Protein kinase G is not essential to NO-cGMP modulation of basal tone in rat pulmonary circulation

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Division of Pulmonary Sciences and Cardiovascular Pulmonary Research Laboratory, University of Colorado Health Sciences Center, Denver, Colorado 80262-0001; Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294-0019; and Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110-1010

Fouty, Brian, Padmini Komalavilas, Masashi Muramatsu, Alan Cohen, Ivan F. McMurry, Thomas M. Lincoln, and David M. Rodman. Protein kinase G is not essential to NO-cGMP modulation of basal tone in rat pulmonary circulation. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H672–H678, 1998.—Nitric oxide (NO) is important in modulating increased pulmonary vascular tone. Whereas in other systems it is believed that the action of NO is mediated through guanosine 3',5'-cyclic monophosphate (cGMP) and protein kinase G (PKG), the validity of this pathway in the pulmonary circulation has not been established. Using isolated salt-perfused normotensive and hypertensive rat lungs, we studied the effects of the soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), and the PKG inhibitors, KT5823, Rp-8-pCPT-cGMPS, and N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide (H-8), on pulmonary vascular resistance. In isolated normotensive lungs, ODQ-mediated inhibition of soluble guanylyl cyclase augmented hypoxic pulmonary vasoconstriction, whereas the PKG inhibitors had no effect. Despite the marked differences in the physiological effect, ODQ and Rp-8-pCPT-cGMPS inhibited PKG activity to a similar degree as determined by a back-phosphorylation assay showing decreased PKG-mediated phosphorylation of serine 1755 on the D-myoinositol 1,4,5-trisphosphate receptor. In hypertensive lungs, inhibition of soluble guanylyl cyclase by ODQ increased perfusion pressure by 101 ± 20% (P < 0.05), an increase similar to that seen with inhibition of NO synthase (NOS), confirming an essential role for cGMPS. In contrast, KT5823, Rp-pCPT-cGMPS, and H-8 (used in doses 5- to 100-fold in excess of their reported inhibitory concentrations for PKG) caused only a small increase in baseline perfusion pressure (14 ± 2%, P = not significant from vehicle control). Effectiveness of PKG inhibition in the hypertensive lungs was also confirmed with the back-phosphorylation assay. These studies suggest that whereas NO-mediated modulation of vascular tone in the normotensive and hypertensive pulmonary circulation is dependent on cGMP formation, activation of PKG may not be essential.

hypoxic pulmonary vasoconstriction

Identification of Nitric Oxide (NO) as an endothelium-derived relaxing factor in the past decade has led to extensive research into its role in regulating vascular tone. Whereas there is some controversy regarding the role of NO in maintaining the low resistance of the normal pulmonary circulation, the importance of NO in modulating increased tone in a variety of acute and chronic pulmonary hypertensive states appears certain. In virtually all species tested, inhibition of NO production leads to augmented hypoxic vasoconstriction (18, 25, 26). In rats with hypoxia-induced pulmonary hypertension, inhibition of NO increases pulmonary vascular resistance (4, 24). Whereas these observations suggest that NO is important in regulating increased vascular tone in the pulmonary circulation, the mechanism through which NO acts has not been established.

The presumed mechanism for NO relaxation of smooth muscle involves guanylate cyclase-catalyzed production of guanosine 3',5'-cyclic monophosphate (cGMP) with subsequent activation of protein kinase G (PKG) (3, 8, 9). This scheme is based largely on studies from systemic vessels (8) or on conduit pulmonary arteries from normotensive animals (3). The principal site of resistance in the pulmonary circulation, however, is in the small arteries and arterioles. In the hypertensive pulmonary circulation, the small arteries undergo medial hypertrophy and the arterioles become muscularized. It is likely the behavior of these peripheral small arteries and arterioles differs from that of normotensive conduit vessels (2, 28).

We therefore tested whether PKG activation was required for NO-cGMP-dependent modulation of vascular tone in the pulmonary circulation. Using physiological and pharmacological tools, we found that despite similar inhibitory effects on PKG activity, the soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), augmented hypoxic pulmonary vasoconstriction (HPV) in normotensive lungs, whereas the PKG inhibitors had no effect. We also found that while inhibition of NO synthase (NOS) or soluble guanylyl cyclase caused vasoconstriction in the hypertensive rat lung, inhibition of PKG did not. We confirmed PKG inhibition in both normotensive and hypertensive lungs by detecting a decrease in PKG-specific phosphorylation of serine 1755 on the D-myoinositol 1,4,5-trisphosphate [Ins(1,4,5)P₃] receptor in lungs treated with the inhibitors. These findings suggest that NO-dependent modulation of vascular tone in the pulmonary circulation, while mediated via cGMP, is not primarily dependent on PKG activation.

Materials and Methods

Chronic hypoxic rat model of pulmonary hypertension. Male Sprague-Dawley rats (250–400 g) were either pulmonary normotensive [kept at Denver’s altitude of 5,280 ft, barometric pressure (Pb) = 630 mmHg] or were made pulmonary hypertensive by 3–5 wk of exposure to a simulated high altitude of 17,000 ft (Pb = 410 mmHg) in a hypobaric chamber, which was continuously flushed with room air to...
prevent accumulation of CO₂, NH₃, and water vapor. The exposure was 24 h/day with 12-h light-dark cycles and was interrupted only briefly to remove a rat for study or for animal care. All rats had free access to standard rat chow and water. The hypoxic exposure reliably induced pulmonary hypertension (mean pulmonary arterial pressure: 43.3 ± 1.9 mmHg; n = 10 rats vs. 20 ± 1.1 mmHg in control; n = 19 rats) (24).

Isolated lung technique. Lungs were isolated from pulmonary hypoxia and hypertensive rats after intraperitoneal injection of 30 mg pentobarbital sodium and intracardiac injection of 200 IU of heparin sodium. Hypertensive lungs were isolated within 1 h of removing rats from the hypobaric chamber. Isolated lungs were ventilated (inspiratory pressure of 9 and expiratory pressure of 2.5 cmH₂O) with a humid air mixture of 21% O₂ and 5% CO₂, 74% N₂ at 60 breaths/min. They were perfused via a pulmonary artery cannula at a constant peristaltic pump flow of 0.04 ml·g body wt⁻¹·min⁻¹. The perfusate, a physiological salt solution, contained the following (in mM): 116.3 NaCl, 5.4 KCl, 0.83 MgSO₄, 19.0 NaHCO₃, 1.04 NaH₂PO₄·2H₂O, 1.8 CaCl₂·H₂O, and 5.5 g-glucose (Earle’s balanced salts, Sigma). Ficoll (4 g/100 ml) was included as a colloid, and sodium medofenamate (3.1 µM) was added to inhibit production of vasodilator prostaglandins. After we cannulated the pulmonary artery and left ventricle, lungs were isolated, flushed of blood with 20 ml physiological salt solution, and placed in a heated, humidified chamber. Effluent perfusate from the left ventricular cannula drained into a reservoir and was recirculated (total volume of 30 ml). Lung and perfusate temperatures were maintained at 38°C, and perfusate pH was kept between 7.35 and 7.45. The lungs were equilibrated for 20 min before vascular responses were measured. Mean perfusion pressure was monitored continuously with transducer and pen recorder, and changes in perfusion pressure were considered to reflect changes in vascular resistance. After the 20-min equilibration period, three hypoxic (0% O₂) inhalation challenges (10 min each) were performed to establish lung vascular reactivity.

The inhibitors Rp-8-pCPT-cGMPS (BioLog Life Science Institute), a membrane-permeant inhibitor of PKG (inhibitory concentration (Ki): 0.5 µM), KT5823 (Calbiochem), a highly specific inhibitor of PKG (Ki: 0.234 µM), or IN-[2-(methylamino)ethyl]-5-isouquinolinesulfonamide dihydrochloride (H-8, Calbiochem), a highly active inhibitor of cyclic nucleotide-dependent protein kinases including PKG (Ki: 0.48 µM), were added to the perfusate to achieve target final concentrations (see RESULTS). The soluble guanylyl cyclase inhibitor, ODQ (Calbiochem), was added to a final concentration of 15 µM (Ki: 3 µM). After 60 min, 100 µM nitro-l-arginine (l-NNA) was added and perfusion pressure measured over 60 min.

To determine whether inhibition of PKG activity would prevent or blunt 8-bromo-guanosine 3’,5’-cyclic monophosphate (8-BrGMP)-induced vasodilation, lungs were constricted with l-NNA (100 µM) in the presence of H-8 (6 µM) or vehicle, and 8-BrGMP was then added to the perfusate in increasing concentrations (1–100 µM). Sodium nitroprusside (SNP) was then added in a concentration (10 µM) that normally completely reverses l-NNA-induced vasoconstriction.

Phosphorylation assay. Back phosphorylation of the Ins(1,4,5)P₃ receptor was performed to determine the effectiveness of PKG inhibition in the perfused lungs according to the method of Komalavilas and Lincoln (17). Isolated perfused lungs were set up as described above. After the 20-min equilibration period, three hypoxic (0% O₂) challenges were performed to establish lung vascular reactivity. For the HPV experiments involving the normotensive lungs, either vehicle, ODQ (15 µM), or Rp-8-pCPT-cGMPS (15 µM) was added to the perfusate, and the lung was exposed to three 10-min cycles of 0% O₂ followed by normoxia while perfusion pressure was monitored. For the hypertensive lungs, H-8 (6 µM) was added to the perfusate and the perfusion pressure was monitored for 30 min. The lungs were then frozen in liquid nitrogen and stored at −80°C. The Ins(1,4,5)P₃ receptor was immunoprecipitated from the rat lung microsome using an anti-Ins(1,4,5)P₃ receptor antibody (antibody raised against an NH₂-terminal peptide from type 1 Ins(1,4,5)P₃ receptor) and phosphorylated in vitro using purified PKG from bovine lung and [γ-³²P]ATP. The samples were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. The Ins(1,4,5)P₃ receptor band was cut out, and the radioactivity incorporated was quantitated. The amount of ³²P incorporated in vitro to the Ins(1,4,5)P₃ receptor depends inversely on the extent of receptor phosphorylation that had occurred in the perfused lung. The amount of ³²P incorporated into the Ins(1,4,5)P₃ receptor band in the H-8-treated rat lung was expressed as a percentage of that in control vehicle-treated lungs, which was considered to be 100%.

RESULTS

Effect of PKG inhibition on HPV in normotensive lungs. Previously, we had reported a two- to threefold increase in HPV in the perfused normotensive lung after NOS inhibition (13). We wanted to determine whether this effect of NO was mediated by cGMP and PKG. Therefore, we tested the effect of soluble guanylyl cyclase and PKG inhibition on HPV in the salt-perfused, normotensive rat lung. ODQ is a selective inhibitor of soluble guanylyl cyclase that is thought to work by competing with NO for the heme site of guanylyl cyclase (27). Inhibition of soluble guanylyl cyclase with ODQ significantly increased HPV compared with control (Fig. 1A). In contrast, we found no effect of PKG inhibition by Rp-8-pCPT-cGMPS on HPV compared with control (Fig. 1A). Similarly, we also found no effect of a second PKG inhibitor, H-8, on HPV (5.1 ± 1.2 vs. 4.3 ± 0.8 mmHg, n = 3 rats, P = not significant).

One possible explanation for the failure of the PKG inhibitors to augment HPV, however, was the inability of the inhibitors to effectively block PKG activity. To address this issue, we attempted to determine in vivo PKG activity using a back-phosphorylation assay of the Ins(1,4,5)P₃ receptor. As described in MATERIALS AND METHODS, this is an indirect method of determining in vivo PKG-specific phosphorylation of serine 1755 on the Ins(1,4,5)P₃ receptor. Increased phosphorylation measured in vitro correlates to decreased (inhibited) phosphorylation occurring in vivo. We compared the degree of in vivo PKG inhibition between ODQ and Rp-8-pCPT-cGMPS using this assay. Both ODQ and Rp-8-pCPT-cGMPS significantly blocked PKG activation compared with control but were not different from each other (Fig. 1B). Therefore, whereas both ODQ and Rp-8-pCPT-cGMPS led to a similar inhibition of PKG activation, only ODQ augmented HPV.

Effect of soluble guanylyl cyclase inhibition on perfusion pressure in hypertensive lungs. We have previously observed that inhibition of NO production by l-NNA
caused vasoconstriction in the hypertensive rat lung (24). Therefore, we attempted to determine whether NO-dependent modulation of basal tone in the hypertensive rat lung was mediated through an increase in cGMP. Addition of ODQ at five times its $K_i$ for guanylyl cyclase caused a progressive and statistically significant ($P < 0.005$) increase in perfusion pressure compared with controls (Fig. 2). The increase in perfusion pressure was similar to that seen following inhibition of NOS, and no additional increase in perfusion pressure was seen after the addition of L-NNA.

Effect of PKG inhibition on perfusion pressure in hypertensive lungs. Next we wanted to determine whether NO-cGMP-dependent modulation of basal tone was mediated through activation of PKG. Using three structurally different PKG inhibitors, we studied the effect of PKG inhibition on basal perfusion pressure in the hypertensive rat lung. Baseline perfusion pressure was not different among the four groups (Fig. 3). After addition of the PKG inhibitors (see Fig. 3 for concentrations) or vehicle (equal volume of deionized water or dimethyl sulfoxide), there was a slow increase in pressure over 60 min that was not different among the groups. This result is consistent with previous findings that a slow increase in pressure occurs over time in the isolated perfused hypertensive rat lung (24). After L-NNA (100 µM) was added, there was a similar significant and sustained increase in pressure in all four groups. The absolute perfusion pressure 60 min after L-NNA was not different among the groups. To minimize the possibility that diffusion limitations prevented adequate tissue levels to effectively block PKG activation in vivo.

Fig. 1. A: inhibition of protein kinase G (PKG) by 15 µM Rp-8-pCPT-cGMPS (n = 5 rats) did not increase hypoxic pulmonary vasoconstriction (HPV) in isolated perfused lungs harvested from pulmonary normotensive rats. Inhibition of soluble guanylyl cyclase with 15 µM 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one (ODQ) significantly increased HPV above control. * $P < 0.05$, n = 4 in each group. B: both Rp-8-pCPT-cGMPS and ODQ increased in vitro D-myo-inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] receptor back phosphorylation compared with controls ($P < 0.04$, n = 4 in each group) but were not different from each other. As noted in text, an increase in in vitro Ins(1,4,5)P3 receptor back phosphorylation correlates with an inhibition of PKG activation in vivo.

Fig. 2. After a 20-min equilibration period, either the soluble guanylyl cyclase inhibitor, ODQ (15 µM), or vehicle (equal volume dimethyl sulfoxide) was added (arrows) to perfusate of an isolated lung harvested from pulmonary hypertensive rats. Inhibition of soluble guanylyl cyclase by ODQ (filled circles, n = 3) significantly increased perfusion pressure in hypertensive lungs (* $P < 0.05$) compared with controls (open circles, n = 6). Little additional increase in pressure was seen following nitric oxide synthase (NOS) inhibition with NG-nitro-L-arginine (L-NNA, 100 µM, second arrow).

Fig. 3. After a 20-min equilibration period, either the PKG inhibitors or vehicle was added to the perfusate (arrows) of isolated lungs from pulmonary hypertensive rats. Vehicle data (water for Rp-8-pCPT-cGMPS and H-8; dimethyl sulfoxide for KT5823) was combined because there was no difference between them on baseline perfusion pressure. Addition of PKG inhibitors, Rp-8-pCPT-cGMPS (15 µM; n = 3; open circles), KT5823 (10 µM; n = 3; filled circles), and H-8 (6 µM; n = 5; open triangles) did not increase mean perfusion pressure above control (n = 7; filled triangles) in isolated perfused hypertensive rat lung. Subsequent increase in perfusion pressure after NOS inhibition by L-NNA (100 µM, second arrow) was not different among the groups.
brane-permeant cGMP analog, 8-BrcGMP, reverses L-

We have previously shown that addition of the mem-

lung. 

PKG activation by ODQ and H-8 in the hypertensive

response compared with vehicle controls. 

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of PKG inhibition, we performed back phosphorylation

pulmonary resistance vessels. To test the effectiveness

H-8 was gaining access to the smooth muscle of the

immediately reversed the PMA-induced vasoconstric-

maximal effective concentration (EC50) tended to be

tended only a small portion (16 4%; P < 0.05) of the

response compared with vehicle controls. 

DISCUSSION

This study tested whether endogenous NO modula-
tion of vascular tone in the pulmonary circulation depend-
on activation of cGMP and PKG. Hasunuma et al. (13) and others (18, 25, 26) have shown that inhibition of NOS augments HPV. We reasoned that if this NO-mediated suppression of HPV required both cGMP and PKG, then pharmacological inhibition of either should mimic pharmacological inhibition of NO and augment HPV. Our initial experiment therefore examined the effect of inhibiting soluble guanylyl cy-
clase and PKG on HPV in isolated lungs from pulmo-
nary normotensive rats.

ODQ is a selective inhibitor of soluble guanylyl cy-
clase that presumably works by oxidizing the pros-
thetic heme group, thus decreasing the ability of NO to
bind and activate the enzyme (27). It does not inhibit particulate guanylyl cyclase (11), and unlike other purported inhibitors of soluble guanylyl cyclase such as methylene blue and LY-83583, it has no known effects on NOS (21, 22). Added in a concentration fivefold greater than the K for guanylyl cyclase, ODQ increased HPV in normotensive lungs threefold, an effect similar to that seen following NOS inhibition with l-NNA. This suggested that the modulating effect of NO on HPV is mediated by its stimulation of guanylyl cyclase with an accompanying increase in cGMP.

Because one of the main intracellular targets for cGMP is PKG, we next studied the effect of inhibiting PKG activation on HPV in normotensive lungs. The Rp analogs of cGMP are the most specific inhibitors of PKG available, and Rp-8-pCPT-cGMPS is the most mem-
brane-permeant isoform (6, 7). Used in concentrations that exceeded the K for PKG by 30-fold, this inhibitor had no effect on HPV. Similar results were observed with H-8, a very effective but less specific PKG inhibi-
tor. Used in a concentration that exceeded the K for PKG by 13-fold, H-8 did not augment HPV. These results suggested that PKG may not be essential to the modulation of HPV by NO-cGMP.

Fig. 4. Autoradiogram of sodium dodecyl sulfate gel showing an increase in in vitro Ins(1,4,5)P3 receptor phosphorylation in H-8-
treated hypertensive lungs. Plus (+) column represents H-8-treated lungs; minus (−) represents vehicle control. Arrow indicates phos-
phorylated Ins(1,4,5)P3 receptor at ∼260 kDa. [Band below is a proteolytic product of Ins(1,4,5)P3 receptor.] This correlates to a
decrease in in vivo phosphorylation as a result of H-8 inhibition of
PKG.
An important possible alternative explanation for our findings, however, was a failure of the pharmacological probes to enter and affect the pulmonary artery myocyte. Whereas the most direct test would be to measure the activation state of PKG in the lung microcirculation, there are no available techniques that allow for preservation of the PKG activation state during the process of tissue isolation and homogenization. To determine whether Rp-8-pCPT-cGMPS effectively inhibited PKG in the isolated lung, we measured PKG-specific phosphorylation of the Ins(1,4,5)P_3 receptor in treated and control lungs using a back-phosphorylation assay previously described in rat aorta (17). An increase in in vitro phosphorylation correlates with a decrease in in vivo phosphorylation. Both ODQ and Rp-8-pCPT-cGMPS caused a significant increase (25%) in in vitro back phosphorylation of the Ins(1,4,5)P_3 receptor compared with controls but were not different from each other. Because we assayed for Ins(1,4,5)P_3 receptor phosphorylation using purified PKG, which only catalyzes the phosphorylation of serine 1755 of the type I Ins(1,4,5)P_3 receptor, the increase in back phosphorylation in treated lungs indicated that a kinase that phosphorylated this residue had been inhibited by Rp-8-pCPT-cGMPS. Thus, although both ODQ and Rp-8-pCPT-cGMPS inhibited PKG activity to a similar degree, only ODQ augmented HPV. This suggested that in the normotensive rat lung, cGMP-mediated mechanisms alternative to PKG are important in modulating HPV.

We have previously shown in the hypertensive rat lung that inhibition of NO production by L-NNA caused a marked and sustained increase in perfusion pressure (24). Using a similar approach, we examined whether this NO-mediated suppression of resting vascular tone required both cGMP and PKG. Added in a concentration fivefold greater than the \( K_i \) for guanylyl cyclase, ODQ caused a sustained increase in perfusion pressure, which was similar to that seen following the inhibition of NOS with L-NNA. Addition of L-NNA did not further increase vasoconstriction, suggesting that the modulating effect of NO in the hypertensive lung is mediated by its stimulation of guanylyl cyclase.

Rp-8-pCPT-cGMPS used in concentrations that exceeded the \( K_i \) for PKG by 30-fold, however, had no effect on resting vascular tone. Subsequent vasoconstriction to L-NNA was unaffected by Rp-8-pCPT-cGMPS pretreatment. Similar results were observed with KT5823, a chemically dissimilar but also selective inhibitor of PKG, and with H-8, a very effective but less specific inhibitor. Used in a concentration that exceeded the \( K_i \) for PKG by 13-fold, H-8 did not increase perfusion pressure and did not attenuate L-NNA vasoconstriction. In a single lung, we tested the effect of exceeding the \( K_i \) for H-8 by 130-fold and again we found no effect. This effect of H-8 was likely not due to nonspecific inhibition of vasoconstriction, because subsequent L-NNA-mediated vasoconstriction was unaffected. These results suggested that PKG inhibition did not mimic the effect of NOS inhibition on basal tone in the hypertensive pulmonary circulation and that PKG may not be essential to modulation of basal tone by NO-cGMP in the hypertensive rat lung.

Although KT5823 has been shown to effectively inhibit SNP-stimulated PKG activity in dispersed gastric smooth muscle cells (23), Rp-8-pCPT-cGMPS to inhibit PKG activation in human platelets (6), and H-8 to inhibit PKG activity in P388 leukemia cells (15), they have not been reported to inhibit PKG activity in intact organ preparations. As previously stated, therefore, an important possible alternative explanation for our findings is a failure of the inhibitors to enter and affect the pulmonary artery myocyte. We have demonstrated the effectiveness of the inhibitor Rp-8-pCPT-cGMPS in the normotensive lung. Next, we studied one of the other inhibitors, H-8, more extensively to determine whether it was effective in the hypertensive lung. First, we took advantage of the relative lack of selectivity of H-8 to establish that the agent was indeed reaching the pulmonary artery myocyte. Whereas 6 \( \mu M \) H-8 affects primarily PKG and PKA, at higher concentrations it can also inhibit PKC. We therefore preconstricted hypertensive lungs with PMA, which causes vasoconstriction by activating PKC. At the peak of PMA vasoconstriction, 35 \( \mu M \) H-8 (twofold greater than the \( K_i \) for PKC) was added, resulting in an immediate reversal of PMA vasoconstriction. This result suggests that H-8 gained rapid access to pulmonary artery myocytes in this model.

To determine whether ODQ and H-8 effectively inhibited PKG in the isolated lung, we measured PKG-specific phosphorylation of the Ins(1,4,5)P_3 receptor in treated and control lungs using the back-phosphorylation assay previously described. Both ODQ and H-8 increased back phosphorylation of the Ins(1,4,5)P_3 receptor in the hypertensive lung by \( \sim 40\% \) compared with controls. This increase in in vitro phosphorylation correlates to a decreased (inhibited) in vivo phosphorylation. As was seen in the normotensive lung, inhibition of soluble guanylyl cyclase by ODQ in the hypertensive lung produced a marked physiological response (increase in baseline perfusion pressure), whereas inhibition of PKG activation alone had no effect.

Using H-8, we also tested whether stimulated vasodilation by the membrane-permeant analog 8-BrcGMP required PKG activation. Addition of 8-BrcGMP to hypertensive lungs preconstricted with L-NNA caused a dose-dependent decrease in perfusion pressure. Pretreatment with H-8 shifted the curve upward but without a significant change in the \( E_{50} \). At maximum doses of 8-BrcGMP and SNP, there was a small (16\%) but significant reduction in maximum vasodilation in the H-8-treated rat lungs. It therefore appears, that although a portion of the stimulated vasodilation in response to increased cGMP is mediated via PKG in the hypertensive lung, the majority is not.

Although our results appear to contradict previous studies (3, 8, 9) that implicate PKG in NO-cGMP-mediated vascular relaxation, our experiments differ in that we studied the pulmonary rather than the systemic circulation (8) and the isolated whole lung rather than single pulmonary artery smooth muscle cells (3).
One of the best descriptions of the effects of PKG in the pulmonary circulation comes from Archer and colleagues (3), who showed that NO-cGMP-mediated relaxation of normotensive rat pulmonary artery rings could be inhibited by the calcium-activated K+ (KCa) channel blocker charybdotoxin. They further demonstrated that the PKG mimetic, Sp-cGMPS, increased whole cell K+ currents in rat pulmonary artery myocytes by activating KCa channels. They suggested that NO-cGMP-mediated vasorelaxation in the pulmonary circulation involves PKG activation of K+ channels. Whereas PKG-mediated activation of KCa channels may indeed be an important mechanism through which pharmacological increases in cGMP affect pulmonary artery SMC activation, our results suggest that an alternative mechanism explains modulation of vascular tone by endogenous NO-cGMP in the pulmonary circulation.

In addition to PKG, known intracellular targets for cGMP include phosphodiesterases (PDE) and membrane-bound ion channels. One or both may be responsible for the cGMP-dependent, PKG-independent vasoconstriction in the rat hypertensive lung. cGMP can act on at least four PDE isoenzymes and can either stimulate (PDE 2) or inhibit (PDE 3) their activity (19). In particular, cGMP inhibits PDE 3, which hydrolyzes adenosine 3',5'-cyclic monophosphate (cAMP). One possibility is that cGMP-dependent vasodilation is mediated through protein kinase A (PKA). However, several lines of evidence suggest that cGMP-dependent vasorelaxation does not work via this pathway (i.e., cause smooth muscle relaxation indirectly by increasing cAMP). First, the Ca2+-lowering effects of cAMP in vascular smooth muscle are mediated primarily through PKG (5). Second, the cGMP analog 8-Br-cGMP, which reverses L-NNA-induced vasoconstriction, does not activate PDEs (19, 29). Finally, at the dose of H-8 used, the K+ for PKA was exceeded by fivefold without observing vasoconstriction. We therefore believe it is unlikely that cGMP is acting via cAMP and PKA.

Recent research in the area of phototransduction has established an alternative mechanism for the action of cGMP, which relies on direct activation of nonselective cation channels rather than on PKG-mediated phosphorylation. Therefore, a third potential explanation for the apparent cGMP-dependent/PKG-independent vasodilation in the hypertensive rat lung could involve direct binding of cGMP to cyclic nucleotide-gated (CNG), nonselective cation channels. Whereas CNG channels homologous to the retinal channel have been found in other tissue, including aorta, whether CNG channels are expressed in the lung or pulmonary circulation is not known. Although the CNG channels cloned from the visual system are cGMP activated (that is, they are opened by increases in cGMP), two cGMP-inhibited CNG ion channels (channels that if inhibited in hypertensive pulmonary artery myocytes would be predicted to cause vasoconstriction) have been recently described in mouse renal epithelium (1) and frog taste bud cells (16). In both cell types, application of cGMP to the internal cell membrane reduced conductance through a nonselective cation channel and decreased Ca2+ influx. In addition we have reported preliminary evidence that messenger RNA coding for a CNG channel is expressed in the pulmonary circulation (10). Unfortunately, no specific inhibitors of CNG channels have been described, and further tests of this hypothesis will require electrophysiological studies beyond the scope of this report.

Our results suggest that PKG activation is not central to NO-cGMP-dependent modulation of vascular tone in the rat lung. Specifically, PKG does not appear to be important in NO-dependent modulation of HPV in the normotensive rat lung or in modulation of the increased vascular tone in the hypertensive rat lung. The mechanism through which NO opposes HPV in the normotensive lung and basal vasoconstriction in the hypertensive lung appears to occur through a cGMP-dependent but PKG-independent pathway. Whether this involves an action of cGMP on PDEs, on membrane-bound ion channels, or through some other mechanism remains to be established.

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