Abstract: Drugs that elevate cAMP levels in human basophils are known to inhibit immunoglobulin E (IgE)-mediated histamine release. We have examined whether cAMP-active agents inhibit the cytosolic Ca\(^{2+}\), [Ca\(^{2+}\)], response that normally accompanies activation of basophils. As previously described, this [Ca\(^{2+}\)]\_response is biphasic, one phase dependent on internal sources of calcium and a second later phase dependent on extracellular calcium, as observed in many cell types. Forskolin and rolipram or their combination had no effect on the initial elevation of cytosolic calcium that follows stimulation with anti-IgE antibody. In contrast, the second phase of the IgE-mediated calcium response was inhibited by these agents. For IgE-mediated responses, the relative efficacy of various cAMP active agents (rolipram = forskolin < dibutryl cAMP < forskolin + rolipram) for the inhibition of histamine release and the second-phase calcium response was similar and roughly paralleled the measured increase in basophil cAMP. In contrast, neither the first nor the second phase of the f-Met-Leu-Phe (fMLP)-induced calcium response was inhibited by any of the cAMP-active agents tested. Indeed, at low concentrations of fMLP, a combination of forskolin and rolipram caused slight enhancement of the calcium response. This result was consistent with the observations that these agents had no effect or caused slight enhancement of histamine or leukotriene release induced by fMLP. Similarly, cAMP-active agents caused no inhibition of C5a or phorbol ester (phorbol myristate acetate)-induced histamine release. These observations suggest that inhibition of the phase of the calcium response that is dependent on extracellular calcium could account for the inhibition of histamine release by these agents. However, these studies also suggested that (1) this is effect is not exerted at the level of the inositol trisphosphate (InsP\(_3\)) receptor or InsP\(_3\) metabolism and (2) the mechanisms that maintain the second-phase calcium response are possibly distinct for IgE- and fMLP-mediated reactions because cAMP-active agents inhibited the second-phase response of only one stimulus. J. Leukoc. Biol. 55: 798-804; 1994.

Key Words: basophils \_ cAMP \_ calcium \_ anti-IgE \_ fMLP

INTRODUCTION

Adenosine 3',5'-cyclic monophosphate is one of the major second messenger systems for the control of membrane signal transduction in many cell types. Before cAMP was discovered, Schild [1] had already observed that histamine release elicited by antigen on sensitized guine pig lung was inhibited by epinephrine. Its participation in the modulation of human basophils has been known since the first report of Lichtenstein and Margolis [2] describing the in vitro inhibitory effect of catecholamines and methylxanthines on antigen-stimulated mixed populations of leukocytes. Many studies published later reported that inhibition of antigen-antibody mediator release from inflammatory cells with elevated levels of cAMP [3] was a common theme. Although inhibition of histamine release from human basophils by cAMP-active agents was first described 25 years ago, the mechanisms by which cAMP elevations affect release remain unknown. However, a number of studies using murine or rat mast cells or cell lines have shown that cAMP-active agents can inhibit the mobilization of calcium that typically follows activation. For example, Foreman et al. [4] first showed that cAMP-active drugs inhibit \(^{45}\)Ca uptake in rat peritoneal mast cells. Similar results have been obtained in other studies [5, 6]. It has been shown that cAMP elevations inhibit one of the inositol trisphosphate (InsP\(_3\)) receptors present on internal cell membranes like the endoplasmic reticulum [7, 8], offering a possible mechanism by which cAMP alters calcium mobilization.

Human basophils respond to two general classes of secretagogues, the immunologically specific immunoglobulin E (IgE)-mediated (anti-IgE, antigen) pathway and receptor activation due to stimuli such as the bacterial peptide formylmethionyl-leucyl-phenylalanine (fMLP) or complement factor C5a [13-16]. Previous studies in this laboratory have shown that anti-IgE antibody and non-IgE-dependent stimuli like fMLP employ different biochemical pathways during basophil stimulation [15-18] while leading to the release of the same mediators with very different kinetics, fMLP activation being faster than anti-IgE antibody [17]. Indeed, drugs that increase cAMP levels inhibit the anti-IgE antibody-induced release of these mediators [19] but poorly inhibit the release induced by fMLP [20]. Because both of these stimuli induce calcium elevations but do not respond similarly to cAMP elevations, fMLP-mediated stimulation

Abbreviations: PIPES, piperazine-N,N'-bis-2-ethanesulfonic acid; HSA, human serum albumin; PAG, PIPES buffer (25 mM) containing human serum albumin and 0.1% glucose; PAGCM, PAG buffer containing 1 mM CaCl\(_2\) and MgCl\(_2\); EGTA, ethyleneeglycol-bis-(a-amino ethyl ether) \(\Lambda,\Lambda\) -tetraacetic acid; anti-IgE, goat polyclonal anti-IgE (PS myeloma) antibody; InsP\(_3\), inositol 1,4,5-trisphosphate; PKC, protein kinase C; fMLP, formylmethionyl-leucyl-phenylalanine; PMA, Phorbol 12-myristate 13-acetate; [Ca\(^{2+}\)]\_free cytosolic calcium.

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provides a useful internal control for any observations of an effect of cAMP elevations on the IgE-mediated calcium signal.

**MATERIALS AND METHODS**

**Buffers**

PAG: 25 mM piperazine-\(N,N\)-bis-2-ethanesulfonic acid (PIPES) (Sigma Chemical Co. St. Louis, MO), 140 mM NaCl, 5 mM KCl, 0.003% human serum albumin (Miles Laboratories, Elkhart, IN), 0.1% glucose. PAGCM: PAG with 1 mM CaCl\(_2\) and 1 mM MgCl\(_2\).

**Reagents**

Goat anti-human IgE was prepared as described previously; the antibody used for these studies represented the IgG fraction of goat serum prepared by DE-52 chromatography [21]. Fura-2AM and Fura-2 (potassium salt) were obtained from Molecular Probes. Rolipram was supplied by Dr. Theodore Torphy at Smith-Kline Beecham. Forskolin, 1,9 dideoxy forskolin (7β-acetoxy-8,13-epoxy-6β-hydroxylabd-14-en-11-one), IBMX (isobutylmethylxanthine), and dibutyryl-cAMP (N6, 2′-O-dibutyryladenosine 3′:5′ cyclic monophosphate) were purchased from Sigma Chemical Co.

**Cell preparation**

Basophils were purified from buffy coat cells, obtained from normal donors undergoing plateletpheresis/leukopheresis, using countercurrent elutriation and Percoll density gradients [22]. In several experiments, when leukopheresis packs were not available, the basophils were purified from Percoll-separated whole blood by affinity chromatography [23], as previously described. As we have not previously noted differences in the behavior of basophils purified by these two techniques, these few experiments were averaged with the majority of experiments in which basophils were purified by elutriation and Percoll gradients. For the calcium studies, the measurements of fMLP-induced mediator release, and the measurements of cellular cAMP levels, basophil purities averaged 83%, ranging from 70 to 95%. For the experiments studying cAMP agents and phorbol myristate acetate (PMA)-induced release, the basophils were enriched by a simple one-step Percoll gradient (1–2% basophils).

**Basophil counting**

Basophils and mast cells were stained with Alcian blue [22] and counted in a Spiers Levy hemocytometer.

**Fura-2AM labeling**

Basophils were labeled with 1 μM Fura-2AM for 20 min at 37°C in RPMI 1640 (Gibco) also containing 0.32 mM EDTA and 2% fetal calf serum (300,000–500,000 cells in 200 μl). After washing once with 200 μl of PAG, the cells were resuspended in 200 μl of PAG for loading in the microscope stage.

**Calcium measurements**

Cytosolic calcium changes were determined by digital video microscopy using techniques previously described in detail [18, 24]. Briefly, 15 μl of cells (20,000–30,000 cells) were loaded onto the siliconized coverslip of the microscope chamber and, after settling, overlaid with 1 ml of buffer. After warming to 37°C, monitoring of the cells was begun, and after four frames (each frame is a single ratio measurement of a field of 30–100 cells) of prechallenge calcium levels were acquired, the cells were challenged with 1 ml of stimulus in buffer and 50–150 frames of data were acquired to determine the subsequent calcium response. Note that for the cells undergoing buffer stimulation, the cells in this area experience the same concentration of stimulus at all time points and this concentration reaches its final state within 10 s [24]. Calibration of the calcium changes was described previously [18] and the same values for the constants were applied to these studies. The plots in this paper represent the average of kinetic curves obtained from several preparations of basophils that themselves were the average of the 30–100 cells viewed under the microscope. We also have found that challenge of basophils with fMLP in RPMI 1640 (Gibco), in which the pH is buffered by HCO\(_3\)-CO\(_2\), leads to the same first- and second-phase [Ca\(^{2+}\)] responses as observed in PAGCM buffers. This indicates that the second-phase [Ca\(^{2+}\)] response we observe is not an artifact of how the pH is buffered or the presence of bicarbonate ions as has been suggested in other studies [25, 26].

**cAMP measurements**

Purified basophils were treated with cAMP-elevating agents, the cells lysed by the addition of ice-cold acidified ethanol (0.9 ml of 86% ethanol, 1 M HCl, 99:1) as described previously [19]. The sample was later thawed and snap frozen in liquid nitrogen, the debris sedimented, the supernatant evaporated to dryness in a vacuum concentrator, and the samples reconstituted in assay buffer. The Amersham cAMP [21] scintillation proximity assay was used to measure cAMP. The data are reported as the stimulation index, the fold increase over basal or control levels.

**Histamine release measurements**

Histamine release for each challenge condition was directly determined by removing 1 ml of the supernatant from the microscope chamber (which contained a total of 2 ml after challenge) after the observation period was complete. Treatment with cAMP-elevating agents was accomplished by preincubating the cells for 10 min with the drug, which was included in the buffer initially overlaid on the 15-μl cell drop (as described above). The total histamine content was obtained by treating 7.5 μl of cells with 200 μl of 8% perchloric acid and bringing the volume to 1 ml with PAGCM.

Some of the histamine release measurements were made in the test tube as described previously [24]. Histamine was determined by the automated method of Siraganian [27] and histamine release was calculated as the ratio of released histamine to total histamine after subtracting spontaneous release from each value. Spontaneous release for the experiments under the microscope and the test tube ranged 5–20%.

Leukotriene release was measured in the same manner except that 100-μl aliquots were analyzed by a leukotriene C\(_4\)-specific radioimmunoassay as described previously [28, 29].

**RESULTS**

Intracellular cAMP levels were increased by several agents known to alter the activity of the adenylate cyclase or phosphodiesterase enzymes. We used 5–10 μM rolipram (RP), a
specific inhibitor of the type IV phosphodiesterase [30] that has a low $K_m$ only for cAMP, in order to avoid some of the pitfalls associated with the use of more general PDE inhibitors like IBMX. Type IV phosphodiesterase has been found to be dominant in human basophils [31]. cAMP levels were also increased with 30 μM forskolin (FK), a specific activator of the catalytic subunit of the adenylyl cyclase [32], and combinations of FK and RP. In several experiments, the effects of the nonspecific phosphodiesterase inhibitor IBMX were also examined, as well as dibutyryl-cAMP. These agents were used because they induce a relatively stable elevation in cAMP so that timing considerations (the time between the addition of agonist and stimulus) could be minimized [19]. In addition, we have found that the inhibitory effects of cAMP-active agents, including compounds that elevate cAMP through membrane receptors (histamine, fenoterol, etc.), appear diminished in purified basophils. In other words, to observe reasonable inhibition of optimally stimulated cells, agents that induce marked elevations in cAMP are required. Lower levels of stimulation could have been used, but to optimize the use of the available purified basophils, an optimal concentration of anti-IgE antibody was desirable.

Effects on IgE-mediated release

Figure 1 shows the [Ca$^{2+}$], response of basophils stimulated with an optimal concentration of anti-IgE antibody in the presence and absence of a combination of forskolin and rolipram. As we have previously described [16, 33], the activation of basophils with anti-IgE antibody results in a biphasic calcium response. An initial peak elevation is attributable to the release of internal stores of calcium, and a second phase coincides with the influx of extracellular calcium (as measured by Mn$^{2+}$ quench experiments [34]). Kinetic curves for cells challenged with buffer alone are not shown and do not show any net changes in [Ca$^{2+}$]. The four data points preceding the challenge with anti-IgE antibody are indicative of these resting levels (see Materials and Methods). The combination of FK plus RP inhibited (paired analysis of individual experiments, $P < .05$) only the second phase of the calcium response. The use of FK (30 μM) or RP (10 μM) alone or dibutyryl-cAMP (2 mM) produced similar results, although to a lesser extent. As an additional control, the use of 1,9-dideoxyforskolin, in place of forskolin, did not cause inhibition. We can also point out that the treatment with FK and RP had no effect on the time lag between the addition of stimulus and the first transient elevation in [Ca$^{2+}$]. We have described the measurement of this time lag in detail in previous studies [24, 33, 34] and a measurement of this parameter was made for each of the cells under observation. For three experiments in which the average [Ca$^{2+}$], response was inhibited 42 ± 10%, the average time lag in the absence of FK and RP was 32 ± 4 s and in their presence it was 32 ± 6 s.

For each kinetic measurement of [Ca$^{2+}$], changes, we also sampled the supernatant at the end of the challenge period to measure histamine release. Therefore, we could establish the relationship between the inhibition of the area under the curve (above resting levels) of the second phase of the calcium response (this measurement being made within the 2- to 10-min time period, during which histamine release occurs at this concentration of anti-IgE antibody) and the inhibition of histamine release. The rank order of efficacy for each of the agents tested was forskolin ≈ rolipram < dibutyryl-cAMP < (forskolin + rolipram) for both the inhibition of histamine release and the second phase of the cytosolic calcium response. On the basis of the drug used, the pattern of this relationship was also roughly related to the elevation in cAMP levels, which are shown in Figure 2. The cAMP measurements were performed as a separate series of experiments using 85-95% basophils.

We should also note that IBMX alone (200 μM) could also partially inhibit the initial transient elevation in [Ca$^{2+}$], and that a combination of FK and IBMX was the most effective inhibitor of both histamine release and the [Ca$^{2+}$] response (both the first and second phases). Direct measurements of intracellular cAMP (see Fig. 2) indicated that this effect may not be entirely related to cAMP because FK and RP together caused higher elevations in cAMP without any effect on the initial transient elevation in [Ca$^{2+}$]. In addition, the combination of IBMX with FK had little further effect on the initial [Ca$^{2+}$], transient while markedly elevating intracellular cAMP (see Fig. 2). At this time, we assume that inhibition of the initial [Ca$^{2+}$], response is due to some effect of IBMX not related to the levels of cytosolic cAMP.

Effects on non-IgE-mediated release

The foregoing experiments indicated that cAMP agents could inhibit both the second-phase [Ca$^{2+}$], response and histamine release. Based on these observations alone, we would predict that these agents would have no effect on histamine release induced by a stimulus like fMLP. This prediction results from our previous observations that histamine release occurs during the initial phase of the calcium response [34]. In contrast, leukotriene release occurs during the second phase of the calcium response [16] and might therefore be inhibited by these agents. Only partially supporting the first prediction, previous studies by other investigators have shown that 10-fold higher concentrations of dibutyryl-cAMP or 3- to 4-fold concentrations ofaminophylline are required to inhibit histamine release induced by fMLP [20]. We initially sought to verify these observations in purified cells and extend them to examine leukotriene release. In the average of seven experiments, neither forskolin (30 μM) nor rolipram (10 μM) nor combinations of both drugs induced a significant inhibition of the response of basophils to fMLP (data not shown). At any concentration of fMLP (10, 100, 300, or 1000 nM), histamine and leukotriene releases were largely unaffected by these agents. For
Fig. 2. Increase in cellular cAMP levels in human basophils treated with cAMP-elevating agents (n = 3). Purified basophils were incubated with several agents for 10 min prior to harvesting for measurement of cellular cAMP. The stimulation index (fold increase in basal CAMP levels) for IBMX (200 µM), RP (rolipram, 10 µM), FK (forskolin, 30 µM), FK + RP (forskolin, 30 µM, + rolipram, 10 µM), and FK + IBMX (forskolin, 30 µM, + IBMX, 200 µM) is shown. Resting levels of cAMP were 117 ± 46 fmol/10^6 basophils.

For example, at 1 µM fMLP, a combination of FK and RP resulted in 120 ± 21% and 110 ± 8% of control (untreated) histamine and leukotriene release, respectively (n = 7) [in cells treated only with the dimethyl sulfoxide carrier (control release), histamine release was 52 ± 9% and control leukotriene release was 7.2 ± 2.3 ng/10^6 basophils].

The absence of inhibition of leukotriene release might be understood when the effects on the calcium response are examined. Figure 3 shows the average [Ca^{2+}]_{i} responses obtained when the cells were stimulated with fMLP in the presence or absence of a combination of FK and RP. As previously described [16], activation with fMLP also generates a biphasic calcium response. Although the kinetic curves shown in Figure 3 are not clearly biphasic, this is a commonly observed curve shape. It is also an average of some curves that show a weak second peak and curves with a clear second phase. In the average control curve, the second phase of the response is not clearly above the first phase of the response but, as we have shown in previous studies, if EGTA is added with the stimulus, the curve consists only of a short-lived initial phase [16]. As we have also previously shown, adding Mn^{2+} with the stimulus results in a quenching (related to Ca^{2+} influx) only 45–60 s after the addition of fMLP, during the later phase of this [Ca^{2+}]_{i} response [16]. Unlike the case of the IgE-mediated response we found no statistically significant inhibition of either phase of the [Ca^{2+}]_{i} response (the errors noted in the figure are the standard errors of the mean of the average data but a paired statistical analysis of the individual preparations of basophils indicated no significant inhibition for this combination of drugs). It was interesting to note that in two of the nine experiments leading to the average curves shown in Figure 3 there was ≈ 30% inhibition of the second phase of the [Ca^{2+}]_{i} response by the combination of FK and RP. However, in these two experiments there was no inhibition of either histamine or leukotriene release. As with IgE-mediated release, there was also no inhibition of the initial transient [Ca^{2+}]_{i} response. Whether the second phase of the [Ca^{2+}]_{i} response has a role in mediator release for this stimulus will be discussed later, but the general lack of an effect on the second phase of the [Ca^{2+}]_{i} response was in agreement with the lack of leukotriene release inhibition with any of the drugs or combination of drugs employed (namely FK plus RP, FK plus IBMX, FK, RP, and dibutyryl-cAMP).

On the basis of studies of cAMP-dependent kinase phosphorylation of nerve cell InsP_{3} receptors, it is possible to envision situations in which high concentrations of InsP_{3} could overcome the inhibitory effect of phosphorylation [7]. With this in mind, one could hypothesize that fMLP induces elevations in InsP_{3} that far exceed those induced by anti-IgE antibody, making it difficult to observe any inhibition by cAMP-elevating agents. To address this possibility, basophils were stimulated with suboptimal concentrations of fMLP (20 and 40 nM) and the effect of a combination of FK and RP on the calcium response was measured. Figure 4 shows the results for 20 nM fMLP stimulation. There was no inhibition of the calcium response and indeed, in both experiments there was enhancement of the calcium response in FK + RP-treated cells. The results with 40 nM fMLP were similar, although the enhancement was not statistically different from the control response. These results indicate that at lower levels of fMLP stimulation, presumably causing lower levels of InsP_{3} generation, elevations in cAMP actually led to an effect opposite that occurring with IgE-mediated stimulation.

Fig. 3. Kinetics of the basophil cytosolic calcium response following challenge with fMLP. Basophils were preincubated with buffer (○) or with 30 µM forskolin and 10 µM rolipram for 10 min (□) and then challenged with 1 µM fMLP (n = 9). The error bars indicate the SEM for these experiments. Average histamine release from untreated cells was 37 ± 10% and it was inhibited 14 ± 18% in treated cells.

Fig. 4. Kinetics of the basophil cytosolic calcium response following challenge with suboptimal fMLP. Basophils were preincubated with buffer (○) or with 10 µM forskolin and 10 µM rolipram for 10 min (□), then challenged with 20 nM fMLP (n = 2). For comparison, the upper curve (△) shows the control response to 1 µM fMLP. Histamine release following 1 µM fMLP averaged 84%; following 20 nM fMLP, 26%; and following FK + RP and 20 nM fMLP, 28%.
We have postulated that an important component of IgE-mediated release in human basophils is the activation of protein kinase C [18, 22]. A drug like PMA can activate PKC in human basophils in the absence of changes in [Ca\(^{2+}\)], and yet cause marked degranulation [18], albeit at a lower rate than IgE-mediated release. Based on the foregoing studies, we would have predicted that cAMP agents would have little effect on PMA-induced histamine release, because they may act on the initial regulation of the [Ca\(^{2+}\)] response. Only histamine release was examined because PMA does not induce the release of leukotriene C\(_4\), in basophils [17]. In five experiments performed with impure basophils, release induced by lower concentrations (1, 10, or 50 ng/ml) of PMA was not inhibited by any of the cAMP-active agents tested, including a combination of FK and RP. We made the paradoxical observation that only the response to the maximum concentration of PMA tested (100 ng/ml, giving maximal release), 81 ± 4% histamine release, was inhibited with RP (to 60 ± 6% histamine release) and the combination of FK plus RP (59 ± 8% histamine release). When considering the entire dose-response curves, these results suggest that these agents produce no effect or a very small inhibition of the response elicited by PMA.

**DISCUSSION**

All the drugs used elevate cAMP for relatively long periods of time [19], and in agreement with previous data, we found inhibition of mediator release produced by immunological activation. However, it should be noted that the elevation in cAMP with these agents was quite large, especially in the context of previous studies in PT-18 mast cells, where activation of cAMP-dependent kinase (PKA) could occur in the absence of detectable increases in whole cell cAMP while inhibiting leukotriene release [6]. It should also be noted, however, that the studies reported here generally examined the effects of these agents on optimal stimulation (for the calcium studies) and as previously shown by Tung and Lichtenstein [35], lower levels of IgE-mediated stimulation are more susceptible to inhibition by cAMP-elevating agents. We have observed this effect in previous studies of purified human basophils as well [36]. We did not find any significant inhibition of mediator release due to elevated cAMP levels in cells activated with fMLP or PMA. Indeed, a breakdown of individual experiments shows that nearly any effect could be observed, from modest inhibition to modest enhancement. This source of this variation remains unclear. With an average effect showing no difference from the control response, this finding confirms previous observations concerning the different signal transduction pathways that monovalent stimuli use to activate basophils, compared with the IgE-mediated activation [16–18, 37]. The results for fMLP differ from those of previous studies [20, 38] in which an IC\(_{50}\) was given for inhibition of fMLP by aminophylline or dibutyryl-cAMP. The implication of providing an IC\(_{50}\) is that histamine release was significantly inhibited by aminophylline or dibutyryl-cAMP, whereas we have not found such inhibition with possibly more selective agents. We used reasonably high concentrations of forskolin and rolipram, and it seems unlikely that significant inhibition could be found at still higher concentrations of these drugs.

The effect of cAMP-elevating agents on the [Ca\(^{2+}\)] response was complex, although some complexity was expected because we now know that the [Ca\(^{2+}\)] response itself is not simple. Studies of human basophils have noted that the biphasic [Ca\(^{2+}\)] response probably results from the mobilization of calcium from different sources (as has been found in many other cell types). We have found that both anti-IgE antibody and iMLP induce the release of an internal store of calcium that is common for both stimuli and is relatively independent of extracellular calcium [34]. The release of this internal store of calcium can occur in the presence of EGTA or La\(^{3+}\). With the exception of IBMX, cAMP-elevating drugs had no effect on this initial transient elevation, whether the stimulus was fMLP or anti-IgE antibody. In the context of a cAMP effect that results in down-regulation of the [Ca\(^{2+}\)] response, this result might be unexpected, because recent studies have noted that at least one InsP\(_3\) receptor (nerve cell) is susceptible to inhibition by cAMP-dependent phosphorylation [8]. Although there is no direct evidence that the initial elevation in [Ca\(^{2+}\)] in human basophils results from the InsP\(_3\)-driven release of intracellular stores, it seems likely that it does. We have shown that the first phase of the [Ca\(^{2+}\)] response is an insufficient signal to initiate degranulation, so that in the context of mediator release, it may not be particularly relevant that there is little effect on the first phase of the [Ca\(^{2+}\)] response. But this leaves a mystery as to the origins of the inhibition of the second-phase [Ca\(^{2+}\)] response, since it is thought that maintenance of the second phase also requires activity of the InsP\(_3\) receptor. Indeed, the lack of an effect on the fMLP-induced second-phase [Ca\(^{2+}\)] response raises the possibility that there are separate mechanisms for the maintenance of prolonged [Ca\(^{2+}\)], responses. For example, if the second phase of the calcium response were sustained by a process similar to the capacitance model described by Putney [39], one would not immediately expect movement of calcium from the outside of the cell to the internal stores to have two different pathways. In fact, in a simple model of the second phase of the [Ca\(^{2+}\)], response, the process of influx is not directly regulated by the stimulus; it is only indirectly regulated by the maintenance of InsP\(_3\) levels, which in turn deplete the internal stores of calcium causing [Ca\(^{2+}\)], influx [40]. However, the differences between the effects of cAMP elevations on fMLP and IgE-mediated stimulation suggest otherwise. The data first suggest that cAMP elevations did not directly affect the InsP\(_3\) receptor function or InsP\(_3\) metabolism, since the IgE- and fMLP-mediated responses were different. This would lead us to upstream events, suggesting a specific effect on InsP\(_3\) generation following IgE-mediated stimulation. However, the absence of an effect on the first phase of the [Ca\(^{2+}\)], response or the lag time for the [Ca\(^{2+}\)], transient suggests that there is little effect on InsP\(_3\) generation (this possibility is being more directly tested by measuring InsP\(_3\) generation). So we are led to the possibility that maintenance of the second phase is affected by cAMP and that this maintenance pathway is under more direct control by the particular stimulus used. This conclusion is particularly puzzling because we have shown that Mn\(^{2+}\) traverses the same influx pathway stimulated by both fMLP and anti-IgE antibody [34], suggesting a similar transport process for these two stimuli.

We have recently concluded that the initial phase of the [Ca\(^{2+}\)] response is insufficient to initiate histamine release [34], and previous studies indicated that it was also insufficient to initiate leukotriene release [16]. However, we have not yet developed a clear picture of the role of the second phase of the response. These studies suggest that it may have an important role in IgE-mediated mediator release. There was a relationship between the inhibition of the second-phase response by different combinations of cAMP-elevating drugs and their inhibition of histamine release. Taken these results together with our recent observations
that IgE-mediated release does not begin until there is a calcium influx (as measured by the quenching of Fura-2 fluorescence by Mn²⁺ and the temporal association between release and the second phase of the response [34]), we presently conclude that these later elevations in cytosolic calcium reflect the mobilization of extracellular calcium and this calcium is, at a minimum, a permissive element in degranulation. But before stronger conclusions can be made, we must also understand the role of calcium oscillations in the basophil response and evaluate their origins and the effects of cAMP-elevating agents on their generation. This is particularly relevant because to some extent the average elevation in cytosolic calcium observed during the second phase of the IgE-mediated response is the result of averaging the oscillations of many individual cells [33]. Many models of calcium oscillations begin with the oscillatory release of intracellular stores of calcium, and changes in the average calcium response may reflect changes in this process, once again focusing attention on the effects of cAMP on the InsP₃ receptor or other components of the processes that release calcium from internal stores. Possibly, the initial transient is not effectively inhibited because the initial release of InsP₃ overcomes some inhibition of the InsP₃ receptor by cAMP and later oscillations involve lesser levels of InsP₃ cycling. Thus, we have begun to examine the effects of cAMP-elevating agents on calcium oscillations in basophils.

The inhibition of the first phase of the response by IBMX remains puzzling. Because the elevation of cAMP by IBMX alone is less than that seen with a combination of forskolin and rolipram, which does not affect this response, we tentatively conclude that IBMX has some other effect on the system. At the high concentrations used, IBMX is known to affect the activity of other systems, such as membrane adenosine receptors [41] or other intracellular pathways [42]. However, the fact that IBMX in combination with FK leads to better inhibition of the second phase of the Ca²⁺ response (compared to FK + RP) might be explained by the apparent inhibition of the InsP₃-driven Ca²⁺ elevation by IBMX (whatever its mechanism of action).

To summarize, the effect of cAMP-elevating agents on the calcium response that follows stimulation of human basophils with IMLP and anti-IgE antibody was examined. Only anti-IgE-mediated calcium elevations were inhibited by the drugs tested and the inhibition was generally restricted to the second phase of the calcium response. The inhibition of the calcium response was associated with inhibition of mediator release induced by anti-IgE antibody. Surprisingly, cAMP-elevating drugs have no effect on the initial release of calcium from internal stores, regardless of the stimulus. In addition, the mechanisms leading to the maintenance of the second phase of calcium response following IMLP were not sensitive to cAMP elevations, suggesting that they were different from the mechanisms for IgE-mediated stimulation.

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