Prednisolone inhibits PDGF-induced nuclear translocation of NF-κB in human pulmonary artery smooth muscle cells

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Ogawa A, Firth AL, Yao W, Rubin LJ, Yuan JX. Prednisolone inhibits PDGF-induced nuclear translocation of NF-κB in human pulmonary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 295: L648–L657, 2008. First published August 15, 2008; doi:10.1152/ajplung.90245.2008.—Pulmonary vascular remodeling, a major cause for the elevated pulmonary vascular resistance in patients with pulmonary arterial hypertension (PAH), is partially due to increased proliferation of pulmonary arterial smooth muscle cells (PASMC) in the media, resulting in vascular wall thickening. Platelet-derived growth factor (PDGF) is a potent mitogen that may be involved in the progression of PAH. Blockade of PDGF receptors has been demonstrated to have therapeutic potential for patients with severe pulmonary hypertension. Prednisolone is an immunosuppressant shown to have anti-inflammatory and antiproliferative effects on PASMC. This study was designed to investigate whether PDGF and prednisolone affect human PASMC proliferation by regulating the nuclear translocation of NF-κB (a transcription factor composed of 2 subunits, p50 and p65). Treatment of human PASMC with PDGF (10 ng/ml) significantly increased nuclear translocation of p50 and p65 subunits. Inhibition of NF-κB activation or nuclear translocation of p50/p65 significantly attenuated PDGF-induced PASMC proliferation (determined by [3H]thymidine incorporation). In the presence of prednisolone (200 μM), the PDGF-induced nuclear translocation of p50 and p65 subunits was markedly inhibited (P < 0.05 vs. the cells treated with PDGF alone). These results indicate that PDGF-induced nuclear translocation of NF-κB may play an important role in stimulating PASMC proliferation (and/or enhancing PASMC survival), whereas prednisolone may exert anti-inflammatory and antiproliferative effects on PASMC by inhibiting NF-κB nuclear translocation. Further experiments show that PDGF inhibits PASMC proliferation.

PULMONARY VASCULAR REMODELING is a major contributor to the elevated pulmonary vascular resistance in patients with pulmonary arterial hypertension. Increased growth factors (e.g., PDGF) and enhanced inflammatory responses (characterized with cytokines and chemokines that can stimulate or activate NF-κB) have recently been demonstrated to be important causes for the development of pulmonary vascular remodeling in patients with idiopathic pulmonary arterial hypertension (IPAH) and chronic thromboembolic pulmonary hypertension (26, 29). Prednisolone is an anti-inflammatory and immunosuppressive drug widely used for treatment of bronchial asthma, chronic obstructive pulmonary disease, autoimmune disease, and leukemia (2, 6, 17, 23, 28). Prednisolone is also routinely used postoperatively in patients with IPAH who have undergone lung transplantation (24, 42). The use of immunosuppressants to treat pulmonary hypertension has shown promising beneficial effects in some patients (4, 19). In addition to its immunosuppressive effects, prednisolone has been shown to inhibit PDGF-stimulated proliferation of pulmonary artery smooth muscle cells (PASMC) from patients with IPAH (26). The antiproliferative effect of prednisolone is believed to be due, in part, to increased expression of p27, an inhibitor of cyclin-dependent kinase (26).

Our laboratory has previously demonstrated that PDGF can stimulate PASMC proliferation by upregulation of canonical transient receptor potential channels (TRPC) and enhancement of receptor-mediated increase in cytosolic Ca2+ concentration ([Ca2+]cyt) via activation of STAT3 and c-Jun (37, 40). Furthermore, upregulation of TRPC channels and cell proliferation in human PASMC by the inflammatory mediator, tumor necrosis factor (TNF)-α, was shown to involve nuclear translocation of NF-κB, a transcription factor known to exert anti-apoptotic effects (38, 39).

Since excessive proliferation of PASMC is a contributory factor to pulmonary vascular remodeling in patients with pulmonary arterial hypertension, it is plausible that immunore sponsive pathways suppressed by prednisolone contribute to this response. Indeed, this may involve inhibition of PDGF-mediated proliferative effects and attenuation of NF-κB nuclear translocation. Therefore, the aim of this study was to test the hypothesis that interaction with PDGF-mediated nuclear transduction of NF-κB is one of the pathways by which prednisolone inhibits PASMC proliferation.

MATERIALS AND METHODS

Cell culture. Human PASMC from normal subjects were purchased from Cambrex (East Rutherford, NJ) and cultured in a humidified 5% CO2 atmosphere at 37°C in DMEM (Invitrogen, Carlsbad, CA) with 10% FBS. After reaching 70% confluency, the cells were subcultured following trypsin/EDTA (0.05%/0.02%) treatment. For experiments, PASMC were grown in 10% FBS containing DMEM to ~80% confluence. Then, on day 1, the culture media were replaced with low-serum culture media (DMEM, 0.1% FBS), and the cells were cultured for another 48 h. On day 3, PDGF-BB (10 ng/ml; Sigma, St. Louis, MO) was added alone or with prednisolone (200 μM) or with prednisolone (200 μM) and PDGF-BB (10 ng/ml). The cells were cultured for 24 h. For experiments testing the effect of PDGF-mediated PASMC proliferation, tyrrolidine dithiocarbamate (PDTC; 50 μM, Sigma) or 4-methyl-N-[3-(phenylpro pyl)benzene-1,2-diamine (JSH-23; 20 μM, EMD Chemicals, Gibbstown, NJ) was added to the media for 24 h on day 3. For experiments involving TNF-α (Biomol International), cells were treated with 30 ng/ml for 30 min. In some experiments, PASMC were prepared from...
transplant lung tissues. Human lung tissues were retrieved after approval from the Human Ethics Committee and the Institutional Review Board of the University of California, San Diego was obtained. Documented written informed consent was obtained from all patients before the procedure. PASMC were isolated using enzymatic dissociation from pulmonary arteries isolated from both IPAH patients (tissues obtained at lung transplantation) and from control patients (non-IPAH). Peripheral arterial segments (<1 mm in outer diameter) were used. Isolated cells were cultured in smooth muscle growth media (Cambrex) containing 10% FBS and 0.1 mg/ml kanamycin in a humidified 5% CO2 atmosphere at 37°C.

Western blot analysis. The cells were gently washed twice in cold PBS, scraped into 0.3 ml of lysis buffer [1% Nonidet P-40 (NP-40), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 100 µg/ml phenylmethylsulfonyl fluoride, and protease inhibitors], and incubated for 30 min on ice. The cell lysates were then sonicated and centrifuged at 12,000 rpm for 10 min, and the insoluble fraction was discarded. Cytoplasmic and nuclear extraction was performed using the protocol by Jobin et al. (16). The cytoplasmic fraction was extracted in cytoplasmic extraction buffer (10 mM Tris·HCl, 60 mM KCl, 1 mM EDTA, 0.4% NP-40, 1 mM dithiothreitol, and protease inhibitors) and concentrated by centrifugation at 2,500 rpm for 3 min. Pellets were resuspended in nuclear extraction buffer (50 mM Tris con 25% glycerol, and protease inhibitors), and incubated for 30 min on ice. The cell lysates were then sonicated and centrifuged at 12,000 rpm for 10 min, and the insoluble fraction was discarded. Protein concentrations were determined by DC Protein assay (Bio-Rad Laboratories, Hercules, CA) using BSA as a standard. For experiments, protein suspensions were electrophoretically separated on SDS-PAGE gels (4–20% or 10%), and protein bands were transferred onto nitrocellulose membranes by electroblot. Bands were transferred onto nitrocellulose membranes by electroblot. The imaging was performed on a Leica DMRB microscope and captured using a Leica DFC300 camera (Leica Microsystems, Bannockburn, IL) and Openlab software (Improvision). The staining intensity of the p65 and p50 was quantified by using Image J software (National Institutes of Health, Bethesda, MD).

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

<table>
<thead>
<tr>
<th>Standard Names (Accession No.)</th>
<th>Size, bp</th>
<th>Predicted Sense/Antisense Location (nt.)</th>
<th>Gene (Chrom.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPC1 (X89066)</td>
<td>369</td>
<td>For regular PCR: 5'-GATTTGGAAAAATTTCTTGGGATG-3'/ 5'-TGATAGCAATTATGAAGGAAAA-3'</td>
<td>3q23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For real-time PCR: 5'-GGACGTCGGTTGGGATG-3'/ 5'-TCAACGTCGCCACACACAGGAC-3'</td>
<td>4q27</td>
</tr>
<tr>
<td>TRPC3 (Y13758)</td>
<td>318</td>
<td>For regular PCR: 5'-TGACCTTTCGGGTGGCTCAAAATG-3'/ 5'-CCTTGGAAAGCTCTTCTTTCTGG-3'</td>
<td>1909–2262</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>For real-time PCR: 5'-CGCCGCTTCTATACAGGT-3'/ 5'-GAGAGTGAGCAGCAGTCCCA-3'</td>
<td>1255–1457</td>
</tr>
<tr>
<td>TRPC4 (U40983)</td>
<td>415</td>
<td>For regular PCR: 5'-GGGGAAATTATCTCTGCGGGAGAATG-3'/ 5'-AAAGCTTGTGGTGGAAATTCGATTG-3'</td>
<td>13q3.13</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>For real-time PCR: 5'-GCGATGTGTTGCTGATATTCTG-3'</td>
<td>790–950</td>
</tr>
<tr>
<td>TRPC6 (AJ006276)</td>
<td>438</td>
<td>For regular PCR: 5'-CTTGGGAGGAGGACGACAAACCC-3'/ 5'-AGAAGTTGCTAGCCAGGAGACTTG-3'</td>
<td>11q2.21</td>
</tr>
<tr>
<td></td>
<td>117</td>
<td>For real-time PCR: 5'-GAGCGAACATGCGGTAGAATGT-3'/ 5'-TGGCTCGAGGATTTGCTG-3'</td>
<td>1626–1742</td>
</tr>
<tr>
<td>GAPDH (AF261085)</td>
<td>243</td>
<td>For regular PCR: 5'-GACGACGAAATTGGGCTACAGC-3'/ 5'-GAGGTTGACTGAGCAAGGTTG-3'</td>
<td>1091–1333</td>
</tr>
<tr>
<td></td>
<td>108</td>
<td>For real-time PCR: 5'-ATGGGGAGGTTGGAAGGTCG-3'/ 5'-GGGGTGCTATTGATGGCAAGAACATA-3'</td>
<td>149–256</td>
</tr>
</tbody>
</table>

The accession numbers in GenBank for the sequence used to design the primers are shown in parentheses. Chrom., chromosome.
and allow intracellular esterases to cleave cytosolic fura-2-AM into active fura-2. Fura-2 fluorescence was detected as 510-nm-wave-length light emission with excitation wavelengths of 340 and 380 nm by use of the digital fluorescence imaging system from Intracellular Imaging (Cincinnati, OH). In all experiments, multiple cells were imaged in a single field, and one arbitrarily chosen peripheral cytosolic area from each cell was spatially averaged. 

\[ \text{[Ca}^{2+}]_{\text{cyt}} \] was expressed as fura-2 fluorescence emission ratio excited at 340 and 380 nm (F/F0).

RT-PCR. Total RNA was isolated from cultured PASMC using TRIzol (Invitrogen) method. One microgram of RNA was treated with DNase I (Invitrogen) before being reverse transcribed to cDNA using Superscript II reverse transcriptase (Invitrogen). The sense and antisense primers were specifically designed from the coding regions of TRPC1, TRPC3, TRPC4, TRPC6, and GAPDH (Table 1). The fidelity and specificity of the sense and antisense oligonucleotides were examined using the BLAST program. PCR was performed by a DNA thermal cycler (MyCycler, Bio-Rad Laboratories) using the Platinum PCR Supermix (Invitrogen). The PCR products were electrophoresed through a 1.5% agarose gel, and amplified cDNA bands were visualized by GelStar nucleic acid stain (Lonza, Switzerland). To semiquantify the PCR products, an invariant mRNA of GAPDH was used as an internal control. The net intensity values of cDNA bands (from electrophoretically separated PCR products) measured by a Kodak electrophoresis documentation system were normalized to the net intensity values of the GAPDH signals; the ratios are expressed as arbitrary units for quantitative comparison.

Real-time RT-PCR was performed by Opticon 2 (MJ Research, Waltham, MA) using a qPCR Mastermix Plus SYBRGreen I kit (Eurogentec). Twenty-five nanograms of cDNA and 0.5 μM sense/antisense primers were used. Cycle conditions were 95°C for 10 min (1 cycle), 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s (45 cycles). Real-time data were normalized to RNA expression level of GAPDH.

Proliferation assay. Cell number was determined using a hemocytometer. Cell count in each of the four 1-mm³ corner squares in the hemocytometer was averaged to calculate total cell number per milliliter in cell suspension. Cell number, normalized by the surface
area of the wells (cells/cm²), was used to compare cell growth rate. Cell viability was determined using trypan blue solution. 

[^3H]thymidine incorporation assay was performed to assess DNA synthesis. PASMC were seeded in 12-well plates and serum-starved for 48 h. On day 3, 1 μCi[^3H]thymidine was added to the conditioned media. Twenty-four hours later, cells were washed with cold PBS once, washed twice with cold 7.5% trichloroacetic acid, and then lysed with 0.5 M NaOH. The radioactivity was measured in a liquid scintillation counter.

Statistical analysis. The composite data are expressed as means ± SE. Differences between groups were examined for statistical significance using Student’s t-test or one-way ANOVA. Differences between controls and drug-treated cells were examined for statistical significance using Fisher’s protected least significant differences test. Differences were considered to be statistically significant when P < 0.05. RESULTS

PDGF induces nuclear translocation of NF-κB. Activation of PDGF receptors in the surface membrane of human PASMC by PDGF may stimulate cell proliferation via intracellular signaling cascades, in addition to activating STAT3 and c-Jun as we previously reported (37, 40). PDGF is also known to cause nuclear translocation of NF-κB, a transcription factor that exerts proliferative and antiapoptotic effect on many cell

![Diagram](http://ajplung.physiology.org/)

Fig. 3. Inhibition of NF-κB nuclear translocation suppresses PDGF-induced PASMC proliferation. Growth-arrested PASMC were incubated in 0.1% FBS-DMEM, PDGF (10 ng/ml), PDGF + PDTC, and PDGF + JSH-23 for 24 h before[^3H]thymidine uptake was measured. A:[^3H]thymidine incorporation (a), measured as counts per minute (cpm), and PDGF-induced[^3H]thymidine uptake (b, normalized to the basal level) in control cells (Basal, incubated in 0.1% FBS-DMEM) and cells treated with PDGF (10 ng/ml, PDGF-Cont), PDGF + PDTC (50 μM), and PDGF + JSH-23 (20 μM). **P < 0.001 vs. PDGF-Cont. **P < 0.01 vs. PDGF-Cont.

B: Western blot analysis shows the expression level of p65 and p50 in the cytoplasmic and nuclear fraction in PASMC before (Basal) and after incubation in the presence of PDGF (10 ng/ml, PDGF-Cont), PDGF + PDTC (50 μM), and PDGF + JSH-23 (20 μM). C: summarized data (means ± SE, n = 3 experiments) show the averaged protein levels of p65 (left) and p50 (right) in the cytoplasmic (Cyt-p65 and Cyt-p50, top) and nuclear (Nuc-p65 and Nuc-p50, middle) fraction and the ratio of cytoplasmic and nuclear p65 and p50 (bottom). *P < 0.05, **P < 0.01 vs. PDGF-Cont.
types (1, 7, 14). As shown in Fig. 1, exposure of human PASMC to PDGF (10 ng/ml) had little effect on expression level of NF-κB subunits p65 and p50 in the cytoplasm, but significantly increased the p65 and p50 protein levels in nuclei (Fig. 1). However, the time course of the effect of PDGF was very different from that of the proinflammatory cytokine, TNF-α. Treatment of human PASMC with TNF-α (30 ng/ml) for 0.5 h caused a significant increase of p65 protein levels in the nuclear fraction. Although treatment with PDGF (10 ng/ml) increased p65 level in the nuclei at 30 min, a similar increase to TNF-α-treated cells in p65 protein level was induced only at 24 h (Fig. 2). These results suggest that PDGF induces PASMC proliferation via multiple pathways, and the enhanced nuclear translocation of NF-κB (p65 and p50) during chronic exposure to PDGF may be one pathway in the mitogenic effect of PDGF.

Inhibition of NF-κB attenuates PDGF-induced PASMC proliferation. To further confirm the involvement of NF-κB in PDGF-mediated PASMC proliferation, we treated the cells with two different NF-κB inhibitors, PDTC and JSH-23 (18, 21, 30, 32). As shown in Fig. 3, inhibition of NF-κB nuclear translocation with 50 μM PDTC or with 20 μM JSH-23 significantly decreased PDGF-induced [3H]thymidine uptake in human PASMC (Fig. 3A). Western blot experiments using cytoplasmic and nuclear proteins show that PDTC and JSH-23 both markedly inhibited the nuclear translocation of p65 and p50 (Fig. 3B). These data indicate that NF-κB nuclear translocation is one of the pathways for PDGF to induce proliferative effect on human PASMC.

Inhibitory effect of prednisolone on PDGF-induced NF-κB nuclear translocation. Prednisolone is an immunosuppressant that has been shown to exert antiproliferative and anti-inflammatory effects on PASM C (26), vascular smooth muscle cells (36), and cardiomyocytes (20, 34). In human PASMC treated with PDGF, concurrent treatment with prednisolone (200 μM) significantly inhibited PDGF-induced nuclear translocation of NF-κB subunits (p65, 80.9 ± 8.1%, P < 0.05; p50, 74.8 ± 8.2%, P < 0.05) (Fig. 4). The cytosolic fraction of p65 and p50, however, remains unaffected by prednisolone.

Consistent with the protein expression, a significant inhibitory effect of prednisolone on the PDGF-induced NF-κB nuclear translocation determined by immunofluorescence staining was observed. As shown in Fig. 5, the fluorescence intensity of p65 and p50 in the nuclear area was significantly increased in cells treated with PDGF [from 122.2 ± 6.5 to 199.9 ± 5.1 arbitrary units (a.u.) for p65 and from 206.4 ± 6.7 to 404.8 ± 8.4 a.u. for p50; P < 0.05]. However, concurrent treatment of the cells with PDGF and prednisolone significantly reduced the increase of p65 (from 199.9 ± 5.1 to 143.7 ± 5.2 a.u., P < 0.05) and p50 (from 404.8 ± 8.4 to 269.0 ± 9.8 a.u., P < 0.01).

In addition to augmenting NF-κB nuclear translocation, PDGF also activated the MAP kinase pathway by stimulating p44/42 phosphorylation. Treatment with PDGF for 1 h significantly increased phosphorylation of p44/42 MAP kinase by 4.4 ± 1.4-fold (P < 0.05); extended treatment for 24 h increased phosphorylated p44/42 MAP kinase by 3.5 ±
0.7-fold \((P < 0.01, \text{Fig. 6})\). Notably, concurrent treatment with prednisolone for 24 h had little effect on the PDGF-induced p44/42 MAP kinase activation (or phosphorylation) (Fig. 6). These results indicate that, although PDGF activates multiple intracellular signaling cascades, prednisolone selectively inhibits the PDGF-mediated NF-κB nuclear translocation.

Effects of prednisolone on store-operated Ca\(^{2+}\) entry and TRPC expression. As PDGF can stimulate PASMC proliferation by upregulation of TRPC, the effects of prednisolone on store-operated Ca\(^{2+}\) entry were investigated. Ca\(^{2+}\) influx was measured as a change in \([\text{Ca}^{2+}]_{\text{cyt}}\) using the membrane-permeable fluorescent probe fura-2. Representative traces showing the time course of changes in \([\text{Ca}^{2+}]_{\text{cyt}}\) in PASMC treated only with PDGF (10 ng/ml) or with prednisolone (200 μM) and PDGF are shown in Fig. 7A. The cyclopiazonic acid (CPA; 10 μM)-induced rise in \([\text{Ca}^{2+}]_{\text{cyt}}\), due to Ca\(^{2+}\) mobilization from the sarcoplasmic reticulum (SR), is reduced by treatment with prednisolone (from 0.61 ± 0.03 to 0.55 ± 0.02 F/F₀, \(P < 0.05\)) (Fig. 7B). However, prednisolone did not show significant effect on the kinetics of the increases in \([\text{Ca}^{2+}]_{\text{cyt}}\) or the capacitative Ca\(^{2+}\) entry (CCE) characteristics (Fig. 7B). The “CCE-plateau” shown in Fig. 7B is calculated by subtracting the fluorescence intensity level before restoration of extracellular Ca\(^{2+}\) (or at the end of 0Ca solution) from the level right before washout of CPA. ATP is also a potent mitogen that plays an important role in the development of pulmonary vascular wall remodeling (10, 41). Extracellular application of ATP (5 mM) did not affect the increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) in PASMC treated with PDGF in the presence of prednisolone compared with those treated only with PDGF (Fig. 7C). The percentage of cells responding to ATP was 50.0% for PDGF treatment and 31.3% for concurrent treatment with prednisolone and PDGF. It is worth noting that all the cells treated with ATP were responsive to CPA when treated after ATP.

Consistent with the results on store-induced Ca\(^{2+}\) influx, prednisolone does not affect expression level of TRPC in PDGF-treated PASMC. Western blot analysis revealed no significant effect of prednisolone on the protein expression level of TRPC1, TRPC3, and TRPC6 in the presence of prednisolone compared to those treated only with PDGF (Fig. 8A). Summarized data show an averaged protein level of TRPC1, TRPC3, and TRPC6 of 105.3 ± 28.2%, 112.8 ± 10.7%, and 130.4 ± 21.9% compared with expression level in PASMC treated only with PDGF, respectively. Messenger RNA expres-
expression of TRPC1, TRPC3, TRPC4, and TRPC6 was also examined by regular (Fig. 8A) and real-time (Fig. 9) RT-PCR. There was no significant difference of TRPC expression levels between PDGF-treated cells and prednisolone and PDGF-treated cells (Figs. 8 and 9).

Inhibitory effect of prednisolone on TNF-α-induced NF-κB nuclear translocation. Treatment of human PASMC with TNF-α (30 ng/ml) for 0.5 h had little effect on the expression level of p65 and p50 in the cytoplasm but caused a significant increase of p65 and p50 protein levels in nuclei (p65, 561.5 ± 138.2%; p50, 288.3 ± 77.6%, P < 0.05) (Fig. 10, A and B). In PASMC treated with TNF-α and prednisolone, TNF-α-induced nuclear translocation of NF-κB (p65) was significantly inhibited (27.2 ± 8.7% of cells treated only with TNF-α, P < 0.01) (Fig. 10, A and C). Little effect of prednisolone on expression level of p65 and p50 in the cytoplasm was observed.

Antiproliferative effect of prednisolone. In PASMC cultured in 0.1% FBS-DMEM, treatment with PDGF (10 ng/ml for 24 h) induced a 1.4-fold increase in cell number. The addition of
prednisolone, at a concentration that caused an inhibitory effect on nuclear translocation of NF-κB, significantly inhibited PDGF-induced proliferation of PASMC by more than 20% (20.5 ± 3%, P < 0.05). These results indicate that prednisolone attenuates PDGF-mediated PASMC proliferation, at least in part, by inhibiting nuclear translocation of NF-κB.

**DISCUSSION**

PDGF is a potent mitogen likely to be involved in the pathogenesis of IPAH (3, 13). It has previously been shown to be significantly increased in lung biopsies for IPAH patients (15, 31), and blockade of PDGF receptors with imatinib mesylate attenuates pulmonary hypertension significantly (11, 31). Previously, it has been reported that prednisolone, a drug that has both anti-inflammatory and immunosuppressive effects (20, 34, 36), inhibits PDGF-induced proliferation in PASMC from patients with IPAH (26). It is important to fully understand the mechanisms by which prednisolone regulates PDGF-induced PASMC proliferation to understand the potential involvement of immunoresponsive pathways in the vascular remodeling associated with pulmonary hypertension. The current research indicates that prednisolone inhibits PDGF-induced nuclear translocation of NF-κB, preventing its stimulation of PASMC proliferation.

Prednisolone is a glucocorticoid (GC), known to affect NF-κB activation (35), a transcription factor controlling many genes important for immunity, inflammation, cell proliferation, and apoptosis. Our data show that, in proliferating PASMC, treatment with PDGF caused nuclear translocation of NF-κB verified by significantly increased p65 and p50 protein levels in nuclei (Figs. 1–3). In human smooth muscle cells, the major component of NF-κB is present as a heterodimer of a 65-kDa (p65) and a 50-kDa (p50) subunit bound to either IκBα or IκBβ (5). In quiescent cells, NF-κB resides in the cytosol in an...
inactive form. Phosphorylation of the IκB inhibitory protein and its subsequent degradation by a proteasome-dependent pathway results in activation of NF-κB (27). NF-κB dimers then translocate to the nucleus and promote transactivation of target genes and release cytokines and adhesion molecules (8, 9). This study also indicates that prednisolone significantly inhibits this PDGF-stimulated nuclear translocation of NF-κB (Figs. 4 and 5). In addition, prednisolone also inhibited TNF-α-induced nuclear translocation of NF-κB (Fig. 10). Prednisolone therefore has potent inhibitory effects on PASMC NF-κB nuclear translocation induced by inflammatory mediators. Such an effect on NF-κB is potentially very important as NF-κB is already indicated in the proliferation of vascular smooth muscle cells (22, 25).

The MAP kinase pathway is also known to be activated by PDGF. In our studies, activation of the MAP kinase pathway by PDGF was verified by detection of p44/42 phosphorylation. However, cotreatment with prednisolone did not significantly affect the PDGF-induced p44/42 activation/phosphorylation (Fig. 6), indicating that, although PDGF activates multiple intracellular signaling cascades, prednisolone selectively inhibits the PDGF-mediated NF-κB nuclear translocation.

In mouse carotid artery smooth muscle cells, however, Mehrhof et al. (22) demonstrated that PDGF- or serum-mediated proliferation was not affected in a knockin mouse model expressing the NF-κB superrepressor IκBαΔN (ΔκBαΔN). The difference between our data and the observations by Mehrhof et al. (22) may be related to the difference of species (mouse vs. human) and/or the difference of systemic artery and pulmonary. It has been demonstrated that systemic and pulmonary arterial smooth muscle cells may respond differently to the same stimuli.

PDGF may additionally stimulate PASMC proliferation by upregulation of TRPC, enhancing receptor-mediated increases in 

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[Ca^{2+}]_{cyt}
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to characteristically such mechanisms in, for example, cells and tissues from patients with pulmonary hypertension.

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