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ISOLATION OF A LIPID-SOLUBLE HISTAMINE RELEASE FACTOR FROM HUMAN PLATELETS

Amanda E. I. Proudfoot, Raffaella Gatto, Timothy N.C. Wells and Kevin B. Bacon#

Glaxo Institute for Molecular Biology, 14 Chemin des Aulx, 1228 Plan-Les-Ouates, Geneva, Switzerland

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Summary. We have isolated a histamine release factor from human platelet supernatants by heparin column chromatography, gel filtration and reversed phase HPLC. Fractions from each chromatographic step were assayed for histamine release factor activity (HRF). A peak of HRF activity was detected at a molecular mass of 60 kDa. Subsequent HPLC purification showed that the factor co-eluted with human serum albumin, but was totally extractable into the lipid phase. Comparison of biological activity with known basophil and eosinophil-activating hydroxyeicoasnoids demonstrated similar activity with 5(S)-hydroperoxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5(S)-HPETE). The identification of this lipid soluble HRF demonstrates the novel potential role of an as yet unidentified lipid as an HRF, in the absence of priming stimuli.

The release of histamine and other inflammatory mediators from human basophils and mast cells occurs in response to a variety of stimuli, including the interaction of antigen with surface-bound IgE, or following stimulation with complement components (1,2). In recent years, many reports have emerged linking cellular immune reactions with basophil and mast cell activation and histamine release, following observations that factors released from mononuclear cells and platelets were able to stimulate histamine release (histamine release factors; HRF; 3-8). While numerous cell sources have been implicated, relatively few of the active moieties have been identified. Proteins of the chemokine superfamily, including IL-8, RANTES and MCP-1 have recently been identified as having important HRF activity (9,10), and certain arachidonic acid metabolites have been shown to be active in conjunction with other priming stimuli (11,12). However, certain high molecular weight factors have also been identified as possessing HRF activity (3-6). We have therefore attempted to identify the nature of a high molecular weight HRF found in platelet supernatants.

Address all correspondence. Present Address: DNAX Institute for Molecular and Cellular Biology, 901 California Ave.,Palo Alto, California 94304.

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We found that the activity co-purifies with human albumin, but was separable only following solubilising in the lipid phase. HRF activity of the lipid factor was comparable with 5(S)-HPETE, a factor known to stimulate histamine release in conjunction with priming stimuli (11,12), however, the factor described in this report was active in isolation.

METHODS

Purification. Platelets were prepared as previously described (13). Previous studies had revealed HRF activity was found in the non-absorbing eluate from the Heparin-Sepharose chromatography. The eluate containing HRF activity was further purified on a Hiload Q 26/10 (Pharmacia) anion exchange column, equilibrated using Tris-HCl (20 mM, pH 8.5) and eluted using a gradient of NaCl from 0 - 0.8 M at 2 ml/min. The active fractions were gel-filtered on a Superdex 75 (26/10) column (Pharmacia) equilibrated in PBS. Active fractions were further purified by reversed phase HPLC on a VarioPrep Nucleosil 300-7 C8 column. Proteins were eluted with a gradient of buffer A (0.1% TFA/water) and buffer B (90% acetonitrile / 10% water / 0.1% TFA) using a gradient of 25-75% buffer B over 40 minutes, and fractions were collected manually. Peptides were sequenced using an Applied Biosystems ABI477A pulsed liquid phase sequencer.

Cells. The human basophilic cell line KU-812 was kindly provided by Dr. K. Kishi (Niigata, Japan), and cultured in RPMI 1640 medium containing 10% heat-inactivated foetal calf serum. At confluence, cells were transformed with human recombinant IL-5 (10 ng/ml; Glaxo IMB), for a minimum of 4 days (14).

Stimuli. PMA (Sigma, Switzerland) was used at a concentration of 10^{-8}M. 5(S)-hydroperoxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5(S)-HPETE), 5(S),15(S)-dihydroxy-6E,8Z,11Z,13Z-eicosatetraenoic acid (5(S),15(S)-diHETE) and 8(S),15(S)-diHETE, were obtained from Socochim (Lausanne, Switzerland). Fractions obtained following chromatography of the platelet supernatants, resuspended in PBS, were diluted in PIPES (25mM; piperazine-N,N'bis[2-ethanesulphonic-1,4-piperazine-diethanesulphonic acid]) buffer containing 1mM EDTA, for HRF assay. Human serum albumin (HSA) was diluted in PBS then PIPES buffer for assay. 10^6 KU-812 cells per treatment were resuspended in buffer containing 25 mM PIPES, 110 mM NaCl, 5 mM KCl, 0.03% HSA, 0.1% glucose, 1 mM $CaCl_2$ and 1 mM $MgCl_2$. All stimuli were added for 20 min at 37°C, the cells then rapidly centrifuged (300 x *g*, 2 min), and the supernatant removed for measurement of histamine content, as previously described (15).

Lipid fractions of the HPLC active fractions were obtained by acidification with sodium acetate (0.1M, pH 3.5) followed by three extractions into ethyl acetate (1:1:1. v/v). The residual aqueous phase waslyophilised, the lipid phase evaporated under a stream of nitrogen and then re-extracted into methanol / N-heptane (1:1:1.5, v/v). The methanolic phase was then dried under a stream of nitrogen and the residue resuspended in PBS prior to assay.

RESULTS

Anion exchange chromatography of the eluate from the heparin column displayed two major UV-absorbing profiles, which contained the main HRF activities (Figure 1). Fractions 10-31 were tested and displayed two major peaks of dilution-related HRF activity, maximal histamine release being 44±8 % of the total histamine content in the IL-5-transformed KU-812 cells (fraction 17) and 26±3 % (fraction 30). PMA (10^{-7}M) stimulated approximately 85 ± 7% total histamine release in this system (n=5; data not shown). We have concentrated on the isolation, purification and identification of the high molecular weight fraction, indicated as HRF-1. On gel filtration of the HRF 1 fraction a major UV-absorbing region corresponded to the major HRF activity (Figure 2). The

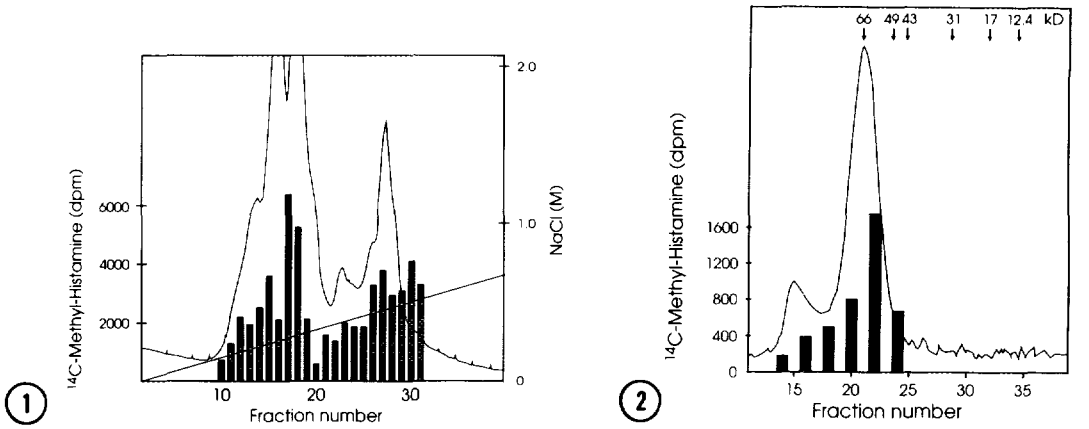


Figure 1. HRF activity in fractions obtained following anion exchange of the heparin column eluate (Hiload Q 26/10; see methods). Histograms show HRF activity obtained using 1/100 dilution of the fractions with IL-5-transformed KU-812 cells and are representative of three separate experiments.

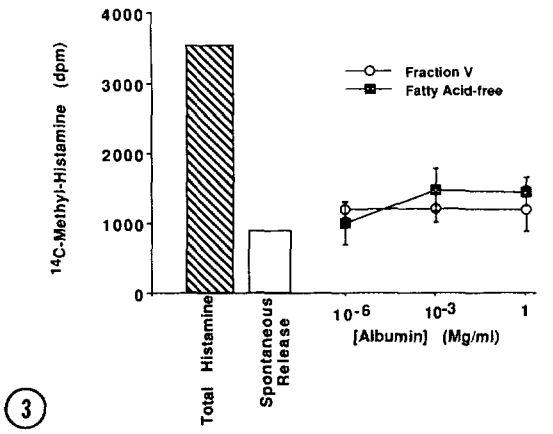
Figure 2. HRF activity obtained following gel filtration chromatography (Superdex 75; see methods) of 10% of the pooled fractions 26-31 from Figure 1. Histograms show HRF activity obtained using 1/500 dilution of the resulting fractions and are representative of four separate experiments.

pooled fraction exhibited dilution-related HRF activity, which was maximal (34±4 % of total) at 1/500 dilution. Similarly, RP-HPLC revealed a single UV-absorbing peak corresponding to maximal HRF activity (37±6 % of total histamine; 1/500 dilution; data not shown).

Sequence analysis of the fractions from gel filtration and RP-HPLC gave a single sequence DAHKSEVAHR...., which shows 100% identity with human albumin. Subsequent analysis of commercially available human serum albumin (Figure 3) for HRF activity failed to reveal biological activity. Since certain lipids have been shown to exhibit HRF activity, 1/5th of the HSA-containing fraction was subjected to solubilisation into aqueous and lipid phases. The dried or lyophilised residues were resuspended in 100 µl PBS prior to bioassay. Figure 4 shows a comparison of HRF activity in these extracts and three lipids, known to activate basophils and eosinophils, 5(S)-HPETE, 5(S),8(S)-diHETE and 5(S),15(S)-diHETE, in the absence of priming or additive stimuli. The lipid-soluble fraction from human platelet supernatants contained HRF activity (42% of total), while 5(S)-HPETE stimulated 30±3 % histamine release. This lipid-soluble HRF activity was again dilution-related, maximal activity occuring at 1/50 dilution of the original extract. The corresponding aqueous fraction following extraction, and a blank extraction of PBS contained no HRF activity.

CONCLUSION

Many reports exist concerning the presence of histamine release factors and inhibitors thereof, in the supernatants generated in mononuclear cell culture (3-6). More recently,



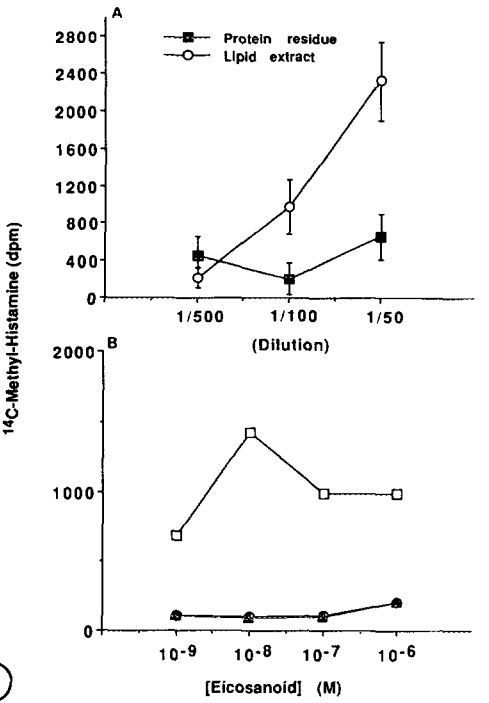


Figure 3. Analysis of HRF activity of commercial albumin. Histograms show total cell histamine and spontaneous release. Standard Fraction V and Fatty-acid free albumin (Sigma) were tested between 1 ng/ml and 1 mg/ml; n=3 experiments.

Figure 4. A) HRF activity in lipid extracts and the resulting protein residues, of the active fraction obtained from gel filtration chromatography (Figure 2). Each point represents the mean ± SE for n=4 experiments. B) HRF activity of eicosanoids 5(S)-HPETE (□), 5(S),8(S)-diHETE (▲) and 5(S),15(S)-diHETE (○). Each point represents the average from two experiments.

work has focused on platelets as a major source of such factors amongst numerous other pro-inflammatory factors (7,8). To date HRFs have been identified as proteins rangeing between 8 and 60kDa in molecular weight, the 8-12 kDa family being identified as members of the chemokine superfamily, such as IL-8, MCP-1, CTAP-III and RANTES (9,10). A few reports exist which detail similar HRF activity induced by certain arachidonic acid metabolites, such as prostaglandin D_2 and 5-HPETE (11,12). In our attempt to identify HRFs in platelet supernatants, we have observed the major activity in the high molecular weight fraction (60kDa) and have attempted to characterize it. This report shows that there appears to be a lipid-soluble factor(s) carried by HSA (since the HPLC solvent system would not allow for separation) which is capable of histamine release activity to levels of up to 40 % of total histamine content. Furthermore, these results also demonstrate that the factor(s) is an HRF in its' own right and that there is no requirement for a priming stimulus. This hypothesis is further supported by the fact that the cell line KU-812 does not express surface IgE (14), hence the histamine release observed occurs via an IgE-independent mechanism.

This study also demonstrates that 5(S)-HPETE is an HRF in the absence of priming stimuli in contrast to the earlier report of Peters et al., (11,12,16). Taken together, the ability of lipid metabolites to function as HRFs in isolation highlights the potential importance of the arachidonic acid metabolic pathway in inflammation. Leukocyte chemotaxis, enzyme release, respiratory burst, and T lymphocyte suppressor cell activity, are stimulated by certain arachidonic acid metabolites, such as LTB_4, 5-, 12-, 15-HETEs and prostaglandin E_2 (17-20), whereas IL-2-dependent T lymphocyte proliferation is inhibited by all HPETE metabolites (19). Arachidonic acid metabolite generation occurs after stimulation of leukocytes by activating stimuli and pro-inflammatory cytokines such as IL-8 and MCP-1, which appear to have numerous effects on all leukocytes (21). Therefore, although the HRF activity due to the lipid mediators is usually no greater than 40% (these experiments), it is possible to envisage positive feedback regulation, or enhancement of the stronger chemokine-induced histamine release (10) in basophils and mast cells, in a pro-inflammatory state where activated leukocytes are present.

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