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Intensity and kinetics of the respiratory burst of human neutrophils in relation to receptor occupancy and rate of occupation by formylmethionylleucylphenylalanine

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Studies on the relationship between the binding of fMet-Leu-Phe and the respiratory response in human neutrophils have been carried out under two different conditions of stimulus presentation, i.e., instantaneously and over a period of time. The main findings are as follows (1) Under the first condition the activation of the respiratory response reaches the maximum value very quickly, when the receptor occupany is less than 20% that at equilibrium. After reaching this maximal value, the activated respiration progressively decreases, while the specific binding of the stimulant continues until equilibrium. (2) Under the second condition, i.e., when the stimulus to neutrophils is presented over a time of 1, 2 or 4 min, the respiratory response (and also the secretory one) is depressed or absent, and the initial rate of the binding (initial V_{ass}) is lower, but the maximal values of the receptor occupancy at equilibrium and of the rate of receptor occupation (maximal V_{ass}) are similar and only slightly lower than those reached under the condition of instantaneous presentation of the stimulus. (3) This form of desensitization is specific for fMet-Leu-Phe and does not consist of the inactivation of the target (NADPH oxidase), since neutrophils desensitized by the slow presentation of the peptide are able to respond to a second challenge with other stimulants. These results indicate that: (1) the efficacy of the stimulus-receptor complexes is short-lived; (2) the intensity of the respiratory response is dependent on the rate of reaching a threshold of binding; (3) when this initial rate is slow, owing to the slow presentation of the stimulus, a specific desensitization takes place, indicating the existence of a molecular mechanism, linked in some way to the initial rate of binding, that modulates the capacity of the stimulus-receptor complexes to transduce signals for cell responses. The physiological role of this type of desensitization is discussed.

Introduction

Synthetic formylated peptides and C5a induce in neutrophils the activation of the processes of aggregation, chemotaxis, secretion and respiration, with production of toxic intermediates of O_2 reduction [1–5]. These responses are initiated by the interaction of the peptide with specific receptors of the cell surface and are regulated by a series of molecular events that at the level of plasma-membrane generate, amplify and transmit specific signals to the target functional system, such as contractile proteins, secretory apparatus and the respiratory system.

Many aspects of ligand-receptor interaction and cell responses have been studied [2,6–12], but the relationships between the formation of ligand-receptor complexes, the transducing events and the

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Abbreviations: fMet-Leu-Phe, formylmethionylleucylphenylalanine; PMA, phorbol myristate acetate.

functional responses of the neutrophils are not well understood. One of the first studies on the stimulus-response coupling in neutrophils, performed in our laboratory, has shown that the respiratory burst, stimulated by the formation of concanavalin A-receptor complexes at the cell surface, can be stopped by addition of sugars known to compete with cell receptors for the lectin [11]. The reversibility of the respiratory burst induced by other stimulants, including formylated peptides, has recently been reinvestigated in our and in other laboratories [10,13–16]. The transient nature of the effects of the ligand-receptor complexes on the respiratory apparatus of neutrophils and the need for continuous binding for maintaining the respiratory system in an activated state has also been shown [11–13].

The intensity of the cell response is influenced by the rate of presentation of the stimulus [17]; in fact, when a chemotactic peptide is presented over a period of time the response of the neutrophil in terms of O_2^- production and of depolarization is depressed with respect to that of the neutrophil stimulated with the same dose of the peptide presented all at once.

In the present paper, studies are reported on the relationship between the kinetics of the respiratory response and that of the binding of the chemotactic peptide in terms of receptor occupancy and rate of receptor occupation and under conditions of different rates of stimulus presentation.

Materials and Methods

Reagents. fMet-Leu-Phe, PMA and cytochalasin B were purchased from Sigma and fMet-Leu-[^{3}H]Phe from New England Nuclear. Preliminary experiments have shown that the biological activity of the labeled peptide is indistinguishable from that of unlabelled one. Stock solutions of fMet-Leu-Phe and of cytochalasin B were made in dimethylsulfoxide and kept at −20°C. All other reagents employed were of the highest available purity.

Preparation of neutrophil suspensions. Neutrophils, more than 98% pure, were prepared from citrate-anticoagulated venous blood obtained from healthy adult donors by dextran sedimentation and centrifugation on Ficoll Hypaque gradients [18]. After hypotonic lysis of contaminating erythrocytes the cells were suspended in Krebs-Ringer phosphate buffer (pH 7.4) (buffer 1) containing 152 mM Na^+, 5 mM K^+, 130 mM Cl^-, 16.6 mM PO_4^{3-}, 1.22 mM Mg^{2+}, 5 mM glucose at a concentration of $(2–4) \cdot 10^7$ cells/ml.

Metabolic studies. The respiratory responses to different stimulants were measured as O_2 consumption at 37°C with a Clark oxygen electrode as previously described [19] using $2 \cdot 10^7$ neutrophils suspended in buffer 1 supplemented with 0.5 mM $CaCl_2$, 5 μg cytochalasin B/ml and 1 mM KCN in order to avoid the inactivation of the chemotactic peptide used as stimulus. The instantaneous velocity of O_2 consumption (V_{ist}) was calculated from angular coefficient of the tangent to the respiration trace at the indicated times. It represents the actual rate of O_2 consumed at each time point $(dO_2/dt) \cdot V_{max}$ is the maximum V_{ist} reached by the respiration.

Secretion assays. $1 \cdot 10^7$ human neutrophils/ml suspended in buffer 1 containing 0.5 mM $CaCl_2$, 1 mM KCN and 5 μg cytochalasin B per ml were incubated for 5 min at 37°C in the plastic chamber used for the measurement of O_2 consumption. Thereafter, fMet-Leu-Phe was added and the cells were incubated at 37°C for a further 5 min from the end of the addition of the stimulant. After this time, the cell suspension was rapidly pelleted by centrifugation at $8000 \times g$ for 30 s in a microcentrifuge (Eppendorf). The supernatants were assayed for released β-glucuronidase [20], as a marker of secretion from azurophilic granules, vitamin B-12-binding protein [21], as a marker of secretion from specific granules, and lactate dehydrogenase [22], as a marker of release of cytosol components. The values of enzyme secretion induced by the stimulant were corrected for spontaneous release and then calculated as percentage of the total cell content measured on sonicated untreated neutrophils.

Addition of the stimulant. fMet-Leu-Phe was added to the neutrophil suspension either instantaneously by injection with a syringe or over different periods of time by driving the peptide solution out of the syringe with an infusion pump. This allowed the addition of the stimulant in the form of a linear time dependent concentration.

fMet-Leu-[^{3}H]Phe binding assay. The binding

of fMet-Leu-[^{3}H]Phe was measured under the same conditions employed for metabolic and secretion studies and using the rapid filtration technique as previously described [23]. Nonspecific binding was defined as the amount of binding not inhibited by 500-fold excess of unlabelled fMet-Leu-Phe and the specific one as the total amount of fMet-Leu-[^{3}H]Phe bound minus the nonspecific binding.

The reversibility of binding was determined by adding a 500-fold excess of unlabelled fMet-Leu-Phe after an appropriate time of incubation of the cells in the presence of 50 nM fMet-Leu-[^{3}H]Phe. The change in cell associated radioactivity after the addition of excess unlabelled fMet-Leu-Phe was used to calculate the reversibly bound fMet-Leu-[^{3}H]Phe, i.e., the ligand-receptor complexes not yet internalized. The amount of nonreversibly bound ligand was calculated from the difference between the specifically bound at the moment of unlabelled peptide addition and that reversibly bound.

Calculation of the rate constants of the binding. The association constant, k_{+1}, was calculated by numerical solution of the following equation:

$$k_1 = k_{obs} \cdot [LR]_e / [L]_T \cdot [R]_T$$

where k_{obs} is the rate constant for the approach to the equilibrium of the binding determined according to Ref. 24, $[LR]_e$ is the concentration of ligand-receptor complexes at equilibrium, $[L]_T$ and $[R]_T$ are the total concentration of the ligand and of the receptors, respectively.

The dissociation constant, k_{-1}, was calculated as $0.693/t_{1/2}$, where $t_{1/2}$ is the half-life for loss of specifically bound radioligand after the additin of 500-fold excess of nonradioactive ligand.

The number of the total receptors, determined previously [23], were 26 000 binding sites/neutrophil, that is a total concentration of $8.6 \cdot 10^{-10}$ M in our cell suspension.

Calculations of the occupancy and the kinetics of ligand-receptor interaction by computer simulation. The combination of the ligand with its receptor is a reversible process which can be written as follows:

$$[L] + [R] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [LR]$$

where [L] is the concentration of the free ligand, [R] of the unoccupied receptors and [LR] of the ligand-receptor complexes.

Thus the rate of the formation of the ligand-receptor complexes (V_{ass}) at a given time can be calculated according to the equation of a second-order reaction:

$$V_{ass} = k_1 \cdot [L] \cdot [R] \quad (1)$$

Eqn. 1 can also be written as follows

$$V_{ass} = k_1 \cdot ([L]_T - [LR]) \cdot ([R]_T - [LR]) \quad (2)$$

for the case in which the all ligand is injected instantaneously and

$$V_{ass} = k_1 (v \cdot t - [LR]) \cdot ([R]_T - [LR]) \quad (3)$$

for the case in which the ligand is injected linearly over a period of time (t_{inf}). In this latter case, the total concentration of the ligand increases with a constant velocity, v (that is, the ratio between the concentration of the ligand at the end the infusion and t_{inf}). Eqn. 3 is true only for $t \leqslant t_{inf}$. So, from the end of the ligand infusion (that is $t > t_{inf}$) Eqn. 2 must be applied.

The rate of the dissociation of the ligand-receptor complexes (V_{diss}) at a given time is given by equation:

$$V_{diss} = k_{-1} \cdot [LR] \quad (4)$$

For the practical application of these equations it is necessary to know [LR] (occupancy) at a given time. This was accomplished by integrating with a computer program (2 ms steps) the following equations:

$$\frac{d[LR]}{dt} = k_1 \cdot ([L]_T - [LR]) \cdot ([R]_T - [LR]) - k_{-1} \cdot [LR] \quad (5)$$

for the case in which the ligand is injected instantaneously, and:

$$\frac{d[LR]}{dt} = k_1 \cdot (v \cdot t - [LR]) \cdot ([R]_T - [LR]) - k_1 \cdot [LR] \quad (6)$$

for the case in which the ligand is injected over a period of time, t_{inf}. In this case Eqn. 6 is true only for $t \leqslant t_{inf}$. For $t > t_{inf}$ Eqn. 5 must be applied.

Results

Fig. 1 shows the time-course of a typical respiratory response of human neutrophils challenged with $5 \cdot 10^{-8}$ M fMet-Leu-Phe. The activated respiration, expressed as V_{ist}, starts about 15 s after the addition of the stimulant and reaches the maximal value in a very short time. The V_{max} of O_2 consumption remains linear for about 20 s and then progressively decreases. By changing the concentration of the stimulus the value of V_{ist} changes, but the kinetics of the respiratory response are not modified.

In terms of receptor binding theory, the question arises whether the respiratory response induced by the chemotactic peptide is related to the occupancy or to the rate of receptor occupation, that is, to V_{ass}. Fig. 1 shows also that the specific binding of the stimulus is very rapid and progressively increases until it reaches a steady level at 6–8 min. The comparison between the respiratory response and the binding of the stimulus demonstrates that while the V_{ist} reaches the maximal value in few seconds and then decreases, the occupancy increases until equilibrium, indicating that there is not a summation of the effect of the ligand-receptor complexes as they form. Furthermore, V_{ist} reaches the maximal value about 30 s after the addition of the stimulus, when the receptor occupancy is less than 20% that at equilibrium. These findings indicate that the intensity of the respiratory response is not directly proportional to the number of the receptors occupied at a given time. Fig. 2 shows the comparison between the respiratory response in term of V_{ist} and the rate of receptor occupation (V_{ass}) and dissociation (V_{diss}). The maximal V_{ass} is reached immediately after the addition of the ligand and then progressively decreases, reaching, within 8 min, a value corresponding to that of V_{diss} (equilibrium). At first sight it seems that the respiratory response, after a short period of time (10 s) necessary for the activation of the transduction mechanisms, correlates with the velocity of the association of fMet-Leu-Phe to the receptors.

It has been recently shown that leukocyte responses can be influenced by the rate of the pre-

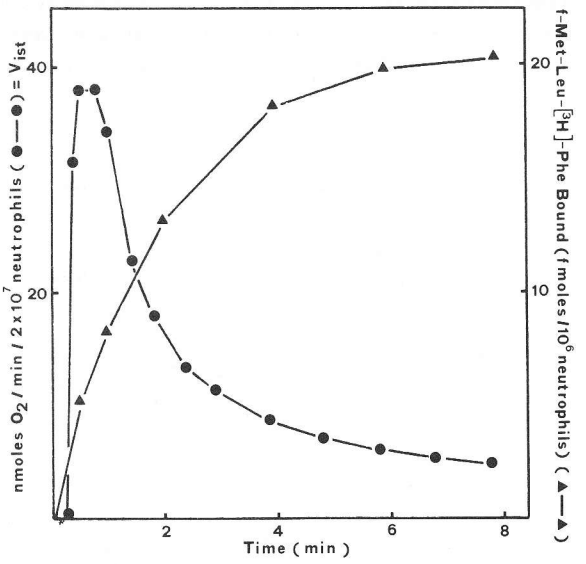

Fig. 1. Kinetics of the specific binding of $5 \cdot 10^{-8}$ M fMet-Leu-[^{3}H]Phe to human neutrophils and of the stimulation of O_2 consumption expressed as V_{ist}. Representative experiment where the measurements were carried out contemporaneously on the same batch of cell.

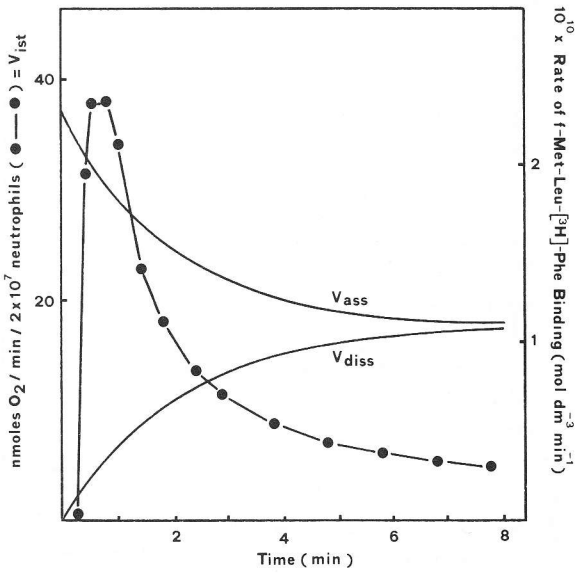

Fig. 2. Comparison between the rate of O_2 consumption (V_{ist}) induced by $5 \cdot 10^{-8}$ M fMet-Leu-Phe in human neutrophils and the rate of fMet-Leu-Phe receptor complexes formation (V_{ass}) and dissociation (V_{diss}). Calculations of V_{ass} and V_{diss} were made by numeric solution of the equations reported in Materials and Methods. Kinetic constants: $k_1 = 5.5 \cdot 10^6\ M^{-1} \cdot min^{-1}$, $k_{-1} = 0.236\ min^{-1}$ and $R_T = 8.6 \cdot 10^{-10}$ M.

sentation of the stimulus. When chemotactic peptides are added to the neutrophil suspension in the form of a linear time-dependent concentration gradient, the cell response in terms of O_2^- production and of change of membrane potential is markedly decreased [17]. Fig. 3 shows the activation of the respiration in neutrophils exposed to $5 \cdot 10^{-8}$ M fMet-Leu-Phe added all at once or over different periods of time (1, 2 or 4 min). It can be

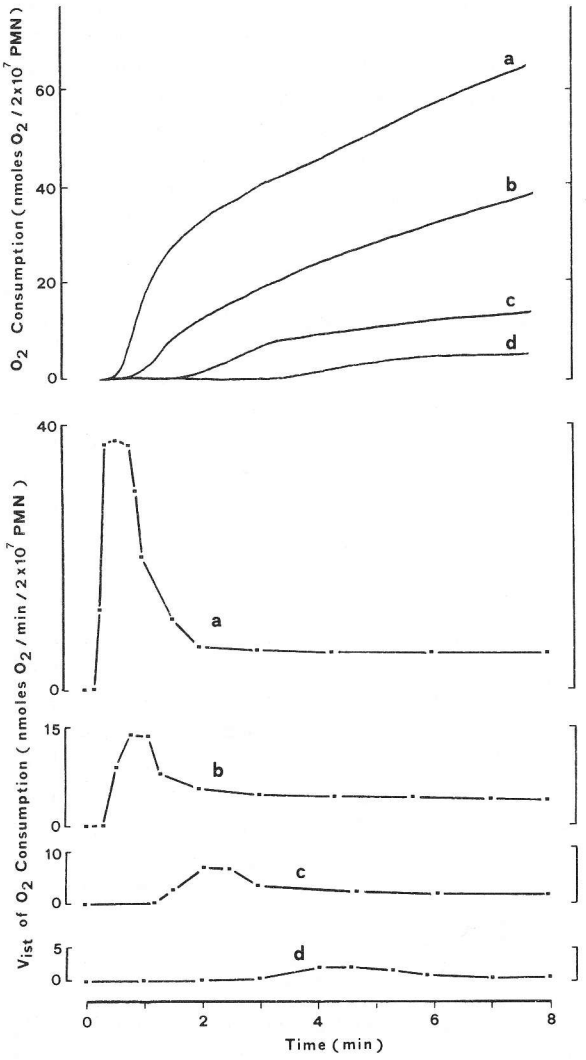

Fig. 3. The respiratory responses of neutrophils (PMN) stimulated by $5 \cdot 10^{-8}$ M fMet-Leu-Phe added all at once (a) or over a period of 1 (b), 2 (c), 4 (d) min. The respiration is expressed as polarographic traces of O_2 consumption (————) and as rate of O_2 consumption (V_{ist}) (· – · – ·).

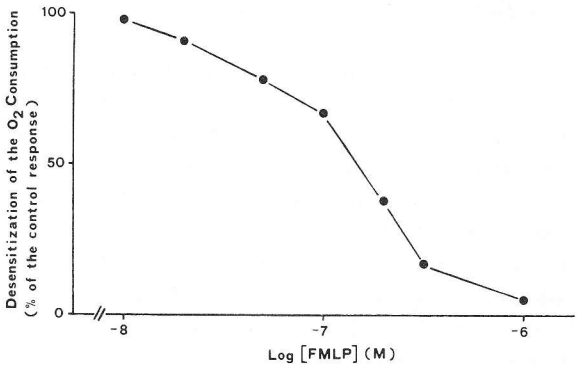

Fig. 4. Desensitization of the respiratory response (O_2 consumption) obtained in human neutrophils by infusing different concentration of fMet-Leu-Phe (FMLP) over 2 min. The desensitization at each concentration of the stimulant is expressed as percent of the control response (stimulant added all at once).

seen that by increasing the time over which the stimulant is infused, the cell response progressively decreases. The infusion of the stimulus over a period of time causes both a decrease in the V_{max} of O_2 consumption and an increase in the lag which precedes the onset of the respiration. With some batches of neutrophils, the respiratory burst was practicaly absent at infusion times of 2–4 min. The results reported in Fig. 4 show that lowest or absent respiratory response occurs at low concentrations of fMet-Leu-Phe, while at high con-

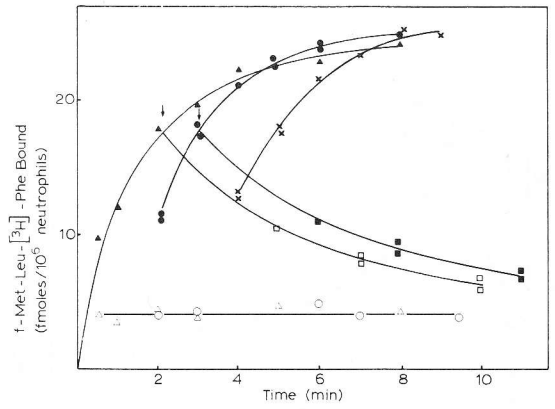

Fig. 5. Time-course and reversibility of the binding of $5 \cdot 10^{-8}$ M fMet-Leu-[^{3}H]Phe to human neutrophils. Total binding in infusion of 2 min (●) and 4 min (×) compared to ligand injected all at once (▲). At the arrows a large excess of unlabelled fMet-Leu-Phe (10 μM) was added and the reversal of binding was measured (□, ■). Unspecific binding infused all at once (△) and during 2 min (○).

centrations of peptide the phenomenon of desensitization by slow infusion progressively decreases. At $3 \cdot 10^{-7}$ M peptide the respiratory response of neutrophils is not modified by the manner of presentation, that is, all at once or over a period of time. Fig. 5 shows the time-course of the binding to the specific receptors when added instantaneously and over 2–4 min. It can be seen that, whatever the rate of presentation of stimulus, for any given dose the same receptor occupancy is reached at equilibrium. Furthermore, the calculation of the association constant (k_{+1}) from the data of the binding after the end of the infusion shows that the value does not change by slowing the presentation of the stimulus. In six experiments, the values of k_{+1} were $(5.63 \pm 0.43) \cdot 10^6$ $M^{-1} \cdot min^{-1}$ when fMet-Leu-Phe was infused all at once and $(5.61 \pm 0.62) \cdot 10^6$ $M^{-1} \cdot min^{-1}$ when the peptide was infused over a period of 2 min. The values of the dissociation constant (k_{-1}) calculated in two experiments were 0.232 and 0.240 min^{-1} for presentation of all the peptide instantaneously or over 2 min, respectively. These results demonstrate that the manner of presentation of the same dose of the ligand does not modify either the number of receptors occupied or their affinity for the ligand.

Fig. 6 reports the time-courses of respiration, of receptor occupancy and of the rate of receptor occupation (V_{ass}) under conditions where $(1, 2, 5) \cdot 10^{-8}$ M fMet-Leu-Phe were added either instantaneously or during 2 min. The values of the occupancy and V_{ass} were calculated by a computer simulation analysis described in the Materials and Methods section. It can be seen that: (a) when the stimulant is infused slowly the maximal value of V_{ass} at the end of the infusion is only slightly lower than that reached when the stimulus is applied all at once; (b) from the end of the infusion the kinetic of the decrease of V_{ass} is similar, whatever the rate of peptide infusion.

In spite of this, the respiratory response to the slow infusion is greatly depressed at each concentration of stimulant.

These findings show that slow infusion of the stimulus does not modify the characteristics of receptor binding, while markedly depressing the efficacy of the ligand receptor complexes.

This desensitization is not due to a different fate of the stimulus receptor complexes, because at equal binding the internalization process (reflected by the amount of nonreversibly bound ligand) was similar when ligand was given at one instant or over a period of time (Fig. 5).

The depression of the respiratory response to the slow presentation of fMet-Leu-Phe is specific. In fact (Fig. 7) neutrophils treated with fMet-Leu-Phe either all at once or over 4 min were able to respond to a second challenge with either PMA or with concanavalin A with similar activation of the respiration. It should be noted that the respiratory response of neutrophils pretreated with fMet-Leu-Phe was similar to that of untreated neutrophils in the case of PMA as second stimulus and higher in the case of concanavalin A as second stimulus. The results obtained with the second stimuli show also that the desensitization by slow presentation of fMet-Leu-Phe is not due to an inactivation of the NADPH oxidase, the terminal respiratory system responsible for the respiratory burst.

The process of desensitization by slow infusion does not occur when PMA is the stimulus. In fact, Fig. 8 shows that the slow infusion of PMA does not depress the respiratory response of neutrophils but only delays the onset of the activated respiration. It is also noticeable that, with PMA as stimulus, the V_{max} of O_2 consumption is maintained for a much longer period than when the stimulus is fMet-Leu-Phe.

Besides the respiratory burst, the secretory response is also influenced by the rate of fMet-Leu-Phe presentation to neutrophils. Table I shows that when the peptide is added to a neutrophil suspension over 4 min the secretion of β-glucuronidase, a marker of lysosomal granules, and of vitamin B12-binding protein, a marker of specific granules, is depressed compared to that induced by the same amount of the peptide added all at once.

Discussion

The results presented in this paper show that the respiratory response of human neutrophils to fMet-Leu-Phe does not correlate with the number of receptor occupied by the ligand. In fact, the maximal V_{ist} of O_2 consumption takes place rapidly when only a small fraction of the receptors are

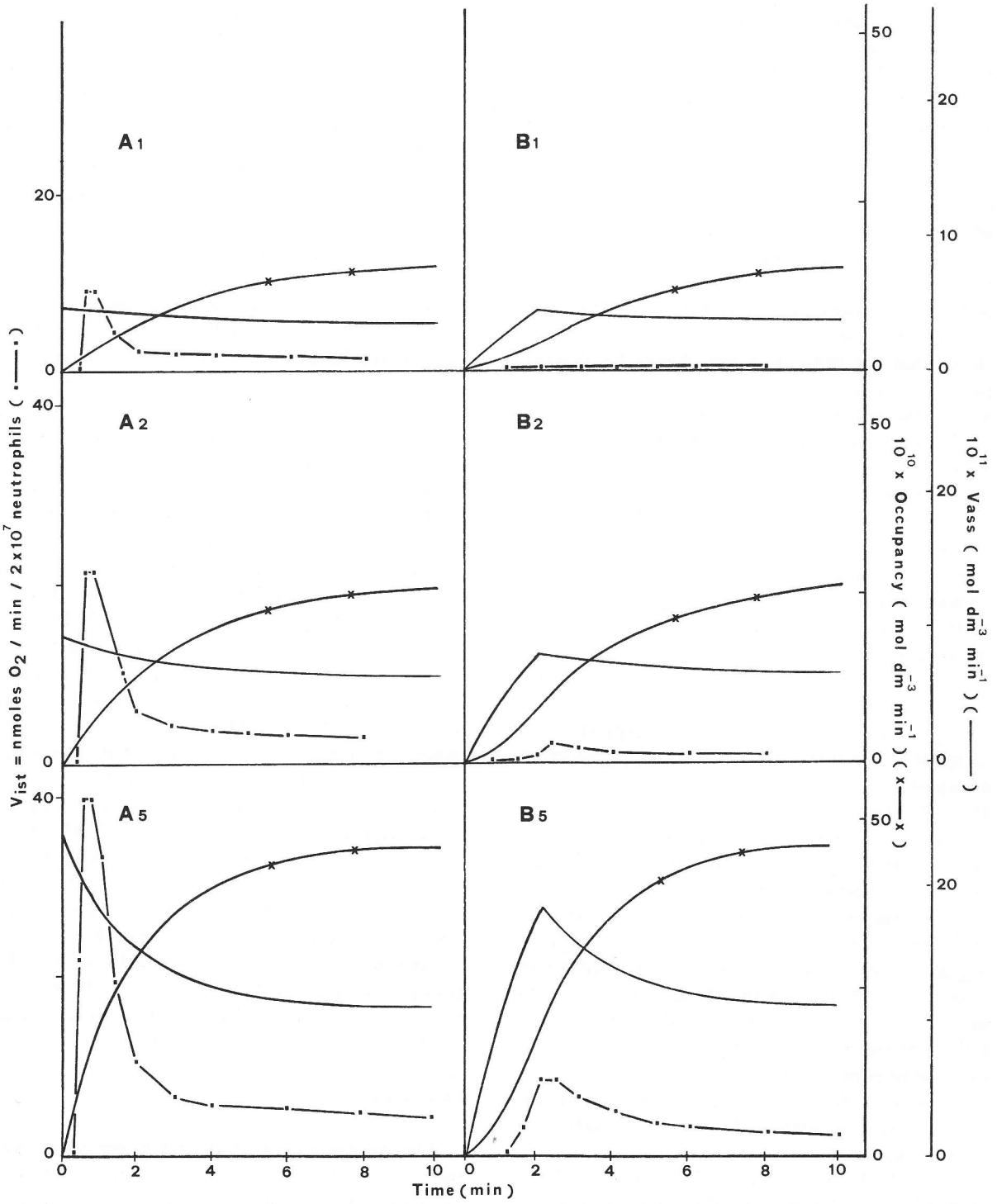

Fig. 6. Relationship between the receptor occupancy, V_{ass} and V_{ist} of O_2 consumption as function of different concentration of fMet-Leu-Phe ($1 \cdot 10^{-8}$ M, $2 \cdot 10^{-8}$ M and $5 \cdot 10^{-8}$ M) added all at once (A_1, A_2, A_5) or over 2 min (B_1, B_2, B_5). Calculation of V_{ass} and occupancy were made by solving the equations reported in Materials and Methods. Kinetic constants: $k_1 = 5.5 \cdot 10^6$ M^{-1} min^{-1}, $k_{-1} = 0.236$ min^{-1} and $R_T = 8.6 \cdot 10^{-10}$ M.

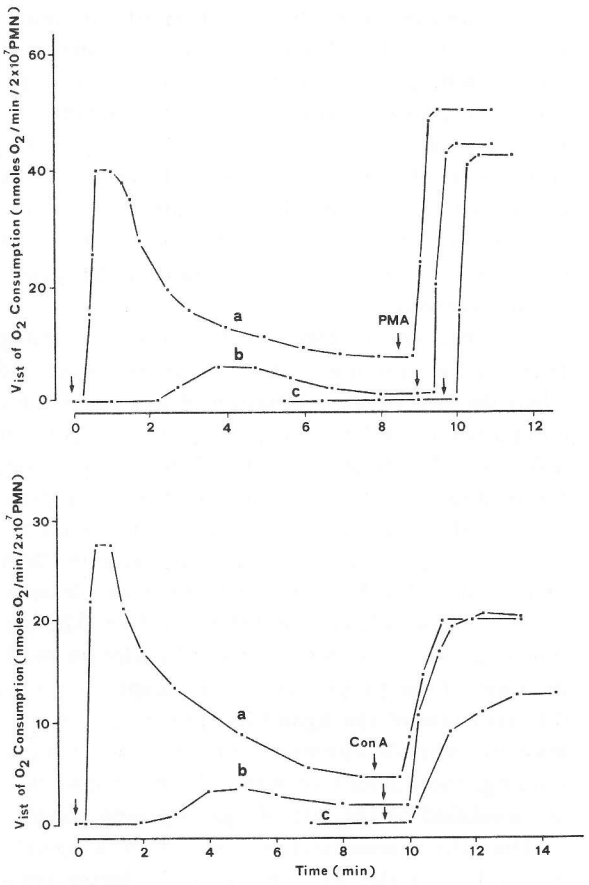

Fig. 7. Rate of O_2 consumption (V_{ist}) induced by $5 \cdot 10^{-8}$ M fMet-Leu-Phe added all at once (trace a) and over 4 min (trace b) in human neutrophils (PMN). Where indicated by the arrows, 20 ng/ml PMA or 100 μg/ml concanavalin A (Con A) were added. Trace c reproesents the control response.

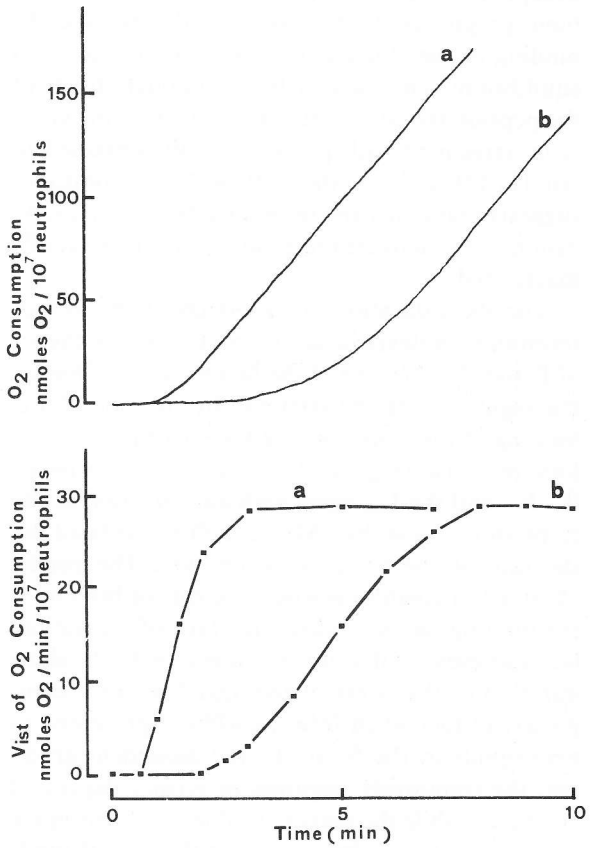

Fig. 8. The kinetics of O_2 consumption of human neutrophils stimulated by 20 ng/ml PMA added all at once (trace a) or over 6 min (trace b). The respiration is expressed as polarographic traces of O_2 consumption (————) and as rate of O_2 consumption (V_{ist}) (·–·–·).

TABLE I

STIMULATION OF O_2 CONSUMPTION, VITAMIN B-12 BINDING PROTEIN AND β-GLUCURONIDASE RELEASE IN HUMAN NEUTROPHILS BY INFUSION ALL AT ONCE AND OVER 4 MIN OF $2 \cdot 10^{-8}$ M AND $5 \cdot 10^{-8}$ M fMet-Leu-Phe

Values represent means ± S.E. of seven experiments for O_2 consumption and four experiments for the β-glucuronidase and B-12 binding protein release.

	$2 \cdot 10^{-8}$ M fMet-Leu-Phe			$5 \cdot 10^{-8}$ M fMet-Leu-Phe		
	Infused all at once	Infused over 4 min	% of desensitization	Infused all at once	Infused over 4 min	% of desensitization
O_2 consumption (V_{max}) [a]	31.2 ± 1.2	4.0 ± 1.3	87.2	49.3 ± 4.1	11.6 ± 2.7	76.5
β-glucuronidase release [b]	11.7 ± 0.38	2.9 ± 0.38	75.2	16.1 ± 1.1	6.5 ± 0.7	59.6
B-12-binding protein release [b]	21.1 ± 1.8	12.3 ± 1.3	41.7	41.6 ± 3.6	28.8 ± 0.35	30.8

[a] nmol O_2/min per $2 \cdot 10^7$ neutrophils.
[b] % of total.

occupied, remains constant for about 20 s and then progressively decreases, while the specific binding of the stimulus continuously increases till equilibrium. This lack of the additional effects of the peptide-receptor complexes as they form, which is in agreement with previous results obtained in our [11,13] and in other [10,14–16], laboratories suggests that either the receptor-effector communication or the activated respiratory system is rapidly inactivated.

The short duration of the effects of the ligand-receptor complexes is, according to the rate theory of Paton [25–27], one of the factors responsible for the regulation of the response by the rate of the binding of the stimulus. The kinetic of the respiratory response (V_{ist}) and the correlation between the V_{ist} and the V_{ass} agree with an activation of the respiratory burst by fMet-Leu-Phe regulated by the rate of the receptor occupation. The results obtained in conditions where the rate of binding is the limiting factor confirm this type of regulation, but also indicate that it is not due only to the short duration of the effect of the ligand-receptor complexes. In fact when fMet-Leu-Phe is presented to neutrophils in the form of time-dependent gradient, the respiratory response in greatly depressed or absent, while the maximal values of the receptor occupancy at equilibrium and of the rate of binding (V_{ass}) at the end of infusion are similar to those under conditions of instantaneous presentation of the ligand (Fig. 6). This means that the same occupancy and the same maximal velocity of occupation can induce different responses depending on the time employed to reach the considered values. Since the maximal respiratory response, in terms of maximal V_{ist}, is reached when only a portion of the receptors is bound (critical binding), the main factor regulating the value of the maximal response is the time necessary to reach the number of receptors occupied corresponding to this threshold; the longer the time, the lower the maximal respiratory response. When the concentration of the stimulus is low or when the presentation of the stimulus to the cells is very slow, the coupling between the stimulus and the response can be completely absent.

Thus, the respiratory response to fMet-Leu-Phe, in terms of maximal V_{ist} is regulated, at least partly, by the time necessary to reach the number of receptors occupied, which represents the maximal stimulus (threshold) at that concentration. After reaching the maximal value, the V_{ist} of respiration progressively decreases and the kinetics of the respiratory response, in terms of V_{ist} throughout the whole duration of the burst, is controlled by the ratio between the V_{ass} and the duration of the efficacy of the ligand receptor complexes (or of the activated state of the target) at any moment.

The mechanism responsible for the uncoupling between the stimulus and the response generated when the rate of the formation of ligand-receptor complexes is under a critical level is at present unknown. Inactivation of the NADPH oxidase, the respiratory system responsible for the respiratory burst, is not involved, since a maximal respiratory response to a second challenge with PMA or concanavalin A occurs in neutrophils desensitized by slow infusion of fMet-Leu-Phe. The desensitization is also not dependent either on modification of the properties of the receptor or on a different fate of the ligand-receptor complexes, at least in terms of internalization. In fact, at equal binding, the amount of internalized complexes is not modified by the rate of ligand presentation.

Thus the desensitization consists of a specific uncoupling at the level of receptor-effector communication. According to Sklar et al. [17], who first described this type of desensitization, a mechanism which inhibits cellular triggering would be effective as long as the binding rate fails to exceed critical values. Since our data show that the rate of the binding (V_{ass}) reaches similar values when the stimulus is injected all at once and when infused over a period of time, this inhibitory mechanism is generated during the initial period of slow rate of binding and it is not removed when the binding rate reaches the critical value at the end of infusion.

The desensitization by slow presentation does not occur when the stimulant is PMA. It has been suggested that this compound acts by activating the protein kinase C by substituting for diacylglycerol [28,29]. If this is the case, the block of the translation of the signal from fMet-Leu-Phe to the target concerns reactions preceding the level of action of PMA, i.e., calcium movements and release [30–34], phospholipid turnover [35–37], cyclic

nucleotide turnover [38–40], etc. It is worthwhile pointing out that this type of desensitization is specific for the stimulus used for the slow presentation, because it does not induce a state of unresponsiveness to PMA or to concanavalin A. This specificity indicates that the molecular mechanism of the uncoupling takes place very near to the receptor or to the ligand-receptor complexes, so that the signal is not translated to the target for the response. It is conceivable that structural modification of the receptors, which retain their binding properties but lack the capacity to transmit signals for the response, is the molecular mechanism of the uncoupling, in analogy with the desensitizationof adenylate cyclase caused by catecholamine in different cells [41,42].

Whatever the mechanism and the transducing level involved, it is clear that it is common also to the secretory response, which is depressed by the slow presentation of the chemotactic peptide.

The physiological meaning of the findings presented in this paper can be discussed. Biological stimuli are more likely to be produced by the cells or by another molecular system, i.e., complement, over a period of time such that their presentation to leukocytes takes place in the form of a concentration gradient increasing with time. Furthermore, this form of interactioin occurs, as discussed by Sklar et al. [17], during chemotaxis when leukocytes move through the concentration gradient of chemoattractant. In this case chemotaxis occurs without release of toxic free radicals and enzymes, a process that can be dangerous for the tissue and for the neutrophil itself.

The modification of the coupling between the stimulus and the responses induced by the slow ligand-receptor interaction is relevant for the modulation of the sensitivity to a stimulus. Thus, in addition to the dose of stimulus, a series of complex but related events, such as the rate of the binding, the time of reaching the threshold value of the binding, and the short duration of the efficacy of ligand-receptor complexes, regulate the intensity and the kinetics of neutrophil responses to chemotactic peptides. Investigations are in progress in our laboratory with the aim to understand better the mechanism of this complex regulation at the molecular level.

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