Nitric oxide induces phosphodiesterase 4B expression in rat pulmonary artery smooth muscle cells

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Busch, Cornelius J., Heling Liu, Amanda R. Graveline, and Kenneth D. Bloch. Nitric oxide induces phosphodiesterase 4B expression in rat pulmonary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 290:L747–L753, 2006. First published November 18, 2005; doi:10.1152/ajplung.00298.2005.—Phosphodiesterases (PDE) metabolize cyclic nucleotides limiting the effects of vasodilators such as prostacyclin and nitric oxide (NO). In this study, DNA microarray techniques were used to assess the impact of NO on expression of PDE genes in rat pulmonary arterial smooth muscle cells (rPASMC). Incubation of rPASMC with S-nitroso-l-glutathione (GSNO) increased expression of a PDE isoform that specifically metabolizes cAMP (PDE4B) in a dose- and time-dependent manner. GSNO increased PDE4B protein levels, and rolipram-inhibitable PDE activity was 2.3 ± 1.0-fold greater in GSNO-treated rPASMC than in untreated cells. The soluble guanylate cyclase (sGC) inhibitor, H1-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, and the cAMP-dependent protein kinase inhibitor, H89, prevented induction of PDE4B gene expression by GSNO, but the protein kinase G (PKG) inhibitors, Rp-8-pCPT-cGMPs and KT-5823, did not. Incubation of rPASMC with IL-1β and tumor necrosis factor-α induced PDE4B gene expression, an effect that was inhibited by L-Nω-(1-iminoethoxy)lysine, an antagonist of NO synthase 2 (NOS2). The GSNO-induced increase in PDE4B mRNA levels was blocked by actinomycin D but augmented by cycloheximide. Infection of rPASMC with an adenovirus specifying a dominant negative cAMP response element binding protein (CREB) mutant inhibited the GSNO-induced increase of PDE4B gene expression. These results suggest that exposure of rPASMC to NO induces expression of PDE4B via a mechanism that requires cGMP synthesis by sGC but not PKG. The GSNO-induced increase of PDE4B gene expression is CREB dependent. These findings demonstrate that NO increases expression of a cAMP-specific PDE and provide evidence for a novel “cross talk” mechanism between cGMP and cAMP signaling pathways.

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DNA microarray. Total RNA was extracted from untreated rPASMC and rPASMC exposed to GSNO for 1, 2, and 4 h using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) followed by further purification using a Qiagen RNeasy total RNA isolation kit (Qiagen, Valencia, CA). Ten micrograms of RNA and dT24 primer were used for generation of first-stand cDNA by reverse transcription followed by incubation with DNA polymerase to synthesize double-strand cDNA using Superscript Choice system (Invitrogen) according to the Affymetrix protocol (Affymetrix, Santa Clara, CA). The synthesized cDNA was used to generate biotin-labeled cRNA by in vitro transcription using the ENZO Bioarray High Yield transcript labeling kit (Affymetrix). Labeled cRNA (40 µg) was fragmented in fragmentation buffer (40 mM Tris, pH 8.1, 100 mM potassium acetate, 30 mM magnesium acetate) for 35 min at 94°C and subsequently hybridized to Affymetrix rat U34A gene chips representing 8,700 rat genes. The chips were washed, stained, and quantified according to Affymetrix protocols. Gene expression is described as absent, present, or marginal and was normalized to 1. The PDE genes represented on the Affymetrix U34A chip were: PDE1C, PDE2A, PDE3A, PDE4B, PDE4D, PDE5A, and PDE7A. Results were analyzed using Genespring software (Genespring 6.1.1, Silicon Genetics). The experiment was repeated with RNA from independently isolated rPASMC.

Quantitative RT-PCR. RNA was isolated from rPASMC using TRIzol reagent, and cDNA was generated with Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) and random primers (Promega). Quantitative PCR was performed with the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) using primers for PDE3B (5'-ATGCCAGGCCCAATGATGTA-3', 5'-CGTGTATGCATCCCTTCGTC-3'), PDE4B (5'-GGACACCTGGCAATCTTACAA-3', 5'-GCAACTGATAGTGTCACCCAGCA-3'), PDE7A (5'-AGAAGAGGTTCCTCCCCGTA-3', 5'-CAACGATACTGTTGGCTTC-3'), and SYBR Green PCR Master Mix (Applied Biosystems). Postamplification dissociation curves were performed to verify the presence of a single amplification product. Ribosomal RNA (18S) was detected with 18S VIC MGB primers (Applied Biosystems) and Taqman Universal PCR Master Mix (Applied Biosystems). Changes in PDE gene expression normalized to 18S ribosomal RNA levels were determined using the relative quantification method (Applied Biosystems).

Immunoblotting. rPASMC were harvested in KHEM buffer containing 50 mM KCl, 50 mM HEPES, 50 mM KOH, 10 mM EGTA, 1.92 mM MgCl2, and 10 µl proteinase inhibitor cocktail (Sigma-Aldrich). Samples were centrifuged at 8,000 g for 15 min at 4°C. Protein concentration of the supernatants was determined by the method of Bradford (5) using bovine serum albumin as the standard. Supernatants (30 µg) were fractionated using 10% SDS-PAGE and transferred to nitrocellulose filters (Osmonics, Minnetonka, MN). Filters were blocked at room temperature for 1 h in PBS, pH 7.4, containing 5% nonfat dry milk (PBS-milk) and then incubated at room temperature for 1 h with a rabbit polyclonal antibody directed against the PDE4B protein (1:500, Fabgenix). Bound antibody was detected after exposure to GSNO for 1, 2, 4, and 8 h using a rabbit polyclonal antibody directed against rabbit immunoglobulin (1:5,000; Cell Signaling, Beverly, MA) and visualized using chemiluminescence (Western Lightning Chemiluminescence kit; PerkinElmer Life Sciences, Boston, MA) and exposure to X-ray film. Films were scanned using a Color Image Scanner (Agfa, Mortsel, Belgium) and NIH Image 1.6 software.

PDE activity. cAMP PDE activity was measured using a modification of the methods of Francis and Corbin (15). After being rinsed in cold PBS, rPASMC were harvested on ice in cold PEM buffer (20 mM sodium phosphate, pH 6.8, 2 mM EDTA, and 25 mM 2-mercaptoethanol) with protease inhibitors and 0.1% Triton X-100. Harvested cells were disrupted by passing five times through a 22-gauge needle. Homogenates were centrifuged at 5,000 g for 10 min at 4°C to remove cellular debris, and supernatants were assayed for PDE activity. Cellular extract (50 µl) was added to a reaction mixture containing 50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 0.1 mg/ml bovine serum albumin, 2.5 µM unlabeled cAMP, and 33 nM [3H]cAMP (Amershams Life Sciences, Arlington Heights, IL) in the presence and absence of 1 µM rolipram to assess the contribution of PDE4 to total cAMP-PDE activity (150 µl final volume). Samples were incubated at 30°C for 10 min. The reaction mixture was cooled to 0°C, and 20 µl of a “stop solution” containing 10 mM GMP, 10 mM cAMP, 50 mM EDTA, 30 mM theophylline, and 100 mM Tris-HCl, pH 7.5, were added. The 5’-nucleoside monophosphate generated in the reaction mixture was converted to adenosine by the addition of 20 µl of a 10 µg/ml solution of Crotalus atrox snake venom 5’-nucleotidase and incubation at 30°C for 10 min. The reaction was terminated by the addition of 1.5 ml of a solution containing 100 µM guanosine, 100 µM adenosine, and 0.15 M EDTA. The reaction mixture was then chromatographed at room temperature using QAE-Sephadex A-25 (Pharmacia Biotech, Piscataway, NJ), previously equilibrated in 20 mM ammonium formate. [3H]Adenosine in the eluate was quantitated by liquid scintillation spectrometry.

Recombinant adenovirus. An adenovirus specifying a dominant negative CREB mutant (Ad.dnCREB) in which Ser133 was changed to alanine was a gift from Dr. William Walker (Dept. of Cell Biology and Physiology, Univ. of Pittsburgh, Pittsburgh, PA). The product of the mutant CREB cannot be activated by phosphorylation and blocks CRE-dependent gene transcription (25). A control adenovirus specifying green fluorescent protein (Ad.GFP) was generously provided by Dr. Anthony Rosenzweig (Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA). Cells were infected with Ad.dnCREB or Ad.GFP for 3 h in RPMI culture medium containing 10% Nuserum [multiplicity of infection (MOI) = 200]. The culture medium was then replaced, and infected cells were incubated for 24 h. Thereafter, cells were incubated in the presence or absence of GSNO and harvested after 2 h. Infection of rPASMC with adenovirus under these conditions leads to gene transduction in 70–80% of cells.

Statistical analysis. Data are reported as means ± SD. After approving the assumption of normality and equal variance across groups, we assessed differences using ANOVA followed by Dunnett’s test. Values were considered significant if P < 0.05. Statistics were performed using the software package SigmaStat (Jandel, San Rafael, CA).

RESULTS

GSNO increases PDE4B gene expression in rPASMC. The Affymetrix rat U34A gene chip used in these studies included DNA sequences corresponding to PDE1 through PDE7. In untreated rPASMC, transcription profiling detected the presence of mRNAs encoding PDE3B, PDE4B, and PDE7A. Exposure of rPASMC to GSNO (100 µM) induced PDE3B gene expression after 2 and 4 h 2.6 ± 1.2- and 2.6 ± 1.5-fold, respectively. PDE4B gene expression was induced at 1, 2, and 4 h 6.2 ± 0.7-, 10.5 ± 1.1-, and 7.8 ± 0.5-fold, respectively, after exposure to GSNO. GSNO decreased PDE7A mRNA levels 2.5 ± 0.1- and 2 ± 0.1-fold after 2 and 4 h, respectively. Quantitative RT-PCR confirmed that GSNO modulated PDE4B (shown) and PDE3B and PDE7A gene expression (data not shown). Because GSNO altered PDE4B mRNA levels to a greater extent than PDE3B and PDE7A mRNA levels, we focused on the mechanisms by which NO regulates PDE4B gene expression.

To investigate the effect of GSNO on PDE4B gene expression, rPASMC were incubated with or without GSNO (100 µM) for 1, 2, 4, 8, and 24 h, and the levels of PDE4B mRNA were measured by quantitative RT-PCR. Consistent with the
microarray results, exposure of rPASMC to GSNO increased PDE4B mRNA levels in a time-dependent fashion (Fig. 1). The increase in PDE4B mRNA levels was observed as early as 1 h after stimulation with GSNO with maximum mRNA levels detected at 2 h. PDE4B mRNA levels remained elevated for 4 h and declined toward basal levels within 8 h. PDE4B gene expression was also induced in a time-dependent manner by a second NO-donor compound, DETA-NONOate (100 μM), with maximum levels detected at 2 h (data not shown). The GSNO-mediated increase in PDE4B gene expression was concentration dependent (Fig. 2). Increased PDE4B mRNA levels were observed in rPASMC exposed to 2 h as little as 10 μM GSNO, and 100 μM GSNO achieved maximum effects.

GSNO increases PDE4B protein expression and rolipram-inhibitable PDE activity in rPASMC. The PDE4B antibody we used detected four proteins in rPASMC that likely represent alternately spliced PDE4B gene products. Incubation of rPASMC with GSNO (100 μM) for 4 and 8 h increased levels of an ~70-kDa PDE4B immunoreactive protein. Densitometric measurement of the PDE4B immunoreactive protein levels is shown.

Induction of PDE4B gene expression by GSNO requires sGC and cAMP-dependent protein kinase. To investigate the role of sGC and cGMP in the induction of PDE4B by NO, rPASMC were pretreated with ODQ (10 μM) for 30 min and were subsequently incubated with or without GSNO (100 μM) for 2 h. Incubation with ODQ alone did not change PDE4B gene expression (Fig. 5). ODQ inhibited the induction of PDE4B expression in GSNO-treated rPASMC. Incubation with the sGC stimulator YC1 (50 μM) induced PDE4B gene expression 3.4 ± 0.1-fold after 2 h (P = 0.001, data not shown). Pretreatment of rPASMC for 30 min with the PKG inhibitors Rp-8-pCPT-cGMPs (10 or 30 μM) or KT-5823 (1 μM) did not inhibit the ability of GSNO to induce PDE4B gene expression (data not shown).

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**Fig. 1.** Incubation with S-nitroso-L-glutathione (GSNO) increases phosphodiesterase (PDE)4B gene expression in rat pulmonary arterial smooth muscle cells (rPASMC) in a time-dependent manner. RNA was extracted from untreated rPASMC and rPASMC incubated with 100 μM GSNO for 1–24 h. Complementary DNA was generated and analyzed for PDE4B gene expression using quantitative PCR; n = 3, means ± SD, *P < 0.05 vs. control. rRNA, ribosomal RNA.

**Fig. 2.** Incubation with GSNO increases PDE4B gene expression in rPASMC in a dose-dependent manner. RNA was extracted from untreated rPASMC and rPASMC incubated with 1, 10, and 100 μM GSNO for 2 h. Complementary DNA was generated and analyzed for PDE4B gene expression using quantitative PCR; n = 3; means ± SD; *P < 0.005 vs. control.

**Fig. 3.** Incubation of rPASMC with GSNO increases PDE4B protein levels. Immunoblots were prepared from extracts of untreated rPASMC and rPASMC incubated with 100 μM GSNO for 4 and 8 h. Blots were reacted with an antibody directed against PDE4B. Incubation with GSNO led to increased expression of an ~70-kDa PDE4B immunoreactive protein. Densitometric measurement of the PDE4B immunoreactive protein levels is shown.

**Fig. 4.** GSNO induces rolipram-inhibitable PDE activity in rPASMC. Protein extracts were prepared from untreated rPASMC and rPASMC exposed to 100 μM GSNO for 4 h (n = 5 for each group). cAMP-metabolizing PDE activity was measured in the presence and absence of the PDE4 inhibitor rolipram (1 μM), and rolipram-inhibitable PDE activity is shown as a percent of activity in untreated rPASMC (*P < 0.03).
Incubation of rPASMC with the adenylate cyclase agonist FK (100 ng/ml) induced PDE4B gene expression, an effect that was blocked by H89. Incubation with H89 alone did not alter PDE4B gene expression in rPASMC. Exposure of rPASMC to 8-bromoadenosine 3',5'-cyclic monophosphate (8-BrcAMP; 1 mM) increased PDE4B mRNA levels 2.9 ± 0.1- and 2.6 ± 0.3-fold after 2 and 4 h, respectively (P < 0.001 for both), whereas 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP; 1 mM) did not significantly alter PDE4B mRNA levels.

NO produced by NOS2 induces PDE4B gene expression. Incubation of rPASMC with a combination of TNF-α (100 ng/ml) and IL-1β (20 ng/ml) for 8 h stimulates NO production by increasing the expression of the inducible NOS isoform, NOS2 (33). We observed that incubation of rPASMC with TNF-α and IL-1β markedly induced PDE4B gene expression (Fig. 7). Inhibition of NOS2 with l-NIL (1 mM) markedly attenuated the induction of PDE4B gene expression by the cytokines. Similarly, ODQ (10 μM) inhibited the cytokine-mediated induction of PDE4B gene expression.

**Induction of PDE4B gene expression requires transcription but not translation.** Pretreatment with actinomycin D, an inhibitor of gene transcription, abolished the ability of GSNO to induce PDE4B gene expression (Fig. 5). Inhibition of mRNA translation in rPASMC with cycloheximide (1 μM) augmented the GSNO-mediated increase of PDE4B gene expression (Fig. 8). Incubation of rPASMC with cycloheximide alone increased PDE4B gene expression approximately twofold.

**Induction of PDE4B expression by GSNO is CREB dependent.** To investigate the role of CREB in the induction of PDE4B gene expression by NO, rPASMC were infected with an adenovirus specifying a dominant negative CREB mutant (Ad.dnCREB) (25) at an MOI of 200. Transduction of dnCREB reduced the ability of GSNO to induce PDE4B gene expression (Fig. 9). Infection of rPASMC with Ad.GFP, as a control, did not affect the ability of GSNO to induce PDE4B gene expression.

**DISCUSSION**

Exposure of rPASMC to NO donor compounds increased PDE4B gene expression as well as protein levels and rolipram-sensitive cAMP-PDE activity. Induction of PDE4B gene expression was dose and time dependent and was mediated by a sGC- and cGMP-dependent mechanism.

We used a transcriptional profiling approach to characterize the expression of genes encoding PDE in rPASMC and to study how expression of these genes is regulated by NO. Expression of three PDE genes (PDE3B, PDE4B, and PDE7A) was detected in rPASMC using DNA microarray techniques, all three of which encode enzymes that metabolize cAMP.

Expression of these PDE genes was detected in rPASMC using DNA microarray techniques, all three of which encode enzymes that metabolize cAMP. Detection of PDE3 and PDE4 is consistent with the findings of Pauvert et al. (30), who reported that PDE3 and PDE4 are the main cAMP-hydrolyzing enzymes in the pulmonary vasculature. Moreover, expression of all three PDE genes...
was modulated by short-term exposure to GSNO with induction of PDE3B and PDE4B and repression of PDE7A. However, there are several important limitations of the transcription profiling approach we employed. First, several PDE genes were not represented on the gene chip we used, including PDE1A and PDE8 through PDE11. Second, DNA microarray techniques may fail to detect genes expressed at low levels in rPASMC. For example, PDE5 mRNA has been detected in rPASMC using RNA blot hybridization and immunoblotting techniques (28), and PDE4D gene expression has been described in rat aortic smooth muscle cells (24).

In this study, we focused on the regulation of PDE4B since PDE4B gene expression was robustly induced by GSNO and because this isoform metabolizes cAMP specifically. Induction of PDE4B gene expression by NO behaved like an early response gene. PDE4B mRNA levels increased only transiently, returning toward baseline within 8 h even in the presence of a NO donor compound with a long half-life (DETA-NONOate, time 1/2 = 20 h), suggesting the existence of a negative-feedback mechanism. Incubation of rPASMC with 100 μM GSNO for 2 h consistently increased PDE4B mRNA levels fourfold or more. In some experiments, PDE4B mRNA levels were nearly 100-fold greater in GSNO-treated cells than in untreated cells. Variation in the magnitude of the PDE4B gene response to GSNO is most likely attributable to small variations in PDE4B mRNA levels in untreated cells. However, subtle differences between rPASMC isolates and changes in rPASMC as they are passaged may also have contributed to variations in the magnitude of GSNO-induced PDE4B gene expression. Concentrations of NO donor compounds sufficient to induce PDE4B gene expression were similar to those required to modulate expression of other genes regulating vascular tone, including RhoA (37), sGC (14), and PKG (39); vascular smooth muscle cell differentiation, including smooth muscle myosin heavy chain or smooth muscle α-actin (4); and vascular smooth muscle cell proliferation, including p21 (17). Incubation of rPASMC with TNF-α and IL-1β induces NOS2 gene expression and increases NO and cGMP levels (40). In the current study, we observed that these cytokines increased PDE4B gene expression and that this induction could be inhibited by L-NIL. At this L-NIL concentration, it is possible that other NOS isoforms would be inhibited if they were present in rPASMC (36). Nonetheless, these findings suggest that concentrations of NO produced endogenously in rPASMC are sufficient to increase PDE4B gene expression.

NO increased expression of an ~70-kDa PDE4B immunoreactive protein. We used the same antibody as Farooqui et al. (13), who found a 66-kDa PDE4B protein in rat testis homogenates. On the basis of the molecular weight of the PDE4B immunoreactive protein in rPASMC, it is likely that NO induced expression either the PDE4B2 or PDE4B4 isoform. Shepherd et al. (38) reported that COS cells transfected with PDE4B cDNA produced an 85-kDa immunoreactive protein, which was ~20 kDa greater than its predicted molecular weight. D’Sa et al. (12) reported that cAMP signaling induced expression of a 65-kDa immunoreactive protein identified as PDE4B2 in cortical neurons.

Rolipram-sensitive cAMP PDE enzyme activity was increased in rPASMC exposed to GSNO. Rolipram inhibits all four PDE4B enzymes, but the IC50 for PDE4A and PDE4B (~1 nM) are much less than those of PDE4C and PDE4D (42). Incubation of rPASMC with GSNO did not alter total cAMP hydrolytic activity. Because PDE4 enzyme activity increased, but total cAMP-PDE activity did not change, it is likely that the activity of another cAMP-metabolizing PDE decreased. It is probable that GSNO decreased activity of PDE3, an enzyme that is inhibited by cGMP. Alternatively, expression of another cAMP-metabolizing PDE, PDE7, was observed to decrease in rPASMC exposed to GSNO.

An inhibitor of sGC enzyme activity, ODQ, prevented the induction of PDE4B gene expression by NO donor compounds or by NOS2-derived NO. Exposure of rPASMC to YC1, a NO-independent activator of sGC (6), induced PDE4B gene expression. These results strongly suggest that the induction of PDE4B gene expression by NO donor compounds is cGMP

Fig. 8. Cycloheximide (CHX) augments the ability of nitric oxide to induce PDE4B expression in rPASMC. After being incubated with and without 1 μM CHX for 30 min, rPASMC were stimulated with and without 100 μM GSNO for 2 h. RNA was extracted from rPASMC, and complementary DNA was generated and analyzed using quantitative PCR; n = 3; means ± SD; *P < 0.001 vs. control; †P < 0.01 vs. GSNO.

Fig. 9. Dominant negative cAMP response element binding protein (CREB) blocks induction of PDE4B gene expression by GSNO. rPASMC were infected with an adenovirus specifying a dominant negative CREB mutant (Ad.dnCREB) or an adenovirus specifying only green fluorescent protein (Ad.GFP; multiplicity of infection of 200). Uninfected and infected cells were stimulated with 100 μM GSNO for 2 h. RNA was extracted from rPASMC, and complementary DNA was generated and analyzed using quantitative PCR; n = 3; means ± SD; *P < 0.001 vs. without GSNO, †P < 0.003 vs. Ad.dnCREB.
increased cGMP levels repressed TNF-
Aizawa et al. (1) observed that in vascular smooth muscle cells, levels can repress PKG gene expression via activation of PKA. and colleagues (6) recently reported that increased cGMP activity has been observed in gastric smooth muscle cells (28). The mechanism by which NO/cGMP leads to PKA-dependent induction of PDE4B2 has been observed in COS cells (38), and PKA-dependent activation of PDE4 enzyme may have unpredictable effects in intact cells (7).

PKA-dependent induction of PDE4B4 gene expression has been observed previously in COS cells (38), and PKA-dependent induction of PDE4B2 has been observed in cortical neurons (12). PKA-dependent activation of PDE4 enzyme activity has been observed in gastric smooth muscle cells (28). The mechanism by which NO/cGMP leads to PKA-dependent activation of PDE4B gene expression is unknown. Brown and colleagues (6) recently reported that cGMP levels can repress PKG gene expression via activation of PKA. Aizawa et al. (1) observed that in vascular smooth muscle cells, increased cGMP levels repressed TNF-α-induced reporter gene expression by inhibiting PDE3 activity leading activation of PKA, presumably via increased cAMP levels. We observed that intracellular cAMP levels did not increase in whole cell extracts of rPASMC incubated with GSNO (0.1–500 μM for 30 min and 100 μM for 20, 40, and 60 min; data not shown). Although these findings appear to support the hypothesis that cGMP directly activates PKA, leading to induction of PDE4B gene expression, it is also possible that local or compartmentalized increases in cAMP levels (mediated by PDE3 inhibition) may have been sufficient to activate PDE4B gene expression but were not detected in whole cell extracts.

NO appears to induce PDE4B gene expression via a transcription-dependent mechanism since pretreatment with actinomycin D blocked induction of PDE4B by GSNO. In contrast, preincubation with the translation inhibitor cycloheximide did not block the NO-induced increase in PDE4B mRNA levels, suggesting that induction of PDE4B gene expression requires gene transcription but not new protein synthesis. Moreover, cycloheximide enhanced the induction of PDE4B gene expression by NO. These latter findings suggest that cycloheximide blocks translation of a protein or proteins that serve to limit the induction of PDE4B gene expression (potentially including PDE4B itself).

PKA activates gene transcription via phosphorylation of CREB (16, 25). It is known that NO regulates CREB activity (9, 21) and that CREB can activate the PDE4B2 promoter in cortical neurons (12), we tested the hypothesis that NO can regulate PDE4B gene expression via CREB. rPASMC were infected with an adenovirus specifying a dominant negative CREB mutant that blocks CRE-dependent gene transcription (25). In cells transduced with dominant negative CREB, the induction of PDE4B expression by NO was markedly repressed, suggesting that CREB is necessary for the induction of PDE4B gene expression by NO in rPASMC.

Interaction between cAMP and cGMP signal transduction pathways has become increasingly appreciated. Cross activation of PKA by cGMP has been reported in smooth muscle cells (2, 10) as well as in human platelets (23). NO can attenuate hormone- and FK-stimulated adenyl cyclase in N18TG2 neuroblastoma cells (22). In cardiac fibroblasts, NO attenuates isoproterenol-induced cAMP levels by increasing PDE2 activity (18). In platelets, NO can increase cAMP, as well as cGMP levels, and induce shape change via activation of PKA (27). Our observations suggest an additional mechanism by which NO/cGMP can modulate cAMP levels: induction of a gene encoding a cAMP-specific PDE, PDE4B.

PDE4 regulates tone of the pulmonary vasculature (30), aorta (24), and femoral arteries (24). PDE4 modulates vascular smooth cell proliferation (34) and vascular endothelial cell migration (29). For example, Phillips et al. (32) reported that inhibition of PDE4 decreased rPASMC proliferation and attenuated hypoxic pulmonary vasoconstriction in rat lungs isolated and perfused in situ. It is conceivable that NO regulates many of these vascular cell functions, in part by modulating PDE4B expression and activity.

In summary, exposure of rPASMC to NO increases PDE4B mRNA levels as well as protein expression and enzyme activity. The NO-induced increase of PDE4B expression is cGMP dependent. Induction of PDE4B gene expression by NO appears to be PKA and CREB dependent. The NO-induced increase of PDE4B expression illustrates a novel mechanism of crosstalk between cAMP- and cGMP-dependent pathways.

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DISCLOSURES

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