# f-MetLeuPhe-INDUCED PHOSPHATIDYLINOSITOL TURNOVER IN RABBIT NEUTROPHILS IS DEPENDENT ON EXTRACELLULAR CALCIUM

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## 1. Introduction

There is an impressive correlation between cell activation phenomena which are mediated by an increase in cytoplasmic $Ca^{2+}$ and stimulated turnover of phosphatidylinositol lipids in those cells [1]. In a number of systems in which cellular activation is clearly dependent on the presence of external $Ca^{2+}$ (e.g., adrenal medulla [2], parotid gland [3–5], mast cells [6]) the agonist-induced phosphatidylinositol responses are calcium independent. The hypothesis has been formulated [7] that phosphatidylinositol breakdown is a direct consequence of receptor activation, and could precede the movements of $Ca^{2+}$ which give rise to increased cytoplasmic $Ca^{2+}$ and consequent cell activation.

We have shown that stimulation of rabbit neutrophils with the synthetic tripeptide *N*-formyl-methionyl-leucyl-phenylalanine (f-MetLeuPhe) in the presence or absence of cytochalasin B is accompanied by an increase in the rate of phosphatidylinositol turnover [8]. This was measured as the incorporation of [$^{32}$P]phosphate into phosphatidylinositol. Here we show that the phosphatidylinositol response in neutrophils is prevented by the omission of extracellular $Ca^{2+}$, under conditions in which cell stimulation still occurs due to the mobilisation of internal $Ca^{2+}$ pools. This observation indicates that the role of phosphatidylinositol turnover, at least in neutrophils, will have to be reconsidered.

## 2. Methods

Neutrophils were obtained from the rabbit peritoneal cavity 4–6 h after the infusion of 250 ml 0.1% glycogen in 0.15 M NaCl. They were suspended at $10^7$ cells/ml in a buffered salt solution as in [8]. Reactions were initiated by adding cells equilibrated at 37°C for 30 min (containing [$^{32}$P]phosphate for phosphatidylinositol studies or prelabelled with $^{45}CaCl_2$ for efflux experiments) to an equal volume of buffer containing f-MetLeuPhe (final conc. as indicated) and $CaCl_2$ (final conc. 1.8 mM) or EGTA (final conc. 10 μM). Reactions were terminated by cooling tubes to 4°C and centrifuging. All the experiments here were done in the presence of cytochalasin B (final conc. 5 μg/ml) to enhance the extent of secretion; we have shown that cytochalasin B does not have any gross effects on binding of [$^3$H]f-MetLeuPhe to neutrophils, stimulated phosphatidylinositol labelling or stimulated $^{45}Ca^{2+}$ efflux [8].

Aliquots of the supernatant were used to measure secreted β-glucuronidase, as in [8]. The phosphatidylinositol response was measured by following the incorporation of [$^{32}$P]phosphate into phosphatidylinositol during 20 min after applying the stimulus [8]. $^{45}Ca^{2+}$ efflux from preloaded cells was measured by centrifuging the cells through Ficoll 5 min after stimulation and measuring the radioactivity in the pellet [8]. Materials were as in [8].

## 3. Results and discussion

Figure 1A shows that f-MetLeuPhe-induced secretion of β-glucuronidase from cytochalasin B-treated rabbit neutrophils is enhanced, but not dependent on the presence of extracellular $Ca^{2+}$. This is in agreement with earlier reports [9]. In addition to increasing the maximal extent of secretion due to optimal concentrations of f-MetLeuPhe, extracellular $Ca^{2+}$ also

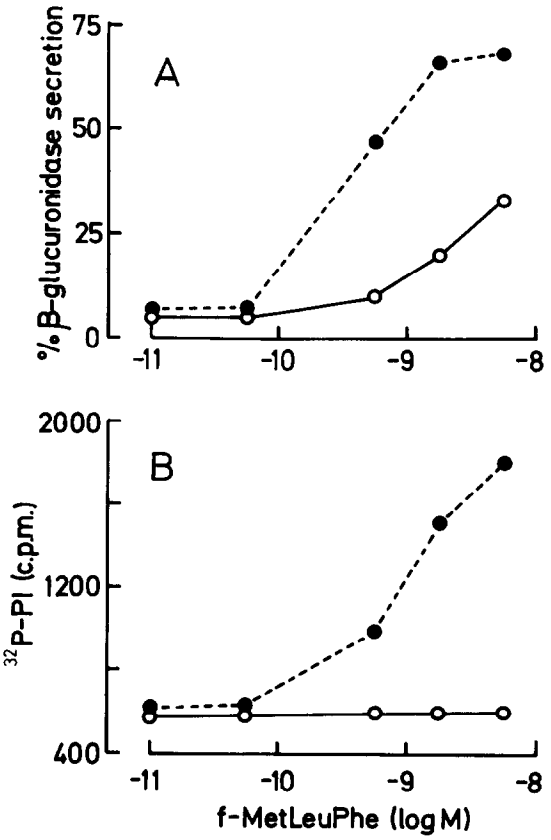


Fig.1. Concentration dependence on f-MetLeuPhe of (A) secretion of β-glucuronidase; (B) incorporation of [$^{32}$P]phosphate into phosphatidylinositol, in the presence and absence of $Ca^{2+}$. The results presented (A,B) are from the same experiment. (o——o) No $Ca^{2+}$; (•- - -•) $Ca^{2+}$ 1.8 mM.

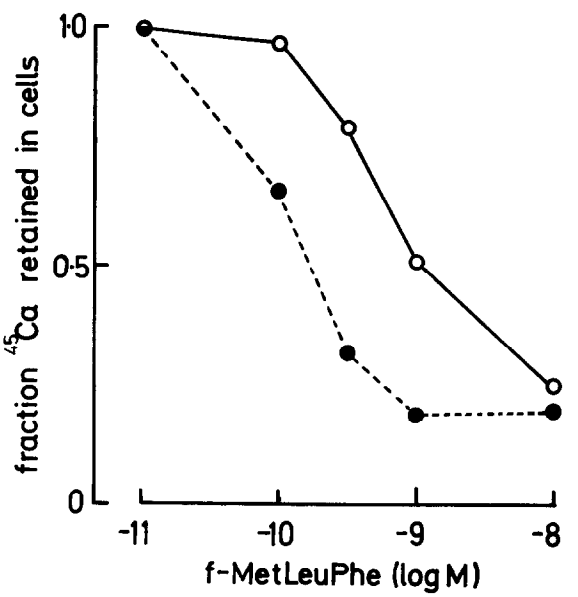


Fig.2. Concentration dependence on f-MetLeuPhe of $^{45}Ca^{2+}$ efflux from preloaded cells in the presence and absence of external $Ca^{2+}$. Secretion of β-glucuronidase was also measured in this experiment and the results were essentially the same as in fig.1(A). (o——o) No $Ca^{2+}$; (•- - -•) $Ca^{2+}$ 1.8 mM.

increases the sensitivity to the ligand so that the onset of secretion occurs at $10^{-10}$ M in the presence of $Ca^{2+}$ and $10^{-9}$ M in its absence. This effect of $Ca^{2+}$ is exerted at a stage subsequent to ligand binding since we were unable to detect any effect of $Ca^{2+}$ on the binding of [$^3$H]f-MetLeuPhe to neutrophils (data not shown).

In contrast to the modulatory effect of external $Ca^{2+}$ on secretion, the experiment shown in fig.1B shows that the stimulation of phosphatidylinositol labelling due to f-MetLeuPhe is absolutely dependent on extracellular $Ca^{2+}$.

Fig.2 shows that in the absence of external $Ca^{2+}$, higher concentrations of f-MetLeuPhe are required to cause $^{45}Ca^{2+}$ efflux. The magnitude of this effect, measured as the shift in effective concentration of the ligand, is comparable to the effect of $Ca^{2+}$ omission on secretion (fig.1A). We previously showed that f-MetLeuPhe stimulated efflux of $^{45}Ca^{2+}$ is modulated to only a small extent due to the presence of cytochalasin B, and that it therefore reflects an early stage in the train of events which leads to cell activation [8]. The enhanced rate of $^{45}Ca^{2+}$ efflux from stimulated cells probably reflects an increase in activity of plasma membrane $Ca^{2+}$ pumps due to increase in intracellular $Ca^{2+}$ from any source. In the absence of external $Ca^{2+}$, the source of $Ca^{2+}$ which initiates $^{45}Ca^{2+}$ efflux presumably corresponds to the membrane bound calcium pool detected by chlortetracycline fluorescence [10,11]. The change in chlortetracycline fluorescence due to addition of f-MetLeuPhe is not sensitive to omission of external $Ca^{2+}$.

An increase in the rate of phosphatidylinositol metabolism has been observed in a wide variety of tissues when stimulated by attachment of ligands to receptors which have the function of mobilising $Ca^{2+}$ [1,7], but the effect of $Ca^{2+}$ on the agonist induced phosphatidylinositol responses have only been reported in a limited number of cases. These are listed in table 1. The realisation that $Ca^{2+}$ dependent functions in some tissues can be initiated by receptors which also mediate $Ca^{2+}$ independent phosphatidyl-

Table 1
Dependence of stimulated phosphatidylinositol turnover on extracellular $Ca^{2+}$

| Tissue | Ligand | Measured tissue function | $Ca^{2+}$ dependence of phosphatidylinositol turnover | Ref. |
|---|---|---|---|---|
| Adrenal medulla (bovine) | Acetylcholine | Catecholamine secretion | None | [2] |
| Anterior pituitary (bovine) | Acetylcholine | Growth hormone secretion | None | [13] |
| Parotid (rat) | Acetylcholine<br>Adrenaline<br>Substance P | $K^+$ efflux | None | [3–5] |
| Lacrimal gland (rat) | Acetyl-β-methylcholine<br>Adrenaline | | None | [12] |
| Salivary gland (blowfly) | 5-hydroxytryptamine | $K^+$ efflux | None | [14] |
| Platelets (human) | ADP | Shape change | None | [15] |
| Mast cells (rat) | Antigens<br>Concanavalin A<br>Chymotrypsin<br>Compound 48/80 | Histamine secretion | None | [6] |
| Pancreas (pigeon) | Acetylcholine | Amylase secretion | Partial | [16] |
| Ileum longitudinal smooth muscle (guinea pig) | Carbamoylcholine | | Partial | [17] |
| Hepatocytes (rat) | Vasopressin<br>Angiotensin<br>Adrenaline | | Partial | [18,19] |
| Synaptosomes (guinea pig, rat) | Acetylcholine | | Partial | [20,21] |
| Iris smooth muscle (rabbit) | Noradrenaline | | Partial | [22] |
| Mast cells (rat) | $ATP^{4-}$ | Histamine secretion | Total | [23] |

inositol responses gave credence to the idea of an intermediary role for phosphatidylinositol linked events in the mobilisation of $Ca^{2+}$. However, in some other tissues (see lower section of table 1) the phosphatidylinositol responses show a partial or total dependence on the presence of extracellular $Ca^{2+}$, and in these cases the existence of a causal relationship between phosphatidylinositol metabolism and $Ca^{2+}$ mobilisation is less clear. In rabbit neutrophils the distinction between agonist induced $^{45}Ca^{2+}$ efflux and secretion on the one hand, and phosphatidylinositol metabolism on the other, is absolute. Here we have a receptor which mobilises $Ca^{2+}$ from internal sources and initiates secretion without any involvement of phosphatidylinositol turnover.

Phosphatidylinositol labelling as measured in the present work probably arises as a secondary consequence of the breakdown of phosphatidylinositol which is initiated by the activated receptor [12]. We have preliminary evidence that the breakdown of phosphatidylinositol in neutrophils stimulated by f-MetLeuPhe is also $Ca^{2+}$ dependent. Our results do not lend support to the general hypothesis concerning a role for phosphatidylinositol breakdown in the mobilisation of $Ca^{2+}$ or even in the mediation of $Ca^{2+}$ dependent cell activation.

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