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PHARMACOLOGICAL MODULATION OF CHEMOTACTIC FACTOR-ELICITED RELEASE OF GRANULE-ASSOCIATED ENZYMES FROM HUMAN NEUTROPHILS

EFFECTS OF PROSTAGLANDINS, NONSTEROID ANTI-INFLAMMATORY AGENTS AND CORTICOSTEROIDS

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Abstract—Human neutrophils demonstrated a selective release of granule-associated β-glucuronidase and lysozyme but not of cytoplasmic lactate dehydrogenase during cell contact with *N*-formyl-methionyl-leucyl-phenylalanine (FMLP). Enzyme discharge was dependent upon treatment of neutrophils with cytochalasin B prior to exposure to FMLP. Prostaglandins (PG) D_2, E_2 and I_2 inhibited enzyme release from cytochalasin B-treated neutrophils incubated with FMLP in phosphate buffered saline, pH 7.4, at 37°. Flurbiprofen, ibuprofen, indomethacin, ketoprofen and benoxaprofen reduced the extrusion of β-glucuronidase and lysozyme from FMLP-stimulated neutrophils; acetylsalicylic acid was inactive. Methylprednisolone sodium succinate, hydrocortisone sodium succinate, prednisolone sodium succinate and triamcinolone acetonide hemisuccinate also demonstrated the capacity to inhibit the selective release of granule-associated enzymes from human neutrophils. Aldosterone hemisuccinate and deoxycorticosterone hemisuccinate were inactive. These studies indicate that certain pharmacological and therapeutic agents may function to alleviate various inflammatory conditions by curtailing the extrusion of degradative enzymes from neutrophils.

Numerous substances, both particulate and soluble, have been reported to induce the release of granule-associated enzymes from neutrophilic leukocytes. In this regard, the synthetic formyl methionyl peptides represent one of the more potent classes of soluble degranulating agents yet described [1, 2]. The tripeptide, *N*-formyl-methionyl-leucyl-phenylalanine (FMLP), in addition to stimulating enzyme extrusion [1–4], has been demonstrated to elicit neutrophil aggregation [5], superoxide production [6] and chemotaxis [7]. Furthermore, the discovery of the similarities in structure between FMLP and an enzyme releasing, bacterial-derived chemotactic factor [8] indicates that FMLP represents a relevant stimulus to be utilized for the study of the regulation of granule enzyme release. The purpose, therefore, of this investigation was to evaluate the capacity of certain prostaglandins, nonsteroid anti-inflammatory agents (NSAIA) and corticosteroids to modulate granule-associated enzyme release from human neutrophils stimulated with the chemotactic peptide, FMLP.

MATERIALS AND METHODS

Preparation of neutrophils. Blood from normal human donors was drawn by venipuncture into one-tenth volume of 3.8% citrate in conical plastic tubes. Neutrophils were purified employing standard techniques of dextran sedimentation, centrifugation on Ficoll/Hypaque, and hypotonic lysis. Final cell suspensions contained a minimum of 98% neutrophils. Viability of the neutrophils was always more than 99 per cent, as determined by trypan blue exclusion.

Incubation conditions. Neutrophils (5×10^6) in 2 ml of phosphate buffered saline (PBS), pH 7.4, containing 138 mM NaCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 0.6 mM $CaCl_2$, 1.0 mM $MgCl_2$ and 0.1% glucose were incubated at 37° in a Dubnoff shaker set at 120 excursions/min according to the various procedures described under Results. After incubation, the samples were centrifuged at 750 *g* (4°) for 3 min and the clear supernatant fractions were assayed for enzyme activities. The net percentage of enzyme activity released was calculated by subtracting the percentage released in buffer alone from the percentage attributable to a given test agent.

Enzyme assays. β-Glucuronidase (EC 3.2.1.31), lysozyme (EC 3.2.1.17) and lactate dehydrogenase activities (EC 1.1.1.27) were determined as described previously [2, 9].

Drug solutions and sources. All solutions of test agents were freshly prepared and were used within 5 min. The agents employed in this study were: acetylsalicylic acid, *N*-formyl-methionyl-leucyl-phenylalanine (Sigma Chemical Co., St. Louis, MO); indomethacin (Merck, Sharp & Dohme, West Point, PA); ketoprofen (Ives Laboratories, Inc.,

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New York, NY); benoxaprofen (Eli Lilly & Co., Indianapolis, IN); flurbiprofen, ibuprofen, methylprednisolone sodium succinate, prednisolone sodium succinate, hydrocortisone sodium succinate, prostaglandins (PG) D_2, E_2 and I_2 (The Upjohn Co., Kalamazoo, MI); triamcinolone acetonide hemisuccinate (E. R. Squibb & Sons, Inc., New Brunswick, NJ); cytochalasin B (Aldrich Chemical Co., Milwaukee, WI).

All NSAIA and *N*-formyl-methionyl-leucyl-phenylalanine were dissolved in dimethylsulfoxide. Solutions of corticosteroids and mineralocorticoids were prepared in PBS except for triamcinolone acetonide hemisuccinate, which was dissolved in ethanol. Prostaglandins D_2 and E_2 and cytochalasin B were prepared in ethanol. Prostaglandin I_2 was dissolved in Tris buffer. All agents were soluble under the defined incubation conditions and they produced no alteration in pH of the incubation media. The small amounts of dimethylsulfoxide and ethanol (final concentration of 0.05%) employed as vehicles did not alter cell viability or enzyme release.

RESULTS

Release of enzymes from cytochalasin B-treated human neutrophils in the presence of FMLP. The data in Fig. 1 demonstrate that FMLP induced a time-dependent discharge of the granule-associated enzymes, β-glucuronidase and lysozyme, from cytochalasin B-treated neutrophils during 15 min of incubation, with maximum release occurring approximately 2 min after cell contact with FMLP. The absence of significant release of cytoplasmic lactate dehydrogenase is indicative of selective granule enzyme extrusion during cell exposure to FMLP.

FMLP-induced release of granule-associated enzymes from human neutrophils in the presence and absence of cytochalasin B. Neutrophils pretreated with cytochalasin B released a maximum of 24.6 ± 2.15 and 38.9 ± 3.25 per cent of total cell activity for β-glucuronidase and lysozyme, respectively, when exposed to FMLP (Table 1). Neutrophils incubated with FMLP (10^{-7} M) in the absence of cytochalasin B, however, released 2.2 ± 0.15 and 6.0 ± 0.54 per cent of total β-glucuronidase and lysozyme activity, respectively.

Effects of prostaglandins (PGD_2, PGE_2 and PGI_2) on the release of β-glucuronidase and lysozyme from human neutrophils. PGD_2, PGE_2 and PGI_2 inhibited FMLP-induced extrusion of β-glucuronidase and lysozyme from cytochalasin B-treated neutrophils (Table 2).

Effects of nonsteroid anti-inflammatory agents on the discharge of β-glucuronidase and lysozyme from human neutrophils. Flurbiprofen, ibuprofen, ketoprofen, indomethacin and benoxaprofen inhibited β-glucuronidase and lysozyme release from cytochalasin B-treated neutrophils in the presence of FMLP (Table 3). Acetylsalicylic acid had no significant effect on FMLP-induced enzyme release.

Effects of corticosteroids on β-glucuronidase and lysozyme release from human neutrophils. Methylprednisolone sodium succinate, hydrocortisone sodium succinate, prednisolone sodium succinate and triamcinolone acetonide hemisuccinate demonstrated a dose-dependent inhibition of FMLP-stimulated release of β-glucuronidase and lysozyme from cytochalasin B-treated neutrophils (Table 4). The relative effects of the corticosteroids indicate that methylprednisolone > triamcinolone acetonide > prednisolone > hydrocortisone. Aldosterone and deoxycorticosterone were inactive.

Recovery of enzyme activities from human neutrophils treated with corticosteroids, nonsteroid anti-inflammatory agents or prostaglandins. Recovery of total lysozyme and β-glucuronidase activities in excess of 95 per cent was obtained from cells and incubation media containing corticosteroids, nonsteroid anti-inflammatory agents or prostaglandins (Tables 5 and 6). Therefore, essentially all enzyme activities were accounted for after all incubations, and the presence of FMLP, cytochalasin B and the

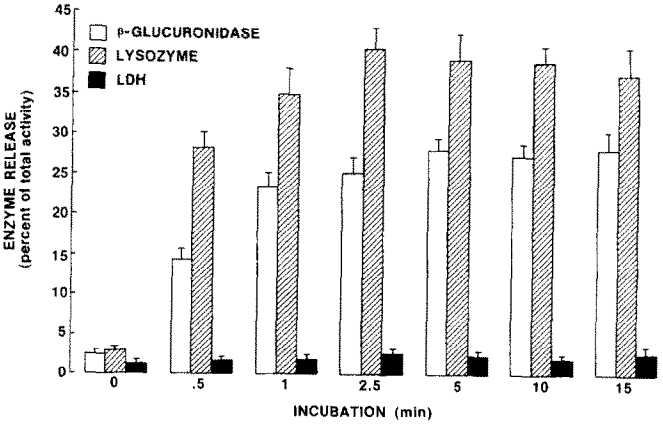

Fig. 1. *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-induced release of β-glucuronidase, lysozyme and lactate dehydrogenase from cytochalasin B-treated human neutrophils. Neutrophils (5×10^6) were preincubated with cytochalasin B (5 μg/ml) for 10 min at 37°. The cells were then incubated with FMLP (10^{-7} M) in PBS for the time periods indicated. Total cell enzyme activities were: 208.8 ± 19.5 μg phenolphthalein/18 hr/5×10^6 cells for β-glucuronidase; 30.4 ± 3.6 μg lysozyme std/3 min/5×10^6 cells for lysozyme; and 430.2 ± 39.7 absorbancy units/min/5×10^6 cells for lactate dehydrogenase. Data represent the mean ± S.E.M. of four experiments.

Table 1. Effect of cytochalasin B on *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-induced release of β-glucuronidase and lysozyme from human neutrophils

Incubation time* (min)	Enzyme release† (% of total activity)			
	With cytochalasin B		Without cytochalasin B	
	β-Glucuronidase	Lysozyme	β-Glucuronidase	Lysozyme
0	1.3 ± 0.05	0.9 ± 0.02	1.1 ± 0.07	0.2 ± 0.08
0.5	15.2 ± 1.23	29.4 ± 2.57	0.5 ± 0.05	1.3 ± 0.11
1	20.6 ± 1.54	35.5 ± 2.98	1.2 ± 0.11	2.1 ± 0.19
2.5	24.6 ± 2.15	38.9 ± 3.25	1.8 ± 0.09	3.6 ± 0.27
5	24.3 ± 1.96	38.7 ± 3.19	2.0 ± 0.14	5.0 ± 0.45
10	24.2 ± 1.75	38.6 ± 3.77	2.1 ± 0.08	5.5 ± 0.52
15	24.3 ± 2.23	38.6 ± 2.84	2.2 ± 0.15	6.0 ± 0.46

* Neutrophils (5×10^6) were preincubated with or without cytochalasin B (5 μg/ml) for 10 min at 37°. The cells were then incubated with FMLP (10^{-7} M) for the time periods indicated.

† Total cell enzyme activities were: 231.7 ± 21.6 μg phenolphthalein/18 hr/5×10^6 cells for β-glucuronidase; and 28.1 ± 2.3 μg lysozyme std/3 min/5×10^6 cells for lysozyme. Each value is the mean ± S.E.M. of three experiments.

Table 2. Effects of prostaglandins on granule enzyme release from human neutrophils*

Agent (μM)	Granule enzyme discharge (% of control)	
	β-Glucuronidase	Lysozyme
Prostaglandin D_2		
100	31.5 ± 2.7 †	33.4 ± 3.9 †
50	49.0 ± 3.8 †	46.6 ± 4.2 †
10	51.6 ± 4.9 †	49.8 ± 4.7 †
5	53.7 ± 5.6 †	53.2 ± 5.4 †
1	61.7 ± 5.9 †	54.9 ± 5.6 †
Prostaglandin E_2		
100	40.6 ± 3.9 †	32.8 ± 2.7 †
50	49.3 ± 3.7 †	51.9 ± 4.7 †
10	55.9 ± 4.8 †	57.9 ± 5.8 †
5	57.4 ± 5.3 †	63.3 ± 6.5 †
1	69.3 ± 6.4 ‡	68.5 ± 5.9 †
Prostaglandin I_2		
100	58.9 ± 4.5 †	58.2 ± 4.9 †
50	66.0 ± 5.7 †	63.2 ± 6.7 †
10	69.8 ± 7.2 †	68.1 ± 5.8 †
5	74.5 ± 7.5 ‡	74.1 ± 7.5 ‡
1	88.6 ± 7.9	96.2 ± 8.9

* Neutrophils (5×10^6) were preincubated with or without the respective agents for 5 min followed by a 10-min incubation with cytochalasin B (5 μg/ml). The cells were then incubated with *N*-formyl-methionyl-leucyl-phenylalanine (10^{-7} M) for 2 min at 37°. Control incubations yielded a value of 65.0 ± 4.6 μg phenolphthalein/18 hr/5×10^6 cells (27.1 per cent of total cell activity) for the release of β-glucuronidase and 12.1 ± 0.9 μg lysozyme std/3 min/5×10^6 cells (42.9 per cent of total cell activity) for release of lysozyme. Each value is the mean ± S.E.M. of five separate experiments.

† Significant at $P < 0.01$ vs control.

‡ Significant at $P < 0.05$ vs control.

Table 3. Effects of nonsteroid anti-inflammatory agents on granule enzyme release from human neutrophils*

Agent (μM)	Granule enzyme discharge (% of control)	
	β-Glucuronidase	Lysozyme
Flurbiprofen		
250	47.4 ± 3.8 †	42.8 ± 3.5 †
100	75.2 ± 6.7 †	63.4 ± 5.4 †
50	84.1 ± 7.4 ‡	72.8 ± 5.9 †
Ibuprofen		
250	50.3 ± 4.5 †	54.4 ± 2.5 †
100	70.0 ± 3.7 †	72.2 ± 6.2 †
50	84.9 ± 4.9 ‡	94.2 ± 8.8
Ketoprofen		
250	50.5 ± 3.4 †	48.1 ± 2.8 †
100	68.4 ± 4.5 †	66.3 ± 6.4 †
50	80.2 ± 5.6 ‡	79.4 ± 6.8 ‡
Indomethacin		
250	10.1 ± 0.4 †	10.6 ± 0.8 †
100	33.9 ± 1.9 †	29.5 ± 2.7 †
50	70.3 ± 6.8 †	73.4 ± 7.7 †
Benoxaprofen		
250	21.2 ± 1.7 †	23.5 ± 1.9 †
100	38.6 ± 1.8 †	34.5 ± 2.7 †
50	65.7 ± 5.4 †	70.3 ± 6.9 †
Acetylsalicylic acid		
250	90.4 ± 6.8	89.9 ± 8.8
100	91.3 ± 8.9	92.5 ± 9.1
50	95.4 ± 7.8	96.4 ± 8.6

* Neutrophils (5×10^6) were preincubated with cytochalasin B (5 μg/ml) for 5 min followed by a 5-min incubation with or without the respective agents. The cells were then incubated with *N*-formyl-methionyl-leucyl-phenylalanine (10^{-7} M) for 2 min at 37°. Control incubations yielded a value of 75.2 ± 6.4 μg phenolphthalein/18 hr/5×10^6 cells (32.7 per cent of total cell activity) for release of β-glucuronidase and 13.4 ± 1.4 μg lysozyme std/3 min/5×10^6 cells (41.4 per cent of total cell activity) for release of lysozyme. Each value is the mean ± S.E.M. of three separate experiments.

† Significant at $P < 0.01$ vs control.

‡ Significant at $P < 0.05$ vs control.

Table 4. Effects of corticosteroids on granule enzyme release from human neutrophils*

Agent (μM)	Granule enzyme discharge (% of control)	
	β-Glucuronidase	Lysozyme
Methylprednisolone sodium succinate		
250	4.8 ± 0.3 †	23.8 ± 1.1 †
100	35.5 ± 1.7 †	38.6 ± 2.1 †
50	58.7 ± 4.5 †	63.5 ± 4.3 †
10	84.9 ± 7.8 ‡	94.8 ± 8.9
Triamcinolone acetonide hemisuccinate		
250	21.3 ± 1.2 †	30.5 ± 1.5 †
100	56.1 ± 2.8 †	59.2 ± 3.4 †
50	68.8 ± 4.2 †	72.5 ± 3.8 †
10	83.8 ± 6.2 ‡	93.3 ± 9.1
Prednisolone sodium succinate		
250	66.6 ± 5.4 †	64.2 ± 5.9 †
100	71.1 ± 7.4 †	64.5 ± 6.3 †
50	74.3 ± 6.7 ‡	71.5 ± 7.4 †
10	78.7 ± 7.5 ‡	84.3 ± 8.1 ‡
Hydrocortisone sodium succinate		
250	68.4 ± 6.9 †	66.3 ± 6.5 †
100	77.4 ± 8.1 †	71.8 ± 7.3 †
50	83.7 ± 8.4 ‡	74.4 ± 6.8 ‡
10	85.7 ± 7.9	78.8 ± 7.8 ‡
Aldosterone hemisuccinate		
100	93.4 ± 9.1	92.3 ± 8.7
50	95.8 ± 7.9	98.5 ± 6.6
Deoxycorticosterone hemisuccinate		
100	95.3 ± 8.4	94.4 ± 9.9
50	99.4 ± 9.5	92.7 ± 7.5

* Neutrophils (5×10^6) were preincubated with or without the respective agents for 20 min followed by a 10-min incubation with cytochalasin B (5 μg/ml). The cells were then incubated with *N*-formyl-methionyl-leucyl-phenylalanine (10^{-7} M) for 2 min at 37°. Control incubations yielded a value of 53.9 ± 5.1 μg phenolphthalein/18 hr/5×10^6 cells (29.7 per cent of total cell activity) for release of β-glucuronidase and 14.6 ± 1.2 μg lysozyme std/3 min/5×10^6 cells (47.1 per cent of total cell activity) for release of lysozyme. Each value is the mean ± S.E.M. of three separate experiments.

† Significant at $P < 0.01$ vs control.

‡ Significant at $P < 0.05$ vs control.

aforementioned test agents did not alter enzyme activities appreciably.

DISCUSSION

The neutrophil represents one of the principal effector cells observed in numerous inflammatory diseases. In response to various stimuli, neutrophils discharge the contents of their cytoplasmic granules into the extracellular space. The enzymatic constituents of these granules, having been released, cause much of the tissue injury associated with the inflammatory process [10–12]. Therefore, the abrogation of neutrophil degranulation would serve to curtail the enzymatic degradation of such tissue components as proteoglycan [13, 14] and collagen [15].

The data presented here show that prostaglandins D_2, E_2 and I_2 inhibit the discharge of β-glucuronidase and lysozyme from neutrophils in contact with FMLP. These results suggest that prostaglandins may function to attenuate the release of deleterious constituents from neutrophils that are known to mediate tissue injury [10, 16]. However, to our knowledge this report demonstrates, for the first time, the capacities of PGD_2 and PGI_2 (prostacyclin) to curtail enzyme release from neutrophils.

There have been numerous reports on the possible role(s) of prostaglandins as mediators of the inflam-

Table 5. Recovery of enzyme activities from human neutrophils treated with prostaglandins

Experimental condition*	Per cent recovery	
	β-Glucuronidase	Lysozyme
5×10^6 Neutrophils (N)	100.5 ± 4.8	100.2 ± 8.5
N + cytochalasin B (CB) + FMLP	100.7 ± 6.6	100.7 ± 4.5
N + prostaglandin D_2 (100 μM) + CB + FMLP	95.6 ± 7.6	100.2 ± 6.9
N + prostaglandin E_2 (100 μM) + CB + FMLP	95.6 ± 6.5	100.5 ± 5.2
N + prostaglandin I_2 (100 μM) + CB + FMLP	100.8 ± 5.5	100.3 ± 3.7

* Incubations of neutrophils were conducted at 37° for 15 min with or without the respective agents [drug, cytochalasin B (5 μg/ml)] and for an additional 2 min with FMLP (10^{-7} M). Samples were then incubated further with Triton X-100–0.04 M Tris acetate, pH 7.4, for 15 min, centrifuged, and the supernatant fractions were then assayed for enzyme activities. Recoveries of enzyme activities were calculated on the basis of control values of total enzyme activities. Values for control total cell enzyme activities were: β-Glucuronidase, 270.4 ± 21.6 μg phenolphthalein/18 hr/5×10^6 cells and lysozyme, 24.9 + 1.2 μg lysozyme std/3 min/5×10^6 cells. Each value is the mean ± S.E.M. of duplicate experiments.

Table 6. Recovery of enzyme activities from human neutrophils treated with corticosteroids or nonsteroid anti-inflammatory agents

Experimental condition*	Per cent recovery	
	β-Glucuronidase	Lysozyme
5×10^6 Neutrophils (N)	100.8 ± 6.5	100.2 ± 9.3
N + cytochalasin B (CB) + FMLP	100.7 ± 5.5	100.5 ± 8.6
N + methylprednisolone sodium succinate (250 μM) + CB + FMLP	100.4 ± 8.8	97.5 ± 7.4
N + hydrocortisone sodium succinate (250 μM) + CB + FMLP	100.8 ± 6.7	100.3 ± 8.5
N + prednisolone sodium succinate (250 μM) + CB + FMLP	100.6 ± 9.2	98.5 ± 8.4
N + triamcinolone acetonide hemisuccinate (250 μM) + CB + FMLP	98.2 ± 7.5	100.5 ± 6.8
N + acetylsalicylic acid (250 μM) + CB + FMLP	100.4 ± 9.3	100.6 ± 8.9
N + benoxaprofen (250 μM) + CB + FMLP	100.6 ± 6.3	100.1 ± 5.9
N + flurbiprofen (250 μM) + CB + FMLP	100.8 ± 8.2	100.9 ± 7.3
N + ibuprofen (250 μM) + CB + FMLP	100.2 ± 5.7	100.3 ± 6.5
N + indomethacin (250 μM) + CB + FMLP	100.8 ± 7.5	100.5 ± 6.3
N + ketoprofen (250 μM) + CB + FMLP	100.7 ± 8.2	100.3 ± 4.9

* Incubations of neutrophils were conducted at 37° for 30 min with or without corticosteroids and cytochalasin B (5 μg/ml) or 10 min with or without nonsteroid anti-inflammatory agents and cytochalasin B (5 μg/ml) and for an additional 2 min with FMLP (10^{-7} M). Samples were incubated further with Triton X-100–0.04 M Tris acetate, pH 7.4, for 15 min, centrifuged, and the supernatant fractions were then assayed for enzyme activities. Recoveries of enzyme activities were calculated on the basis of control values of total enzyme activities. Values for control total cell enzyme activities were: β-Glucuronidase, 207.5 ± 15.4 μg phenolphthalein/18 hr/5×10^6 cells and lysozyme, 27.6 ± 0.9 μg lysozyme std/3 min/5×10^6 cells. Each value is the mean ± S.E.M. of duplicate experiments.

matory process. For example, Willoughby and DiRosa [17] found that prostaglandin-like substances were associated with various experimentally induced models of inflammation. Willis [18] showed that prostaglandin E_2 could be isolated from inflammatory exudates in the rat. Conversely, our data, together with the findings of others showing that prostaglandins inhibit phagocytosis [19–21], lymphokine secretion [22], and histamine release from basophils [23, 24], suggest that prostaglandins may function as modulators of the inflammatory process by curtailing the extrusion of mediators of inflammation and regulating other inflammatory cellular activities.

The capacity of FMLP to stimulate granule enzyme release from human neutrophils was also inhibited by the therapeutically effective NSAIA, flurbiprofen, ibuprofen, ketoprofen, indomethacin and benoxaprofen; acetylsalicylic acid was inactive. The rank order of effectiveness of these agents (indomethacin > benoxaprofen > ketoprofen > flurbiprofen = ibuprofen) correlates with their ability to suppress the inflammatory process in experimental models of inflammation [25–28] in which granule-associated enzymes have been localized at the site of tissue injury [29, 30]. The mechanism(s) by which NSAIA retard FMLP-elicited enzyme release, however, remains conjectural. We reported previously that NSAIA inhibited calcium inophore (A23187)-elicited enzyme release by interfering with calcium association with cells [31]. We have also indicated that FMLP-stimulated release of granule enzymes from human neutrophils requires extracellular calcium [2]. Because calcium has been demonstrated to play an integral role in membrane fusion [32–34], it is possible that NSAIA inhibit FMLP-induced enzyme release by modulating the association of calcium with neutrophils. Our data appear to correspond with and support the findings which demonstrate that NSAIA inhibit FMLP-elicited superoxide radical generation [35], which is a calcium-dependent process [6, 36]. In addition, NSAIA have been shown to curtail phagocytosis [9, 21] and chemotaxis [37], which are calcium-mediated events [7, 38]. These data, together with our findings, suggest that NSAIA, in addition to inhibiting prostaglandin synthesis, may exert their anti-inflammatory effects by regulating other activities of inflammatory cells.

The nonsteroid anti-inflammatory agents employed in this study were evaluated at concentrations that may be somewhat higher than the free plasma levels achieved in man with therapeutic doses of these drugs. The free plasma levels of these agents, however, may not be indicative of the effective doses of these drugs. In this regard, Graf *et al.* [39] and Brune *et al.* [40] showed that the concentration of acidic nonsteroid agents was three times greater in the inflammed area of a carrageenan-induced inflammatory reaction than the control values, which signifies preferential accumulation of drug at the site of inflammation. Furthermore, agents such as indomethacin are bound to plasma proteins in excess of 90 per cent [41]. Whether or not the pK_a and/or structure of these agents, as well as the pH at the site of inflammation, contribute to the concentration of these drugs in the diseased area [39, 40, 42, 43] remains a subject of speculation at the present time. The concentrations of prostaglandins (1–10 μM) demonstrated to inhibit enzyme release may also parallel the concentrations achieved at the site of tissue injury. Willis [18] has reported microgram amounts of prostaglandins in inflammatory exudates; and neutrophils [44], as well as macrophages [45], have been demonstrated to synthesize and release prostaglandins. The accumulation of these cell types at the site of an inflammatory reaction could facilitate the attainment of a concentration of prostaglandins that would surpass the blood levels of these agents. Therefore, the concentrations of nonsteroid anti-inflammatory agents and prostaglandins required to effectively curtail granule enzyme release may approximate the concentrations of these agents at their sites of action *in vivo*.

We report here that methylprednisolone sodium succinate, hydrocortisone sodium succinate, prednisolone sodium succinate and triamcinolone acetonide hemisuccinate inhibit FMLP-induced release of β-glucuronidase and lysozyme from human neutrophils. The relative effectiveness of these corticosteroids (methylprednisolone > triamcinolone acetonide > prednisolone > hydrocortisone) in inhibiting enzyme extrusion approximates the rank order of anti-inflammatory activity of these agents in human and laboratory animals. Therefore, the effects of these agents on an inflammatory cell such as the neutrophil appear to correlate with their therapeutic effects. It is relevant to note that aldosterone hemisuccinate and deoxycorticosterone hemisuccinate, which are mineralocorticoids and do not possess anti-inflammatory activities, had no significant effect on granule enzyme release. Therefore, the curtailment of enzyme discharge appears to be specific for those steroids demonstrating anti-inflammatory activity. These data are supported by reports demonstrating that corticosteroids suppress other mechanisms of degranulation from human neutrophils [46–49].

Numerous hypotheses have been presented concerning the mechanism(s) by which corticosteroids abrogate granule enzyme release. In this regard, it is important to note that a soluble stimulus such as FMLP appears to elicit enzyme discharge via exocytosis, as opposed to phagocytic release described by Weissmann *et al.* [19]. Exocytosis entails the translocation of cytoplasmic granules to the plasmalemma, whereby an interaction and subsequent fusion of plasma and granule membranes occur, resulting in the discharge of granule constituents. Corticosteroids, because of their lipophilicity, may complex with the plasma and/or granule membranes and interfere with the fusion process, which would lead to inhibition of exocytosis. This effect of corticosteroids could be related to their capacity to stabilize granule membranes and thus prevent the release of the granule constituents [50–52].

We have demonstrated that the chemotactic peptide, FMLP, induces selective extracellular release of granule-associated enzymes from human neutrophils. The observation that enzyme discharge is dependent upon the pretreatment of cells with cytochalasin B is consistent with and confirms, with

human neutrophils, reports concerning rabbit neutrophils [1], as well as indicating that FMLP-elicited extrusion of granule-associated enzymes is analogous to the mechanism of frustrated endocytosis [53].

In summary, we have described the capacity of naturally occurring prostaglandins to curtail granule enzyme release from human neutrophils resulting from cell contact with the tripeptide, FMLP. We have also indicated that certain therapeutically effective corticosteroids and nonsteroid anti-inflammatory agents have the capacity to inhibit FMLP-induced granule enzyme release. Furthermore, the recovery of total β-glucuronidase and lysozyme activities in excess of 95 per cent, from neutrophils treated with the respective agents, indicates that these agents exert selective effects on enzyme release. The fact that these agents can modulate the activity of FMLP, which is structurally and functionally similar to an endogenous mediator of inflammation, suggests that degradative enzyme discharge stimulated with FMLP represents a valuable model for both the development of more effective therapeutic agents and the elucidation of the mode of action of existing drugs on the cellular process of exocytosis. In addition, an evaluation of the activities of endogenous participants in inflammation, such as prostaglandins, may enhance our knowledge of the pathogenesis of various inflammatory diseases.

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