Phosphatidylinositol Metabolism in Rat Hepatocytes Stimulated by Glycogenolytic Hormones

EFFECTS OF ANGIOTENSIN, VASOPRESSIN, ADRENALINE, IONOPHORE A23187 AND CALCIUM-ION DEPRIVATION

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1. The effects on phosphatidylinositol metabolism of three Ca\(^{2+}\)-mobilizing glycogenolytic hormones, namely angiotensin, vasopressin and adrenaline, have been investigated by using rat hepatocytes. 2. All three hormones stimulate both phosphatidylinositol breakdown and the labelling of this lipid with \(^{32}\)P. 3. The response to angiotensin occurs quickly, requires a high concentration of the hormone and is prevented by [1-sarcosine, 8-isoleucine]angiotensin, a specific angiotensin antagonist that does not prevent the responses to vasopressin and to adrenaline. This response therefore seems to be mediated by angiotensin-specific receptors. 4. [1-Deaminocysteine,2-phenylalanine,7-(3,4-didehydroproline),8-arginine]vasopressin, a vasopressin analogue with enhanced antidiuretic potency, is relatively ineffective at stimulating phosphatidylinositol metabolism. This suggests that the hepatic vasopressin receptors that stimulate phosphatidylinositol breakdown are different in their ligand selectivity from the antidiuretic vasopressin receptors that activate renal adenylate cyclase. 5. Incubation of hepatocytes with ionophore A23187, a bivalent-cation ionophore, neither mimicked nor appreciably changed the effects of vasopressin on phosphatidylinositol metabolism, suggesting that phosphatidylinositol breakdown is not controlled by changes in the cytosol Ca\(^{2+}\) concentration. This conclusion was supported by the observation that hormonal stimulation of phosphatidylinositol breakdown and resynthesis persists in cells incubated for a substantial period in EGTA, although this treatment somewhat decreased the phosphatidylinositol response of the hepatocyte. The phosphatidylinositol response of the hepatocyte therefore appears not to be controlled by changes in cytosol [Ca\(^{2+}\)], despite the fact that this ion is thought to be the second messenger by which the same hormones control glycogenolysis. 6. These results may be an indication that phosphatidylinositol breakdown is an integral reaction in the stimulus–response coupling sequence(s) that link(s) activation of \(\alpha\)-adrenergic, vasopressin and angiotensin receptors to mobilization of Ca\(^{2+}\) in the rat hepatocyte.

A specific increase in the metabolism of phosphatidylinositol is a widespread cellular response to activation of certain types of cell-surface receptor for hormones and neurotransmitters, particularly receptors in whose actions Ca\(^{2+}\) ions are strongly implicated. One possible explanation of this association could be that phosphatidylinositol breakdown, the reaction controlled by receptor activation, is somehow implicated in the mechanism(s) through which various types of receptor control the mobilization of Ca\(^{2+}\) ions within cells (Michell, 1975, 1979; Jones & Michell, 1978a; Jones et al., 1979). Most studies of this phenomenon have so far been carried out in various types of tissue fragments, particularly from secretory tissues and smooth muscles. More recently, however, it has become clear that rat hepatocytes possess at least three populations of hormone receptors in whose actions Ca\(^{2+}\) ions are intimately involved, namely those for \(\alpha\)-adrenergic ligands, vasopressin and angiotensin (Stubbs et al., 1976; Keppens et al., 1977; Hems et al., 1978; Assimacopoulos-Jeannet et al., 1977; Van de Werve et al., 1977; Blackmore et al., 1978; Chen et al., 1978). It has been known for some years that \(\alpha\)-adrenergic stimulation brings about enhanced phosphatidylinositol metabolism in a variety of tissues, possibly including rat liver (De Torrentegui & Berthet, 1966; Jones & Michell, 1978a), and consideration of the effects of vasopressin and angiotensin on smooth muscles suggested to us that their actions might also involve phosphatidylinositol metabolism.
metabolism (Michell et al., 1977a). Subsequently it was found that isolated rat hepatocytes show a rapid increase in the labelling of their phosphatidyl-
inositol with $^{32}$P when exposed to vasopressin (Kirk et al., 1977, 1978, 1979; Billah & Michell, 1978) or angiotensin (Billah & Michell, 1978). The present paper is concerned with detailed description of the response to angiotensin, with demonstration of the fact that the phosphatidylinositol responses of hepatocytes involve phosphatidylinositol breakdown and with the relationship between the simultaneous changes in cellular Ca$^{2+}$ status and in phosphatidyl-
inositol metabolism that these hormones provoke.

Materials and Methods

Materials

Bovine plasma albumin (fraction V; fatty acid-
poor, dialysed against 0.154m-NaCl for at least 24h) was from Miles Laboratories., Stoke Court, Stoke Poges, Slough SL2 4LY, Berks., U.K.

Adrenaline, collagenase (type 1) and synthetic vasopressin (grade V) were from Sigma Chemical Co., Poole, Dorset BH17 7NH, U.K.

Synthetic angiotensin octapeptide and [1-sarcosine-
8-isoleucine]angiotensin were from Peninsula Laboratories Inc., P.O. Box 1111, San Carlos, CA 94070, U.S.A.

Bivalent-cation ionophore A23187 was a gift from Eli-Lilly, Indianapolis, IN, U.S.A.

Sagatal (pentobarbitone sodium; 60mg/ml) was from May and Baker, Dagenham, Essex, U.K.

[1-Deaminocysteine,2-phenylalanine,7-(3,4-dide-
hydropoline),8-arginine]vasopressin was a gift from Dr. Roderich Walter, Department of Biochemistry, University of Chicago, Chicago, IL, U.S.A.

Isolation of rat hepatocytes

Hepatocytes were isolated from fed male Wistar rats (230–260g) essentially by the method of Berry & Friend (1969). A rat was anaesthetised with 0.4ml of Sagatal and the liver was cannuilated via the portal vein. After washing out the blood with Ca$^{2+}$-free Krebs–Ringer bicarbonate buffer, pH 7.4, the liver was subjected to recirculating perfusion with 100ml of the same buffer containing 30mg of collagenase. The perfusion was at 37°C and the medium was constantly gassed with O$_2$/CO$_2$ (19:1, v/v). After 20–30min, the collagenase-treated liver was gently stirred in a plastic beaker with a plastic rod. The separated cells were washed 3–4 times by suspension in Krebs–Ringer–Hepes [4-(2-hydroxyethyl)-1-
piperazine-ethanesulphonic acid; 25mm] buffer,

ph 7.4, containing 1mm-Ca$^{2+}$, so as to remove debris and broken cells. When viewed by phase-contrast microscopy, approx. 90% of the cells remained non-
aggregated and phase-dense and also excluded Trypan Blue. Cells isolated from 24h-starved rats by this method synthesized glucose from 10mm-pyruvate or 10mm-alanine at rates comparable with those reported by other workers (e.g. Berry & Friend, 1969).

Incorporation of $^{32}$P into hepatocyte lipids in vitro

Isolated washed cells were diluted to a dry weight of approx. 20mg/ml with Krebs–Ringer–Hepes buffer containing 1mm-Ca$^{2+}$ and 4% (w/v) bovine serum albumin (unless otherwise stated). A portion (0.5ml) of this suspension was then added by using an automatic pipette to plastic vials each containing 0.5ml of the same buffer containing 40μCi of $[^{32}P]$P, and appropriate other additions (e.g. hor-
mones). The incubations were usually for 15min and incorporation was stopped by adding 3.75ml of chloroform/methanol (1:2, v/v). Lipids were extracted and separated by paper chromatography and the separated phosphatidylinositol and, in some experiments, the combined spot containing most other phospholipids were analysed for radioactivity (see Jones et al., 1979). The labelling of the lipids in stimulated cells was expressed as a percentage of the value in incubations without added hormones. In each experiment, two to four replicate assays were carried out under each set of incubation conditions.

Studies of phosphatidylinositol breakdown

For some of the experiments on phosphatidylin-
ositol breakdown, prelabelled hepatocytes were isolated from rats that had received an intraperitoneal injection of approx. 0.7mCi of $[^{32}P]$P, about 24h previously. Incubation conditions were identical with those in experiments for $^{32}$P incorporation. Both in control and stimulated cells, the separated phosphatidylinositol was then analysed for radioactivity. In some experiments, both with radioactive and non-radioactive cells, the phosphate content of the lipids was estimated. The results were expressed as percentage changes in the $^{32}$P or phosphate contents of the lipids relative to controls run in parallel. In each experiment, six to 18 replicate assays were done under each set of incubation conditions.

Results

Stimulation of phosphatidylinositol labelling by angiotensin

Incubation of hepatocytes with $[^{32}P]$P in the presence of angiotensin produced a dose-dependent increase in the labelling of phosphatidylinositol
half-maximum stimulation occurred at about 50nM-angiotensin, a much higher concentration than is needed for half-maximum stimulation of glycogen phosphorylase (Keppens & De Wulf, 1976). When hepatocytes were incubated with $^{32}$P for 5 min and angiotensin was then added, an increase in phosphatidylinositol labelling was seen within 1 min and the maximum stimulated rate of incorporation was achieved in less than 5 min (Fig. 2).

When individual preparations of hepatocytes were incubated with 1µM-angiotensin, vasopressin (10µ-units/ml; 23nM) and 100µM-adrenaline, concentrations of ligands that give close to maximum stimulation of phosphatidylinositol labelling, we routinely observed that the stimulation in phosphatidylinositol labelling during 15 min incubations was higher with vasopressin (5–8-fold) than with angiotensin or adrenaline (both 3–5-fold) (e.g. Table 2).

De Torrentegui & Berthet (1966) and Kirk et al. (1977) have shown that the adrenaline response is susceptible to blockade by $\alpha$-adrenergic antagonists.

**Stimulation of phosphatidylinositol breakdown**

There are now a considerable number of situations in which it has been clearly shown that receptor-stimulated phosphatidylinositol metabolism involves phosphatidylinositol breakdown, and it seems likely that this is the initial reaction in all such responses (see Jones et al., 1979). This also appears to be the case in the responses of hepatocytes. Table 1 summarizes the results of a considerable number of experiments in which we investigated the effects of hormones on either (a) the $^{32}$P content of phosphatidylinositol in prelabelled hepatocytes or (b) the total phosphatidylinositol content of cells.
most of the experiments a decrease of about 4-8% was seen in the amount or radioactivity of phosphatidylinositol. In the majority of the experiments the replicate assays were sufficiently precise for this small decrease to be statistically significant. There was no change in either the labelling or amount of lipid in the major phospholipids during these incubations (results not shown). The majority of the breakdown experiments were done with vasopressin, since this gave the largest stimulation of phosphatidylinositol labelling, but the limited information on angiotensin and adrenaline suggests that they also provoked phosphatidylinositol breakdown (Table 1).

**The role of Ca\(^{2+}\) in stimulation of phosphatidylinositol metabolism**

We used two different approaches to investigate whether changes in cytosol Ca\(^{2+}\) concentration are involved in provoking the phosphatidylinositol responses; first, by admitting Ca\(^{2+}\) to cells by using the ionophore A23187, and, secondly, by depleting cells of environmental Ca\(^{2+}\).

In experiments with the ionophore, albumin was omitted from the incubation medium to prevent it from being bound in an ineffective form, and the ionophore was presented to the cells 9 min before the addition of Ca\(^{2+}\): albumin omission caused a very slight increase in the vasopressin-stimulated incorporation of \(^{32}\)P into phosphatidylinositol (results not shown). There is abundant evidence that addition of ionophore A23187 to cells at concentrations of 0.5-10 \(\mu\)M is able to effectively activate glycogenolysis (Pointer et al., 1976; Keppens et al., 1977; Assimacopoulos-Jeannet et al., 1977) and to inhibit pyruvate kinase (Chan & Exton, 1978). By contrast, these concentrations of ionophore A23187 had only a slight stimulatory effect on the labelling of phosphatidylinositol [the means for two experiments showed rises of 15% at 0.6 \(\mu\)M (not significantly different from control), 45% at 2 \(\mu\)M (\(P<0.05\)) and 55% at 6 \(\mu\)M (\(P<0.05\)]. In addition, 2 \(\mu\)M-ionophore A23187 had no effect, either stimulatory or inhibitory, on the enhancement of phosphatidylinositol labelling caused by any of the three Ca\(^{2+}\)-mobilizing hormones.

![Graph](image)

**Fig. 3. Inhibition of angiotensin-stimulated phosphatidylinositol breakdown by [1-Sarcosine, 8-isoleucine]angiotensin**

Hepatocytes were incubated with 1 \(\mu\)M-angiotensin for 15 min, together with various amounts of [1-sarcosine, 8-isoleucine]angiotensin. The results plotted are means for triplicate determinations in one of two similar experiments.

<table>
<thead>
<tr>
<th>Table 1. Breakdown of phosphatidylinositol in hepatocytes exposed to Ca(^{2+})-mobilizing hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells, either (a) (^{32})P-labelled or (b) unlabelled, were incubated with or without hormones for 15 min and then their phosphatidylinositol content was estimated either radiochemically or by phosphate assay. Results from individual experiments were means ± S.E.M. for six to 18 incubations; the effects of hormones were then assessed by Student's (t) test. The mean radioactivity or content of phosphatidylinositol in unstimulated cells was in each case set at 100 and the hormone-treated values were expressed as a percentage (±S.E.M.) of this value. From this treatment came the presented means derived from the number of experiments indicated in parentheses. Each group of experiments was then examined by the use of a paired (t) test. Abbreviation: n.s., not significant.</td>
</tr>
</tbody>
</table>

(a) Breakdown of \(^{32}\)P-labelled phosphatidylinositol

<table>
<thead>
<tr>
<th>Phosphatidylinositol content or radioactivity (% of control)</th>
<th>Significance of difference from controls for individual experiments by (t) test</th>
<th>Significance of differences between control and stimulated groups by paired (t) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin (10\text{m-units/ml})</td>
<td>94.9 ± 1.2 (7)</td>
<td>(P&lt;0.01) (3), (P&lt;0.05) (3), n.s. (1)</td>
</tr>
<tr>
<td>Angiotensin (1 (\mu)M)</td>
<td>97.2 ± 0.3 (4)</td>
<td>(P&lt;0.05) (2), n.s. (2)</td>
</tr>
<tr>
<td>Adrenaline (100 (\mu)M)</td>
<td>94.9 ± 1.6 (4)</td>
<td>(P&lt;0.01) (3), n.s. (1)</td>
</tr>
</tbody>
</table>

(b) Decrease in phosphatidylinositol content

<table>
<thead>
<tr>
<th>Phosphatidylinositol content or radioactivity (% of control)</th>
<th>Significance of difference from controls for individual experiments by (t) test</th>
<th>Significance of differences between control and stimulated groups by paired (t) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin (10\text{m-units/ml})</td>
<td>95.1 ± 0.6 (5)</td>
<td>(P&lt;0.01) (3), (P&lt;0.05) (1), n.s. (1)</td>
</tr>
</tbody>
</table>

1979
These results suggested that changes in cytosol Ca\textsuperscript{2+} concentration probably play no essential role in the stimulation of phosphatidylinositol metabolism by these hormones. This point of view gained further support from the observation that, as with phosphatidylinositol labelling, the addition of 2\mu M-ionophore A23187 did not either mimic or interfere with vasopressin-stimulated breakdown of phosphatidylinositol. In two independent experiments, the mean phosphatidylinositol content of ionophore A23187-treated cells was 100.4\% of the control value (no significant difference), whereas the cells treated with vasopressin (94.5\% of control) or with vasopressin and ionophore A23187 (93.7\%) were both significantly different from the control value \( (P < 0.01) \), but not from each other.

Results from the Ca\textsuperscript{2+}-deprivation experiments were somewhat less clear-cut. Cells were isolated in a nominal Ca\textsuperscript{2+}-free medium and each batch of cells was then divided, one batch being washed in buffer containing 0.2mM-EGTA and the other in buffer with 1mM-Ca\textsuperscript{2+}. When stimulated with vasopressin, angiotensin or adrenaline the Ca\textsuperscript{2+}-depleted cells still responded with an increase in phosphatidylinositol labelling, but the magnitude of this stimulation was considerably less than in Ca\textsuperscript{2+}-replete cells (Table 2). In our hands, unstimulated cells incubated in Ca\textsuperscript{2+}-free medium showed greater labelling of phosphatidylinositol than did those incubated with Ca\textsuperscript{2+}, so that the decrease in the hormone-stimulated labelling was clearly apparent both in absolute terms and by comparison with the appropriate controls. This pattern differs somewhat from that reported by Kirk et al. (1978), who found decreased labelling in unstimulated Ca\textsuperscript{2+}-free cells, but we do not know why.

Vasopressin-stimulated phosphatidylinositol breakdown appeared still to occur in Ca\textsuperscript{2+}-depleted cells (Table 3), as was expected from the observation that at least some of the vasopressin-stimulated phosphatidylinositol labelling persisted in these cells. In view of the difficulty of measuring the small decrease in cellular phosphatidylinositol content that was produced by hormonal stimulation, we could not be sure whether the magnitude of the hormone-stimulated phosphatidylinositol breakdown was unchanged after Ca\textsuperscript{2+}-deprivation. An unexpected feature of these experiments was that the phosphatidylinositol content of Ca\textsuperscript{2+}-depleted cells appeared always to be appreciably higher than that of Ca\textsuperscript{2+}-replete cells, irrespective of whether they had been exposed to vasopressin (results not shown). The most likely explanation of this observation appears to be trivial, namely that the absence of Ca\textsuperscript{2+} from the medium in which cells were washed before incubation changed the way in which they packed during centrifugation, but we have not completely ruled out more important effects of Ca\textsuperscript{2+}-depletion either on the phosphatidylinositol content of the cells or on the efficiency of our lipid extraction procedure.

**The ligand specificity of the vasopressin receptor**

The most active known vasopressin analogue, at least in terms of its antidiuretic potency, is [1-

<table>
<thead>
<tr>
<th>Radioactivity in phosphatidylinositol (d.p.m.)</th>
<th>1 mM-Ca\textsuperscript{2+}</th>
<th>0.2 mM-EGTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additions</td>
<td>6920±580</td>
<td>8590±900</td>
</tr>
<tr>
<td>+ Angiotensin (1 \mu g/ml)</td>
<td>23900±90</td>
<td>12340±570</td>
</tr>
<tr>
<td>+ Vasopressin (100 units/ml)</td>
<td>37810±610</td>
<td>19600±360</td>
</tr>
<tr>
<td>+ Adrenaline (100\mu g)</td>
<td>29290±850</td>
<td>13590±1130</td>
</tr>
</tbody>
</table>

Table 2. Effect of Ca\textsuperscript{2+}-deprivation on stimulated labelling of phosphatidylinositol

Hepatocytes were incubated in \( ^{32}\)P-labelled Ca\textsuperscript{2+}-containing or Ca\textsuperscript{2+}-free media for 15 min with or without hormones. Results are expressed as means± S.E.M. for triplicate incubations in one of two experiments that gave similar results.

<table>
<thead>
<tr>
<th>Phosphatidylinositol content (% of Ca\textsuperscript{2+}-containing control)</th>
<th>Significance of difference from appropriate control for individual experiments by ( t ) test</th>
<th>Significance of difference between control and stimulated groups by paired ( t ) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM-Ca\textsuperscript{2+}+10m-units of vasopressin/ml</td>
<td>94.4±1.0 (3)</td>
<td>( P &lt; 0.005 ) (2)</td>
</tr>
<tr>
<td>0.2 mM-EGTA</td>
<td>107±3.5 (3)</td>
<td>( P &lt; 0.05 ) (1)</td>
</tr>
<tr>
<td>0.2 mM-EGTA+10m-units of vasopressin/ml</td>
<td>98.5±0.2 (3)</td>
<td>( P &lt; 0.001 ) (2)</td>
</tr>
</tbody>
</table>

Table 3. Vasopressin-stimulated phosphatidylinositol breakdown in cells deprived of extracellular Ca\textsuperscript{2+}

Cells were incubated in Ca\textsuperscript{2+}-free conditions as described in the text. Methods and presentation are otherwise as described in Table 1. For statistical analysis, each vasopressin-treated group was compared with the otherwise identical control group containing Ca\textsuperscript{2+} or EGTA. Abbreviation used: n.s., not significant.

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deaminocysteine, 2-phenylalanine, 7-(3,4-didehydro-
proline), 8-arginine] vasopressin, which is about 26
more times more potent than [8-arginine] vasopressin
(Smith & Walter, 1978). This synthetic molecule was
designed to interact with maximum efficiency with the
adenylate cyclase-coupled vasopressin receptor of
kidney, so it seemed possible that it might dis-
criminate against a Ca^{2+}-mobilizing vasopressin
receptor. This was one of the vasopressin analogues
tested by Kirk et al. (1979), and they indeed found it
to be a partial agonist of low efficacy when used to
stimulate phosphatidylinositol metabolism. We have
obtained independent confirmation of this result. In
our hands this analogue produced no appreciable
effect on phosphatidylinositol metabolism at con-
centrations of less than approx. 50–100 nM, and even
at 1–10 μM it only produced a 2–4-fold increase in
phosphatidylinositol labelling.

Discussion

As a result of the studies reported in the present
paper and by others (De Torrentegui & Berthet, 1966; Kirk et al., 1977, 1978), it is clear that phos-
phatidylinositol metabolism in rat hepatocytes can
be stimulated by activation of at least three types of
receptor, namely those sensitive to α-adrenergic
stimuli, to vasopressin and to angiotensin, but that
it is not affected by activation of glucagon receptors
(Kirk et al., 1977). The three effective types of
receptor all share an ability to activate glycogenolysis
by a mechanism that does not involve cyclic AMP
and in which an increase in cytosol Ca^{2+} concentration
appears to be a key intracellular effector of the
hormone effects (Stubbs et al., 1976; Keppens et al.,
1977; Assimacopoulos-Jeannet et al., 1977; Van de
Thus these results fulfilled our expectation, based on
the idea that phosphatidylinositol breakdown might
be a reaction essential to all situations involving
receptor-controlled Ca^{2+} mobilization, that these
three receptors in the hepatocyte should all cause
activation of phosphatidylinositol metabolism (see
the introduction).

In addition, the unusual ligand selectivity of the
vasopressin receptors responsible for initiating
phosphatidylinositol breakdown (Kirk et al., 1979;
the present study) suggests that these receptors
differ markedly from the vasopressin receptors in
kidney, which control adenylate cyclase and thus
inhibit diuresis. In some other situations in which
small ligands (e.g. adrenaline, histamine) exert
control over their target cells in these two distinct
ways it has become clear this is achieved by the exis-
tence of two independent families of receptors, with
one controlling adenylate cyclase (e.g. β-adrenergic
and H_{2}-histaminergic receptors) and the other
controlling Ca^{2+} mobilization (e.g. α-adrenergic and
H_{1}-histaminergic receptors) (see Michell, 1979).
The simplest interpretation of the present observa-
tions is to suggest that vasopressin, like catechol-
amines and histamine (see Michell, 1979), can
interact with two different classes of cellular receptors,
one of them coupled to adenylate cyclase and the
other to a Ca^{2+}-mobilizing mechanism. Although
there is already pharmacological evidence that
demonstrates that cells can possess more than one
type of receptor responsive to the same peptide
ligand (e.g. for opioid peptides; Lord et al., 1977),
vasopressin appears to be the first peptide ligand for
which one can also identify the type of cellular control
that is achieved by each class of receptor.

In other systems that have been studied, the
accumulated evidence supports the idea that hormone-stimulated phosphatidylinositol meta-
bolism is always initiated through phosphatidylino-
sitol breakdown (Michell et al., 1977a; Jones et al.,
1979), and the experiments reported in the present
paper indicate that this reaction also initiates the
increase in phosphatidylinositol metabolism that
occurs in hepatocytes exposed to any of the Ca^{2+}-
mobilizing glycogenolytic hormones.

There seems little doubt that removal of Ca^{2+}
from the medium surrounding hepatocytes for any
appreciable time can prevent most or all glycogenoly-
tic responses to vasopressin and angiotensin, and
probably also to adrenaline (e.g. Stubbs et al., 1976;
Keppens et al., 1977), even though a response can
still be evoked by these hormones in cells in which
Ca^{2+} withdrawal occurs only just before addition of
any of these hormones (Blackmore et al., 1978). In
addition, the glycogenolytic effects of these hormones
can be mimicked by using ionophore A23187, a
bivalent-cation ionophore, to admit Ca^{2+} to the cells
(Keppens et al., 1977; Assimacopoulos-Jeannet et al.,
1977) or to mobilize Ca^{2+} from intracellular
stores (Pointer et al., 1976). This suggests that an
increase in the cytosol concentration of free Ca^{2+} is
alone sufficient to activate glycogenolysis, probably
through direct activation of phosphorylase kinase by
Ca^{2+} (Vandenheede et al., 1977).

However, the evidence accumulated in the present
studies and by Kirk et al. (1978) points clearly to the
opposite conclusion for hormone-stimulated break-
down of phosphatidylinositol and for hormone-
stimulated phosphatidylinositol labelling; these
events seem not to be markedly influenced by changes
in cytosol Ca^{2+} concentration. Perhaps the most
convincing of the items amongst this evidence is
the inability of the ionophore A23187 to have any ap-
preciable affect on phosphatidylinositol metabolism:
it did not produce phosphatidylinositol breakdown
or a substantial stimulation of labelling, nor did it
change the responses of these two parameters to
activation by hormones. This type of situation, in

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which stimulated phosphatidylinositol metabolism is found to be a Ca\(^{2+}\)-insensitive response to receptors whose physiological effects are mediated through Ca\(^{2+}\), is now familiar (Trifaro, 1969; Jones & Michell, 1975, 1976, 1978b; Oron et al., 1975; Abdel-Latif, 1976; Bicknell et al., 1979; Fain & Berridge, 1978, 1979; Berridge & Fain, 1979; Jones et al., 1979) and it was this type of observation that originally provoked the suggestion that phosphatidylinositol breakdown might be a reaction essential to stimulus–response coupling at such receptors (Michell, 1975, 1979; Michell et al., 1977a,b; Jones & Michell, 1978b).

Although the simplest interpretation of the previous information on the control of glycogenolysis by Ca\(^{2+}\)-mobilizing hormones was to suggest that the main change brought about by activation of appropriate hormone receptors is an increase in cell-surface Ca\(^{2+}\) permeability (e.g. Keppens et al., 1977), there is more recent information that may point to a more complex situation. In particular, Chen et al. (1978) and Blackmore et al. (1978) have both found that there is a rapid net efflux of Ca\(^{2+}\) from cells activated by the Ca\(^{2+}\)-mobilizing hormones, suggesting initiation by these hormones of the mobilization of Ca\(^{2+}\) ions from some intracellular pool, maybe in mitochondria. This would of course require the existence of some intermediary signal to carry the hormone-activation message to the site within the cell from which Ca\(^{2+}\) was to be mobilized.

Our original suggestion relating to the function to receptor-stimulated phosphatidylinositol breakdown was that it produced inositol cyclic phosphate (Michell & Lapetina, 1972; Lapetina & Michell, 1973), and it is conceivable that this could play a messenger role in a pathway leading to intracellular Ca\(^{2+}\) mobilization. However, no clear evidence has yet been forthcoming that this phosphate ester has an essential role as a messenger at any of the receptors that control phosphatidylinositol breakdown (Michell, 1975; Freinkel & Dawson, 1973; Lapetina & Zieher, 1976), and this has led us more recently to emphasize possible ways in which phosphatidylinositol breakdown might control the permeability of the plasma membrane more directly (Michell et al., 1977a). There is sufficient uncertainty about Ca\(^{2+}\) mobilization in the hepatocyte for either type of role for phosphatidylinositol breakdown to be considered seriously. If phosphatidylinositol breakdown is the receptor-coupled initiating event in Ca\(^{2+}\) mobilization (in either manner) then it must occur at the plasma membrane; the cellular site at which this reaction occurs is therefore the next essential point that must be established.

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