Differential effects of phosphodiesterase PDE-3/PDE-4-specific inhibitors on vasoconstriction and cAMP-dependent vasorelaxation following balloon angioplasty

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Submitted 26 April 2006; accepted in final form 9 February 2007

Zhao H, Quilley J, Montrose DC, Rajagopalan S, Guan Q, Smith CJ. Differential effects of phosphodiesterase PDE-3/PDE-4-specific inhibitors on vasoconstriction and PDE-dependent vasorelaxation following balloon angioplasty. Am J Physiol Heart Circ Physiol 292: H2973–H2981, 2007. First published February 9, 2007; doi:10.1152/ajpheart.00419.2006.—It is known that cAMP and cGMP are important for vasorelaxation, and cyclic nucleotide phosphodiesterases (PDEs) regulate their levels. Balloon angioplasty (BAL) is associated with reduced cAMP and cGMP levels, and inhibition of PDE-3 reduces restenosis. In this study, we found that BAL increased PDE-3 activity, which affected vasoreactivity of rat aortic rings 24-h post-BAL; these were compared with intact (INT) and ex vivo endothelium-denuded rings (RUB) from sham rats. In BAL and RUB rings, vasorelaxant responses to ACh were abolished. The EC50 for phenylephrine (PE) was 1.8-fold less in RUB than in INT or BAL, whereas the maximal contractile effect of PE was greater in BAL than in INT or RUB. PDE-3 inhibitors reduced the maximal response to PE by >65% in BAL compared with 10–30% in INT and RUB; the reduction of the maximal response to U-46619 was 37% in BAL compared with 8% in INT with no reduction in RUB. PDE-4 inhibitors reduced PE-induced tone by <30% in an endothelium-dependent manner. Vasorelaxant responses to agonists that utilize cAMP were greatly impaired in BAL and RUB rings, and inhibition of PDE-3 enhanced the vasorelaxant responses in BAL or RUB. Inhibition of PDE-4 increased vasorelaxant responses to isoproterenol (ISO) to a much lesser degree. Thus PDE-3 and PDE-4 inhibitors exhibited differential effects on PE-induced tone and vasorelaxant responses to ISO. Inhibition of PDE-3 also produced a greater increase in cAMP in BAL than INT or RUB rings. These results suggest that increased PDE-3 activity after BAL may promote a vasospastic state and that the reduction in cAMP may, possibly, influence vessel remodeling.

Ballooning aorta; cyclic adenosine 3’,5’-cyclic monophosphate
The balloon was then inflated to 750–850 mmHg, and the catheter was partially withdrawn to the branch of the femoral artery. This process was repeated three times. The catheter was removed, the femoral artery sutured, and the incision clipped. For sham rats, the uninflated catheter was inserted only once into the left femoral artery without entering the aorta.

Preparation of isolated aortic rings. Preliminary studies from this laboratory showed that PDE-3 and PDE-4 expression and activity started to increase as early as 24 h after BAL, and the increase lasted 4–7 days; thus the 24-h time point was chosen for the experiments to examine the early effects of PDEs on the blood vessel. Following laparotomy and thoracotomy, the aorta from the heart to just above the kidneys was excised, flushed with Krebs solution (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4·7H2O, 2.5 mM CaCl2·2H2O, 10 mM dextrose, 25 mM sodium bicarbonate) to remove blood, and dissected free of perivascular tissue. For contractility studies, the aorta was cut into 2.5-mm rings and kept in ice-cold Krebs until mounted onto hooks in a tissue bath chamber. For ex vivo removal of endothelium from sham rings (RUB), a toothpick was inserted into the ring and rolled gently for two revolutions. Hematoxylin and eosin staining confirmed that endothelium was removed in both BAL and RUB rings (data not shown).

Vascular contractility studies. Aortic vascular reactivity was measured using the Myothrac System, and data from six to eight channels (6–8 rings) were collected using the MP100 Data Acquisition System (both from World Precision Instruments, Sarasota, FL). Rings stretched to 2 g of tension (baseline) were equilibrated for 60 min in 10-ml oxygenated Krebs buffer at 37°C. Experiments were initiated by measuring contractions to KCl (10–100 mM or 80 mM). Then contractile responses to the α1-agonist phenylephrine (PE) (1–3 μM) were examined. After a maximal PE contraction was established, rings were relaxed with acetylcholine (ACH) (3–3 μM). Subsequently, relaxant responses to one of the following drugs were evaluated in rings precontracted with PE (3 μM): isoproterenol (ISO), a nonselective β-adrenergic agonist; carbachol [propylcine (PGI2) analog]; forskolin (FSK) to directly activate adenylate cyclase; or PDE-3 inhibitor OPC3911. Next, effects of PDE-3 inhibitors [OPC3911, cilostazol (OPC3103), and milrinone] or PDE-4 inhibitors (Ro20-1724 and cilomilast) were studied; rings were incubated with inhibitors for 10 min before eliciting a maximal contraction to PE. Contractile responses to U-46619 (thromboxane A2 receptor agonist, 100 nM) in rings incubated with OPC3911 (1 μM) for 25 min were also studied. PDE inhibitors cannot be easily washed out, and, therefore, only one inhibitor could be tested on each ring. In addition, maximum PE contractions were different in the three types of rings. Consequently, relaxant responses to receptor agonists were expressed in two ways: 1) as the percentage of the initial PE-induced contraction (%PE contraction) to compare ISO sensitivity, and 2) as absolute changes in tension. Since FSK cannot be easily removed after application, only one dose response could be obtained for each ring. Therefore, rings incubated with OPC3911 were compared with untreated control rings. Finally, the response to KCl (80 mM) was tested again at the end of the experiment to ensure that there was no temporal change in reactivity. Aortic rings were washed at least three times with Krebs solution, and tension was allowed to return to baseline after each set of treatments in two or three replicate rings, and the rings reequilibrated for at least 20 min before the next drug addition. Initial experiments showed no differences in the contractile responses of the proximal and distal parts of the aorta, and in subsequent experiments rings were randomized.

All of the PDE-3 and PDE-4 inhibitors compete for the active site of the enzyme. The reported IC50 values for selective inhibition of vascular PDE-3 activity are as follows: OPC3911 0.03 μM; milrinone 0.49 μM; and cilostazol 0.2 μM (27, 51). The reported IC50 for cilomilast inhibition of PDE-4 in U937 cells is 30 nM (19) and 0.4–0.7 μM for Ro20-1724 for recombinant human PDE-4B/4D (19, 56). Our studies typically used 0.1 μM OPC3911 (3.3 times IC50) and 0.5 μM cilostazol (2.5 times IC50). Since the effects of PDE-4 inhibitors were not as pronounced in preliminary studies, relatively higher concentrations were used vs. the PDE-3-selective drugs: 0.5 μM cilomilast (16.7 times IC50) and 10 μM Ro20-1724 (14.3 to 25 times IC50).

Medial SMC preparation and PDE activity assay. Medial SMC were prepared as described (37). Briefly, aortas were rapidly excised from the animal, rinsed in medium 199 with Hank’s balanced salt solution, and incubated for 25 min in the same medium plus 1% collagenase, 0.25% elastase, and 1% soy bean trypsin inhibitor. After incubation, the adventitia and endothelium were rapidly stripped away from the media. Medial SMC were snap-frozen in liquid nitrogen and stored at −70°C. Tissues were then powdered and homogenized on ice with homogenate buffer (290 mM sucrose, 10 mM MOPS, 1.0 mM EGTA, 3.0 mM Na2SO4, 1.0 mM DTT, 3.0 mM benzamidine, 10 μg/ml pepstatin, 10 μg/ml leupeptin, 10 μg/ml antipain, 0.8 mM PMSF and polytronized for 10 s periods. The samples were then subjected to differential centrifugation at 1,000 g for 10 min, 7,500 g for 10 min followed by 40,000 g for 60 min at 4°C. The 40,000 g supernatants, the cytosol fractions, were used for PDE assay.

For the cAMP PDE activity assay, 1H-cAMP was added at 100,000 dpm to buffer containing 50 mM HEPES, 0.1 mM EGTA, 8.3 mM MgCl2, and 0.1 μM cAMP in 200–1 μl volumes. Various concentrations of the test protein sample in 100 μl were added to start the reaction, and the mixture was incubated at 30°C for 15 min. The reaction was stopped by adding 100 μl stop mix (7.5 mM CAMP/3.3 mM 8′-AMP in 0.17 N HCl). Samples were neutralized with 100 μl buffer containing 250 mM Tris and 250 mM NaOH, pH 8.0. Then 100 μl venom mix (0.1875% Crotilus atrox in 100 mM Tris, pH 8.0) was added, and samples were incubated at 30°C for 20 min. The sample was then applied to DEAE-Sephadex A-25 column, and the eluate was collected in scintillation vials with 10 ml Beckman Readi-Gel. The column was washed with 3.6 ml H2O, and the eluate was also collected in the same vial. The radioactivity was measured in a liquid scintillation counter. PDE-3 or PDE-4 activities were determined as the activities inhibited by OPC3911 (1.5 μM) or Ro20-1724 (15 μM), respectively, added to the assay mix.

Western blot analysis of protein kinase A phosphorylation. For Western blot analysis, aortic rings were frozen in liquid nitrogen, homogenized in homogenate buffer, and centrifuged at 16,000 g for 20 min. Forty microliters of the supernatants were mixed with 10 μl of 5× sample buffer (312.5 mM Tris-HCl, pH 6.8, 10% SDS, 25% glycerol, 0.05% bromophenol blue), incubated at 95°C for 5 min, and then subjected to Western blot analysis with Bio-Rad Hercules, CA) system and a protein kinase A (PKA) substrate antibody from Cell Signaling (no. 9621, Beverly, MA) at 1:3,000 dilution.

 Determination of cAMP and cGMP levels in aorta. Aortic rings (3.5–4 mm) were incubated in Krebs buffer at 37°C for 10 min and then incubated with different drugs for another 10 min. The rings were then frozen in liquid nitrogen, homogenized in 5% TCA, and centrifuged at 16,000 g for 20 min. Forty microliters of the supernatants were mixed with 10 μl of 5× sample buffer (312.5 mM Tris-HCl, pH 6.8, 10% SDS, 25% glycerol, 0.05% bromophenol blue), incubated at 95°C for 5 min, and then subjected to Western blot analysis with Bio-Rad (Hercules, CA) system and a protein kinase A (PKA) substrate antibody from Cell Signaling (no. 9621, Beverly, MA) at 1:3,000 dilution.

Statistical analysis. The results shown are means ± SE. Data were analyzed by the Student’s t-test with Excel (single point) or two-way ANOVA with SigmaStat 2.0 (whole curve). P < 0.05 was considered as significant. Curve fitting with SigmaPlot V6 (1-Site Ligand model, log scale of drug concentration) was used to estimate IC50 values. The drug maximum reaction (Rmax) was the value for either contraction above baseline, or the relaxation of PE-induced contraction, measured at the highest drug concentration tested.

Drugs and chemicals. NaCl, MgSO4, CaCl2·2H2O, dextrose, and L-ascorbic acid were obtained from Fisher Scientific (Suwanee, GA), and carbachol from Biomol (Plymouth Meeting, PA). OPC3911 and PDE INHIBITORS AFFECT VASOREACTIVITY AFTER ANGIOPLASTY

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PDE INHIBITORS AFFECT VASOREACTIVITY AFTER ANGIOPLASTY

VOL 292 • JUNE 2007 • www.ajpheart.org

AJP-Heart Circ Physiol • VOL 292 • JUNE 2007 • www.ajpheart.org

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cilostazol were generous gifts from Dr. Junichi Kambayashi (Otsuka, Rockville, MD). Cilomilast (SKB 207499) was generously provided by Dr. Theodore J. Torphy (formerly affiliated with SmithKline Beecham). All others were obtained from Sigma (St. Louis, MO).

RESULTS

Differential effects of BAL and ex vivo endothelium denudation on contractile response to PE. To compare the effects of BAL and ex vivo removal of the endothelium, rings from BAL rats and two sets of rings from sham rats (INT and RUB) were studied. Contractile responses to PE differed between the INT and the other two groups (Fig. 1A). The EC\textsubscript{50} for PE was 1.8-fold less in RUB (52 nM) vs. INT (95 nM) (P < 0.001), while the R\textsubscript{max} contraction at 3 μM PE was unchanged by acute removal of endothelium (Fig. 1A). In contrast, the EC\textsubscript{50} for PE in BAL (92 nM) was similar to that for INT, but the R\textsubscript{max} was greater in BAL (*P < 0.005 vs. INT). Contractile responses to KCl were similar in INT, RUB, and BAL rings (Fig. 1B). The responses to 80 mM KCl were not different among the three groups of rings at the beginning and end of the contractile studies and were unaffected by the inhibitors, although the contractile response to KCl was increased by 12–13% in all three groups of rings at the end of the study (data not shown). It should be noted that, when the contractile response to PE was expressed as a percentage of the response to KCl (80 mM), the R\textsubscript{max} in RUB rings was greater than that in INT (P < 0.05) but was not different from that in BAL. ACh-induced relaxation of PE contracted rings was markedly impaired in both BAL and RUB rings compared with INT rings (data not shown).

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seen in the other groups (*P < 0.01 BAL vs. INT or RUB). A
time control for a second PE concentration curve without any
inhibitor showed no difference in INT and RUB rings. BAL
showed a slight increase of 12–13% compared with the first
contraction at maximum PE concentration (3 μM) (data not
shown).

To further clarify the effect of PDE-3 inhibitors on vaso-
constriction, rings from the three groups were contracted with
PE (3 μM), and then cumulative concentration-response curves
were generated for OPC3911. As shown in Fig. 2B, the curve
for OPC3911-induced vasorelaxation was shifted to the left in
BAL rings compared with INT rings (*P < 0.05, INT vs.
BAL). The concentration response in RUB rings was similar to
that in INT.

**Effect of PDE-3 inhibitors, OPC3911 and cilostazol, on
ISO-induced relaxation.** BAL, RUB, and INT rings were
contracted with PE (3 μM), and then cumulative concentra-
tion-response curves were generated for ISO. The rings were
then washed, incubated with the PDE-3 inhibitor, OPC3911
(0.1 μM) or cilostazol (0.5 μM), contracted with PE, and
relaxed with ISO again as above. As shown in Fig. 3, ISO-
duced relaxation was impaired in both BAL and RUB rings
compared with INT rings (*P < 0.001, BAL or RUB vs. INT,
whole curve). When normalized to corresponding PE contrac-
tions, after treatment with OPC3911, sensitivity to ISO in both
BAL and RUB rings was indistinguishable from that of INT
rings (Fig. 3A). R_{max} with ISO (3 μM) was greatly improved in
both BAL and RUB; in BAL, with OPC3911 (0.1 μM), only
8.96% PE contraction remained, compared with 61.9% without
OPC3911, and, in RUB, it was 4.54 vs. 64.3% (Fig. 3A). In
terms of change in tension, as shown in Fig. 2, PE-induced
contraction was greatly reduced in BAL by OPC3911 (Fig. 3B)
such that a minimal effect of ISO was required to produce
maximum relaxation. In contrast, PE-induced contraction in
INT and RUB was only slightly affected by OPC3911, but the
dose-response curve to ISO in RUB was markedly shifted to
the left (Fig. 3B). Another PDE-3 inhibitor, cilostazol, was
tested for the effects on the ISO dose-response curve. Cilostazol
(0.5 μM, Fig. 3C) showed similar leftward shifting of the
ISO curve as OPC3911 (0.1 μM). Cilostazol improved the ISO
sensitivity (*P < 0.001, BAL or RUB vs. INT without inhibi-
tors, whole curve) in both BAL and RUB rings to near that of
INT rings, and R_{max} for ISO (3 μM) was greatly enhanced in
both BAL and RUB. With cilostazol (0.5 μM) in BAL, only
13.0% PE contraction remained, compared with 59.0% without
cilostazol, and, in RUB, the values were 19.8 vs. 63.7%.
Another PDE-3 inhibitor, milrinone, showed similar results as
cilostazol (data not shown).

**Effect of PDE-3 inhibitor, OPC3911, on carbacyclin- and
FSK-induced relaxation.** BAL, RUB, and INT rings were
contracted with PE (3 μM), and then cumulative concentra-
tion-response curves were generated for carbacyclin (Fig. 4A)
and FSK (Fig. 4B). For carbacyclin, the rings were then
washed, incubated with PDE-3 inhibitor OPC3911 (0.1 μM),
contracted with PE, and relaxed with carbacyclin again. Since
FSK cannot be washed from the rings after application, a
separate set of rings was incubated with OPC3911 and com-
pared with their untreated controls. Carbacyclin- and FSK-
duced relaxation was impaired in both BAL and RUB rings
compared with INT rings (*P < 0.001, BAL or RUB vs. INT,
whole curve). OPC3911 (0.1 μM) improved ISO sensitivity in
both BAL and RUB rings to near that of INT rings for both
carbacyclin and FSK. R_{max} for carbacyclin (3 μM) was af-
fected as follows; in BAL treated with OPC3911 (0.1 μM),
31.3% PE contraction remained compared with 90.2% without
OPC3911; in RUB, the values were 31.7 vs. 98.7%. FSK fully
relaxed the rings with or without OPC3911, so no change of
R_{max} for FSK (3 μM) was observed.

**Effect of PDE-3 inhibitor, OPC3911, on U-46619-induced
contraction.** The effect of PDE-3 inhibitor, OPC3911, on
vasoconstriction induced by U-46619 was studied. Again,
the contraction in the presence of OPC3911 (1 μM; \( \Delta T_{\text{inhibitor}} \)) was
compared with a prior U-46619 contraction of the same ring
without the inhibitor (\( \Delta T_{\text{without}} \)). As shown in Fig. 5, incubation
with OPC3911 reduced the tension differentially in the three
types of rings (*P < 0.05, BAL vs. INT or RUB). The tension
was reduced by 37% in BAL rings, whereas it was reduced by
only 8% in INT rings with no reduction in RUB rings. How-
PDE inhibitors affect vasoreactivity after angioplasty

Effect of PDE-3 inhibitor, OPC3911 (OPC, 0.1 μM), on relaxant responses to carbacyclin (A) and forskolin (FSK, B) in INT (squares) and RUB (triangles) aortic rings and those from rats following BAL (circles). For carbacyclin, n = 4 per group. For FSK, n = 4–7 for INT and RUB; n = 6–10 for BAL. Rings were precontracted with PE (3 μM). Dotted lines and open symbols, untreated rings; solid lines and symbols, with inhibitors. *P < 0.001, BAL or RUB vs. INT.

Effect of PDE-4 inhibitors, Ro20-1724 and cilomilast, on ISO-induced relaxation. The PDE-4 inhibitors, Ro20-1724 and cilomilast, were tested for their effects on responses to ISO in all three types of aortic rings (*P < 0.001, BAL or RUB vs. INT, whole curve). Both inhibitors increased the sensitivity to ISO. However, even with 10 μM of Ro20-1724 (Fig. 6A) or 0.5 μM of cilomilast (Fig. 6B), the ISO response in both BAL and RUB rings was less sensitive compared with that in INT rings (*P < 0.001, BAL or RUB vs. INT for both inhibitors, whole curve). Ro20-1724 (10 μM) moderately increased Rmax for ISO (3 μM) in BAL and RUB; in BAL, 40.5% PE contraction remained compared with 51.0% without Ro20-1724; in RUB rings, the values were 36.5 vs. 69.1%. Similar results were obtained for cilomilast (0.5 μM): in BAL with and without cilomilast, the respective values for the remaining PE contraction were 46.1 vs. 67.6% and in RUB, 46.5 vs. 73.4%.

PDE-3 and PDE-4 activity and cAMP-cGMP level in BAL and control aorta. PDE-3 and PDE-4 activity was measured in the cytosolic fraction of aortic smooth muscle from rats 24 h after BAL or sham operation. As shown in Fig. 7A, PDE-3 activity was increased 65.8% (*P < 0.005, BAL vs. sham), and PDE-4 activity was increased 52.1%, although not significantly (P = 0.076, BAL vs. sham).

Increased PDE-3 activity should affect cAMP and, perhaps, cGMP levels. We thus measured these levels in BAL and INT and RUB aorta rings under basal conditions, or after incubation with PE, or after incubation with different combinations of PDE-3 and PDE-4 inhibitors and FSK (Fig. 7, B and C). We did not observe a difference in basal cAMP levels. In the presence of PE, cAMP levels tended to be lower in BAL than in INT, but the difference did not reach significance (P = 0.083), although the difference was significant between RUB and INT (†P < 0.05). When the PDE-3 inhibitor OPC3911 was added, there was a significant increase in cAMP in BAL (*P < 0.05, drug treatment vs. basal level), which was higher than in INT or RUB (‡P < 0.05, BAL vs. INT; and ‡P < 0.05, BAL vs. RUB). Dual inhibition of PDE-3 (OPC3911) and PDE-4 (Ro20-1724) further increased cAMP levels in all groups of rings (*P < 0.01, drug treatment vs. basal level), with BAL higher than RUB but not INT (‡P < 0.05, BAL vs. RUB). RUB showed lower cAMP levels than INT (†P < 0.05). When FSK was added with the dual inhibitors, cAMP levels increased in all groups of rings (*P < 0.05, drug treatment vs. basal level), but greater cAMP levels were seen in BAL compared with INT or RUB rings (‡P < 0.05, BAL vs. INT; and ‡P < 0.05, BAL vs. RUB). Dual inhibition of PDE-3 (OPC3911) and PDE-4 (Ro20-1724) further increased cAMP levels in all groups of rings (*P < 0.01, drug treatment vs. basal level), with BAL higher than RUB but not INT (‡P < 0.05, BAL vs. RUB). With OPC3911 alone but not dual inhibitors with or without FSK increased cGMP levels in INT and BAL (*

PKA phosphorylation in BAL and CON aortic rings. Western blot analysis of PKA phosphorylation was carried out with an antibody raised against the PKA serine phosphorylation site. The signal strength of the bands between 50 and 150 kDa was quantified. Quantification was also carried out for Ponceau S staining for the similar region, and the ratio was used to
estimate PKA phosphorylation levels. As shown in Fig. 8, A and B, the two bands around the 85-kDa marker contributed most to the quantitation, and the phosphorylation was weaker in BAL than in CON. The phosphorylation level in BAL is ~68% of that in CON (Fig. 8C, *P < 0.05).

**DISCUSSION**

We have shown that PDE-3 activity is increased following BAL and that PDE-4 activity tended to increase but did not reach significance. We thus hypothesized that changes in activity would modify vascular responsiveness by influencing the levels of cyclic nucleotides. We showed that BAL modifies vascular responsiveness via mechanisms in addition to removal of the endothelium, which include increased activity of PDE-3.

We compared aortic rings from rats subjected to BAL to rings from sham rats. Because BAL results in the removal of the endothelium, we also used rings from sham rats where the endothelium was removed by rubbing. The differences between BAL and RUB rings are readily apparent when considering PE- or U-46619-induced contraction in the presence of PDE-3 inhibitors. Inhibition of PDE-3 reduced the contractile response to PE or U-46619 in a similar degree in INT and RUB rings but produced a much greater effect in BAL rings, whereby the maximal contractile response to PE or U-46619 was markedly reduced. This is consistent with increased PDE-3 activity in BAL rings, where inhibition of PDE-3 in BAL rings would result in a relatively greater increase in cAMP to counter the contractile effects of PE or U-46619. As responses to PE or U-46619 in RUB and INT rings were much less affected by inhibitors of PDE, this is consistent with the interpretation that PDE is at basal levels and, therefore, inhibition produces a smaller increase in cAMP. This idea was further supported by the observations that the PDE-3 inhibitor OPC3911, alone or with a PDE-4 inhibitor and FSK, induced a greater increase in cAMP levels in BAL vs. INT or RUB. We did not observe a difference in the basal cAMP level between BAL and INT or RUB aortic rings despite increased cytosolic cAMP response to PE or U-46619 in BAL compared to INT or RUB.
Fig. 8. Decreased PKA phosphorylation in homogenates of rat aorta 24 h after BAL compared with sham-operated CON rats. A: Western blots showing PKA phosphorylation levels in BAL or CON aorta. B: Ponceau S staining of the blot showing protein loading. C: relative PKA phosphorylation level expressed as the ratio of PKA blot intensity to Ponceau S intensity in BAL (shaded bar, n = 3) and CON (open bar, n = 3) normalized to CON. The signal strengths of similar regions between 50 and 150 kDa for PKA blot or Ponceau S blot were quantified. *P < 0.05, BAL vs. CON.

The change in cAMP is subtle and/or is localized or that the sensitivity of the cAMP assay is not enough to elucidate the difference. We thus examined PKA phosphorylation by Western blot with an antibody raised against PKA serine phosphorylation site. The PKA phosphorylation level was lower in BAL than control, but the difference did not achieve significance. Furthermore, the inhibitors of PDE and FSK had a limited effect on cGMP levels, and we suggest that the effects of PDE-3 inhibitors on contractile responses to PE were primarily via effects on cAMP levels.

The effect of PDE-3 on vasoreactivity after BAL is specific for this PDE family, as the effect of inhibition of PDE-4 was much less pronounced, suggesting that this isoform plays a minor role in hydrolyzing cyclic nucleotides that are increased in response to contraction. In addition, in INT rings, although inhibitors of both PDE-3 and PDE-4 moderately reduced PE-induced contraction, the effects of PDE-4 inhibitors are endothelium dependent, while those of PDE-3 inhibitors are not. This is consistent with previous studies showing that relaxation of PE-contracted rat aortic rings by PDE-3 inhibitors was endothelium independent (27), whereas relaxation induced by PDE-4 inhibitors was endothelium dependent (24). These different effects of PDE-3 and PDE-4 inhibitors may relate to different cellular locations for PDE-3 and PDE-4 (38). The hydrophobic NH2-terminal regions of PDE-3 are important for its membrane localization (53), while the distribution of PDE-4 is affected by interaction with B-arrestins (45) or A kinase anchoring proteins (2).

The different PE responses in BAL, INT, and RUB rings were agonist selective, since KCl-induced contractions were comparable in all groups before and after the inhibitors, whereas the PDE-3 inhibitors moderately reduced the response to PE in INT and RUB rings but produced a marked reduction in BAL rings. The maximal response to PE was greater in BAL rings than that in INT or RUB rings without PDE-3 inhibitors, but the difference between BAL and RUB was not apparent when the responses were expressed as a percentage of the response to KCl. We speculate that any increase in the maximal response to PE in BAL rings may result from increased activity of PDE, with a resultant reduction of cyclic nucleotides that may moderate contractile mechanisms. Surprisingly, the 50% effective dose for PE-induced contraction in RUB rings was approximately one-half of that for INT rings and was similar for BAL and INT rings. These findings do not agree with those of Lippolis et al. (29), who reported reduced maximal contractile responses to PE and KCl in BAL rat carotid artery. However, Heijenbrok et al. (17) found increased sensitivity to PE in the rat carotid artery immediately after injury, whereas contractile responses to KCl were unchanged, and Accorsi-Mendonca et al. (1) reported that the maximal response to PE was reduced in the injured rat carotid artery but enhanced in the contralateral vessel 4–7 days after BAL. Possible explanations for these variable results include different sources of vascular tissue (57), the degree of damage to the vessel (36), or different times of testing after injury.

We also studied the effects of PDE-3 inhibitors on cAMP-dependent vasorelaxant responses to ISO, carbacyclin, and FSK. cAMP-dependent vasorelaxant responses were greatly impaired in both BAL and RUB rings contracted with PE, which may relate to removal of endothelial NO and a reduction in cGMP, which inhibits PDE-3 activity (for reviews, see Refs. 9, 34). Therefore, removal of the endothelium and NO could be expected to increase PDE-3 activity with a resultant decrease in cAMP levels in response to PDE-3 inhibitors. This was supported by the results that, in the presence of PE, cAMP and cGMP levels were lower in RUB and also tended to be lower in BAL than that in INT, but the levels were similar in RUB and BAL. It may also explain why PE contractions in both BAL and RUB were different from those in INT. When normalized to maximum PE-induced contractions, responses to cAMP-dependent vasorelaxants were enhanced similarly in BAL and RUB rings by PDE-3 inhibitors to that observed in INT rings. However, as mentioned above, PDE-3 inhibitors greatly reduced PE-induced tension in BAL, and, therefore, the additional decrease in tension to produce maximal relaxation in response to an agonist was relatively small. This is consistent
with the notion that only a small further increase in cAMP in response to an agonist is required for maximal relaxation in BAL. In contrast, PE-induced contraction in INT and RUB rings was much less affected by inhibition of PDE-3, and the leftward shift in the dose-response curve to agonists was much less in INT. In RUB rings, the effects of vasorelaxant agonists were markedly enhanced in the presence of PDE-3 inhibitors, such that the dose-response curves were similar to those of INT rings. Thus there is a synergistic interaction in RUB that is clearly different from BAL. Inhibitors of PDE-4 also enhanced the impaired vasorelaxant activity of ISO in RUB and BAL rings, but the effect was much less marked than that for inhibition of PDE-3, again suggesting a minor role for this isoform in vasorelaxation.

The ability of PDE-3 inhibitors to shift to the left the dose-response curves for all vasorelaxant agents including FSK, which directly activates adenylate cyclase, in RUB rings suggests that a combination of PDE-3 inhibitors with cAMP-dependent agonists could result in a better vasorelaxation. This could be useful in pathological conditions such as atherosclerosis and hypertension. Indeed, previous studies showed that milrinone restored the impaired ISO-response curve in hypoxia-induced pulmonary hypertension, whereas the PDE-4 inhibitor rolipram was much less effective (55). Furthermore, low doses of PDE inhibitors amplified the pulmonary vasodilatory effects of inhaled prostacyclin in rabbits (48, 49) and humans (13) with pulmonary hypertension.

Increased PDE-3 activity can account for our observations of altered vasoreactivity in BAL rings, and we found that PDE-3 activity was significantly increased following BAL. PDE-3 can be activated as a result of phosphorylation by PKA or Akt (PKB) (23, 33, 54, 58), and Akt can be activated by PDGF through phosphatidylinositol 3-kinase (5, 12), a pathway that is involved in regulating the vascular SMC phenotype (15). In rat carotid artery, PDGF was upregulated 6 h after BAL injury, and the PDGF receptors were upregulated 1–2 wk after BAL injury (32). We postulate that upregulation of PDGF could activate Akt, which, in turn, activates PDE-3. PDE-4 activity was increased after BAL, albeit insignificantly, and PDE-4 can be activated by PKA phosphorylation or ERK-mediated phosphorylation (8, 18). Chronic regulation of PDE-3 and PDE-4 expression by a cAMP-dependent feedback stimulus also has been demonstrated (7, 30, 33, 52), and the mechanism underlying upregulation of PDEs by BAL remains to be determined.

In summary, increased PDE-3 activity after BAL modifies vascular responsiveness, which may have important functional implications for angioplasty.

GRANT

This study is supported by National Heart, Lung, and Blood Institute Grant 1R01HL069061 and a grant from the American Diabetes Association.

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PDE INHIBITORS AFFECT VASOREACTIVITY AFTER ANGIOPLASTY

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