Consequences of Chemotactic Peptide Receptor Modulation for Leukocyte Orientation

SALLY H. ZIGMOND
Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT To exhibit chemotaxis, the orientation of locomotion along a chemical gradient, cells sense differences in concentrations of a chemotactic factor by detecting some difference in the occupancy of their chemotactic receptors. Thus chemotaxis is sensitive to the number of receptors present and might be used to evaluate the consequences of receptor down-regulation.

The ability of rabbit peritoneal polymorphonuclear leukocytes (PMNs) to orient to a standard gradient at various concentrations of N-formylnorleucyleucylphenylalanine (FNLLP) was examined. The observed orientation was compared to that expected if the directional signal were proportional to a difference in the absolute number or the fractional number of receptors occupied. The receptor occupancy in varying gradients was calculated from the binding constant of FNLLP, 2 X 10^-8 M (Zigmond and Sullivan, 1979, J. Cell Biol. 82:517-527), and the receptor number (a) present initially or (b) present after down-regulation (Sullivan and Zigmond, 1980, J. Cell Biol. 85:703-711). The observed concentration dependence of cell orientation is similar to the change in the number of receptors occupied, the receptor number being corrected for down-regulated cells. The net effect of receptor loss appears to be a decreased sensitivity to gradients at high concentrations of peptide.

Incubation of cells with a variety of different ligands results in the decrease of the number of receptors for that ligand on the cell surface. This process of ligand-induced receptor modification has been called down-regulation (1). In most cases the consequences of this receptor loss for subsequent cell function are not clear.

When leukocytes are incubated at 37°C with chemotactic peptides, the number of their peptide receptors is reduced (2). This reduction is dose dependent, being greatest when cells are incubated in relatively high concentrations of peptide. When incubated in concentrations ~10 times the dissociation constant, Kd, of the receptor for the peptide, the receptor number is reduced to ~20% of control levels. The remaining receptors exhibit the same affinity for the peptide as do the original receptors (2). The receptor loss occurs rapidly after peptide addition, reaching a plateau level within 15 min. It has previously been demonstrated that the ability of human PMNs to orient in various gradients of the chemotactic peptide N-formylmethionylmethionylmethionine (fMetMetMet) is similar to that expected if the magnitude of the directional signal is a result of the difference in the number of receptors occupied over some distance in the gradient (3). From this one can predict that cells should orient optimally in a standard gradient (e.g., a 10-fold gradient across a 1-mm distance), when the concentration of peptide is at the Kd for its receptor. The orientation should decline symmetrically on a log plot of concentrations above and below the Kd.

We have characterized the ability of the chemotactic peptide N-formylnorleucyleucylphenylalanine (FNLLP) to bind and to induce orientation in rabbit PMNs. The optimal orientation occurs at concentrations below the Kd. The accuracy of the orientation decreases more rapidly at high concentrations of peptide than at low concentrations. This asymmetry was also seen with fMetMetMet.

Cells responding to a chemotactic peptide in vitro assays (and presumably also in vivo) are exposed to the peptide for extended periods of time, e.g., several hours. Thus, the cells in these circumstances will have the number of their receptors reduced according to the concentration of peptide present. After correction for this receptor loss, the magnitude of the directional signal based on the change in the number of receptors occupied now fits closely with the orientation behavior observed experimentally. By extrapolating back to estimate the orientation if there were no receptor loss, we find that the net consequence of receptor loss is a decreased sensitivity to gradients at high concentrations of peptide.
MATERIALS AND METHODS

Cells

Rabbit peritoneal exudate cells were collected 4 h after injection of 0.1 g of shellfish glycogen (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 3 x 10^6 cells/ml. The cells in the center microscope field of the bridge were observed with a ×40 phase objective (field diameter, 0.4 mm) between 15 and 30 min after assembling the chamber. The direction of locomotion of the PMNs was judged morphologically; the front of a locomoting cell was identified by its pseudopod and the rear by its knoblike tail. Cells were scored as moving either toward the high concentration of a chemotactic factor or toward the low concentration. Only cells with pseudopods and tails and whose direction of movement could be evaluated were scored. Thus, immobile cells and cells moving perpendicular (±10°) to the gradient were not counted. The unscored population was usually ~20% of the PMNs present. The bridge was scanned across the slide (in the direction normal to the gradient) until at least 50 cells were scored. The results were expressed as the number of cells moving toward the high concentration divided by the total number of scoreable cells (the sum of those scored as moving toward and away from the chemotactic factor) × 100.

FIGURE 1 Plot of the receptors occupied as a function of concentration of FNLLP at 4°C, (solid line), or after receptor loss at 37°C, (dashed line). The 4°C curve (no down-regulation) was computed from information presented previously (2, 4). Receptor occupancy, [R], at any concentration [C] is equal to [C]/([C] + Kd) x [R0]. The dissociation constant Kd is 2 x 10^-8 M and [R0], the total number of surface receptors, is 5 x 10^4/cell (2). The curve for receptor occupancy after down-regulation was computed in the same way using the reduced values of [R0] indicated in the text; the Kd remains at 2 x 10^-8 M.

Binding Assay

Petri dishes containing 10^5 cells were incubated in 0.5 ml of Hank's with the appropriate concentration of tritiated (125 Ci/mM) FNLLP. After incubation, this medium was aspirated and the dish washed quickly (6 s) and vigorously in two baths of 4°C saline. The reversibility of binding was measured by varying the time of the wash in chilled saline. Cell-associated radioactivity was measured by adding 0.5 ml of 0.1 N NaOH to each dish. The cells were scraped into this fluid with a rubber policeman, and then each dish was washed with 0.5 ml of distilled water. This 1 ml of cell suspension was counted in 15 ml of scintillation fluid Formula 963 (New England Nuclear, Boston, Mass.) with an efficiency of ~40%. In preliminary experiments, Lowry assays were done to confirm that cells were not lost from any of the dishes during the treatment. In all experiments the dishes were monitored visually for cell loss.

Down-regulation

Cells were preincubated with unlabeled FNLLP for various lengths of time at 37°C or 4°C. The percent receptors lost at various concentrations of peptide are: at 1 x 10^-5 M, 85 ± 8%; at 3 x 10^-7 M, 80 ± 2%; at 3 x 10^-6 M, 56 ± 5%; at 3 x 10^-9 M, 37 ± 5%; at 3 x 10^-9 M, 10 ± 10%; and at 1 x 10^-9 M, 4 ± 10%.

The affinity of the receptors before and after receptor loss is the same: Kd = 2 x 10^-8 M (2). Thus the number of receptors occupied at various concentrations of FNLLP at 37°C, i.e., after down-regulation, and at 4°C, before down-regulation, are shown in Fig. 1. These are idealized curves for the noncooperative peptide binding with a Kd of 2 x 10^-8 M in both cases (2, 4). The loss of receptors, which is particularly prominent at high concentrations, decreases the extent of binding to about one-fourth of that present originally.

As seen in Fig. 2, the ability of rabbit peritoneal PMNs to orient in various 10-fold gradients of FNLLP across a 1-mm bridge in a visual assay is optimal when the mean concentration is ~1 x 10^-9 M. Although there is scatter to the data, several features appear consistently. The peak orientation occurs below the Kd of binding and the curve is not symmetrical but falls off more rapidly at high concentrations than at low concentrations. The asymmetry of the curve is also apparent when a different peptide fMetMetMet and human peripheral blood PMNs are used, as has been published previously (3).

RESULTS

The extent of receptor loss after 20 min at 37°C in various concentrations of FNLLP has now been determined in >40 different experiments. The percent receptors lost at various concentrations of peptide are: at 1 x 10^-9 M, 85 ± 8% (mean ± SEM); at 3 x 10^-7 M, 80 ± 2%; at 3 x 10^-6 M, 56 ± 5%; at 1 x 10^-8 M, 37 ± 5%; at 3 x 10^-9 M, 10 ± 10%; and at 1 x 10^-9 M, 4 ± 10%.

The affinity of the receptors before and after receptor loss is the same: Kd = 2 x 10^-8 M (2). Thus the number of receptors occupied at various concentrations of FNLLP at 37°C, i.e., after down-regulation, and at 4°C, before down-regulation, are

FIGURE 2 The percentage of PMNs oriented into the 180° sector toward the higher concentration of FNLLP is plotted vs. the mean peptide concentration over the 1-mm bridge. All tests were done with a 10-fold increase in concentration across the 1-mm bridge. The data is plotted as mean ± SEM.
occupied over a 10-µm distance at the center of a 10-fold gradient before and after down-regulation, as in Figs. 3 and 4 but this time to scale. The difference between the two curves illustrates the effect of down-regulation: there is a decrease in sensitivity at high concentrations. Down-regulation has little effect on the directional signal at low concentrations.

**DISCUSSION**

Leukocytes respond to chemotactic peptides as many cells respond to peptide hormones, by decreasing the number of receptors available for further binding (2). The significance of the decreased receptor number for the chemotactic peptides is unclear. It may serve to limit certain leukocyte responses, such as increased adhesiveness, to situations where there is a dra-

---

**Figure 3** Comparison of orientation behavior in peptide gradient with various parameters of receptor occupancy existing before receptor loss. The number of receptors occupied at each concentration (dot-dashed line, a) is replotted from Fig. 1. The cell orientation observed at each concentration in a 10-fold gradient over 1 mm (solid line, b) is replotted from Fig. 2. The calculated change in the number of receptors occupied after a 10-µm displacement up the gradient from the center of a 10-fold linear gradient over 1 mm is plotted as a dotted line (c). This 10-µm displacement would result in ~2% increase in the mean concentration of peptide. Thus this is the difference in the number of receptors occupied between two concentrations, i.e., \([R_{C2}] - [R_{C1}]\). For example, the difference in number of receptors occupied between a cell at concentrations of 1.02 \(\times 10^{-8}\) M and one at 1.00 \(\times 10^{-8}\) M would be 16,887 - 16,667, or 220, receptors. The fractional change in the number of receptors occupied over the same change in concentration, i.e., \(([R_{C2}] - [R_{C1}])/([R_{C1}] - [R_{C2}])\), is shown in curve d. For a change in concentration between 1.0 \(\times 10^{-8}\) M and 1.02 \(\times 10^{-8}\) M FNLLP, the fractional change would be 220/16,667 or a 1.32% change. This can also be considered as the signal resulting from the change in the ratio of the receptors occupied at the two concentrations, because \((([R_{C2}] - [R_{C1}])/[R_{C1}]) = (R_{C2}/R_{C1}) - (R_{C1}/R_{C2}) = (R_{C2}/R_{C1}) - 1\).

**Figure 4** Comparison of orientation behavior in peptide gradient with various parameters of receptor occupancy that would exist after receptor loss. The number of receptors occupied at each concentration is the dot-dashed line (a; replotted from Fig. 1). The cell orientation observed at each concentration in a 10-fold gradient over 1 mm is the solid line (b; replotted from Fig. 2). The calculated change in the number of receptors occupied after a 10-µm displacement up the gradient from the center of a 10-fold linear gradient over 1 mm is shown in curve c. In contrast to Fig. 3 where the number of receptors occupied was calculated using \([R_{a}] = 5 \times 10^4\) receptors/cell, here \([R_{a}]\) is corrected for the receptor loss that occurs at each concentration as given in the text. For example, in 1 \(\times 10^{-8}\) M FNLLP, where a 37% loss of receptors leaves ~2.3 \(\times 10^4\) receptors/cell, the change in receptor number between a cell at 1.02 \(\times 10^{-8}\) M and one at 1.09 \(\times 10^{-8}\) M is 10,639 - 10,500, or 139, receptors.

**Figure 5** The calculated change in receptor occupancy over 10 µm in a 10-fold gradient with various mean concentrations before (dashed line) and after (solid line) down-regulation. The two curves are drawn to scale to illustrate that the net effect of down-regulation is a decrease in sensitivity at high concentrations.

---

The dashed curve in Fig. 3 is included to examine the possibility that a cell does not detect a gradient through differences in the number of occupied receptors but rather through fractional changes in the number of occupied receptors, i.e., through changes in the ratio of occupied receptors (5). As can be seen, this would result in an extremely high sensitivity at low concentrations that would continually decrease with increasing concentrations. This clearly differs from the observed behavior. Thus PMNs as well as bacteria appear to detect a gradient through a change in the number of receptors occupied (5).
romatic increase in the concentration of the peptide. This could contribute to cells adhering to endothelial cells when suddenly exposed to chemotactic factors. The decreased receptor number could also limit the amount of an intracellular messenger to a small range around some sensitive threshold level. Alternatively, the decrease in receptor number may play no constructive role in the response but merely be a consequence of the relative rates of receptor-mediated peptide uptake and receptor reinsertion. From this study it seems unlikely that the receptor loss increases the chemotactic ability of cells. PMNs appear to sense the gradient by detecting a difference in the number of receptors occupied. The ability of cells to orient in a peptide gradient corresponds closely to that calculated for cells having undergone receptor loss. The orientation is markedly decreased at high concentrations of peptide (concentration greater than or equal to $K_d$) from that expected were there no receptor loss. The orientation data presented fit well with the directional signals calculated to be present.

The calculations assume a linear gradient across the bridge. We have previously demonstrated that an approximately linear gradient is established by $\sim 15$ min for a molecule with a molecular weight similar to that of the peptide (3). However, we have also noted that cells on the bridge appear to inactivate the peptide. It seems unlikely that inactivation of the peptide would lead to an orientation optimal at apparent concentrations lower than the $K_d$; rather, because the inactivation would cause the concentrations present to be less than those calculated, one might expect orientation to require higher concentrations. If digestion is playing a significant role, the down-regulation may not completely account for the observed behavior. Other peptide effects, such as alterations in the rate of locomotion by different concentrations of peptide, that greatly affect the outcome of many chemotaxis assays have little effect here because the orientation is independent of the rate of locomotion.

Berg and Purcell (6) have argued on theoretical grounds that little improvement in the ability to sense a concentration is achieved by increasing the receptor number above $a/s$ specific receptors, where $a$ is the radius of the cell and $s$ is the radius of the binding patch on the membrane. For a PMN, $a$ is $\sim 10 \mu m$ and $s$ can be estimated to be $\sim 10^4$. Thus $a/s$ is $\sim 10^4$. It is interesting that even after maximal receptor loss there are still $\sim 10^4$ receptors (after 85% loss, there are $\sim 0.7 \times 10^4$).

That the orientation correlates with a change in number of receptors occupied over a given distance (e.g., $10 \mu m$) does not define the specific details of how the cell senses a gradient. A cell could compare the receptor occupancy across its dimensions, for example, by comparing the density of receptor occupancy on its left and right. Alternatively, the cell could detect a change over time in the number of receptors occupied on small portions of its membrane. For example, if a pseudopod extends up a gradient the number of receptors occupied on its surface would increase. In either case, we can begin to get a feeling for the change in receptor occupancy that occurs when 90% of the cells viewed are moving into the $180^\circ$ sector toward the well containing the higher peptide concentration. If we assume an equal distribution of the receptors on the cell surface, the number of receptors occupied on one-fourth of the cell surface can be compared with the number occupied by a similar portion of the cell surface $10 \mu m$ away. 90% orientation occurs in a 10-fold gradient between $2 \times 10^{-8}$ M and $2 \times 10^{-8}$ M over a 1-mm bridge; the concentration at the midpoint of the bridge is $1.1 \times 10^{-7}$ M. The concentration $10 \mu m$ away is $1.12 \times 10^{-8}$ M. At $1.1 \times 10^{-8}$ M, 35.48% of the receptors are occupied, whereas at $1.12 \times 10^{-8}$ M, 35.90% of the receptors are occupied. Here we assume there is no change in receptor number between these two concentrations and that $\sim 60\%$ of the original $5 \times 10^6$ receptors are present, i.e., $3 \times 10^4$ receptors/cell or $7.5 \times 10^3$ receptors per one-fourth of the cell surface. This results in a change of 32 receptors ($2,693 - 2,661$) being occupied. At $10^{-9}$ M, where a 10-fold gradient gives $\sim 70\%$ orientation, a change of only $\sim 10$ receptors would be expected. The changes during the extension of a 3-$\mu m$ pseudopod would be even less. Clearly, the cell response depends on a relatively small change in receptor occupancy.

I thank Dr. Doug Lauffenberger and Susan Sullivan for helpful discussion and Robin Hargrove for excellent technical assistance.

The work was supported by grants from the National Science Foundation (PCM 77-4442) and the National Institutes of Health (HL 15835) to the Pennsylvania Muscle Institute.

Received for publication 20 June 1980, and in revised form 9 December 1980.

REFERENCES

1. Catt, K. J., J. P. Harwood, G. Aguiar, and M. L. Durand. 1979. Hormonal regulation of peptide receptors and target cell responses. Nature (Lond.). 280:109-116.
2. Sullivan, S. J., and S. H. Zigmond. 1980. Chemotactic peptide receptor modulation in polymorphonuclear leukocytes. J. Cell Biol. 82:517-527.
3. Zigmond, S. H. 1977. The ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. J. Cell Biol. 75:606-616.
4. Zigmond, S. H., and S. J. Sullivan. 1979. Sensory adaptation of leukocytes to chemotactic peptides. J. Cell Biol. 82:517-527.
5. Dahlkeias, P. W.; P. A. Elwell, and P. S. Lovely. 1976. Studies of bacterial chemotaxis in defined concentration gradients. J. Supramol. Struc. 4:329-342.
6. Berg, H. C., and E. M. Purcell. 1977. Physics of chemoreception. Biophys. J. 20:190-219.