Stimulation of Human Neutrophil Leukocyte Aerobic Glucose Metabolism by Purified Chemotactic Factors

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ABSTRACT The interaction of human neutrophils adherent to plastic petri dishes with the purified chemotactic factors C5a and kallikrein increased their rate of aerobic glycolysis 25–120% and the activity of their hexose monophosphate shunt (HMPS) 100–600%, reaching a plateau after 2 hr at 37° C. The stimulation of either pathway required a chemotactically active stimulus since neither C5 nor prekallikrein or inactivated kallikrein could enhance metabolic activity. Marked suppression of the neutrophil chemotactic response by preincubation with a chemotactic factor to achieve deactivation, 5×10^{-7} M diisopropyl fluorophosphate, or the neutrophil immobilizing factor (NIF) did not prevent the stimulation of HMPS activity or glycolysis by chemotactic factors. The metabolic inhibitors iodoacetate and 6-aminonicotinamide at concentrations which blocked enhancement of glycolysis or HMPS activity, respectively, partially suppressed the chemotactic response of neutrophils to the chemotactic factors. The capacity of a chemotactic factor to stimulate glucose metabolism of human neutrophils is associated with a maximal chemotactic response, but this stimulation is not alone sufficient for chemotaxis.

INTRODUCTION

The overall rate of consumption of glucose by polymorphonuclear leukocytes, its metabolism by the glycolytic pathway, and especially its degradation by way of the hexose monophosphate shunt (HMPS)[1] are markedly increased during phagocytosis (1, 2). Not only phagocytosis by polymorphonuclear leukocytes but also their directed migration in response to specific chemotactic factors is suppressed by inhibitors of glucose metabolic pathways (3, 4). Both these leukocyte functions are also inhibited by other agents which influence glucose transport or metabolism but act at diverse sites, such as colchicine (5, 6), corticosteroids (7, 8), and cytochalasin B (9, 10). In contrast, the neutrophil immobilizing factor (NIF), which prevents both random and directed mobility without affecting leukocyte adherence or phagocytic capacity, does not prevent the stimulation of the HMPS by an active chemotactic principle (11). These studies have been extended to show that neutrophil glycolytic activity is increased by activation with a chemotactic factor and to compare the effects of NIF, diisopropyl fluorophosphate (DFP), and deactivation by chemotactic factors, which all suppress chemotaxis, on both HMPS and glycolytic response to chemotactic factor stimulation.

METHODS

Acrylic radiochemotactic chambers (Neuro Probe, Inc., Bethesda, Md.) were assembled with 3-μ and 8-μ pore size micropore filters (Millipore Corp., Bedford, Mass.) as previously described (12). Hanks' solution and Medium 199 with or without phenol red (Microbiological Associates, Inc., Bethesda, Md.), ovalbumin five times recrystallized (Miles-Seravac, Kankakee, Ill.), dextran, Sephadex, and Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), sodium diatrizoate (Hypaque, Winthrop Laboratories, N. Y), two times recrystallized trypsin and soybean trypsin inhibitor (Worthington Biochemical Corp. Freehold, N. J.), sodium [^{51}Cr]chromate, [1-^{14}C]glucose, and [6-^{14}C]glucose (Amersham-Searle Corp., Arlington Heights, Ill.), sodium lauryl sulfate and (L)-ascorbic acid (Fisher Scientific Co., Medford, Mass.), lactic acid dehydrogenase

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[1] *Abbreviations used in this paper:* ANIF, NIF stimulated by acid pH; AU, absorbency units; DFP, diisopropyl fluorophosphate; ENIF, NIF stimulated by endotoxin followed by low K^+ medium incubation; HMPS, hexose monophosphate shunt; KRPG-ovalbumin, Krebs-Ringer phosphate glucose solution made 0.1 g/100 ml in ovalbumin; LDH, lactic acid dehydrogenase; NAD, nicotinamide adenine dinucleotide; NIF, neutrophil immobilizing factor; PhNIF, NIF formed during phagocytosis; SBTI, soybean trypsin inhibitor; SLS, sodium lauryl sulfate; TCA, trichloroacetic acid.

(LDH), (L)-lactic acid as a 0.40 mg/ml solution, and nicotinamide adenine dinucleotide (NAD) (Sigma Biochemicals Inc., St. Louis, Mo.), diisopropyl fluorophosphate (Aldrich Chemical Co., Inc., Milwaukee, Wisc.), 6-aminonicotinamide (Mann Research Laboratories, Orangeburg, N. Y.), iodoacetate (Eastman Kodak Co., Rochester, N. Y.), and plastic 35 × 10 mm petri dishes (Falcon Plastics, Oxnard, Calif.) were obtained from the manufacturers. Rice starch (Whittaker, Clark and Daniels, Inc., Plainfield, N. J.) was washed five times in distilled water, incubated with autologous fresh human plasma for 1 h at 37°C, and washed again three times in saline before use.

NIF was derived from 1×10^7 human leukocytes which had been incubated at 37°C in 1 ml of buffer alone (control for NIF), or stimulated by acid pH (ANIF) or by endotoxin followed by low K^+ medium incubation (ENIF), or engaged in phagocytosis (PhNIF), and was partially purified by heating and by gel filtration on Sephadex G-25 (13).

Gamma radiation from ^{51}Cr-containing micropore filters was measured with a dual channel gamma well counter, and beta radiation from [^{14}C]glucose solutions was quantitated with Brays fluid in a liquid scintillation counter (Nuclear-Chicago, Des Plaines, Ill.). Lactate concentration in leukocyte supernatants was measured by precipitation of the protein with trichloroacetic acid (TCA) (Fisher Scientific Co., Medford, Mass.) at a final concentration of 5%, centrifugation at 400 *g* for 10 min, and addition of 0.5 ml of the 2.0 ml clear supernatant solution to 2.2 ml of reagent solution containing 0.05 ml LDH, 1.15 ml distilled water, 5 mg NAD, and 2.0 ml 0.2 M glycine buffer (pH 9.1). After a 30 min incubation of each reaction mixture at 37°C, the optical density at 340 nM was measured against a blank containing 2.2 ml of reagent solution and 0.5 ml 5% TCA, and the lactate concentration was derived from a standard curve obtained with (L)-lactate in 5% TCA at concentrations from 1 to 10 mg/100 ml (14). Phagocytosis by human neutrophils of starch particles previously incubated in serum was carried out as previously described (13).

Leukocyte chemotaxis. Chemotaxis of human leukocytes was assayed by a radiochemotactic method (12) which utilizes Boyden chambers (13, 15) modified to hold a layer of two micropore filters of 8 μ and 3 μ pore size between the ^{51}Cr-labeled leukocyte suspension containing $5–7 \times 10^6$ leukocytes per ml and the chemotactic stimulus. Human peripheral leukocytes from normal donors were isolated from citrated whole blood by dextran sedimentation of erythrocytes and lysis of residual erythrocytes in 0.84% NH_4Cl (13). Neutrophils and mononuclear leukocytes were purified by centrifugation on Ficoll-Hypaque cushions (16). The chemotactic factors employed were human C5a, generated by tryptic digestion of highly purified C5 (17), and human kallikrein, isolated directly or produced from purified prekallikrein by activation with purified Hageman factor fragments (18, 19). Kinin-generating activity was quantitated by incubation of 10–20 μl of the kallikrein solution with 0.2 ml heat-inactivated plasma with assessment of the bradykinin generated by its ability to contract the guinea pig ileum utilizing a purified bradykinin standard (Sandoz Pharmaceuticals, Basel, Switzerland) (18). The chemotactic chambers were incubated for 4–4½ h at 37°C, and the neutrophil chemotactic response in duplicate chambers was calculated as net percent radioactivity = Rs-Rc/Rt-Rc, where Rs represents the counts per 4 min in the bottom filter of stimulated chambers, Rc the counts per 4 min in the bottom filter of control chambers without a stimulus, and Rt the counts per 4 min in the initial 0.5 ml of leukocyte suspension added to each chamber.

Glucose metabolism by adherent leukocytes. 1 ml portions of suspensions of leukocytes or purified neutrophils containing $4–6 \times 10^6$ neutrophils in Hanks' solution without added protein were layered in duplicate 35-mm diameter plastic petri dishes and incubated for 30 min at 37°C. The nonadherent leukocytes were decanted, and the adherent leukocytes were washed with three 2-ml portions of Hanks' solution and covered with 1 ml of Krebs-Ringer phosphate glucose solution (20) made 0.1 g/100 ml in ovalbumin (KRPG-ovalbumin).

The activity of the HMPS of adherent leukocytes was determined by measuring the rate of conversion of [1-^{14}C]-glucose to $^{14}CO_2$. After the addition of 0.4 μCi of [1-^{14}C]-glucose, the adherent neutrophils were interacted with C5a or kallikrein, or allowed to phagocytose starch particles for varying time intervals at 37°C. The reaction was stopped and $^{14}CO_2$ driven off into the gaseous phase in the sealed dishes by addition of three drops of 2 N HCl. The $^{14}CO_2$ trapped in the ensuing 10 min in a 2 N NaOH-saturated pledget was quantitated by use of 10 ml of Brays solution per vial in a liquid scintillation counter. The counts per minute (cpm) of the $^{14}CO_2$ from each dish were divided by the optical density at 280 nM of a solution of the adherent leukocytes in 3% sodium lauryl sulfate (SLS) to arrive at the cpm per 0.2 absorbency unit (AU) 280. Stimulation of the HMPS expressed as net cpm/0.2 AU 280 was calculated by subtraction of the mean value for duplicate unchallenged dishes from the mean value for stimulated dishes. No correction was made for conversion of [1-^{14}C]glucose to $^{14}CO_2$ by other pathways, since the liberation of $^{14}CO_2$ from [6-^{14}C]glucose did not exceed 5% of that from [1-^{14}C]glucose (21).

The glycolytic activity of adherent neutrophil layers was assessed by measuring the lactate concentration in KRPG-ovalbumin after varying incubation intervals at 37°C with the LDH assay (14) described above. 0.5 ml KRPG-ovalbumin was mixed with an equal volume of 10% TCA to stabilize the lactate concentration. The lactate concentration expressed as micrograms lactate per dish was standardized using the AU 280 of an SLS solution of the adherent leukocytes in each dish. Stimulation of neutrophil glycolytic activity expressed as net micrograms lactate per 0.2 AU 280 was calculated by subtraction of the mean value for duplicate unchallenged dishes from that for duplicate stimulated dishes.

The effect of DFP, NIF, metabolic inhibitors, or chemotactic factors employed to achieve deactivation on the glucose metabolic rates of neutrophil layers and chemotactic activity of neutrophil suspensions was expressed as a percent of the respective activity observed with cells preincubated with buffer alone or with an identical dilution of control for NIF. The influence of DFP and soybean trypsin inhibitor (SBTI) on the capacity of kallikrein to stimulate metabolic and chemotactic activities was expressed as a percent of that stimulation seen with native kallikrein at an identical concentration. Each experiment was performed at least three times utilizing leukocytes from different donors on each occasion.

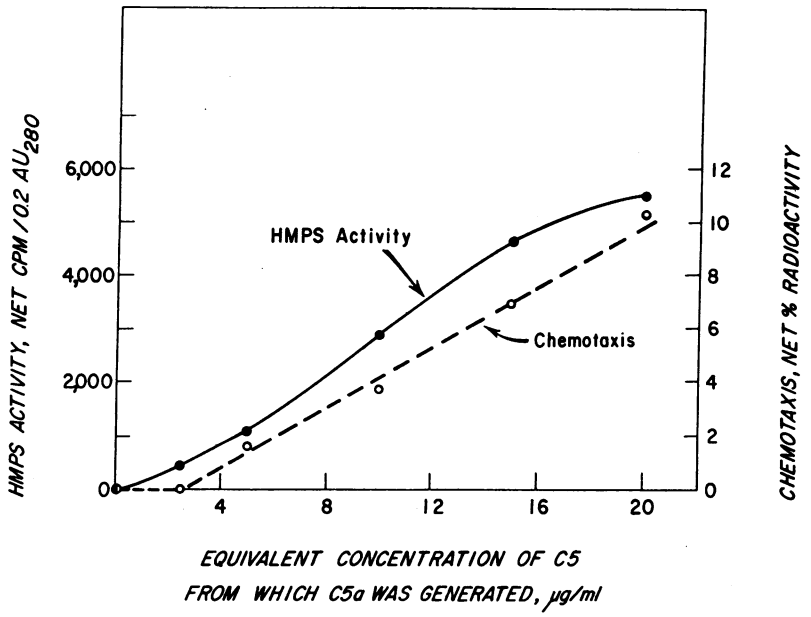

FIGURE 1 Dose-response of C5a stimulation of human leukocyte HMPS activity and chemotaxis. HMPS activity is expressed as net increase in activity of leukocytes of which 80–85% were neutrophils; the base line activity was 1324 cpm/0.2 AU 280 after 80 min at 37°C. C5a was produced by digestion of C5 with 5 μg trypsin per 100 μg C5 in 1 ml for 30 min at 37°C, followed by the addition of a 1.4-fold molar excess of soybean trypsin inhibitor and was diluted in KRPG-ovalbumin as indicated. Chemotaxis was determined as described in Methods.

RESULTS

Stimulation of the HMPS by purified chemotactic factors

It had been previously demonstrated that fewer than 10% of leukocytes adherent to plastic petri dishes became detached after up to 80 min of incubation at 37°C in the presence of chemotactic factors, and thus in studies of stimulation of aerobic glucose metabolism by chemotactic factors adherent human peripheral leukocyte or purified neutrophil layers were used to minimize the metabolic contribution of alterations in leukocyte motility (11). As shown in Fig. 1, C5a produced a dose-response increase in conversion of the first carbon of glucose to $^{14}CO_2$ which rose as high as six times the base line conversion rate. Identical doses of C5a demonstrated a linear increase in chemotactic activity over the range of concentrations studied. The lowest concentrations of C5a or kallikrein (not shown) which consistently showed a 25–50% enhancement of CO_2 production did not necessarily stimulate chemotactic migration.

Both the time course of enhanced CO_2 production and its relation to HMPS activity were next studied by using adherent layers of purified neutrophils. A chemotactically active dose of either C5a or kallikrein resulted in stimulation of $^{14}CO_2$ production from [1-^{14}C]glucose which reached a plateau at four times the unstimulated neutrophil activity of 1263 cpm/0.2 AU 280 after 80 min of incubation at 37°C (Fig. 2). In contrast, the production of $^{14}CO_2$ from [6-^{14}C]glucose was never more than 5% of that from [1-^{14}C]glucose, indicating that stimulation of glucose metabolism by either chemotactic factor was proceeding by way of the HMPS. The $^{14}CO_2$ production from [1-^{14}C]glucose by adherent neutrophils engaged in the phagocytosis of starch particles reached a plateau of nearly seven times base line value by 40 min after the introduction of starch (Fig. 2).

Requirement for an active chemotactic factor. The chemotactic activity of purified human kallikrein is dependent on the integrity of a serine esterase active site which is not expressed in the chemotactically inactive precursor molecule prekallikrein and which can be inhibited by diisopropyl fluorophosphate or soybean trypsin inhibitor (19). Activation of a prekallikrein preparation by Hageman factor fragments devoid of any activity resulted in an increase of 200% in kinin-generating activity, 90% in HMPS-stimulating activity, and 500% in chemotaxis as compared to the base line values yielded by the spontaneously activated kallikrein in the prekallikrein preparations (Fig. 3). When the kinin-generating activity of purified kallikrein was inhibited by preincubation with SBTI or DFP, there was a concomitant dose-

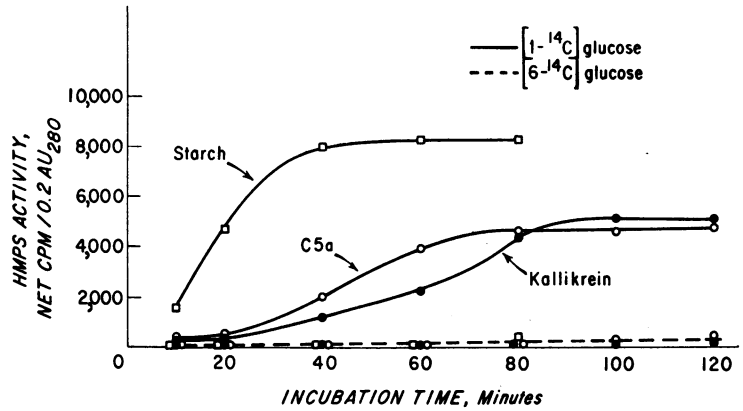

FIGURE 2 Kinetics of stimulation of human neutrophil HMPS by chemotactic factors. HMPS stimulation is expressed as net cpm/0.2 AU 280 as in Fig. 1. The C5a employed was obtained by tryptic digestion of C5 as in Fig. 1 and was used at a final concentration equivalent to 12.5 μg of C5 per ml. Kallikrein derived from prekallikrein was at a concentration which released 5 μg bradykinin per ml from 0.2 ml heat-inactivated plasma. Starch particles were at a 6 : 1 initial ratio with leukocytes.

response suppression of both the HMPS-stimulating and chemotactic activities of kallikrein (Fig. 4A and B). Thus, the capacity of kallikrein to stimulate HMPS activity is dependent upon the activation of the precursor and the maintenance of the active site.

Effect of chemotactic deactivation. Neither exposure of leukocytes to DFP alone nor preincubation of leukocytes with untreated kallikrein and then DFP followed by washing of the leukocyte layers to remove these agents resulted in a reduction in the leukocyte HMPS stimulation after the introduction of active kallikrein (Fig. 4C). In contrast, either procedure resulted in suppression of the chemotactic responsiveness of the leukocytes to kallikrein.

The time course of deactivation of human leukocytes after exposure to a single kallikrein stimulus is depicted in Fig. 5. Chemotactic deactivation is established by the demonstration that cells exposed to a single stimulus, washed twice, and rechallenged in chemotactic chambers within 2 min of the first stimulus failed to respond to a second stimulus at that point or for up to 90 min after the initial interaction with kallikrein. De-

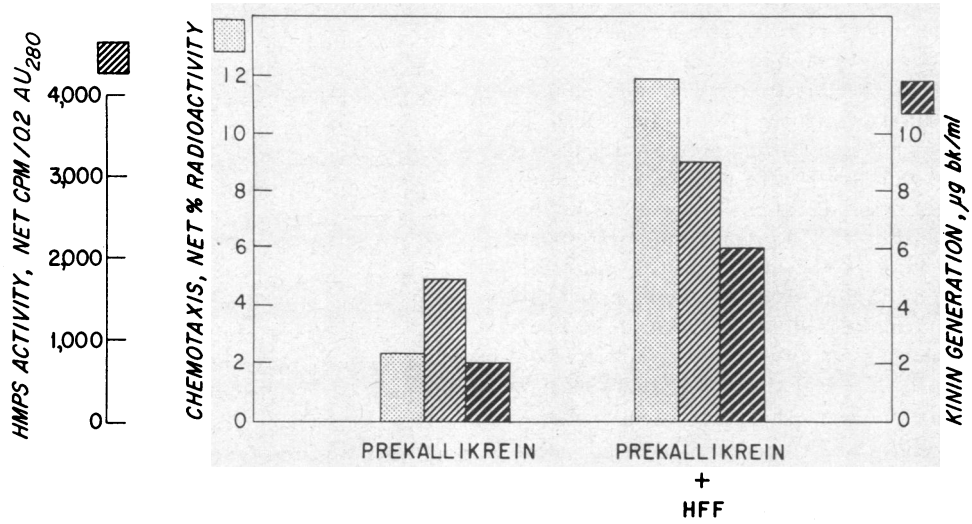

FIGURE 3 Enhancement by kallikrein of neutrophil HMPS activity after activation of prekallikrein with Hageman factor fragments (HFF). Kinin-generating activity was expressed as μg bradykinin per ml generated from 0.2 ml heat-inactivated plasma. HMPS activity was calculated as in Fig. 1 with an 80 min incubation at 37°C. Chemotaxis is expressed as in Fig. 1.

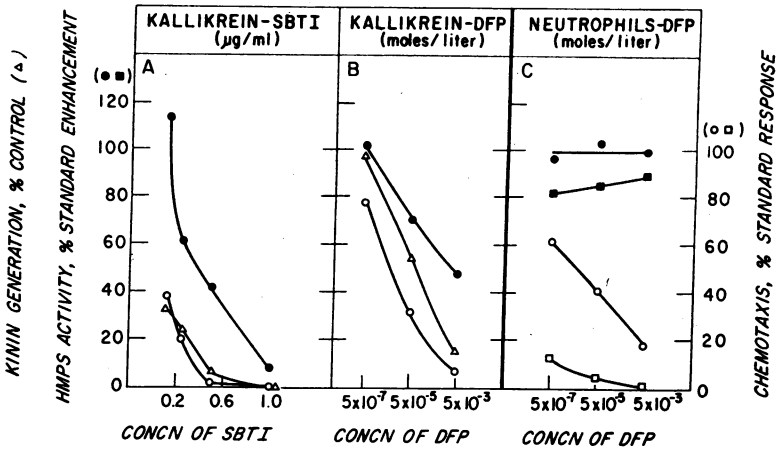

FIGURE 4 Inhibition of human leukocyte kallikrein-stimulated HMPS activity and chemotaxis. HMPS activity, expressed as percent of standard enhancement, was calculated by dividing the net HMPS stimulation observed with modified kallikrein or leukocytes by the net HMPS stimulation achieved with native kallikrein and untreated leukocytes. Chemotaxis is expressed as a percent of standard response derived from net percent radioactivity with modified kallikrein or leukocytes divided by net percent radioactivity with native stimulus and leukocytes. Kinin generation is also expressed as the percent of activity of the uninhibited enzyme. (A) Kallikrein at a concentration which generated 5 μg/ml bradykinin from 0.2 ml heat-inactivated plasma was incubated for 10 min at 37°C with SBTI before interaction with untreated leukocytes. (B) Kallikrein at the same concentration as A was incubated for 2 h at 37°C with DFP and then dialyzed for 24 h at 4°C, as was the native control kallikrein. (C) Leukocyte layers or suspensions were incubated for 20 min at 37°C with DFP and then washed three times before stimulation with kallikrein at the same concentration as in A (●, ○). Another set of leukocyte samples was incubated with kallikrein for 15 min at 37°C and then with DFP for 20 min at 37°C, followed by washing three times before restimulation with kallikrein (■, □).

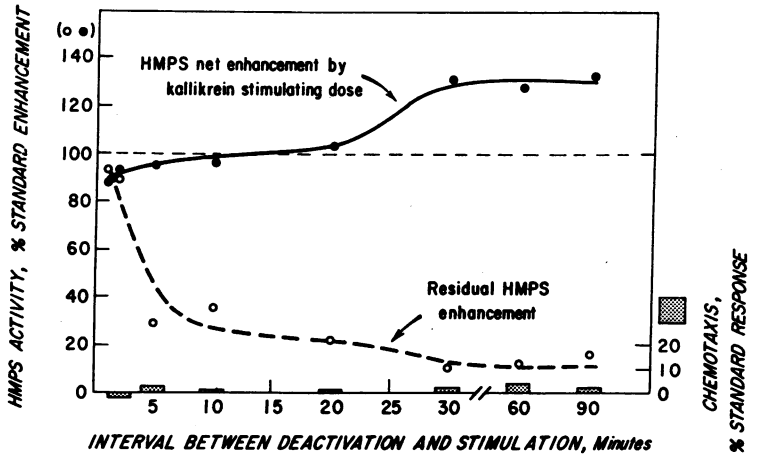

FIGURE 5 Effect of kallikrein chemotactic deactivation on leukocyte HMPS stimulation. HMPS activity and chemotaxis were calculated as in Fig. 4, and the kallikrein concentration was identical with that in Fig. 4. HMPS activity of leukocyte layers deactivated by exposure to a single dose of kallikrein (----) is plotted as the percent of standard enhancement achieved by a dose of kallikrein not followed by washing. The HMPS activity of deactivated leukocytes subjected to restimulation with kallikrein (———) was corrected for the residual activity from the first kallikrein stimulus. Chemotaxis is the response of deactivated leukocytes to restimulation with kallikrein as a percent of standard response of untreated leukocytes to the same stimulus.

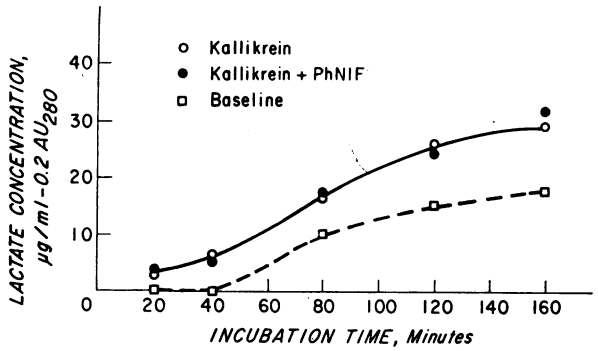

FIGURE 6 Kinetics of stimulation by kallikrein of human neutrophil glycolytic activity. The kallikrein concentration generated 10 μg bradykinin per ml from 0.2 ml heat-inactivated plasma. Purified PhNIF diluted 1/64 was preincubated with the neutrophils for 10 min in Krebs-Ringer phosphate buffer before the addition of glucose and kallikrein; this concentration of PhNIF suppressed the chemotactic responsiveness of neutrophils to kallikrein by 79% in a standard assay.

activation was apparent at a time when the response of the HMPS to the initial stimulus was still maximal. Furthermore, deactivation persisted despite the fact that the response of the HMPS to a second stimulus remained normal.

Stimulation of the glycolytic pathway by purified chemotactic factors

The exposure of adherent layers of purified neutrophils to chemotactic doses of kallikrein (Fig. 6) resulted in a 70% increase in glycolytic activity by 2–2½ h as assessed by lactate concentrations in the supernatants. Comparable data were obtained for C5a, and the range of stimulation in three experiments with each factor was 25–120%. Neither prekallikrein, DFP-inactivated kallikrein, nor C5 incubated with SBTI-inactivated trypsin had any glycolytic enhancing activity. The neutrophil immobilizing factor (NIF), which blocks the migration of polymorphonuclear leukocytes in response to chemotactic factors, had no suppressive effect on enhanced glycolysis (Fig. 6).

TABLE I
Effect of Chemotactic Inhibitors on Human Leukocyte Glucose Metabolism

Inhibitor*	Concentration	% Viability‡	Chemotaxis§	HMPS§	Glycolysis§
	M		*% of standard response*		
DFP	5×10^{-4}	86	8	89	−65
	5×10^{-5}	93	13	94	−47
	5×10^{-7}	95	22	99	61
PhNIF	1/16	81	28	88	72
	1/64	85	41	102	87
	1/256	89	69	97	71
	1/1,024	93	92	96	76

* Mixed leukocyte layers or suspensions were preincubated with the inhibitors for 20 min at 37°C and then washed in the case of DFP, and [1-^{14}C] glucose and kallikrein were added.
‡ Viability was assessed as the percent of 300 neutrophils which excluded trypan blue dye after 30 min at 37°C.
§ Kallikrein at the same concentrations as Fig. 2 was used as the stimulus for chemotaxis, HMPS, and glycolysis. The percent of standard response for chemotaxis and the percent of standard enhancement for HMPS activity were calculated as in Fig. 5; the percent of standard enhancement for glycolysis was calculated from the enhancement achieved by kallikrein with untreated leukocytes which was set at 100%.

Effect of inhibitors on chemotaxis and glucose metabolism

Chemotactic inhibitors. Preincubation of human leukocytes with DFP profoundly suppressed their chemo-

TABLE II
Effect of Metabolic Inhibitors on Neutrophil Chemotaxis

Inhibitor*	Concentration	% Viability‡	HMPS§	Glycolysis§	Chemotaxis§
	M			*% of standard response*	
6-aminonicotinamide	10^{-3}	72	−8.3±10.7	0.6±2.7	−36.9±27.1
	10^{-5}	91	16.0±5.2	95.3±17.1	66.0±11.2
	10^{-8}	96	106.1±59.1	109.0±12.6	82.7±19.4
Iodoacetamide	10^{-3}	58	−84.1±41.6	0.2±4.0	−107.1±27.8
	10^{-6}	89	−39.2±21.8	9.3±7.6	34.2±16.9
	10^{-8}	95	15.9±7.4	61.4±20.1	62.5±12.6

* Layers or suspensions of purified neutrophils were preincubated for 20 min at 37°C with inhibitors before stimulation with kallikrein.
‡ Viability was assessed with trypan blue dye as in Table I.
§ These values are expressed as the percent of standard response as in Table I, and represent the mean ±1 SD for three experiments. C5a derived from 5 μg C5 per ml was used in one experiment, and kallikrein at a concentration which yielded 3 μg bradykinin per ml from 0.2 ml heat-inactivated plasma was used in two experiments.

tactic and glycolytic response to kallikrein without affecting viability or stimulation of their HMPS by an identical concentration of kallikrein (Fig. 4 C, Table I). Leukocytes treated with partially purified NIF from phagocytosis supernatants (PhNIF) exhibited a normal viability and dose-response suppression of their chemotactic response to kallikrein without inhibition of HMPS or glycolytic stimulation to the same agent. Thus, although the suppression of the chemotactic response to kallikrein by NIF differs from the suppression achieved by DFP in that NIF does not affect glycolysis, both agents are inhibitory without influencing the capacity of chemotactic factors to stimulate the HMPS.

Metabolic inhibitors. Purified neutrophils which had been preincubated with 6-aminonicotinamide, an inhibitor of the HMPS and, at high concentrations, of glycolysis (*22*), demonstrated a dose-related inhibition of HMPS stimulation by purified chemotactic factors (Table II). An intermediate concentration (10^{-5} M), which suppressed HMPS but not glycolytic enhancement, reduced chemotactic responsiveness by 23–45%. Iodoacetamide, a broad spectrum inhibitor which interacts predominantly with sulfhydryl and hydroxyl functional groups, suppressed HMPS and glycolytic stimulation by chemotactic factors and the chemotactic response.

DISCUSSION

The resting activities of the human neutrophil hexose monophosphate shunt (HMPS) and glycolytic pathway were both stimulated by an interaction of neutrophils with purified chemotactic factors (Figs. 1, 2, and 6). The stimulation occurred in a dose-related fashion and plateaued between 1½ and 2½ h. The ability of chemotactic factors to enhance aerobic utilization of glucose by purified neutrophils in either pathway was dependent upon the specific chemotactic activity of the factors and therefore developed only after the cleavage of C5a from C5 and the conversion of prekallikrein to kallikrein by active Hageman factor fragments (Fig. 3). The activity of kallikrein was lost when the active site responsible for its other functions, kinin-generation and chemotaxis, was inactivated by treatment with SBTI or DFP (Fig. 4 A and B).

Despite the capacity of an active chemotactic factor to initiate both chemotactic stimulation and metabolic enhancement, metabolic enhancement was not found to be sufficient for chemotaxis. Leukocytes exposed to a single chemotactic stimulus, washed, and immediately introduced into a chemotactic chamber for exposure to the same stimulus failed to migrate, a circumstance termed chemotactic deactivation (*23*). Immediate chemotactic deactivation occurred despite the fact that companion studies revealed maximal stimulation of the HMPS from the original stimulus at the time the cells were introduced into the chamber, and the leukocyte deactivation persisted although their capacity to respond metabolically to a second stimulus was normal throughout the 90 min period (Fig. 5). Further, DFP and NIF yielded a dose-related suppression of chemotaxis (Fig. 4 C, Table I) without impairment of the HMPS stimulation to an identical stimulus. The differences in isolation and activation of the two chemotactic factors indicate that the dissociation of HMPS stimulation from chemotactic responsiveness is not due to common impurities in the chemotactic factors (*17–19*). Finally, even the combination of deactivation and DFP inhibition of chemotaxis did not diminish the capacity of the cell to demonstrate stimulation of the HMPS by kallikrein (Fig. 4 C).

Although stimulation of HMPS and glycolysis was not sufficient for chemotactic migration, stimulation of glucose metabolism did appear to be necessary for maximum chemotactic response. Profound inhibition of HMPS stimulation with maintenance of glycolytic activity by 10^{-5} M 6-aminonicotinamide or 10^{-8} M iodoacetate was associated with 23–50% suppression of chemotaxis, and suppression of both HMPS and glycolytic stimulation by 10^{-6} M iodoacetate gave a 49–83% suppression of neutrophil chemotaxis (Table II). As the effect of these inhibitors on chemotaxis and on stimulation of glucose metabolism exhibited a similar dose-response curve, it seems unlikely that they are mediated by distinctly different actions of the inhibitors, but this possibility has not been excluded. The action of DFP on neutrophils is more complex since it inhibits both enzymes in the glycolytic pathway and neutrophil membrane-bound serine esterases which are critical to the chemotactic response (*24*). At high concentrations (5×10^{-5} M) DFP suppressed chemotaxis and glycolytic enhancement, and at lower concentrations (5×10^{-7} M) DFP showed similar profound suppression of chemotaxis with only mild inhibition of glycolytic enhancement by chemotactic factors (Table I).

Previous work (*11, 13*) showed that irreversible inhibition of human neutrophil mobility by NIF was not lethal to the cells and was functionally specific, since neither neutrophil phagocytosis nor adherence to surfaces was altered by NIF. The concomitant finding that NIF did not suppress stimulation of the HMPS during phagocytosis or by the introduction of chemotactic factors (*11*) has now been extended to show that it also did not suppress the enhanced glycolytic activity associated with exposure to chemotactic factors (Fig. 6, Table I).

The measurement of glycolytic activity by lactate production is a direct and reliable method, while the assessment of HMPS activity by $^{14}CO_2$ generation from [1-

^{14}C]glucose is indirect and subject to several assumptions. Glycolysis may contribute more label from [1-^{14}C]glucose than [6-^{14}C]glucose to $^{14}CO_2$ if the triose-phosphate isomerase reaction is not at complete equilibrium or glyceraldehyde-3-phosphate is in part shunted into other reactions and not oxidized to CO_2 in the Krebs cycle (21). These effects may be counterbalanced if fructose-6-phosphate is cycled from the HMPS to glycolysis without the 1-^{14}C label present in the starting [1-^{14}C]-glucose. These phenomena are usually quantitatively trivial, and a more significant problem relating to the rate of generation of $^{14}CO_2$ from [1-^{14}C]glucose by the HMPS is due to recycling of unlabeled fructose-6-phosphate formed from pentose phosphate which dilutes the intracellular [1-^{14}C]glucose in proportion to the degree of recycling. Despite these objections, the rate of production by neutrophils of $^{14}CO_2$ from [1-^{14}C]glucose generally bears a constant if not absolute relationship to HMPS activity (21).

A situation analogous to the chemotactic factor-enhancement of glucose metabolism exists in the process of phagocytosis by human neutrophils (4). Both neutrophil functions result in a 25–100% stimulation of glycolytic activity as assessed by lactate production (4; Fig. 6) and a much more striking stimulation of HMPS activity assessed by $^{14}CO_2$ generation from [1-^{14}C]glucose (4; Figs. 1 and 2). The very early increases in the instantaneous rate of particle uptake and the cumulative rate of particle uptake which plateaus after 5–6 min of phagocytosis correlate temporally with increases in the rate of neutrophil glucose metabolism (25). By analogy, the slower and more sustained stimulation of glucose metabolic rates of neutrophils produced by chemotactic factors (Figs. 2 and 6) may correlate with the chemotactic stimulation of leukocyte mobility which increases over a period of 2–5 h (12). Finally, when neutrophils were incubated with nonphagocytosable Mycoplasma organisms (26) or when particle uptake was blocked by pretreatment of the neutrophils with corticosteroids,[2] there was no suppression of the usual phagocytic stimulation of the HMPS. The analogies in chemotaxis are the failure of deactivation by chemotactic factors and inhibition with DFP or NIF to suppress the stimulation of the HMPS.

ACKNOWLEDGMENTS

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