British Journal of Pharmacology (2000) 130, 457–463

www.nature.com/bjp

Inhibition of pulmonary eosinophilia and airway hyperresponsiveness in allergic mice by rolipram: involvement of endogenously released corticosterone and catecholamines

*,[1]T.T. Kung, [1]Y. Crawley, [1]B. Luo, [1]S. Young, [1]W. Kreutner & [1]R.W. Chapman

[1]Department of Allergy, Schering-Plough Research Institute Kenilworth, New Jersey, NJ 07033, U.S.A.

1 This study investigates the role of adrenal-derived catecholamines and corticosterone on the inhibition by rolipram, a phosphodiesterase (PDE)-4 inhibitor, of pulmonary eosinophilia and airway hyperresponsiveness (AHR) in allergic mice.

2 The following experimental groups were studied in mice sensitized and challenged with ovalbumin (OVA): normal, adrenalectomized, propranolol (β-adrenoceptor antagonist) and metyrapone (corticosterone synthesis inhibitor) treated. These interventions were studied both in the absence and in the presence of rolipram. Eosinophil numbers in the bronchoalveolar lavage (BAL) and AHR to methacholine were measured 24 h after OVA challenge.

3 Treatment of sensitized mice with rolipram (0.3–10 mg kg^{-1}, p.o.), inhibited pulmonary eosinophilia and the AHR to methacholine in OVA-challenged mice.

4 Adrenalectomy increased the number of eosinophils in the BAL of OVA-challenged mice but had no effect on AHR to methacholine. Adrenalectomy attenuated both the rolipram-induced inhibition of BAL eosinophilia and AHR to methacholine in OVA challenged mice. Propranolol (10 mg kg^{-1}, p.o.) had no effect on the inhibition of eosinophilia by rolipram but attenuated the inhibition of AHR to methacholine in OVA challenged mice. On the other hand, metyrapone (10 mg kg^{-1}, p.o.) attenuated the inhibition of eosinophilia by rolipram but had no effect on the inhibition of AHR to methacholine in OVA challenged mice. Metyrapone-treatment alone increased the number of eosinophils in the BAL of OVA-challenged mice.

5 These results identify an important role for adrenal-derived catecholamines and corticosterone on the inhibition of pulmonary eosinophilia and AHR by rolipram in allergic mice.
British Journal of Pharmacology (2000) **130,** 457–463

Keywords: Adrenals; airway; catecholamines; eosinophils; hyperresponsiveness; metyrapone; phosphodiesterase-4 inhibitor; rolipram

Abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; HPA, hypothalamo-pituitary-adrenal axis; i.p., intraperitoneal; i.v., intravenous; MC, methylcellulose; OVA, ovalbumin; PDE, phosphodiesterase; PD_{100}, provocative dose of methacholine increasing respiratory resistance by 100% above baseline; Rrs, respiratory resistance

Introduction

Phosphodiesterase (PDE) isoenzymes are found ubiquitously throughout the body and regulate various biological responses, some of which are potentially important in the pathogenesis of pulmonary diseases. For example, PDE-4 inhibitors increase cyclic AMP in many types of cells, relax airway smooth muscle tone (Palfreyman & Souness, 1996), inhibit the release of neuropeptides from pulmonary 'C' fibres (Undem *et al*., 1994) and decrease airway microvascular leakage in response to provocation with inflammatory mediators (Raeburn & Karlsson, 1993; Howell *et al*., 1995). PDE-4 inhibitors also suppress eosinophil and neutrophil migration into pulmonary tissue (Underwood *et al*., 1994; 1998; Turner *et al*., 1994; Gozzard *et al*., 1996) and potently inhibit the release of cytokines and mediators from inflammatory cells (Dent *et al*., 1994; Kambayashi *et al*., 1995; Crocker *et al*., 1996; Pettipher *et al*., 1997; Torphy, 1998). Therefore, PDE-4 inhibitors may have therapeutic utility for treatment of inflammatory lung diseases (Torphy, 1998).

It is well established that PDE-4 inhibitors attenuate both the influx of eosinophils into the lungs and the airway hyperresponsiveness (AHR) in response to bronchoprovocation with allergic or inflammatory stimuli in animals (Turner *et al*., 1994; Underwood *et al*., 1994; Gozzard *et al*., 1996; Holbrook *et al*., 1996; Danahay & Broadley, 1997). A number of studies have identified an interaction of PDE inhibitors with the hypothalamo-pituitary-adrenal (HPA) axis where augmented release of catecholamines and corticosteroids from the adrenal glands has been described (Hadley *et al*., 1996; Cheng *et al*., 1997; Kumari *et al*., 1997; Pettipher *et al*., 1996; 1997; Underwood *et al*., 1997; Sato *et al*., 1998). Underwood *et al*. (1997) found that the inhibition of antigen-induced bronchoconstriction in guinea-pigs by PDE-4 inhibitors was abolished by prior administration of β-adrenoceptor antagonists or adrenalectomy. Furthermore, Griswold *et al*. (1993) reported that β-adrenoceptor antagonists abolish the anti-inflammatory effects of a PDE4 inhibitor in mice. On the other hand, Hughes *et al*. (1996) found that the antiinflammatory activity of a PDE-4 inhibitor against IL-5-induced pleural eosinophilia was unaffected by adrenalectomy or by pre-treatment with β-adrenoceptor antagonist in rats.

Previous studies in mice sensitized and challenged with aerosolized OVA demonstrate pulmonary inflammation characterized by an influx of eosinophils into the lungs and AHR (Kung *et al*., 1994; Hessel *et al*., 1995). The effect of PDE-4

*Author for correspondence.

inhibitors has not been assessed in this model, and it was the aim of this study to determine the effects of rolipram, a selective PDE-4 inhibitor (Torphy, 1998) on the pulmonary eosinophilia and AHR in allergic mice. Experiments were also performed to assess the role of adrenals on the actions of rolipram by studying the effect of adrenalectomy, treatment with metyrapone, a corticosterone synthesis inhibitor (De Bie *et al*., 1996), and treatment with propranolol, a β-adrenoceptor antagonist (Underwood *et al*., 1997).

Methods

Studies were performed on normal (Jackson Labs., Bar Harbor, ME, U.S.A.) and adrenalectomized (Charles River, MA, U.S.A.) male, B6D2F1 mice (25–30 g). The mice were fasted overnight before study, but given water *ad libitum*. The adrenalectomized mice were given free access to a supply of isotonic saline and were sensitized to ovalbumin 2 weeks after the adrenalectomy procedure.

Sensitization and challenge procedure

The mice were sensitized by intraperitoneal (i.p.) injection of 15 μg of OVA adsorbed onto 2 mg of alum gel (Kung *et al*., 1994). Non-sensitized mice received i.p. injection of the alum gel. On day 5, the mice were given a booster injection of the same alum or OVA/alum mixture intraperitoneally. Twelve days after sensitization, the mice were challenged with aerosolized OVA (0.5%) that was generated by an ultrasonic nebulizer (DeVilbiss, Somerset, PA, U.S.A., model Ultra-Neb 99). The aerosol was circulated through a large plexiglass chamber and mice were placed into the chamber for 1 h in the morning and again in the afternoon (6 h apart) of a single day. Twenty-four hours after OVA aerosol challenge, the mice were prepared for the collection of BAL fluid or for the assessment of AHR to methacholine.

Collection and assessment of BAL fluid

The mice were sacrificed by CO_2 inhalation and the trachea was catheterized. Two separate 0.3 ml aliquots of isotonic saline were injected into the tracheal cannula and collected as the BAL fluid sample. Total cell counts were done by haemocytometry. Slides were prepared on a Shandon cytospin (Pittsburgh, PA, U.S.A.; model Cytospin 2) at 250 r.p.m. for 10 min. The slides were fixed with methanol and stained with Leukostat (Fisher Scientific, Pittsburgh, PA, U.S.A.). Differential counts on at least 200 cells were done to identify the number of eosinophils in each sample (Kung *et al*., 1994). Mice were prepared for either the collection of BAL fluid or for the measurement of AHR to methacholine (see below).

Measurement of respiratory mechanics and airway hyperresponsiveness to methacholine

The mice were anaesthetized with i.p. injection of ketamine (100 mg kg^{-1}) and xylazine (10 mg kg^{-1}) and prepared with tracheal and jugular venous catheters. The animals were artificially ventilated with a computer assisted ventilator (FlexiVent SAV: Scientific Respiratory Equipment, Montreal, Canada) using a tidal volume of 0.5 ml and a rate of 130 breaths per minute.

Respiratory resistance (Rrs) was measured with the same system by the forced oscillation technique (Schuessler & Bates, 1995). Briefly, the ventilator was used to deliver two cycles of a 2 Hz sinusoidal volume waveform to the airway. Airway pressure and delivered volume were measured continuously and airflow was calculated from the derivative of volume. A two element series resistance-elastance model for the airway was fitted to the flow and pressure data to yield values of Rrs.

Rrs was measured during intravenous (i.v.) injection of increasing doses (0.1–1 mg kg^{-1}) of methacholine and the peak value of Rrs produced by each injection was recorded. Each methacholine dose was given in a volume of 70 μl, approximately 4 min apart. Response to the methacholine was calculated as per cent increase above baseline Rrs. A four-parameter logistic equation was fitted to the dose-response curve (GraphPad Prism, San Diego, CA, U.S.A.) and from this the provocative dose of methacholine that increased Rrs by 100% above baseline (PD_{100}) was calculated.

Effect of adrenalectomy on the activity of rolipram

Rolipram was evaluated for its effects on the pulmonary eosinophilia and AHR to methacholine in both normal and adrenalectomized OVA challenged mice. Rolipram was given orally to mice on two separate occasions 2 h before each challenge of aerosolized OVA. Control animals received methylcellulose vehicle (MC) 2 h before both OVA challenges.

Effect of propranolol on the activity of rolipram

To study the role of endogenous catecholamines acting *via* β-adrenoceptors on the activity of rolipram, mice were treated with propranolol, a β-adrenoceptor antagonist. Propranolol has a high first-pass metabolism that may potentially limit its activity with time. To minimize this factor, a relatively high dose of propranolol (10 mg kg^{-1}) was given one half hour before each dose of rolipram. Rolipram was given 2 h before each OVA challenge. This dose of propranolol has been previously used to block β-adrenoceptor stimulation in mice (Elenkov *et al*., 1995).

Effect of metyrapone on the activity of rolipram

To study the role of endogenous corticosteroids on the activity of rolipram, mice were treated orally with metyrapone (10 mg kg^{-1}), a compound that reduces plasma corticosterone levels in mice (DeBie *et al*., 1996), which was given on three separate occasions. The first metyrapone dose was given 20 h before the first OVA challenge. The two subsequent metyrapone doses were given 1 h before each dose of rolipram that in turn was given 2 h before each OVA challenge. Control groups received oral MC at the times mentioned above.

Statistical analysis

Data are presented as the mean $\pm$ s.e.mean. Statistically significant effects between the different treatment groups were determined by Analysis of Variance and Fisher's least protected difference (StatView, Abacus Concepts In., Berkeley, CA, U.S.A.). A P-value less than 0.05 was accepted as statistically significant.

Drugs

The following drugs were used in this study: rolipram, methacholine chloride, ($\pm$) propranolol hydrochloride and ovalbumin (Sigma Chemical Co., St. Louis, MO, U.S.A.), metyrapone (Aldrich Chemicals, Milwaukee, WI, U.S.A.) and aluminum hydroxide gel (alum) (Reheis, Berkley Heights, NJ, U.S.A.).

Animal care and use

This study was conducted with prior approval from the Animal Care and Use Committee of Schering-Plough Research Institute that is a facility accredited by the American Association for the accreditation of Laboratory Animal Care.

Results

Effects of rolipram in intact mice

OVA challenge to sensitized mice increased the number of total cells and eosinophils in the BAL fluid 24 h after the challenge. Rolipram (0.3–3 mg kg^{-1}, p.o.) dose-dependently inhibited the number of total cells and eosinophils in the BAL fluid of sensitized, challenged mice (Figure 1). No greater inhibition was produced by 10 mg kg^{-1} of rolipram.

Intact, non-sensitized mice challenged with aerosolized OVA had a baseline value for Rrs of 1.17 ± 0.08 cmH_2O ml^{-1} s^{-1} ($n=10$). Bronchoprovocation with i.v. methacholine at 0.1, 0.3 and 1.0 mg kg^{-1} increased Rrs values of 1.74 ± 0.07, 2.39 ± 0.28 and 3.12 ± 0.36 cmH_2O ml^{-1} s^{-1} (or 49, 104 and 167% increase above baseline), respectively (Figure 2). OVA challenge to sensitized mice produced AHR that was manifest as a significant increase in methacholine-induced brochoconstriction compared to mice that were sensitized but challenged with aerosolized saline or to mice that were non-sensitized and challenged with aerosolized OVA (Figure 2, Table 1). In these comparisons, the PD_{100} values for methacholine-induced bronchial responses were significantly lower in mice that were sensitized and challenged with OVA (Table 1) and numerically this amounted to a 3 fold increase in AHR. AHR to methacholine in OVA-challenged, sensitized mice was significantly reduced by rolipram at oral doses of 2 and 10 mg kg^{-1} (Table 1).

Effect of adrenalectomy on the activity of rolipram

OVA challenge increased the number of BAL eosinophils in both sham-operated and adrenalectomized sensitized mice although significantly more eosinophils were found in the BAL fluid of adrenalectomized animals after OVA challenge (Figure 3). Rolipram (10 mg kg^{-1}, p.o.) reduced the number of BAL eosinophils in both sham-operated and adrenalectomized, sensitized mice although the inhibition by rolipram in adrenalectomized mice (30% inhibition, not statistically significant) was less than that in sham-operated controls (73% inhibition, $P<0.05$) (Figure 3).

AHR to methacholine was not different between OVA-challenged adrenalectomized and sham-operated, sensitized mice (Table 2). However, in adrenalectomized mice, rolipram (10 mg kg^{-1}, p.o.) did not inhibit AHR to OVA-challenge (Table 2).

Effect of propranolol on the activity of rolipram

Propranolol (10 mg kg^{-1}, p.o.) had no effect on the number of BAL eosinophils in sensitized, OVA-challenged mice. Furthermore, propranolol had no effect on the inhibition by rolipram (10 mg kg^{-1}, p.o.) of the BAL eosinophilia produced by OVA challenge in sensitized mice (Figure 4).

Propranolol (10 mg kg^{-1}, p.o.) had no significant effect on AHR to methacholine in both non-sensitized (data not shown) and sensitized OVA-challenged mice, but significantly

Table 1 Effect of rolipram on airway hyperresponsiveness in allergic mice

Experimental group	*Challenge*	*Treatment* (mg kg^{-1}, p.o.) *BID*	*PD_{100} of methacholine*†* (mg kg^{-1})
Sensitized	Saline	MC	0.44 ± 0.08‡
Non-sensitized	OVA	MC	0.38 ± 0.07‡
Sensitized	OVA	MC	0.13 ± 0.02
Sensitized	OVA	Rolipram 0.2	0.19 ± 0.02
Sensitized	OVA	Rolipram 2.0	0.46 ± 0.11‡
Sensitized	OVA	Rolipram 10.0	0.47 ± 0.13‡

*PD_{100} : Provocative dose of methacholine increasing Rrs by 100% above baseline. †Values are mean ± s.e.mean ($n=5-8$ per group). ‡$P<0.05$ compared to sensitized, challenged with OVA and treated with MC vehicle.

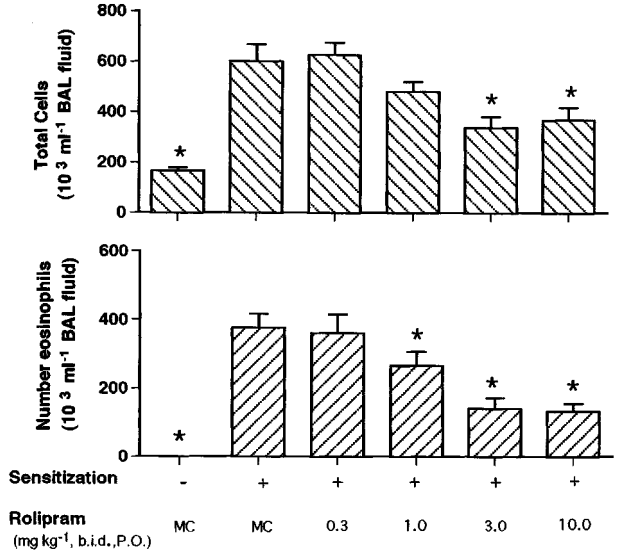

Figure 1 Effect of rolipram on the number of total cells and eosinophils in the BAL fluid of antigen challenged allergic mice. All groups were challenged with OVA. Values represent the mean ± s.e.mean ($n=6$ per group). *$P<0.05$ compared to sensitized, MC-treated group.

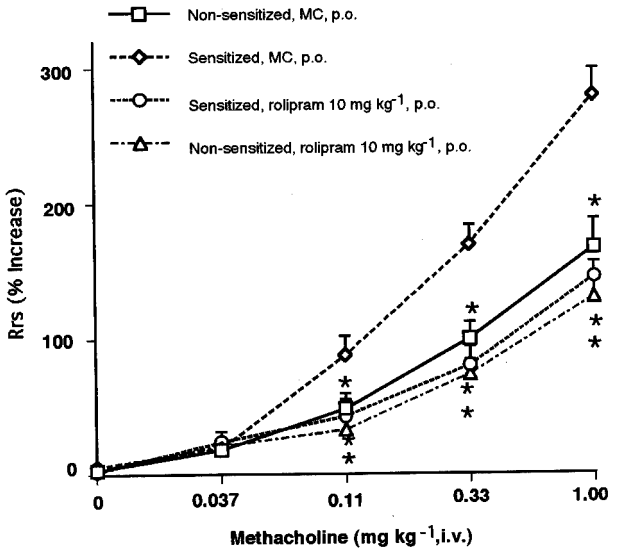

Figure 2 Effect of rolipram on airway hyperresponsiveness to methacholine in antigen challenged allergic mice. All groups were challenged with OVA. Values represent mean ± s.e.mean ($n=6$ per group) of per cent increase Rrs over baseline. *$P<0.05$ compared to sensitized, MC-treated group.

($P<0.05$) attenuated the inhibition by rolipram (10 mg kg^{-1}, p.o.) of the airway hyperresponsiveness to OVA challenge in sensitized mice (Table 3).

Effect of metyrapone on the activity of rolipram

OVA challenge to sensitized mice increased the number of BAL eosinophils in both vehicle and metyrapone (10 mg kg^{-1}, p.o.) treated animals although significantly more eosinophils were found in the BAL fluid of the metyrapone-treated mice (Figure 5). Rolipram (10 mg kg^{-1}, p.o.) reduced the number of BAL eosinophils in both vehicle and metyrapone treated mice although the inhibition by rolipram in metyrapone-treated mice (23% inhibition, not statistically significant) was less than that produced by rolipram in vehicle treated animals (52% inhibition, $P<0.05$).

Metyrapone (10 mg kg^{-1}, p.o.) had no effect on AHR to methacholine in both non-sensitized (data not shown) and sensitized mice that were challenged with OVA (Table 3). Furthermore, metyrapone had no effect on the inhibition by rolipram of the AHR to OVA challenge in sensitized mice (Table 3).

Discussion

Rolipram is a selective inhibitor of the PDE-4 isoenzyme that inhibits allergen-induced pulmonary eosinophilia and AHR in guinea-pigs, rabbits and monkeys (Turner *et al*., 1994; Underwood *et al*., 1994; Gozzard *et al*., 1996; Holbrook *et al*., 1996; Danahay & Broadley, 1997). In this study, rolipram inhibited the pulmonary eosinophilia and AHR produced by antigen challenge in allergic mice. Although we did not measure blood levels of catecholamines or corticosterone, the results of this study suggest a significant involvement with adrenal-derived mediators on the inhibitory action of rolipram. Previous studies have shown that PDE inhibitors may stimulate the release of catecholamines and corticosteroids from the adrenals which may account for part of their anti-inflammatory activity (Hadley *et al*., 1996; Cheng *et al*.,

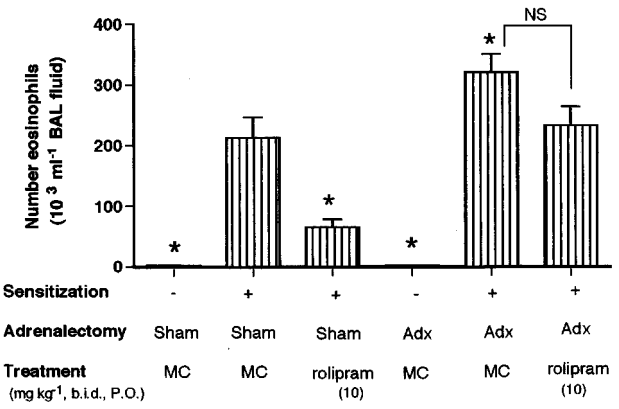

Figure 3 Effect of adrenalectomy on the inhibition of pulmonary eosinophilia by rolipram in OVA challenged allergic mice. Values represent the mean ± s.e.mean ($n=6$ per group). *$P<0.05$ compared to sensitized, sham-operated, MC-treated group. NS = not significant.

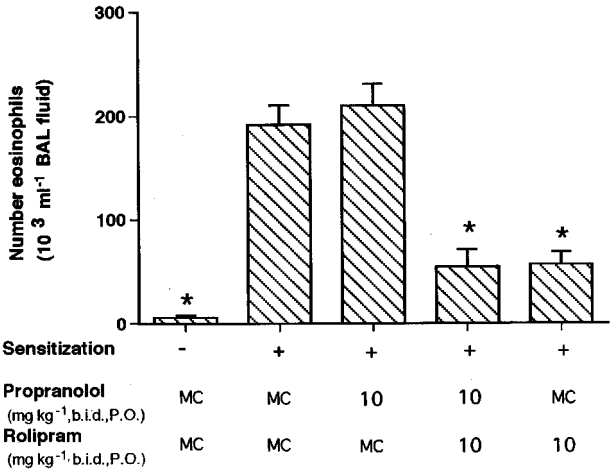

Figure 4 Effect of propranolol on the inhibition of pulmonary eosinophilia by rolipram in OVA challenged allergic mice. Values represent the mean ± s.e.mean ($n=6$ per group). *$P<0.05$ compared to sensitized, MC-treated group.

Table 3 Effect of propranolol and metyrapone on the inhibition by rolipram of airway hyperresponsiveness in allergic mice

Sensitization	*Treatment*	*Dose* (mg kg^{-1}, p.o.)	*PD_{100} of methacholine*†* (mg kg^{-1})
Non-sensitized	MC Vehicle	–	0.47 ± 0.10‡
Sensitized	MC Vehicle	–	0.21 ± 0.10§
Sensitized	Rolipram	10	0.49 ± 0.05‡
Sensitized	Propranolol	10	0.17 ± 0.01§
Sensitized	Rolipram + Propranolol	10	0.24 ± 0.02§
Sensitized	Metyrapone	10	0.23 ± 0.02§
Sensitized	Rolipram + Metyrapone	10	

*PD_{100} : Provocative dose of methacholine increasing Rrs by 100% above baseline. †Values are mean ± s.e.mean ($n=5$–10 per group). ‡$P<0.05$ compared to sensitized, challenged with OVA and treated with MC vehicle. §$P<0.05$ compared to sensitized, challenged with OVA and treated with rolipram 10 mg kg^{-1}

Table 2 Effect of adrenalectomy on the inhibition by rolipram of airway hyperresponsiveness in allergic mice

Animal	*Sensitization*	*Treatment*	*Dose* (mg kg^{-1}, p.o.)	*PD_{100} of methacholine*†* (mg kg^{-1})
Sham-operated	Non-sensitized	MC Vehicle	–	0.41 ± 0.07‡
	Sensitized	MC Vehicle	–	0.18 ± 0.02
	Sensitized	Rolipram	10	0.49 ± 0.05‡
Adrenalectomized	Non-sensitized	MC Vehicle	–	0.50 ± 0.06‡
	Sensitized	MC Vehicle	–	0.19 ± 0.04
	Sensitized	Rolipram	10	0.15 ± 0.02

PD_{100} : Provocative dose of methacholine increasing Rrs by 100% above baseline. †Values are mean ± s.e.mean ($n=5$–10 per group). ‡$P<0.05$ compared to sensitized, challenged with OVA and treated with MC vehicle.

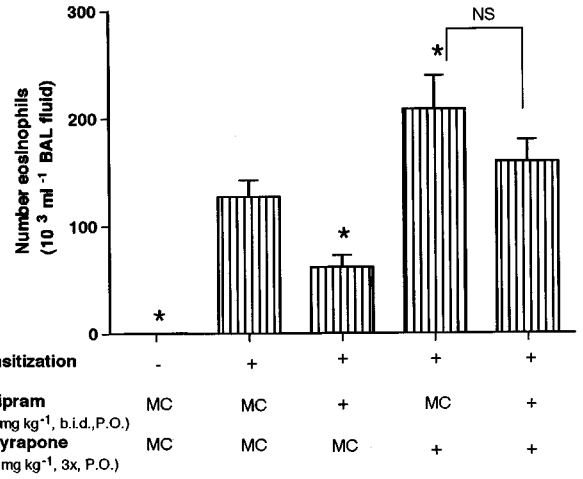

Figure 5 Effect of metyrapone on the inhibition of pulmonary eosinophilia by rolipram in OVA challenged allergic mice. Metyrapone (10 mg kg^{-1}, p.o.) was given on three separate occasions (see Methods). Values represent the mean ± s.e.mean ($n=6$ per group). *$P<0.05$ compared to sensitized, MC-treated group. NS = not significant.

1997; Kumari *et al*., 1997; Sato *et al*., 1998). However, the results in this study are the first to show an interaction between rolipram and the adrenal glands on the inhibition of pulmonary eosinophilia and AHR after antigen challenge in allergic mice.

Pulmonary eosinophilia is a characteristic feature of human asthma that may contribute to the pathology of this disease (Kay, 1991). Corticosteroids are effective drugs for the treatment of the inflammatory component of asthma and are known to reduce pulmonary eosinophilia associated with antigen challenge in mice (Chapman *et al*., 1998). Endogenous corticosteroids are also anti-inflammatory and may serve an important protective function in conditions of acute and chronic lung inflammation (DeBie *et al*., 1996). Recent studies in rodents have ascribed an involvement of endogenous corticosterone to the antiinflammatory activity of rolipram and other PDE-4 inhibitors (Hadley *et al*., 1996; Pettipher *et al*., 1997; Kumari *et al*., 1997; Sato *et al*., 1998) and in our study, the inhibitory effect of rolipram against the pulmonary eosinophilia after antigen challenge was suppressed in adrenalectomized and in metyrapone-treated mice. These results strongly suggest involvement of endogenous corticosterone in this response. It is unlikely that circulating catecholamines participate in the inhibition of pulmonary eosinophilia by rolipram because propranolol, a β-blocker, had no effect. These results confirm the observations made by Underwood *et al*. (1997) who found that β-blockers had no effect on the inhibition of antigen-induced pulmonary eosinophilia by rolipram in guinea-pigs.

Airway hyperresponsiveness is another important feature of human asthma and is demonstrated in allergic mice after antigen-challenge (Hessel *et al*., 1995). In this study, a 3 fold increase in AHR to methacholine was observed 24 h after antigen challenge. Treatment of sensitized mice with rolipram suppressed the antigen-induced AHR. Importantly, the inhibitory effects of rolipram on the AHR was ameliorated in both adrenalectomized and propranolol-treated mice; but was unaffected by metyrapone treatment. These results identify an important role for endogenous catecholamines, acting through β-adrenoceptors, on the inhibition of AHR by rolipram in allergic mice. The fact that propranolol suppressed the inhibition of AHR by rolipram but had no effect on the inhibition of pulmonary eosinophilia confirms previous observations in animals that eosinophil numbers in the lungs do not always correlate with the presence of AHR (Mauser *et al*., 1993; Lilly *et al*., 1996; Tournoy *et al*., 2000). Furthermore, the inability of metyrapone to affect the inhibition of AHR by rolipram suggests that endogenous corticosterone is not involved in this response. These results clearly indicate that the inhibitory activities of rolipram against eosinophilia and AHR are regulated through different adrenal factors.

The exact mechanism by which rolipram interacts with the adrenal glands remains to be elucidated, but several explanations can be postulated. PDE inhibitors increase hypothalamo-pituitary-adrenocortical (HPA) activity in rodents (Hadley *et al*., 1996; Kumari *et al*., 1997; Sato *et al*., 1998). Hadley *et al*. (1996) found that the PDE-4 inhibitor denbufylline caused an increase in plasma corticosterone in rats by stimulating the release of ACTH from the anterior pituitary. Kumari *et al*. (1997) confirmed these findings and also reported that other PDE-4 inhibitors, such as rolipram and BRL 61063, activated the HPA axis in the rat at both the anterior pituitary and the hypothalamus. Therefore, rolipram may stimulate the release of corticosterone in mice which in turn ameliorates the influx of eosinophils into the lungs following antigen challenge. PDE inhibitors, such as theophylline and aminophylline, increase the levels of circulating catecholamines in humans and this effect may be important for the anti-asthma efficacy of these drugs. (Higbee *et al*., 1982; Vestal *et al*., 1983). PDE-inhibitors increase tissue levels of cyclic AMP and may potentiate the actions of endogenous catecholamines at effector cells (Torphy, 1998). The latter has been proposed as the mechanism of action for rolipram to inhibit antigen-induced bronchoconstriction in guinea-pigs (Underwood *et al*., 1997), and this may be important for inhibition of AHR after antigen challenge in our study.

In conclusion, the results of this study identify a significant involvement with adrenal-derived catecholamines and corticosterone on the inhibitory actions of rolipram against pulmonary eosinophilia and AHR in allergic mice. These results added to a growing list of observations that identifies an important role for the HPA axis on the pharmacological actions of PDE-4 inhibitors. However, as suggested previously, the adrenal involvement of PDE-4 inhibition may be a condition that is unique to rodents and not seen in humans (Pettipher *et al*., 1996). If so, then experiments in rodents may overestimate the efficacy of PDE-4 inhibitors in humans.

The authors thank Ms Carol Battle for the preparation of this manuscript.

References

CHAPMAN, R.W., SEHRING, S.J., GARLISI, C.G., FALCONE, A., KUNG, T.T., STELTS, D., MINNICOZZI, M., JONES, H., UMLAND, S., EGAN, R.W. & KREUTNER, W. (1998). Anti-inflammatory activity of inhaled mometasone furoate in allergic mice. *Arzneim.-Forsch. Drug Res.,* **48,** 384 – 391.

CHENG, J.B., WATSON, J.W., PAZOLES, C.J., ESKRA, J.D., GRIFFITHS, R.J., COHAN, V.L., TURNER, C.R., SHOWELL, H.J. & PETTIPHER, E.R. (1997). The phosphodiesterase type 4 (PDE4) inhibitor CP-80, 633 elevates plasma cyclic AMP levels and decreases tumor necrosis factor-α (TNF-α) production in mice: effect of adrenalectomy. *J. Pharmacol. Exp. Ther.,* **280,** 621 – 626.

CROCKER, C., TOWNLEY, R.G. & KHAN, M.M. (1996). Phosphodiesterase inhibitors suppress proliferation of peripheral blood mononuclear cells and interleukin-4 and-5 secretion by human T-helper type 2 cells. *Immunopharmacology,* **31,** 223 – 225.

DANAHAY, H. & BROADLEY, K.J. (1997). Effect of inhibitors of phosphodiesterase, on antigen-induced bronchial hyperreactivity in conscious sensitized guinea pigs and airway leukocyte infiltration. *Br. J. Pharmacol.,* **120,** 289 – 297.

DEBIE, T.T., HESSEL, E.M., VAN ARK, I., VAN ESCH, B., HOFMAN, G., NIJKAMP, F.P. & VAN OOSTERHOUT, A.J.M. (1996). Effect of dexamethasone and endogenous corticosterone on airway hyperresponsiveness and eosinophilia in the mouse. *Br. J. Pharmacol.,* **119,** 1484 – 1490.

DENT, G., GIEMBYCZ, M.A., EVANS, P.M., RABE, K.F. & BARNES, P.J. (1994). Suppression of human eosinophil respiratory burst and cyclic AMP hydrolysis by inhibitors of type IV phosphodiesterase: interaction with the β-adrenoceptor agonist albuterol. *J. Pharmacol. Exp. Ther.,* **271,** 1167 – 1174.

ELENKOV, I.J., HASKÓ, G., KOVÁCS, K.J. & VIZI, E.S. (1995). Modulation of lipopolysaccharide-induced tumor necrosis factor-α production by selective α-and β adrenergic drugs in mice. *J. Neuroimmunol.,* **61,** 123 – 131.

GOZZARD, N., EL-HASHIM, A., HERD, C.M., BLAKE, S.M., HOLBROOK, M., HUGHES, B-., HIGGS, G.A. & PAGE, C.P. (1996). Effect of the glucocorticosteroid budesonide and a novel phosphodiesterase type 4 inhibitor CDP840 on antigen-induced airway responses in neonatally immunized rabbits. *Br. J. Pharmacol.,* **118,** 1201 – 1208.

GRISWOLD, D.E., WEBB, E.F., BRETON, J., WHITE, J.R., MARSHALL, P.L. & TORPHY, T.J. (1993). Effect of selective phosphodiesterase type IV inhibitor, rolipram, on fluid and cellular phase of inflammatory response. *Inflammation,* **17,** 333 – 344.

HADLEY, A.J., KUMARI, M., COVER, P.O., OSBORNE, J., POYSER, R., FLACK, J.D. & BUCKINGHAM, J.C. (1996). Stimulation of the hypothalamo-pituitary-adrenal axis in the rat by the type 4 phosphodiesterase (PDE-4) inhibitor, denbufylline. *Br. J. Pharmacol.,* **119,** 463 – 470.

HESSEL, E.M., VAN OOSTERHOUT, A.J.M., HOFSTRA, C.L., DE BIE, J.J., GARSSEN, J., VAN LOVEREN, H., VERHEYEN, A.K.C.P., SAVELKOUL, H.F.J. & NIJKAMP, F.P. (1995). Bronchoconstriction and airway hyperresponsiveness after ovalbumin inhalation in sensitized mice. *Eur. J. Pharmacol.,* **294,** 401 – 412.

HIGBEE, M.D., KUMAR, M. & GALANT, S.P. (1982). Stimulation of endogenous catecholamine release by theophylline: a proposed additional mechanism of action for theophylline effects. *J. Allergy Clin. Immunol.,* **70,** 377 – 382.

HOLBROOK, M., GOZZARD, N., JAMES, T., HIIGGS, G. & HUGHES, B. (1996). Inhibition of bronchospasm and ozone-induced airway hyperresponsiveness in the guinea pig by CDP840, a novel phosphodiesterase type 4 inhibitor. *Br. J. Pharmacol.,* **118,** 1192 – 1200.

HOWELL, R.E., JENKINS, L.P. & HOWELL, D.E. (1995). Inhibition of lipopolysaccharide-induced pulmonary edema by isozyme-selective phosphodiesterase inhibitors in guinea pigs. *J. Pharmacol. Exp. Ther.,* **275,** 703 – 709.

HUGHES, B., HOWAT, D., LISLE, H., HOLBROOK, M., JAMES, T., GOZZARD, N., BLEASE, K., HUGHES, P., KINGABY, R., WARRELLOW, G., ALEXANDER, R., HEAD, J., BOYD, E., EATON, M., PERRY, M., WALES, M., SMITH, B., OWENS, R., CATTERALL, C., LUMB, S., RUSSELL, A., ALLEN, R., MERRIMAN, M., BLOXHAM, M. & HIGGS, G. (1996). The inhibition of antigen-induced eosinophilia and bronchoconstriction by CDP840, a novel stereo-selective inhibitor of phosphodiesterase type-4. *Br. J. Pharmacol.,* **118,** 1183 – 1191.

KAMBAYASHI, T., JACOB, C.O., ZHOU, D., MAZUREK, N., FONG, M. & STRASSMANN, G. (1995). Cyclic nucleotide phosphodiesterase type IV participates in the regulation of IL-10 and in the subsequent inhibition of TNF-α and IL-6 release by endotoxin-stimulated macrophages. *J. Immunol.,* **155,** 4909 – 4916.

KAY, A.B. (1991). Asthma and inflammation. *J. Allergy Clin. Immunol.,* **87,** 893 – 910.

KUMARI, M., COVER, P.O., POYSER, R.H. & BUCKINGHAM, J.C. (1997). Stimulation of the hypothalamo-pituitary-adrenal axis in the rat by three selective type-4 phosphodiesterase inhibitors: in vitro and in vivo studies. *Br. J. Pharmacol.,* **121,** 459 – 468.

KUNG, T.T., JONES, H., ADAMS III G.K., UMLAND, S.P., KREUTNER, W., EGAN, R.W., CHAPMAN, R.W. & WATNICK, A.S. (1994). Characterization of murine model of allergic pulmonary inflammation. *Int. Arch. Allergy Immunol.,* **105,** 83 – 90.

LILLY, C.M., CHAPMAN, R.W., SEHRING, S.J., MAUSER, P., EGAN, R.W. & DRAZEN, J.M. (1996). Effects of interleukin 5-induced pulmonary eosinophilia on airway reactivity in the guinea pig. *Am. J. Physiol.,* **270,** L368 – L375.

MAUSER, P.J., PITMAN, A., WITT, A., FERNANDEZ, X., ZURCHER, J., KUNG, T., JONES, H., WATNICK, A.S., EGAN, R.W., KREUTNER, W. & ADAMS III G.K. (1993). Inhibitory effect of the TRFK-5 anti-IL-5 antibody in a guinea pig model of asthma. *Am. Rev. Respir. Dis.,* **148,** 1623 – 1627.

PALFREYMAN, M.N. & SOUNESS, J.E. (1996). Phosphodiesterase type IV inhibitors. In *Progress in Medicinal Chemistry*. ed. Ellis, G.P. & Luscombe, D.K., **Vol. 33,** pp. 1 – 52. Amsterdam: Elsevier Science BV.

PETTIPHER, E.R., ESKRA, J.D. & LABASI, J.M. (1997). The inhibitory effect of rolipram on TNF-α production in mouse blood ex vivo is dependent upon the release of corticosterone and adrenaline. *Cytokine,* **9,** 582 – 586.

PETTIPHER, E.R., LABASI, J.M., SALTER, E.D., STAM, E.J., CHENG, J.B. & GRIFFITHS, R.J. (1996). Regulation of tumor necrosis factor production by adrenal hormones in vivo: insights into the anti-inflammatory activity of rolipram. *Br. J. Pharmacol.,* **117,** 15430 – 1534.

RAEBURN, D. & KARLSSON, J.A. (1993). Effects of isoenzyme-selective inhibitors of cyclic nucleotide phosphodiesterase on microvascular leak in guinea pig airways *in vivo*. *J. Pharmacol. Exp. Ther.,* **267,** 1147 – 1152.

SATO, J., HORI, S. & KAWAMURA, M. (1998). Effect of theophylline, caffeine and dimethylxanthines on endogenous glucocorticoid levels in mice. A possible mechanism of anti-inflammatory activity of theophylline. *Pharm. Pharmacol. Commun.,* **4,** 499 – 501.

SCHUESSLER, T.F. & BATES, J.H.T. (1995). A computer-controlled research ventilator for small animals: design and evaluation. *IEEE Trans. Biomed. Eng.,* **42,** 860 – 866.

TORPHY, T.J. (1998). Phosphodiesterase isozymes. Molecular targets for novel antiasthma agents. *Am. J. Respir. Crit. Care Med.,* **157,** 351 – 370.

TOURNOY, K.G., KIPS, J.C., SCHOU, C. & PAUWELS, R.A. (2000). Airway eosinophilia is nor a requirement for allergen-induced airway hyperresponsiveness. *Clin. Exp. Allergy.,* **30,** 79 – 85.

TURNER, C.R., ANDRESEN, C.J., SMITH, W.B. & WATSON, J.W. (1994). Effects of rolipram on responses to acute and chronic antigen exposure in monkeys. *Am. J. Respir. Crit. Care Med.,* **149,** 1153 – 1159.

UNDEM, B.J., MEEKER, S.N. & CHEN, J. (1994). Inhibition of neurally mediated nonadrenergic, noncholinergic contractions of guinea pig bronchus by isozyme-selective phosphodiesterase inhibitors. *J. Pharmacol. Exp. Ther.,* **271,** 811 – 817.

UNDERWOOD, D.C., BOCHNOLOICZ, S., OSBORN, R.R., KOTZER, C.J., LUTTMANN, M.A., HAY, D.W.P., GORYCKI, P.D., CHRISTENSEN, S.B. & TORPHY, T.J. (1998). Antiasthmatic activity of the second-generation phosphodiesterase 4 (PDE 4) inhibitor SB 207499 (Airflo) in the guinea pig. *J. Pharmacol. Exp. Ther.,* **287,** 988 – 995.

UNDERWOOD, D.C., KOTZER, C.J., BOCHNOWICZ, S., OSBORN, R.R., LUTTMAN, M.A., HAY, D.W.P. & TORPHY, T.J. (1994). Comparison of phosphodiesterase III, IV and dual III/IV inhibitors on bronchospasm and pulmonary eosinophil influx in guinea pigs. *J. Pharmacol. Exp. Ther.,* **270,** 250 – 259.

UNDERWOOD, D.C., MATTHEWS, J.K., OSBORN, R.R., BOCHNOWICZ, S. & TORPHY, T.J. (1997). The influence of endogenous catecholamines on the inhibitory effects of rolipram against early- and late-phase response to antigen in the guinea pig. *J. Pharmacol. Exp. Ther.,* **280,** 210–219.

VESTAL, R.E., EIRIKSSON, C.E., MUSSER, B., OZAKI, L.K. & HALTER, J.B. (1983). Effect of intravenous aminophylline on plasma levels of catecholamines and related cardiovascular and metabolic responses in man. *Circulation,* **67,** 162–171.

(Received September 16, 1999
Revised February 8, 2000
Accepted February 16, 2000)