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Chemoattractant receptor functions in human polymorphonuclear leukocytes are divergently altered by membrane fluidizers

(chemotaxis/fMet-Leu-Phe receptor/*n*-butanol)

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ABSTRACT The chemotactic factor receptor on leukocytes initiates several cellular responses including chemotaxis, lysosomal enzyme secretion, and O_2^- production. The latter two responses require approximately 10–100 times more chemoattractant than is required for chemotaxis. We determined the effects of membrane fluidizers on the binding characteristics and the functional activities of the oligopeptide fMet-Leu-Phe chemotactic factor receptor on polymorphonuclear leukocytes. Fluidization was induced by aliphatic alcohols and monitored by diphenylhexatriene fluorescence polarization. Low doses of *n*-butanol (0.25%) and *n*-pentanol (0.1%) were nontoxic to the leukocytes yet reduced their diphenylhexatriene-induced polarization, indicating increased membrane fluidity. At these doses of alcohols, the affinity of the fMet-Leu-Phe receptor was enhanced from $K_d = 25.5 \pm 7.6$ nM to $K_d = 5.2 \pm 0.9$ nM and $K_d = 6.0 \pm 0.9$ nM, respectively. Chemotaxis was also increased, as indicated by the decrease, by a factor of approximately 1/3 in the ED_{50} for fMet-Leu-Phe, as well as by a 1.5-fold increase in the maximal distance of migration in the presence of 0.25% butanol or 0.1% pentanol. In contrast to chemotaxis, the alcohols depressed fMet-Leu-Phe stimulation of O_2^- production by 90% although they had no effect on phorbol 12-myristate 13-acetate-induced O_2^- production. Secretion of lysozyme was also inhibited. Thus, the affinity of the fMet-Leu-Phe receptor can be modulated by membrane fluidizers. The higher affinity state of the receptor induced by the alcohols is more efficient in transducing chemotactic signals but is deficient in mediating O_2^- production or secretion. Thus, the transduction mechanisms for the various biological activities of the chemotactic factor receptor are heterogeneous and can be differentially manipulated by membrane fluidizers.

Leukocyte chemotactic responses are initiated by the binding of chemoattractants to specific cell surface receptors (1–5). Certain synthetic *N*-formylated methionyl oligopeptides, which may be analogous to the NH_2-terminal degradation products of bacterial proteins, are potent chemotactic factors for polymorphonuclear leukocytes (PMNs) (6). The same factors, at higher doses than are required for chemotaxis, stimulate other biological activities in leukocytes, including the production of superoxide anions (7, 8) and the secretion of lysosomal enzymes (9). Though the existence of highly specific receptors for *N*-formylated oligopeptides on leukocytes has been established, little is known about the factors that regulate receptor binding or the transduction of its signals leading to different biological responses.

In human PMNs, agents that stimulated low levels of lysosomal granule caused an increase in the *N*-formylated peptide receptor number and an apparent decrease in the affinity of the binding sites (10, 11). In guinea pig macrophages, chemoattractant receptors exist in more than one affinity state and it has been suggested that receptor affinity reflects its functional activity (12, 13). PMNs developed increased binding to oligopeptide chemoattractants on treatment with aliphatic alcohols and the data were interpreted as showing cryptic receptors in the cells (11, 14). In other studies, interaction of chemotactic factors with leukocytes were found to alter the phospholipid metabolism of the cells (15–18), and it was suggested that such alterations in the lipid composition of the membrane could affect its physical features in the vicinity of the occupied receptors and thereby influence receptor activity.

In a broad spectrum of cellular systems, changes in averaged membrane fluidity have been shown to modify the binding characteristics of receptors (19, 20) and receptor-induced metabolic processes (21, 22). Because aliphatic alcohols augment the averaged membrane fluidity in a nonspecific manner (23), we determined the effects of such alcohols on the binding and functional activities of oligopeptide chemoattractant receptors on human PMNs.

MATERIALS AND METHODS

Chemicals. *N*-Formylmethionylleucylphenylalanine (fMet-Leu-Phe) and 4β-phorbol 12α-myristate 13α-acetate (PMA) were from Sigma. fMet-Leu-[^{3}H]Phe (46.7 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was from New England Nuclear.

Cells. Purified PMNs (>95%) were isolated from the blood of healthy volunteers by using dextran and Ficoll-Hypaque gradients (2).

fMet-Leu-[^{3}H]Phe Binding to PMNs. Cells were suspended in incubation buffer (24) and, where indicated, with the alcohols for 15 min at 25°C. The alcohols were present during the assays. Binding with fMet-Leu-[^{3}H]Phe was carried out with 7×10^5 cells in 150-μl volumes in the absence (total binding) and presence (nonspecific binding) of 10 μM fMet-Leu-Phe at either 25°C for 60 min or at 0°C for 120 min. The difference between total and nonspecific binding was defined as specific binding. Modeling of the data was carried out with a nonlinear least-squares fitting computer program developed by Hancock *et al.* (25), the algorithms of which are based on Feldman's (26) rigorous treatment of the mass action law.

Chemotaxis Assay. Migration of PMNs into 8-μm nitrocellulose filters (Millipore) in 0.2-ml blind-well chambers (27) was assayed by the leading front technique as described (28). Where indicated, cells were incubated for 15 min at 25°C with the al-

Abbreviations: PMN, polymorphonuclear leukocyte; PMA, 4β-phorbol 12α-myristate 13α-acetate; P_i/NaCl, phosphate-buffered saline; DPH, 1,6-diphenyl-1,3,5-hexatriene.

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cohol prior to the chemotaxis assay, during which time the alcohols were present in equal concentrations in both compartments of the chemotaxis chambers.

Superoxide Anion Production. PMNs were first incubated for 15 min at 25°C with buffer or with 0.1% or 0.25% *n*-butanol. Reaction mixtures of 1.5 ml consisted of 5×10^5 cells, 80 μM cytochrome *c* (type VI; Sigma), various doses of *n*-butanol, and either fMet-Leu-Phe or PMA in Hanks' balanced salt solution (pH 7.0) and were incubated for 15 min at 37°C. The absorbance at 550 nm of the supernatant was determined. Results were recorded as absorbance of the stimulated system minus that of the appropriate nonstimulated one.

Lysozyme Release. PMNs (2.5×10^6) in 1 ml of phosphate-buffered saline (pH 7.4) (P_i/NaCl) were incubated with 10 μM cytochalasin B (Sigma) in the absence or presence of buffer or of 0.1% or 0.25% *n*-butanol. Then, either buffer, various doses of fMet-Leu-Phe, or Triton X-100 to a final concentration of 0.2% was added, and the mixture was incubated for 15 min at 37°C. The reaction was terminated by centrifugation at 1,500 × g for 15 min at 4°C. A 0.2-ml aliquot of the supernatant was added to 0.8 ml of *Micrococcus lysodeikticus* (Sigma) suspension (0.3 mg/ml), this mixture was incubated for 15 min at 37°C, and 0.5 ml of absolute ethanol was added to terminate the assay. Light scattering at 450 nm was measured, and results were recorded as $100 \times [A_{450}$ (experimental) $- A_{450}$ (control)$]/[A_{450}$ (Triton X-100) $- A_{450}$ (control)$]$.

Steady-State Fluorescence Polarization. PMNs were fluorescently labeled by incubating 1×10^6 cells with 1 μM 1,6-diphenyl-1,3,5-hexatriene (DPH) suspension in P_i/NaCl for 15 min at 25°C. Alcohols were added for a further 15 min of incubation. The unincorporated DPH was removed by two washes with P_i/NaCl. Fluorescence polarization was measured at 25°C with a Microviscosimeter MV-1 (Elscint, Israel) and recorded in terms of P (29) only for values remaining constant at successive dilutions [1:3 (vol/vol) with P_i/NaCl] to avoid light scattering and signal-to-noise effects on the polarization measurements. Results are expressed by the dimensionless microviscosity parameter defined as $[(r_0/r) - 1]^{-1}$, where r is the anisotropy calculated from the measured polarization according to $r = 2P/(3 - P)$ and r_0 is the limiting value of r at infinite viscosity (29). The microviscosity parameter is a relative and uncalibrated presentation of the membrane lipid core viscosity wherein DPH is embedded.

RESULTS

The Effect of Various Alcohols on the Steady-State Binding of fMet-Leu-[^{3}H]Phe. The steady-state binding of fMet-Leu-[^{3}H]Phe to human PMNs was studied first at a constant 2.5% (vol/vol) concentration of the primary alcohol series. The steady-state binding of fMet-Leu-[^{3}H]Phe to cells previously incubated with the alcohols for 30 min at 25°C is shown in Fig. 1. Ethanol had no significant effect on binding, whereas *n*-propanol and *n*-butanol enhanced binding. In contrast, *n*-pentanol totally abolished fMet-Leu-[^{3}H]Phe binding. Cell viability as determined by trypan blue exclusion revealed no toxic effects for ethanol and *n*-propanol whereas *n*-butanol and *n*-pentanol caused 85% and 100% cell mortality, respectively.

To quantitate the physical effects of the alcohols on whole PMN membranes, studies of DPH fluorescence polarization were carried out. The results (Fig. 2) revealed a monotonous increase in PMN membrane fluidization with elongation of the primary alcohols that reached an extreme fluidized state for the *n*-pentanol-treated cells. The latter state had a microviscosity parameter value far below that of one of the most fluid liposome systems made of asolectin—i.e., 0.55 (30). This suggests that

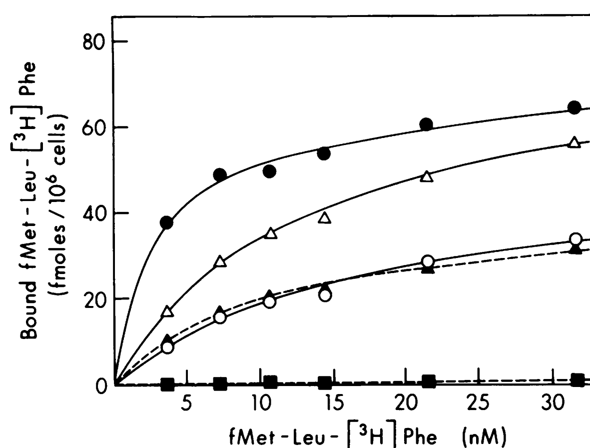

FIG. 1. Specific binding of fMet-Leu-[^{3}H]Phe to PMNs previously incubated with buffer (○) or with 2.5% ethanol (▲), *n*-propanol (△), *n*-butanol (●), or *n*-pentanol (■). Binding was carried out at 0°C for 2 hr. Each point represents the mean of two determinations of a pair of total and nonspecific systems.

2.5% *n*-pentanol actually disrupted the cell membrane structure, thereby causing loss of the fMet-Leu-Phe binding capacity.

Further studies of the alcohol effects were made with *n*-butanol as a model system at concentrations that maintained full cell viability. To verify that the effects of *n*-butanol were due to its ability to alter the physical state of the membrane lipid core, concentrations of *n*-pentanol that produced equivalent membrane fluidization were also examined. The dose-dependent fluidization effects of *n*-butanol and *n*-pentanol at concentrations that maintained >95% PMN viability as measured by trypan blue exclusion are shown in Fig. 3. Whole cell membrane fluidization was complete 2 to 3 min after introduction of the alcohols, and this effect was reversed on the same time scale by their removal (data not shown).

Isotherms of total and nonspecific binding of fMet-Leu-[^{3}H]Phe in the absence or presence of either 0.25% *n*-butanol or 0.1% *n*-pentanol are presented in Fig. 4. The data fitted a single-receptor mass-action-law model. The alcohols produced a shift of the receptor affinities (K_d ± SEM) from 25.7 ± 7.6 nM for the nontreated cells to 5.2 ± 0.9 and 6.0 ± 0.9 nM for the *n*-butanol- and *n*-pentanol-treated cells, respectively. The maximal binding capacities were estimated to be 57 ± 6, 40 ± 3, and 43 ± 5 fmol per 10^6 cells for control, *n*-butanol-treated

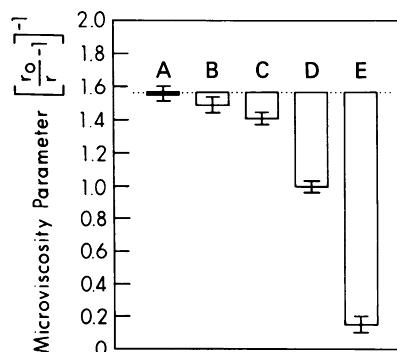

FIG. 2. Reduction of whole PMN membrane microviscosity by 2.5% aliphatic alcohols. The microviscosity parameter was derived from the steady-state fluorescence polarization of DPH determined at 25°C. Bars: A, nontreated; B, ethanol; C, *n*-propanol; *D*, *n*-butanol; E, *n*-pentanol. Bars represent means ± SD of four PMN preparations, each measured twice.

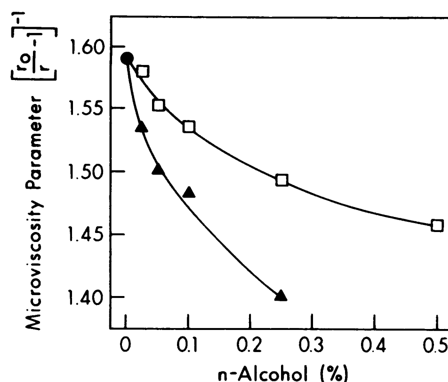

FIG. 3. PMN membrane microviscosity response to low concentrations of n-butanol (□) and n-pentanol (▲). Each point represents duplicate readings, the reproducibility of which was ±0.01.

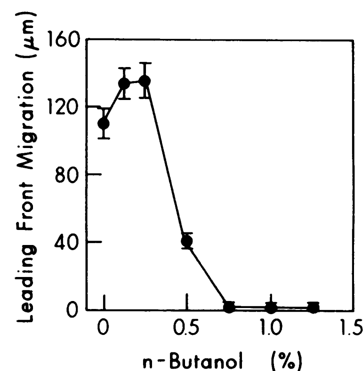

FIG. 5. Dose-dependent effect of n-butanol on fMet-Leu-Phe-stimulated PMN chemotaxis. Cells were incubated with n-butanol and then applied to blind-well chemotaxis chambers containing 10 nM fMet-Leu-Phe and the indicated doses of n-butanol in the lower compartment. Migration was allowed to proceed for 35 min at 37°C. Results represent means ± SD of four independent determinations.

and n-pentanol-treated cells, respectively. These estimations should be considered as approximate, since the whole cell steady-state-associated fMet-Leu-Phe cannot be accounted for exclusively as surface receptor bound (31, 32). The alcohols did not increase the internalization of fMet-Leu-[^{3}H]Phe, since the residual bound fraction after a 60-min dissociation with 500-fold excess fMet-Leu-Phe was not increased in the alcohol-treated cells.

The Effect of n-Butanol and n-Pentanol on the Chemotaxis of Human PMNs. fMet-Leu-Phe is known to induce a maximum chemotactic response in human PMNs at a dose of 10 nM, beyond which the response decreases sharply (2, 33). Low concentrations of n-butanol elevated the peak chemotactic response whereas, at higher concentrations of the alcohol, inhibition of chemotaxis was observed (Fig. 5). The latter trend is correlated with, and is assumed to precede, the cellular toxicity dose-dependent effect of n-butanol.

There was an increased sensitivity by the alcohol-treated PMNs to submaximal stimulating doses of fMet-Leu-Phe, with an average shift of the chemotactic response to a fMet-Leu-Phe dose lower by a factor of approximately 1/3 for the maximal effect of the alcohols (Fig. 6). No effect of the alcohols was noted on chemotactic desensitization. The paired doses of alcohols that produced similar effects on the apparent microviscosity and fMet-Leu-[^{3}H]Phe binding (i.e., 0.05% n-pentanol ≃ 0.1% n-butanol and 0.1% n-pentanol ≃ 0.25% n-butanol) also caused similar effects on the PMN chemotactic responses.

The time dependence of PMN migration at the maximal enhancing alcohol concentrations is shown in Fig. 7. In untreated PMNs, the fMet-Leu-Phe-stimulated migration deflected from the random migration after about 10 min. The stimulated migration rate of the leading front increased from the apparent linear random migration rate of 1.8 μm/min to about 3.1 μm/min. Neither n-butanol nor n-pentanol significantly changed the maximal fMet-Leu-Phe-stimulated migration (i.e., 3.3 μm/min); however, this rate was initiated instantaneously rather than after a 10-min delay. Similar effects were seen in a morphological polarization assay (34, 35), which does not use filters or require cell adherence to a substrate (data not shown).

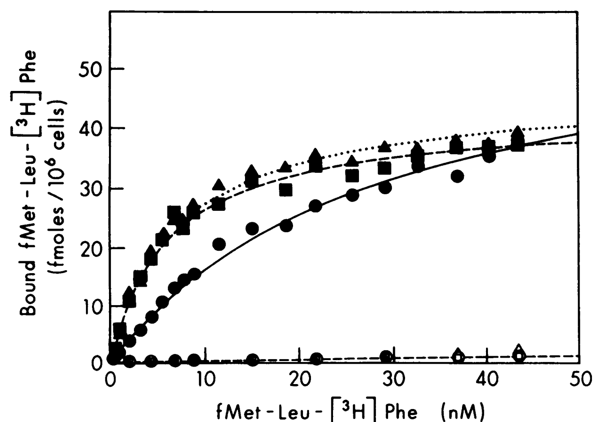

FIG. 4. Total and nonspecific binding of fMet-Leu-[^{3}H]Phe to PMNs in the absence (○, ●) and presence of either 0.25% n-butanol (□, ■) or 0.1% n-pentanol (△, ▲). Binding was carried out in the absence (●, ■, ▲) and in the presence (○, □, △) of 10 μM fMet-Leu-Phe at 25°C for 60 min. The curves were produced by computerized fitting of the data to a monoaffinity receptor model. Paired Student's t tests of the data obtained from three duplicated experiments revealed no significant difference between the n-butanol- and n-pentanol-treated systems ($P \gg 0.5$), both of which are significantly different from the control system ($P < 0.01$ up to 20 nM fMet-Leu-[^{3}H]Phe).

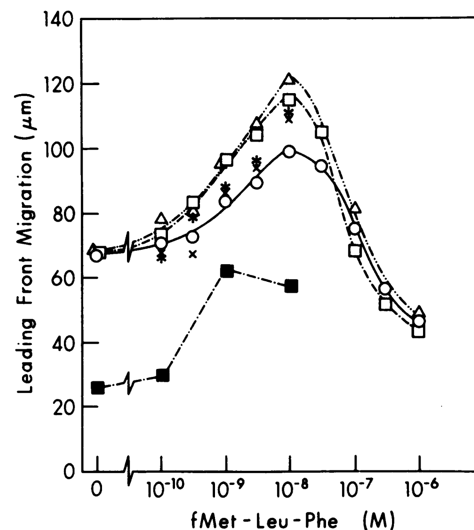

FIG. 6. Effects of n-butanol and n-pentanol on fMet-Leu-Phe dose-dependent PMN chemotaxis. Before assay, cells were incubated with buffer (○), with 0.1% (×), 0.25% (□), or 0.5% (■) n-butanol, or with 0.05% (*) or 0.1% (△) n-pentanol. Cell migration was assayed for 35 min at 37°C. Each point represents the mean of three to six experiments each of triplicate filters. SD values were <10%. Paired Student's t tests revealed highly significant effects for alcohol-treated migrating PMNs in response to ≤10 nM fMet-Leu-Phe ($P \ll 0.01$) but no differences between the pairs 0.05% n-pentanol vs. 0.1% n-butanol and 0.1% n-pentanol vs. 0.25% n-butanol ($P > 0.5$).

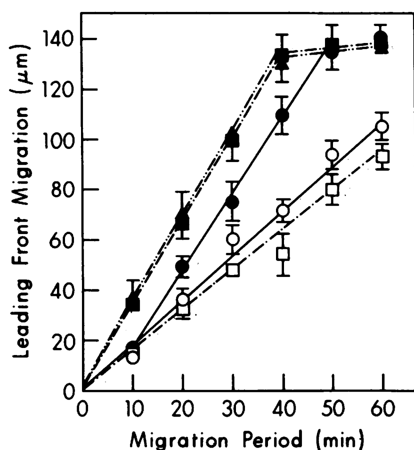

FIG. 7. Effects of *n*-butanol and *n*-pentanol on the time course of PMN migration. Before assay, cells were incubated with buffer (○, ●), 0.25% *n*-butanol (□, ■), or 0.1% *n*-pentanol (▲). Random migration (○, □) and chemotaxis (●, ■, ▲) were assayed in the absence or presence of 10 nM fMet-Leu-Phe in the lower compartment of the chemotaxis chamber. Results are means ± SD of three experiments with triplicate filters. The 140-μm plateau represents the far surface of the filter.

The Effect of *n*-Butanol on fMet-Leu-Phe-Stimulated O_2^- Production and Lysozyme Secretion. Chemotactic factors stimulate superoxide dismutase-inhibitable O_2^- production and the secretion of lysosomal enzymes by PMNs. Both activities were tested in alcohol-treated PMNs. *n*-Butanol at doses of 0.1% and 0.25% inhibited fMet-Leu-Phe-stimulated O_2^- production of PMNs by about 50% and 85%, respectively (Fig. 8*A*). A shift to higher fMet-Leu-Phe doses (approximately fivefold) of the ED_{50} accompanied the *n*-butanol inhibition effects. Similar inhibition was recorded for the corresponding *n*-pentanol concentrations using 1 μM fMet-Leu-Phe stimulation (data not

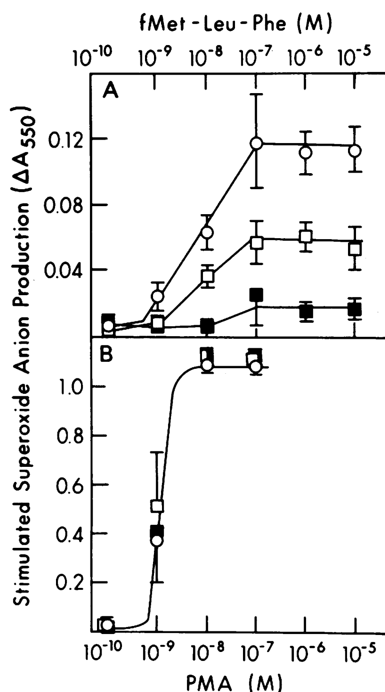

FIG. 8. Effect of *n*-butanol on superoxide anion production. PMNs previously incubated with buffer (○) or with 0.1% (□) or 0.25% (■) *n*-butanol were stimulated by fMet-Leu-Phe (*A*) or PMA (*B*). Stimulated O_2^- production is expressed as A_{550}(experimental) − A_{550}(control) and presented as mean ± SD of four experiments performed in duplicate.

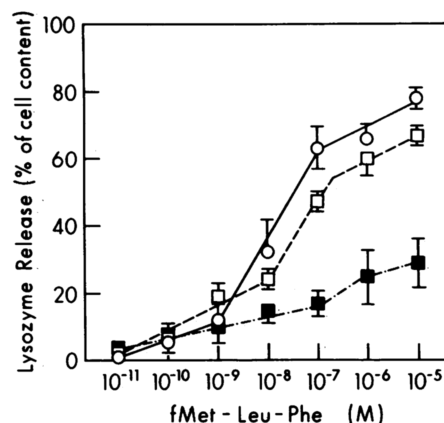

FIG. 9. Effect of *n*-butanol on lysozyme release from PMNs. Cells previously incubated with buffer (○) or with 0.1% (□) or 0.25% (■) *n*-butanol were incubated with the indicated doses of fMet-Leu-Phe. Released lysozyme was assayed for disruption of *M. lysodeikticus* in suspension by measurement of light scattering. Results represent percentage of the potential decrease in A_{450} of the PMNs obtained in four experiments performed in duplicate.

shown). The effect of the alcohol was not due to nonspecific toxicity, since O_2^- production evoked by PMA remained unaltered (Fig. 8*B*), implying that the alcohol affected the chemotactic factor receptor transduction mechanism of O_2^- production rather than the oxidase. In other experiments, treatment of PMNs with 1 μM cytochalasin B enhanced the O_2^- production stimulated by 0.1 μM fMet-Leu-Phe to the level achieved by the maximal dose of PMA—i.e., ΔA_{550} = 0.97 ± 0.04 and 1.08 ± 0.02, respectively. Under these conditions, 0.25% *n*-butanol was equally effective in inhibiting fMet-Leu-Phe-stimulated O_2^- production (i.e., ΔA_{550} was reduced to 0.04 ± 0.02). In addition, the fMet-Leu-Phe-stimulated, but not the PMA-stimulated, respiratory burst, as measured by O_2 consumption, was inhibited in butanol-treated PMNs in the presence or absence of cytochalasin B (data not shown). The effects of the alcohols in inhibiting O_2^- production by fMet-Leu-Phe could not be due to a "scavenging" effect, because the alcohols did not depress PMA-stimulated O_2^- production and blocked the respiratory burst stimulated by fMet-Leu-Phe but not that stimulated by PMA (36).

The effect of fMet-Leu-Phe on the stimulation of lysosomal enzyme secretion was inhibited by about 15% and 65% by 0.1% and 0.25% *n*-butanol, respectively, together with a ED_{50} shift of more than an order of magnitude to higher fMet-Leu-Phe doses (Fig. 9). The spontaneous lysozyme release as well as the total lysozyme activity of the PMNs were unaffected by the alcohols.

DISCUSSION

The ability of leukocytes to perceive and migrate along minute gradients of chemoattractants suggests that this response must be finely regulated. This complex cellular response is initiated by the binding of the chemoattractants to highly specific receptors (37) that evoke a number of other important biological responses, including secretion of lysosomal enzymes and production of superoxide anions. The latter two activities, however, require up to 100-fold more chemoattractant than is needed for chemotaxis stimulation (7–9). While chemotactic factor receptor occupancy is necessary for initiating these responses, the actual mechanisms of the transduction and regulation of the receptor are as yet poorly defined.

The data presented here suggest that alcohols increase fMet-Leu-Phe specific binding in accord with their capability to in-

duce membrane fluidization. Similar enhanced binding of chemoattractants to rabbit and human PMNs treated with *n*-butanol was interpreted as evidence for the presence of cryptic receptors in these cells (11, 14). However, our data, based on analysis of detailed binding isotherms, are more consistent with the contention that aliphatic alcohols enhance the affinity rather than the numbers of chemoattractant receptors on PMNs. The mechanism of this enhancement requires the integrity of the membrane structure (e.g., no binding with 2.5% *n*-pentanol-treated PMNs) but not necessarily viable cells (e.g., enhanced binding with 2.5% *n*-butanol-treated PMNs) and thus requires no active metabolic process. Doses of the alcohols that fully preserved cell viability and caused apparent mild membrane fluidization shifted the fMet-Leu-[^{3}H]Phe apparent binding affinity of whole PMN receptors to a higher state. The hypothesis that the binding affinity shift is correlated with the degree of membrane fluidization was supported by the almost identical effect on binding obtained for the fluidizing-synchronized concentrations of *n*-butanol and *n*-pentanol.

The most profound finding in this study is that the increase in the apparent binding affinity due to the alcohols is associated with divergent alterations of the biological activities initiated by the chemotactic factor receptor. Interestingly, chemotaxis, which responds optimally to low doses of fMet-Leu-Phe, was amplified, whereas superoxide anion production and lysozyme secretion, which respond at more elevated doses of fMet-Leu-Phe, were inhibited. Yet, all of the above response mechanisms—i.e., maximal cellular migration rate, respiratory burst, and total cellular lysozyme activity—retained their full capacity in the alcohol-treated cells. It is not yet clear how the alcohols enhanced PMN chemotactic responses but it is unlikely that this was due to effects on motility itself because neither random migration nor the maximally stimulated PMN movement rate were altered. The alcohols rather appeared to decrease the lag time required for the PMNs to begin responding to the chemotactic gradient. The foregoing phenomenon together with the strong correlation of the effects of the alcohols with the physical state of the cell membranes leads to the conclusions that the alcohol effects are confined to the receptor or to the interaction of the receptor with those transduction mechanisms that are associated with the membranous lipid core structure. The ability of the alcohols to simultaneously affect various fMet-Leu-Phe-stimulated responses in strikingly diverse ways strongly suggests that certain chemoattractant receptor transduction mechanisms are distinct and can be regulated by the physical state of the cell membrane. A model that arises from these data divides the transduction mechanisms of the chemotactic factor receptor into at least two categories, those associated with a higher binding affinity and others associated with the lower binding affinity. The former transduces directional cell movement while the latter initiates secretion and the respiratory burst. This concept may be supported by the recent observation that fMet-Leu-Phe receptors on PMN isolated membranes appear to exist in more than one affinity state (24, 38).

Among the implications of the data presented herein are that one can differentially modify the biological functions of human PMNs by exposing them to agents that affect physical parameters of membrane function. The ability to pharmacologically modify leukocyte function in regard to chemotactic responsiveness, secretion, and superoxide anion production could have theoretical implications in regard to treatment of immunodeficiency, inflammatory, and neoplastic disorders.

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