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Evidence for a Role of Phosphatidylinositol Turnover in Stimulus–Secretion Coupling

STUDIES WITH RAT PERITONEAL MAST CELLS

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Histamine secretion and phosphatidylinositol turnover were compared in antigen-sensitized rat peritoneal mast cells stimulated with a number of different ligands. A small and variable increase in the incorporation of [^{32}P]P_i and of [^{3}H]inositol into phosphatidylinositol was observed when the cells were treated with immunoglobulin E-directed ligands (antigens and concanavalin A), and this was accompanied by a low amount of secretion (< 10% of total cell histamine). In the presence of added phosphatidylserine, the addition of immunoglobulin E-directed ligands invariably led to an enhanced rate (approx. 4-fold) of labelling of phosphatidylinositol and, in the presence of Ca^{2+}, this was accompanied by the secretion of histamine. The labelling of phosphatidylinositol and histamine secretion were also stimulated by chymotrypsin and compound 48/80. Whereas the phosphatidylinositol response did not require the presence of extracellular Ca^{2+}, the secretion of histamine was either enhanced or dependent on extracellular Ca^{2+} (depending on the ligand used). The dependence on ligand concentration for the phosphatidylinositol response and histamine secretion were similar. The increased isotopic incorporation into phosphatidylinositol continued for about 1 h although histamine secretion (elicited with concanavalin A) stopped within 2 min. These results support the proposition that metabolic events involving phosphatidylinositol play a necessary intermediate role in the regulation of Ca^{2+} channels by ligand-activated receptors.

The attachment of appropriate ligands to receptors on cell surfaces sets in train a sequence of events that ultimately results in the expression or inhibition of cellular activity. In many secretory and contractile tissues it is the entry of Ca^{2+} ions that triggers the final response (Douglas, 1968; Rubin, 1970; Hurwitz & Suria, 1971; Gomperts, 1976), but the actual mechanism whereby the binding of an agonist to a receptor is translated into an increase in membrane permeability to Ca^{2+} is not yet understood for any cell type. In seeking the determining features of the Ca^{2+}-flux event, one must look for structural or metabolic changes that fulfil some or all of the following criteria. (1) They should be dependent on relevant interaction between specific ligands and their receptors. (2) The induced changes should occur in the absence of external Ca^{2+}. (3) They should be a universal accompaniment of receptor-activated processes involving movement of Ca^{2+} into cells.

The only known response that largely fulfils these criteria is the breakdown of phosphatidylinositol. When ligands are applied to Ca^{2+}-mobilizing receptors on cells that have been preincubated in the presence of [^{32}P]P_i or of [^{3}H]inositol, this early metabolic event may be detected as a result of the increase in labelling of phosphatidylinositol due to its consequent resynthesis. A strong case for the involvement of phosphatidylinositol breakdown in the control of Ca^{2+} channels in a wide variety of different cell types has been made (Michell, 1975; Michell *et al.*, 1976*a*,*b*, 1977; Jafferji & Michell, 1976*a*–*c*).

The secretion of histamine from rat peritoneal mast cells can be triggered by specific antigen (Mota, 1957; Austen *et al.*, 1965), anti-immunoglobulin (Humphrey *et al.*, 1963) or concanavalin A (Keller, 1973). These all bind to, and cross-link, cell-surface IgE (Levine, 1965; Ishizaka & Ishizaka, 1968; Lawson *et al.*, 1975; Magro & Bennich, 1977). Secretion can also be triggered by a number of non-IgE-directed ligands, and in this category may be included chymotrypsin (Uvnas & Antonsson, 1963; Saeki, 1964; Sasaki, 1975; Lagunoff *et al.*, 1975) and the polycation compound 48/80 (Diamant & Uvnas, 1961; Bloom & Haegermark, 1965). Histamine secretion by these agents is a well-documented example of a secretory process in which the final stimulus to secretion certainly involves movements of Ca^{2+} and probably requires an increase in the chemical

Abbreviation used: IgE, immunoglobulin E.

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activity of Ca^{2+} in the cytosol (Douglas & Ueda, 1973; Gomperts, 1976). There are a variety of ligands that stimulate histamine secretion, and a large number of conditions and drugs that are known to inhibit histamine release by preventing Ca^{2+} fluxes. It has therefore been suggested that the mast cell would be a useful system in which to further our understanding of the relationship that appears to exist between phosphatidylinositol turnover and the regulation of Ca^{2+} channels (Gomperts, 1976; Michell *et al.*, 1977).

In the present paper we report on the existence and some characteristics of phosphatidylinositol turnover in mast cells on stimulation with a number of IgE-directed and non-IgE-directed ligands.

Materials and Methods

Materials

Larvae of *Nippostrongylus brasiliensis* were kindly provided by Dr. Bridget Ogilvie, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. Crude *N. brasiliensis* antigen and a partially purified allergen (Jarrett & Stewart, 1973) were gifts from Dr. Ellen E. E. Jarrett, Wellcome Research Laboratories for Experimental Parasitology, Veterinary Hospital, University of Glasgow, Glasgow, Scotland, U.K. Pertussis vaccine B.P. and compound 48/80 were obtained from Dr. L. G. Garland, Burroughs Wellcome, Beckenham, Kent, U.K. α-Chymotrypsin (type II) and ovalbumin (grade V) were obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Phosphatidylserine was purchased from Lipid Products, Nutfield, Surrey, U.K.

Methods

Sprague–Dawley rats (either sex) were mainly used, though on occasion we used rats of the Wistar and hooded Lister strains. The animals were immunized to ovalbumin by intramuscular injections (0.25ml into each hind leg) of a suspension of *Bordetella pertussis* (8×10^8 organisms/ml) in a solution of egg-white protein (50mg/ml). Alternatively, the animals were immunized to the antigens of the helminth parasite *Nippostrongylus brasiliensis* by subcutaneous injection of 4×10^3 larvae. After 15–30 days, a cell suspension containing 2–5% mast cells was obtained by peritoneal lavage with iso-osmotic saline (0.9% NaCl) containing heparin (25i.u./ml). The mast cells were purified to about 95% homogeneity by using the method of Cooper & Stanworth (1976) with minor modifications. We obtained about 10^6 cells/animal; four to six animals were used for each experiment.

The cells were suspended at $(1–2)\times10^6$ cells/ml in a phosphate-free buffered salt solution (pH7.5) containing 137mM-NaCl, 2.7mM-KCl, 1mM-$MgCl_2$, 1.8mM-$CaCl_2$, 10μM-EGTA, 20mM-4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid, 5.6mM-glucose, 20μM-phosphatidylserine and 1mg of bovine serum albumin/ml. In experiments where the effect of Ca^{2+} was studied, $CaCl_2$ was omitted at this stage. The purpose of adding phosphatidylserine to the buffered solution was to increase the degree of secretion on stimulation with IgE-directed ligands (Goth *et al.*, 1971).

The basic experimental design was to incubate the cells in the presence of either [^{3}H]inositol (about 50μCi/ml) or [^{32}P]P_i (about 20–30μCi/ml) for 30min to label intracellular precursor pools. Cells [80μl, i.e. $(1–2)\times10^5$ cells] were then transferred to tubes containing the various ligands and Ca^{2+} as indicated (final vol. 100μl). The incubations were continued for a further 15min or as indicated. The incubations were stopped by adding ice-cold 0.9% NaCl and the cells sedimented by centrifugation at 4°C (5min at 400*g*). The supernatants were removed for the determination of histamine and the cells were retained for the extraction of lipids. A separate sample of cells was heated at 100°C for 5min to release the total cell histamine. Histamine was measured fluorimetrically essentially by the method of Shore *et al.* (1959) with modifications as described by Fewtrell & Gomperts (1977).

The amount of histamine released into the extracellular fluid as a consequence of applying a stimulus is expressed as a percentage of the total cell histamine. Spontaneous (i.e. non-induced) release of histamine was also measured and the amount deducted from the stimulus-induced quantity. Spontaneous secretion was always <5% of total cell histamine.

The extraction of cell lipids and the separation of phosphatidylinositol by descending chromatography on formaldehyde-treated papers was as described by Michell & Jones (1974) and Jafferji & Michell (1976*a*). Lipid prepared from rat livers was added during the extraction to act as a carrier and to enable the localization of the phosphatidylinositol spot by the use of I_2 vapour. The area containing the phosphatidylinositol was cut from the paper and placed in either 10ml of scintillation fluid for the counting of ^{3}H, or into 10ml of water for counting ^{32}P (Cerenkov radiation). Samples were counted for radioactivity for 20min and at least 500 counts were accumulated above background values of 9 ± 2 and 6 ± 2 (mean±S.D.) c.p.m. for ^{3}H and ^{32}P respectively. It should be added that low counts were only obtained occasionally and that such data do not form the basis for substantive conclusions. In general it was possible to accumulate in the region of 2000 counts above background. The results are presented as average d.p.m. for duplicate cell samples, which were always within 10% of each other.

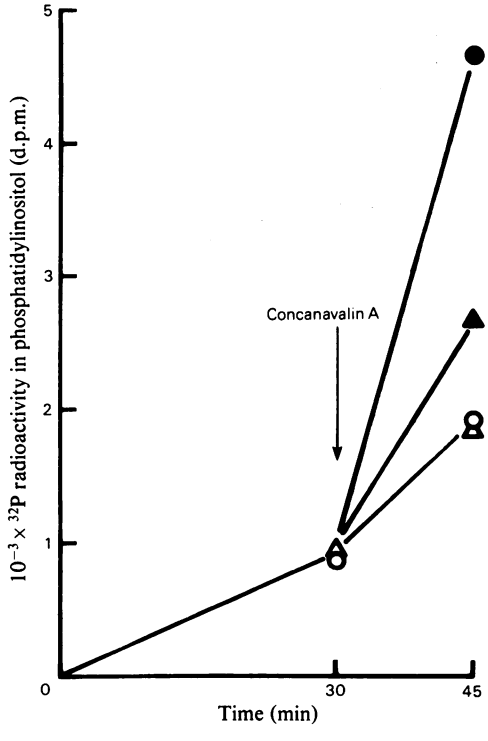

Fig. 1. *Stimulation of* [^{32}P]P_i *incorporation into mast-cell phosphatidylinositol by concanavalin A* (10μg/ml) *and its potentiation by phosphatidylserine* (20μM) *in the presence* (● *and* ○) *and absence* (▲ *and* △) *of phosphatidylserine*
Open symbols indicate controls; filled symbols indicate the presence of concanavalin A. Histamine release was 9% without phosphatidylserine and 52% with phosphatidylserine. Mast cells from hooded Lister rats were used for this experiment. The results presented are means for duplicate incubations and are from one of three similar experiments.

Results

Stimulation of phosphatidylinositol labelling and histamine secretion by IgE-directed ligands, and the effect of phosphatidylserine

We have used phosphatidylserine to enhance the degree of histamine secretion due to IgE-directed ligands. In its absence, histamine secretion occurs to a much lower extent (Goth *et al.*, 1971) and in our hands was often zero: in its presence we were invariably able to obtain 30–40% histamine secretion. It was therefore important at the outset to investigate possible effects of phosphatidylserine on phosphatidylinositol metabolism in resting and stimulated cells.

Fig. 1 shows the rate of incorporation of [^{32}P]P_i into mast-cell phosphatidylinositol in the presence and absence of phosphatidylserine, and the increased rate due to adding concanavalin A (10μg/ml) after 30min. Whereas phosphatidylserine does not appear to affect the basal rate of [^{32}P]P_i incorporation, a pronounced increase in the rate of labelling occurs on adding the ligand, and this is potentiated by phosphatidylserine. Though the concanavalin A-induced increase is greater in the presence of phosphatidylserine (4.2-fold greater than the control, compared with 1.9-fold greater than the control in the absence of phosphatidylserine), the most obvious effect of phosphatidylserine is on the extent of histamine release, which increased in this experiment from 9 to 52%. All other experiments reported were carried out with phosphatidylserine present in the incubation buffer solutions.

The results in Table 1 record the histamine secretion and the increment in phosphatidylinositol labelling with [^{3}H]inositol when the mast cells were stimulated with antigen (ovalbumin). The increment in labelling is expressed as the ratio of counts (stimulated/control values) measured over the entire period of incubation (0–45min) and also over the period of

Table 1. *Antigen-stimulated histamine secretion and labelling of phosphatidylinositol with* [^3H]*inositol*
Mast cells were preincubated with [^{3}H]inositol for 30min. At this time some incubations were stopped, and the remaining cells were further incubated for 15min in the presence or absence of the antigen (ovalbumin). Results are means for duplicate incubations (± range) and are from one of four similar experiments.

		Increase in phosphatidylinositol labelling during entire period of incubation (0–45min)		Increase in phosphatidylinositol labelling during period of stimulation (30–45min)	
	Histamine secretion (%)	^{3}H radioactivity (d.p.m.)	Ratio of stimulated/ control values	^{3}H radioactivity (d.p.m.)	Ratio of stimulated/ control values
Preincubation	0	57±3			
Control	0	102±3	1.0	45	1.0
Antigen	40	235±12*	2.3	178	3.9

* Significantly different from equivalent incubation without antigen ($P<0.01$).

stimulation (30–45 min). From Table 1, it can be seen that the stimulated rate of increase of [^{3}H]inositol incorporation is similar to the stimulated rate of [^{32}P]P_i incorporation in the experiment of Fig. 1 (measured in the presence of phosphatidylserine). This 4-fold increase in ^{3}H-labelling was accompanied by the release of 40% of the mast-cell histamine.

To simplify the procedure in subsequent experiments, the measured increases in labelling were derived from samples taken at the start and end of the experiment (i.e. no sample was taken at the time of adding the stimulating ligand).

Effect of Ca^{2+} on phosphatidylinositol labelling and histamine secretion induced by IgE- and non-IgE-directed ligands

Table 2 shows the effects of a number of different ligands and the effect of external Ca^{2+} on both histamine secretion and phosphatidylinositol labelling. The non-IgE-directed ligands that we have used induce a normal exocytotic (non-lytic) degranulation of mast cells, morphologically similar to the events initiated by IgE-directed ligands (Kagayama & Douglas, 1974; Lagunoff *et al.*, 1975).

The dependence of histamine secretion on the presence of Ca^{2+} varies with the ligand used to elicit the response. Thus the partially purified allergen from *N. brasiliensis* and concanavalin A are both highly dependent on the presence of Ca^{2+}, and the effect of chymotrypsin is doubled in the presence of Ca^{2+}. The secretion induced by the crude *N. brasiliensis* antigen, and by the polycation compound 48/80 (see also Fig. 2) are also affected to a small extent. By contrast, at no time did we detect any effect of Ca^{2+} on the extent of phosphatidylinositol labelling induced by any of the triggering ligands.

The effect of Ca^{2+} on secretion induced by compound 48/80 is variable, ranging from zero effect to a high degree of dependence when the cells have been incubated in EDTA to deplete internal stores. It has been suggested that whereas depleted cells stimulated with compound 48/80 require external Ca^{2+} for secretion, fresh cells are able to mobilize Ca^{2+} from internal sources (Douglas & Ueda, 1973). In our experiments, the cells were exposed to EGTA (10 μM) during the 30 min preincubation period with radioactive isotopes, and this might account for the enhancement due to Ca^{2+} that we observed. Once again, however, no effect of Ca^{2+} on the labelling of phosphatidylinositol could be detected at any concentration of compound 48/80 (Fig. 2).

Dose–response and time course of phosphatidylinositol labelling and histamine secretion

The dependence on ligand concentration of both phosphatidylinositol labelling and histamine secre-

Table 2. *Effect of Ca^{2+} on histamine secretion and on incorporation of [^{3}H]inositol and [^{32}P]P_i into phosphatidylinositol in mast cells treated with various IgE-directed and non-IgE-directed ligands*

Mast cells were preincubated with either [^{3}H]inositol (*a*) or with [^{32}P]P_i (*b*) for 30 min. At this time samples were taken and added to solutions of various stimulating ligands as indicated, and the incubations continued for a further 15 min. The secreted histamine and the incorporation of isotopes into phosphatidylinositol were determined as described under 'Methods'. The results presented (which derive from two separate experiments, *a* and *b*) are means for duplicate incubations, and are representative of seven similar experiments.

(*a*)

			Increase in phosphatidylinositol labelling during period of incubation (0–45 min)	
	Ca^{2+} (1.8 mM)	Histamine secretion (%)	^{3}H radioactivity (d.p.m.)	Ratio of stimulated/control values
Control		0	230*	1.0
N. brasiliensis antigen	−	46.4	541	2.35
	+	54	472	2.05
N. brasiliensis allergen	−	10.5	464	2.0
	+	57.5	494	2.15
Concanavalin A (10 μg/ml)	−	0	497	2.15
	+	36.5	518	2.25
Compound 48/80	−	40	619	2.7

(*b*)

			Increase in phosphatidylinositol labelling during period of incubation (0–45 min)	
	Ca^{2+} (1.8 mM)	Histamine secretion (%)	^{32}P radioactivity (d.p.m.)	Ratio of stimulated/control values
Control	−	0	8400*	1.0
Chymotrypsin (100 μg/ml)	−	17	12690	1.5
	+	35	12700	1.5

* Significantly different from equivalent incubations with ligands at $P<0.001$.

tion is very similar for stimulation by concanavalin A (Fig. 3), which cross-links cell-surface IgE or compound 48/80 (Fig. 2).

Fig. 4 illustrates the time course of histamine secretion and of phosphatidylinositol labelling due to the addition of concanavalin A (10μg/ml) to sensitized mast cells. Whereas histamine secretion is complete within 2min of applying the ligand, the increase in phosphatidylinositol labelling had only just commenced at this time. The increased rate of incorporation is then maintained for about 50min.

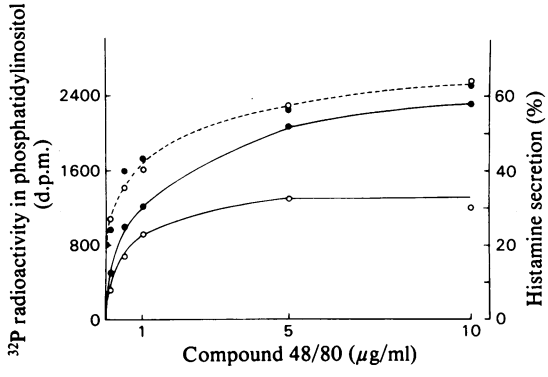

Fig. 2. *Dose-response effects of compound* 48/80 *on histamine secretion and* [^{32}*P*]P_i *incorporation into mast-cell phosphatidylinositol in the presence and absence of* Ca^{2+} (1.8*m*M)

○——○, Histamine secretion, no Ca^{2+}; ●——●, histamine secretion, +Ca^{2+}; ○----○, ^{32}P incorporation, no Ca^{2+}; ●----●, ^{32}P incorporation, +Ca^{2+}. The results presented are means for duplicate incubations and are from one of three similar experiments.

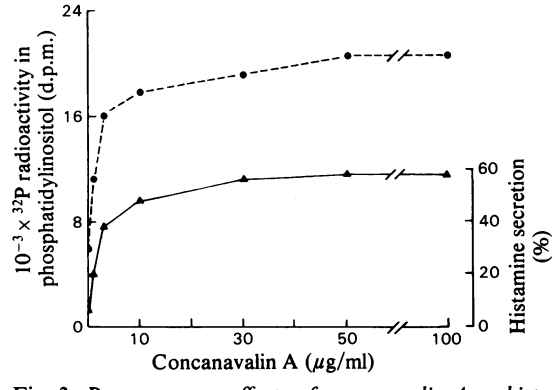

Fig. 3. *Dose-response effects of concanavalin A on histamine secretion* (▲——▲) *and incorporation of* [^{32}*P*]P_i *into mast-cell phosphatidylinositol* (●----●)

The results presented are means for duplicate incubations and are from one of three similar experiments.

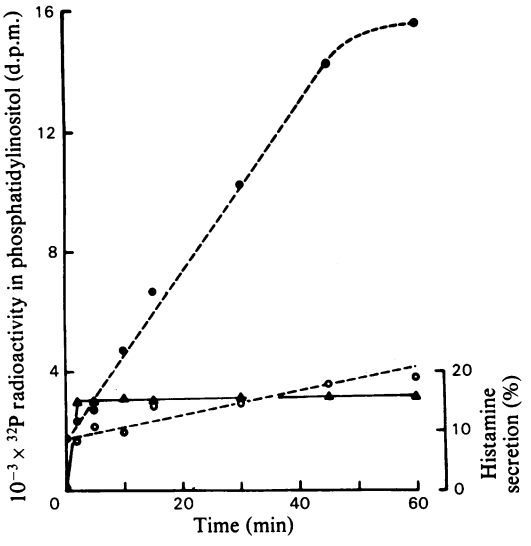

Fig. 4. *Time course of histamine secretion and labelling of mast-cell phosphatidylinositol with* [^{32}*P*]P_i *after stimulation with concanavalin A* (10μg/ml)

▲——▲, Histamine secretion; ○----○, incorporation of ^{32}P in unstimulated cells; ●----●, incorporation of ^{32}P in stimulated cells. The results presented are means for duplicate incubations and are from one of three similar experiments.

Discussion

It has been suggested that rat peritoneal mast cells would be a good system in which to study the role of the phosphatidylinositol response in receptor-mediated events (Gomperts, 1976; Michell *et al.*, 1977). Mast cells are readily obtainable in homogeneous suspension, and when stimulated they rapidly secrete a self-limited amount of an easily measurable product. Among other such 'mediator' cells (which would include the platelets, neutrophils, macrophages etc.) the mast cells offer certain advantages. Thus it is known that stimulation through the IgE system requires only that receptor pairs be cross-linked (Segal *et al.*, 1977) by the stimulating ligand. The consequent degranulation is a localized affair, taking place in the proximity of the cross-linked receptor (Lawson *et al.*, 1978) and this implies that Ca^{2+} enters the cytosol in the neighbourhood of the stimulated receptor. It is therefore likely that any precursor reactions leading to enhanced membrane permeability also occur in that vicinity.

We have studied phosphatidylinositol turnover and histamine secretion in mast cells in response to a number of different ligands, both IgE-directed and non-IgE-directed. The secretion of histamine induced by these ligands ranges from being highly dependent on external Ca^{2+} (e.g. concanavalin A, *N. brasiliensis*

allergen) to being merely enhanced or almost independent of external Ca^{2+}.

We have used phosphatidylserine to enhance the degree of histamine secretion from mast cells stimulated with the IgE-directed ligands. It is known that phosphatidylserine prolongs the duration of antigen-induced Ca^{2+} channels (Foreman & Garland, 1974), but since on occasion the dependence on the presence of phosphatidylserine was absolute it seems likely that phosphatidylserine can have an effect on the initiation of the secretory process as well. In agreement with others (Baxter & Adamik, 1978) we have found that dependence on phosphatidylserine is a feature of mast cells deriving from Sprague–Dawley rats: with cells obtained from Wistar and hooded Lister rats the effect of phosphatidylserine is generally to potentiate the secretory response due to IgE-directed ligands (see Fig. 1). A total dependence on added phosphatidylserine has also been observed for the stimulation of mast-cell secretion with high-molecular-weight dextrans (Garland & Mongar, 1974).

We found clear evidence for potentiation of phosphatidylinositol labelling by phosphatidylserine in mast cells stimulated with IgE-directed ligands. Since ligand-induced labelling occurs in the absence of added Ca^{2+}, we suggest that phosphatidylserine can act synergistically with the ligand in the control of Ca^{2+} channels and hence secretion. Previous work suggests that phosphatidylserine does not affect ligand binding to receptors (IgE) (Mongar & Svec, 1972) and the mechanism of potentiation remains an enigma. A more critical study will be needed to establish the specific point of interaction of phosphatidylserine with phosphatidylinositol metabolism. The main purpose of reporting these observations in the present context is to demonstrate that experiments carried out in its presence differ in degree, but not in kind, from when it is omitted.

The dose–response relationships of two ligands were examined. It was clear that the phosphatidylinositol-turnover response and the histamine secretion due to both concanavalin A and compound 48/80 were so similar that they would be superimposable if the ordinate scales in Figs. 2 and 3 were suitably adjusted. This is in marked contrast with the situation with muscarinic cholinergic receptors. In that case a much higher concentration of the cholinergic ligand is required to maximize the extent of phosphatidylinositol labelling than is needed to initiate optimal expression of tissue activity (Michell *et al.*, 1976*a*). This is probably due to the presence of spare receptors. Whereas stimulation of only a small proportion of muscarinic receptors sufficiently increases membrane permeability to Ca^{2+} to maximize physiological responses (contraction or secretion), events involving phosphatidylinositol, being more intimately linked to the cell-surface receptors, become maximally involved only when all receptors are occupied. It is known that the maximal secretory response of mast cells can be achieved without involving all the IgE (Lawson *et al.*, 1975), so there are spare receptors. However, the excess of receptors over the number that become involved under conditions of maximal stimulation is rather low [i.e. 2- or 3-fold compared with about 200-fold for smooth muscle (Birdsall & Hulme, 1976; Burgen & Spero, 1968)].

In no case could we detect any effect of external Ca^{2+} on ligand-induced phosphatidylinositol labelling. In this respect, the phosphatidylinositol response in mast cells resembles cholinergic and α-adrenergic stimulation as exemplified by studies on parotid tissue (Jones & Michell, 1975; Oron *et al.*, 1975), pancreas (Hokin, 1966), adrenal medulla (Trifaro, 1969) and iris smooth muscle (Abdel-Latif, 1976). Ca^{2+}-independent phosphatidylinositol responses have also been reported in hepatocytes (Billah & Michell, 1978; Kirk *et al.*, 1978), substance P-stimulated rat parotid (Jones & Michell, 1978) and 5-hydroxytryptamine-stimulated blowfly salivary glands (Fain & Berridge, 1978). These events can be counted among the criteria that one might hope to fulfil in seeking to identify receptor-mediated events that are proximal to induced Ca^{2+} movements.

It has been suggested that when mast cells are stimulated with the polycation compound 48/80, the Ca^{2+} required for the exocytotic degranulation can be derived from intracellular sources (Douglas & Ueda, 1973). In our experiments, the application of compound 48/80 initiated a phosphatidylinositol response comparable in magnitude with that induced by the IgE-directed ligands and by chymotrypsin. Thus it would appear that if phosphatidylinositol turnover is indeed a necessary intermediate step in permitting Ca^{2+} flux, then it must also be involved in releasing Ca^{2+} from intracellular stores. It is interesting that compound 48/80 causes secretion of histamine from mast cells even in the presence of 10μM-EGTA, but that under similar conditions, the ionophore A23187 is without effect (Foreman *et al.*, 1973; S. Cockcroft & B. D. Gomperts, unpublished work). It would thus appear that compound 48/80 is able to mobilize intracellular Ca^{2+} stores that are inaccessible to the ionophore.

The phosphatidylinositol response has been studied by measuring the increase in labelling of phosphatidylinositol with either [^{3}H]inositol or with [^{32}P]P_i. The degree of ligand-induced labelling with both isotopes was similar. The labelling is believed to be a secondary consequence of the breakdown of phosphatidylinositol initiated by the ligand-activated receptor (Michell, 1975). We have not been able to study the chemical breakdown of phosphatidylinositol owing to the paucity of the cells. Nevertheless it is clear from the time course of concanavalin A-induced secretion that breakdown would have to

occur within 2min of applying the ligand if it has any essential role in the regulation of cell-surface Ca^{2+} channels. The time course of labelling is similar to that observed in other tissues such as guinea-pig ileum smooth muscle on stimulation with carbachol (Jafferji & Michell, 1976*a*).

The main evidence underlying the role of phosphatidylinositol breakdown as a necessary precursor of Ca^{2+} influx in tissues comes from the association of this response with those receptors that are known to mobilize Ca^{2+} (Michell, 1975; Michell *et al.*, 1976*a*, 1977). Our results clearly suggest an intimate relationship between phosphatidylinositol metabolism and the regulation of Ca^{2+}-mediated secretion in mast cells. We have also shown that phosphatidylserine, which potentiates $^{45}Ca^{2+}$ flux (Foreman *et al.*, 1977) and Ca^{2+}-dependent secretion due to IgE-directed ligands (Goth *et al.*, 1971), also potentiates the phosphatidylinositol response. We think that this is direct evidence for a role of phosphatidylinositol metabolism in the regulation of subsequent Ca^{2+}-dependent events.

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