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INHIBITION OF IgE-MEDIATED RELEASE OF HISTAMINE AND PEPTIDE LEUKOTRIENE FROM HUMAN BASOPHILS AND MAST CELLS BY FORSKOLIN*

GIANNI MARONE†, MICHELE COLUMBO†, MASSIMO TRIGGIANI†, RAFFAELE CIRILLO†, ARTURO GENOVESE† and SALVATORE FORMISANO‡

†Department of Medicine, and ‡Department of Molecular and Cellular Biology and Pathology, University of Naples II School of Medicine, Via S. Pansini 5, 80131 Naples, Italy

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Abstract—Forskolin, a diterpene compound isolated from the roots of *Coleus forskohlii*, activates adenylate cyclase in membranes from a variety of mammalian tissues. We found that forskolin (10^{-7} to 3×10^{-5} M) caused a concentration-related inhibition of IgE-mediated release of histamine and peptide leukotriene C_4 (LTC_4) from human basophils and lung mast cells. There was a significant linear correlation between the per cent inhibition of histamine and LTC_4 release from both cell types. However, in both systems forskolin exerted a significantly greater inhibitory effect on LTC_4 release than on histamine release. The concentration–response inhibition curve was paralleled by a forskolin-induced rise in cAMP levels in human leukocyte and mast cell preparations. The relationship between the effect of forskolin and the cAMP concentration was supported by the finding that forskolin inhibited the "first stage" of antigen-induced histamine release, but not the release caused by the Ca^{2+} ionophore, A23187. Propranolol, a competitive β-receptor antagonist, did not block the inhibition of mediator release or the cAMP accumulation caused by forskolin. These data suggest that forskolin modulates the release of mediators of immediate hypersensitivity reactions via the activation of adenylate cyclase in human basophils and mast cells.

The interaction of specific allergens and IgE antibodies bound to receptors on mast cells and basophils leads to a complex sequence of biochemical events that bring about cellular activation and mediator release [1, 2]. *In vitro* studies have clarified the biochemical mechanisms of the release process [2, 3], and the mediators produced by human basophils and mast cells, which include histamine, chemotactic factors, enzymes, platelet-activating factor, and the slow-reacting substance of anaphylaxis (SRS-A) [4, 5]. SRS-A consists of a series of metabolically related products of arachidonic acid metabolism: leukotrienes C_4 (LTC_4), D_4 (LTD_4), and E_4 (LTE_4) [6, 7]. These peptide leukotrienes are potent constrictors of smooth muscle, they enhance vascular permeability, stimulate airways mucus production, and may have important immunoregulatory functions [8].

Considerable effort has been devoted to the study of the pharmacological control of the basophil/mast cell release reactions. There is increasing evidence that cyclic adenosine 3′-5′-monophosphate (cAMP) participates in both the initiation and modulation of the IgE-mediated release reactions of these cells [9–11]. Exogenous cAMP and cAMP phosphodiesterase inhibitors suppress IgE-mediated histamine release from human basophils and mast cells [9, 12, 13]. Furthermore, catecholamines, adenosine, prostaglandins of the E series, and histamine itself all inhibit the IgE-mediated release reaction from human basophils [10, 12–15]. Their inhibitory effects are mediated by activation of cell surface receptors linked to adenylate cyclase, resulting in increased intracellular cAMP [12–16]. Analogs of these substances are utilized to treat obstructive airways diseases [17, 18].

Forskolin, a diterpene isolated from the roots of the plant *Coleus forskohlii* [19], activates adenylate cyclase in membranes from a variety of mammalian tissues [20]. Forskolin and its diterpene derivatives bind to a single binding site on the catalytic unit of adenylate cyclase [21]. Recently it has been shown that *in vitro* forskolin relaxes guinea-pig trachea and inhibits antigen-induced histamine release from guinea-pig lung [22, 23]. *In vivo* it inhibits antigen-induced bronchospasm [22] and reduces metacholine-induced bronchoconstriction in asthmatics [24].

In the light of these observations, it appeared of interest to determine the effect of forskolin on IgE-mediated release of histamine and of LTC_4 from basophil leukocytes of allergic donors and from mast cells isolated from human lung. Forskolin was found to inhibit IgE-mediated histamine and LTC_4 release. It is postulated that forskolin exerts its inhibitory effect by activating the adenylate cyclase in the human basophils and mast cells, thereby causing an increase in the intracellular cAMP level.

MATERIALS AND METHODS

Leukocyte donors. Venous blood was obtained from allergic subjects, aged 20–42 yr. The use of human volunteers was approved by the Committee

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of Clinical Investigations of the University of Naples, II School of Medicine, and informed consent was always obtained.

Buffers. The PIPES buffer (P) used in these experiments was made up of 25 mM PIPES, 110 mM NaCl, 5 mM KCl, pH 7.35; buffer PC was P with the addition of 2.0 mM $CaCl_2$ [16]. Tyrode's buffer contains (g/l): NaCl, 8.0; KCl, 0.2; NaH_2PO_4, 0.05; $CaCl_2 \times 2H_2O$, 0.26; $MgCl_2 \times 6H_2O$, 0.25; and glucose, 1.0; pH was titrated to 7.4 with sodium bicarbonate [17].

Histamine release from human basophils. Leukocytes were isolated from blood samples by dextran sedimentation as previously described [25]. Cells were allowed to sediment for 90 min at 22°. The leukocyte-rich upper layer was drawn off, pelleted and washed as previously described [10]. All cell preparations were >95% viable as assessed from the ability to exclude erythrocin B. Aliquots (0.4 ml) of the cell suspension were placed in Falcon 12 × 75 mm polyethylene tubes and warmed to 37°; 0.2 ml of the prewarmed (37°) releasing stimuli were added, and incubation was continued at 37° for 45 min. After centrifugation the cell-free supernatants were assayed for histamine with an automated fluorometric technique [26]. The net percentage release was calculated from the histamine released spontaneously (≃10%) from the unstimulated aliquots less the total histamine release from cell aliquots lysed with 2% perchloric acid [15]. To study the two stages ("first" and "second") of histamine release, cells were incubated with antigen for 2 min in the absence of calcium, washed twice with cold P, and resuspended in PC as previously described [10, 13]. All experiments were carried out with the cells of at least three separate donors and each experiment was performed in duplicate or triplicate, with a less than 10% variation between replicates.

Histamine release from human lung. Human lung tissue obtained during surgical resection was processed as previously described [13]. Macroscopically normal lung tissue, obtained within 1 hr of resection, was dissected free of major bronchi and blood vessels, chopped with scissors into samples of 100–150 mg wet weight and washed 5 times in buffer P. Replicate samples were passively sensitized in 1.0 ml of a 1:20 dilution of serum from a patient highly allergic to ragweed E for 18 hr at 22°, washed and placed in 5.0 ml of PC. To assess histamine release, duplicate samples of human lung tissue were prewarmed to 37° and challenged by incubation with antigen E for 20 min. Spontaneous histamine release was determined in duplicate tubes without antigen. Total histamine was measured in parallel triplicate tubes boiled in 2% perchloric acid for 10 min to extract tissue histamine [13].

Isolation of human lung mast cells. Macroscopically normal lung tissue was dissected free of major bronchi and blood vessels and chopped finely with scissors. The fragments were washed at 22° and dispersed into their cellular elements by incubation with the protease, pronase, chymopapain, collagenase, and elastase. These preparations (2% to 12% mast cells) were purified further by countercurrent centrifugation elutriation [27], followed by centrifugation over discontinuous Percoll gradients [28]. The experiments reported here were performed with preparations containing 20% to 96% of mast cells. Mast cell preparations were contaminated with various proportions of eosinophils, neutrophils, monocytes/macrophages, lymphocytes, basophils, type II pneumocytes, Clara cells, and endothelial cells. The cells were washed, and a portion was removed for staining with alcian blue to determine total cell number [29], after which the cells were resuspended in PC for release studies.

cAMP assay. cAMP was assayed with the method of Brown *et al.* [30], slightly modified as previously described [31].

Radioimmunoassay and high performance liquid chromatography. The radioimmunoassay for LTC_4 was performed as described previously [32], using dextran-coated charcoal as the separation technique. The rabbit anti-LTC_4 antiserum has previously been characterized [32]. Cross-reactivity for various heterologous ligands was as described [32]. HPLC was performed using a Beckman liquid chromatography, equipped with two model 114 A pumps, a 420 system controller, a 210 sample injector with 50 mcl injection loop and a 163 UV-visible wavelength detector. All HPLC procedures were performed with unlabeled internal standards (which were detected by u.v. absorption at the appropriate wavelength) as previously described [17].

Materials. PIPES, chymopapain, elastase type 1 and propranolol were purchased from Sigma Chemical Company (St. Louis. MO). Perchloric acid (60%) was purchased from J. T. Baker Chemical Company (Deventer, The Netherlands), forskolin, pronase, DNAse, and ionophore A23187 were from Calbiochem-Behring Corporation (La Jolla, CA), Percoll and dextran 70 were from Pharmacia Fine Chemicals (Uppsala, Sweden) and collagenase was from Worthington (Freehold, NJ). (^{3}H)-cAMP (34.5 Ci/mmol) and (^{3}H)-LTC_4 (35.7 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Antigen E was a kind gift from Dr A. Kagey-Sobotka, The Johns Hopkins University, Baltimore, MD; rye I was kindly provided by Dr D. Marsh, The Johns Hopkins University, Baltimore, MD. LTC_4, LTD_4, and LTE_4 and the rabbit anti-LTC_4 were a generous gift from Dr J. Rokach (Merck Frosst Canada, Montreal).

Statistical analysis. The data were subjected to linear regression analysis by the least squares method ($y = a + bx$), where the slope of the line and y-axis intercept were b and a, respectively. The standard deviation of the slope (Sb) and y-axis intercept (Sa), and the comparison vs a constant (b vs 1.0 and a vs 0.0) were obtained by standard statistical methods [33].

RESULTS

The effect of forskolin on IgE-mediated histamine and peptide leukotriene release from human basophils

It has been demonstrated that the histamine release process can be divided into at least two stages, a Ca^{2+}-independent "first stage" in which antigen or anti-IgE binds to the cell surface IgE antibodies and cell activation occurs, and a Ca^{2+}-dependent "second stage" in which histamine release

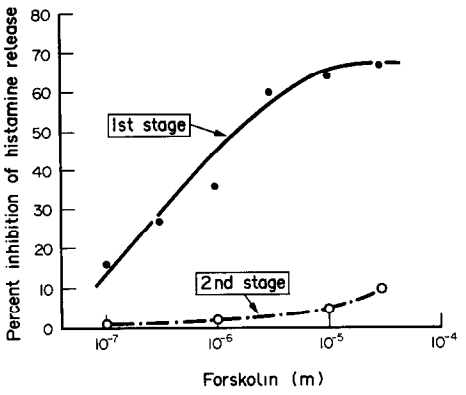

Fig. 1. Effect of different concentrations of forskolin on the "first" and "second" stages of histamine release from the leukocytes of an allergic donor. Cells were preincubated 10 min with forskolin before antigen addition. The control percentages of histamine release were 36.4 ± 3.4 (first stage) and 38.1 ± 2.8 (second stage). Each point represents the mean of duplicate determinations.

occurs [34]. PGE_2, isoproterenol, adenosine and other adenylate cyclase agonists inhibit the release of histamine only in the first stage of the process [10, 13, 15]. Data on the staging of the effect of forskolin on antigen-induced histamine release from human basophils are presented in Fig. 1. In a series of 12 experiments, forskolin (10^{-7} to 3×10^{-5} M) caused concentration-related inhibition of antigen-induced histamine release from human basophils of allergic donors. Figure 1 shows a representative experiment: forskolin caused marked inhibition during the "first stage" of histamine release, but had no effect during the "second stage". The inhibition ranged from about 15% at 10^{-7} M to 50–90% at 3×10^{-5} M. Within this concentration range forskolin had no effect on cell viability nor did it cause spontaneous histamine release.

The peptide leukotrienes (leukotriene C_4, D_4 and E_4; formerly referred to as SRS-A) have long been felt to be important in the pathogenesis of allergic disorders [7, 8]. It has been reported that specific antigen or anti-IgE causes the release of SRS-A from preparations of mixed leukocytes [35]. More recently, these studies have been extended to show that enriched preparations of basophils (40–70% pure) also release immunoreactive peptide leukotrienes after an IgE-mediated stimulus [36]. These observations suggest that the basophil is, in fact, the cell responsible for the IgE-mediated release of LTC_4 in mixed leukocyte populations. Therefore, it appeared important to evaluate the effect of forskolin on *de novo* synthesis of LTC_4.

LTC_4 was detected in the cell-free supernatants of human peripheral blood cells challenged with antigen. Table 1 shows that a concentration of forskolin as low as 3×10^{-8} M effectively inhibited basophil leukotriene release. The concentration of forskolin producing 50% inhibition (IC_{50}) was between 8×10^{-8} and 6×10^{-7} M. It is clear from these results that forskolin inhibits the release of leukotriene more effectively than it does histamine secretion. The radioimmunoassay we employed to detect LTC_4 also detects LTD_4 and LTE_4 [32]. Therefore, it is possible that the apparent inhibition of LTC_4 release was due to an increased rate of conversion of LTC_4 to the less cross-reactive metabolites, LTD_4 and LTE_4. To rule out this possibility, supernatants from challenged controls and forskolin (10^{-5} M)-treated cells were extracted and analyzed on HPLC [17]. Fractions corresponding to the known retention times of LTC_4, LTD_4 and LTE_4 were evaluated. Forskolin did not enhance the proportion of LTD_4 and LTE_4 (data not shown). Therefore it is unlikely that the inhibitory effect of forskolin was due to an increased catabolism of LTC_4.

Agents that act on membrane-bound adenylate cyclase to raise the intracellular cAMP levels inhibit histamine secretion during the first stage of the release process and have no effect on the second stage [10, 13, 15]. The data shown in Fig. 1, therefore, suggested that forskolin might raise the level of intracellular cAMP in human basophil leukocytes. Figure 2 shows that the forskolin concentrations that inhibited histamine release did in fact cause a concentration-dependent increase in leukocyte cAMP levels. The effect of forskolin became detectable at 10^{-7} M and reached a maximum at approximately 3×10^{-5} M. Similar results were obtained with leukocytes from four donors.

In these experiments cells were preincubated

Table 1. The effect of forskolin on IgE-mediated histamine and leukotriene C_4 (LTC_4) release from human basophils*

	Exp. 1		Exp. 2		Exp. 3	
Forskolin, M	LTC_4	Histamine	LTC_4	Histamine	LTC_4	Histamine
No drug	32	58	47	42	84	48
3×10^{-8}	26	59	N.D.	N.D.	72	47
10^{-7}	14	53	32	36	49	34
10^{-6}	0	37	16	22	26	23
10^{-5}	0	24	12	15	13	18
3×10^{-5}	0	18	0	8	0	5

* Rye I antigen final concentration 10^{-1} mcg/ml (Exp. 1), 3×10^{-2} mcg/ml (Exp. 2) and 4.8×10^{-3} mcg/ml (Exp. 3). Cells were preincubated with forskolin for 10 min before the addition of Rye I antigen. Values are expressed as ng LTC_4-equivalents/10^6 basophils and percent histamine release. Each value represents the mean of duplicate determinations.

N.D.; not done.

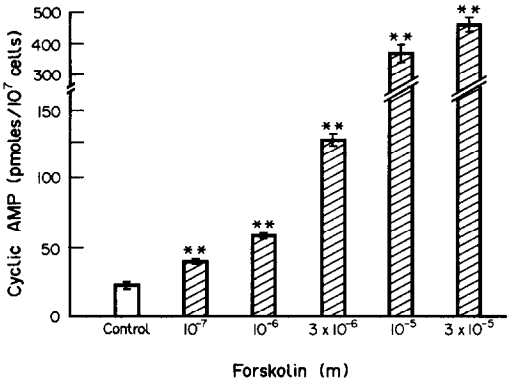

Fig. 2. Effects of different concentrations of forskolin on the levels of intracellular cyclic AMP in human leukocytes. Cells were incubated with the indicated concentrations of forskolin for 10 min, then the medium was aspirated and the intracellular cAMP levels were measured as described under "Materials and Methods". Each symbol represents the mean ± SEM of triplicate determinations. **P < 0.001 when compared with control.

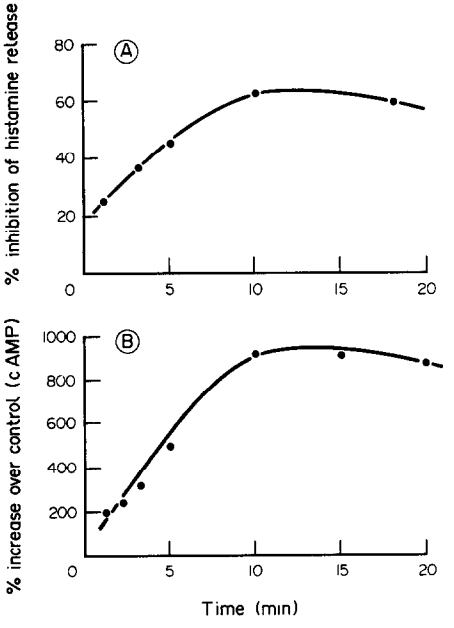

Fig. 3. (A) Inhibition of antigen-induced secretion during the first stage of histamine release from the leukocytes of an allergic donor. Cells were preincubated for different intervals with forskolin (10^{-5} M) before antigen addition. The control percentage of histamine release was 34.5. (B) Effect of forskolin (10^{-5} M) on intracellular accumulation of cyclic AMP in human leukocytes. Each point represents the mean of duplicate determinations in each section.

10 min with forskolin before the addition of the antigen. Inhibition was significantly lower with 2–5 min of preincubation. Figure 3 shows the kinetics of the inhibition of antigen-induced histamine release (A) and of cAMP accumulation produced by 10^{-5} M forskolin (B).

Lack of inhibition of the effects of forskolin by propranolol

Forskolin raises intracellular cAMP levels by acting directly on adenylate cyclase, without cell surface mediation [20, 37]. Therefore, its effects on both cAMP metabolism and histamine release are probably not attributable to an interaction with β-adrenergic receptors present on human leukocytes [9, 13]. However, to explore this possibility, cells were preincubated for 5 min with or without various concentrations of propranolol, a competitive β-receptor antagonist, before the addition of forskolin. These concentrations of propranolol had no effect on histamine release, and they did not block either the forskolin-induced inhibition of histamine release, or the accumulation of cAMP in leukocytes (data not shown).

Table 2. The effect of forskolin on ionophore A23187-induced histamine release from human basophils*

Forskolin, M	% Histamine release	
	Exp. 1	Exp. 2
No drug	46.8 ± 2.8	43.2 ± 3.8
10^{-7}	48.9 ± 3.0	41.5 ± 2.6
10^{-6}	42.6 ± 2.4	40.8 ± 3.4
10^{-5}	43.5 ± 3.8	44.3 ± 3.9
10^{-4}	42.0 ± 3.4	39.6 ± 4.1

* Ionophore final concentration 1×10^{-1} mcg/ml (Exp. 1) or 2×10^{-1} mcg/ml (Exp. 2). Cells were preincubated with forskolin for 5 min (Exp. 1) or 10 min (Exp. 2) before the addition of A23187. Each value represents the mean ± SEM of triplicate determinations.

The effects of forskolin on A23187-induced histamine release from human basophils

The mechanism of histamine release from human basophils induced by the calcium ionophore A23187 is similar to the final biochemical stages of antigen-induced histamine release [13, 38]. Agents that act on membrane-bound adenylate cyclase to increase cAMP levels within the cells have no effects on A23187-induced histamine release [10, 13, 38]. Table 2 shows that forskolin (10^{-7}–10^{-4} M) had no effect on A23187-induced histamine release when basophils were preincubated for 5 or 10 min with the drug before stimulation. Similar results were obtained in two other experiments in which cells from two different donors were used.

The effect of forskolin on IgE-mediated mediator release from human lung mast cells

The effect of forskolin on IgE-mediated mediator release from lung mast cells was examined in three series of experiments. In a first series, human lung was passively sensitized with serum from an allergic patient. Chopped lung fragments were preincubated (10 min) with forskolin (10^{-7}–3×10^{-5} M) and then challenged with antigen E (5×10^{-1}mcg/ml). Figure 4 shows the results of three such experiments. Forskolin induced a concentration-related inhibition of antigen-induced histamine secretion from human lung *in vitro*. The effect of forskolin on histamine

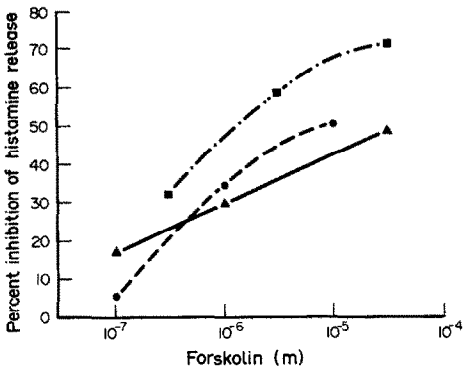

Fig. 4. Inhibition of antigen-induced secretion from human lung mast cells. Human chopped lung fragments were preincubated with forskolin (10 min) before antigen E (5×10^{-1} mcg/ml) addition. Each symbol represents the results of a single experiment. The control percentage of histamine release was between 15 and 29%.

Table 4. The effect of forskolin on intracellular cyclic AMP levels in mast cells purified from human lung*

	Exp. 1	Exp. 2	Exp. 3
Forskolin, M	Cyclic AMP (pmoles/10^7 cells)		
No drug	9.7 ± 0.3	21.4 ± 0.5	15.8 ± 0.9
10^{-7}	15.1 ± 1.3†	25.6 ± 1.3‡	25.6 ± 1.1†
10^{-6}	22.4 ± 1.4†	42.2 ± 0.4†	30.5 ± 2.9†
10^{-5}	27.8 ± 1.5†	63.4 ± 5.9†	47.1 ± 4.9†
3×10^{-5}	34.4 ± 2.4†	81.5 ± 4.1†	68.2 ± 6.1†

* Experimental conditions were as in Fig. 2. Each value represents the mean ± SEM of triplicate determinations. Lung mast cell purity was 91% (Exp. 1), 86% (Exp. 2), and 96% (Exp. 3).

† $P < 0.01$ when compared with the corresponding value of no drug.

‡ $P < 0.05$ when compared with the corresponding value of no drug.

release from human lung was not affected by propranolol (data not shown).

In a second group of experiments, the effect of forskolin on the release of histamine and LTC_4 from purified human lung mast cells was examined. *In vitro* studies indicate that mast cells challenged with anti-IgE are the major, and perhaps the only, source of LTC_4 in human lung [39]. Table 3 shows that forskolin inhibited the anti-IgE-induced release of histamine and LTC_4 in a concentration-dependent fashion. The IC_{50} for the release of histamine was between 10^{-6} and 10^{-5} M, and the IC_{50} for the release of LTC_4 was between 5×10^{-7} and 10^{-6} M. Forskolin (10^{-7}–3×10^{-5} M) had no effect on A23187-induced mediator release from purified lung mast cells (data not shown).

In a final series of experiments we evaluated the possibility that forskolin might raise the level of intracellular cAMP in human lung mast cells. Cells of a 91 ± 2.9% purity were used. As shown in Table 4 the concentrations of forskolin that inhibited mediator release did in fact cause a concentration-dependent increase in lung mast cell cAMP levels. Propranolol had no effect on forskolin-induced cAMP accumulation in lung mast cells (data not shown).

Correlation between the inhibition of histamine and LTC_4 release from human basophils and mast cells

We next compared the inhibition of histamine release to that of LTC_4 release in human basophils and mast cells challenged with anti-IgE. We found a significant linear correlation between the inhibition of histamine and LTC_4 release from both basophils ($r = 0.94$; $P < 0.001$) and mast cells ($r = 0.92$; $P < 0.001$). The linear regression lines for basophils and mast cells were superimposable, both slopes (b) and y-axis intercepts (a) not being statistically different (b: 1.05 and 0.99, respectively; a: 20.83 and 22.35, respectively). Therefore, we pooled the data for both cells in a single regression line (Fig. 5). The slope of this line (1.02 ± 0.06; mean ± SD) was not significantly different from 1.0, whereas the y-axis intercept (21.77 ± 2.97; mean ± SD) was significantly different from 0 ($t = 7.34$; $P < 0.001$). These results indicated a more marked inhibition of LTC_4 release (approximately 22%) than histamine, at all levels of inhibition caused by forskolin.

DISCUSSION

The present results indicate that forskolin, a selective agonist of adenylate cyclase, is a potent inhibitor of IgE-mediated mediator release from human baso-

Table 3. The effect of forskolin on IgE-mediated histamine and leukotriene C_4 (LTC_4) release from human lung mast cells*

	Exp. 1		Exp. 2		Exp. 3	
Forskolin, M	LTC_4	Histamine	LTC_4	Histamine	LTC_4	Histamine
No drug	120	36	78	12	94	18
10^{-7}	89	32	64	9	82	14
10^{-6}	48	26	41	7	46	9
10^{-5}	32	18	10	3	21	5
3×10^{-5}	0	8	5	2	0	2

* Anti-IgE final concentration 3 mcg/ml (Exp. 1 and 3) or 5 mcg/ml (Exp. 2). Cells were preincubated with forskolin for 10 min before the addition of anti-IgE. Values are expressed as ng LTC_4 equivalent$_s$/10^6 basophils and percent histamine release. Each value represents the mean of duplicate determinations.

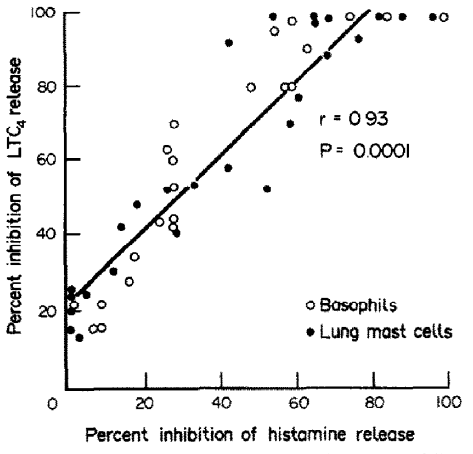

Fig. 5. Correlation between forskolin-induced inhibition of histamine and LTC_4 release from human basophils (O) and lung mast cells (●). The linear regression line was calculated by the least squares method ($y = a + bx$) where the slope of the line was $b = 1.02$ and the y-axis intercept $a = 22.35$. The correlation coefficient, $r = 0.93$, was highly significant ($P < 0.0001$).

phils and lung mast cells. The inhibitory effect *in vitro* becomes detectable at a level as low as 10^{-7} to 3×10^{-7} M and peaks at about 3×10^{-5} M.

Forskolin appears to inhibit the secretion of preformed mediators such as histamine, and of *de novo* synthesized mediators such as peptide leukotrienes. The latter are synthesized by two inflammatory cells, i.e. basophil leukocytes and lung mast cells, that probably play a distinct and relevant role in allergic disorders [40, 41]. Peptide leukotrienes (LTC_4, LTD_4, and LTE_4) have long been believed to be important in the pathophysiology of allergic diseases [8]. Bridging of IgE-receptors on human basophils and lung mast cells causes the release of LTC_4 [36, 39]. The *in vivo* release of immunoreactive LTC_4 after antigen challenge suggests that peptide leukotrienes may play an important role in immune allergic reactions [42]. Previous investigations have shown that peripheral basophils and lung mast cells are the major, and perhaps the only, source of LTC_4 in mixed leukocytes and lung mast cells, respectively, challenged with anti-IgE [36, 39].

Our finding that forskolin is a potent *in vitro* inhibitor of both histamine and LTC_4 release from human basophils and lung mast cells suggests that direct activation of adenylate cyclase in these inflammatory cells might have a bearing on the *in vivo* pharmacological control of allergic reactions.

The mechanism of histamine release from human basophils can be divided into at least two distinct stages [10, 34]. Inhibitory agents such as adenosine, fenoterol, histamine and PGE_2 act only during the first stage, and inhibition of histamine release is associated with an increase in intracellular cAMP [10, 12, 13, 15, 40, 43]. The present data indicate that forskolin is an effective inhibitor of basophil histamine release during this first stage and that it raises leukocyte cAMP levels. Inhibition of histamine release is first observed at about 10^{-7} M and peaks at about 3×10^{-5} M; forskolin-induced changes in leukocyte cAMP occur over the same concentration range. Furthermore, the kinetics of inhibition of histamine release and of the increase in leukocytes are the same.

It should be pointed out that our results were obtained with mixed leukocyte samples. It may be objected that the effect of forskolin on basophil cAMP metabolism can only be ascertained on highly purified preparations. However, with highly purified lung mast cells we found that forskolin-induced changes in cAMP levels occur over the same concentration range that inhibited IgE-mediated release of histamine and LTC_4. The involvement of cAMP in forskolin-induced inhibition is also suggested by the finding that all the agents known to inhibit histamine release by enhancing intracellular cAMP levels, including forskolin, have no effect on A23187-induced histamine release [10, 13, 15, 38]. Therefore, forskolin could inhibit histamine secretion by activating adenylate cyclase on the basophil/mast cell membrane which, in turn, results in the accumulation of cAMP. It is unlikely that the effect of forskolin is mediated by activation of the β-adrenergic receptor since the substance's effects on inhibition of mediator release and cAMP accumulation are not blocked by propranolol, a selective β-blocker.

Forskolin was a potent inhibitor of release of both preformed and *de novo* synthetized chemical mediators from human basophils and mast cells. Our results demonstrated a significant linear correlation between the per cent inhibition of histamine and LTC_4 release from both human basophils and lung mast cells. However, in both systems forskolin had a greater inhibitory effect on LTC_4 release than on histamine secretion. Although the reasons for this phenomenon are still unclear, the fact that it occurred in both basophils and mast cells suggests that a metabolic step common to basophils and mast cells exerts a more marked effect in the modulation of LTC_4 release than in the secretion of histamine. This may be related to differences in sensitivity to increased intracellular cAMP levels induced by forskolin. However, other compounds that presumably do not affect intracellular cAMP levels also inhibit the release of LTC_4 more completely than the release of histamine [44].

Forskolin inhibits IgE-mediated release of histamine from guinea-pig lung fragments [22, 23]. It also relaxes guinea-pig trachea [22, 23] and prevents both antigen- and histamine-induced bronchospasm in guinea pigs [22]. In addition the substance reverses metacholine-induced bronchoconstriction in asthmatics [24]. We now show that it is a potent inhibitor of the IgE-mediated release of chemical mediators from human basophils and lung mast cells, and that this effect is probably mediated by a rise in the cAMP level in these cells. The IgE-mediated release of histamine and leukotrienes from human basophils and lung mast cells plays a central role in the pathophysiology of human allergic and inflammatory disorders [8, 42]. Therefore, our results obtained in two *in vitro* models relevant to human anaphylaxis [2, 45] might have a bearing on the pharmacological control of this condition.

One possible pathophysiological mechanism in asthma may be a reduction in the number of β-adrenergic receptors [46, 47]. Patients with asthma are subsensitive to the effect of catecholamines through down-regulation of β-adrenergic receptors [48, 49], and treatment with β-agonists may further down-regulate receptors [46, 50]. Forskolin acts directly on the catalytic unit of adenylate cyclase, as suggested by evidence that the adenylate cyclase of a genetic variant of S-49 cells, deficient in the regulatory component of the enzyme [51], remains sensitive to forskolin [52]. Therefore, the efficacy of forskolin in inhibiting mediator relase would not be influenced by the number of β-receptors because the substance bypasses the β-adrenergic receptors. This biochemical property could be exploited in asthmatic patients who are subsensitive to the pharmacological effects of β-adrenergic drugs because of a reduced number of β-adrenergic receptors.

Extracts of *Coleus forskohlii* have been used in Hindu herbal medicine for many centuries to treat various respiratory and cardiovascular disorders [53]. Recent trials have demonstrated that intravenous forskolin exerts positive inotropic and chronotropic effects [54]. The present results suggest a mechanism whereby forskolin might exert an anti-inflammatory effect. Chemical mediators released by IgE-dependent immunological activation of mast cells and basophils probably play an essential role in the pathogenesis of allergic disorders in humans [1]. Therefore, forskolin and its diterpene analogs [21] represent a new class of drugs that may prevent antigen-induced allergic reactions by inhibiting mediator release from basophils and mast cells.

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