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# The fatty acid composition of phosphatidylinositol, phosphatidate and 1,2-diacylglycerol in stimulated human neutrophils

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The fatty acid composition of phosphatidylinositol (PtdIns), phosphatidate (PtdOH) and 1,2-diacylglycerol (DG) was determined in cytochalasin B-treated human neutrophils in the presence and in the absence of formylmethionyl-leucyl-phenylalanine. The compositions of PtdOH and DG in stimulated cells resemble closely that of PtdOH in control cells and are quite different from the composition of PtdIns. DG appears to be produced as a subsequent metabolite of PtdOH and not as an intermediate in the conversion of PtdIns into PtdOH. We conclude that PtdOH is produced directly from a small pool of newly synthesized PtdIns in stimulated neutrophils.

A characteristic of inositol lipids from animal tissues is that they exhibit a predominantly 1-stearoyl,2-arachidonyl fatty acid composition on the *sn*-glycerol backbone (Holub & Kuksis, 1978). This is in marked contrast with the composition of PtdOH, which, despite being a close metabolic precursor of PtdIns, generally possesses relatively low contents of stearate and arachidonate. Holub & Kuksis (1978) have shown that at least in liver the PtdIns acquires its characteristic composition by fatty acid exchange after its synthesis *de novo* rather than by selection of a particular preformed pool of PtdOH for its synthesis, so that newly synthesized PtdIns would be expected to have a fatty acid composition similar to that of PtdOH and distinctly different from that of bulk PtdIns.

In addition to its precursor role in the synthesis of PtdIns *de novo*, PtdOH may also appear in cells as a product of inositol lipid breakdown in the wide range of tissues that turn over their inositol lipids in response to stimulation by an extracellular agonist (Michell *et al.*, 1981). Results with stimulated platelets (Broekman *et al.*, 1981) and with pancreas (Geison *et al.*, 1976) seem to indicate that the new PtdOH has a fatty acid composition very similar to that of bulk PtdIns, rich in stearate and arachidonate. It is widely believed that the new PtdOH is produced by phosphorylation of DG that is formed by phospholipase C attack on inositol lipids (Michell *et al.*, 1981).

In contrast, using neutrophils we have recently reported that fMet-Leu-Phe stimulates the direct conversion of PtdIns into PtdOH by a $Ca^{2+}$-dependent mechanism possibly involving phospholipase D (Cockcroft, 1984). Measurements of chemical and radiochemical loss of PtdIns (Cockcroft *et al.*, 1981; Cockcroft, 1982, 1984) in these cells have suggested that it is a newly synthesized pool of PtdIns that is agonist-sensitive, and on this basis we might predict that, unlike the situation in pancreas and platelets, the fatty acid composition of the resulting PtdOH may not resemble that of bulk PtdIns. The results now presented here confirm this prediction by showing that the PtdOH synthesized in human neutrophils stimulated with fMet-Leu-Phe has a fatty acid composition very similar to that of PtdOH in unstimulated cells and quite different from that of bulk PtdIns. We suggest that the new PtdOH is derived from a small pool of newly synthesized PtdIns that is distinct from the main PtdIns pool in these cells. We have also investigated the concentrations of DG and its fatty acid profile, and have demonstrated that the DG concentrations do not alter at 10s, when generation of PtdOH and enzyme secretion are still proceeding. The DG concentration does increase at 2min, but this is fully accounted for by a parallel decrease in PtdOH concentrations.

Abbreviations used: PtdIns, phosphatidylinositol; PtdOH, phosphatidate; DG, 1,2-diacylglycerol; fMet-Leu-Phe, formylmethionyl-leucyl-phenylalanine.

## Methods

Preparation of human neutrophils was as described previously (Cockcroft, 1984). Neutrophils were suspended at $10^8$ cells/ml, and 2–3ml of cells was used per incubation. Cytochalasin B (a drug that enhances secretion while inhibiting the motile responses of the cell) was added to cells (final concentration 5$\mu$g/ml) before the addition of fMet-Leu-Phe. Cells were stimulated with fMet-Leu-Phe (final concentration 100nM) added in a small volume of 0.15M-NaCl. Samples were quenched with 10ml of ice-cold 0.15M-NaCl, and the cells were sedimented by centrifugation at 300*g* at 4°C. Samples (50$\mu$l) of supernatant were removed for the determination of the secreted $\beta$-glucuronidase as described previously (Bennett *et al.*, 1980). The remainder of the supernatant was discarded, and the cell pellet was retained for extraction of cell lipids as described previously (Cockcroft, 1984). Phospholipids were separated by two-dimensional t.l.c. and lipid phosphorus was measured as described previously (Cockcroft, 1984). DG was separated by t.l.c. and the fatty acids of DG, PtdOH and PtdIns were analysed by g.l.c. as described previously (Allan & Cockcroft, 1983).

## Results and discussion

On the addition of fMet-Leu-Phe to neutrophils there is a loss of approx. 7nmol of PtdIns per $10^8$ cells, equivalent to about 15% of the total PtdIns pool. Most of this PtdIns decrease can be accounted for at 10s by an increase in PtdOH alone and at 2min by the combined concentrations of PtdOH and DG (6–8nmol total per $10^8$ cells), and this evidence, together with radiolabelling data already published (Cockcroft, 1984), strongly suggests that PtdIns is the direct source of the new PtdOH. However, as is shown in Table 1, the fatty acid composition of the bulk PtdIns is considerably different from that of the PtdOH formed on stimulation. Whereas the PtdIns has a typical profile for mammalian inositides, with high contents of stearate and arachidonate and only a small amount of palmitate, the PtdOH that is formed is comparatively rich in palmitate, oleate and linoleate, in this respect resembling the endogenous PtdOH of unstimulated cells. These results are consistent with the idea, outlined above, that the PtdOH formed in stimulated cells is derived from a small pool of PtdIns that itself is undergoing rapid turnover, and that consequentially has an unusual

Table 1. *Fatty acid composition of PtdIns, PtdOH and DG from control and fMet-Leu-Phe-stimulated human neutrophils*
For experimental details see the text. Values are given as means±S.D., with the numbers of experiments in parentheses. Abbreviation: N.D., not determined.

| Fatty acid | Content (mol/100mol) Control | fMet-Leu-Phe (10s) | fMet-Leu-Phe (2min) |
|---|---|---|---|
| PtdIns | | | |
| $C_{16:0}$ | 4±1 (4) | N.D. | 4±1 (3) |
| $C_{18:0}$ | 36±5 | | 41±4 |
| $C_{18:1}$ | 14±1* | | 25±3* |
| $C_{18:2}$ | 6±1 | | 6±1 |
| $C_{20:4}$ | 39±4† | | 24±3† |
| Concn. (nmol/$10^8$ cells) ... | 50.1±1.3 (4) | 43.3±1.7 (4) | 43.3±2.0 (4) |
| PtdOH | | | |
| $C_{16:0}$ | 27±3 (3) | 20±2 | 20±3 |
| $C_{18:0}$ | 20±1 | 19±1 | 21±2 |
| $C_{18:1}$ | 32±2 | 34±3 | 30±1 |
| $C_{18:2}$ | 15±4 | 17±5 | 18±4 |
| $C_{20:4}$ | 5±2 | 10±2 | 10±2 |
| Concn. (nmol/$10^8$ cells) ... | 1.7±0.1 (3) | 9.5±0.4 (3) | 7.6±0.4 (3) |
| DG | | | |
| $C_{16:0}$ | 26±4 (4) | 27±2 | 20±1 |
| $C_{18:0}$ | 16±2 | 16±1 | 15±1 |
| $C_{18:1}$ | 35±1 | 34±2 | 40±1 |
| $C_{18:2}$ | 12±2 | 13±3 | 13±1 |
| $C_{20:4}$ | 10±3 | 8±2 | 10±3 |
| Concn. (nmol/$10^8$ cells) ... | 2.0±0.4 (4) | 1.9±0.3 (4) | 5.0±0.5 (4) |

* Statistically significant: $P = 0.02$.
† Statistically significant: $0.05 > P > 0.02$.

fatty acid composition similar to that of precursor PtdOH.

A pronounced change in the overall fatty acid composition of PtdIns was also seen in each experiment after incubation with fMet-Leu-Phe for 2min (Table 1). The loss of arachidonate (also observed by Yano *et al.*, 1983) and an increase in oleate content could be due to a process of deacylation/re-acylation resulting from activation of phospholipase $A_2$, previously demonstrated to be present in neutrophils (Victor *et al.*, 1981; Bormann *et al.*, 1984).

We have also assessed the concentration of DG at both 10s and 2min. There was no change in the DG concentration at 10s, and this result complements earlier findings by ourselves (Cockcroft, 1984) and others (Walsh *et al.*, 1983) that in cells prelabelled with [$^3$H]glycerol or with [$^3$H]arachidonate there is no formation of labelled DG at early times. However, at 2min an increase in the DG concentration is observed, and a late increase has been reported by Takenawa *et al.* (1983) as well. This increase can be accounted for by the observed decrease in PtdOH concentrations between 10s and 2min. At each time point the fatty acid composition of the DG was similar to that of PtdOH and quite different from the composition of PtdIns. These observations suggest that DG only accumulates in neutrophils as a consequence of PtdOH accumulation and not as a result of inositide breakdown by a phospholipase C-type mechanism. In support of this conclusion, we have not been able to detect in stimulated neutrophils any increased concentrations of the inositol phosphates that would be expected to be produced by phospholipase C attack on inositides (Cockcroft & Allan, 1984). This is despite the undoubted presence in neutrophil plasma membranes of a polyphosphoinositide phosphodiesterase (Cockcroft *et al.*, 1984), which could potentially degrade inositides if it were activated.

Our results therefore are not consistent with the now commonly accepted mechanism for agonist-stimulated breakdown of inositides (Michell *et al.*, 1981; Berridge, 1984) whereby polyphosphoinositides are degraded to DG and inositol phosphates and where PtdOH appears as a product of DG phosphorylation. Rather, in the human neutrophil it seems that stimulation with fMet-Leu-Phe promotes the breakdown of a specific minor pool of PtdIns to give PtdOH directly, presumably by a phospholipase D-type mechanism, and that DG appears only as a subsequent product of PtdOH metabolism. However, the possibility remains that there is a small pool of inositol lipid that breaks down as a direct consequence of receptor occupancy but is masked by the larger mass changes reported here, which could be associated with subsequent steps in cellular activation.

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