Stimulation of Phosphatidylinositol Turnover in various Tissues by Cholinergic and Adrenergic Agonists, by Histamine and by Caerulein

By Lynne M. JONES, Shamshad COCKCROFT,*† and Robert H. MICHELL
*Department of Biochemistry, University of Birmingham, P.O. Box 363,
Birmingham B15 2TT, U.K.*

(Received 8 February 1979)

Studies are reported of the biochemical and pharmacological characteristics of the stimulation of phosphatidylinositol metabolism that is produced in appropriate target tissues by stimulation of various receptors that use Ca^{2+} as their second messenger. (1) Muscarinic cholinergic and α -adrenergic phosphatidylinositol responses were observed in rat lacrimal gland, and a response to caerulein was detected in the longitudinal smooth muscle of guinea-pig ileum. (2) The muscarinic cholinergic phosphatidylinositol response of rat lacrimal gland, like that of several other tissues, is not dependent on the availability of extracellular Ca^{2+} . (3) Three phosphatidylinositol responses, namely to histamine in guinea-pig ileum smooth muscle, to α -adrenergic stimulation in rat vas deferens and to muscarinic cholinergic stimulation in rat lacrimal gland, were all found to involve phosphatidylinositol breakdown. (4) The stereospecificity of the muscarinic receptor responsible for the phosphatidylinositol response of guinea-pig pancreas was tested by using the two stereoisomeric forms of acetyl- β -methylcholine; the *S*-isomer was very much more active than the *R*-isomer in provoking both phosphatidylinositol breakdown and its labelling with ^{32}P , as it is in provoking other physiological responses such as contractility or secretion. (5) Pilocarpine, a muscarinic partial agonist, provoked a significantly smaller phosphatidylinositol breakdown in rat parotid fragments than did carbamoylcholine, a potent muscarinic agonist. (6) All of these results are consistent with, but do not prove, a previously offered hypothesis that suggests that phosphatidylinositol breakdown is a reaction essential to stimulus-response coupling at a variety of cell-surface receptors that mobilize Ca^{2+} from and through the plasma membranes of target tissues.

There are a variety of cell-surface receptors that, when activated, trigger a rise in the cytosol Ca^{2+} concentration and an increase in phosphatidylinositol metabolism in target tissues. The initial reaction in stimulated phosphatidylinositol metabolism appears to be phosphatidylinositol breakdown. It has been suggested that this stimulated phosphatidylinositol breakdown might have an essential role in the mobilization of Ca^{2+} from and through the plasma membrane (Michell, 1975, 1979*a,b*; Michell *et al.*, 1976*a,b*, 1977*a,b*; Jafferji & Michell, 1977; Jones & Michell, 1978*a,b*; Billah & Michell, 1978, 1979). The formulation of this hypothesis was an attempt to explain several types of observation. First, phosphatidylinositol metabolism appears to be stimulated by all receptors that utilize Ca^{2+} as their second messenger. Secondly, stimulated phosphatidylinositol metabolism may be a response that occurs in all tissues bearing appropriate receptors; this is best docu-

mented for the muscarinic cholinergic receptor. Thirdly, the pharmacological characteristics of receptors responsible for stimulating phosphatidylinositol metabolism and for controlling other cellular responses appear very similar, if not identical. Fourthly, stimulation of phosphatidylinositol metabolism does not appear to be caused by an increase in the intracellular Ca^{2+} concentration, despite the fact that Ca^{2+} appears to be the second messenger used by these receptors for controlling other cellular responses. Fifthly, the degree of stimulation of phosphatidylinositol metabolism appears, at least for the muscarinic cholinergic receptor, to be proportional to the fraction of the total receptor population occupied by agonist molecules, whereas many physiological responses are fully stimulated by occupation of fewer receptors.

Control of phosphatidylinositol breakdown by receptors has not so far been reproducibly achieved in broken-cell systems, and therefore it is not yet possible to investigate the molecular mechanism of the proposed functional link between phosphatidylinositol breakdown and Ca^{2+} mobilization. How-

* Née Jafferji.

† Present address: Department of Experimental Pathology, University College Hospital Medical School, University Street, London WC1E 6JJ, U.K.

ever, the hypothesis that proposes that these two events are coupled in some essential manner can be either strengthened or disproved by studies that test the general applicability of any of the conclusions listed above. Several studies that have been reported since the original formulation of the hypothesis (Michell, 1975) have served to extend the correlation between stimulation of phosphatidylinositol metabolism and Ca^{2+} mobilization (Jafferji & Michell, 1976*a,b,c*, 1977; Lo *et al.*, 1976; Abdel-Latif, 1976; Lapetina *et al.*, 1976; Kirk *et al.*, 1977, 1978, 1979; Billah & Michell, 1978, 1979; Fain & Berridge, 1978, 1979*a,b*; Jones & Michell, 1978*b*; Pickard & Hawthorne, 1978; Berridge & Fain, 1979; Young *et al.*, 1979; Bicknell *et al.*, 1978; Cockcroft & Gomperts, 1979). In only some of these studies has any emphasis been put on the detailed pharmacological characteristics of the receptors being studied, of the possible occurrence of phosphatidylinositol breakdown and of its Ca^{2+} -independence. In the preceding paper (Billah & Michell, 1979) we reported a detailed study of the phosphatidylinositol response that is evoked in rat hepatocytes by Ca^{2+} -mobilizing glycogenolytic stimuli, and in the present paper we report some further investigations of these points in various tissues stimulated with cholinergic and adrenergic agonists, with histamine and with caerulein.

Materials and Methods

Materials were mainly from the same sources as used previously (Michell & Jones, 1974; Jones & Michell, 1974, 1975; Jafferji & Michell, 1976*a,b*). The synthetic *R*- and *S*-isomers of acetyl- β -methylcholine were kindly given by Dr. D. J. Triggles, Department of Biochemical Pharmacology, University of Buffalo, NY, U.S.A., and the racemic form of this compound was from Sigma (London) Chemical Co. Ltd., Kingston upon Thames, Surrey KT2 7BH, U.K., as was caerulein. Pancreases and the longitudinal smooth muscle from ileum were obtained from 400 g guinea pigs of either sex. Lacrimal glands and parotid glands were from male Wistar rats of about 250 g. Vasa deferentia came from male rats of about 350–400 g.

Methods for the preparation and incubation of tissue fragments, and for the extraction and analysis of their lipids, were mainly identical to those used previously (Michell & Jones, 1974; Jones & Michell, 1974, 1975; Jafferji & Michell, 1976*a,b,c*); precise details of the periods of incubation, quantities of ^{32}P , concentrations of drugs etc., are given in legends to the Figure and Tables. Vasa deferentia were cut into slices crosswise. Briefly, tissue fragments were incubated with ^{32}P -labelled medium for 30–45 min so as to label lipid precursors, then they were stimulated for 10–20 min; the degree of labelling of their

lipids, and sometimes the phosphatidylinositol concentration, were then determined. In view of the approximately constant rate of ^{32}P incorporation in control incubations throughout the entire incubation period, it should be noted that a doubling of the specific radioactivity of phosphatidylinositol on stimulation would reflect a 3–5-fold increase in the rate of ^{32}P incorporation during the period of stimulation.

Results and Discussion

Responses of the rat lacrimal gland to cholinergic and adrenergic stimuli

One of the simplest approaches to testing the idea that phosphatidylinositol breakdown and Ca^{2+} mobilization are functionally coupled in some way is to search in new tissues for the responses that must occur if this hypothesis is correct. In recent years, it has become clear that the physiology of the lacrimal gland is in many ways similar to that of salivary glands. For example, the rat exorbital lacrimal gland exhibits protein secretion and a K^+ efflux when stimulated by muscarinic cholinergic or α -adrenergic agonists in the presence of extracellular Ca^{2+} (Herzog *et al.*, 1978; Keryer & Rossignol, 1976; Putney *et al.*, 1977; Herman *et al.*, 1978). These responses are accompanied by Ca^{2+} influx into the gland (Keryer & Rossignol, 1976), and the electrophysiological changes evoked by muscarinic cholinergic stimulation are essentially the same as those seen in the parotid gland (Iwatsuki & Petersen, 1978). In addition, there has been a preliminary indication that phospholipid metabolism in the lacrimal gland can be stimulated cholinergically (Chambaut-Guerin *et al.*, 1978).

The information in Tables 1 and 2 shows clearly that both cholinergic and adrenergic stimuli produced a marked activation of phosphatidylinositol metabolism in this tissue. The cholinergic response involved both breakdown and labelling of phosphatidylinositol, was effectively provoked by acetyl- β -methylcholine, which is primarily a muscarinic agonist, and was inhibited by atropine, a muscarinic antagonist. In addition, there was no change in the effectiveness of stimulation by acetyl- β -methylcholine when Ca^{2+} was removed from the extracellular medium, despite the fact that this abolishes protein secretion and decreases the cholinergically stimulated K^+ efflux (Putney *et al.*, 1977; Herman *et al.*, 1978).

As in other tissues (see Jones & Michell, 1978*a*), the adrenergic phosphatidylinositol response of the lacrimal gland appears to be mediated by α -adrenergic receptors, since it was inhibited by 2-bromo-*NN*-dimethyl-2-phenylethylamine and not by pindolol (Table 2, and see Jones & Michell, 1978*a*, for references to antagonists).

Table 1. *Muscarinic cholinergic stimulation of phosphatidylinositol metabolism in rat lacrimal gland*

Lacrimal gland fragments were incubated in labelled medium (20 μ Ci of [32 P]P_i/ml) containing 1 mM-Ca²⁺ for 45 min. They were then collected, washed with appropriate unlabelled medium (with or without Ca²⁺), and then incubated in fresh labelled medium (with 1 mM-Ca²⁺ and 0.2 mM-EGTA or with 0.2 mM-EGTA) for a further 15 min during which agonists and/or antagonists were present. Results are means $\pm$ S.E.M. with the number of determinations shown in parentheses, and are combined from two or three experiments.

	³² P incorporation into phosphatidylinositol (d.p.m./nmol)		Ratio of phospholipid content (phosphatidylinositol/other phospholipids)	
	With Ca ²⁺	Ca ²⁺ -free	With Ca ²⁺	Ca ²⁺ -free
No additions	23 $\pm$ 1 (7)	22 $\pm$ 1 (12)	0.102 $\pm$ 0.002 (12)	0.103 $\pm$ 0.002 (16)
+1 mM-Acetyl- β -methylcholine	86 $\pm$ 3 (7)*	96 $\pm$ 8 (11)*	0.086 $\pm$ 0.003 (12)*	0.092 $\pm$ 0.002 (17)†
+1 mM-Acetyl- β -methylcholine and 100 μ M-atropine	31 $\pm$ 1 (7)†‡		0.101 $\pm$ 0.002 (11)‡	

* Significantly different from incubations with no additions ($P < 0.001$).

† Significantly different from incubations with no additions ($P < 0.01$).

‡ Significantly different from incubations with acetyl- β -methylcholine ($P < 0.001$).

Table 2. *Adrenaline-stimulated phosphatidylinositol turnover in rat lacrimal gland*

Lacrimal gland fragments were incubated for 60 min in medium containing 20 μ Ci of [32 P]P_i/ml. Tissue fragments were then transferred to unlabelled medium containing antagonists for 25 min and adrenaline was present for the final 15 min. Results are means $\pm$ S.E.M. with the number of determinations in parentheses, expressed as percentages of the appropriate control value in each of two or three identical experiments. Abbreviation: DMPEA, 2-bromo-*NN*-dimethyl-2-phenylethylamine.

	Specific radioactivity of phosphatidylinositol (% of control)
No additions	100 $\pm$ 11 (10)
+ 100 μ M-Adrenaline	192 $\pm$ 22 (12)*
+ 10 μ M-DMPEA	123 $\pm$ 18 (10)
+ 100 μ M-Adrenaline and 10 μ M-DMPEA	139 $\pm$ 11 (12)†
+ 10 μ M-Pindolol	120 $\pm$ 16 (8)
+ 100 μ M-Adrenaline and 10 μ M-pindolol	194 $\pm$ 19 (13)*

* Significantly different from the control value ($P < 0.01$).

† Significantly different from incubations in the presence of adrenaline alone ($P < 0.05$).

Table 3. *Stimulation of phosphatidylinositol metabolism by caerulein in fragments of ileum smooth muscle*

Muscle fragments were incubated for 30 min in the presence of [3 H]inositol (1 μ Ci/ml), then a further 30 min in the presence of [3 H]inositol and drugs. The values given are means $\pm$ S.E.M. for the number of replicate assays shown in parentheses, and they come from one of four similar experiments.

	[3 H]Inositol incorporation (d.p.m./nmol of tissue phospholipid)
Control	88 $\pm$ 13 (5)
+ 50 ng of caerulein/ml	160 $\pm$ 21 (8)*
+ 100 μ M-Carbamoylcholine	174 $\pm$ 4 (5)*

* Significantly different from control incubations ($P < 0.001$).

(Jafferji & Michell, 1976*a,b,c*). This muscle also shows Ca²⁺-dependent contraction in response to pancreozymin and, with even greater sensitivity, to related peptides such as caerulein (Erspamer *et al.*, 1967). It is well known that these peptide ligands stimulate phosphatidylinositol metabolism in pancreas (e.g. Hokin-Neaverson, 1974, 1977; Baudhuin *et al.*, 1971; Baudhuin & Cantraine, 1972), and it is shown in Table 3 that caerulein is also an effective stimulant to phosphatidylinositol metabolism in fragments of the longitudinal smooth muscle from guinea-pig ileum. Unlike most of our experiments, this series used [3 H]inositol as the labelled precursor with which to follow effects on phosphatidylinositol metabolism, so the effect of carbamoylcholine is also presented for comparison; the stimulations produced by both ligands were similar.

Response of ileum smooth muscle to caerulein

It has already been shown that the longitudinal smooth muscle of guinea pig ileum exhibits enhanced phosphatidylinositol metabolism in response to at least four Ca²⁺-mobilizing stimuli that elicit contraction, namely muscarinic cholinergic, H₁-histaminergic, 5-hydroxytryptamine and depolarization by elevation of the extracellular K⁺ concentration

Phosphatidylinositol breakdown as the initiating reaction in phosphatidylinositol metabolism stimulated by histamine or noradrenaline

There have been reports that histamine (acting through H_1 -receptors) and noradrenaline (acting through α -receptors) stimulate incorporation of ^{32}P into phosphatidylinositol in the smooth muscles of respectively the guinea-pig ileum (Jafferji & Michell, 1976b) and rat vas deferens (Canessa de Scarnatti & Lapetina, 1974). Studies of other tissues have suggested that the reaction that initiates such an increase in phosphatidylinositol turnover is normally phosphatidylinositol breakdown. The same also appears to be the case in these two situations, since in both cases there was a significant decrease in the tissue phosphatidylinositol content on stimulation (Table 4). It has been shown previously that the α -adrenergic responses of parotid fragments (Jones & Michell, 1975) and iris smooth muscle (Abdel-Latif, 1976) involve phosphatidylinositol breakdown, but this does not appear to have been demonstrated in the previous studies of phosphatidylinositol metabolism stimulated by H_1 -histaminergic receptors.

Pharmacological characteristics of the muscarinic cholinergic responses

The tentative conclusion that the cholinergic receptor population responsible for triggering the phosphatidylinositol response is likely to be the same muscarinic receptor population as is responsible for evoking other cellular responses has arisen through the observation of superficial similarities between the pharmacological characteristics of the

cholinergic receptors that control all of the studied phosphatidylinositol responses. They are responsive to relatively specific muscarinic agonists such as acetyl- β -methylcholine, carbamoyl- β -methylcholine and pilocarpine (Gerber *et al.*, 1973; Jones & Michell, 1974; Lapetina *et al.*, 1976; Jafferji & Michell, 1976a, Pickard *et al.*, 1977), and much less sensitive to nicotinic agonists (Jones & Michell, 1975; Pickard *et al.*, 1977). In addition, all are inhibited by muscarinic antagonists, e.g. atropine or propylbenzilylcholine mustard, but not by nicotinic antagonists, e.g. tubocurarine, mecamylamine or surugatoxin (Hokin, 1968; Jones & Michell, 1975; Lapetina *et al.*, 1976; Jafferji & Michell, 1976a; Hokin-Neaverson, 1977; Pickard *et al.*, 1977; Young *et al.*, 1979). However, the only detailed study of the pharmacological potency of any of these ligands in modifying phosphatidylinositol metabolism has been that of Miller (1977), who showed that the apparent affinity constant of atropine for the muscarinic receptors that control phosphatidylinositol metabolism in synaptosomes is very similar to the affinity constant calculated from atropine inhibition of other muscarinic responses.

One of the most marked features of the ligand selectivity of muscarinic receptors responsible for physiological responses such as smooth-muscle contraction is a high degree of discrimination between stereoisomeric agonists. We have therefore tested the ability of the *R*-, *S*- and racemic forms of acetyl- β -methylcholine to evoke an increase in phosphatidylinositol labelling in guinea-pig pancreas fragments; this tissue was chosen because it exhibits a very high degree of stimulated ^{32}P incorporation. It is realised that labelling is a secondary response that may not directly mirror the extent of phosphatidylinositol breakdown, but it does not seem unreasonable to expect that in different samples of tissue fragments a particular amount of submaximal stimulation of phosphatidylinositol labelling (e.g. 50% of the maximum observed increase) might always reflect approximately the same degree of stimulation of phosphatidylinositol breakdown.

The results of this experiment (Fig. 1) show that the stereoselectivity of the receptors that elicit the phosphatidylinositol response is qualitatively similar to the stereoselectivity of the receptors that elicit other responses, with the *S*-isomer much more potent than the *R*-isomer and racemic acetyl- β -methylcholine intermediate in potency. There is even some indication in our data that the apparent selectivity of the receptor responsible for the phosphatidylinositol response (about 10000-fold in favour of the *S*-isomer) might be even greater than that of the receptors that trigger other responses (about 250–1000-fold in favour of the *S*-isomer in provoking contraction of various smooth muscles; Triggle, 1971; Chang & Triggle, 1973).

Table 4. Breakdown of phosphatidylinositol in (a) histamine-treated guinea-pig ileum smooth muscle and (b) adrenaline-treated rat vas deferens

In the experiments with ileum smooth muscle, incubations were for a total of 60 min, with histamine present during the final 30 min (Jafferji & Michell, 1976b). Vas-deferens fragments were incubated for a total of 75 min, with adrenaline present for the final 15 min. Values given are means $\pm$ S.E.M. for the number of determinations shown in parentheses, and are combined from two independent experiments.

	Ratio of phosphate content (phosphatidylinositol/other phospholipids)
(a) Guinea-pig ileum smooth muscle	
Control	0.075 $\pm$ 0.003 (8)
+ 100 μ M-Histamine	0.064 $\pm$ 0.003 (7)*
(b) Rat vas deferens	
Control	0.069 $\pm$ 0.002 (11)
+ 1 mM-Adrenaline	0.055 $\pm$ 0.002 (14)*

* Significantly different from appropriate control group ($P < 0.05$).

Fig 1. Stimulation of pancreatic phosphatidylinositol labelling with isomeric forms of acetyl- β -methylcholine

Pancreas fragments were incubated for 30 min in ^{32}P -labelled media, recovered by filtration, distributed into an appropriate number of individual flasks and incubated for a further 10 min in the presence of various concentrations of acetyl- β -methylcholine together with $10\text{ }\mu\text{M}$ -eserine. Incubations were in triplicate in each experiment, and the values plotted are means $\pm$ S.E.M. for four experiments with the racemic compound, or means with range for two experiments with *R*- and *S*-isomers. The phosphatidylinositol content of the tissue fragments was also measured and at all points indicated by asterisks there was a significant ($P < 0.01$) agonist-stimulated loss of phosphatidylinositol, as assessed by the ratio of phosphatidylinositol/other phospholipids (see Jones & Michell, 1974).

It is particularly interesting to compare these dose-response curves for the acetyl- β -methylcholine isomers with the dose-response curves for acetylcholine and carbamoylcholine (see Michell *et al.*, 1976a) and with the acetyl- β -methylcholine-binding curves given by Ward & Young (1977). When this is done, it is immediately apparent that the acetyl- β -methylcholine dose-response curves for the phosphatidylinositol response are qualitatively different from those of the other agonists in that they do not show the marked flattening that is also characteristic of the receptor-binding curves for potent muscarinic agonists. They therefore differ from the flattened binding curves for the two acetyl- β -methylcholine isomers given by Ward & Young (1977). Despite this difference, however, there is much closer correspondence between the concentrations of the individual acetyl- β -methylcholine isomers required to give half-maximal ligand binding and phosphatidylinositol turnover than between physiological responsiveness and phosphatidylinositol turnover; for the

S-isomer, half-maximal specific binding, phosphatidylinositol turnover and contraction occur respectively at around $10\text{ }\mu\text{M}$ (Ward & Young, 1977), $\leq 1\text{ }\mu\text{M}$ (Fig. 1) and $0.03\text{--}0.06\text{ }\mu\text{M}$ (Ward & Young, 1977; Chang & Triggle, 1973), whereas the equivalent values for the *R*-isomer are $\geq 1\text{ mM}$ (Ward & Young, 1977), around 10 mM (Fig. 1) and 0.06 mM (Chang & Triggle, 1973). In view of the probability that each flattened agonist-binding curve is the sum of the binding curves for two functionally interconvertible receptor subpopulations (Birdsall *et al.*, 1978) and of the continuing uncertainties attending the use of phosphatidylinositol labelling as a quantitative measure of the phosphatidylinositol response (see Michell, 1975; Michell *et al.*, 1976a), the available information does not allow a definite conclusion as to how precise is the suggested relationship between receptor occupancy and the evocation of the phosphatidylinositol response (Michell *et al.*, 1976a).

In previous studies it has been reported that phosphatidylinositol turnover can be stimulated both by potent muscarinic agonists such as acetylcholine or carbamoylcholine and by pilocarpine, which is a partial agonist (Gerber *et al.*, 1973; Jafferji & Michell, 1976a). In the studies by Jafferji & Michell (1976a) it was found that a high concentration of pilocarpine (1 mM , incorrectly quoted in the published paper as $1\text{ }\mu\text{M}$) produced an increase in the labelling of phosphatidylinositol in ileum smooth muscle that was similar to the maximum increase provoked by high concentrations of potent agonists such as acetylcholine. As subsequently pointed out by Birdsall & Hulme (1976), this result would be very difficult to reconcile with a role for phosphatidylinositol breakdown in stimulus-response coupling if it can be assumed that the maximal stimulation of phosphatidylinositol labelling produced by any two agonists can be interpreted to mean that they provoke phosphatidylinositol breakdown at the same rate. Since phosphatidylinositol breakdown is somewhat easier to assess in parotid fragments than in ileum smooth-muscle fragments, we have measured the extent of phosphatidylinositol breakdown and of phosphatidylinositol labelling in parotid fragments that were incubated with high and equal concentrations of pilocarpine and carbamoylcholine (Table 5); these two drugs have approximately equal affinities for muscarinic receptors, despite their different efficacies as agonists. Both provoked phosphatidylinositol labelling, but carbamoylcholine was significantly more effective. In addition, carbamoylcholine produced a highly significant breakdown of phosphatidylinositol, whereas pilocarpine had only a slight effect on the tissue phosphatidylinositol concentration, thus leaving the concentration of phosphatidylinositol in the pilocarpine-treated tissue significantly higher than in that treated with carbamoylcholine.

Conclusions

In general, the experiments reported in the present paper, in the preceding paper (Billah & Michell, 1979) and in several of the papers cited in the introduction have all given substantial support to the hypothesis that receptor-stimulated phosphatidylinositol breakdown is an essential reaction in stimulus-response coupling at muscarinic cholinergic, α -adrenergic and H_1 -histaminergic receptors and at other receptors that utilize Ca^{2+} as their second messenger. The studies of the lacrimal gland provide further evidence of the ubiquity of phosphatidyl-

inositol responses to the former two receptors. The pharmacological studies on the pancreas and parotid provide further evidence compatible with the view that cells probably possess a single population of muscarinic receptors that control both phosphatidylinositol breakdown and other cellular responses (e.g. protein secretion). If the same receptors were not responsible for controlling the two sets of events then the two postulated populations of receptors would have to show similar stereoselectivity and pilocarpine would have to be a partial agonist at both types of site; this would obviously be a less parsimonious, and therefore less probable, explanation of the available

Table 5. *Stimulation of phosphatidylinositol metabolism by pilocarpine and by carbamoylcholine in rat parotid fragments*
Tissue fragments were incubated for 1 h in labelled medium ($5 \mu\text{Ci}$ of $[^{32}\text{P}]\text{P}_i/\text{ml}$) and were then stimulated during a further 15 min incubation (see the Materials and Methods section). Results are means $\pm$ S.E.M. for the number of observations shown in parentheses.

	^{32}P incorporated into phosphatidylinositol (c.p.m./nmol)	Ratio of phosphate content (phosphatidylinositol/ other phospholipids)
Control	104 ± 8 (8)	0.070 ± 0.003 (8)
$100 \mu\text{M}$ -Carbamoylcholine	308 ± 23 (8)*	0.053 ± 0.001 (8)*
$100 \mu\text{M}$ -Pilocarpine	160 ± 11 (8)†§	0.063 ± 0.002 (8)‡

* Value significantly different from corresponding control value at $P < 0.001$.

† Value significantly different from corresponding control value at $P < 0.01$.

‡ Significance of difference from corresponding control value $0.1 > P > 0.05$.

§ Value significantly different from corresponding carbamoylcholine-treatment value at $P < 0.001$.

|| Value significantly different from corresponding carbamoylcholine-treatment value at $P < 0.01$.

Table 6. *Situations in which there is evidence that phosphatidylinositol breakdown is involved in the initiation of receptor-stimulated phosphatidylinositol metabolism*

Receptor	Tissue	References
Muscarinic cholinergic	Avian salt gland	Hokin & Hokin (1964)
	Pancreas	Hokin-Neaverson (1977)
	Cerebral cortex	Lunt & Pickard (1975)
	Parotid gland	Jones & Michell (1974, 1975, 1976)
	Iris smooth muscle	Abdel-Latif (1976)
	Ileum smooth muscle	Jafferji & Michell (1976a)
α -Adrenergic	Lacrimal gland	The present paper
	Parotid gland	Jones & Michell (1975)
	Iris smooth muscle	Abdel-Latif (1976)
	Vas-deferens smooth muscle	The present paper
	Hepatocytes	Billah & Michell (1979)
H_1 -Histaminergic	Ileum smooth muscle	The present paper
Pancreozymin	Pancreas	Hokin-Neaverson (1977)
Substance P	Parotid gland	Jones & Michell (1978b)
Vasopressin	Hepatocytes	Billah & Michell (1978, 1979)
Angiotensin	Hepatocytes	Billah & Michell (1979)
5-Hydroxytryptamine	Blowfly salivary gland	Fain & Berridge (1978, 1979a,b); Berridge & Fain (1979)
Conditions favouring cell proliferation	Fibroblasts, lymphocytes	Ristow <i>et al.</i> (1973); Diringer & Koch (1973); Koch & Diringer (1973); Diringer & Friis (1977)
Glucose or mannose	Islets of Langerhans	Freinkel <i>et al.</i> (1975); Clements & Rhoten (1976)
Electrical depolarization	Synaptosomes	Pickard & Hawthorne (1978)

information. Support for the conclusion that phosphatidylinositol responses are initiated through phosphatidylinositol breakdown has come both from the experiments with lacrimal glands and from the studies of the ileum and vas deferens smooth muscles. Table 6 provides an up-to-date summary of the numerous situations that have provided evidence that phosphatidylinositol breakdown is involved in receptor-stimulated phosphatidylinositol metabolism. Finally, the idea that receptor-stimulated phosphatidylinositol breakdown is not a consequence of a rise in cytosol $[Ca^{2+}]$ has received further support from the studies with the lacrimal gland.

We are grateful to the Medical Research Council and the University of Birmingham for financial support.

References

- Abdel-Latif, A. A. (1976) in *Function and Metabolism of Lipids in the CNS and PNS* (Porcellati, G., Amaducci, L. & Galli, G., eds.), pp. 227–256, Plenum Press, New York
- Baudhuin, H. & Cantraine, F. (1972) *Biochim. Biophys. Acta* **270**, 248–253
- Baudhuin, H., Rochus, L., Vincent, D. & Dumont, J. E. (1971) *Biochim. Biophys. Acta* **252**, 171–183
- Berridge, M. J. & Fain, J. (1979) *Biochem. J.* **178**, 59–69
- Bicknell, R. J., Young, P. W. & Schofield, J. G. (1979) *Mol. Cell. Endocrinol.* **13**, 167–180
- Billah, M. M. & Michell, R. H. (1978) *Biochem. Soc. Trans.* **6**, 1033–1035
- Billah, M. M. & Michell, R. H. (1979) *Biochem. J.* **182**, 661–668
- Birdsall, N. J. M. & Hulme, E. C. (1976) *J. Neurochem.* **27**, 7–16
- Birdsall, N. J. M., Burgen, A. S. V. & Hulme, E. C. (1978) in *Cholinergic Mechanisms and Psychopharmacology* (Jenden, D. J., ed.), pp. 25–33, Plenum Press, New York
- Canessa de Scarnatti, O. & Lapetina, G. (1974) *Biochim. Biophys. Acta* **360**, 298–305
- Chambaut-Guerin, A.-M., Muller, P. & Rossignol, B. (1978) *J. Biol. Chem.* **253**, 3870–3876
- Chang, K. J. & Triggle, D. J. (1973) *J. Med. Chem.* **16**, 718
- Clements, R. S. & Rhoten, W. B. (1976) *J. Clin. Invest.* **57**, 684–691
- Cockcroft, S. & Gomperts, B. D. (1979) *Biochem. J.* **178**, 681–687
- Diringer, H. & Friis, I. (1977) *Cancer Res.* **37**, 2979–2984
- Diringer, H. & Koch, M. A. (1973) *Biochem. Biophys. Res. Commun.* **51**, 967–971
- Erspermer, V., Bertaccini, G., De Caro, G., Endean, R. & Impicciatore, M. (1967) *Experientia* **23**, 702–703
- Fain, J. & Berridge, M. J. (1978) *Biochem. Soc. Trans.* **6**, 1038–1039
- Fain, J. & Berridge, M. J. (1979a) *Biochem. J.* **178**, 45–58
- Fain, J. N. & Berridge, M. J. (1979b) *Biochem. J.* **182**, 655–661
- Freinkel, N., El-Younsi, C. & Dawson, R. M. C. (1975) *Eur. J. Biochem.* **59**, 245–252
- Gerber, D., Davies, M. & Hokin, L. E. (1973) *J. Cell Biol.* **56**, 736–745
- Herman, G., Busson, S., Ovracht, L., Maurs, C. & Rossignol, B. (1978) *Biol. Cell.* **31**, 255–264
- Herzog, V., Sies, H. & Miller, F. (1976) *J. Cell Biol.* **70**, 692–706
- Hokin, L. E. (1968) *Int. Rev. Cytol.* **23**, 187–208
- Hokin, M. R. & Hokin, L. E. (1964) in *Metabolism and Physiological Function of Lipids* (Dawson, R. M. C. & Rhodes, D. N., eds.), pp. 423–424, John Wiley, London
- Hokin-Neaverson, M. R. (1974) in *Secretory Mechanisms of Exocrine Glands* (Thorne, N. A. & Petersen, O. H., eds.), pp. 701–712, Munksgaard, Copenhagen
- Hokin-Neaverson, M. R. (1977) in *Function and Biosynthesis of Lipids* (Bazan, N. G., Brenner, R. R. & Giusto, N. M., eds.), pp. 429–446, Plenum Press, New York
- Iwatsuki, N. & Petersen, O. H. (1978) *J. Physiol. (London)* **275**, 507–520
- Jafferji, S. S. & Michell, R. H. (1976a) *Biochem. J.* **154**, 653–657
- Jafferji, S. S. & Michell, R. H. (1976b) *Biochem. Pharmacol.* **25**, 1429–1430
- Jafferji, S. S. & Michell, R. H. (1976c) *Biochem. J.* **160**, 397–399
- Jafferji, S. S. & Michell, R. H. (1977) *Biochem. Soc. Trans.* **505**, 104–106
- Jones, L. M. & Michell, R. H. (1974) *Biochem. J.* **142**, 583–590
- Jones, L. M. & Michell, R. H. (1975) *Biochem. J.* **148**, 479–485
- Jones, L. M. & Michell, R. H. (1976) *Biochem. J.* **158**, 505–507
- Jones, L. M. & Michell, R. H. (1978a) *Biochem. Soc. Trans.* **6**, 673–688
- Jones, L. M. & Michell, R. H. (1978b) *Biochem. Soc. Trans.* **6**, 1035–1037
- Keryer, G. & Rossignol, B. (1976) *Am. J. Physiol.* **230**, 99–104
- Kirk, C. J., Verrinder, T. R. & Hems, D. A. (1977) *FEBS Lett.* **83**, 267–271
- Kirk, C. J., Verrinder, T. R. & Hems, D. A. (1978) *Biochem. Soc. Trans.* **6**, 1031–1033
- Kirk, C. J., Rodriguez, L. & Hems, D. A. (1979) *Biochem. J.* **178**, 493–496
- Koch, M. A. & Diringer, H. (1973) *Biochem. Biophys. Res. Commun.* **55**, 305–311
- Lapetina, E. G., Brown, W. G. & Michell, R. H. (1976) *J. Neurochem.* **26**, 649–651
- Lo, H., Lehotay, D. C., Katz, D. & Levey, G. S. (1976) *Endocrine Res. Commun.* **3**, 377–385
- Lunt, G. G. & Pickard, M. R. (1975) *J. Neurochem.* **24**, 1203–1208
- Michell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–147
- Michell, R. H. (1979a) in *Companion to Biochemistry* (Bull, A. T., Lagnado, J. T., Tipton, K. & Thomas, J. O., eds.), vol. 2, pp. 205–228, Longmans, London
- Michell, R. H. (1979b) in *Cellular Receptors* (Shulster, D. & Levitzki, A., eds.), in the press, John Wiley, London
- Michell, R. H. & Jones, L. M. (1974) *Biochem. J.* **138**, 47–52
- Michell, R. H., Jafferji, S. S. & Jones, L. M. (1976a) *FEBS Lett.* **69**, 1–5
- Michell, R. H., Jones, L. M. & Jafferji, S. S. (1976b) in *Stimulus-Response Coupling in the Gastrointestinal Tract* (Case, R. M. & Goebell, H., eds.), pp. 89–105, MTP Press, Lancaster

- Michell, R. H., Jafferji, S. S. & Jones, L. M. (1977a) in *Function and Biosynthesis of Lipids* (Bazan, N. G., Brenner, R. R. & Giusto, N. M., eds.), pp. 447-464, Plenum Press, New York
- Michell, R. H., Jones, L. M. & Jafferji, S. S. (1977b) *Biochem. Soc. Trans.* **5**, 77-82
- Miller, J. C. (1977) *Biochem. J.* **168**, 549-555
- Pickard, M. R. & Hawthorne, J. N. (1978) *J. Neurochem.* **30**, 145-155
- Pickard, M. R., Hawthorne, J. N., Hayashi, E. & Yamada, S. (1977) *Biochem. Pharmacol.* **26**, 448-450
- Putney, J. W., Parod, R. J. & Marier, S. H. (1977) *Life Sci.* **20**, 1905-1912
- Ristow, H.-J., Franke, W. & Frohlich, M. (1973) *Z. Naturforsch. Teil B* **28**, 188-194
- Triggle, D. J. (1971) *Neurotransmitter-Receptor Interactions*, Academic Press, New York
- Ward, D. & Young, J. M. (1977) *Br. J. Pharmacol.* **61**, 189-197
- Young, P. W., Bicknell, R. J. & Schofield, J. G. (1979) *J. Endocrinol.* **80**, 203-213