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Met-Ile-Phe-Leu derivatives: full and partial agonists of human neutrophil formylpeptide receptors

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Abstract

The biological action of a series of Met-Ile-Phe-Leu analogues was analyzed on human neutrophils, to evaluate their ability to interact with formylpeptide receptors and to induce the related neutrophil responses. Three in vitro assays were carried out: receptor binding, chemotaxis and superoxide anion release. Our results demonstrate that formyl-Met-Ile-Phe-Leu derivatives act as more potent full agonists than formyl-Met-Leu-Phe, the tripeptide normally used as a model chemoattractant for the study of cell functions. On the other hand, the presence of *N*-ureidoisopropyl substituent in tetrapeptides imparts weak partial agonist properties. It has furthermore been demonstrated that the C-terminal methyl esterification or amination weakly influences the properties of tetrapeptide homologues. Finally, *t*-Boc-Met-Ile-Phe-Leu derivatives do not appear able to interact with formylpeptide receptors.

Keywords: Neutrophil, human; Formylpeptide receptor; Full agonist, Partial agonist, Neutrophil functionality

1. Introduction

Polymorphonuclear neutrophils are phagocytic cells involved in the defense against infections (Prossnitz and Ye, 1997). In this context, the CHO-Met-Leu-Phe-OH (formyl-Met-Leu-Phe-OH) peptide, derived from bacterial sources or from disrupted mitochondria, was identified as a potent chemoattractant for phagocytes (Carp, 1982; Marasco et al., 1984). This discovery led to the identification on neutrophils of a G-protein-coupled formylpeptide receptor which has since been cloned (Koo et al., 1983; August et al., 1997). The binding of CHO-Met-Leu-Phe-OH to formylpeptide receptor triggers multiple biochemical responses by neutrophils, including adhesion, chemotaxis and free radical generation. These effects constitute the physiological defense against bacterial infections and tissue damage (Prossnitz and Ye, 1997). Formylpeptide receptor antagonists could have beneficial effects as therapeutic agents on the treatment of inflammation disorders (Smith, 1994; Behar and Porcelli, 1995; Meera et al., 1999) Consequently, the study and development of potent and selective formylpeptide receptor antagonists is of considerable interest.

Agonist or antagonist activities of peptide derivatives on human neutrophils appear to be dependent on their primary sequence and on the N- and C-terminal substituents (Freer et al., 1980; Derian et al., 1996; Higgins et al., 1996; Dalpiaz et al., 1999). As an example, it has been reported that the formyl group of CHO-Met-Leu-Phe is essential for adequate biological activity of agonists (Freer et al., 1980; Derian et al., 1996). On the other hand, it has been demonstrated that *N*-*t*-Boc or *N*-ureido-ethyl, *n*-propyl and isopropyl substituents in the Met-Leu-Phe chain induce an antagonist behaviour (Higgins et al., 1996).

The *t*-Boc-Phe-D-Leu-Phe-D-Leu-Phe-OH derivative has been reported as displaying an appreciable formylpeptide receptor antagonist activity on rabbit and human neutrophils (Aswanikumar et al., 1977; Dalpiaz et al., 1999). Moreover, it has recently been verified that *N*-ureido-Phe-D-Leu-Phe-D-Leu-Phe-OH peptide derivatives can enhance

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the noticeable antagonist activity of the *t*-Boc-homologue (Higgins et al., 1996; Dalpiaz et al., 1999). All these pentapeptide derivatives have been reported to be more active on human neutrophils than the C-terminal methyl-ester ones (Dalpiaz et al., 1999).

As for tetrapeptide derivatives, CHO-Met-Ile-Phe-Leu-OH has been recognised as a full chemotactic agonist on human monocytes (Rot et al., 1987) although its properties on neutrophils have not been extensively analysed. It could be of interest to evaluate if *t*-Boc or *N*-ureido-aliphatic substituents in the tetrapeptide chain can induce an antagonist behaviour on human neutrophils. In this context, the present paper reports the synthesis and biological analysis of nine Met-Ile-Phe-Leu analogues (Fig. 1). In particular, the *t*-Boc, formyl and isopropylureido substituents have been included at the N-terminal of the peptides, which were all obtained as free acid, methyl ester and amido derivatives. The biological properties of the peptide derivatives were evaluated in three "in vitro" assays: receptor binding, superoxide anion release and chemotaxis. The aim of the present study was an extensive analysis of the properties of Met-Ile-Phe-Leu derivatives on human neutrophils. Starting from an evaluation of the biological activity of CHO-Met-Ile-Phe-Leu, the effects of C-terminal esterification and amination were subsequently examined. Moreover, the consequences of N-terminal isopropylureido and *t*-Boc substitutions—which convert the agonist CHO-Met-Leu-Phe to an antagonist (Derian et al., 1996; Higgins et al., 1996)—were evaluated. Our conclusions indicate that formyl and isopropylureido derivatives are respectively more and less potent agonists than CHO-Met-Leu-Phe. On the other hand, the presence of the *t*-Boc substituent appears detrimental for the activity of the tetrapeptide derivatives.

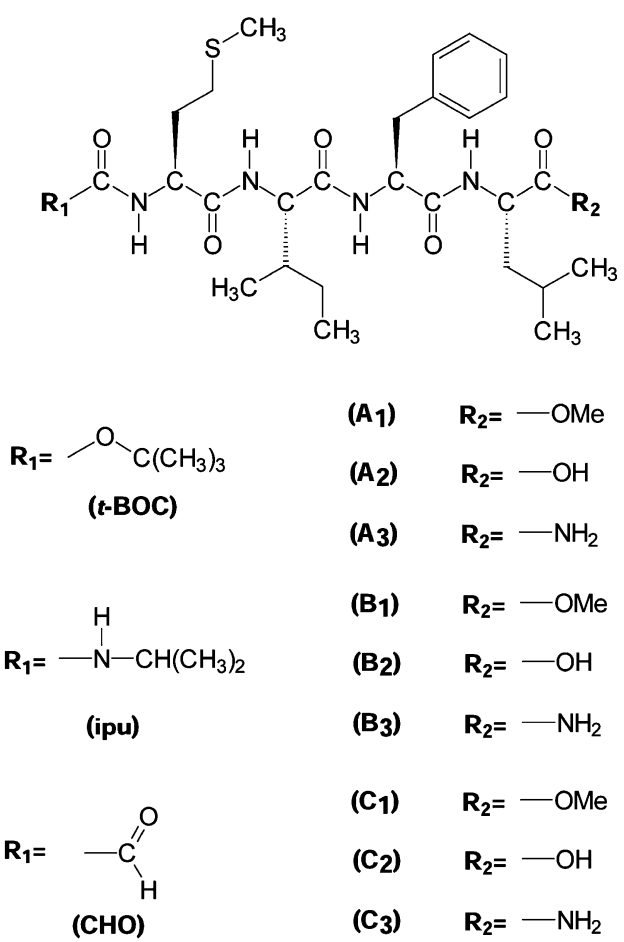

Fig. 1. Chemical formulas of the nine tetrapeptide derivatives analysed.

2. Materials and methods

2.1. Peptides

t-Boc-Met-OH (Sigma Aldrich-Fluka, Milan, Italy) was coupled to HOSu (*N*-hydroxysuccinimide, Fluka) using DCC (*N,N′*-dicyclohexylcarbodiimide, Fluka) in DMF (*N,N*-dimethylformamide, Carlo Erba, Milan, Italy) at 0°C (1 h) and kept at room temperature (12 h). The resulting *t*-Boc-Met-OSu was coupled to H-Ile-OH (Fluka) in sodium bicarbonate solution (pH 8) as previously described (Anderson et al., 1964). The resulting dipeptide *t*-Boc-Met-Ile-OH was coupled to H-Phe-Leu-OMe using EDCI (*N*-dimethylaminopropyl,*N*-ethylcarbodiimide, Calbiochem-Novabiochem, Läufelfingen, Switzerland) and HOBt (hydroxybenzotriazole, Fluka) in DMF at 0°C (1 h) and kept at room temperature (12 h) in order to obtain the tetrapeptide *t*-Boc-Met-Ile-Phe-Leu-OMe ($\mathbf{A}_1$).

The dipeptide H-Phe-Leu-OMe was prepared coupling *t*-Boc-Phe-OH to H-Leu-OMe using the previous coupling reaction and the removal of *t*-Boc group was performed by treatment with a 1:1 mixture of trifluoroacetic acid–chloroform at room temperature. The tetrapeptide ($\mathbf{A}_1$) was in part saponified with 1 N NaOH and in part dissolved in methanol at 0°C in the presence of NH_3 gas (Bodanszky and Bodanszky, 1984) in order to obtain the C-terminal free acid ($\mathbf{A}_2$) and the C-terminal amido ($\mathbf{A}_3$) homologues, respectively.

Tetrapeptide ($\mathbf{A}_1$) was moreover used as amino deblocked for the synthesis of alkyl-ureido peptide ($\mathbf{B}_1$) by reaction with isopropyl-*N*-succimidyl carbamate as previously described (Dalpiaz et al., 1999). The peptide derivative ($\mathbf{B}_1$) was in part saponified and in part used to obtain the C-terminal free acid ($\mathbf{B}_2$) and the amido ($\mathbf{B}_3$) homologues, respectively.

For the synthesis of compounds ($\mathbf{C}_1$), ($\mathbf{C}_2$), ($\mathbf{C}_3$) the tetrapeptide derivative ($\mathbf{A}_1$) was unprotected by treatment with 98% formic acid. The resulting formate salt of the peptide was directly treated with EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, Fluka) and kept at room temperature for 3 h (Lajoie and Kraus, 1984). The resulting peptide derivative ($\mathbf{C}_1$) was treated, using the previous described reactions, in order to obtain the free acid ($\mathbf{C}_2$) and C-terminal amido ($\mathbf{C}_3$) homologues.

All peptides were purified by silica gel column and were analyzed by reverse phase liquid chromatography on a Vydac C_{18} column with acetonitrile gradient elution. The compounds were characterized by mass spectrometry analysis, melting point determination and polarimetric measurements.

Stock solutions, 10^{-2} M of CHO-Met-Leu-Phe (Sigma, St. Louis MO, USA) and 10^{-3} M of tetrapeptide derivatives, were prepared in dimethylsulfoxide (DMSO, Sigma) and diluted using Krebs–Ringer-phosphate containing 0.1% w/v glucose (KRPG), pH 7.4, before use. KRPG was made up as a stock solution of the following composition: NaCl, 40 g/l; KCl, 1.875 g/l; $Na_2HPO_4.2H_2O$, 0.75 g/l; KH_2PO_4, 0.125g/l; $NaHCO_3$, 1.25g/l; glucose, 10 g/l. This solution was five times working strength. 1 mM $MgCl_2$ and $CaCl_2$ were supplemented to the buffer before the biological test. At the concentrations used, DMSO did not interfere with any of the biological assays performed.

2.2. Cell preparation

Cells were obtained from heparinized (10 U/ml) peripheral blood of fasting healthy subjects. Twenty milliliters of blood was supplemented with 12 ml of a solution consisting of 6% (by weight) Dextran T70 and was settled onto a 50 ml polypropylene tube at room temperature for 45 min. The turbid upper supernatant containing leukocytes was carefully removed and layered onto 10 ml of Ficoll-Paque (Pharmacia, density gradient for lymphocyte isolation), centrifuged at $250 \times g$ for 20 min at room temperature. The pellet containing neutrophils was further purified by erythrocytes hypotonic lysis (0.86% NH_4Cl for 10 min). The cells were washed twice and resuspended using KRPG, pH 7.4, at a final concentration of 50×10^6 cells/ml and used immediately. The percentage of neutrophils was 98–100% pure and $\geq 99\%$ viable, as determined by the Trypan blue exclusion test. The study was approved by the local Ethics Committee, and informed consent was obtained from all participants.

2.3. Receptor binding assays

Binding assays were carried out essentially according to Dalpiaz et al. (1999) and Spisani et al. (1996). Briefly neutrophils (5×10^6) were incubated in 200 μl of KRPG, supplemented with 1 mM $MgCl_2$ and $CaCl_2$, at 37°C for 15 min at 37°C and for 90 min at 4°C, according to previous time course analysis. Displacement experiments were performed in the presence of 10 nM [^{3}H]CHO-Met-Leu-Phe (specific activity = 71.5 Ci/mmol; NEN Research Products, Du Pont de Nemours, Milan, Italy) using at least six different concentrations of cold drugs added with [^{3}H]CHO-Met-Leu-Phe before addition of neutrophils to the experimental system. Non-specific binding was defined as binding in the presence of 100 μM CHO-Met-Leu-Phe and was about 20% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/C glass-filters using a Micro Mate 196 Cell Harvester (Packard Instrument). The filter-bound radioactivity was counted on Top Count (efficiency 57%) with Micro Scint-20 (30 μl in 96-well plates). All the values obtained are means of three independent experiments performed in duplicate.

2.4. Random locomotion and chemotaxis

Random locomotion and chemotaxis were evaluated using a 48-well microchemotaxis chamber (BioProbe, Milan, Italy), with a 3-μm pore size membrane filter (Millipore, Milan, Italy), estimating the distance in micrometers that the leading-front of the cell migrated, and using the method of Zigmond and Hirsch (1973). Chemoattractant activity was determined by adding CHO-Met-Leu-Phe or the tetrapeptide derivatives to the lower compartment of the chemotaxis chamber and it was expressed in terms of chemotactic index (C.I.), which is the ratio:

$$\text{C.I.} = \frac{\text{migration toward test attractant} - \text{migration toward the buffer}}{\text{migration toward the buffer}}$$

Migration in the presence of buffer alone was 30 μm ± 4 S.E. ($n = 6$). All the values obtained are means of six independent experiments performed in duplicate.

2.5. Superoxide anion production

O_2^- production was measured by the superoxide dismutase (0.5 mg/ml)-inhibitable reduction of ferricytochrome *c* (Torrini et al., 1996) modified for microplate-based assays. The tests were carried out in a final volume of 200 μl containing 4×10^5 neutrophils, 100 nmol of cytochrome *c* (Sigma) and KRPG. At zero time, different amounts (ranging from 10^{-12} to 10^{-5} M) of each peptide derivative were added, and the plates were incubated in a microplate reader (Ceres 900, Bio-TeK instruments) with the compartment temperature set at 37°C.

Absorbance was recorded at wavelengths of 550 and 468 nm. Differences in absorbance at the two wavelengths were used to calculate nanomoles of O_2^- produced, using a molar extinction coefficient for cytochrome *c* of 18.5 mM^{-1} cm^{-1}. This value has been calculated by the difference between the molar extinction coefficients from reduced ($\varepsilon_{550} = 27.6 \times 10^3$ M^{-1} cm^{-1}) and oxidized ($\varepsilon_{550} = 9.1 \times 10^3$ M^{-1} cm^{-1}) states of cytochrome *c* (Margoliash and Frohwirt, 1959). Neutrophils were preincubated in the presence of 5 μg/ml cytochalasin B (Sigma) for 5 min prior to activation by CHO-Met-Leu-Phe or the tetrapeptide derivatives. Results were expressed as net nanomoles. The net nanomoles were calculated using the formula: stimulated neutrophils − resting neutrophils alone. All the values obtained are means of six independent experiments performed in duplicate.

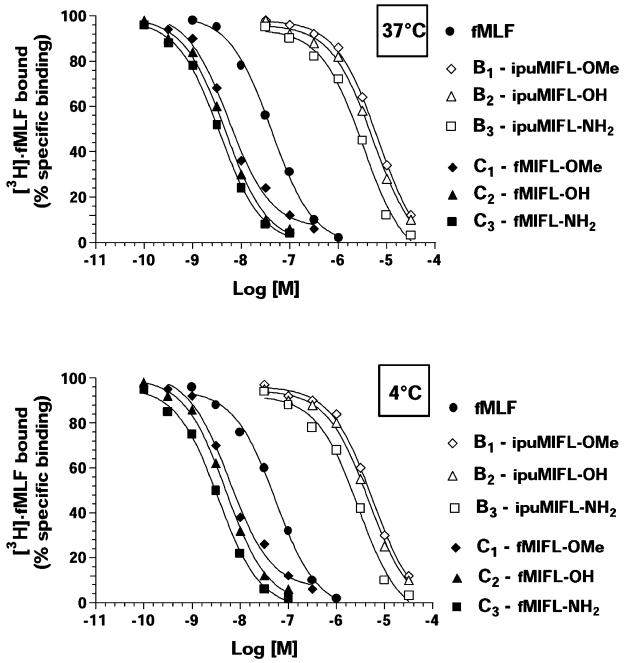

Fig. 2. Competition experiments of CHO-Met-Leu-Phe (fMLF), CHO-(fMIFL) and *N*-isopropylureido (ipuMIFL) tetrapeptide derivatives for specific [^{3}H]fMLF binding carried out at 37°C and 4°C on human neutrophils. These are single representative experiments carried out in duplicate.

2.6. Data analysis

The cold drug concentrations displacing 50% of labelled ligand (IC_{50}) were obtained by computer analysis of displacement curves.

For determination of EC_{50} values [EC_{50} = concentration of peptide required to produce 50% of the maximum effect as determined from the concentration–effect curve], data were expressed as a percentage of the maximal response obtained with each individual peptide derivatives. EC_{50} values were calculated from the mean of the individual dose–response curves fitting using response data increasing with concentration of peptide derivatives. All data were analysed using the non-linear regression curve fitting computer program Graph Pad Prism (Graph Pad, San Diego, CA, USA).

Analysis of variance was performed with SigmaStat (version 2.0, Jandel Scientific software). Difference was considered statistically significant at P values < 0.05.

3. Results

Fig. 2 shows the results from [^{3}H]CHO-Met-Leu-Phe displacement experiments carried out at 37°C and 4°C using CHO-Met-Leu-Phe, *N*-formyl- and *N*-isopropylureido tetrapeptide derivatives. The CHO-Met-Ile-Phe-Leu and *N*-isopropylureido-Met-Ile-Phe-Leu derivatives appear to bind with respectively higher and lower affinity toward formylpeptide receptors than CHO-Met-Leu-Phe. The IC_{50} values are presented in Table 1. In particular, the affinities of CHO-Met-Ile-Phe-Leu tetrapeptides ($3.6\ nM \leq IC_{50} \leq 5.6\ nM$ at 37°C and $2.9\ nM \leq IC_{50} \leq 6.1\ nM$ at 4°C) are one order of magnitude higher than CHO-Met-Leu-Phe affinity (41 nM at 37°C and 45 nM at 4°C). On the other hand, the IC_{50} values of *N*-isopropylureido-Met-Ile-Phe-Leu derivatives ($3200\ nM \leq IC_{50} \leq 6500\ nM$ at 37°C and $2900\ nM \leq IC_{50} \leq 6000\ nM$ at 4°C) indicate that their affinities are two orders of magnitude lower than that of CHO-Met-Leu-Phe. The IC_{50} values of tetrapeptide derivatives reported in Table 1 do not appear to change considerably with the different C-terminal substituents. Very weak affinities have been found for *t*-Boc derivatives: their IC_{50} values exceeded 1 mM at 37°C and 4°C (data not shown).

Chemotactic responses of human neutrophils to CHO-Met-Leu-Phe, CHO-Met-Ile-Phe-Leu and *N*-isopropy-

Table 1
Receptor binding and related effects (chemotaxis and superoxide anion production) of CHO-Met-Leu-Phe (fMLF), CHO-(fMIFL) and *N*-isopropylureido (ipuMIFL) tetrapeptide derivatives on human neutrophils

Peptide	Receptor binding		Chemotaxis		O_2^- superoxide release	
	IC_{50} (nM), 37°C	IC_{50} (nM), 4°C	EC_{50} (nM)	Efficacy	EC_{50} (nM)	Efficacy
fMLF	41 ± 3	45 ± 4	0.049 ± 0.003	1.25 ± 0.11	23 ± 2	43 ± 5
B$_1$ (ipuMIFL-OMe)	6500 ± 400	6000 ± 500	4.7 ± 0.4 nM	0.60 ± 0.05	2000 ± 100	27 ± 3
B$_2$ (ipuMIFL-OH)	5200 ± 300	4800 ± 400	3.3 ± 0.2 nM	0.53 ± 0.04	2100 ± 100	30 ± 3
B$_3$ (ipuMIFL-NH$_2$)	3200 ± 200	2900 ± 200	7.6 ± 0.5 nM	0.58 ± 0.05	1150 ± 60	37 ± 4
C$_1$ (fMIFL-OMe)	5.6 ± 0.3	6.1 ± 0.3	0.0056 ± 0.0003	1.29 ± 0.12	1.20 ± 0.05	48 ± 6
C$_2$ (fMIFL-OH)	4.7 ± 0.2	5.2 ± 0.3	0.0065 ± 0.0005	1.23 ± 0.10	1.59 ± 0.08	44 ± 5
C$_3$ (fMIFL-NH$_2$)	3.6 ± 0.2	2.9 ± 0.2	0.0030 ± 0.0002	1.32 ± 0.12	2.1 ± 0.1	42 ± 5

Efficacy data of chemotaxis are expressed as chemotactic index; efficacy data of superoxide anion production are expressed as net nanomoles of $O_2^-/1 \times 10^6$ cells/5 min. ±S.E. are reported. Differences between results obtained with ipuMIFL derivatives or fMIFL derivatives are not systematically significant for each response.

Receptor binding values are means of three independent experiments performed in duplicate.

Chemotaxis and superoxide O_2^- anion release values are means of six independent experiments performed in duplicate.

lureido-Met-Ile-Phe-Leu derivatives are shown in Fig. 3. The dose–response curves rise to a peak and then decline to zero, with ligand concentrations higher than the optimum value. This behaviour appears typical of chemoattractants (Rot et al., 1987; Vertuani et al., 1987). It can be observed that the optimum concentration value for chemotactic response is around 10^{-9} M for CHO-Met-Leu-Phe, whereas the efficacy values are around 10^{-10} and 10^{-7} for CHO-Met-Ile-Phe-Leu and *N*-isopropylureido-Met-Ile-Phe-Leu derivatives, respectively. Moreover, the chemotactic index peaks, attributed to CHO-Met-Leu-Phe and CHO-tetrapeptides, are similarly high. On the other hand, the peaks obtained for *N*-isopropylureido-derivatives indicate that their maximal responses are reduced in comparison to the other peptides. The results are quantitatively reported in Table 1, which includes EC_{50} and efficacy values of the chemotactic index for the compounds reported in Fig. 3. EC_{50} values of CHO-Met-Ile-Phe-Leu tetrapeptides ($3\ pM \leq EC_{50} \leq 6.5\ pM$) are one order of magnitude lower than CHO-Met-Leu-Phe value (49 pM). As for efficacy, the chemotactic index values of these compounds are distributed in a narrow range around 1.25. These results indicate that the CHO-tetrapeptide derivatives are full agonists, like CHO-Met-Leu-Phe, albeit more potent. Moreover, their ability to induce chemotaxis does not significantly change with the different C-terminal substituents. Similarly, the EC_{50} ($3.3\ nM \leq EC_{50} \leq 7.6\ nM$) and efficacy ($0.53 \leq$ efficacy ≤ 0.60) values of *N*-isopropylureido derivatives are not markedly influenced by these C-terminal substitutions, whereas the same derivatives appear to be weak partial agonists with respect to CHO-Met-Leu-Phe.

No significant chemotactic response is induced by any *t*-Boc-Met-Ile-Phe-Leu derivatives (data not shown) in the range of concentration investigated (from 10^{-13} to 10^{-5} M).

Fig. 4 shows the ability of CHO-Met-Leu-Phe, CHO-Met-Ile-Phe-Leu and *N*-isopropylureido-Met-Ile-Phe-Leu derivatives to trigger superoxide anion production in human neutrophils. Also in this case, dose–response curves present a peak. The concentrations corresponding to the peaks range within three orders of magnitude. In particular, optimal responses for CHO-Met-Ile-Phe-Leu derivatives are obtained around 10^{-8} M, whereas for CHO-Met-Leu-Phe and *N*-isopropylureido-Met-Ile-Phe-Leu derivatives, such responses are obtained around 10^{-6} M and near 10^{-5} M, respectively. The peaks of moles of O_2^-, attributed to CHO-Met-Leu-Phe and CHO-tetrapeptides, have similar height, whereas the peaks of *N*-isopropylureido derivatives appear shorter. Table 1 includes the quantitative results from superoxide anion production experiments. It can be observed that the EC_{50} values of CHO-tetrapeptides ($1.20 \leq IC_{50} \leq 2.10$ nM) are significantly lower than the value of CHO-Met-Leu-Phe (23 nM), whereas their efficacy ranges around that of CHO-Met-Leu-Phe value (44 nmol of O_2^-). It can be concluded that, as far as superoxide anion production is concerned, CHO-tetrapeptide derivatives are more potent full agonists than CHO-Met-Leu-Phe. On the other hand, the EC_{50} ($1.15 \leq EC_{50} \leq 2.1$ μM) and efficacy ($27 \leq$ efficacy ≤ 37 nmol O_2^-) values of *N*-isopropylureido derivatives indicate that these compounds are weak partial agonists.

The ability of all tetrapeptide derivatives reported in Table 1 to trigger O_2^- production does not considerably change with the different C-terminal substituents.

No O_2^- production is triggered by any of the *t*-Boc-Met-Ile-Phe-Leu derivatives (data not shown) in the range of concentration investigated (from 10^{-12} to 10^{-5} M).

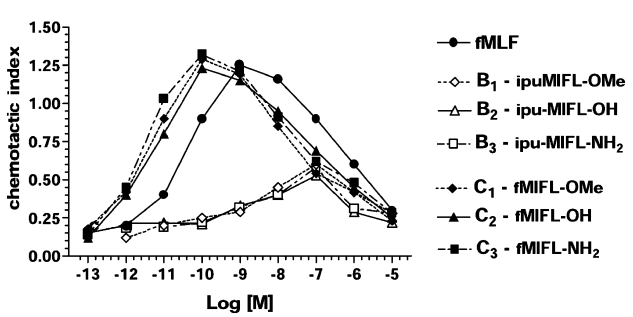

Fig. 3. Chemotactic index of CHO-Met-Leu-Phe (fMLF), CHO-(fMIFL) and *N*-isopropylureido (ipuMIFL) tetrapeptide derivatives toward human neutrophils. These are single representative experiments carried out in duplicate.

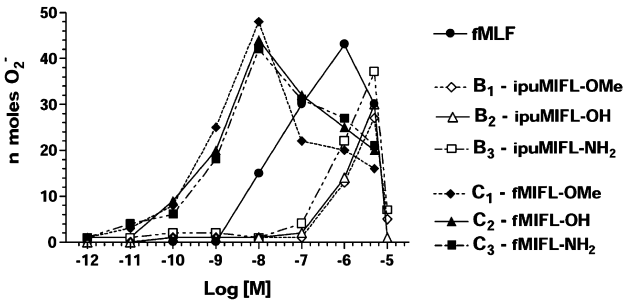

Fig. 4. Superoxide anion production of CHO-Met-Leu-Phe (fMLF), CHO-(fMIFL) and *N*-isopropylureido (ipuMIFL) tetrapeptide derivatives toward human neutrophils. Data are expressed as net nmol/1×10^6 cells/5 min. These are single representative experiments carried out in duplicate.

4. Discussion

We synthesised a series of Met-Ile-Phe-Leu derivatives with the aim of evaluating their affinity and their agonist or antagonist power toward formylpeptide receptors of human neutrophils. The biological properties of tetrapeptides (Fig. 1) were compared with those of CHO-Met-Leu-Phe. Previous studies have demonstrated that CHO-Met-Ile-Phe-Leu-OH acts as a full chemotactic agonist on human monocytes (Rot et al., 1987): the IC_{50} value of the same compound, obtained by competition experiments us-

ing fluoresceinated CHO-Met-Leu-Phe-Lys, was 9 ± 2 nM. As reported in Table 1, the IC_{50} values we obtained on human neutrophils for the binding of the same compound to formylpeptide receptors were 4.7 ± 0.2 nM at 37°C and 5.2 ± 0.2 nM at 4°C. To the best of our knowledge, this is the first report demonstrating a significantly higher affinity of CHO-Met-Ile-Phe-Leu with respect to CHO-Met-Leu-Phe ($IC_{50} = 41 \pm 3$ nM at 37°C and $IC_{50} = 45 \pm 5$ nM at 4°C, Table 1).

At physiological temperatures, receptor processing events such as its internalization can influence the results of the binding studies (Hoffman et al., 1996a,b). For this reason, the displacement experiments have been performed not only at 37°C, but also at 4°C. In fact, at this latter temperature the receptor is not internalized (Jesaitis et al., 1984). The results reported in Fig. 2 and in Table 1 suggest that at both incubation temperatures the compounds have the same effectiveness in displacing [^{3}H]CHO-Met-Leu-Phe from its binding site. A similar result was previously obtained using CHO-Met-Leu-Phe-OMe derivatives (Fabbri et al., 2000).

Figs. 2–4 allow us to observe that CHO-Met-Ile-Phe-Leu derivatives act as full agonists more potent than CHO-Met-Leu-Phe. In fact, they show efficacy properties similar to those of CHO-Met-Leu-Phe and higher affinity toward formylpeptide receptors (Table 1).

It has been noted that the presence of N-terminal *t*-Boc or branched aliphatic substituents convert the agonist CHO-Met-Leu-Phe to weak antagonists for human neutrophils (Derian et al., 1996; Higgins et al., 1996). Besides, while CHO-Phe-D-Leu-Phe-D-Leu-Phe has been reported to be a potent chemoattractant, its homologue *t*-Boc-Phe-D-Leu-Phe-D-Leu-Phe has been shown to be an antagonist for rabbit and human neutrophils (Aswanikumar et al., 1977; Dalpiaz et al., 1999). These observations were the stimulus for us to synthesise the tetrapeptide derivatives, including N-terminal *t*-Boc and isopropylureido substituents (Fig. 1), with the aim of verifying whether their presence can influence the biological properties of Met-Ile-Phe-Leu derivatives. The affinity toward formylpeptide receptors of tetrapeptides appears to be strongly affected by N-terminal substituents. As shown in Fig. 2, the capacity of *N*-isopropylureido-derivatives to inhibit 10 nM [^{3}H]CHO-Met-Leu-Phe binding is drastically reduced with respect to that of *N*-formyl derivatives. In particular, as reported in Table 1, the IC_{50} values of *N*-isopropylureido-derivatives are three orders of magnitude higher than those of the formyl ones. Moreover, the presence of the N-terminal *t*-Boc group seems to extinguish the affinity toward formylpeptide receptors of these tetrapeptides: their IC_{50} values have been estimated as greater than 1 mM. On the other hand, the agonist CHO-Met-Ile-Phe-Leu derivatives do not become antagonists in the presence of *N*-isopropylureido substituents. In fact, the results reported in Figs. 3 and 4 allow us to observe that *N*-isopropylureido-derivatives are able to induce chemotaxis and trigger superoxide anion production in human neutrophils, even if they show a weak partial agonist behaviour (Table 1). It is therefore important to note that the presence of the *N*-isopropylureido substituent reduces the affinity and the efficacy of CHO-Met-Ile-Phe-Leu derivatives to formylpeptide receptors but does not cause the transformation of the tetrapeptides from agonists to antagonists.

As observed from affinity and activity data (Table 1), the agonist properties of Met-Ile-Phe-Leu derivatives on human neutrophils do not seem noticeably influenced by C-terminal methyl esterification or by the conversion to the corresponding amide. A similar behaviour on human neutrophils has been described for CHO-Met-Leu-Phe derivatives (Dentino et al., 1991) and it is shared with *N*-isopropylureido-Met-Ile-Phe-Leu derivatives but not with other formylpeptide receptor agonists or antagonists. In fact, it has been reported that the presence of the methyl ester function at the carboxy terminus of ethyl-Met-Leu-Phe and *n*-Boc-Met-Leu-Phe, respectively, induces a significant enhancement and increase of formylpeptide receptor affinity on human neutrophils (Derian et al., 1996). It has been reported that the methyl ester derivative of CHO-Met-Leu-Phe is more potent than the parent compound on rabbit neutrophils (Iqbal et al., 1984; Dentino et al., 1997).

The properties of Met-Ile-Phe-Leu derivatives seem to conform to formylpeptide receptor models proposed and discussed by several authors (Freer et al., 1982; Edmundson and Ely, 1985; August et al., 1997; Mills et al., 1998). Accordingly, the *N*-formyl group has been suggested to form a hydrogen bond with a phenolic hydroxyl group of a tyrosine residue. Methionine in ligand position 1 appears optimal for the binding whereas position 2 is less critical. An aromatic residue like phenylalanine is optimal in position 3. It was also observed that the receptor has sufficient "room" to accommodate even a fourth amino acid residue. Moreover, the results obtained from the present work allow us to suggest that C-terminal methyl esterification, or the conversion to the corresponding amide, weakly influences the formylpeptide receptor agonist properties of peptide derivatives.

In conclusion, the CHO-Met-Ile-Phe-Leu peptide interactions with human neutrophils have been characterized by receptor binding, chemotaxis and superoxide anion production assays. The results provide new information relative to structure–activity relationships and formylpeptide receptor: C-terminal modification has little effect upon biological activity and the substitution of the *N*-formyl group with the *N*-isopropylureido one induces their conversion to weak partial agonists. The *t*-Boc-Met-Ile-Phe-Leu derivatives do not appear to be capable of interacting with human neutrophil formylpeptide receptors.

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