Combination of nonspecific PDE inhibitors with inhaled prostacyclin in experimental pulmonary hypertension

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Schermuly, Ralph Theo, Axel Roehl, Norbert Weissmann, Hossein Ardeschir Ghofrani, Hanno Leuchte, Friedrich Grimminger, Werner Seeger, and Dieter Walmrath. Combination of nonspecific PDE inhibitors with inhaled prostacyclin in experimental pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 281: L1361–L1368, 2001.—Inhalation of aerosolized prostacyclin (PGI2) exerts selective pulmonary vasodilation, but its effect is rapidly lost after termination of nebulization. Amplification of the vasodilatory response to inhaled PGI2 might be achieved by phosphodiesterase (PDE) inhibitors to stabilize its second messenger, cAMP. We established stable pulmonary hypertension in perfused rabbit lungs by continuous infusion of U-46619. Short-term (10-min) aerosolization maneuvers of PGI2 effected a rapid, moderate decrease in pulmonary arterial pressure, with post-PGL2 vasorelaxation being lost within 10–15 min, accompanied by a marginal reduction in shunt flow. Preceding administration of subthreshold doses of the PDE inhibitors theophylline, dipyridamole, and pentoxifylline via the intravascular or inhalational route, which per se did not influence pulmonary hemodynamics, caused more than doubling of the immediate pulmonary arterial pressure drop in response to PGI2 and marked prolongation of the post-PGL2 vasorelaxation to >60 min (all PDE inhibitors via both routes of application). This was accompanied by a reduction in shunt flow in the case of aerosolized theophylline (27.5%), pentoxifylline (30.5%), and dipyridamole (33.4%). Coaerosolization of PGI2 and PDE inhibitors may be considered as a therapeutic strategy in pulmonary hypertension.

INHALED VASODILATORS HAVE BEEN SHOWN to achieve selective pulmonary vasorelaxation and supraselective vasodilation in well-ventilated (i.e., inhaled vasodilator-accessible) regions within the lung in experimental and clinical studies. Inhalation of gaseous nitric oxide (NO) (14, 26) and nebulization of prostacyclin (PGI2) (13, 30, 31, 33) were indeed noted to improve ventilation-perfusion matching and to lower pulmonary arterial pressure (Ppa) in patients suffering from acute respiratory distress syndrome and chronic pulmonary hypertension. The prostaglandin PGI2 does, however, possess a very short biological half-life (2–3 min) at a physiological pH, and after inhalation of aerosolized PGI2, the pulmonary vasodilatory effect is lost within <30 min both under experimental conditions and when tested in patients (13, 18). Because the vasodilatory effect of the prostaglandins is exerted via enhanced cAMP generation, blocking the catabolism of this second messenger might offer amplification of the pulmonary vasodilatory effects of these agents. The hydrolysis of the cyclic nucleotides proceeds via a group of phosphodiesterase (PDE) isoforms (25), and the presence of isoforms 1 and 3–5 has been demonstrated for the lung parenchyma (15). PDE3 and PDE4 preferentially hydrolyze cAMP, whereas PDE5 possesses a high affinity for cGMP (25). In a recent study in intact rabbits with acute pulmonary hypertension (18), subthreshold intravenous doses of monoselective PDE3, PDE4, and PDE5 inhibitors were noted to augment and prolong the pulmonary vasodilatory response to inhaled PGI2 while limiting the hypotensive effect to the pulmonary circulation. We presently employed clinically approved, mostly nonspecific PDE inhibitors in a model of thromboxane-mediated pulmonary hypertension in perfused rabbit lungs. Theophylline, pentoxifylline, and dipyridamole were either admixed to the lung perfusate or administered by aerosolization. Low doses of each agent were chosen, guaranteeing that the PDE inhibitors per se would not affect lung hemodynamics and gas exchange as assessed by the multiple inert gas elimination technique (MIGET). All agents, whether infused or nebulized, strongly enhanced the vasodilatory response to a subsequently performed PGI2 aerosolization maneuver. In addition, coadministration of the PDE inhibitors via the inhalational route resulted in a marked improvement of the gas exchange abnormalities, in particular the increase in shunt flow, accompanying the thromboxane-elicited pulmonary hypertension. We conclude that low-dose PDE inhibitors may offer amplification of the selective pulmonary vasodilatory effects of aerosolized prostaglandins and that...
the inhalational route might be particularly suitable for such an approach.

METHODS

Materials

PGI₂ (Epostenol) was supplied by Wellcome (London, UK), and the thromboxane A₂ mimetic U-46619 was from Paesel-Lorei (Frankfurt, Germany). Sterile Krebs-Henseleit hydroxethylamlylopectine buffer (KHBB) was obtained from Serag-Wiesen (Naila, Germany). Theophylline (Euphyllin) was supplied by Byk Gulden (Constance, Germany), pentoxifylline (Trental) was from Hoechst Marion Roussel (Bad Soden, Germany), and dipyridamole (Persantin) was from Boehringer (Ingelheim, Germany). The ultrasonic nebulizer Pulmo Sonic 5500 was obtained from DeVilbiss Medizinische Produkte (Langen, Germany). All other chemicals were purchased from Merck (Darmstadt, Germany).

Isolated Lung Model

The isolated lung model has been previously described in detail (22). Briefly, rabbits weighing 2.6–2.9 kg were deeply anesthetized with intravenous ketamine-xylazine and anticoagulated with heparin (1,000 U/kg). A tracheostomy was performed, and the animals were ventilated with room air with a Harvard respirator (tidal volume, 9–13 ml/kg; frequency, 10 breaths/min; positive end-expiratory pressure, 1 mmHg). After a midsternal thoracotomy, catheters were placed in the pulmonary artery and left atrium, and perfusion with KHBB was started and slowly increased to a final flow of 115 ml/min. Left atrial pressure was set at 1.2 mmHg in all experiments, and weight gain was recorded. Pressures in the pulmonary artery, left atrium, and trachea were continuously registered (zero referenced at the hilum). Perfusate samples (total perfusate volume, 500 ml) were taken from the arterial and venous parts of the system. Gas samples were taken from the outlet of an expiration gas mixing box.

Aerosolization

PGI₂ and PDE inhibitors were nebulized with an ultrasonic device (mass median aerodynamic diameter, 4.5 μm; geometric SD, 2.6; Pulmo Sonic 5500). The nebulizer was located between the ventilator and the lung to be passed by the inspiration gas; the nebulization system was previously described in detail (20). For the given ventilator setting (tidal volume, 9–13 ml/kg; frequency, 10 breaths/min; positive end-expiratory pressure, 1 mmHg), this nebulization system resulted in a deposition fraction of 0.25 ± 0.02 as determined by a laser photometric technique (21).

Ventilation-Perfusion Ratio Determination in Isolated Lungs by MIGET

The distribution of ventilation and perfusion was determined by the MIGET as described by Wagner et al. (27). Six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) were dissolved in KHBB and continuously infused (0.5 ml/min). After an equilibration period of at least 40 min, 10-ml perfusate samples were drawn from the left atrium and pulmonary artery. A corresponding 30-ml gas sample was collected from an expiration gas mixing box. The dissolved gases in the perfusate were extracted by equilibration (40 min) with nitrogen in a shaking water bath. The gas phases and exhaled gases were analyzed by gas chromatography. The ratios of left atrial to venous partial pressures (retention) and of expired to mixed venous partial pressures (excretion) were calculated for each gas. With a computer program, the retention and excretion resulted in a ventilation-perfusion ratio (Vα/Q) distribution determined with least squares analysis with enforced smoothing. The residual sum of squares (RSS) was the result of testing the compatibility of the inert gas data to the derived Vα/Q distribution with the least squares method. An indication of acceptable quality of the Vα/Q distributions is a RSS of 5.348 or less in half of the experimental runs (50th percentile) or 10.645 or smaller in 90% of the experimental runs (90th percentile) (28). In the present study, 93.8% of RSS were <5.348 and 99.1% were <10.645.

Determination of cAMP

As previously described (19), cAMP was measured with a radioimmunoassay kit (Immunotech, Marseilles, France). Briefly, duplicate samples of 500 μl of perfusate were collected at 0, 30, 45, 60, 75, 105, and 135 min and incubated with 125I-labeled cAMP in antibody-coated tubes. After incubation, bound radioactivity was counted, and values were calculated with a standard curve. The cAMP level is given as picomoles per milliliter.

Experimental Protocols

As previously described (29), a sustained increase in Ppa from 7.2 ± 0.2 to 32.7 ± 1.1 mmHg was achieved by a continuous infusion of 25–55 ng·kg⁻¹·min⁻¹ of U-46619; individual titration was performed. This level of pulmonary hypertension was then maintained for at least 150 min, with a variation in Ppa of <3 mmHg. In preceding experiments, increasing doses of aerosolized PGI₂ were applied via the inhalational route, and a dose of 10 ng·kg⁻¹·min⁻¹ was found to decrease Ppa by ~4.0 mmHg when administered over a 10-min aerosolization period. The efficacy of the PDE inhibitors was assessed in dose-effect curves for theophylline, pentoxifylline, and dipyridamole; these agents were either bolus injected into the recirculating buffer fluid or nebulized over a 10-min period. In separate experiments, a subthreshold dose of the PDE inhibitor, with no effect on Ppa and gas exchange per se, was applied either intravenously or via the inhalational route over a 10-min administration period, and subsequent inhalation of the PGI₂ standard dose (10 ng·kg⁻¹·min⁻¹ over 10 min; see time schedules in Figs. 1, 2, 4, and 5) was performed. The following experimental groups were used.

Control lungs. After termination of the steady-state period, Vα/Q measurements were performed at 30, 45, 60, 75, 105, and 135 min (n = 6 lungs); no interventions were undertaken.

U-46619-treated lungs. After termination of the steady-state period, U-46619 was continuously infused over 135 min to provoke an increase in Ppa to 32.7 ± 1.2 mmHg (n = 6 lungs). Vα/Q measurements were performed 30, 45, 60, 75, 105, and 135 min after the beginning of the U-46619 application.

Dose-effect curve of inhaled and infused PDE inhibitors. U-46619 was titrated and then continuously infused as described in U-46619-treated lungs, establishing a stable pulmonary hypertension. Increasing doses of the PDE inhibitors were either bolus injected into the recirculating medium or nebulized within 10-min aerosolization periods (cumulative dose-effect curves; n = 4 lungs/group). The doses were 2, 4, 6, 10, 20, and 30 μg/ml iv and 2, 10, and 20 μg·kg⁻¹·min⁻¹ (nebulization) for theophylline; 0.1, 1, 2, 10, and 100 μg·ml⁻¹ iv and 30, 60, and 300 μg·kg⁻¹·min⁻¹ (nebulization) for pen-
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toxifylline; and 1, 5, 50, 500, and 2,500 pg/ml iv and 2, 10, and 20 ng·kg⁻¹·min⁻¹ (nebulization) for dipyridamole.

PGI₂ aerosolization. U-46619 was administered as described in U-46619-treated lungs. Forty-five minutes after the U-46619 infusion was started, PGI₂ was aerosolized for 10 min at a dose of 10 ng·kg⁻¹·min⁻¹. VA/Q measurements were performed after 30, 45, 60, 75, 105, and 135 min (n = 6 lungs).

PGI₂ aerosolization combined with intravenous or inhaled PDE inhibitors. U-46619 was infused as described in U-46619-treated lungs, and after 30 min, a subthreshold dose of one of the PDE inhibitors was either infused or nebulized (n = 6 lungs/group). The dose was taken from the dose-effect curve established in the preceding experiments. The doses employed in these experiments were 4 μg/ml and 2 μg·kg⁻¹·min⁻¹ for theophylline, 2 μg/ml and 30 μg·kg⁻¹·min⁻¹ for pentoxifylline, and 1 ng/ml and 2 ng·kg⁻¹·min⁻¹ for dipyridamole for infusion and nebulization, respectively. Fifteen minutes after subthreshold PDE administration, PGI₂ nebulization was performed as described for the PGI₂ group (10 ng·kg⁻¹·min⁻¹ over a 10-min period). The times for the measurement of ventilation-perfusion distribution corresponded to the preceding experiments.

Data Analysis

All values are given as means ± SE or coefficients of variation (SD/mean in percent). A one-way analysis of variance for repeated measures was used to evaluate significant differences among conditions, and where significance was found, post hoc analysis was performed with the Student-Newman-Keuls test. Significance was considered to exist at P < 0.05. These analyses were performed with WinSTAT for Windows, version 3.1 (Kalmia, Cambridge, MA).

RESULTS

Baseline

All lungs displayed Ppa values in the range between 5 and 7 mmHg after termination of the steady-state period. VA/Q measurements under these baseline conditions revealed a unimodal, narrow distribution of perfusion and ventilation to the midrange VA/Q regions (0.1 < VA/Q < 10) throughout (Table 1). Shunt flow (VA/Q < 0.005; Fig. 1) and perfusion of poorly ventilated areas (0.005 < VA/Q < 0.1) were extremely low (total sum <2%), with no perfusate flow to high VA/Q regions (10 < VA/Q < 100) being detected. Dead space ventilation (VA/Q > 100) amounted to ≈50% in this system of isolated lung ventilation via tracheal tubing. The initial weight of the lungs was 11.4 ± 1.3 g for all groups. At the end of the experiments, the weight of the control lungs increased by 12.4 ± 1.3% (Fig. 2).

Continuous infusion of U-46619 (mean dose 41.0 ± 13.9 ng·kg⁻¹·min⁻¹) provoked an increase in Ppa to 32.7 ± 1.0 mmHg within 25 min, followed by plateauing of pulmonary hypertension. Also, delayed-onset lung weight gain was noted, and shunt flow progressively increased to 54.9 ± 4.6% of total lung perfusion within 135 min (Table 1, Fig. 1). In addition, a marked broadening of flow dispersion and ventilation distribution in the midrange VA/Q areas was observed in response to the U-46619 infusion (Table 1). Dead space increased by 15.1% (data not given). The total weight of the isolated organs was increased to 123 ± 17% of the initial weight (Fig. 2).

Dose-Inhibition Curves of PDE Inhibitors Administered in the Absence of PGI₂

All PDE inhibitors presently investigated relieved the U-46619-elicted pulmonary hypertension in a dose-dependent manner via both the intravenous and inhalational routes (Fig. 3). The dose-effect curves for dipyridamole ranged at markedly lower quantities for

Table 1. Gas-exchange variables in response to PGI₂ inhalation in combination with PDE inhibitors

<table>
<thead>
<tr>
<th></th>
<th>Shunt, %Q</th>
<th>Low VA/Q, %Q</th>
<th>Normal VA/Q, %Q</th>
<th>Log SDQ</th>
<th>Log SDV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VA/Q&lt;0.005</td>
<td>0.005&lt;VA/Q&lt;0.1</td>
<td>1&lt;VA/Q&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 min</td>
<td>135 min</td>
<td>0 min</td>
<td>135 min</td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>1.5±0.5</td>
<td>1.8±0.9</td>
<td>0.1±0.3</td>
<td>0.0±0.2</td>
<td>98.5±0.9</td>
</tr>
<tr>
<td>U-46619</td>
<td>1.6±0.5</td>
<td>54.9±4.6</td>
<td>0.3±0.3</td>
<td>0.3±0.2</td>
<td>1.7±1.4</td>
</tr>
<tr>
<td>U-46619-PGI₂ aer-Theo iv</td>
<td>1.6±0.2</td>
<td>46.7±5.5</td>
<td>0.3±0.2</td>
<td>3.1±1.5</td>
<td>98.1±0.4</td>
</tr>
<tr>
<td>U-46619-PGI₂ aer-Theo aer</td>
<td>1.2±0.4</td>
<td>42.3±14.7</td>
<td>1.0±0.4</td>
<td>3.8±1.6</td>
<td>97.9±0.2</td>
</tr>
<tr>
<td>U-46619-PGI₂ aer-Theo aer</td>
<td>1.7±0.7</td>
<td>27.5±3.9a</td>
<td>1.28±0.68</td>
<td>5.3±1.5</td>
<td>97.2±0.1</td>
</tr>
<tr>
<td>U-46619-PGI₂ aer-Theo aer</td>
<td>0.9±0.3</td>
<td>41.9±9.3</td>
<td>0.5±0.43</td>
<td>0.5±0.5</td>
<td>98.6±0.4</td>
</tr>
<tr>
<td>U-46619-PGI₂ aer-Theo aer</td>
<td>1.2±0.15</td>
<td>30.5±6.2a</td>
<td>0.1±0.1</td>
<td>1.6±1.3</td>
<td>98.7±0.1</td>
</tr>
<tr>
<td>U-46619-PGI₂ aer-Dipyrr aer</td>
<td>0.2±0.1</td>
<td>49.4±8.1</td>
<td>1.0±0.3</td>
<td>1.6±1.0</td>
<td>98.8±0.1</td>
</tr>
<tr>
<td>U-46619-PGI₂ aer-Dipyrr aer</td>
<td>1.1±0.3</td>
<td>33.4±7.3a</td>
<td>1.0±0.43</td>
<td>5.8±3.0</td>
<td>97.9±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. PGI₂, prostacyclin; aer, aerosol; PDE, phosphodiesterase; VA/Q, alveolar ventilation; Q, perfusion; Theo, theophylline; Pent, pentoxifylline; Dipyrr, dipyridamole; log SDQ and log SDV, dispersion of perfusion and ventilation, respectively. All data were obtained by multiple inert gas elimination technique. Distribution of flow to shunt, low ventilation-perfusion ratio (VA/Q), and normal VA/Q areas amounted to virtually 100% in all experiments; the minor fraction of flow to high VA/Q areas is not shown. a P < 0.05 compared with U-46619-PGI₂ aer group.
both modes of application compared with those for theophylline and pentoxifylline.

**Effect of Aerosolized PGI₂**

At the chosen dose of PGI₂ nebulization (10 ng·kg⁻¹·min⁻¹ over 10 min), a moderate decrease in the Ppa values that were elevated in response to U-46619 infusion was noted, with a maximum pressure decline of 3.5 ± 0.6 mmHg at the end of the aerosolization period (P < 0.05; Fig. 4). The vasodilatory effect of PGI₂ nebulization commenced within 2 min (data not shown in detail) and immediately leveled off after termination of the aerosol maneuver; prenebulization Ppa values were again reached within 15 min. The pronounced increase in intrapulmonary shunt occurring in the lungs with U-46619 infusion was moderately but not significantly reduced by the 10-min PGI₂ nebulization maneuver (Fig. 1). Broadening of flow dispersion and ventilation distribution in the midrange Vₐ/Q regions as assessed after 135 min was not different from the abnormalities encountered in the U-46619-infused lungs in the absence of PGI₂ aerosolization (Table 1), and no impact on lung weight gain was noted (121 ± 14%; Fig. 2).

**Combination of PGI₂ Nebulization With Subthreshold Administration of PDE Inhibitors**

Based on the dose-effect curves of the different PDE inhibitors via either the intravascular or inhalational route, subthreshold doses of all agents were chosen, which per se did not affect the pulmonary hypertension and gas exchange abnormalities elicited by U-46619 infusion. When applied before the PGI₂ nebulization maneuver, however, marked amplification of the response to the aerosolized PGI₂ was noted.

**Theophylline.** In the presence of inhaled theophylline, the PGI₂-induced maximum Ppa decrease was augmented from 3.5 ± 0.6 to 7.4 ± 0.5 mmHg (P < 0.05), whereas intravascularly applied theophylline ef-
fected only a minor augmentation of the immediate PGI₂-induced Ppa drop. A prolonged pressure decline in the subsequent perfusion period was, however, observed for both routes of application; even after 135 min, the preceding U-46619-elicited pressure plateau was not yet reestablished (Fig. 4). The increase in weight gain was not significantly influenced (Fig. 2) by both modes of theophylline administration. There was a significant reduction in shunt flow in the aerosolized theophylline and PGI₂ lungs from 46.7 ± 6.5 to 30.5 ± 6.2% (P < 0.05; Fig. 1) but not by intravenous application of this agent (41.9 ± 9.3%). Dispersion of perfusion (log SDQ) was significantly reduced (1.03 ± 0.07 vs. 1.49 ± 0.13; P < 0.01) in the lungs with pentoxifylline aerosolization. The percent increase in initial weight gain was mark-

Pentoxifylline. Subthreshold doses of inhaled and infused pentoxifylline significantly enhanced the maximum Ppa decrease in response to the subsequent PGI₂ nebulization from 3.7 to 9.2 (intravenous) and 9.9 (aerosolized) mmHg and prolonged the post-PGI₂ vasorelaxation to >60 min (Fig. 4). Intrapulmonary shunt flow was significantly reduced by aerosolization of pentoxifylline (46.7 ± 5.5 to 30.5 ± 6.2%; P < 0.05; Fig. 1) but not by intravenous application of this agent (41.9 ± 9.3%). Dispersion of perfusion (log SDQ) was significantly reduced (1.03 ± 0.07 vs. 1.49 ± 0.13; P < 0.01) in the lungs with pentoxifylline aerosolization. The percent increase in initial weight gain was mark-
PDE inhibitors and PGI2 in pulmonary hypertension

Dipyridamole. Administration of dipyridamole significantly prolonged (>60 vs. 15 min) the vasodilatory efficacy of PGI2 nebulization (Fig. 4). Intravenous application failed to reduce the intrapulmonary shunt flow (49.4 ± 8.1 vs. 46.7 ± 5.5%), whereas this decreased in the aerosolized dipyridamole and PGI2 lungs (33.4 ± 7.3%; P < 0.05; Table 1). The percent increase in initial weight gain was reduced in both groups (Fig. 2), but a significant decrease was only noted in the aerosolized dipyridamole and PGI2 lungs (76 ± 24 vs. 123 ± 17%; P < 0.05). No significant impact on the broadening of the perfusion dispersion in the midrange V/VQ areas was observed in both groups with dipyridamole administration.

Ventilation Pressures

No significant change in peak airway pressure during constant-volume ventilation was noted in any of the experimental groups (data not shown in detail).

Measurement of cAMP

As shown in Fig. 5, the cAMP concentration in control experiments with U-46619 infusion increased slowly to 4.7 ± 0.9 pmol/ml. Aerosolization of PGI2 resulted in a significant increase in cAMP to 8.3 ± 1.4 pmol/ml (P < 0.05). The combination of inhaled PGI2 with aerosolized pentoxifylline and aerosolized theophylline significantly enhanced the PGI2-elicited cAMP liberation (from 8.3 ± 1.4 to 14.6 ± 1.2 and 12.3 ± 0.9 pmol/ml, respectively). In the presence of inhaled dipyridamole, a cAMP concentration of 11.0 ± 2.3 pmol/ml was measured (not significant).

DISCUSSION

When continuously infused, the thromboxane mimetic U-46619 is suitable for establishing stable pulmonary hypertension in isolated rabbit lungs, and this model has repeatedly been employed for analyzing the pulmonary vasodilatory effect of various agents (10, 16, 19, 29). When dose-effect curves for the pulmonary vasodilatory effect of the presently employed PDE inhibitors in this model were established, largely comparable efficacy was noted for theophylline and pentoxifylline, with dipyridamole being effective in a three orders of magnitude lower concentration range. This is in line with available pharmacological data characterizing the methylxanthines theophylline and pentoxifylline as nonselective PDE1–5 inhibitors, with IC50 values ranging between 50 and 200 μM (2, 12), whereas dipyridamole inhibits PDE5, -6, -8, and -10, with IC50 values ranging between 1 and 5 μM (1, 17, 24). The relief of pulmonary hypertension by various PDE inhibitors, when applied in sufficiently high doses, has previously been demonstrated in several animal models (3, 5, 7, 18); however, under clinical conditions, such an approach is hampered by the severe side effects caused by these agents at higher concentration ranges. Notably, when the total amount of substance either admixed to the perfusion fluid or offered via inhalation was calculated, corresponding vasodilatory effects were caused by lower quantities of all three PDE inhibitors in the case of the inhalational application. This is even more impressive when considering the fact that, at best, approx 30% of the total nebulized material is definitely deposited in the alveolar space (20, 29). Thus the predominant mode of action of the aerosolized PDE inhibitors appears to be “regional” vasodilation rather than “systemic” vasodilation, which might occur after transit of the agent from the alveolar space into the recirculating perfusate contained in the vascular compartment.

Aerosolized PGI2 was previously demonstrated to be a potent pulmonary vasodilator in the model of U-46619-elicited pulmonary hypertension (29). Predominant relief of precapillary and a moderate reduction of postcapillary vascular resistance were shown to underlie this prostanoid effect. In the present investigation, the dose and duration of PGI2 nebulization were limited to establish a moderate Ppa decrease response to the prostanoid (3.5 ± 0.6 mmHg), which...
was rapidly reversible after cessation of the aerosolization maneuver. A prominent finding of the present study is the fact that the preceding administration of subthreshold doses of the methylxanthines theophylline and pentoxifylline, whether undertaken via the intravascular or inhalational route, resulted in a marked amplification of the pulmonary vasodilatory effect of nebulized PGI₂, with post-PGI₂ vasorelaxation being prolonged to >60 min. These data are in line with recent studies (18, 19) in intact rabbits or perfused rabbit lungs with U-46619-induced pulmonary hypertension, in which an enhanced vasodilatory response to nebulized PGI₂ occurred after the preceding administration of monoselective or dual-selective PDE3 or -4 inhibitors. Increased cAMP accumulation in response to the prostanoitid was demonstrated in these studies, and stabilization of PGI₂-induced cAMP due to inhibition of cAMP-hydrolyzing PDEs by the nonselective PDE inhibitors theophylline and pentoxifylline indeed offers a most plausible explanation for the efficacy of subthreshold doses of these agents. cAMP via activation of protein kinase A results in phosphorylation of the myosin light chain kinase, thereby effecting vascular smooth muscle relaxation. When coadministration of dipyridamole and PGI₂ was tested in a corresponding fashion, this PDE inhibitor was also noted to markedly prolong the post-PGI₂ vasorelaxation, although the overall efficacy in amplifying the PGI₂ response was somewhat less prominent than that of the methylxanthines. In preceding investigations addressing pulmonary hypertension (9, 32), dipyridamole has been noted to amplify pulmonary vasodilation induced by NO in the ovine fetus and to attenuate the rebound pulmonary hypertension occurring on withdrawal of NO under conditions of cardiac surgery, which may be easily attributed to the stabilization of cGMP arising due to NO-related stimulation of guanylate cyclase. These data corroborate the study by Iechinose et al. (8), who investigated the effect of inhaled NO in the presence of the PDE5 inhibitor zaprinast. They found amplification and prolongation of the NO-induced vasodilation by zaprinast via increased transpulmonary cGMP levels. The intracellular cGMP content is mainly controlled by PDE5, with the role of the recently described cGMP-hydrolyzing PDE9 (23) not yet being fully settled. The present finding of enhanced efficacy of the PGI₂-cAMP axis in the presence of dipyridamole does, however, suggest close interaction between cAMP- and cGMP-mediated vasodilatory pathways. Inhibition of the cGMP-sensitive PDE3 by increased cGMP levels arising in the presence of dipyridamole is one well-known mechanism of this type of interaction as similarly suggested from a study (6) in isolated vascular strips.

When employing the MIGET for detailed analysis of gas exchange conditions, physiological ventilation-perfusion matching in the absence of any significant shunt flow was encountered in control lungs as previously described (29). In parallel with the pulmonary hypertensive response, severe gas exchange abnormalities, characterized by a prominent increase in shunt flow to >50%, were encountered in lungs undergoing the U-46619 challenge. The maneuver of limited-dose and short-term PGI₂ aerosolization caused a moderate reduction in shunt flow that was not significantly different from the nontreated lungs. Interestingly, transbronchial administration of subthreshold doses of the methylxanthines and, to some minor extent, of dipryridamole before PGI₂ nebulization resulted in a marked reduction in shunt formation, with corresponding maintenance of normal V/AQ areas. Such an efficacy was, however, missing on intravascular administration of the PDE inhibitors.

Three mechanisms may underlie the impressive beneficial effect of the inhaled PDE inhibitors on the U-46619 elicited gas exchange disturbances. 1) The inhaled PDE inhibitors might be effective by limiting lung edema formation. Significantly lower lung weight as assessed at the end of the experiments was indeed noted in lungs with coaerosolization of PGI₂ and pentoxifylline or dipryridamole, with the effects of pentoxifylline greater than those of dipryridamole. This observation is in line with previous reports on the inhibitory effects of pentoxifylline on microvascular leakage in models of acute lung injury (4, 11). 2) Coaerosolization of the PDE inhibitors might possess a higher overall vasodilatory potency than intravascular administration of these agents. A previous study (29) of the gas exchange abnormalities in the present model demonstrated that the strength of the pulmonary hypertensive response is correlated with the severity of V/AQ mismatch and, in particular, the extent of shunt flow even before onset of marked lung edema formation. This finding suggests that the increased Ppa forces perfuse flow through poorly or nonventilated lung areas, existing even in nonedematous lungs, or perfusion of some type of “preformed shunt vessels” that are excluded from perfusion under conditions of normal intravascular pressure. 3) Coaerosolization of the PDE inhibitors might improve ventilation-perfusion matching via selective pulmonary vasodilation in well-ventilated lung areas. This interpretation suggests that combining aerosol-driven distribution of both the directly vasorelaxant PGI₂ and the PDE inhibitor for second messenger stabilization is the most efficient approach to restrict the vasodilatory response to aerosol-accessible, i.e., well-ventilated, lung areas, with preferred distribution of flow to these lung regions.

In conclusion, the pulmonary vasodilatory effect of aerosolized PGI₂ is significantly amplified by coadministration of the clinically approved PDE inhibitors theophylline, pentoxifylline, and dipryridamole at doses that per se do not exert any hemodynamic effect. Both the intravascular and inhalational routes of PDE inhibitor administration may be employed for this purpose, with the methylxanthines being somewhat more potent than dipryridamole via both routes. Prolongation of the half-life of the second messenger cAMP by the subthreshold doses of the PDE inhibitors is suggested as the underlying mode of action. Relief of pulmonary hypertension by PGI₂ nebulization and coaerosolization but not by confussion with the methylxanthines
causes a marked improvement in ventilation-perfusion matching, with a reduction in shunt flow, suggesting that use of the inhalational route of application for both PGI2 and the PDE inhibitor is most effective in targeting the vasorelaxant properties to well-ventilated lung regions for maintenance of gas exchange.

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