Vol. 103, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1981 Pages 766-773

DEACTIVATION OF THE EFFECTS OF F-MET-LEU-PHE AND LEUKOTRIENE B_4 ON CALCIUM MOBILIZATION IN RABBIT NEUTROPHILS

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Received October 15,1981

SUMMARY: Preincubation of rabbit neutrophils with the synthetic chemotactic factor f-Met-Leu-Phe has been found to diminish the ability of these cells to mobilize calcium upon subsequent stimulation by f-Met-Leu-Phe or by leukotriene B_4. The preexposure of the neutrophils to leukotriene B_4 on the other hand results in a diminished subsequent response to itself but an unaltered response to f-Met-Leu-Phe. These results demonstrate that deactivation can be observed at the level of calcium mobilization, strengthen the postulated second messenger role of calcium in neutrophils and imply that neutrophil activation by chemotactic factors can bypass the arachidonic acid metabolic pathway.

INTRODUCTION

The similarities between the effects of chemotactic factors such as the synthetic peptide formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe) and the complement factor $C5_a$, and those of arachidonic acid and its lipoxygenase metabolite 5(S),12(R)dihydroxy-6,8,10,14(cis-trans-trans-cis)-eicosatetraenoic acid (leukotriene B_4) on neutrophil functional responsiveness and calcium handling have been stressed recently. It has been found for example that: 1) several neutrophil functions could be elicited by the exogenous addition of these fatty acids (1-3), 2) arachidonic acid was released from phospholipids upon stimulation by chemotactic factors (4), 3) phospholipase and lipoxygenase inhibitors antagonized

ABBREVIATIONS: f-Met-Leu-Phe: formyl-methionyl-leucyl-phenylalanine. leukotriene B_4: 5(S),12(R)-dihydroxy-6,8,10,14(cis-trans-trans-cis)-eicosatetraenoic acid.

0006-291X/81/220766-08$01.00/0

neutrophil responsiveness (5-8) and 4) arachidonic acid and leukotriene B_4 mimicked most of the effects of f-Met-Leu-Phe on calcium handling by neutrophils (9-11).

Based on these, and other, results it has been suggested that arachidonic acid mobilization and subsequent metabolism occupies a central place in the excitation response coupling sequence in the neutrophils.

We wish now to describe recent studies in which the effects of preincubation with f-Met-Leu-Phe or leukotriene B_4 on the subsequent calcium response to these two stimuli was examined. The results obtained significantly strengthen the postulated second messenger role of calcium and force a reevaluation of the above mentioned role of arachidonic acid in the activation of the neutrophils.

MATERIALS AND METHODS

Rabbit peritoneal neutrophils obtained and handled as previously described (12) were used throughout these experiments. They were suspended at 1×10^7 cells/ml in magnesium and protein free modified Hanks' balanced salt solution buffered with 20 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid), pH 7.3. A 20 minute preincubation at 37°C preceded all further experimental manipulation.

Calcium transport was measured using the rapid sampling silicone oil method previously described in detail (5,9,10,11,13).

Uptake experiments measured the time course of the association of ^{45}Ca with the cells immediately following the addition of the radioisotope and the appropriate stimuli. In the case of steady-state experiments the cells were incubated with ^{45}Ca for 50 minutes following which the various manipulations were performed in media of the same ^{45}Ca specific activity.

Deactivation was achieved by a 5 minute preincubation of neutrophil suspensions with 10^{-7} M f-Met-Leu-Phe or 2.6×10^{-7} M leukotriene B_4 followed by two washes with a volume equal to that of the original suspension of media lacking the stimuli and a 5 minute reequilibration period. The various wash solutions were maintained at 37°C and in the steady-state experiments were of the same ^{45}Ca specific activity as the incubation media.

Leukotriene B_4 was purified and characterized as previously described (14). F-Met-Leu-Phe was obtained from Peninsula Labs (San Carlos, CA) and ^{45}Ca (as $CaCl_2$ in water) from New England Nuclear (Boston, MA). All other reagents were analytical grade.

RESULTS

Preincubation of rabbit neutrophils with high concentrations of chemotactic factors substantially diminishes their subsequent responsiveness to the same or other stimuli; this phenomenon has been termed deactivation (15). Originally described with peptide chemotactic factors (bacterial factors, $C5_a$ and f-Met-

Leu-Phe) this phenomenon has recently been extended to include the neutrophils active fatty acids arachidonic acid and leukotriene B_4 (16,17). The behavior of the stimulated calcium movements during these various manipulations had not yet been examined. As f-Met-Leu-Phe and leukotriene B_4 act either at different steps along the activation sequence of the neutrophils, or possibly through separate receptors, we have tested now for homo- and heterologous deactivation to f-Met-Leu-Phe and leukotriene B_4. The purpose of these experiments was to further define the mechanisms underlying the deactivation phenomenon and to investigate the similarities and differences among stimulated calcium movements, chemotaxis and lysosomal enzyme release.

1. Effect of preincubation with f-Met-Leu-Phe or leukotriene B_4 on the subsequent calcium uptake response to the addition of f-Met-Leu-Phe and leukotriene B_4

We have first examined, in the calcium uptake assay, the effects of deactivation (see Methods Section for protocol) by f-Met-Leu-Phe and leukotriene B_4 on the subsequent ability of the neutrophils to respond to each of these two stimuli.

As shown in Figure 1, the previous exposure to 10^{-7} M f-Met-Leu-Phe severely diminishes the ability of the neutrophils to respond to further stimulation by f-Met-Leu-Phe or leukotriene B_4 by an enhanced rate of ^{45}Ca uptake. Both homo- and heterologous deactivation are apparent under these conditions.

Preincubation with leukotriene B_4 produces, on the other hand, only homologous deactivation. The response to f-Met-Leu-Phe is totally unaffected by the previous exposure to the fatty acid (Figure 2).

2. Effect of preincubation with f-Met-Leu-Phe or leukotriene B_4 on the subsequent intracellular calcium redistribution induced by f-Met-Leu-Phe and leukotriene B_4

Increases in the rate of ^{45}Ca uptake may be due either to an increase in permeability or an elevation in the level of intracellular exchangeable calcium. The events that follow the stimulation of cells suspended in the absence of added calcium and in steady-state with respect to the medium ^{45}Ca specific activity

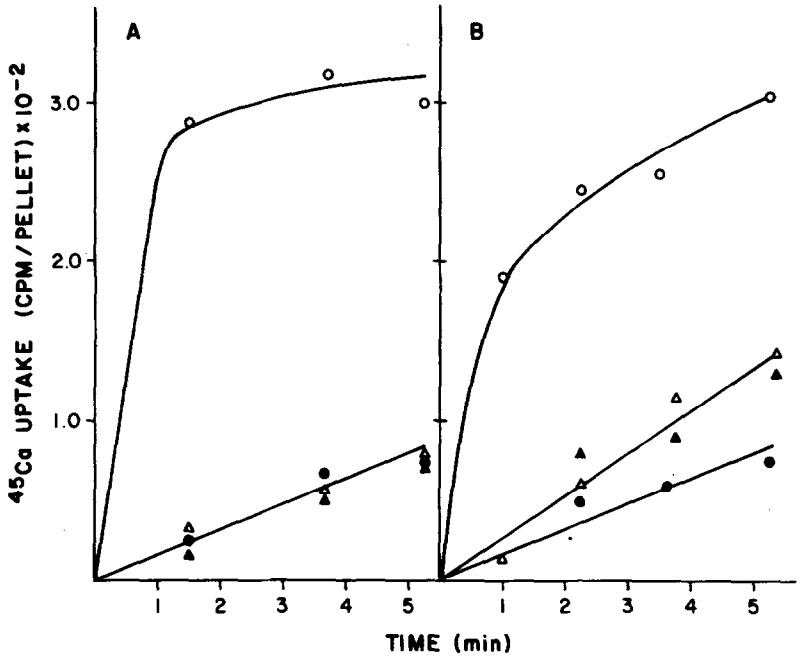

Figure 1: Effect of preincubation with f-Met-Leu-Phe on the ability of f-Met-Leu-Phe and leukotriene B_4 to enhance the rate of ^{45}Ca uptake in rabbit neutrophils upon subsequent stimulation. The experimental manipulations are described in the Materials and Methods section. The symbols are as follows: control cells (●); control deactivated cells (▲); f-Met-Leu-Phe (10^{-9} M) (panel A) or leukotriene B_4 (6×10^{-8} M) (panel B) (○); f-Met-Leu-Phe (10^{-9} M) (panel A) or leukotriene B_4 (6×10^{-8} M) (panel B) added to deactivated cells (△).

reflect, on the other hand, the mobilization of intracellular calcium (18,19). The results of a representative experiment in which the effect of preincubation with 10^{-7} M f-Met-Leu-Phe on the subsequent mobilization of calcium by f-Met-Leu-Phe and leukotriene B_4 was examined are illustrated in Figure 3 (the details of the experimental manipulations are described in the Methods section). It is clear that the responses to both stimuli were significantly blunted by the pre-exposure to the chemotactic peptide. The behavior of this additional parameter of cellular calcium handling is thus similar to that of calcium uptake depicted in Figure 1.

The pattern of the response of the intracellular calcium mobilization to the previous exposure to leukotriene B_4 is similar to that of calcium uptake under

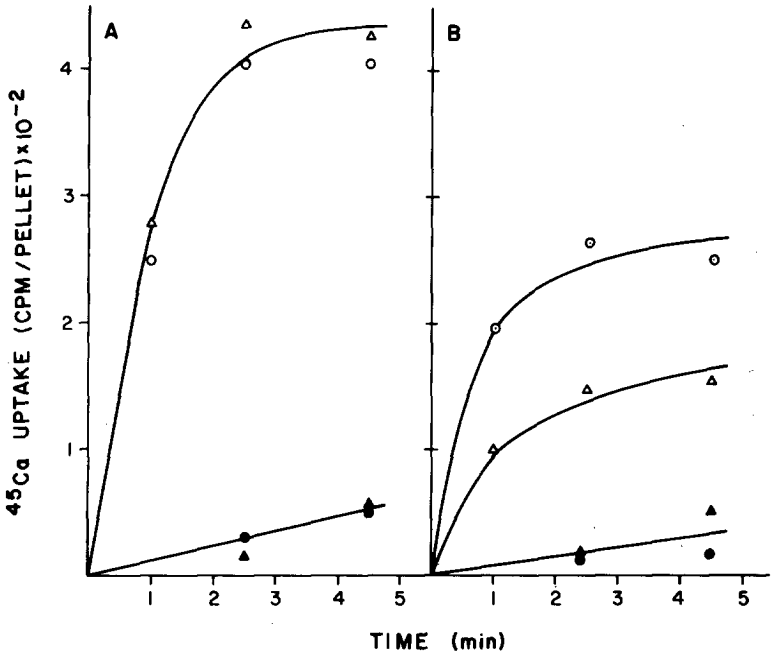

Figure 2: Effect of preincubation with leukotriene B_4 on the ability of f-Met-Leu-Phe and leukotriene B_4 to enhance the rate of uptake of ^{45}Ca in rabbit neutrophils upon subsequent stimulation. The experimental manipulations are described in the Materials and Methods section. Symbols as in Figure 1.

the same conditions (Figure 2). Cells pretreated with leukotriene B_4 show a significantly diminished response to a subsequent challenge with that fatty acid and an essentially unaltered response to f-Met-Leu-Phe (homo- but not heterologous deactivation) (Figure 4).

DISCUSSION

The results of the experiments described above in which calcium mobilization as induced by two classes of cytotaxins under deactivation conditions was examined allow several important conclusions related to neutrophil activation to be drawn. The basic finding is that preincubation with f-Met-Leu-Phe, a synthetic peptide thought to be related to the naturally occurring bacterial factor, diminishes the subsequent responses to itself and to the chemotactic fatty acid leukotriene B_4. Preexposure to leukotriene B_4 on the other hand, only produces homologous deactivation and leaves unaffected the subsequent response to f-Met-Leu-Phe. These

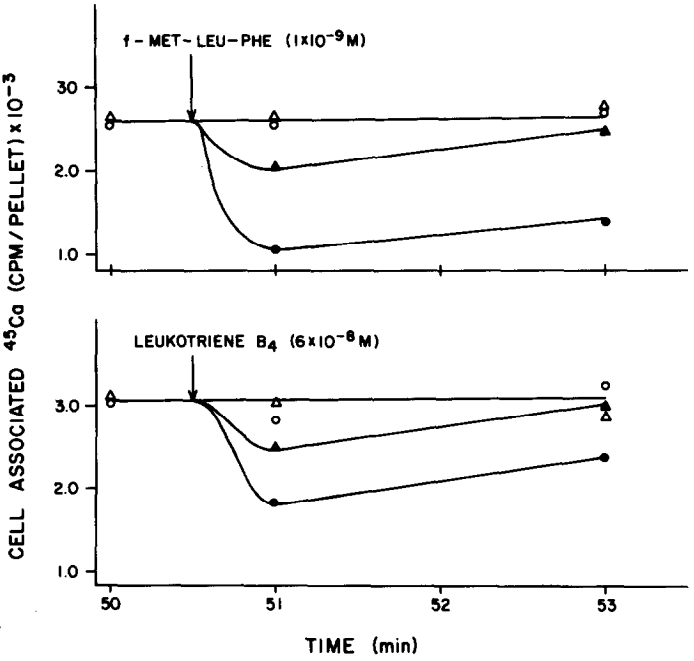

Figure 3: Effect of preincubation with f-Met-Leu-Phe on the subsequent ability of f-Met-Leu-Phe and leukotriene B_4 to mobilize intracellular calcium. The experimental manipulations are described in the Materials and Methods section. The symbols are as follows: control cells (O); control deactivated cells (Δ); f-Met-Leu-Phe (10^{-9} M) (upper panel) or leukotriene B_4 (6×10^{-8} M) (lower panel) (●); f-Met-Leu-Phe (10^{-9} M) (upper panel) or leukotriene B_4 (6×10^{-8} M) (lower panel) added to deactivated cells (▲).

findings were found whether one monitors intracellular calcium redistribution or altered membrane permeability to calcium was monitored.

The two parameters of calcium mobilization examined here behave with respect to the deactivation phenomenon in a manner essentially similar to lysosomal enzyme release (16). These results are therefore strongly supportive of the previously postulated causal relationship between elevated calcium levels and the initiation of neutrophil responsiveness (13,19). As the present calcium studies were conducted in the absence of cytochalasin B and thus of degranulation and its potential side effects, they offer perhaps the best evidence for a second messenger role of calcium in the neutrophils.

This study demonstrates, for the first time, that deactivation can be observed not only at the level of the cell's functional responses but also at that of some

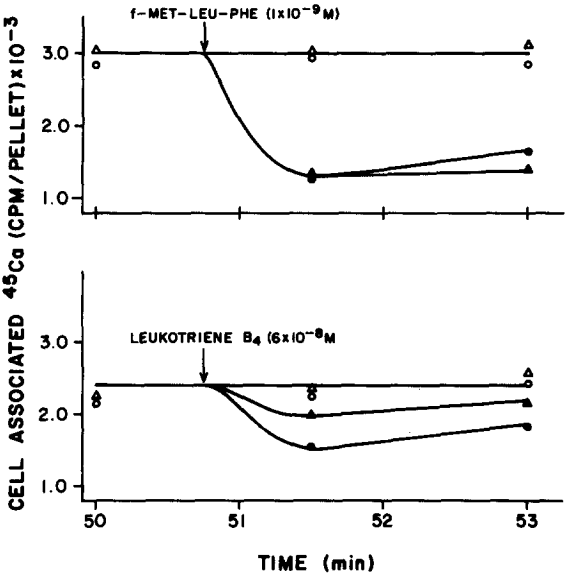

Figure 4: Effect of preincubation with leukotriene B_4 on the subsequent ability of f-Met-Leu-Phe and leukotriene B_4 to mobilize intracellular calcium. The experimental manipulations are described in the Materials and Methods section. Symbols as in Figure 3.

of the early events of neutrophil activation, namely calcium mobilization. Although these results do not bear directly on the biochemical basis of deactivation, they do offer additional support to the idea that there are at least two levels at which deactivation occurs: receptor down-regulation and unidentified intracellular events (see reference 20 for review of neutrophil deactivation data).

Perhaps the most important conclusion to be drawn from the present results concerns the relationship between arachidonic acid mobilization and neutrophil activation by chemotactic factors. Previous results had implicated an almost tight linkage between these two events. The diminished response to leukotriene B_4 upon the preexposure to f-Met-Leu-Phe supports the contention that the synthetic peptide does indeed involve the arachidonic acid metabolic pathway. The lack of effect of the preincubation with leukotriene B_4 on the subsequent f-Met-Leu-Phe responses stongly argues on the other hand that the latter can effectively bypass the arachidonic acid pathway. As such these results invite a reevaluation of the activation pathways available to the neutrophils.

ACKNOWLEDGEMENT: The work was supported in part by NIH grant AI-13734 and by the National Cancer Institute of Canada.

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