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Studies of receptor-stimulated inositol lipid metabolism should focus upon measurements of inositol lipid breakdown

Many hormones and other stimuli enhance the metabolism of inositol lipids (the 'phosphatidylinositol response'). Although the cellular role of this response is not yet understood, it is probably related to some widespread mechanism that increases the cytosolic Ca^{2+} concentration (see Michell & Kirk, 1981, for a review).

Stimulation of inositol lipid metabolism is usually detected as an increase in the labelling of phosphatidylinositol in stimulated cells, and it is still not uncommon for studies to use phosphatidylinositol labelling as their only index of the 'phosphatidylinositol response'. However, the initiating reaction in stimulated inositol lipid metabolism is almost certainly inositol lipid breakdown, with enhanced labelling of phosphatidylinositol (and of phosphatidate, its metabolic precursor) a delayed secondary event. Most evidence has suggested that the initiating event is phosphatidylinositol breakdown (Jones *et al.*, 1979), but recent studies of iris muscle and of hepatocytes have raised the possibility that phosphatidylinositol breakdown might itself be secondary to the receptor-stimulated breakdown of phosphatidylinositol 4,5-bisphosphate (Abdel-Latif *et al.*, 1977; C. P. Downes, J. A. Creba, C. J. Kirk & R. H. Michell, unpublished results). Breakdown of phosphatidylinositol 4,5-bisphosphate in response to a rise in cytosolic $[\text{Ca}^{2+}]$ has also been reported in several cells (see Downes & Michell, 1981, for references) and this leads secondarily to enhanced labelling of phosphatidate and/or phosphatidylinositol. Enhanced phosphatidylinositol labelling can also occur: (a) when any other lipid breaks down to yield diacylglycerol which can then be converted to phosphatidate and phosphatidylinositol (e.g. triacylglycerol breakdown in response to cytosolic Ca^{2+} ; Allan & Michell, 1977); or (b) when synthesis of phosphatidylinositol *de novo* is enhanced (e.g. during the redirection of lipid synthetic pathways in cells exposed to amphiphilic cations; Brindley *et al.*, 1975).

Stimulated phosphatidylinositol labelling is

therefore a phenomenon which, though useful, is an inconsistent guide to the prior occurrence of receptor-stimulated inositol phospholipid breakdown. Moreover, there is no reason to expect any close quantitative relationship between primary inositol phospholipid breakdown and secondary phosphatidylinositol labelling. This may sometimes occur (e.g. Kirk *et al.*, 1981), but in other cases stimulated phosphatidylinositol breakdown may not be followed by any increase in phosphatidylinositol labelling (e.g. Berridge & Fain, 1979). In view of this variability, we suggest that it should become standard practice to include quantitative measurements of inositol lipid breakdown (and preferably measurements on each inositol lipid) in all studies which aim to probe the mechanism or role of stimulated inositol lipid metabolism. Such measurements are most rigorously made by chemical assay of tissue inositol lipid concentrations, but similar results seem generally, though not always (e.g. Fain & Berridge, 1979), to be obtained in studies which measure the stimulated disappearance of lipids prelabelled to 'equilibrium' with radioactive P_i or inositol.

The need for a rigorous approach to quantitative measurements of stimulated inositol lipid breakdown has been brought into focus by the emergence of two important controversies. First, there is disagreement between laboratories, sometimes using different cellular systems, as to whether or not stimulated inositol lipid breakdown is mediated by changes in the cytosolic $[\text{Ca}^{2+}]$ (see Cockcroft *et al.*, 1980; Michell & Kirk, 1981, for references). Secondly, different laboratories disagree as to whether the dose-response curves for stimulated inositol lipid metabolism in various tissues stimulated with appropriate hormones coincide with the curves describing ligand binding to receptors or are displaced towards lower ligand concentrations than the receptor-occupation curves (see, for example, Michell *et al.*, 1976; Weiss & Putney, 1981; Kirk *et al.*, 1981). There is at present no way to determine to what extent these disagreements arise from real differences between the tissues and hormones under study in different laboratories and to what extent they are a result of differences in the experimental techniques used to measure changes in inositol lipid metabolism (e.g. phosphatidylinositol labelling as compared with phosphatidylinositol breakdown). What is clear, however, is that future

studies from which important conclusions are drawn about the mechanism or function of inositol lipid breakdown should never rely solely on measurements of phosphatidylinositol labelling: important observations should always receive confirmation from studies of inositol lipid breakdown. With regard to the function of this breakdown, two outstanding problems are to decide which inositol lipid is the first to be degraded on stimulation and by what route. Facilitation of this may call for the development of better methods for obtaining early kinetic measurements of that breakdown, maybe by analysing the inositol phosphates released during breakdown of inositol lipids.

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- Abdel-Latif, A. A., Akhtar, R. A. & Hawthorne, J. N. (1977) *Biochem. J.* **162**, 61–73
- Allan, D. & Michell, R. H. (1977) *Biochem. J.* **164**, 389–397
- Berridge, A. J. & Fain, J. N. (1979) *Biochem. J.* **180**, 655–661
- Brindley, D. N., Allan, D. & Michell, R. H. (1975) *J. Pharm. Pharmacol.* **27**, 462–464
- Cockcroft, S., Bennett, J. P. & Gomperts, B. D. (1980) *Nature (London)* **288**, 275–277
- Downes, C. P. & Michell, R. H. (1981) *Biochem. J.* **198**, 133–140
- Fain, J. N. & Berridge, M. J. (1979) *Biochem. J.* **180**, 655–661
- Jones, L. M., Cockcroft, S. & Michell, R. H. (1979) *Biochem. J.* **182**, 669–676
- Kirk, C. J., Michell, R. H. & Hems, D. A. (1981) *Biochem. J.* **194**, 155–165
- Michell, R. H. & Kirk, C. J. (1981) *Trends. Pharmacol. Sci.* **2**, 86–89
- Michell, R. H., Jafferji, S. S. & Jones, L. M. (1976) *FEBS Lett.* **69**, 1–10
- Weiss, S. J. & Putney, J. W. (1981) *Biochem. J.* **194**, 463–468