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SIMILARITIES IN THE MECHANISMS BY WHICH FORMYL-METHIONYL-LEUCYL-PHENYLALANINE, ARACHIDONIC ACID AND LEUKOTRIENE B_4 INCREASE CALCIUM AND SODIUM INFLUXES IN RABBIT NEUTROPHILS

T.F.P. Molski*, P.H. Naccache**, P. Borgeat† and R.I. Sha'afi*

Departments of Physiology* and Pathology**
University of Connecticut Health Center
Farmington, CT 06032
and
Centre de Recherches en Endocrinologie Moleculaire†
Le Centre Hospitalier de l'Universite Laval
Sainte-Foy, Quebec, Canada

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SUMMARY: The chemotactic factors f-Met-Leu-Phe, arachidonic acid and leukotriene B_4 induce a rapid stimulation of both Ca^{2+} and Na^+ influx in rabbit neutrophils. In the three cases the stimulation is rapid and the effects are not additive. Furthermore in all cases the stimulation of Na-influx but not of Ca-uptake is inhibited by the potassium-sparing diuretic amiloride. Preincubation with high concentrations of the chemotactic factor f-Met-Leu-Phe followed by washing of rabbit neutrophils reduces significantly the stimulation of calcium uptake induced by arachidonic acid, leukotriene B_4 and f-Met-Leu-Phe. These results strongly suggest that the exogenous addition of arachidonic acid or of leukotriene B_4 leads to the activation of the same permeation pathways as do better defined chemotactic factors.

INTRODUCTION

It has been shown recently that exogenously added arachidonic acid and leukotriene B_4 (a lipoxygenase metabolite of arachidonic acid) mimic many of the effects of the chemotactic factor formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe) on neutrophils (1-12). Human, rat and rabbit neutrophils can be induced to move, aggregate and, usually in the presence of cytochalasin B, degranulate upon the addition of arachidonic acid or leukotriene B_4 in vitro (1-12). F-Met-Leu-Phe, arachidonic acid and leukotriene B_4, also profoundly modify the permeability of the plasma membrane of rabbit neutrophils to sodium and calcium and mobilize a common pool of intracellular Ca^{2+} (13 - 17). These changes

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in membrane permeability are thought to be intimately involved in the sequence of events which is initiated by the binding of chemotactic factors to their plasma membrane receptors and which eventually leads to neutrophil activation.

Very little is known about the mechanism(s) underlying the observed enhancement in membrane permeability. One outstanding question in this regard is whether these three stimuli share a common mechanism responsible for the observed changes in membrane permability. We wish now to report the results of experiments in which this question has been examined. The results to be reported clearly demonstrate that the ultimate biochemical and/or biophysical changes in membrane permeability induced by f-Met-Leu-Phe, arachidonic acid and leukotriene B_4 are the same.

MATERIALS AND METHODS

Rabbit peritoneal neutrophils collected, handled and suspended in modified Hanks' balanced salt solution (no Mg^{2+}, no protein, pH: 7.4) as previously described were used throughout (13-17).

Cation uptake was measured using the rapid sampling silicone oil method previously described in detail (13-17). The cells were allowed to thermally equilibrate for 10-20 minutes at 37°C before the beginning of the experiments. In the experiments designed to test for deactivation, the cells were incubated with high concentrations of f-Met-Leu-Phe (10^{-7} M) for five minutes, washed extensively and tested for response to each of the three stimuli (f-Met-Leu-Phe, arachidonic acid and leukotriene B_4) in parallel with cells which had undergone similar experimental manipulations except for the exposure to f-Met-Leu-Phe. After the cells were washed free of f-Met-Leu-Phe they were incubated for five minutes prior to the addition of ^{45}Ca and the subsequent stimulus.

The chemotactic factor formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe) was a generous gift of Dr.R.J. Freer, Medical College of Virginia, Richmond, VA., amiloride was kindly supplied by Merck, Sharp and Dohme, Rahway, N.J., ^{22}Na and ^{45}Ca were obtained from New England Nuclear, Boston, Mass., and arachidonic acid was obtained from the Sigma Chemical Company(St. Louis, Mo). Leukotriene B_4 was prepared from swine blood as previously described (16). All other reagents were analytical grade.

RESULTS AND DISCUSSION

The chemotactic factor f-Met-Leu-Phe causes a rapid increase in the membrane permeability of rabbit neutrophils to calcium and sodium. The chemotactic factor-dependent increase in membrane permeability to calcium is not inhibitable by the potassium-sparing diuretic amiloride whereas the corresponding increase for sodium is (18). In order to compare the effect of f-Met-Leu-Phe to those

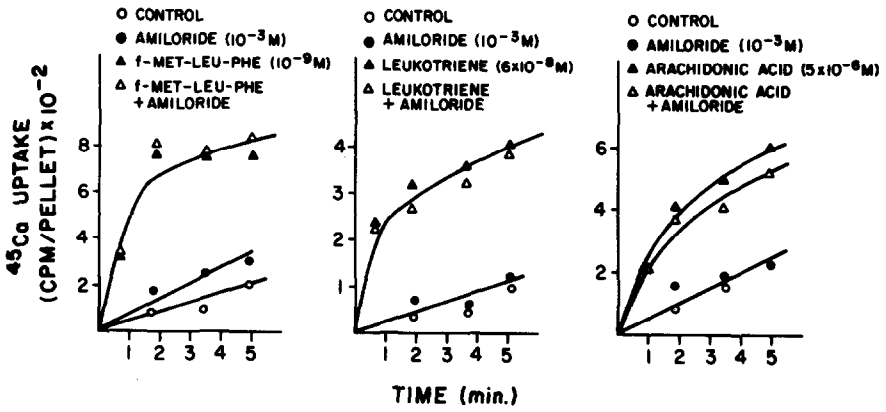

Figure 1: Effect of the chemotactic peptide f-Met-Leu-Phe, arachidonic acid and leukotriene B_4 on Ca-uptake in rabbit neutrophils in the presence and absence of amiloride.

of arachidonic acid and leukotriene B_4 on calcium uptake we have examined the effects of amiloride on the enhancement of calcium uptake by the three stimuli. The results which are summarized in Figure 1 clearly illustrate the similarities among the actions of the three stimuli on Ca-uptake; in each case, the stimulation of ^{45}Ca uptake is rapid and amiloride insensitive. In another set of experiments, we have also found that the simultaneous addition of arachidonate or leukotriene B_4 and f-Met-Leu-Phe causes no significant greater enhancement in Ca-influx than did f-Met-Leu-Phe by itself (results not shown).

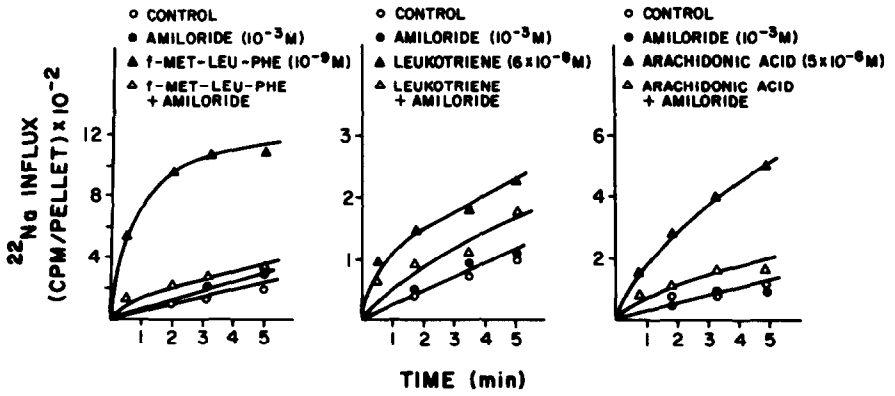

Figure 2: Effect of f-Met-Leu-Phe, arachidonic acid and leukotriene B_4 on Na-influx in rabbit neutrophils in the presence and absence of amiloride.

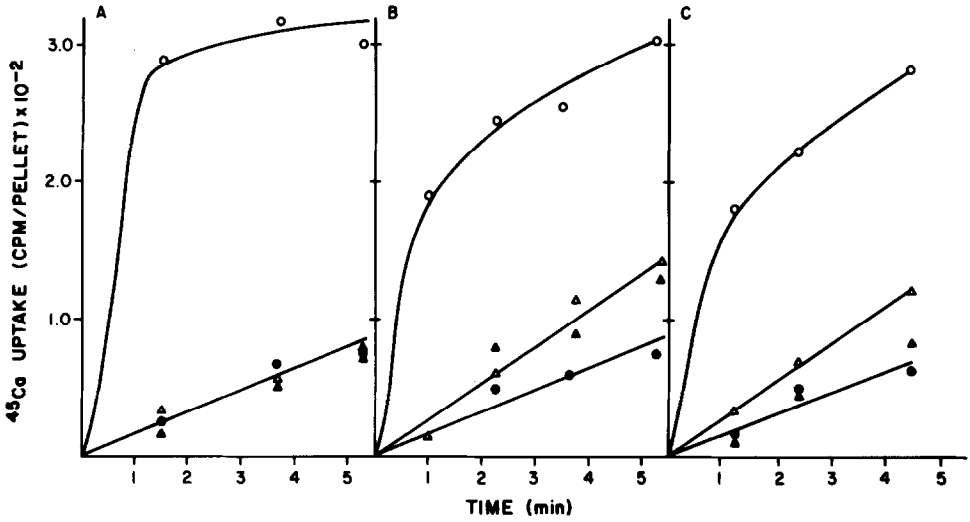

Figure 3: Effect of deactivation by f-Met-Leu-Phe on the subsequent stimulation of Ca-uptake in rabbit neutrophils by f-Met-Leu-Phe, arachidonic acid and leukotriene B_4. In these experiments, the cells were preincubated with 10^{-7} molar f-Met-Leu-Phe for 5 minutes, washed extensively and tested for response to each of the three stimuli. The second stimulus in panel A is f-Met-Leu-Phe (10^{-9} M), in panel B, arachidonic acid (2 x 10^{-6} M) and in panel C, leukotriene B_4 (6 x 10^{-8} M). ●-● normal control cells, O-O normal stimulated cells, ▲-▲ deactivated control cells, Δ-Δ deactivated stimulated cells.

We examined next the effect of amiloride on the stimulus dependent enhancement in Na-influx. The results are summarized in Figure 2. Once again the similarities among the three stimuli are striking. They all produce a rapid increase in Na-influx which is inhibitable by amiloride.

It is generally found that preincubation of rabbit neutrophils with high concentrations of the chemotactic factors f-Met-Leu-Phe or C_{5a} followed by washing reduces significantly the responsiveness (chemotaxis and lysosomal enzyme release) of these cells to subsequent addition of the same stimulus (19). This phenomenon has been referred to as deactivation. As the behavior of the stimulus-dependent change in calcium uptake in these cells with respect to deactivation is not known, and in order to further probe the similarities among events initiated by f-Met-Leu-Phe, arachidonic acid and leukotriene B_4, we have examined the effect of preincubation with f-Met-Leu-Phe on the subsequent stimulation of Ca-uptake by the three stimuli. The results of a representative experiment (at least three different experiments, each on a different cell preparation, were carried out)

is illustrated in Figure 3. The results summarized in this figure show clearly that cells preincubated with f-Met-Leu-Phe show significant decrease in responsiveness to subsequent stimulation with f-Met-Leu-Phe, arachidonic acid and leukotriene B_4; i.e., these cells show both homo- and heterologous deactivation. The homologous deactivation is much more pronounced than the heterologous deactivation. In addition to showing, for the first time, deactivation at the level of stimulated calcium movements, the results of these experiments once again emphasize the striking similarities among the permeability changes induced by the above three stimuli.

Based on the results just described one can conclude that the three stimuli (f-Met-Leu-Phe, arachidonic acid and leukotriene B_4) share a common mechanism which is responsible for the profound modification in the permeability of the plasma membrane of rabbit neutrophils to calcium and sodium ions which is observed. This conclusion is based on the following points:

(1) The effects of the three stimuli on Na^+ and Ca^{2+} influx are rapid.
(2) In all three cases the stimulus-dependent Na-increase is inhibitable by amiloride but not the stimulus-dependent Ca-increase.
(3) The effects of the three stimuli are not additive.
(4) Cells pretreated with f-Met-Leu-Phe lose their ability to respond to arachidonic acid, leukotriene B_4, as well as to f-Met-Leu-Phe.

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