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CRYPTIC RECEPTORS FOR CHEMOTACTIC PEPTIDES IN RABBIT NEUTROPHILS

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SUMMARY

Specific binding of chemotactic peptides to receptors in rabbit neutrophils was enhanced in the presence of aliphatic alcohols - especially n-propanol and n-butanol. The effect was concentration dependent with maximum increases in specific binding of 2.6 and 3.0 fold in the presence of 2.5% n-propanol and n-butanol respectively. The results indicate that up to 65% of the receptors are cryptic and unavailable for ligand interactions. It is suggested that minute to minute modulation of chemotactic responsiveness could occur by controlling at the membrane level the number of receptors exposed to chemotactic stimuli.

INTRODUCTION

Chemotaxis, the directed migration of cells in response to a chemical gradient, is a property of a variety of cell types including bacteria, slime mold and leukocytes. We have previously described a family of N-formylated peptides which are extremely potent chemoattractants for rabbit polymorphonuclear leukocytes (1). The most active is N-formyl-methionyl-leucyl-phenylalanine (CHO.Met.Leu.Phe.OH) which shows half maximal stimulation of chemotaxis (rabbit neutrophils) at 7×10^{-11}M. In addition to stimulating chemotaxis these peptides were equally effective in initiating a wide variety of other physiological and biochemical events in these cells (2,3).

The suggestion (1) that this group of peptides produced this myriad of effects by interaction with a specific receptor was confirmed following synthesis of an intrinsically labelled analog, N-formyl-norleucyl-leucyl-p-^{3}H-phenylalanine (CHO.Nle.Leu.p.^{3}H.Phe.OH) (4). Using this analog, a specific and saturable receptor was unequivocally demonstrated (5). This receptor was present at concentrations of 10^{5} sites/cell and its calculated K_d (1.5×10^{-9}M)

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was in close agreement with the biological data (ED_{50} chemotaxis, 6.6×10^{-10}M) for the identical, unlabelled CHO.Nle.Leu.Phe.OH.

The data cited above were obtained following incubation at 4°C to avoid destruction of labelled and unlabelled peptides by neutrophil proteases. This low temperature almost certainly affects the physical properties of the cell membrane and, hence, may alter the interaction of ligand with the receptor. This suspicion was confirmed when it was noted that use of the primary alcohol, n-butanol, as a solvent in the radioreceptor assay enhanced binding of CHO.Nle.Leu.p.^{3}H.Phe.OH. We have expanded this initial observation and have studied the effects of a series of aliphatic alcohols on specific binding in rabbit neutrophils. The results are reported below.

MATERIALS AND METHODS

Rabbit neutrophils were obtained as follows. Albino rabbits (2.5-3.5 kg) were injected intraperitoneally with 150 ml of sterile saline containing 0.1% glycogen. Sixteen hours later an additional 200 ml of heparinized (2 units/ml) sterile saline was given intraperitoneally and the neutrophil-rich exudate collected, on ice, by gravity drainage through a polyethylene cannula. The exudate was strained through cheesecloth, centrifuged at 250 x g for 5 min and the packed cells resuspended in 100 ml of isotonic buffered NH_4Cl. After 5 min the cells were again centrifuged at 250 x g for 5 min. The packed cells were washed once with Gey's balanced salt solution containing 0.015M Hepes, pH 7.4 and, after centrifugation, were resuspended in the same solution to a final concentration of 2.2×10^{6} cells/ml. The cells were treated with 0.1mM of tosyl-L-phenylalanyl chloromethane and kept in an ice bath until used.

The radioreceptor assay was carried out essentially as described by Aswanikumar et al. (5). Each tube contained 70-90,000 cpm of CHO.Nle.Leu.p.^{3}H-Phe.OH and increasing concentrations (10^{-10}-10^{-5}M) of unlabelled CHO.Nle.Leu-Phe.OH. The incubation was initiated by addition of 2 ml of cell suspension and carried out at 4°C for 60 min. The incubation was terminated by rapid filtration (Hoeffer filtration apparatus) onto Whatman GF/F glass fiber filters. Each tube was rapidly washed with 2 ml of ice-cold Gey's balanced salt solution and the filters washed with 2 x 10 ml of ice-cold phosphate-buffered saline (pH 7.4, 0.02M). The filters were placed into vials containing 10 ml of scintillation fluid (ACS™, Amersham) and counted in a Beckman LS100C liquid scintillation spectrophotometer.

When alcohols were tested, a constant amount was added before the addition of 2 ml neutrophil suspension. Also some experiments were carried out at 24°C instead of 4°C.

CHO.Nle.Leu.p.^{3}H.Phe.OH and CHO.Nle.Leu.Phe.OH were prepared as described previously (4). Tosyl-L-phenylalanyl chloromethane (TPCK) was obtained from Calbiochem Corp. All other solvents, salts and buffers were of reagent grade quality.

RESULTS AND DISCUSSION

The initial observation in this study was made while testing a n-butanol extract of Escherichia coli (E. coli) culture filtrate for binding inhibition. This is known to contain a substance which is both chemotactic (6) and competes for the same binding site as the formylated peptides (5). It was noted, however, that in the solvent control [2.5% (v/v) n-butanol] specific binding was consistently enhanced by approximately 2-3 fold. A careful evaluation of this phenomenon was then undertaken to determine to what degree this effect was concentration-dependent. The results are shown in Fig. 1. As can be seen there was a clear concentration dependency with as little as 0.025% (v/v) n-butanol producing a consistent, although not statistically significant, increase in total binding to the neutrophil. Increases to 0.25% and 2.5% (v/v) n-butanol, however, produced clear changes in total binding amounting to 2.1 and 3.0 fold increases, respectively. Higher concentrations of n-butanol produced no greater increases in binding.

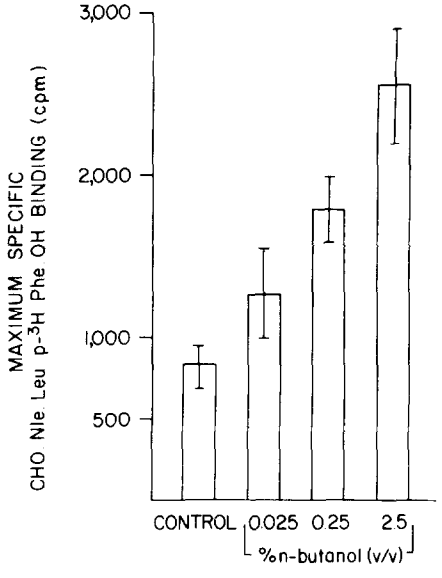
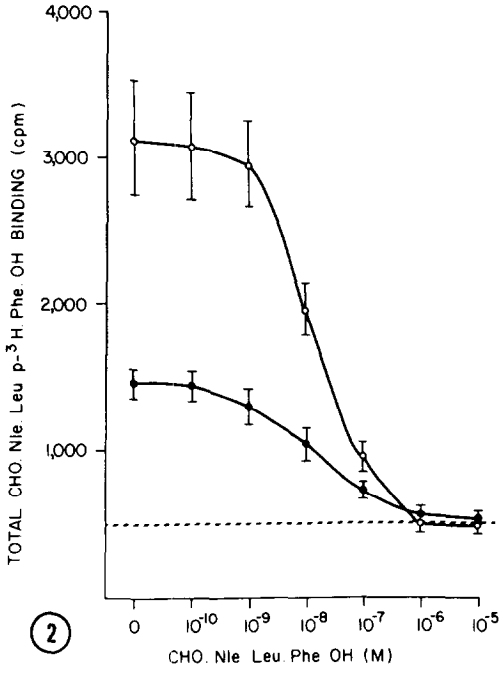


Fig. 1. Effect of n-butanol on maximum specific binding of CHO.Nle.Leu.p.-^{3}H.Phe.OH to rabbit neutrophils. Incubation carried out at 4°C for 60' under control conditions and in the presence of increasing concentrations of n-butanol. Each value is the average ± SEM of 5-8 determinations.



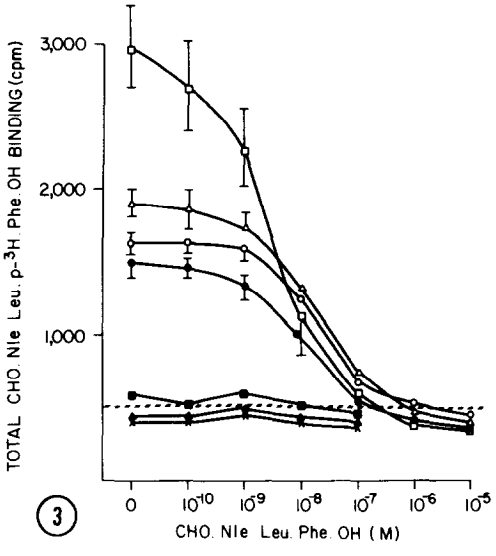


Fig. 2. Effect of n-butanol on binding inhibition curve for CHO.Nle.Leu.Phe.OH. Binding inhibition by increasing amounts (10^{-10}-10^{-5}M) of concentrations of CHO.Nle.Leu.Phe.OH determined in the absence (●—●) and presence of 2.5% n-butanol (v/v) (○—○). Incubations were carried out at 4°C for 60' and each point represents the average ± SEM of 5-8 determinations. The horizontal dashed line represents non-specific binding to filters.

Fig. 3. Effect of aliphatic alcohols on specific binding of CHO.Nle.Leu.p.-^{3}H.Phe.OH to rabbit neutrophils. Control (●—●) and in the presence of 2.5% (v/v) each of methanol (○—○), ethanol (△—△), n-propanol (□—□), n-pentanol (×—×), n-hexanol (▲—▲) and n-heptanol (■—■). Incubations were carried out at 4°C for 60' and each point represents the average ± SEM of 5-16 determinations. The horizontal dashed line represents non-specific binding to filters.

The nature of the binding inhibition curve in the presence and absence of 2.5% (v/v) n-butanol was also investigated (Fig. 2). Inspection of these curves indicates that all of the increased binding is due to increases in specific receptor binding. There was no change in non-specific binding - most of which, in our system at least, is due to binding of radiolabelled peptide to filters rather than cells. Also calculation of the ID_{50} (i.e. the concentration of unlabelled CHO.Nle.Leu.Phe.OH required to reduce specific binding by 50%) showed no differences between control (1.05×10^{-8}M) and n-butanol (1.1×10^{-8}M).

In an effort to determine if this was specific for n-butanol or whether other solvents produced this same dramatic increase in receptor binding a number of other alcohols were tested. These were a series of linear aliphatic alcohols beginning with methanol and up through heptanol. The results are shown in Fig. 3 and indicate some gross structure-activity relationships. For example both methanol and ethanol [2.5% (v/v)] were weak but consistent enhancers of specific binding. Propanol [2.5% (v/v)], however, produced a marked increase in binding (2.6 fold) which was equivalent to that seen with the same concentration of n-butanol (Fig. 2). As was the case with n-butanol (Fig. 2) all of the additional bound counts were displaceable by unlabelled CHO.Nle.Leu.Phe.OH. It is interesting that 2.5% (v/v) n-pentanol, n-hexanol and n-heptanol all totally eliminated peptide binding to the neutrophils.

Binding experiments were also carried out at 24°C to determine if the n-butanol effect was peculiar to the low temperature (4°C) normally used for the assay. Qualitatively the same results were observed - that is the presence of n-butanol markedly enhanced specific binding. Quantitatively, however, the absolute amount of binding was reduced, probably due to destruction of the peptide by neutrophil proteases.

It is clear from these studies that the neutrophil receptor for the chemotactic peptides exists in two states. One population, which represents approximately 33-50% of the total binding sites, is readily accessible to ligand while the remainder of the receptors are relatively or absolutely unavailable for interaction with the labelled peptide. Clearly, however, by the use of either n-propanol or n-butanol we can "expose" all the receptors to ligand. The rapidity of the alcohol effect supports the notion that these represent pre-existing receptor sites, although their exact location is not known. While it would be convenient to assume that they reside in the plasma membrane the possibility that they represent binding sites on some internal organelles cannot be ruled out at present.

In view of the effects of the series of alcohols we assume that this is due simply to a solvent effect on the membrane. It would appear that

as the alcohols increase in hydrophobicity they become more effective in exposing the cryptic receptor sites. This trend continued up to n-pentanol at which point all binding was lost. We do not know the reason(s) for this but it may be that the larger alcohols either destroy the receptor or disrupt the membrane to the point where the receptor is solubilized. Both possibilities are under investigation.

The broader implications of this study are two fold. First, is that our previously published (5) data on the number of binding sites in neutrophils is a low estimate in that it represents only those receptors available for ligand interaction. In light of these new data there would appear to be 2-3 times more receptor sites than one can normally observe. The second, and probably more important consideration is the fact that neutrophils can regulate their responsiveness to chemotactic stimuli (7). At a receptor level this could occur by changes in affinity for the ligand or the absolute number of receptors. We propose herein a third possibility - that is that the neutrophil could possibly modulate responsiveness by regulating the proportion of its total receptor population available to chemotactic stimuli.

ACKNOWLEDGEMENT

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