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

Cornelius J. Busch,1,2 Heling Liu,2 Amanda R. Graveline,2 and Kenneth D. Bloch2

1Department of Anesthesia and Critical Care and 2Cardiovascular Research Center of the Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts

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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, 1H-[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-(l-argininomethyl)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.

NITRIC OXIDE (NO) regulates the catalytic subunit, which phosphorylates target proteins. The released catalytic subunit may also translocate to the nucleus (19) where it can phosphorylate transcription factors, including members of the CREB family. PKA-dependent phosphorylation of CREB at Ser133 modulates transcription of genes containing cAMP response element (CRE) promoter sequences.

There are 11 PDE families with differences in their selectivities for cAMP and cGMP, kinetics, tissue distribution, regulation, and pharmacological inhibition (reviewed in Ref. 35). Four of 11 PDE families exclusively hydrolyze cAMP: PDE3, PDE4, PDE7, and PDE8. There are four PDE4 genes (PDE4A, PDE4B, PDE4C, and PDE4D) (20). The PDE4 genes encode >16 isoforms, each with a unique NH2-terminal region. Specific PDE4 isoforms regulate distinct intracellular processes, depending on their intracellular location and compartment (20).

We used a DNA microarray approach to characterize the expression of PDE genes in rat pulmonary arterial smooth muscle cells (rPASMC) exposed to NO and observed that one PDE4 gene, PDE4B, was markedly induced in rPASMC exposed to NO. We report that exposure to NO increases PDE4B gene expression, protein expression, and enzyme activity via a cGMP- and CREB-dependent mechanism.

MATERIALS AND METHODS

Reagents. S-nitroso-l-glutathione (GSNO), KT-5823, (z)-1-[N-(2-aminoethyl)-N-(2-aminoethyl)aminol]-diazan-1-ium-1, 2 diolate (DETA-NONOATE), and 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole (YC1) were obtained from Alexis (San Diego, CA). Forskolin (FK) was purchased from Sigma-Aldrich (St. Louis, MO). L-Nω-(l-argininomethyl)lysine (l-NIL), 1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one (ODQ), and H89 were obtained from Calbiochem-Novabiochem (La Jolla, CA). Actinomycin D, cycloheximide, and Rp-8-PCPT-cGMPs were purchased from Biomol Research Laboratories (Plymouth Meeting, PA). IL-1β and TNF-α were obtained from R&D Systems (Minneapolis, MN). Antibodies directed against PDE4B were obtained from Fabgenix (Shreveport, LA).

Cell culture. Cultures of primary rPASMC were prepared from explants of endothelium- and adventitia-stripped pulmonary arteries of adult Sprague-Dawley rats, as described previously (40). Cells were maintained in RPMI 1640 medium supplemented with 10% NuSerum (Collaborative Biomedical Products, Bedford, MA), 112 U/ml penicillin, and 112 U/ml streptomycin. Cells passed between 6 and 12 times were used for experiments. All animal experiments were conducted under protocols reviewed and approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital.

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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 μM 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: PDE1B, PDE2A, PDE3B, 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′-ATGCAAGGCG-CAATGATGTA-3′, 5′-CTGTATGCATTCTTCCCGG-3′), PDE4B (5′-GGCACATGGCAGCTTCTTCAAGA-3′, 5′-GACCTGTAGT- GTGCTAAGGACA-3′), PDE7A (5′-GAAGAGGCTTCCCGGTA3′, 5′-CAGACATACTTGCTTGGTCC-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 μM/mL protease 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 by incubation with 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 7.6 ± 0.3-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 manner (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 for 2 h to 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.

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.

rPASMC with GSNO (100 μM) for 4 and 8 h increased levels of an ~70-kDa PDE4B-immunoreactive protein (Fig. 3). Rolipram-inhibitable cAMP-metabolizing PDE activity increased in rPASMC incubated with GSNO (100 μM) for 4 h (Fig. 4), whereas total cAMP hydrolysis was not significantly changed.

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).

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) for 2 h. RNA was extracted from rPASMC, and complementary DNA was generated and analyzed using quantitative PCR; n = 3; means ± SD; *P < 0.01 vs. control, †P < 0.001 vs. GSNO.

Pretreatment with the PKA inhibitor H89 (50 mM) inhibited the ability of GSNO to induce PDE4B gene expression (Fig. 6). Incubation of rPASMC with the adenylate cyclase agonist FK (1 μM) 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. Detection of PDE3 and PDE4 in rPASMC 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 rat PASMC. For example, PDE5 mRNA has been detected in rat PASMC 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 isozyme 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 PASMC 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 PASMC isolates and changes in PASMC as they are passaged may also have contributed to variations in the magnitude of GSNO-induced PDE4B gene response. 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 PASMC 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 PASMC (36). Nonetheless, these findings suggest that concentrations of NO produced endogenously in PASMC 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 PASMC, it is likely that NO induced expression either the PDE4B2 or PDE4B4 isozyme. 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 PASMC 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 PASMC 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 PASMC 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 PASMC 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 dependent.
dependent. One of the major target enzymes for cGMP is PKG (10, 33). Inhibiting PKG with Rp-8-pCPT-cGMPs or KT-5823 did not prevent the induction of PDE4B by GSNO, suggesting that induction of PDE4B gene expression by NO is not PKG dependent. On the other hand, inhibition of PKA with H89 prevented the induction of PDE4B gene expression. Moreover, we observed that the PKA activator, 8-BrcAMP, increased PDE4B mRNA levels, whereas the PKG activator, 8-BrcGMP, did not. We hypothesize that NO-stimulated cGMP accumulation induces PDE4B gene expression via direct stimulation of PKA or via inhibition of PDE3 activity leading to activation of PKA by cAMP. It has been reported that cGMP is a more potent PDE3 inhibitor than is 8-BrcGMP (3). One potential limitation of these studies is that they rely on pharmacological inhibitors that may have nonspecific effects. For example, H89 can inhibit protein kinases other than PKA (11), and KT-5823 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 induction of PDE4B2 has been observed in COS cells (38), and PKA-dependent activation of PDE4 enzyme activity has been directly stimulated of PKA by cAMP. It has been reported that cGMP is a more potent PDE3 inhibitor than is 8-BrcGMP (3). One potential limitation of these studies is that they rely on pharmacological inhibitors that may have nonspecific effects. For example, H89 can inhibit protein kinases other than PKA (11), and KT-5823 may have unpredictable effects in intact cells (7).

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). Because 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 transfected 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 adenylyl 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 PDE4 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 cross talk between cAMP- and cGMP-dependent pathways.

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Present address of C. J. Busch: Department of Anesthesiology, Ruprecht-Karls-University, Heidelberg, Germany.

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DISCLOSURES

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