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N-Formyl-methionyl-leucyl-phenylalanine induced accumulation of inositol phosphates indicates the presence of oligopeptide chemoattractant receptors on circulating human lymphocytes

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Using [^{3}H]-inositol-labeled human lymphocytes, formation of inositolphosphates was found as a specific response to the chemotactic formylpeptide, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), in a fashion similar to the effects of fMLP in human granulocytes. Inositol phosphate formation could be significantly augmented by lithium ions. The results suggest that fMLP-mediated hydrolysis of phosphoinositides is involved in its chemotactic effects on human lymphocytes.

Chemotaxis; Formylpeptide receptor; Inositol phosphate; Lithium ion; (Human lymphocyte)

1. INTRODUCTION

Migration of granulocytes is mediated by a variety of chemotactic factors of which the chemoattractant oligopeptide fMLP is one of the most potent [1,2]. In spite of the large amount of information accrued on granulocyte chemotaxis, relatively few data are available on lymphocyte chemotaxis, except a few reports about the stimulation of human lymphocyte migration by fMLP [3,4]. However, very little is known about activation of lymphocytes by fMLP and the possible biochemical mechanisms involved.

In contrast, profound evidence indicates that fMLP-mediated granulocyte activation results from an interaction of fMLP with a specific formylpeptide receptor which activates the breakdown of polyphosphoinositides via a G-protein coupled phospholipase C which finally leads to elevated cytosolic Ca^{2+} concentrations [2,5,6]. Accordingly, we studied the possible presence of a similar transducing mechanism on circulating human lymphocytes by investigating the possible effect of agonist stimulation with fMLP on the accumulation of inositol phosphates in lymphocytes in the presence and the absence of lithium ions.

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Abbreviations: fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; IP1, inositol monophosphate; IP2, inositol biphosphate; IP3, inositol triphosphate; IP4, inositol tetrakisphosphate

2. MATERIALS AND METHODS

2.1. *Materials*

[^{3}H]Inositol (spec. act. 20 Ci/mmol) was from NEN (Dreieich, FRG). Medium 199 and fMLP were obtained from Sigma-Chemie (Deisenhofen, FRG). All other chemicals used were of analytical grade. Incubation media were always freshly prepared and filtered through sterile Millex-GS 0.22 μm filter units (Millipore SA; Molsheim, France) before use. Cells were counted on a Coulter Counter T 540.

2.2. *Lymphocyte preparation*

Human lymphocytes were isolated from fresh blood (EDTA containers) of healthy adult donors (aged between 23 and 35 years) using Ficoll-Paque (Pharmacia, Uppsala, Sweden) according to the method of Böjum [7] and the directions provided by the manufacturer. The washed lymphocytes were finally suspended in medium 199 containing 5% autologous serum at a concentration of 10^7 cells/ml. Viability, as determined by the Trypan blue exclusion test, was 95% or more and contamination with granulocytes was less than 2%. The pH of the incubation medium was adjusted to 7.35.

2.3. *Evaluation of inositol phosphate turnover*

For radiolabeled inositol incorporation, lymphocyte suspensions were incubated with [^{3}H]inositol (5 μCi/10^7 cells) for 24 h at 37°C in a shaking water bath. [^{3}H]Inositol-labeled cells were separated by centrifugation and resuspended in a modified Hank's balanced salt solution (pH 7.35) containing (in mmol/l): NaCl 124, KCl 4, Na_2HPO_4 0.64, KH_2PO 0.66, $NaHCO_3$ 15.2, Hepes 10, $MgCl_2$ 0.2, $CaCl_2$ 0.5 and glucose 5.6. When LiCl was added, the NaCl content was corrected to achieve isotonicy. 0.18 ml of the cell suspension, about 3×10^6 cells, were incubated in plastic tubes with fMLP after preincubation with various concentrations of LiCl for the indicated times. Incubations were stopped by trichloroacetic acid 60% (final concentration 10%) and brief sonication. After centrifugation for 10 min at 10000 × *g*, the supernatants were removed and assayed for inositol phosphates. After removing trichloroacetic acid by diethylether extraction (four times with 0.3 ml), the supernatants were freeze dried and the residues dissolved in 100 μl water. The inositol phosphates

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were separated by anion exchange chromatography [8] with some modifications. Briefly, 100 μl samples were applied to columns (15 × 0.28 cm) of Dowex 1X8-200 in the formate form. The columns were washed with 5 ml water and 16 ml of 5 mM sodium tetraborate/60 mM sodium formate to separate labeled inositol and glycerophosphoinositol. IP1 was eluted with 10 ml of 0.2 M ammonium formate/0.1 M formic acid, IP2 with 12 ml of 0.6 M ammonium formate/0.1 M formic acid and higher inositol phosphates (e.g. IP3 and IP4) with 12 ml of 1.8 M ammonium formate/0.1 M formic acid. The recovery of IP1 was greater than 95% and that of IP2, IP3 and IP4 was about 70%, as determined with ^{3}H-labeled standards. The resultant fractions were evaporated and the residues were dissolved in 2 ml (IP1) or 4 ml (IP2) and 8 ml (IP3 + IP4) water. After the addition of 16 ml Quickzint 402 (IP1) or 12 ml (IP2) and 24 ml (IP3 + IP4), Quickzint Flow 306 radioactivity was determined on a Beckman liquid scintillation counter and transformed to dpm.

3. RESULTS

Initial experiments indicated that the incubation of circulating human lymphocytes with [^{3}H]inositol leads to an incorporation of [^{3}H]inositol into cell lipids, which is maximal after 24 h of incubation (data not shown). Maximal incorporation of [^{3}H]inositol at a medium concentration of about 8 μCi/ml was 4×10^4 dpm/10^6 cells. All further experiments were performed with lymphocytes incubated with [^{3}H]inositol for 24 h.

When lymphocytes were incubated in the presence of LiCl together with fMLP at a concentration of 100 nM which gives maximal stimulation of inositol phosphate accumulation in human granulocytes [2,6,9], intracellular levels of the different inositol phosphates were significantly elevated. The time course of the fMLP-stimulated increase of intracellular concentrations of IP_1, IP_2, and IP_3 + IP_4 is given in fig.1. Maximal levels of IP_3 + IP_4 were observed slightly before and maximal levels of IP_2 slightly after a stimulation period of 1 min, while maximal accumulation of IP_1 was only observed after 7–10 min of incubation (fig.1). Accumulation of inositol phosphates in all three fractions was strongly dependent on the medium concentration of fMLP (figs 2 and 3). When stimulation was performed for 1 min, maximal effects were observed at fMLP concentrations of about 1000 nM and half-maximal effects at about 30–50 nM for all three fractions (fig.2). When lymphocytes were incubated for 10 min, fMLP was significantly more potent in stimulating IP_1 accumulation, with maximal effects seen at 100 nM and half-maximal effects at about 5 nM (fig.3). Other neuroreceptor agonists including epinephrine, histamine, serotonine, carbachol and several other cholinergic agonists (oxotremoxine, Mc-A-343) were inactive in stimulating the accumulation of inositol phosphates in human lymphocytes.

The accumulation of inositol phosphates in all fractions was profoundly dependent on the medium con-

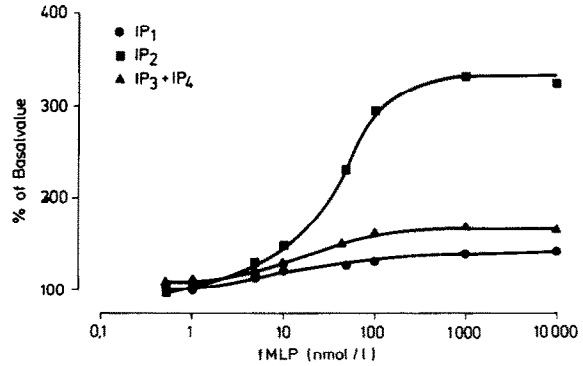

Fig.2. Effects of increasing fMLP concentration on inositol phosphate accumulation. [^{3}H]Inositol-labeled lymphocytes were stimulated for 60 s with fMLP at the concentrations indicated after preincubation with 10 mM lithium chloride. Values are means of duplicates of one representative experiment. The experiment was replicated two times.

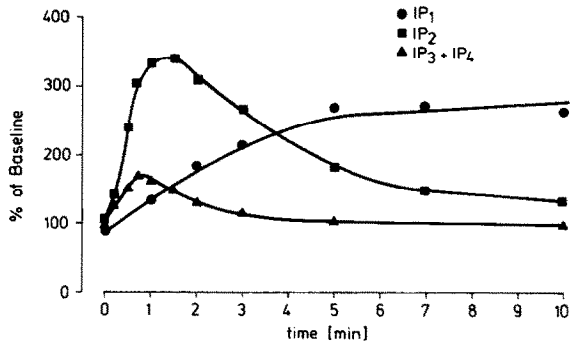

Fig.1. Time course of [^{3}H]inositol phosphate formation after stimulation with 100 nM fMLP. Cells were preincubated with lithium chloride (10 mM) for 15 min. Baseline levels of [^{3}H]IP_1, [^{3}H]IP_2 and [^{3}H]IP_3 + [^{3}H]IP_4 were 2414, 816 and 1710 dpm/10^6 lymphocytes, respectively. Values are means of duplicates of one representative experiment. The experiment was replicated three times.

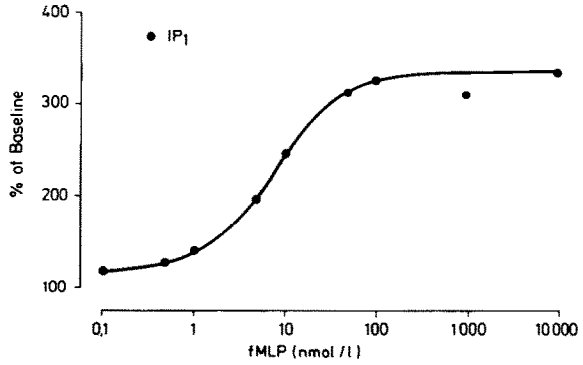

Fig.3. Effects of increasing fMLP concentration on inositol phosphate accumulation. [^{3}H]Inositol-labeled lymphocytes were stimulated for 10 min with fMLP at the concentrations indicated after preincubation with 10 mM lithium chloride. The baseline level of [^{3}H]IP_1 was 6148 dpm/10^6 lymphocytes. Values are means of duplicates of one representative experiment. The experiment was replicated three times.

centration of LiCl when investigated at the time points of maximal fMLP stimulation as demonstrated in fig.1. Under control conditions, i.e. without LiCl, accumulation of all three fractions ranged between 80 and 120% over the baseline concentrations (fig.4). However, accumulation of all three fractions was profoundly increased by LiCl in a concentration-dependent fashion, with maximal effects observed at medium concentrations of about 10 mM LiCl and half-maximal effects of LiCl at about 0.5 mM (fig.4). Maximal effects of LiCl on inositol phosphate accumulation ranged from 200 to 300% over baseline concentrations (fig.1).

4. DISCUSSION

The experiments reported strongly suggest the presence of formylpeptide receptors on circulating human lymphocytes which, like similar receptors on human granulocytes, are linked to a phospholipase C-using transducing mechanism. The properties of this formylpeptide receptor as indicated by the time course of inositol phosphate stimulation, by the ED_{50} of fMLP, as well as by its maximal effective concentration show close similarities to the formylpeptide receptor-linked transducing system of human granulocytes [2,6,9] and strongly suggest that fMLP-mediated chemotaxis of human lymphocytes [3,4] might be mediated by mechanisms similar to those present in granulocytes [1,2,5,6]. Our negative findings with several neuroreceptor agonists confirm negative findings about the possible activation of guanylate cyclase in human lymphocytes with similar agents [10].

However, the effects of lithium on inositol phosphate accumulation induced by fMLP are much more pronounced in the case of human lymphocytes than in the case of granulocytes, where even maximal stimulation only resulted in inositol phosphate levels of about 100% over baseline [9] or where lithium-induced amplification of agonist-induced inositol phosphate accumulation ranged only between 100 and 150% over baseline stimulation without lithium [2]. The reported effects of lithium on fMLP-induced accumulation of inositol phosphates in human lymphocytes are quite similar to the profound effects of lithium on the amplification of carbachol-, serotonine-, histamine- and phenylephrine-induced stimulation of inositol phosphates in slices of rat brain and parotid glands [4,8]. Moreover, the ED_{50} observed for lithium in the present communication (about 0.5 mM) comes very close to its half-maximal inhibitory concentration for *myo*inositol-L-phosphatase in vitro (about 0.7 mM) [12] and is even lower than its ED_{50} in the two rat tissues already mentioned above (about 1.0 mM) [8,11]. Accordingly, fMLP-induced accumulation of inosotol phosphates in human lymphocytes represents an excellent model to study lithium effects in man at therapeutic concentrations.

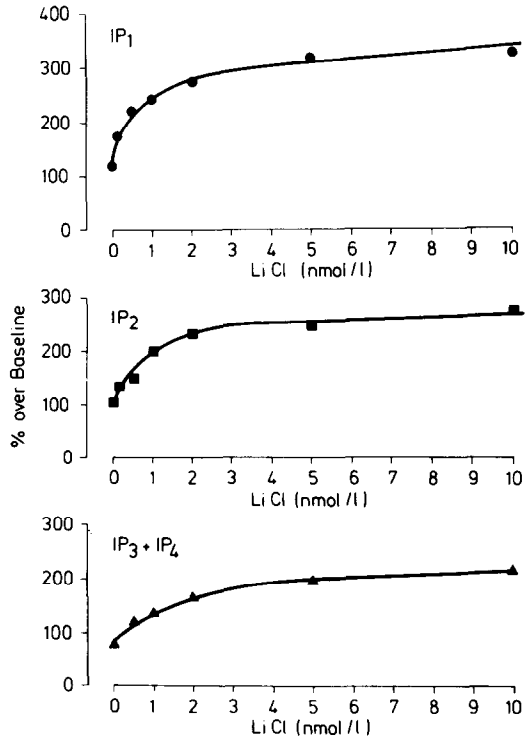

Fig.4. Dose-response curves for lithium-induced enhancement of inositol phosphate generation. Lymphocytes were preincubated with the indicated lithium concentrations for 3 h, after which time uptake of lithium ions into lymphocytes is maximal [10]. Cells were stimulated with 100 nM fMLP for 10 min in the case of IP_1 or for 1 min in the case of IP_2 and higher inositol phosphates (IP_3 + IP_4). Values are means of duplicates of one representative experiment. The experiment was replicated two times.

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