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Phosphodiesterase Inhibitors Prevent Endothelin-1-Induced Vasoconstriction, Bronchoconstriction, and Thromboxane Release in Perfused Rat Lung

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Endothelin-1 (ET-1) causes broncho- and vasoconstriction in the rat isolated perfused lung and induces the release of thromboxane and prostacyclin. Pharmacological inhibition of phosphodiesterases (PDE) is known to relax airway and vascular smooth muscle and it attenuates the release of pro-inflammatory mediators. Therefore, we examined whether and how rolipram (specific for PDE IV) and motapizone (specific for PDE III) affect ET-1-elicited changes in lung function. 5μM motapizone attenuated broncho- and vasoconstriction to a greater extent than 5μM rolipram. Simultaneous pretreatment with both PDE inhibitors protected completely. Thromboxane release was suppressed by rolipram, but not by motapizone. Prostacyclin release was neither influenced by single, nor by combined pretreatment with either compound. We conclude that combined inhibition of PDE III and IV counteracts ET-1-elicited pressor- and inflammatory actions in the lung.

Endothelins are discussed as pathological mediators in a number of airway diseases such as asthma, ARDS or pulmonary hypertension [1]. The acute pulmonary actions of endothelins include broncho- and vasoconstriction as well as release of inflammatory mediators such as TNF, thromboxane A_2 (TX) and prostacyclin (PGI) [1, 2, 3]. Endothelin-1 (ET-1) induces bronchoconstriction partly by initiating thromboxane release and partly by binding to ET_B-receptors on airway smooth-muscle [4, 5]. The ET-1-induced constriction of the pulmonary artery appears to be mainly mediated by ET_A-receptors [1, 3].

Elevation of cytosolic cyclic-AMP (cAMP) levels by inhibition of phosphodiesterases (PDE) has been shown to suppress release of pro-inflammatory mediators, e.g. TNFα release from alveolar macrophages [6]. PDE-inhibitors also relax airways [7] and pulmonary vessels [8, 9, 10] and are therefore considered as useful anti-inflammatory drugs against e.g. asthma or ARDS [6, 11, 12]. The pharmacological effects of PDE-inhibitors on endothelin-induced alterations in lung function, however, has not been investigated yet, in spite of the possible causal relationship between endothelin and pulmonary diseases. This prompted us to investigate the effects of motapizone (PDE III-specific) and rolipram (PDE IV-specific) on the ET-1-induced pressor responses and eicosanoid release in the model of the isolated perfused rat lung.

MATERIALS AND METHODS

Materials. Female Wistar rats (210-240g, Harlan-Winkelmann GmbH, Borchen, Germany) were used as lung donors. Pentobarbital sodium (Nembutal) was purchased from the Wirtschaftsgenossenschaft Deutscher Tierärzte (Hannover, Germany), bovine albumin from Serva (Heidelberg, Germany) and ET-1 from Boehringer Mannheim (Mannheim, Germany). Rolipram was a gift from Schering AG (Berlin, Germany) and motapizone from Nattermann-Rhone Poulenc-Rorer (Cologne, Germany).

Isolated perfused rat lung preparation. The rat lungs were prepared and perfused essentially as described recently [13]. Briefly, lungs were perfused at constant hydrostatic pressure (12 cm H_2O) through the pulmonary artery which resulted in a flow rate of about 30 ml/min. As a perfusion medium we used Krebs-Henseleit buffer (37°C) that contained 2% albumin, 0.1% glucose and 0.3% HEPES. The total amount of recirculating buffer was 100 ml. The lungs were suspended by the trachea and were ventilated by negative pressure ventilation with 80 breaths/min and a tidal volume between 1.95 and 2.05 ml. Every 5 min a hyperinflation (−16 cm H_2O) was performed. Artificial thorax chamber pressure was measured with a differential pressure transducer (Validyne DP 45-14), and air flow velocity with a pneumotachometer connected to a differential pressure transducer (Validyne DP 45-15). The lungs respired humidified air. The perfusate flow (Q) as well as the arterial (Part) and venous pressure (Pven) were continuously monitored. The pH of the perfusate before entering the lung was kept at 7.4 by constant gassing of the buffer with carbogen. All data were transmitted to a Computer via an analog pulmonary-mechanics-analyser (Buxco Electronics Inc., Sharon,

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Abbreviations: ET-1, endothelin-1; PDE, phosphodiesterase; TX, thromboxane A2; PGI, prostacyclin.

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USA) which calculated pulmonary resistance, pulmonary compliance and tidal volume. Vascular resistance was calculated under LS-12 (Buxco Electronics Inc., Sharon, USA) on the computer. Simultaneously, chamber pressure, airflow velocity, tidal volume, dynamic compliance, airway resistance and perfusate flow rate were recorded on a Graphtec Linearrecorder WR3500. The data for pulmonary and vascular resistance are presented as their inverse values, i.e. as pulmonary and vascular conductance.

Measurement of thromboxane and prostacyclin. Samples taken from the perfusate were stored at −20°C. Thromboxane A_2 was assessed as the stable by-product thromboxane B_2 by EIA (Cayman, Ann Arbor, US.) The cross-reactivity of the detecting antibody was TXB_2, 100%; 2,3-dinor TXB_2, 8.2%; prostaglandins (e.g., PGD_2, PGE_2, 6-keto-$PGF_{1\alpha}$) < 0.5%. Prostacyclin was assessed as the stable by-product 6-keto-$PGF_{1\alpha}$ by EIA (Cayman). The cross-reactivity of the detecting antibody was 6-keto-$PGF_{1\alpha}$, 100%; 2,3-dinor-6-keto-$PGF_{1\alpha}$, 8.7%; $PGF_{2\alpha}$, 2.1%; PGE_2 0.92%; $PGF_{1\alpha}$, 0.8%, other prostaglandins ⩽ 0.1%.

Experimental design. ET-1 was dissolved in 10μl 1% acetic acid and further diluted with H_2O. Rolipram and motapizone were dissolved in phosphate buffered saline. To obtain a stable baseline, all lungs were perfused for 40 min before a bolus of 1 nmol ET-1 was injected into the pulmonary artery. Rolipram and motapizone were added 10 min before administration of ET-1. Within these 10 min neither compound induced any alterations in lung function. 5 μM of either inhibitor were used in order to obtain both selective and (nearly) complete inhibition of the respective isoenzyme [14]. After administration of ET-1, the lungs were perfused and ventilated for another 110 min, or until breathing ceased as a result of the ET-1 treatment.

Statistics. Data in the figures are given as mean ± SEM. For pulmonary and vascular conductance, the area under the curve was used for the statistical analysis. Data were analysed by ANOVA. In case of differences among the groups, Newman-Keuls-test was performed (GraphPad Prism; GraphPad Software, Inc.; San Diego; USA). $P<0.05$ was considered to be significant.

RESULTS

Bronchoconstriction. Bolus injection of 1 nmol ET-1 into the pulmonary artery caused a strong decrease in pulmonary conductance (G_L) and tidal volume (not shown), which resulted in the cessation of breathing after 40 minutes (Fig. 1a). Pretreatment of the lungs with 5 μM rolipram 10 minutes before ET-1 challenge attenuated the ET-1-elicited bronchoconstriction. A similar pretreatment with 5 μM motapizone provided a more potent protection than rolipram, resulting in a decrease in pulmonary conductance of only about 50% after 110 minutes. Despite the relative weak action of rolipram alone, administration of 5 μM of both PDE-inhibitors together resulted in full protection from the ET-1 induced bronchoconstriction. The time course of pulmonary conductance was similar to the one of untreated (control) lungs.

Vasoconstriction. For the ET-1 elicited vasoconstriction, a similar pattern of intervention as for the bronchoconstriction was observed. ET-1 induced strong vasoconstriction in the IPL resulting in complete cessation of perfusate flow after 40 min (Fig. 1b). Both 5 μM rolipram and 5 μM motapizone attenuated this vasoconstriction; again, motapizone was more effective. Dual inhibition of PDE III and IV by combined pretreatment with rolipram and motapizone nearly completely prevented the ET-1-elicited vasoconstriction.

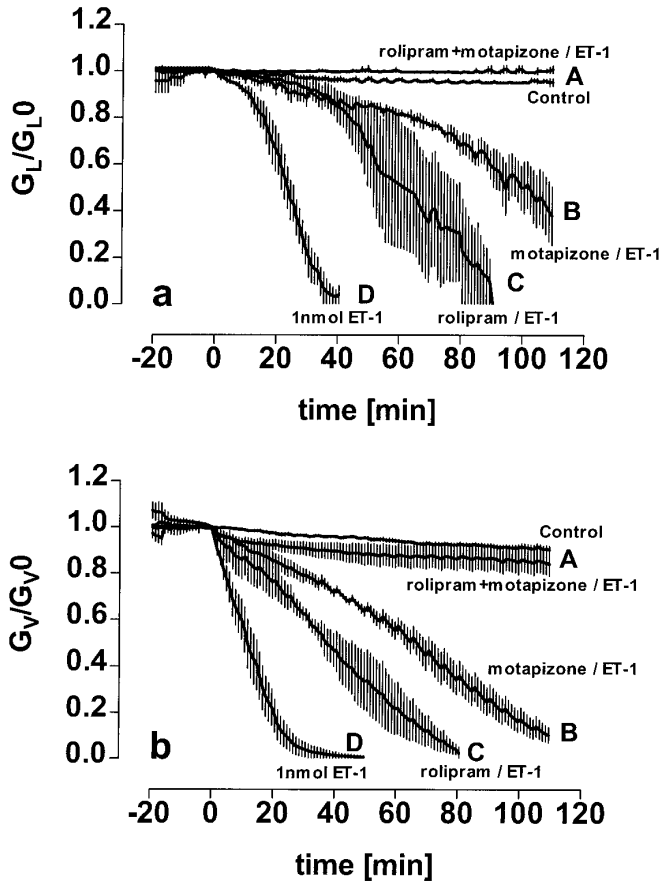

FIG. 1. Time course of ET-1-induced changes in airway (a) and vascular (b) conductance in rat lungs pretreated with either 5μM rolipram (n=3) or 5μM motapizone (n=3) or 5μM of both compounds (n=4) 10 min before bolus-injection of 1 nmol ET-1 (n=4) at time point 0. Control indicates untreated lungs (n=4). G/G_0, conductance normalized to time 0. Data (solid lines) are means ± SEM. Data were analyzed by Newman-Keuls test for multiple comparisons, based on the area under the curve (AUC) of each treatment. The capital letters indicate the statistical groupings, i.e. different capital letters indicate groups that were significantly different from all other groups at $p<0.05$.

Release of thromboxane. Since the ET-1-induced bronchoconstriction is partially mediated by thromboxane, we examined the effects of rolipram and motapizone on the production of thromboxane (Fig. 2a). 30 min after administration of ET-1, perfusate TXB_2 (the stable byproduct of TXA_2) was increased by about 80 pg/ml. Motapizone did not significantly suppress this TX release, whereas rolipram completely eliminated the ET-1-elicited TX release into the perfusate. Pretreatment with both compounds was not different from rolipram alone.

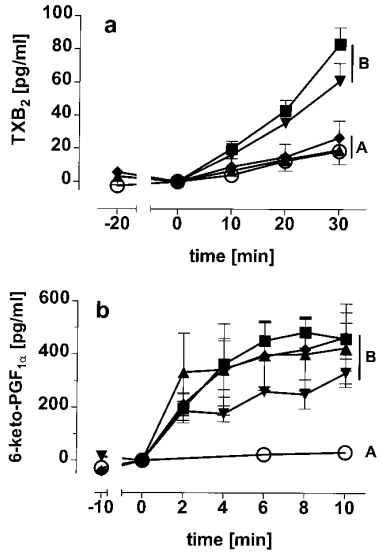

FIG. 2. Time course of ET-1-induced release of thromboxane B_2 (a, n=4) or 6-keto-$PGF_{1\alpha}$ (b, n=3) into the perfusate (■). Lungs were pretreated with 5μM rolipram (▲), 5μM motapizone (▼) or 5μM of both compounds (♦), 10 min before injection of 1 nmol ET-1. Control indicates untreated lungs (○; n=3 for TXB_2, n=7 for 6-keto-$PGF_{1\alpha}$). Data are shown as means ± SEM and represent the difference to the concentration at the time of addition of ET, i.e. time 0. At this time the absolute concentrations of perfusate TXB_2 and 6-keto-$PGF_{1\alpha}$ were 209±102 (±SD) and 24±11 (±SD), respectively. At time point 30 min (TXB_2) respectively 10 min (6-keto-$PGF_{1\alpha}$) data were analyzed by the Newman-Keuls test for multiple comparisons. The capital letters indicate the statistical groupings, i.e. different capital letters indicate groups that were significantly different from all other groups at $p<0.05$.

Release of prostacyclin. 6-keto-$PGF_{1\alpha}$, the stable byproduct of PGI_2, was measured in the perfusate. A rapid release of the prostaglandin was observed after injection of ET-1, resulting in an increase in 6-keto-$PGF_{1\alpha}$ concentrations of about 500 pg/ml after 10 min. However, neither rolipram nor motapizone nor combined pretreatment with both compounds affected 6-keto-$PGF_{1\alpha}$ release (Fig. 2b).

DISCUSSION

In spite of a mutually causative interaction of PDE-inhibitors and endothelins in the treatment of lung diseases such as asthma or ARDS the pulmonary pharmacology of these compounds has not been investigated before. The present study demonstrates the potential of PDE-inhibitors to prevent ET-induced vaso- and bronchoconstriction in rat lungs. Our findings demonstrate also that the release of thromboxane, but not of prostacyclin, following ET-1 challenge is effectively suppressed by PDE-inhibitors.

Motapizone and rolipram are well established inhibitors of PDE III and IV, respectively. Here these drugs were used at a concentration of 5 μM, because this concentration inhibition of PDE III respectively PDE IV is nearly complete [14]. At this concentration, rolipram, but not motapizone eliminated thromboxane release, suggesting that PDE IV is the isoenzyme involved in the regulation of ET-1-elicited formation of thromboxane. This finding is in line with studies addressing tissues other than the lung which demonstrate the predominant role of PDE IV in cells which release pro-inflammatory mediators. For example, inhibition of PDE IV but not PDE III impedes LPS-induced formation of TNFα in human blood monocytes *in vitro* [15]. Notably, rolipram was more potent in attenuating thromboxane production in guinea-pig neutrophils than ibudilast, a selective inhibitor of PDE III [16]. And finally, PDE IV, but not PDE III, is engaged in the control of endotoxin-induced thromboxane release in perfused rat lungs [17]. In contrast to their effect on thromboxane release, the ET-1-induced prostacyclin release was not affected by the PDE-inhibitors. In contrast, PGI-release in the circulation of LPS-treated conscious sheep [18] and from mouse hepatocytes [19] was attenuated in the presence of PDE-inhibitors.

Though rolipram completely inhibited thromboxane release, motapizone showed significantly better protection against the ET-1-induced bronchoconstriction. This can be explained by the observation that thromboxane is responsible for only a small part of the ET-1-induced bronchoconstriction [5]. Full protection against the ET-1-induced pressor responses required simultaneous pretreatment with both inhibitors. This is in keeping with previous studies, where inhibition of PDE III and PDE IV was shown to act in an additive or synergistic manner, with PDE III being the more important enzyme in regulating airway smooth-muscle tone [14, 20, 21].

Vascular tone displayed a similar pattern of PDE III/IV inhibition as the airways: rolipram attenuated ET-1-triggered vasoconstriction only weakly, while motapizone was more effective. However, complete protection again required combined treatment with both PDE-inhibitors. A predominant role of PDE III in pulmonary vasculature has been described for rat and humans [8]. Further studies report that PDE III acts synergistically with PDE V, e.g. in human pulmonary arteries [8] and in *in situ* perfused rabbit lung [22]. Nevertheless, in a rat IPL model inhibition of PDE V or PDE IV turned out to have little effect on relaxing the vasculature [23].

Our major result is that combined inhibition of PDE III and IV completely prevents ET-1-induced broncho- and vasoconstriction as well as the release of thromboxane. As endothelins are thought to act as mediators in lung diseases, the potential therapeutic value of PDE-inhibitors is likely to include antagonism of endothelin action as a hitherto not recognized pharmacological principle.

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