lung injury (in this case, triggered by LPS treatment). Therefore, it is possible that the increased PAI-1 in CTEPH patients is due to enhanced p75NTR activity following the vascular injury that initiated formation of the fibrotic clot.

In the human lung, neurotrophins and their receptors (high-affinity Trk and low-affinity p75) have been detected in various cell types, including epithelia, airway and vascular smooth muscle, alveoli, and neurons (32). Interestingly, p75NTR is detectable only in ganglionic neurons and pulmonary artery (PA) SMCs (PASMCs) in human lungs (32). Rat PASMCs also exhibit similar specific immunoreactivity for p75NTR (31). Furthermore, expression of all neurotrophin receptors, including p75NTR, is enhanced in smooth muscle (both bronchial and PASMCs) from spontaneously hypertensive rats compared with normotensive

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animals (31). Pulmonary vasconstriction, such as that observed in pulmonary hypertensive subjects, is due to contraction of PASMCs within the vascular wall. Based on these findings, we hypothesized that p75NTR may have a novel role in regulating PA vasoreactivity. In the present study, we evaluated the agonist-mediated vasconstriction and nitric oxide-mediated vasodilatation of pulmonary arteries isolated from wild-type (WT) and p75NTR knockout (p75−/−) mice. Our findings suggested that p75NTR deletion selectively augments the development of active tension of the PA in response to agonist-induced capacitative Ca2+ entry (CCE) and to endothelin-1 (ET-1). Vasoreactivity to other vasoactive compounds such as serotonin (5-HT), U-46619 (U4; a thromboxane A2 analog), 4-aminopyridine (4-AP; a K+ channel blocker), and thrombin appeared not to be altered by p75NTR deletion.

MATERIALS AND METHODS

Animals and isolation of PA rings. Male 5- to 8-wk-old p75−/− mice (19) were from a C57Bl/6 background and purchased from The Jackson Laboratory. Balb-c mice and C57Bl/6J mice were used as controls. Use of mice for the experiments presented in this study was approved by the Institutional Animal Care and Use Committee of the University of California-San Diego. After mice had been decapitated using a procedure approved by the Institutional Animal Care and Use Committee, the lungs were quickly removed from the mouse. The right and left branches of the main PA and the first order of intrapulmonary arteries were isolated within 45 min of tissue retrieval. Adipose and connective tissues were carefully removed, and the remaining arteries were cut into 1- to 2-mm-long rings.

Tension measurements. Two tongue connected rings (75 μm diameter) were carefully inserted through the lumen of PA rings. One hook was mounted on the bottom of a perfusion chamber, and the other hook was connected to an isometric force transducer (Harvard Apparatus). Isometric tension was continuously monitored and acquired using Datalab software (Datalab Instruments). Resting passive tension was set at 300 mg (43), and rings were equilibrated for 1 h at resting tension and then challenged three times with 40 mM KCl to obtain a stable contractile response. The optimal resting (or basal) tension (300 mg) was determined by our previous study (43) showing that increasing the basal tension from 100 to 300 mg significantly augmented the 40 mM K+-induced active tension in isolated mouse PA rings. To compare active tensions in different arterial rings, the ability to normalize active tension to vessel weight or length is an important factor in interpreting the results. The PA rings we used in this study were too small for us to obtain an accurate weight, so the agonist-induced pulmonary vasocostriction was compared in different arterial rings based on the amplitude of active tension (determined by the difference between the total tension and basal tension).

Isolated PA rings were superfused with modified Krebs solution (MKS; at 37°C) consisting of (in mM) 138 NaCl, 1.8 CaCl2, 4.7 KCl, 1.2 MgSO4, 1.2 NaH2PO4, 5 HEPES, and 10 glucose (pH 7.4). For Ca2+-free solution (0Ca-MKS), CaCl2 was replaced by equimolar MgCl2, and 1 mM EGTA was added to chelate residual Ca2+. In the high-K+ solution, NaCl was replaced by equimolar KCl to maintain osmolarity. The active tension induced by agonists was normalized by the basal tension and expressed as the net increase in tension (in mg).

For small vessels like the mouse PA rings used in this study, it is difficult to insert hooks into the lumen without damaging ECs. Our previous histological data showed that ECs were present in the PA rings after contraction experiments (43). Perfusion of bradykinin, an endothelium-dependent vasodilator, caused a marked relaxation in mouse PA rings contracted by 40 mM K+ (43). These data indicated that small vessels can be mounted with a functional endothelium. In this study, however, endothelial function (e.g., bradykinin-mediated vasodilatation) was not determined for each vessel. The comparison of agonist-induced pulmonary vasocostriction and vasodilatation between WT and p75−/− mice might be affected by the potential damage of endothelial function in the isolated vessels.

Histological preparation. After the contraction experiment, PA rings were fixed overnight in 10% neutral buffered formalin. The formalin-fixed rings were serially cut for microscopic examination. The vessel tissues were processed and embedded in paraffin blocks in an automatic tissue processor (Sakura Tissue-Tek VIP, Sakura Finetek). Paraffin-embedded tissues were cut into 5-μm-thick sections for staining with either lectin (endothelium specific) or α-actin (smooth muscle specific).

p75NTR immunohistochemistry. Isolated PA rings were embedded into OCT, frozen, sectioned on a cryostat, and placed onto glass slides. Tissue sections were then fixed in methanol for 7 min at −20°C. After being washed in PBS (3 washes for 5 min each), sections were blocked in 3% BSA in PBS plus 0.1% Triton for 30 min, followed by three 5-min washes in PBS. Antibodies against p75NTR (goat anti-p75NTR, 1:10 dilution, Santa Cruz Biotechnology), CD31 (rat polyclonal, 1:10 dilution, BD Pharmingen), and desmin (goat polyclonal, 1:10 dilution, Santa Cruz Biotechnology) were diluted in 1% BSA in PBS plus 0.1% Triton and incubated overnight at 4°C. Primary antibodies were washed off with PBS (3 washes for 5 min each), and secondary antibodies (donkey anti-goat FITC, 1:200 dilution; donkey anti-rabbit Cy3, 1:200 dilution; and anti-mouse aminomethyl-coumarin, 1:200 dilution) were incubated for 30 min at room temperature. Tissue sections were washed in PBS (3 times for 5 min each) and mounted with Slowfade (Molecular Probes). Images were acquired on an Axioplan II epifluorescence microscope (Carl Zeiss Microimaging) using dry Plan-Neofluar lenses using ×10 (0.3 numerical aperture (NA)), ×20 (0.5 NA), or ×40 (0.75 NA) objectives equipped with an AxioCam HRc digital camera and the Axiovision image-analysis system.

Smooth muscle α-actin staining and immunofluorescence. Frozen lung tissue sections prepared from WT and p75−/− mice were thawed, fixed in 4% paraformaldehyde-PBS for 10 min, and then washed in PBS. Sections were incubated 1 h at room temperature in a blocking solution composed of PBS supplemented with 2% BSA, 0.1% Triton X-100, and 2% normal serum (Santa Cruz Biotechnology). Sections were incubated overnight at 4°C with anti-smooth muscle α-actin monoclonal antibody (1:400 dilution, Sigma) diluted in blocking solution. Sections were washed three times (10 min each) in PBS and incubated 1 h at room temperature with FITC-conjugated secondary antibodies diluted in blocker. Sections were incubated in 4′,6-diamidino-2-phenylindole (DAPI; 5 μM) staining solution for 5 min and washed in PBS before mounted in anti-fade mounting solution. Fluorescence images were taken from a DeltaVision RT Deconvolution Microscope System using a 10 objective. The number of vessels per field was obtained by averaging the numbers of vessels from 8 to 10 images.

RT-PCR. Total RNA was extracted from brains, hearts, and lungs of WT and p75−/− mice with TRIzol reagent (Invitrogen). RNA (1 μg) was first treated with DNase I (Invitrogen) before being transcribed to cDNA with Superscript II reverse transcriptase (Invitrogen). Canonical transient receptor potential (TRPC1-7) channel cDNA was amplified by PCR on a Bio-Rad thermal cycler using Platinum Blue PCR SuperMix (Invitrogen). The total volume of each reaction tube was 25 μL. PCR primer sequences are shown in Table 1. Specificities of sense and antisense oligonucleotides were examined using the National Center for Biotechnology Information BLAST program. PCR products were separated by agarose gel electrophoresis. β-Actin was used as an internal positive control for semiquantitative. The net intensity values of cDNA bands was measured by Image J software, and PCR products were normalized to the GAPDH product from the same cDNA sample PCR and run on the same gel.

Chemicals. All drugs were from Sigma unless otherwise indicated. 4-AP, phenotolamine (Phen), sodium nitroprusside (SNP; Fluka), and 5-HT were dissolved directly into MKS on the day of use. U4 was
prepared as a stock in ethanol. Phenytoine (PE) and ET-1 were prepared as concentrated stock solutions in distilled water. Cyclopiazonic acid (CPA) was dissolved in DMSO to make a stock solution of 1,000 μM. All stock solutions were aliquoted and kept frozen at −20°C until use. On the day of experiments, aliquots of the stock solutions were dissolved in MKS to the proper concentrations. The pH values of all solutions were measured after the addition of the drugs and readjusted to 7.4. DAPI was prepared as a 10 μM stock solution in an antibody buffer containing 500 mM NaCl, 20 mM Tris-HCl (pH 7.4); it was diluted (1:100) in PBS before use.

Statistics. The composite data are expressed as means ± SE. Statistical analysis was performed using paired or unpaired Student’s t-tests or ANOVA and post hoc tests (Student-Newman-Keuls) where appropriate. Differences were considered to be significant at P < 0.05.

**RESULTS**

Expression of p75NTR in PA rings. Isolated PA rings from WT mice were stained with a p75 antibody, desmin, and CD31 to identify p75NTR expression in SMCs (desmin marked) and ECs (CD31 marked) within the vessel wall. As shown in Fig. 1, p75NTR was detected not only in the smooth muscle (Fig. 1, A, B, and D) but also in the endothelium (Fig. 1, A–C). Double staining for p75NTR and desmin, and p75NTR and CD31, demonstrated the colocalization of p75NTR in both ECs (Fig. 1E) and SMCs (Fig. 1F), respectively. This was confirmed by triple staining with p75NTR, desmin, and CD31 (Fig. 1E). Double staining for desmin and CD31 showed little colocalization, as expected (Fig. 1G).

Mouse lung p75NTR expression. Lung sections obtained from WT and p75−/− mice were fixed and mounted. Immunohisto-

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**Table 1. Oligonucleotide sequences of the primers used for RT-PCR**

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<th>GenBank Accession No.</th>
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<td>NM_011643</td>
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GenBank accession numbers are accession numbers in GenBank for the sequence used in designing the primer. TRPC, canonical transient receptor potential Ca2+ channels.

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Fig. 1. Expression of the p75 neurotrophin receptor (p75NTR) in the pulmonary artery (PA). A: immunohistochemical analysis of p75NTR expression (green) in isolated mouse PA rings using CD31 (blue) and desmin (red) as markers of endothelial cells and smooth muscle cells, respectively. Inset, triple staining revealed areas of colocalization between p75NTR and CD31 (yellow arrowhead) and p75NTR and desmin (red arrowheads). The box outlines the area magnified further in B–G. B–D: higher-magnification images of p75NTR staining alone (B; green staining), CD31 alone (C; blue staining), and desmin alone (D; red staining). E and F: double staining for p75NTR and CD31 again showed colocalization (E; greenish-blue staining), as did double staining for p75NTR and desmin (F; light brown staining). G: double staining for desmin and CD31 showed little colocalization.
Fig. 2. Active tension induced by active store depletion-induced capacitative Ca\(^{2+}\) entry (CCE) is enhanced by p75\textsuperscript{NTR} deletion. A: fixed and mounted lungs slices from wild-type (WT; left) and p75\textsuperscript{NTR} knockout (p75\textsuperscript{−/−}; right) mice were stained with 4',6-diamidino-2-phenylindole (DAPI; blue) and p75\textsuperscript{NTR} antibody (red). Insets (magnified boxes from top images) show p75\textsuperscript{NTR} staining in the wall of PAs of WT animals only. B: representative traces showing tension generated by exposures of WT and p75\textsuperscript{−/−} PA rings to 40 mM K\(^{+}\) (40K). C: summary of active tension induced by 40K in WT (n = 8) and p75\textsuperscript{−/−} (n = 8) PA rings. D: representative tension traces of rings exposed to phenylephrine (PE) and phentolamine (Phen) in the absence or presence of p75\textsuperscript{−/−}. E: summary of active tension induced by 40K and CCE in PA rings exposed to cyclopiazonic acid (CPA) in the absence and presence of phenylephrine (PE; middle) and by receptor-operated Ca\(^{2+}\) influx (PE + Ca; right) according to the protocol shown in D. ROC, receptor-operated Ca\(^{2+}\) influx. *P < 0.05 vs. WT.

Fig. 3. Active tension induced by passive store depletion-induced CCE is not affected by p75\textsuperscript{NTR} deletion. A: representative tension tracings from WT and p75\textsuperscript{−/−} PA rings exposed to cyclopiazonic acid (CPA) in the absence and presence of extracellular Ca\(^{2+}\), with the latter used to stimulate CCE (shaded region) as a result of passive Ca\(^{2+}\) leakage from the SR to the cytosol. B: summarized data (means ± SE) showing active tension induced by 40K and CPA-induced CCE in PA rings from WT (n = 8) and p75\textsuperscript{−/−} (n = 6) mice.

AJP-Heart Circ Physiol • VOL 295 • OCTOBER 2008 • www.ajpheart.org
agonist-mediated increases in [Ca$$^{2+}$$]$$_{cyt}$$ include Ca$$^{2+}$$ entry through receptor-operated Ca$$^{2+}$$ channels (ROCs) activated by diacylglycerol and PKC. Therefore, agonist-induced smooth muscle contraction is primarily caused by a rise in [Ca$$^{2+}$$]$$_{cyt}$$ due to Ca$$^{2+}$$ release, CCE through SOCs, and Ca$$^{2+}$$ influx via ROCs, although membrane depolarization and Ca$$^{2+}$$ influx through VDCCs are also involved in agonist-induced vasoconstriction.

The next set of experiments was designed to examine whether CCE-induced contraction was affected by p75$$^{NTR}$$. As shown in Fig. 2D, left, application of the $\alpha_1$-adrenergic agonist PE to PA rings in the absence of extracellular Ca$$^{2+}$$ resulted in a small transient contraction, which was due obviously to Ca$$^{2+}$$ release from intracellular stores. After the intracellular Ca$$^{2+}$$ store has been depleted, the $\alpha$-adrenergic antagonist Phen was applied to PA rings (for $\sim$10 min) to block $\alpha$-receptors and return the level of intracellular second messengers (e.g., diacylglycerol) to the baseline level. The reintroduction of extracellular Ca$$^{2+}$$ (1.8 mM) in the presence of PE and Phen caused a sustained elevation in active tension, which was apparently caused by CCE (Fig. 2D, shaded area). The amplitudes of Ca$$^{2+}$$ release-mediated PA contraction were comparable in WT and p75$$^{-/-}$$ mice (Fig. 2, D and E, left), whereas the amplitudes of CCE-induced PA contraction were significantly greater in PA rings from p75$$^{-/-}$$ mice than in rings from WT mice (Fig. 2, D and E, middle). Removal of Phen from the media (in the continued presence of PE and Ca$$^{2+}$$) resulted in a large contraction, which was believed to be due to Ca$$^{2+}$$ influx through ROCs (and VDCCs). The amplitudes of ROC-mediated contraction were, however, not significantly different between PA rings from WT and p75$$^{-/-}$$ mice (Fig. 2, D and E, right).

These observations suggest that p75$$^{NTR}$$. activation regulates agonist-mediated pulmonary vasoconstriction by selectively inhibiting CCE-mediated contraction. p75$$^{NTR}$$ deletion, however, appeared to have no significant effect on PE-induced pulmonary vasoconstriction due to Ca$$^{2+}$$ influx via ROCs and VDCCs (see Fig. 2B).

Fig. 5. Active tension induced by serotonin (5-HT), thromboxane A2 analog U-46619 (U4), and 4-aminoypyridine (4-AP) is not affected by p75$$^{NTR}$$ deletion. A–C: representative tracings (a), summarized absolute tension (b), and summarized active tension (c) in WT and p75$$^{-/-}$$ PA rings exposed to 5 $\mu$M 5-HT (A; n = 8), 10 $\mu$M U4 (B; n = 8), and 5 mM 4-AP (C; n = 8) in the presence of extracellular Ca$$^{2+}$$. **P < 0.01 and ***P < 0.001 vs. Cont.
reversible inhibitor of the SR Ca\(^{2+}\) pump [sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase] that “passively” depletes Ca\(^{2+}\) from intracellular stores in the absence of extracellular Ca\(^{2+}\) (34). As shown in Fig. 3, CPA induced a small contraction in the absence of extracellular Ca\(^{2+}\) due to Ca\(^{2+}\) leakage from the SR to the cytosol (Fig. 3A). After intracellular stores had been depleted, restoration of extracellular Ca\(^{2+}\) caused a large contraction due to CCE. However, the contractions caused by the passive store depletion-mediated CCE were not significantly different between PA rings from WT and p75\(^{-/-}\) mice (Fig. 3, A and B). These results indicate that, in contrast to CCE induced by active store depletion (e.g., by PE), p75\(^{\text{NTR}}\) deletion does not affect contraction mediated by CCE due to passive store depletion. Our next experiments examined the possibility that p75\(^{\text{NTR}}\) can modulate CCE triggered by mechanisms other than SR Ca\(^{2+}\) depletion.

\(p75^{\text{NTR}}\) is involved in regulating ET-1-mediated pulmonary vasoconstriction. ET-1 is a potent vasoconstrictor that exerts its contractile action on PA rings by activating ET type A and B receptors (8, 21). As shown in Fig. 4, the application of 0.1 \(\mu\)M ET-1 caused a transient contraction in PA rings isolated from WT and p75\(^{-/-}\) mice (Fig. 4A). However, the amplitude of ET-1-induced active tension in PA rings isolated from p75\(^{-/-}\) mice was much greater than in rings from WT mice (Fig. 4, A–C). In addition to the amplitude of the transient contraction, the kinetics of ET-1-mediated contraction in PA rings from p75\(^{-/-}\) mice seemed to be different from those in rings from WT mice. The ET-1-induced PA contraction in p75\(^{-/-}\) mice contained an initial transient contraction followed by a sustained contraction before ET-1 was washed out, whereas the ET-1-induced contraction in WT mouse PA rings only had a transient contraction (Fig. 4A). These results suggest that p75\(^{\text{NTR}}\) is involved in regulating ET-1-induced PA contraction; the activation (or presence) of p75\(^{\text{NTR}}\) inhibits ET-1-mediated pulmonary vasoconstriction.

Effect of \(p75^{\text{NTR}}\) on pulmonary vasoconstriction induced by 5-HT, a thromboxane analog, and 4-AP. In addition, we examined the effects of a variety of vasoactive stimulants that target membrane receptors and/or ion channels. Stimulation with 5 \(\mu\)M 5-HT, 10 \(\mu\)M U4 (a thromboxane A\(_2\) analog), and 5 mM 4-AP (a K\(^+\) channel inhibitor) all caused the contraction of PA rings from WT and p75\(^{-/-}\) mice (Fig. 5). The amplitudes of active tension and time to peak contraction kinetics did not appear to be affected by the p75\(^{\text{NTR}}\) deletion.

In related experiments, the extracellular application of a high concentration (10 U/ml) of thrombin elicited reversible transient contractions of WT and p75\(^{-/-}\) PA rings that were not apparent at a lower concentration (0.1 U/ml; Fig. 6, A and B). The active tension generated by 10 U/ml thrombin in PA rings, although slightly large, was not statistically different in p75\(^{-/-}\) (\(P = 0.11\)) mice compared with WT controls (Fig. 6C). Our findings suggest that normal p75\(^{\text{NTR}}\) activity may differentially regulate vasoconstrictor signaling and, more specifically, that intact p75\(^{\text{NTR}}\) signaling may be involved in suppressing ET-1-mediated vasoconstriction while having little effect on pulmonary vasoconstriction induced by 5-HT and U4.

\(p75^{\text{NTR}}\) deletion does not alter SNP-induced vasodilation. Previous experiments have focused primarily on excitation-contraction coupling using vasoconstrictors. We next tested whether vasodilation in response to the nitric oxide donor SNP was altered by p75\(^{\text{NTR}}\) deletion. PA rings were precontracted by an exposure to 25 mM K\(^+\) and then exposed to increasing doses of SNP (1 pM–10 \(\mu\)M). In PA rings from both WT and p75\(^{-/-}\) mice, SNP caused progressive dose-dependent relaxation (Fig. 7, A and B). However, based on the results shown in Fig. 7C, which depicts the percent active tension generated by 25 mM K\(^+\) as a function of SNP concentration, SNP-induced pulmonary vasodilation in PA rings from p75\(^{-/-}\) mice was not significantly different from that in rings from WT mice. These data suggest that p75\(^{\text{NTR}}\) deletion does not alter the vasodilatory response to SNP.

\(p75^{\text{NTR}}\) deletion alters TRPC expression. CCE function is linked to the expression of TRPC channels. Having observed a change in CCE in PA rings isolated from p75\(^{-/-}\) mice, we examined and compared TRPC expression in tissues isolated from WT and p75\(^{-/-}\) mice. Extracts from the brain, heart, and lungs from WT and p75\(^{-/-}\) mice were evaluated for total mRNA expression of seven TRPC isoforms. Brain tissues from WT and p75\(^{-/-}\) animals exhibited similar levels (not statistically different) of all TRPC subunits (Fig. 8A). TRPC1 was more highly expressed in the p75\(^{-/-}\) heart, but the increase was not statistically significant compared with WT (Fig. 8B); only TRPC1–3 and TRPC6 isoforms were detected in the heart. In the lungs, both TRPC1 and TRPC2 were more highly expressed (\(P < 0.05\) and \(P < 0.01\), respectively) in p75\(^{-/-}\) tissues (Fig. 8C). TRPC3 and TRPC6 expression were also higher, but not significantly, in p75\(^{-/-}\) lungs. The data suggest that p75\(^{\text{NTR}}\) may suppress the expression of TRPC subunits underlying CCE and receptor-operated Ca\(^{2+}\) entry in pulmonary tissues.
Three main factors contribute to the development of pulmonary hypertension: enhanced and sustained vasoconstriction, vascular remodeling (in the form of intimal and medial hypertrophy), and in situ thrombosis, all of which can ultimately lead to the narrowing and/or obliteration of the vascular lumen and increased pulmonary vascular resistance. Having ascertained the increased contractility of PA rings from p75NTR knockout animals, we investigated whether pulmonary vascular remodeling might also be evident in the knockout animals. Figure 9A shows smooth muscle α-actin in lung cross sections from WT and p75NTR−/− mice. Increased wall thickness was evident in p75NTR−/− sections. In some segments, obliteration of the vascular lumen was well under way, suggesting that these animals likely developed pulmonary hypertension. We quantified the remodeling by evaluating the wall thickness and diameter of vessels in lung cross sections. In p75NTR−/− mice, the PA wall was thickened (P < 0.001) and the external diameter was decreased compared with WT mice (Fig. 9B). The ratio of thickness to diameter was also significantly increased in p75NTR−/− mice. We also verified the extent of vascularization in the lungs. Figure 9C shows the numbers of vessels counted per field (at ×10 magnification) in WT and p75NTR−/− lung cross sections; vascular pruning was evident in the lungs of p75NTR−/− mice. Taken together, these observations suggest that p75NTR−/− mice exhibit histological and pathological changes consistent with pulmonary hypertension.

**DISCUSSION**

Excitation-contraction coupling in vascular smooth muscle is primarily initiated by an increase in [Ca^{2+}]_{cyt} in vascular SMCs. A rise in [Ca^{2+}]_{cyt} in PASMCs is a major trigger for pulmonary vasoconstriction and thus an important intracellular signaling process for excitation-contraction coupling in PAs. Smooth muscle contraction and relaxation can be regulated by changes in membrane potential in SMCs. When the SMC membrane is depolarized (e.g., with high-K^+ perfusate or by blockade of K^+ channels in the plasma membrane), Ca^{2+} influx through VDCCs is a major recourse for the elevated [Ca^{2+}]_{cyt} and serves as an important mechanism for SMC contraction.

Smooth muscle contraction and relaxation can also be regulated by mechanisms independent of changes in membrane potential. The elevated [Ca^{2+}]_{cyt} in SMCs, upon activation of membrane receptors, is mainly caused by Ca^{2+} influx through ROCs and/or SOCs and by Ca^{2+} release from intracellular Ca^{2+} stores, such as the SR or ER. However, many contractile agonists cause vasoconstriction by a complex mechanism involving Ca^{2+} release from different intracellular stores and...
Ca\(^{2+}\) influx through different channels (e.g., ROCs, SOCs, and VDCCs).

As shown in this study, pulmonary vasoconstriction induced by membrane depolarization, as a result of high-K\(^+-\)mediated shift of the K\(^+\) equilibrium potential or 4-AP-mediated inhibition of voltage-gated K\(^+\) channels on the surface membrane of PASMCs, was not significantly different in PA rings from WT and p75\(^{-/-}\) mice (Figs. 2, B and C, and SC). These results suggest that p75\(^{NTR}\) is probably not involved in membrane depolarization-induced Ca\(^{2+}\) influx through VDCCs in PASMCs.

The amplitude of active tension induced by 5-HT (Fig. 5A), the thromboxane A\(_2\) analog (U4; Fig. 5B), and thrombin (Fig. 6) appeared to be comparable in PA rings from WT and p75\(^{-/-}\) mice. However, ET-1-induced active tension in PA rings from p75\(^{-/-}\) mice was significantly greater than in rings from WT mice (Fig. 4). These results indicate that p75\(^{NTR}\) and/or its downstream signaling cascades selectively regulate ET-1-induced pulmonary vasoconstriction. It is still unclear how p75\(^{NTR}\) interferes with ET-1 and endothelin receptor signaling in PASMCs to inhibit PA smooth muscle contraction. p75\(^{NTR}\) is known to function as a coreceptor for several receptors, such as TrkA (3), NogoR (40), and PAR3 (6), and regulate their downstream signaling. One of the possibilities is that p75\(^{NTR}\) selectively interacts with the ET-1 signaling pathway to mediate Ca\(^{2+}\) mobilization (or depletion) from intracellular stores (i.e., IP\(_3\)-sensitive SR).

Pulmonary vasoconstriction induced by active store depletion-mediated CCE, induced by the α-receptor agonist PE, was significantly enhanced by p75\(^{NTR}\) deletion (Fig. 2, D and E), whereas smooth muscle contraction induced by passive store depletion-induced Ca\(^{2+}\) influx through SOCs was comparable in PA rings from WT and p75\(^{-/-}\) mice (Fig. 3). Taken together, these data suggest that p75\(^{NTR}\) may constitutively inhibit the production of IP\(_3\) or activation of IP\(_3\) receptors on the SR membrane. The inhibitory effect of p75\(^{NTR}\) seems to be selective because it only affects ET-1- and PE-induced pulmonary vasoconstriction.

p75\(^{NTR}\) has been primarily characterized in the regulation of cellular differentiation and/or survival in the nervous system. Recent studies have identified novel biological roles of p75\(^{NTR}\) outside of the nervous system in the regulation of lung fibrosis (33) and inflammation (31) and liver regeneration (27). Few studies have evaluated its importance in smooth muscle physiology, and none have evaluated its putative role in regulating muscular contraction. Ricci et al. (32) demonstrated the expression of p75\(^{NTR}\) mRNA in human PASMCs. We confirmed the predominant expression of p75\(^{NTR}\) in PASMCs in our murine model. Whole animal knockout of p75\(^{NTR}\) abolished its expression in lung arteries. However, membrane depolarization-induced vasoconstriction (i.e., by 40 mM K and 4-AP) was not significantly altered by p75\(^{NTR}\) deletion, unlike agonist-induced contraction.

Colocalization of p75\(^{NTR}\) with CD31 also suggested the expression of p75\(^{NTR}\) in ECs from WT animals. This is a novel observation, as very few studies have evaluated its importance in vascular ECs. In a recent study, Caporali et al. (5) demonstrated that gene transfer-induced expression of p75\(^{NTR}\) impairs the survival, proliferation, migration, and adhesion capacities of cultured ECs and endothelial progenitor cells. Intramuscular p75\(^{NTR}\) delivery also impaired angiogenesis and neovascularization in human umbilical vein ECs. p75\(^{NTR}\) in cultured porcine aortic ECs has also been associated with EC migration and angiogenesis (29). In an experimental autoimmune encephalomyelitis model (murine model of multiple sclerosis), p75 expression was linked to altered cellular infiltration and interaction of activated ECs with cells of the immune system (18); a similar role may be involved in brain injury and regulation of the blood-brain barrier (24). In our experiments, the endothelium was intact in the PA rings, and no work was conducted on endothelium-denuded vessels, so we cannot evaluate the potential impact of endothelial p75\(^{NTR}\) expression of pulmonary vascular function. However, judging from the excessive wall hypertrophy and vascular lumen obliteration we observed in p75\(^{-/-}\) tissues (Fig. 9), we can hypothe-
esize that the decreased p75\textsuperscript{NTR} expression may somehow have contributed to either 1) increased EC proliferation or 2) increased PASMC proliferation due to infiltration of mitogenic and proliferative factors from the vascular lumen subsequent to impairment of the endothelial barrier. Whether this is truly the case remains to be investigated.

p75\textsuperscript{NTR} can interact directly with phosphodiesterase 4A to target cAMP degradation to the plasma membrane (33). In the lung, cAMP downregulation by p75\textsuperscript{NTR} results in decreased proteolytic activity of tissue plasminogen activator and increased pulmonary fibrosis. As it relates to vascular tone, cAMP is an intracellular second messenger that usually causes increased pulmonary vasodilation (12, 28); therefore, cAMP downregulation by p75\textsuperscript{NTR} results in decreased pulmonary vasoconstriction. However, ET-1-induced pulmonary vasoconstriction was significantly enhanced in PA rings from p75\textsuperscript{−/−} mice; that is, p75\textsuperscript{NTR} exerts an inhibitory effect on ET-1-mediated PA contraction. These observations suggest that p75\textsuperscript{NTR}-modulated cAMP production and/or degradation is not involved in controlling ET-1- and PE-induced pulmonary vasoconstriction. In addition, p75\textsuperscript{NTR} deletion did not have a significant effect on SNP-induced pulmonary vasoconstriction, further indicating that p75\textsuperscript{NTR}-mediated changes in intracellular second messengers (e.g., cAMP and cGMP) may not directly affect agonist-induced vasoconstriction and vasodilation.

We and others have previously demonstrated that CCE can modulate pulmonary vascular tone in rat PAs (14, 17, 22, 35, 39, 42). CCE is often associated with store depletion-induced Ca\textsuperscript{2+} influx via SOCs (4). We showed that CCE induced by PE (i.e., by passive store depletion) was not altered by p75\textsuperscript{NTR} deletion. However, agonist (e.g., PE)-induced CCE, or CCE induced by active store depletion, was significantly enhanced by p75\textsuperscript{NTR} deletion, although even this response varied with the nature of the agonist. Active tension induced by PE and ET-1 was significantly enhanced in p75\textsuperscript{−/−} mouse PAs, whereas tension generated by 5-HT, U4, 4-AP, and thrombin remained unaffected. ET-1 is the most potent vasoconstrictor of PAs. The indication that its vasoactive functions are suppressed by p75\textsuperscript{NTR} expression suggests that p75\textsuperscript{NTR} may be an important regulator not only of pulmonary fibrosis (33) but also of pulmonary vascular tone.

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26. Park JA, Lee JY, Sato TA, Koh JY. Co-Induction of p75\textsuperscript{NTR} and p75\textsuperscript{NTR}\textsuperscript{−/−} associated death executor in neuronal and proliferative factors from the vascular lumen subsequent to impairment of the endothelial barrier. Whether this is truly the case remains to be investigated.
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