Is phospholipase A₂ activation regulated by G-proteins?

Shamshad Cockcroft, Christopher P. Nielson and Jane Stutchfield

Department of Physiology, University College London, London WC1E 6JJ, U.K.

Introduction

Many different cell types, including neutrophils, release arachidonic acid in response to receptor-mediated stimulation. The fatty acid can act either as an intracellular second messenger or it can be metabolized to biologically active eicosanoids. The key enzyme responsible for mobilizing arachidonate is phospholipase A₂. Release of arachidonate is from phospholipids, principally phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol.

Many receptors activate three phospholipases (C, A₂ and D), when stimulated by an agonist. The coupling of phospholipase C to receptors via G-proteins is well documented, but our knowledge of the regulation of phospholipase A₂ is less well understood. Early studies suggested that phospholipase A₂ activation was a consequence of second messengers generated by phospholipase C activation. Phospholipase A₂ is stimulated in vitro by Ca²⁺ in the millimolar range, but, nevertheless, it has been commonly assumed that Ca²⁺ is the major regulator of activity in vivo. Synergistic activation of arachidonate release by micromolar levels of Ca²⁺ and the protein kinase C activator, phorbol 13-myristate 12-acetate (PMA), has also been documented for many cell types. However, more recent work indicates that phospholipase A₂ activation is regulated via a G-protein analogous to phospholipase C regulation.

The evidence that phospholipase A₂ may be regulated by G-proteins has been accumulating for a number of years (for recent reviews see [1, 2]). Initial studies were done in intact cells, where it was demonstrated that pertussis toxin pretreatment led to inhibition of phospholipase A₂ activation. Pertussis toxin is known to ADP-ribosylate the G-proteins of the G₁ family as well as G₂. From this observation, it was suggested that the receptor was coupled to phospholipase A₂ via a G-protein, designated G₄ [3]. Another indicator that phospholipase A₂ is regulated by G-proteins was the observation that activators of G-proteins, such as GTP analogues or fluoride, were able to stimulate arachidonate release. Table 1 summarizes the evidence supporting a role for G-protein regulation of phospholipase A₂.

Many receptors that are known to activate phospholipase A₂ are also coupled to phospholipase C. Since the products of phospholipase C activation may, in principle, stimulate phospholipase A₂, it is important to discriminate between sequential activation as opposed to parallel activation of the two lipases. The following evidence supports parallel activation: (i) pertussis toxin selectively inhibits phospholipase A₂ activation without impairing phospholipase C activation [3, 4]; (ii) neomycin selectively inhibits phospholipase C activation (by substrate sequestration) [3] without impairing phospholipase A₂; and (iii) PMA, an activator of protein kinase C, inhibits phospholipase C activation without affecting the stimulation of phospholipase A₂ [3, 5].

Because protein kinase C activation itself is a consequence of phospholipase C activation, and PMA can substitute for the endogenously generated diacylglycerol, experiments with PMA do not exclude the obligatory requirement for stimulation of phospholipase C in order to switch on phospho-
Biochemical Society Transactions

Table I
Systems where it has been shown that phospholipase A2 can be regulated by a G-protein

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Agonist</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cells</td>
<td>GTP[S]</td>
<td>[13, 14]</td>
</tr>
<tr>
<td>Rat basophilic cells</td>
<td>GTP[S]</td>
<td>[15]</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>GTP[S]</td>
<td>[16]</td>
</tr>
<tr>
<td>Fluoride</td>
<td></td>
<td>[7]</td>
</tr>
<tr>
<td>HL60 cells</td>
<td>GTP[S] (unpublished work)*</td>
<td></td>
</tr>
<tr>
<td>(differentiated)</td>
<td>fMet-Leu-Phe</td>
<td>(unpublished work)*</td>
</tr>
<tr>
<td>FRTL-5 thyroid cells</td>
<td>α-Adrenergic, GTP[S]</td>
<td>[3]</td>
</tr>
<tr>
<td>Swiss 3T3 fibroblasts</td>
<td>Bradykinin, GTP[S]</td>
<td>[4]</td>
</tr>
<tr>
<td>Rod outer segments</td>
<td>Light, βy-Subunits</td>
<td>[18, 19]</td>
</tr>
<tr>
<td>Aplysia sensory neurons</td>
<td>FMRFamide, GTP[S]</td>
<td>[20]</td>
</tr>
<tr>
<td>Platelets</td>
<td>H1-Histamine, GTP[S]</td>
<td>[21, 22]</td>
</tr>
<tr>
<td>Fluoride</td>
<td></td>
<td>[23]</td>
</tr>
<tr>
<td>Mesangial cells</td>
<td>Lipopolysaccharide</td>
<td>[6]</td>
</tr>
</tbody>
</table>

lipase A2. Only the experiment using neomycin demonstrates conclusively that the activation of the two lipases is independent. Are there any agonists that are exclusively linked to phospholipase A2 activation? Lipopolysaccharide appears to fall into this category. It stimulates phospholipase A2 in mesangial cells in a pertussis-toxin-sensitive manner without affecting phospholipase C activation [6].

To study the relative contribution by these two mechanisms, we have examined the regulation of phospholipase A2 activity in streptolysin-O-permeabilized neutrophils and HL60 cells. Our findings suggest that phospholipase A2 activation is under dual regulation. Although Ca2+ and protein kinase C activation can regulate phospholipase A2 activity, it is apparent that a G-protein is also involved. The contribution by the two pathways with a physiological stimulus would suggest that at least 50% of the arachidonate released could be accounted for by direct coupling of G-protein to phospholipase A2. However, a requirement for Ca2+ in the micromolar range was obligatory and this would suggest that a prior activation of phospholipase C was necessary for phospholipase A2 activation to occur.

Regulation of phospholipase A2 by Ca2+ alone

Studies with intact neutrophils and HL60 cells clearly indicate that a rise in cytosolic Ca2+ induced by a physiological agonist is not sufficient to activate phospholipase A2 [24]. The evidence can be summarized as follows. (a) ATP and fMet-Leu-Phe can both stimulate arachidonate release as

[Figure 1: Phospholipase A2 activation stimulated by Ca2+, GTP[S] and fMet-Leu-Phe from permeabilized neutrophils in the presence (a) or absence (b) of MgATP]

Human neutrophils were labelled with 3H-labelled arachidonic acid for 1 h and washed. After 30 min of preincubation, the cells were metabolically inhibited for 5 min. The cells were transferred to tubes containing the permeabilizing agent, streptolysin O and other additions as indicated. After 10 min incubations, the cells were quenched with ice-cold 0.9% (w/v) NaCl, centrifuged and the supernatant sampled for released arachidonate. The results are expressed as the proportion of arachidonate mobilized as a percentage of the total radioactivity incorporated in the cell determined after lysis with 1% (v/v) Triton X-100. O, No addition; ●, fMet-Leu-Phe (1 μM) and △, GTP[S] (60 μM).
well as phospholipase C activation in intact neutrophils. The optimal rise in cytosolic Ca\(^{2+}\) achieved by both agonists is similar, yet fMet-Leu-Phe is better than ATP in stimulating arachidonate release. (b) Some analogues of ATP (e.g. 8-Bromo-ATP) were able to interact with the ATP receptor and to activate phospholipase C leading to a rise in cytosolic Ca\(^{2+}\), but were unable to stimulate phospholipase A\(_2\) activation. (c) Phospholipase C activation by ATP or fMet-Leu-Phe could be inhibited by PMA treatment; in contrast, release of arachidonate by ATP and fMet-Leu-Phe was either enhanced (by ATP) or unaffected (by fMet-Leu-Phe).

The effect of Ca\(^{2+}\) was examined in permeabilized human neutrophils (Figs. 1a and b). Streptolysin O was used to permeabilize the cells. Before permeabilization, the cells were metabolically inhibited. Under these conditions, intact cells do not respond to external stimulation. This is probably related to inhibition of phospholipase C activation owing to substrate depletion for this enzyme. Raising the Ca\(^{2+}\) concentration from pCa 8 to pCa 5 results in the release of arachidonate. Ca\(^{2+}\), 1 \(\mu\)M, was ineffective, but 10 \(\mu\)M induced a response. Arachidonate release, secondary to protein kinase C activation, with 10 \(\mu\)M-Ca\(^{2+}\) could be ruled out as the response was independent of MgATP (cf. Figs. 1a and b). Ca\(^{2+}\)-dependent activation has also been demonstrated in membranes and again 10 \(\mu\)M-Ca\(^{2+}\) was necessary to observe a response [25].

**Is phospholipase A\(_2\) activation regulated by G-proteins**

G-proteins can be directly activated using non-hydrolysable analogues of GTP or fluoride. GTP analogues, guanosine 5'-[\(\gamma\)-thio]triphosphate (GTP[S]), guanosine 5'-[\(\beta,\gamma\)-imido]triphosphate (GppNHp) and guanosine 5'-[\(\alpha,\beta\)-methylene]-triphosphate (GppCH\(_3\)p) were found to be potent activators of phospholipase A\(_2\) in streptolysin-O-permeabilized neutrophils and HL60 cells (Fig. 2a). GTP analogues were able to stimulate arachidonate release, secondary to protein kinase C activation, with 10 \(\mu\)M-Ca\(^{2+}\) could be ruled out as the response was independent of MgATP (cf. Figs. 1a and b). Ca\(^{2+}\)-dependent activation has also been demonstrated in membranes and again 10 \(\mu\)M-Ca\(^{2+}\) was necessary to observe a response [25].

![Figure 2](image-url)

(a) Stimulation of arachidonate release by GTP analogues, GTP[S], GppNHp and GppCH\(_3\)p from permeabilized neutrophils. (b) Fluoride-stimulated arachidonate from intact neutrophils.
from metabolically inhibited cells stimulated by GTP[S] is dependent on exogenously added MgATP. GTP[S]-mediated stimulation of phospholipase A$_2$ was reduced substantially in the absence of MgATP implying a role for phospholipase C activation. This role could be the provision of sufficient diacylglycerol for protein kinase C activation and the subsequent protein phosphorylation. The role of inositol trisphosphate in raising Ca$^{2+}$ is obviated here, since Ca$^{2+}$ is provided in the form of Ca$^{2+}$ buffers. It is not known whether phospholipase D activation would occur under these experimental conditions and whether any products of this pathway have any influence on phospholipase A$_2$ activity.

fMet-Leu-Phe can also stimulate phospholipase A$_2$ activation in permeabilized human neutrophils, providing that Ca$^{2+}$ in the micromolar range is available (Fig. 1a). In contrast to GTP[S], the response to fMet-Leu-Phe is only reduced by 50% when MgATP is excluded (cf. Figs. 1a and b). Because the fMet-Leu-Phe-induced responses are inhibited by treatment with pertussis toxin, it is concluded that the response stimulated with fMet-Leu-Phe in the absence of MgATP must be regulated by a G-protein belonging to the G$_i$ family.

It is clear that there is a component of phospholipase A$_2$ activation that appears to be controlled by Ca$^{2+}$/protein kinase C. The contribution by this pathway can be viewed as positive feedback, analogous to the Ca$^{2+}$-dependent stimulation of phospholipase C. Arachidonic acid is a potential protein kinase C activator [9] and has the ability to release cytosolic Ca$^{2+}$ [10]. A possible mechanism regulating phospholipase A$_2$ activation by protein kinase could be phosphorylation of an inhibitory constraint. Touqui et al. [11] have put forward a model of phospholipase A$_2$ activation whereby it is suggested that phosphorylation of lipocortin removes an inhibitory constraint from the phospholipase A$_2$, thus allowing it to be activated. Lipocortins have been shown to be substrates for protein kinase C in human neutrophils [12].

**Conclusions**

Phospholipase A$_2$ activation in neutrophils is under dual regulation. fMet-Leu-Phe can activate arachidonate release by two mechanisms: a G-protein-dependent pathway and a Ca$^{2+}$/protein-kinase-C-regulated pathway. The relative contributions of these pathways in fMet-Leu-Phe-stimulated phospholipase A$_2$ activation appear to be equal. It is important to stress, however, that in intact cells, activation of phospholipase A$_2$ may be subsequent to phospholipase C activation, since 1 μM-Ca$^{2+}$ appears to be required for fMet-Leu-Phe to stimulate phospholipase A$_2$.

We are grateful to the Wellcome Trust and the Lister Institute for support. S.C. is a Lister Institute Fellow.


Received 17 December 1990