The Influence of Endogenous Catecholamines on the Inhibitory Effects of Rolipram Against Early- and Late-Phase Response to Antigen in the Guinea Pig

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ABSTRACT

Selective inhibitors of the low-$K_m$ cAMP-specific phosphodiesterase (PDE4) inhibit inflammatory cell function and relax airway smooth muscle. Thus PDE4 inhibitors may be useful in the therapy of asthma. The present study was conducted to determine whether the in vivo activity of rolipram, a prototypical PDE4 inhibitor, is due to its ability to potentiate the anti-inflammatory effects of prostaglandins or catecholamines, endogenous activators of adenylyl cyclase, in models of the early-and late-phase response to antigen. Rolipram, administered i.v. to anesthetized, paralyzed and ventilated ovalbumin-sensitized guinea pigs, inhibited i.v. antigen-induced bronchoconstriction with an ID$_{50}$ value of 0.2 mg/kg. Pretreatment with either of the beta adrenocceptor antagonists propranolol and nadolol (0.5 mg/kg i.v.), enhanced the bronchial reactivity to antigen and abolished the inhibitory activity of rolipram (0.1–10 mg/kg i.v.). In addition, the inhibitory activity of three structurally dissimilar PDE4 inhibitors was nearly abolished by propanolol. Cyclooxygenase inhibition by indomethacin slightly enhanced the reactivity to antigen but did not affect the inhibitory activity of rolipram. Plasma catecholamine concentrations were not altered by rolipram (0.3 or 1 mg/kg i.v.), which indicates that there was no stimulation of catecholamine release. Bilateral adrenalectomy reduced plasma epinephrine concentrations (from 1700 pg/ml to 400 pg/ml), significantly enhanced airway reactivity to antigen and substantially reduced the inhibitory activity of rolipram (3 mg/kg i.v.). Pretreatment of conscious guinea pigs with the beta adrenocceptor antagonist nadolol, 2 mg/kg p.o., enhanced aerosol antigen-induced bronchoconstriction and pulmonary eosinophil influx measured by bronchoalveolar lavage. Nadolol reduced the inhibitory effect of rolipram against antigen-induced bronchoconstriction but not eosinophil influx. The inhibitory effect of rolipram was unaffected by indomethacin. The present data suggest that circulating catecholamines play an important protective role against antigen-induced bronchoconstriction in the guinea pig. Moreover, the inhibitory activity of PDE4 inhibitors against antigen-induced bronchoconstriction, but not eosinophil influx, is reduced by beta adrenergic blockade or adrenalectomy. Thus the inhibitory activity of PDE4 inhibitors against antigen-induced bronchoconstriction may be related to their synergism with endogenous catecholamines to suppress mast cell degranulation.

Increases in cellular concentrations of cAMP or cGMP mediate relaxation of airway smooth muscle (Torphy, 1994; Giembycz and Raeburn, 1991). In addition, cAMP acts as a second messenger in inflammatory cells to inhibit chemotaxis, cytotoxicity and degranulation (Bourne et al., 1974; Plaut et al., 1980; Kuehl et al., 1987; Torphy and Undem, 1991). Cyclic AMP is formed from ATP by the catalytic action of adenyl cyclase. The activity of this enzyme is stimulated by endogenous hormones and autacoids, such as epinephrine and prostaglandin E$_2$, that act via cognate cell-surface receptors (Robison et al., 1971). Metabolic degradation and subsequent biologic inactivation of cyclic nucleotides occurs through hydrolytic cleavage of the 3’-phosphodiester bond, a reaction catalyzed by a family of PDE isozymes (Beavo, 1977; Beavo and Reifsnyder, 1990). These isozymes are encoded by distinct genes and differ with respect to their physical and kinetic characteristics, substrate preference, tissue distribution and sensitivity to various classes of isozyme-selective inhibitors (Beavo and Reifsnyder, 1990).

Several laboratories have described PDE isozyme profiles in airway smooth muscle from various species (Silver et al., 1988; Torphy and Cieslinski, 1990; Elliott et al., 1990; De Boer et al., 1992; Torphy et al., 1993). A similar isozyme categorization for inflammatory cells has been summarized (Torphy and Undem, 1991; Giembycz and Dent, 1992). On the basis of the putative role of cAMP in inflammatory cells and airway smooth muscle, coupled with the key role played by PDE4 in regulating cAMP content in these cells, PDE4

ABBREVIATIONS: cGMP, guanosine 3’-5’-cyclic monophosphate; PDE, phosphodiesterase; PDE4, cAMP-specific PDE; BAL, bronchoalveolar lavage; OA, ovalbumin; PEG, polyethylene glycol 400; sGaw, specific airway conductance.
In vitro studies have described the bronchodilatant and inflammatory cell-stabilizing properties of PDE4 inhibitors (Torphy and Undem, 1991; Nielson et al., 1991; Torphy et al., 1992), including the ability of these compounds to suppress activation of eosinophils (Souness et al., 1991; Dent et al., 1991).

Szentivanyi (1968) initially hypothesized that beta adrenoceptor function is implicated in airways disease. Several more recent findings suggest an important role of beta adrenoceptor function and, as a consequence, cAMP in regulating airway reactivity in bronchial asthma. These findings are as follows: 1) airway smooth muscle hypofunction in asthmatics (Barnes et al., 1980; Szentivanyi et al., 1984; Spina et al., 1989); 2) decreased beta adrenoceptor numbers on lymphocytes of asthmatics (Brooks et al., 1979); 3) nocturnal fluctuations in plasma epinephrine concentrations (Barnes et al., 1980; Bates et al., 1994); 4) frank bronchoconstriction (McNeill, 1964; Skinner et al., 1975; Benson et al., 1977) or increased airway sensitivity in asthmatics after the administration of beta adrenoceptor-blocking drugs (Zaid and Beall, 1966; Bouhuys et al., 1971); 5) mutations or genotypic variations of the beta-2 adrenoceptor among normal and asthmatic human airway smooth muscle cells that confer differential desensitization or down-regulation (Reihsaus et al., 1993; Green et al., 1995) and 6) the isolation of autoantibodies to the beta-2 adrenoceptor and the demonstration of their functional significance in asthma (Venter et al., 1980; Fraser et al., 1981; Turki and Liggett, 1995).

Administration of antigen to sensitized animals results in acute bronchoconstriction mediated by extrusion of mast cell contents (Collier and James, 1967). This acute response to antigen is regulated by catecholamines in at least two ways. First, activation of beta adrenoceptors by circulating catecholamines suppresses mast cell degranulation (Barnes, 1986). Second, the responsiveness of airway smooth muscle to a number of mast cell-derived bronchoconstrictors is reduced by the presence of endogenous catecholamines.

A fundamental tenet of cyclic nucleotide action is that activators of adenyl cyclase act synergistically with PDE inhibitors. Thus it has been proposed that the antiasthmatic effects of PDE4 inhibitors are at least partly due to their ability to amplify the actions of substances such as epinephrine and PGE2 (Torphy and Undem, 1991). Against this backdrop, the purpose of the present study was twofold: 1) to describe the increased airway reactivity to antigen produced by cyclooxygenase inhibition, beta adrenoceptor blockade or adrenalectomy in the guinea pig, and 2) to determine whether the in vivo activity of rolipram, a PDE4 inhibitor, is due to its ability to potentiate the anti-inflammatory effects of prostaglandins or catecholamines in models of the early- and late-phase response to antigen.

Materials and Methods

All animal protocols in this manuscript were approved by the Animal Care and Use Committee at SmithKline Beecham Pharmaceuticals, King of Prussia, PA.

Sensitization procedure. Male Hartley guinea pigs (200–250 g; Hazelton Research Animals, Denver, PA) were sensitized by i.m. injections of 0.35 ml of a 5% (w/v) OA/saline solution into each thigh (0.7 ml total) on days 1 and 4. Guinea pigs were ready for use after day 25.

Measurement of bronchoconstriction in anesthetized guinea pigs. Male Hartley guinea pigs (600–800 g), actively sensitized to ovalbumin, were anesthetized with sodium pentobarbital (40 mg/kg i.p.) approximately 10 to 15 min before surgery. The jugular vein, carotid artery and trachea were cannulated (PE tubing 50, 60 and 260, respectively) for drug administration, blood pressure monitoring and ventilation. Bilateral vagotomy was performed to minimize cholinergic influence. Animals were paralyzed (pancuronium bromide, 0.1 mg/kg i.v.) and ventilated (45 breaths/min) with a Harvard Rodent Respirometer (model 683, Harvard Apparatus, South Natick, MA). Airway pressure changes were measured via a side-arm of the tracheal cannula with a Druck PDCR 10/2L, 70 mBar transducer (Druck Incorporated, New Raifield, CT). The ventilatory stroke volume was set to produce a side-arm pressure of 8 cm of H2O (approximately 5 cc room air). Blood pressure was measured with a Statham P23XL Physical Pressure Transducer (Viggo-Spectramed, Oxnard, CA). Pressures were recorded on a Grass Model 7D Polygraph (Grass Instrument Co., Quincy, MA). The animals were kept on a heated surgical table throughout the experiment to maintain body temperature. Bronchoconstriction in the anesthetized guinea pig model was measured as changes in insufflation pressure (Δ cm H2O) and expressed as a mean ± S.E.M. percentage of the maximum bronchoconstriction elicited by KCl administration.

Effect of indomethacin, beta adrenoceptor antagonists or rolipram on OA-induced bronchoconstriction. After surgical preparation for the above airway mechanics protocol, a 10-min stabilization period was observed. Saline vehicle, indomethacin (10 mg/kg i.v.) or racemic (+/-) propranolol (0.5 mg/kg i.v.) was administered. Ten minutes later, animals were challenged with the low dose of OA, 0.1 mg/kg i.v. A second dose of 0.2 mg/kg (0.3 total) was administered at the peak of the airway response to the lower dose of antigen. Previous studies indicate that this second dose of OA resulted in near-maximal antigen-induced bronchoconstriction (Underwood et al., 1993, 1996a). After a peak airway response to the second dose of OA had been reached, a saturated KCl solution, 1 ml/kg i.v., was administered, which produced maximal bronchoconstriction and the death of the animal.

In a separate group of animals, we studied the effect of saline, indomethacin (10 mg/kg i.v.) or propranolol (0.5 mg/kg i.v.) on the inhibitory activity of rolipram. Five minutes after the administration of saline, indomethacin or propranolol, rolipram was administered i.v. at doses of 0.1, 0.3, 1.0 or 3.0 mg/kg (saline group); 0.1, 0.3 or 1.0 (indomethacin group) or 0.1, 0.3, 1.0, 3.0 or 10 mg/kg (propranolol group). Percent inhibition was calculated by comparison of the airway response to the high dose of OA (0.3 mg/kg i.v.) in rolipram-treated animals with the response to the high-dose OA challenge in a control, sensitized animal group corresponding to each treatment regime. Each animal received only one treatment regime and one dose of rolipram.

In a protocol similar to that of the aforementioned studies, saline or one of four different beta adrenoceptor antagonists was administered at a dose of 0.5 mg/kg i.v., 5 min before the administration of rolipram (1.0 mg/kg i.v.). The four beta adrenoceptor antagonist were 1) the less active isomer of propranolol, R(-)-propranolol, 2) the more active isomer of propranolol, S(+) propranolol, 3) racemic (+/-) propranolol and 4) nadolol. The percent inhibition by rolipram was calculated by comparison of the airway response to the high dose of OA (0.3 mg/kg i.v.) in rolipram-treated animals with the response to the high-dose OA challenge in a parallel control, sensitized animal group.

Effect of cimetidine on activity of rolipram. In an additional set of animals, we assessed the effects of the H2 histamine receptor antagonist cimetidine (10 mg/kg i.v., 10 min before OA challenge) on OA-induced bronchoconstriction, as well as on the inhibitory effects of rolipram (0.3 mg/kg i.v.) against OA-induced bronchoconstriction.

Studies with other PDE4 inhibitors. The effects of beta adrenoceptor blockade on the ability of three structurally distinct PDE4 inhibitors to suppress antigen-induced bronchoconstriction was as-
cessed by administering the inhibitors in the presence and absence of propranolol (0.5 mg/kg i.v.) as in the previous experiments with rolipram. In addition to the (R) enantiomer of rolipram, we tested three other PDE4 inhibitors: BRL 61063 (Buckle et al., 1994), WAY-PDA 641 (Heaslip et al., 1994) and SB 201609 (Forster et al., 1994). The dose selected for each PDE4 inhibitor was at least 5-fold greater than the ID$_{50}$ value against OA, which had been previously determined in the antigen-induced bronchoconstriction model in our laboratory. ID$_{50}$ values are as follows: (R)rolipram, 0.2 mg/kg; BRL 61063, 0.03 mg/kg; SB 201609, 0.03 mg/kg; WAY-PDA-641, 0.3 mg/kg.

Effect of rolipram on catecholamine levels. In a separate study, the effect of rolipram, 0.3 or 1.0 mg/kg i.v., on plasma catecholamine levels in the guinea pig was assessed before and during the airway reaction to OA. Accompanying the measurement of airway reactivity to OA, arterial blood samples were taken 1) during the stabilization period, 2) 10 min after the administration of vehicle or rolipram (0.3 or 1.0 mg/kg i.v.) and 3) at the peak of the airway response to the cumulative 0.3 mg/kg OA.

Catecholamine measurement. One milliliter of arterial blood was drawn into a syringe containing 0.1 cc heparin solution (300 U/ml saline). The blood was replaced with 1 ml of isotonic saline. The blood samples were centrifuged at 10,000 rpm (12,000 × g) for 10 min, and plasma was immediately frozen for catecholamine analysis. Fractional catecholamine analyses were performed by alumina extraction and HPLC electrochemical detection by SmithKline Biotechnology, Pittsburgh, PA. Where necessary, catecholamine concentrations were converted to logarithmic scale to achieve normality in distribution for optimal statistical analysis. Cortisol concentrations were measured via radioimmunoassay by SmithKline Beecham Bioscience Clinical Laboratories, Collegeville, PA.

Effect of adrenalectomy on the inhibitory activity of rolipram. In adrenalectomized animals or in sham-operated animals, the inhibitory activity of rolipram, 3 mg/kg i.v. (≥10 × the ID$_{50}$ in this model) was assessed against OA-induced bronchoconstriction. As in the previous rolipram study, arterial blood samples were drawn 1) during base line, 2) after rolipram or vehicle administration and 3) at the peak of the airway response to antigen.

Adrenalectomy procedure. As has been previously described (Underwood et al., 1996a), animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.). The animals were shaved, and a paracostal incision approximately 3 cm long was made through the skin and the abdominal musculature. A small sponge moistened with warm saline was packed into the abdominal cavity to hold the intestine out of the field. Each adrenal gland was isolated with a sterile cotton swab. The adrenal gland was incised using a sterile glass Pasteur pipette with its tip broken off and filed to provide a sharp dissecting instrument. The pipette was attached to a vacuum pump aspirator (GOMCO, Allied Healthcare Products, St. Louis, MO), and each adrenal was suctioned out from the surrounding cortical capsule and replaced with a small piece of Gelfoam. The skin layers were clipped closed to retain heat. The procedure for sham-operated animals lacked only the adrenal incision and suction. Animals were then surgically prepared for pulmonary mechanics measurements as outlined in the previous section. The technique has previously been shown preferentially to remove the medulla, leaving much of the cortex intact (Underwood et al., 1996a).

Conscious animal body plethysmography and BAL. The characteristics of the early- and late-phase response to antigen challenge in conscious guinea pigs was reported previously (Underwood et al., 1993, 1994). Conscious guinea pigs (500–600 g) were placed into a double-flow body plethysmograph (Penn-Century, Philadelphia, PA) consisting of a nasal (head) chamber and a thoracic (body) chamber. Each chamber was equipped with a pneumotachograph consisting of six fine (325)-mesh screens stacked in series. A bias air flow of 250 ml/min was passed into the nasal chamber to provide continuous fresh air, and a bias air flow of 125 ml/min was passed into the thoracic chamber to maintain a comfortable ambient temperature in the plethysmograph. A seal was made between the two chambers by placing a magnetic collar containing a slab of black rubber sandwiched between two pieces of thin latex around the guinea pig’s neck. The magnet-containing collar secured the guinea pig such that little or no air flowed between chambers. Each chamber was connected to a Buxco Noninvasive Respiratory Analyzer (Buxco Electronics, Sharon, CT) via a Validyne differential pressure transducer (± 2 cm) to determine measurement of tidal volume, minute volume, tidal airflow, respiratory rate, resistance and sGaw.

All data were recorded on a Grass model 7D polygraph (Grass Instruments, Quincy, MA) and digitized by a Buxco Data Logger (Buxco Electronics, Sharon, CT). Readings were taken continuously and averaged over 15 sec. After a 10-min stabilization period, animals were exposed to aerosols of 0.5% solutions of OA generated by a DeVilbiss Pulmosonic nebulizer (Breathing Services, Ephrata, PA) and delivered for 5 sec at a rate of 250 ml/min via a nose cone built into the plethysmograph.

In all animals, results were calculated as percent change in sGaw from base-line readings taken just before the initial antigen challenge and reported as peak change (mean ± S.E.M.) every 30 sec for at least 10 min after OA challenge.

Twenty-four hours after OA challenge, guinea pigs were killed by cervical dislocation and exsanguinated by severing the axillary arteries. The lungs were lavaged with 50 ml of Dulbecco’s phosphate-buffered saline (5 aliquots of 10 ml), which was aspirated after gentle chest massage. The BAL fluid was centrifuged at 2200 rpm (1100 × g) for 10 min, the supernatant was aspirated, and the pellet was resuspended in 5 ml 0.25% NaCl to lyse residual erythrocytes. This dispersion was centrifuged at 2200 rpm (1100 × g) for 10 min, the supernatant was aspirated, and the pellet was resuspended with 5 ml 0.9% NaCl. Total cell counts were done by hemocytometry using trypan blue stain. Slides were prepared on a Shandon Cytospin 2 (Pittsburgh, PA) at 300 rpm for 5 min, fixed and stained. Differential counts on at least 200 cells were done using standard morphologic criteria to classify cells as eosinophils, neutrophils or mononuclear cells, and the results were expressed as percentages of total cells counted.

Effect of indomethacin or rolipram on the inhibitory activity of rolipram in the conscious guinea pig bronchoconstriction/eosinophil influx model. The effects of the cyclooxygenase inhibitor indomethacin (10 mg/kg i.p.) or the long-acting, orally efficacious beta adrenoceptor antagonist nadolol (2 mg/kg p.o.), each administered 1 hr before antigen challenge, were assessed on acute bronchoconstriction and the eosinophil influx measured in the BAL fluid 24 hr after antigen challenge. The effects of indomethacin and nadolol on the inhibitory activity of rolipram (10 mg/kg p.o.) were also assessed in this model.

Statistical analysis. Bronchoconstriction results were compared among multiple-treatment groups with analysis of variance (ANOVA) followed by Dunnett’s test or Fisher’s protected least squares (PLSD) test. Catecholamine concentration data were analyzed with ANOVA or, where appropriate, with ANOVA for repeated measures followed by a Student-Newman-Keuls test for variability. Statistical significance was determined at a minimum 95% level of confidence. Statistical analysis for bronchoconstriction and inflammatory cell influx was performed using Statview 512+ software (Brainpower Inc., Agoura Hills, CA) on a Macintosh computer. Statistical analysis of catecholamine concentrations was performed on a SAS main frame statistical analysis system.

Drugs. OA (chicken egg, grade V), indomethacin and S(-)-, R(+)- and racemic (+/-) propranolol were purchased from Sigma Chemical Co. (St. Louis, MO). Nadolol was a generous gift from the Bristol Myers-Squibb Co. (Princeton, NJ). Racemic and (R)-rolipram, BRL 61063, SB 201609, WAY-PDA-641 and cimetidine were synthesized by Dr. Siegfried Christensen and colleagues in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA).
Results

Effect of indomethacin or propranolol on inhibitory activity of rolipram. In anesthetized, OA-sensitized guinea pigs, i.v. administration of OA produced a dose-related increase in pulmonary inflation pressure (fig. 1). In a recent study, we have shown that the administration of 0.1 mg/kg OA produces a threshold airway pressure response, and the subsequent addition of 0.2 mg/kg (0.3 mg/kg total) produces a near-maximal response (Underwood et al., 1996a). In the vehicle-treated animals in the present study, the 0.1-mg/kg dose of OA produced a change in airway pressure of 5.3 ± 1.7 cm H$_2$O ($n = 10$), which represented 6.1 ± 2.3% of the maximal airway response to KCl (fig. 1), and the 0.3-mg/kg dose of OA (total dose) produced an increase of 73.4 ± 3.7 cm H$_2$O, which was 90.6 ± 3.3% of the KCl-induced maximal airway response (fig. 1).

Pretreatment with indomethacin, 10 mg/kg i.v., 10 min before the antigen challenge, had no significant effect on the low-dose OA-induced airway responses when compared with the effect on vehicle-treated animals ($n = 9$, fig. 1). However, pretreatment with propranolol (0.5 mg/kg i.v.) significantly enhanced (3- to 4-fold increase, $n = 9$) the airway response to the low dose of OA ($P < .01$, Dunnett’s test). There were no significant differences in airway responses to the high-dose OA among all treatment groups ($P > .05$, ANOVA) (fig. 1). There were also no differences in the KCl-induced maximal airway responses among the vehicle or treated groups (vehicle = 82.3 ± 6.1 cm H$_2$O, indomethacin = 78.2 ± 5.2 cm H$_2$O and propranolol = 80.4 ± 2.8 cm H$_2$O).

When rolipram was administered 5 min after saline administration and 10 min before antigen challenge, it produced a dose-related inhibition of the airway response to the high-dose OA when compared with a corresponding group of animals that had received only saline (fig. 2). The ID$_{50}$ value of rolipram against OA-induced bronchoconstriction was approximately 0.2 mg/kg i.v., with abolition (≥90% inhibition) of the response at 1 mg/kg. Similarly, when rolipram was administered to animals that had received indomethacin, 10 mg/kg i.v., the ID$_{50}$ value was determined to be 0.2 mg/kg i.v., with at least 90% inhibition at the 1-mg/kg rolipram dose (fig. 2). However, when rolipram was administered to animals that had received 0.5 mg/kg propranolol, it did not inhibit the OA-induced bronchoconstriction, even at a dose of 10 mg/kg, which represented 50 times the ID$_{50}$ value in this model (fig. 2).

Effect of other beta adrenoceptor antagonists on activity of rolipram. In addition to racemic (±) propranolol, S(-)-propranolol, we studied nadolol (0.5 mg/kg i.v.) and the less potent R(-)-enantiomer of propranolol to determine the effect of these beta adrenoceptor antagonists on the inhibitory activity of rolipram (fig. 3). After the administration of saline vehicle, rolipram (1 mg/kg i.v.) abolished the anti-
gen-induced bronchoconstriction (93 ± 3% inhibition). However, the inhibitory effect of rolipram was virtually abolished by prior administration of racemic (+/−) propranolol, S−−propranolol or nadolol. Pretreatment with R−−−propranolol reduced, but did not abolish, the effect of rolipram (fig. 3).

Studies with other PDE4 inhibitors. When (R)-rolipram or any of three other PDE4 inhibitors (BRL 61063, SB 201609 and WAY-PDA-641) was administered at doses 5- to 10-fold greater than its previously determined i.v. ID₅₀ values, the OA-induced bronchoconstriction was inhibited by 83% to 95% (P < .01, Dunnett’s test; n = 4 each) (fig. 4). However, in animals that received propranolol (0.5 mg/kg) 5 min before administration, PDE inhibitors failed to significantly alter the response to antigen (fig. 4).

Effect of rolipram on catecholamine levels. To determine the effect of rolipram on plasma catecholamine levels, arterial blood samples were drawn 1) during the base-line equilibration period, 2) 9 min after i.v. administration of vehicle or rolipram (0.3 or 1.0 mg/kg i.v., 1 min before antigen challenge) and 3) at the peak of the airway response to antigen (fig. 5). Rolipram administered to the same animals at a dose of 0.3 or 1.0 mg/kg inhibited OA-induced bronchoconstriction by 77 ± 9% and 90 ± 4%, respectively (n = 7–12, fig. 5A). Rolipram treatment (0.3 or 1 mg/kg) did not alter plasma epinephrine concentrations from the corresponding baseline (P > .05, ANOVA with repeated measures, n = 8–10). Upon antigen challenge, plasma epinephrine levels rose approximately 5-fold in vehicle-treated animals (fig. 5B), which was significantly different from baseline and treatment levels (P < .05; ANOVA with repeated measures and Student-Newman-Keuls test). In contrast, plasma epinephrine levels in animals pretreated with rolipram, 0.3 and 1 mg/kg i.v., rose only 2.2-fold and 1.7-fold, respectively (fig. 5B). These values were not significantly different from their own base-line or pretreatment measurements but were significantly different from vehicle-treated animals upon antigen challenge (P < .05; ANOVA and Student-Newman-Keuls test).

In these animals we also measured dopamine and norepinephrine concentrations (table 1). Neither dose of rolipram significantly altered plasma concentrations of dopamine or norepinephrine when compared with their own base-line values. Upon antigen challenge, norepinephrine concentrations rose 2- to 3-fold in vehicle-treated animals and in both dosage groups of rolipram-treated animals (P < .05; table 1). Dopamine concentrations were not altered.

Effect of cimetidine on activity of rolipram. In this set of animals, cimetidine (10 mg/kg i.v.) did not affect the bronchoconstrictor response to OA. The maximal increase in airway pressure to OA was 83.4 ± 3.9 cm H₂O in vehicle-treated animals and 87.1 ± 5.1 cm H₂O in cimetidine-treated animals. In addition, pretreatment with cimetidine (10 mg/kg i.v.) did not alter the inhibitory activity of rolipram (0.3 mg/kg i.v.) against OA-induced bronchoconstriction when compared with vehicle/rolipram alone. Percent inhibition of the OA-induced bronchoconstriction was as follows: cimetidine/rolipram, 82.8 ± 0.9%; vehicle/rolipram, 96.2 ± 27.5% (P > .05, Student’s unpaired t test, n = 6; data not shown in graphical form).
TABLE 1
Plasma catecholamines in anesthetized guinea pigs before treatment (Base line), after vehicle or rolipram treatment and during OA-induced bronchoconstriction peak (antigen challenge)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Base Line</th>
<th>Treatment</th>
<th>Antigen Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dopamine</td>
<td>Norepinephrine</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Vehicle</td>
<td>231.4 ± 28.3</td>
<td>293.6 ± 120.5</td>
<td>220.4 ± 76.9</td>
</tr>
<tr>
<td>Rolipram (0.3 mg/kg i.v.)</td>
<td>320.4 ± 36.1</td>
<td>228.0 ± 31.3</td>
<td>302.4 ± 34.4</td>
</tr>
<tr>
<td>Rolipram (1.0 mg/kg i.v.)</td>
<td>264.4 ± 43.8</td>
<td>298.4 ± 44.5</td>
<td>246.4 ± 36.3</td>
</tr>
</tbody>
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Effect of adrenalectomy on inhibitory activity of rolipram. The airway responses to both doses of OA were compared in four groups: 1) sham-operated/vehicle-treated, 2) adrenalectomized/vehicle-treated, 3) sham-operated/rolipram-treated and 4) adrenalectomized/rolipram-treated (fig. 6A). In the sham-operated guinea pigs, the 0.1-mg/kg dose of OA produced a small change in airway pressure of (3 ± 1%) of the maximal airway pressure change elicited by KCl (fig. 6A).

In both vehicle-treated and rolipram-treated animals that had been adrenalectomized, the response to the 0.1-mg/kg dose of OA was markedly enhanced. This response was significantly different compared from that of the corresponding group of sham-operated animals (Fisher’s PLSD; P < 0.05; fig. 6A).

Rolipram (3 mg/kg i.v.) abolished the high-dose OA-induced bronchoconstriction in sham-operated animals (97 ± 1% inhibition, fig. 6A). In contrast, the inhibitory effect of rolipram was markedly attenuated in animals that had been adrenalectomized (28 ± 9% inhibition, fig. 6A). There were no significant differences in the airway responses to the higher dose of OA among sham-operated/vehicle (84% of KCl maximum), adrenalectomized/vehicle (98%) and adrenalectomized/rolipram groups (69%) (P > .05; ANOVA). The KCl-induced maximal responses were not significantly different among the four groups (data not shown).

In the same animals, plasma catecholamine concentrations were measured in blood drawn during the initial 5-min stabilization (base line), 5 min after rolipram or vehicle treatment (treatment) and during the peak airway response to the high-dose (0.3 mg/kg, total) OA (antigen challenge). Base-line epinephrine concentrations were significantly lower in adrenalectomized/vehicle (337 ± 65 pg/ml) and adrenalectomized/rolipram animals (440 ± 107 pg/ml) vs. sham/vehicle (1482 ± 188 pg/ml) and sham/rolipram (1879 ± 282 pg/ml) (fig. 6B). Treatment with vehicle or rolipram (3.0 mg/kg i.v.) did not significantly alter the plasma epinephrine concentrations of any of the four groups when compared with their corresponding base-line values (fig. 6B, ANOVA with repeated measures). Plasma epinephrine concentrations in blood taken at the peak of the antigen response were also significantly greater in sham-operated/vehicle (7459 ± 762 pg/ml) and sham/rolipram animals (3876 ± 961 pg/ml) when compared with their corresponding adrenalectomized group (adrenalectomy/vehicle = 648 ± 157 pg/ml and adrenalectomy/rolipram = 943 ± 97 pg/ml; fig. 6B).

In addition to epinephrine levels, we measured norepinephrine and dopamine concentrations (table 2). Dopamine concentrations did not markedly change in any treatment group or even in response to antigen challenge. Although rolipram did not raise norepinephrine concentrations in sham-operated animals, there was a significant rise after rolipram treatment in adrenalectomized animals. As in the previous experiment, norepinephrine levels rose upon OA challenge in all groups (table 2). However, much larger increases occurred in adrenalectomized animals (table 2).

Cortisol concentrations also were measured in the plasma...
5 min after pretreatment with vehicle or rolipram. The cortisol concentrations (µg/dl) were as follows: sham/vehicle 34.8 ± 6.5; sham/rolipram, 38.8 ± 2.3; adrenalectomy, 26.5 ± 7.5; adrenalectomy/rolipram, 31.8 ± 6.5. The concentrations were not significantly different among the treatment groups (P > .05, ANOVA, n = 4–5).

**Effect of indomethacin or nadolol on inhibitory activity of rolipram in the conscious guinea pig bronchoconstriction/eosinophil influx model.** OA, administered by nebulized aerosol (0.5% for 5 sec) to conscious guinea pigs, produced a rapid (1–2 min) decrease in sGaw that persisted for up to 10 min after antigen challenge (fig. 7A). The mean maximal decrease in sGaw in vehicle-treated animals was 41 ± 4% from prechallenge baseline (fig. 7A). Pretreatment with the cyclooxygenase inhibitor indomethacin, 10 mg/kg i.p., slightly enhanced the airway response to antigen (maximal decrease in sGaw was 51 ± 7%; fig. 7A). Indomethacin significantly enhanced OA-induced eosinophil influx (n = 8; P < .01; Fisher PLSD; fig. 7B). Pretreatment with rolipram (10 mg/kg p.o.) significantly inhibited both the antigen-induced bronchoconstriction (maximal decrease in sGaw was 22 ± 3%) and the subsequent eosinophil influx (2.7 ± 1.8% of total recovered cells; n = 5; P < .01; Fisher’s PLSD; fig. 7A and B). Pretreatment with indomethacin (10 mg/kg i.p.) did not alter the inhibitory activity of rolipram on either the bronchoconstriction or the eosinophil influx (Fisher’s PLSD test; fig. 7, A and B).

Pretreatment with the orally active beta adrenoceptor antagonist nadolol (2 mg/kg p.o.) so enhanced the OA-induced acute bronchoconstriction that the first four guinea pigs that received this treatment died within 4 min after challenge. Consequently, no other nadolol alone-treated animals were challenged with OA. Nadolol (2 mg/kg p.o.) abolished the inhibitory effect of rolipram on the OA-induced bronchoconstriction (maximal decrease in sGaw was 60 ± 6%; n = 6, P < .01, Fisher’s PLSD). In contrast, nadolol had no effect on the ability of rolipram to inhibit eosinophil influx (fig. 7B). Baseline sGaw in any of the treatment groups was not significantly different from that in the vehicle-treated group, which suggests that there was no inherent bronchomotor activity of the compounds tested and no obvious measurable cyclooxygenase product- or beta adrenoceptor-mediated airway tone. The base-line sGaw values [sec⁻¹ (cm H₂O)⁻¹] for the treatment groups were as follows: vehicle, 0.12 ± 0.02; indomethacin, 0.12 ± 0.03; rolipram, 0.10 ± 0.03; indomethacin and rolipram, 0.12 ± 0.03; nadolol, 0.11 ± 0.03; nadolol and rolipram, 0.14 ± 0.02.

The deaths of the animals in the nadolol-alone group precluded any comparison of bronchoconstrictor or chemotactic activity. However, to show that nadolol indeed enhances

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**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Base Line</th>
<th>Treatment</th>
<th>Antigen Challenge</th>
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<td></td>
<td>Dopamine</td>
<td>Norepinephrine</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Sham/vehicle</td>
<td>244.4 ± 34.9</td>
<td>495.2 ± 87.4</td>
<td>175.8 ± 35.1</td>
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<td>Adrenalectomy/vehicle</td>
<td>290.7 ± 58.3</td>
<td>893.2 ± 87.9</td>
<td>316.0 ± 86.7</td>
</tr>
<tr>
<td>Sham/rolipram</td>
<td>248.0 ± 39.6</td>
<td>580.4 ± 198.0</td>
<td>325.0 ± 91.4</td>
</tr>
<tr>
<td>Adrenalectomy/rolipram</td>
<td>290.0 ± 30.3</td>
<td>816.0 ± 96.0</td>
<td>447.2 ± 76.1</td>
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</table>

**Fig. 7. Effects of indomethacin or nadolol on the inhibitory effects of rolipram against OA-induced bronchoconstriction (A) and eosinophil influx (B) in the conscious guinea pig.** Indomethacin (10 mg/kg i.p.) or nadolol (2 mg/kg p.o.) was administered immediately before vehicle or rolipram (10 mg/kg, p.o.). Bronchoconstriction (panel A) and eosinophil influx measured by bronchoalveolar lavage (panel B) were elicited by inhaled aerosol OA (0.5% for 5 sec) in conscious guinea pigs. n = 6–8. *Significantly different from vehicle; †significantly different from indomethacin alone; P < .05 Fisher’s PLSD.
antigen-induced bronchoconstriction and eosinophil influx, we pretreated nadolol-treated and vehicle-treated animals with the H1 histamine antagonist chlorpheniramine (0.1 mg/kg s.c.), 15 min before antigen challenge. Antigen challenge produced a 2-fold greater drop in sGaw (−76%) and a 2-fold increase in eosinophilia (42% of total cells) in nadolol/chlorpheniramine-treated animals compared with vehicle/chlorpheniramine-treated animals (−34% sGaw, 18% of total cells). Again, nadolol (2 mg/kg p.o.) blocked the inhibitory activity of rolipram (10 mg/kg p.o.) on the OA-induced bronchoconstriction but not on the subsequent eosinophil influx (data not shown).

Discussion

The purpose of this study was to evaluate the role of endogenous circulating catecholamines and prostanoids, activators of adenylyl cyclase, in the antiasthmatic actions of PDE4 inhibitors. The major new findings of the present study can be summarized as follows: 1) Beta adrenoceptor antagonists enhance airway reactivity to antigen in the guinea pig. 2) The potent inhibitory effects of PDE4 inhibitors against antigen-induced bronchoconstriction are abolished by prior administration of a beta adrenoceptor antagonist. 3) Rolipram, at doses that abolish the airway response to antigen, do not alter circulating levels of epinephrine. 4) The inhibitory activity of rolipram against antigen is abrogated in adrenalectomized animals. 5) In contrast to the effect of beta adrenoceptor antagonist pretreatment on the inhibitory activity of rolipram against antigen-induced bronchoconstriction, the inhibitory effects of rolipram on the antigen-induced eosinophil influx were not diminished in conscious guinea pigs.

Several laboratories, including our own, have demonstrated an enhanced airway responsiveness to exogenous spasmogens or antigen after treatment with beta adrenoceptor antagonists (Ney, 1983; Hulbert et al., 1985; Underwood et al., 1996a). It has been suggested that this increase in responsiveness is due to a decrease in the adrenergic tone generated by circulating catecholamines acting at beta-2 adrenoceptors (Diamond, 1972; Bongrani et al., 1983). Other studies have shown an increase in airway reactivity to selected spasmogens (Bongrani et al., 1983) or antigen (Underwood et al., 1996a) in adrenalectomized animals. This enhanced reactivity corresponds to a reduced level of circulating catecholamines (Underwood et al., 1996a). These findings are confirmed in the present study.

We have previously shown that the PDE4 inhibitor rolipram dose-dependently inhibits antigen-induced airway bronchoconstriction and subsequent inflammatory cell influx in the guinea pig (Underwood et al., 1993). The present study demonstrates that the inhibitory activity of a number of structurally diverse PDE4 inhibitors against antigen-induced bronchoconstriction in the guinea pig depends in large part on the activation of beta adrenoceptors by circulating catecholamines. Two lines of evidence support this conclusion. First, treating animals with any one of a variety of beta adrenoceptor antagonists abolished the protective activity of PDE4 inhibitors. This effect is not compound-specific; it was observed regardless of which beta adrenoceptor antagonist was used. Second, adrenalectomy results in an increased responsiveness to antigen and a diminution of the inhibitory activity of rolipram. Both of these activities corresponded to significantly lower circulating concentrations of epinephrine in the guinea pigs.

We also show in this study that the beta adrenoceptor antagonist-induced diminution of the effectiveness of rolipram is not limited to propranolol, which has been shown to have ancillary activities such as a membrane-stabilizing/local anesthetic effect (Levy, 1967), in that nadolol, S-(-)-propranolol and, to a much lesser extent, the less active isomer of propranolol, R-(+)-propranolol, all reduce the effectiveness of rolipram. We have previously shown that compared with other beta adrenoceptor antagonists, R-(+)-propranolol is much less active at enhancing antigen-induced bronchoconstrictor activity (Underwood et al., 1996a), which probably corresponds to its poor potency at the beta-2 adrenoceptor (Howe and Shanks, 1966; Kaumann and Blinks, 1967). Bongrani and colleagues (1983) have shown the requirement for a higher dose of R-(+)-propranolol (compared with S-(-)-propranolol or racemic propranolol) to exacerbate leukotriene C4-induced bronchospasm in the guinea pig. Attenuation of the anti-inflammatory response of rolipram by nadolol has also been demonstrated in an arachidonic acid-induced ear edema model (Grisswold et al., 1993). Moreover, the ability of beta adrenoceptor antagonists to abolish the efficacy of rolipram is not compound-specific, because the activities of three other structurally distinct PDE4 inhibitors [BRL 61063, a xanthine (Buckle et al., 1994), SB 201609, an oxamide (Forster et al., 1994), and WAY-PDA-641, an oxime carbamate (Heaslip et al., 1994)], are also diminished by pretreatment with propranolol.

Having demonstrated the ability of PDE4 inhibitors to suppress antigen-induced bronchoconstriction, we next wanted to address the issue of whether these compounds release catecholamines or potentiate the actions of catecholamines at effector cells. The data clearly support the latter scenario. Rolipram had no effect on base-line plasma epinephrine levels. In fact, epinephrine levels in antigen-challenged animals were actually less in those that received rolipram than in those that were treated with vehicle. This result probably stemmed from the ability of rolipram to protect animals from the anaphylactic insult.

Bilateral adrenalectomy mimicked the effect of beta adrenoceptor blockade. The increase in reactivity to antigen and the abrogation of the inhibitory activity of rolipram coincided with a precipitous drop in the levels of circulating epinephrine. The highest dose of rolipram given to adrenalectomized animals, 3 mg/kg, significantly increased norepinephrine concentrations. As we found in a previous study, norepinephrine concentrations were considerably higher in adrenalectomized than in sham-operated animals, especially during antigen challenge (Underwood et al., 1996a). It is tempting to speculate that epinephrine plays a regulatory role in neural norepinephrine release or, alternatively, that a greater antigen-induced mast cell activation after adrenalectomy results in a higher level of neuronal stimulation. Weinreich and Undem (1987) demonstrated an increased synaptic activity as a result of antigenic stimulation of sympathetic ganglia in the guinea pig, which suggests that immunologic activation of mast cells can directly potentiate neurotransmission of sympathetic ganglia.

In contrast to the role of catecholamines, this study demonstrates that the inhibitory activity of rolipram in the guinea pig is not dependent on the activity of cyclooxygenase.
products (i.e., PGE₂) that could serve as activators of adenylyl cyclase to drive this system of second messenger amplification. However, there appeared to be a slight enhancement of the bronchoconstrictor and eosinophil chemotactic response to antigen in the presence of indomethacin, which suggests that upon antigen stimulation, cyclooxygenase products produce a modest bronchodilatory and/or inflammatory cell quiescent “tone”. Alternatively, cyclooxygenase inhibition could shunt arachidonic acid toward the lipoxygenase pathway in the guinea pig to form leukotrienes, agents with potent bronchoconstrictor and chemotactic activity. It has been demonstrated that H₂ antihistamines inhibit histamine-induced increases in cAMP concentrations in inflammatory cells and thus block histamine-induced inhibition of further release of histamine (Lichtenstein and Gillespie, 1973 and 1975). However, the present finding that the H₂ antihistamine cimetidine has no effect on either the OA-induced bronchospasm or the inhibitory activity of rolipram against this bronchospasm suggests that H₂ receptor activation plays a relatively minor role in the amplification of cAMP concentrations and the diminution of antigen-induced bronchospasm by a PDE4 inhibitor in the present in vivo setting.

The ability of n-adrenaline to abrogate the inhibitory effect of rolipram on OA-induced bronchoconstriction in conscious animals mirrors the results in the anesthetized guinea pigs. The results with indomethacin were similar in both models. However, the lack of effect of either indomethacin or n-adrenaline on the inhibitory activity of rolipram in the eosinophil influx suggests that the activity of PDE4 inhibitors on this inflammatory process is not dependent on endogenous cathecolamines. Indeed, PDE4 inhibitors appear to alter eosinophil trafficking by a mechanism that does not involve the suppression of mast cell degranulation. We have previously shown that rolipram is more potent and effective at blocking the formation and release of a newly formed lipid mediator (prostaglandin D₂) than of preformed mediators such as histamine, a dominant acute bronchoconstrictor released upon antigen challenge and presumably after mast cell degranulation had already occurred. It is not known whether the effect of rolipram on cytokines that may be involved in chemotaxis of eosinophils parallels the formation of PGD₂. We have previously shown that LTD₄, another lipid mediator resulting from mast cell activation, is an important chemotactic agent for eosinophils into the guinea pig airway and that the action of LTD₄ may be dependent on another mediator, the cytokine interleukin-5 (Underwood et al., 1996b). One could speculate that the cAMP requirement for inhibition of newly formed mediators may be less than the concentration necessary to diminish preformed histamine release. This speculation is consistent with the finding that elevations in isolated mast cell cAMP are more effective at inhibiting lipid mediator release (LTC₄ and PGD₂) than histamine release (Undem et al., 1988, 1990). However, the in vivo action of rolipram on other cells involved in the allergic inflammatory response, such as T-cells, should not be neglected.

In addition, the potential synergistic activity of rolipram and endogenous catecholamines on airway smooth muscle should not be ignored. Although it has been shown that rolipram itself is a poor bronchodilator (Underwood et al., 1993; Howell et al., 1993) and, in the present study, that base-line sGaw did not differ between vehicle- and rolipram-treated animals, it has also been shown that upon antigen challenge in the guinea pig, large amounts of catecholamines are released from the adrenal medulla to counteract the resulting bronchoconstriction (Underwood et al., 1996a). The ability of rolipram to amplify the cAMP activity of catecholamines released by antigen challenge may serve to potentiate bronchorelaxant activity.

In conclusion, PDE4 inhibitors act in concert with endogenous catecholamines to suppress mast cell degranulation in conscious and anesthetized guinea pigs. In contrast, the ability of these compounds to inhibit pulmonary eosinophil influx is not dependent on beta adrenoceptor activation. Additional studies are required to determine which, if any, additional activities (i.e., cytokine formation, pulmonary or airway vascular reactivity) of PDE4 inhibitors are influenced by endogenous activators of adenylyl cyclase.

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References

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