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THE RELATIONSHIP BETWEEN CYCLIC AMP CHANGES AND HISTAMINE RELEASE FROM BASOPHIL-RICH HUMAN LEUCOCYTES

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Abstract—Histamine release and changes in cyclic AMP levels induced by a variety of stimuli have been measured in isolated human leucocytes from a patient with 40–70% basophilia. Adenosine and sodium fluoride induced early monophasic rises in cyclic AMP which peaked at 1 min, but they did not release histamine. 2′,5′-Dideoxyadenosine (DDA) caused a transient fall in cyclic AMP levels. Anti-IgE, polylysine and calcium ionophore A23187 induced a slow release of histamine commencing 2–5 min after addition of secretagogue. With polylysine and A23187, release was still proceeding 45 min after challenge. In contrast, the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (f-met-leu-phe) induced a rapid secretion of histamine which was complete within 2 min. Anti-IgE induced a rapid monophasic rise in cyclic AMP which reached a maximum at 45 sec and was inhibited by pretreatment with DDA. Cyclic AMP rises induced by polylysine and f-met-leu-phe were kinetically similar but smaller in magnitude. A23187 caused a later rise in cyclic AMP which peaked 3 min after challenge. A high concentration (50 μM) of compound 48/80 induced a slow cytotoxic release of histamine which was not accompanied by changes in cyclic AMP levels. The inconsistent quantitative and kinetic relationships of histamine release and cyclic AMP production suggest that changes in cyclic AMP levels may not play a key role in the biochemical events leading to mediator secretion from human basophil leucocytes.

Cyclic adenosine 3′,5′-monophosphate (cyclic AMP) plays an important role as an intracellular messenger in the regulation of many secretory processes. Pharmacological agents which elevate cellular levels of cyclic AMP, such as β-adrenoceptor agonists, methylxanthines or dibutyryl-cyclic AMP, are inhibitory to IgE-dependent release of mediators from both mast cells and basophils [1]. However, direct measurements of cyclic AMP following cell activation for histamine secretion do not support the theory of a simple inhibitory role for cyclic AMP. Stimulation of rat purified peritoneal mast cells with specific antigen, anti-rat IgE, divalent anti-IgE Fc receptor antibody or concanavalin A, all of which are thought to activate mast cells by cross-linking IgE receptors, induces an early monophasic rise in cellular cyclic AMP levels which in peaking at 5–15 sec precede histamine release [2–4]. In contrast, mediator release induced by non-immunological stimuli which by-pass the IgE-receptor, such as compound 48/80 and calcium ionophore A23187, is preceded by a fall in cyclic AMP levels [5, 6], possibly resulting from activation of calcium-dependent phosphodiesterase rather than by inhibition of adenylate cyclase [7, 8].

In basophils, the relationship between cyclic AMP and mediator release is less well-defined. In a rat basophil leukaemic cell line (RBL-1) which has a functional adenylate cyclase as demonstrated by sodium fluoride stimulation no detectable changes in cyclic AMP levels occur upon cell activation with anti-IgE or anti-IgE-Fc receptor antibody [9]. In a previous single study on basophils from a patient with basophil leukaemia, cyclic AMP levels rose 2 to 3-fold 15–30 sec after anti-IgE challenge but fell below baseline before the onset of mediator secretion 5–10 min later [10].

We have had the opportunity to study leucocytes from a subject with a 40–70% basophilia and have examined the kinetic relationship between cellular levels of cyclic AMP and histamine release following challenge of the cells immunologically and with artificial secretagogues.

MATERIALS AND METHODS

Materials. The following chemicals were used: dextran (average MW 110,000, Fisons); human serum albumin (HSA, Sigma); deoxyribonuclease (DNase, bovine pancreas, Sigma); ethylenediaminetetraacetic acid (EDTA, Sigma); trichloroacetic acid (TCA, Sigma); absolute ethanol (BDH); triethylamine (BDH); acetic anhydride (BDH); adenosine 3′,5′-cyclic phosphoric acid 2′-O-succinyl-3-[^{125}I]iodotyrosine methyl ester ([^{125}I]cyclic AMP, 600 Ci/mmole) [11]; rabbit anti-cyclic AMP-BSA serum (Miles-Yeda); bovine gamma globulin (Cohn fraction II, Sigma); polyethyleneglycol (approximate MW 4000, Sigma).

Agents used to stimulate leucocytes were: sodium fluoride (Sigma); adenosine (Sigma); 2′,5′-

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dideoxyadenosine (DDA, P.L. Biochemicals); heat-inactivated goat anti-human IgE [12]; poly-lysine (poly-l-lysine, approximately 180 residues, average MW 40,000, Sigma); formyl-methionyl-leucyl-phenylalanine (f-met-leu-phe, Sigma); calcium ionophore A23187 (Lilly); and compound 48/80 (Sigma).

The patient. The patient was a 77-year-old female who was presented with a progressive anaemia. Her haemoglobin varied between 8 and 10 g/dl and her total leucocyte count between 4.7 and 14.7×10^9/l. Differential leucocyte counts on stained blood films suggested that basophils constituted 16–20% of nucleated cells whereas electron microscopy indicated 40–70% basophils, many of which were partially degranulated. Repeated haemotological investigations failed to reveal a cause for the basophilia. At no time did blood or bone marrow analysis show evidence of a myeloproliferative disorder. After 7 months of non-specific symptoms and weight loss, a disseminated erythematous pruritis rash appeared on the trunk and limbs which was associated with an increase in the number of circulating degranulated basophils and grossly elevated plasma histamine levels (13.6–41.8 nmoles/l) assayed by a micro-enzymatic radiotransfer assay [12]. Her pruritis responded only to high doses of chlorpheniramine (64 mg/day). Fourteen months after initial presentation she developed splenomegaly, pancytopenia and died.

Separation and challenge of leucocytes. Throughout the last 9 months of her illness six venous blood samples were obtained for basophil studies. The leucocytes were separated by dextran sedimentation [11], washed three times in calcium and magnesium-free Tyrode solution containing 0.03% human serum albumin (HSA), and finally resuspended in complete Tyrode solution containing HSA and 0.02% DNase. Attempts to purify the basophils using Percol gradient centrifugation [13] were unsuccessful because of the fragile nature of the the absence of passive sensitization, the basophils were unresponsive to anti-IgE stimulation. However, after incubation for 2 hr at 37° with 10% serum from an atopic donor, challenge with anti-IgE, 1/10,000–1/30 dilutions, gave a bell-shaped concentration–response curve with a maximum histamine release of 12–15% at a 1/3000 dilution. Passively sensitized cells were used in all subsequent experiments.

To assess release of basophil histamine, duplicate 0.9 ml aliquots of leucocyte suspension, containing 5×10^5 nucleated cells, were challenged with 0.1 ml of secretagogue or Tyrode solution (spontaneous control) at 37°. Release reactions were stopped at various times after challenge by addition of 1 ml ice-cold 0.5 M EDTA, and the cell pellets and supernatants separated by centrifugation at 400 *g* at 4° for 10 min. The supernatants were acidified by addition of 0.1 ml 55% TCA before histamine estimation. Total histamine was assessed in duplicate tubes in which unchallenged cells had been disrupted by addition of 0.1 ml 55% TCA.

For measurement of cellular cyclic AMP levels, duplicate 0.45 ml aliquots of leucocyte suspension containing 3×10^6 nucleated cells were challenged by addition of 0.05 ml of secretagogue or Tyrode solution (control) at 37°. At various times after challenge, reactions were terminated by addition of 0.5 ml ice-cold ethanol and vortex-mixing. The disrupted cell debris was precipitated by centrifugation at 400 *g* for 10 min at 4° and 0.5 ml of the separated supernatant stored at −20° until assay.

Assays. Histamine was assayed spectrofluorimetrically [12]. Net basophil release of histamine induced by secretagogue was calculated as a percentage of total histamine and the value corrected for spontaneous release in the absence of secretagogue.

Cyclic AMP was measured by a single antibody radioimmunoassay [14]. Duplicate 0.1 ml aliquots of the ethanol supernatants were evaporated to dryness and the residue was dissolved in 0.1 ml of 0.05 M sodium acetate buffer (pH 6.2) at 4°. All subsequent steps were carried out at this temperature. Cyclic AMP was acetylated by addition of 5 μl of a freshly prepared mixture of triethylamine and acetic anhydride (2:1) while vortex-mixing, and the tubes were allowed to stand for 15 min. One hundred microlitres of [^{125}I]cyclic AMP was added to each tube, and the mixtures were allowed to stand for 15 min before addition of 0.1 ml of rabbit anticyclic AMP-BSA serum. After incubation for 12–18 hr at 4°, protein-bound cyclic AMP was precipitated by addition of 0.05 ml 20 mg/ml bovine gamma globulin, in sodium acetate buffer, followed 15 min later by 1 ml 16% polyethylene glycol. The precipitate was separated by centrifugation at 1500 *g* for 30 min and the radioactivity of bound [^{125}I]cyclic AMP determined using a gamma radioactivity counter (Packard). Cyclic AMP in the test samples was calculated by reference to a standard curve formed by a logit-regression analysis. The assay had a sensitivity of 15 fmole cyclic AMP with intra- and inter-assay coefficients of variation of 7 and 9%, respectively. Previous use of this assay with rat mast cells demonstrated an 83–100% recovery of cyclic AMP from cell extracts [3].

RESULTS

Demonstration of functional adenylate cyclase

The resting cyclic AMP level in unstimulated leucocytes was 7.85 ± 0.21 pmole/10^7 nucleated cells (mean ± S.E.M.) in nine experiments.

The functional integrity of the adenylate cyclase in the basophil-rich leucocytes was examined by addition of sodium fluoride, an agent which stimulates adenylate cyclase by inhibiting the hydrolysis of GTP in the regulatory-subunit of the enzyme complex [15] and of adenosine which interacts with specific cell surface receptors to stimulate adenylate cyclase and elevate cyclic AMP [16]. In a single experiment, sodium fluoride, 5 mM, induced an early monophasic rise in cellular cyclic AMP levels of 177.8% at 15 sec after addition and a smaller rise of 66.7% 1 min after stimulation (Fig. 1a). Cyclic AMP levels returned to baseline within 15 min of sodium fluoride addition. Adenosine, 100 μM in two experiments, induced a similar rise in cyclic AMP reaching a peak of 179.0 ± 26.2% above baseline at 30 sec,

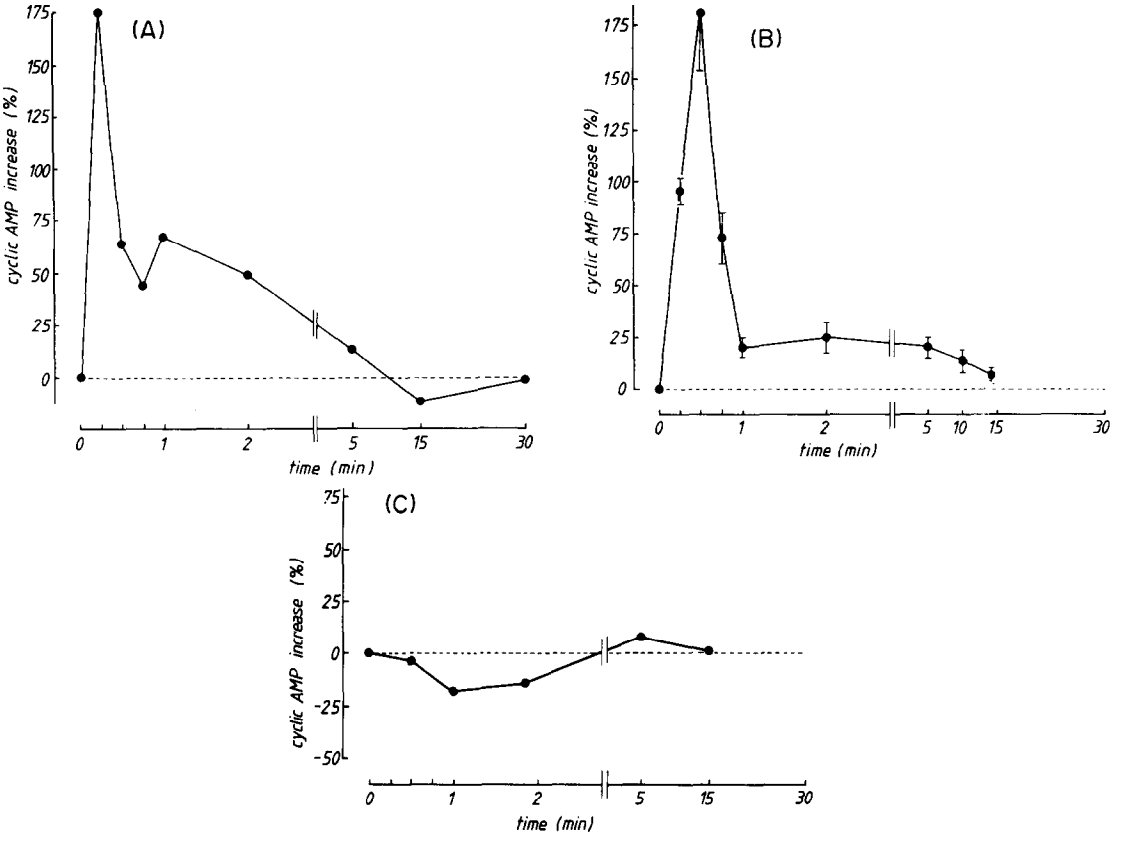

Fig. 1. Cyclic AMP changes induced by (a) sodium fluoride, 5 mM, (b) adenosine, 100 μM, and (c) DDA, 0.5 mM, in basophil-rich human leucocytes. Results for adenosine are mean ± S.E.M. of two experiments. Results for sodium fluoride and DDA are from single experiments.

after which the level rapidly returned to baseline (Fig. 1b).

In one experiment, 2',5'-dideoxyadenosine (DDA), 0.5 mM, a ribose modified purine analogue of adenosine which interacts with an inhibitory 'P'-site on the catalytic sub-unit of adenylate cyclase [16], caused a 16.7% fall in cyclic AMP levels at 1 min with baseline levels being restored by 5 min (Fig. 1c).

Sodium fluoride, adenosine and DDA all failed to induce basophil histamine release.

Anti-IgE induced histamine release and cyclic AMP changes

Incubation of basophil leucocytes with goat anti-human IgE, 1/3000 final dilution in three experiments, induced a slow secretion of histamine. This started 2 min after challenge, was maximal in rate between 2 and 15 min, and had reached 13.2 ± 2.4% of the total available histamine 45 min after stimulation (Fig. 2). Challenge with anti-IgE induced an early monophasic rise in cellular levels of cyclic AMP

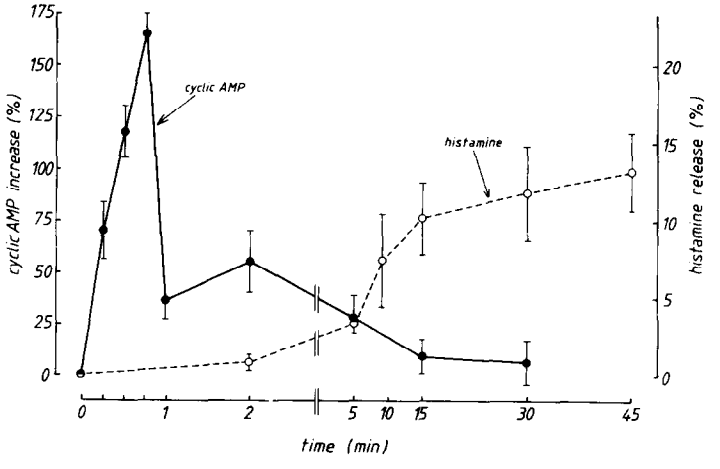

Fig. 2. Histamine release and cyclic AMP changes induced by anti-IgE, 1/3000 dilution, in basophil-rich human leucocytes. Results are mean ± S.E.M. of three experiments.

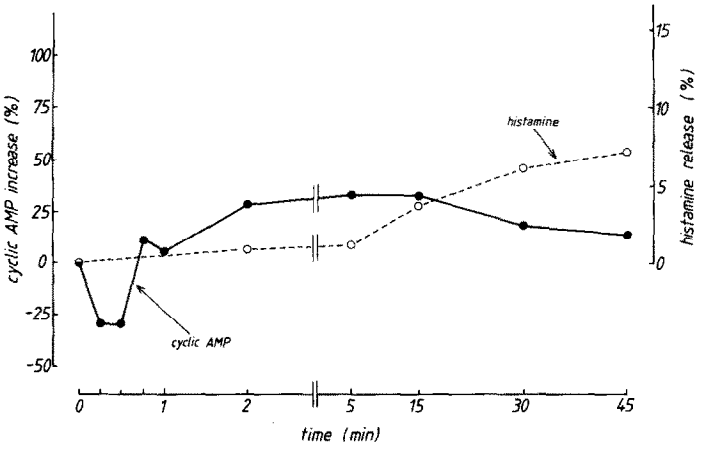

Fig. 3. Effect of 15 min preincubation with 0.5 mM DDA on histamine release and cyclic AMP changes induced by anti-IgE, 1/3000 dilution, in basophil-rich human leucocytes. Results are from a single experiment.

which reached a mean maximum of 165.0 ± 9.8% above baseline levels at 45 sec. This was followed by a smaller secondary rise of 55.0 ± 13.0% 2 min after challenge with levels falling to baseline within 15 min. At no time did cyclic AMP levels fall below baseline values after cell activation with anti-IgE.

In one experiment, preincubation of leucocytes with 0.5 mM DDA for 15 min prior to challenge reduced histamine release induced by anti-IgE, 1/3000 dilution, by 46.2% compared to untreated cells without apparent effect upon the kinetics of release (Fig. 3). Whereas anti-IgE alone stimulated an early rise in cyclic AMP (Fig. 2), this was completely abolished by DDA pretreatment (Fig. 3), only a small later rise of 33.3% being observed 5–15 min after challenge.

Histamine release and cyclic AMP changes induced by non-immunological stimuli

Polylysine, 0.1 μM in two experiments, caused a slow release of histamine which started 5 min after addition of secretagogue and was still increasing at 45 min when 15.6 ± 1.2% of the total available histamine had been released (Fig. 4a). As with anti-IgE stimulation, release of histamine was preceded by an early monophasic rise in cyclic AMP which reached a maximum of 43.7 ± 5.8% at 15 sec with levels returning to baseline within 15 min. Unlike anti-IgE, there was no later rise in cyclic AMP levels.

Basophil histamine release induced by the neutrophil chemotactic peptide f-met-leu-phe, 1 μM in two experiments, was more rapid and quantitatively greater than that obtained with either anti-IgE or polylysine (Fig. 4b). Histamine was detectable in the supernatant 15 sec after addition of the secretagogue and release was complete by 2 min. At 45 min 67.9 ± 0.7% of the cell-associated histamine had been released. F-met-leu-phe induced an early monophasic rise of cyclic AMP levels in parallel with histamine release. Cyclic AMP levels reached a maximum of 41.3 ± 7.6% at 30 sec and returned to baseline within 2 min. This was followed by a second smaller rise in cyclic AMP levels of 20.6 ± 3.9% 15 min after addition of secretagogue.

Calcium ionophore A23187, 1 μM in two experiments, induced a slow release of histamine starting 2–5 min after challenge and still progressing at 45 min when 15.2 ± 6.5% of the total available histamine had been released (Fig. 4c). However, in contrast with the other secretory stimuli, A23187 did not induce an early monophasic rise in cyclic AMP but only a later rise which reached 13.8 ± 5.1% above resting levels at 3 min. By 5 min the level of cyclic AMP had fallen to marginally below baseline, where it remained.

A high concentration of compound 48/80, 50 μg/

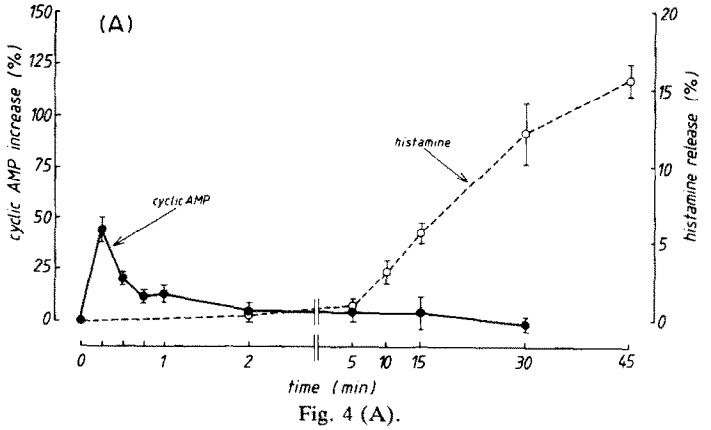

Fig. 4 (A).

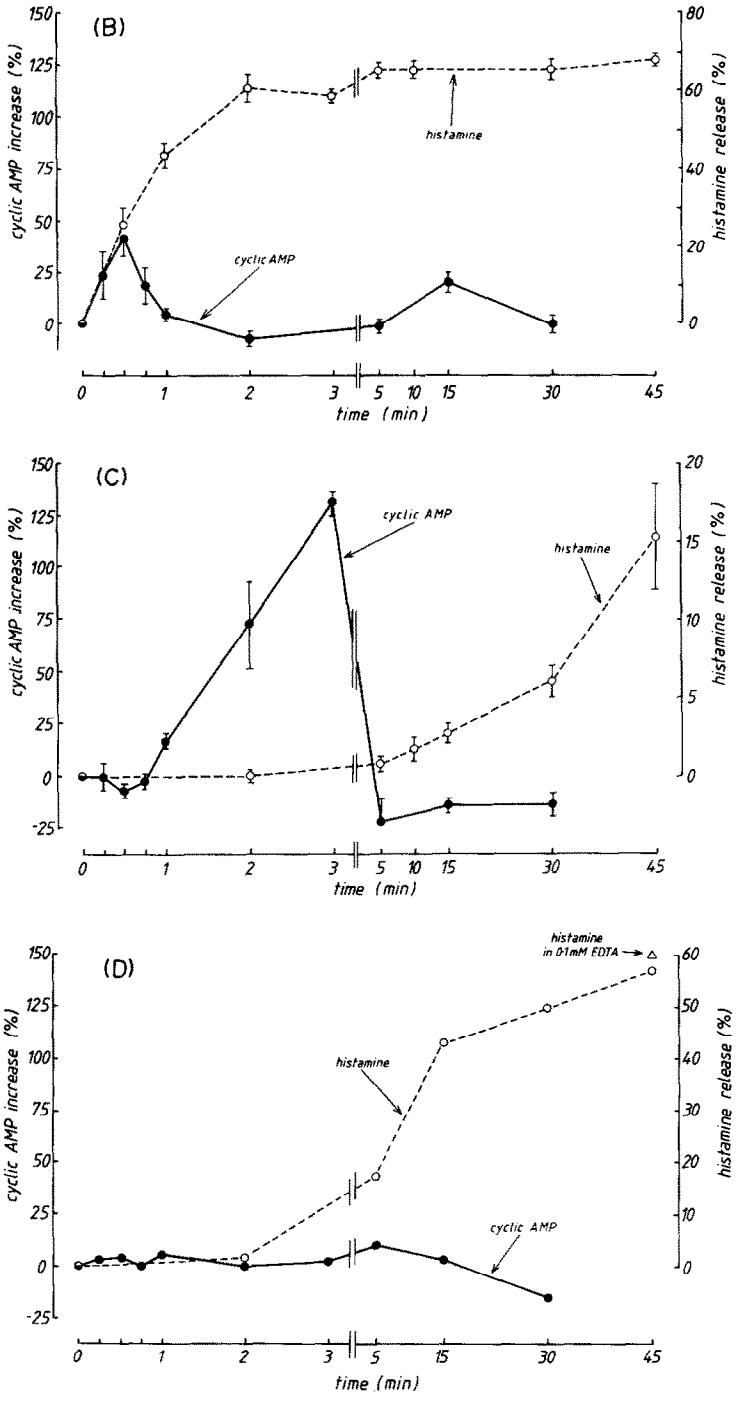

Fig. 4. Histamine release and cyclic AMP changes induced by (a) polylysine, 0.1 μM, (b) f-met-leu-phe, 1 μM, (c) calcium ionophore A23187, 1 μM, and (d) compound 48/80, 50 μg/ml, in basophil-rich human leucocytes. Results for polylysine, f-met-leu-phe and A23187 are mean ± S.E.M. of two experiments each. Results for compound 48/80 are from a single experiment in which release at 45 min was also determined in the presence of 0.1 mM EDTA.

ml, induced a slow release of histamine which at 45 min had reached 57.1% of the total available for release (Fig. 4d). Chelation of extracellular calcium with 0.1 mM EDTA failed to inhibit 48/80 induced histamine release, indicating that its mechanism was cytotoxic [11]. There were no changes in cyclic AMP levels following exposure to compound 48/80.

DISCUSSION

Using isolated leucocytes separated from the blood of a patient in which basophils constituted 40–70% of the nucleated cells, we have examined the relationship between basophil histamine release and the production of cyclic AMP using different secretory

stimuli. Our results demonstrated that there was no consistent relationship between these events, suggesting that an increase in cyclic AMP is not an obligatory event in mediator secretion from basophils.

As the leucocytes were obtained from a basophilia patient it was a necessary prerequisite to demonstrate that the basophils were morphologically and functionally similar to cells from normal donors. Electron microscopic sections showed the basophils to represent 40–70% of the leucocytes population, a percentage larger than that observed using the light microscope. The reason for this discrepancy was that many basophils were partially degranulated and were mistaken for neutrophils on light microscopy. Evidence of high plasma histamine levels, symptoms of hyperhistaminaemia and the observation that histamine content of the basophils, 0.8 $\mu g/10^6$ cells, was lower than published estimates of 0.9–1.9 $\mu g/10^6$ basophils [10, 13, 17] support this view. The cyclic AMP content of unstimulated cells, 7.85 ± 0.21 pmole/10^7. nucleated cells, agrees with published levels of 8.3 pmole/10^7 cells for leukaemic basophils [10] and 8.6 pmoles/10^7 cells for normal mixed leucocytes [18]. Freshly separated cells failed to release histamine on incubation with goat anti-human IgE, but following passive sensitization responded with the typical bell-shaped dose–response curve observed with normal basophils [19, 20]. The optimal concentration of anti-IgE for histamine release and the maximum release achieved were both lower than those observed with basophils separated from blood of normal individuals [21], although release induced by f-met-leu-phe was comparable [22]. This suggests that the basophils from the basophilia patient were immunologically immature rather than functionally unresponsive.

The presence of a functional receptor-linked adenylate cyclase system within the leucocytes was demonstrated by using sodium fluoride, adenosine and DDA. Sodium fluoride, which stimulates adenylate cyclase by inhibiting the hydrolysis of GTPase by the regulatory sub-unit of the enzyme [15], induced an early monophasic rise in cyclic AMP. Adenosine, which interacts with purinergic receptors on the cell membrane, raised rather than lowered intracellular cyclic AMP levels, thus providing evidence that the predominant receptor present in basophil-rich human leucocytes is of the A2 (or Ra site) type [16, 23]. Conversely, DDA, which inhibits adenylate cyclase by interaction with intracellular P-sites on the enzyme [16], lowered cyclic AMP levels.

The agents used to induce histamine secretion from human basophils activate the cells by different mechanisms. Immunological stimulation with anti-IgE induced a slow release of histamine which reached 13.2 ± 2.4% at 45 min. Polylysine, a polybasic peptide which induces secretion by direct interaction with the cell membrane [24, 25], produced a similar release response. F-met-leu-phe, however, which induces secretion by a mechanism closely related to that of the anaphylatoxins C_{3a} and C_{5a} [22], induced release of 70% of the available histamine within 2 min of addition. Release induced by introduction of calcium through the cell membrane by ionophore A23187 [26] commenced only after a 5–10 min lag and then proceeded only slowly. The characteristics of histamine release induced by all these agents conform to their reported activities in leucocyte preparations from normal patients [19, 20, 22, 24, 27]. In contrast, compound 48/80, a histamine releaser in rat peritoneal mast cells, does not release histamine from human basophils in low concentrations [24]. As the presence of extracellular calcium is obligatory for basophil mediator release [11], the failure of calcium deprivation to inhibit histamine release induced by 50 μg/ml of compound 48/80 in our experiments is highly suggestive of cytotoxicity, but further tests for cytotoxicity were not performed.

Anti-IgE induced an early monophasic release of cyclic AMP which reached a maximum 45 sec after challenge and a later smaller rise peaking at 2 min. These results are consistent with a previous report on anti-IgE induced cyclic AMP changes in leucocytes from a basophil leukaemia patient with 77% basophils [10]. Although our cell preparation contained many cell types capable of immunological interaction with anti-IgE including basophils [17], eosinophils [28], lymphocytes and monocytes [29], the cyclic nucleotide response was almost identical to that observed in highly purified rat peritoneal mast cells [30]. In these cells, the early rise cyclic AMP arises as a direct result of immunological stimulation of IgE-receptor linked adenylate cyclase, whereas the later rise is secondary to the action of released prostaglandins [31]. The observation that DDA blocked the early rise in cyclic AMP induced by anti-IgE in our leucocyte preparation is highly suggestive of an IgE-receptor linked stimulation of adenylate cyclase. The stimulus for the small later rise in cyclic AMP remains undetermined as granule-associated histamine, the only inflammatory mediator measured, was not released until well after this peak had occurred.

Polylysine and f-met-leu-phe also produced early monophasic rises in cyclic AMP, but the degree of nucleotide production was less than with anti-IgE despite equivalent or greater histamine release. As the cyclic nucleotide respose with f-met-leu-phe was similar to that reported for human neutrophils [32], it is possible that these cells contributed to the cyclic AMP changes observed in our basophil-rich leucocyte preparation.

In rat peritoneal mast cells, histamine release induced by A23187 is accompanied by a fall in cyclic AMP levels [6], probably occurring as a result of phosphodiesterase activation [7, 8]. In basophil-rich leucocytes we found a rise in cyclic AMP which occurred later and persisted for longer than with other stimuli. Although the mechanism of this rise is not known, it may have resulted secondarily to activation of phospholipase A_2 [33] and production of arachidonic acid metabolites from different cell types, e.g. prostaglandins E and I_2 [3, 34]. The failure of the high concentration of 48/80 to alter cyclic AMP levels indicates that neither this compound nor the cytotoxicity induced by it results in the production of this nucleotide.

The involvement of changes in cyclic AMP levels in the coupling of mast cell and basophil activation

to secretion of mediators is still debated. Our finding that at no time did the level of cyclic AMP fall below baseline following immunological challenge would refute the argument that a decrease in cyclic AMP is the stimulus for histamine release [1]. Conversely, the temporal separation of the early anti-IgE induced changes in cyclic AMP and histamine release, and the observation that adenosine and sodium fluoride did not induce histamine release despite inducing similar cyclic AMP changes suggest that cyclic AMP initiated biochemical events such as activation of protein kinases [35] are not, in isolation, the trigger for IgE-dependent mediator secretion. Furthermore, the finding that DDA completely abolished the early cyclic AMP rise induced by anti-IgE whereas it reduced histamine release by only 46% also throws doubt on the obligatory role of cyclic AMP in IgE-dependent mediator release. The inconsistent quantitative and kinetic relationship between cyclic AMP production and histamine release using a variety of stimuli leads to the conclusion that, although possibly having a modulatory function, changes in cylic AMP levels may not play a key role in the biochemical processes leading to histamine secretion from human basophils.

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