vation of hepatic glycogen phosphorylase is abolished in the presence of EGTA (Keppens et al., 1977; Hems et al., 1978). These results are consistent with the hypothesis that enhanced phosphatidylinositol turnover represents an early effect of vasopressin on the liver cell that may precede hormone-stimulated Ca²⁺ uptake and subsequent changes in glycogen phosphorylase activity.

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Stimulation of the Breakdown and Resynthesis of Phosphatidylinositol in Rat Hepatocytes by Angiotensin, Vasopressin and Adrenaline

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Phosphatidylinositol breakdown, and secondarily its labelling with ³²P or [³H]inositol, is stimulated in cells exposed to hormones and neurotransmitters that utilize Ca²⁺ ions as their second messenger, and it has been suggested that this reaction may be intrinsic to the mechanism of stimulus–response coupling at such receptors (Michell, 1975, 1979; Michell et al., 1976, 1977a,b; Jones & Michell, 1978a). The glycogenolytic responses of rat hepatocytes to vasopressin, to α-adrenergic stimuli and to angiotensin II all appear to utilize Ca²⁺ as their essential second messenger (Assimacopoulos-Jeannet et al., 1977; Keppens et al., 1977; Hems et al., 1978), and Kirk et al. (1977, 1978) have shown that both vasopressin and adrenaline stimulate phosphatidylinositol turnover in isolated rat hepatocytes. Our experiments have been designed: (1) to see if angiotensin provokes a similar phosphatidylinositol response in these cells, (2) to determine whether the hormonal stimulation of phosphatidylinositol labelling depends on or is independent of the intracellular Ca²⁺ ion concentration, and (3) to determine whether phosphatidylinositol breakdown is the initial reaction stimulated by the hormones. Hepatocytes were isolated from rat liver in Ca²⁺-free media (Berry & Friend, 1969) and were then incubated with or without hormones in a Heps (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid)/Ringer buffer containing 11 mm-glucose (and sometimes 2% bovine serum albumin), usually for 15 min. In labelling experiments the media contained [³²P]Pi, (approx. 40 μCi/ml), and for the breakdown experiments prelabelled hepatocytes were isolated from rats that had received an intraperitoneal injection of approx. 0.7 mCi of [³²P]Pi, about 24 h earlier.

The labelling of phosphatidylinositol during a 15 min incubation was increased 6–8-fold by 10 munits of vasopressin/ml, and 3–4-fold by either 100 μM-adrenaline or 1 μM-angiotensin II. Half-maximal stimulation by angiotensin was at about 50 nM, a much higher concentration than is needed for half-maximal activation of glycogen phosphorylase (Keppens & De Wulf, 1976). The response to angiotensin was abolished
by low concentrations of [Sar\(^1\), Ile\(^8\)]angiotensin II (Sar, sarcosine), a specific angiotensin II antagonist; this compound did not decrease the responses to either vasopressin or adrenaline. The response to angiotensin was easily detected within 1 min.

The responses to the three hormones were compared in cells incubated in either a medium containing 1 mM-Ca\(^{2+}\) or a Ca\(^{2+}\)-free medium containing 0.2 mM-EGTA. The labelling of phosphatidylinositol in unstimulated cells incubated in the absence of Ca\(^{2+}\) was more rapid than labelling in tissue exposed to Ca\(^{2+}\), as has sometimes been observed with other tissues (e.g. Jones & Michell, 1975; Jafferji & Michell, 1976). The three hormones all stimulated phosphatidylinositol labelling in the Ca\(^{2+}\)-deprived cells, but the magnitude of the increase in labelling was in each case markedly decreased relative to the response in the presence of Ca\(^{2+}\). A second method of investigating whether the phosphatidylinositol response was initiated by an increase in the intracellular Ca\(^{2+}\) concentration was by the use of the bivalent-cation ionophore A23187. This compound is effective in stimulating a Ca\(^{2+}\)-triggered activation of glycogen phosphorylase in rat hepatocytes (Keppens et al., 1977; Assimacopoulos-Jeannet et al., 1977). Ionophore A23187 at concentrations of 1 \(\mu\)g/ml or more produced a small (but inconsistent) increase in lipid labelling; this was not, however, confined to phosphatidylinositol. Each hormone effectively provoked its normal increase in phosphatidylinositol labelling when it was added to cells incubated in the presence of 1 \(\mu\)g of ionophore A23187/ml. On balance, these results suggest, as do those of Kirk et al. (1978), that the increase in phosphatidylinositol metabolism that is produced in hepatocytes by angiotensin, vasopressin or adrenaline is not a result of the increase in intracellular Ca\(^{2+}\) concentration that these hormones bring about. Similar observations have previously been made with three different stimuli in the parotid gland (Oron et al., 1975; Jones & Michell, 1975, 1976, 1978b), with acetylcholine in adrenal medulla (Trifaro, 1969), with histamine and carbamoylcholine in ileum smooth muscle (Jafferji & Michell, 1976), and with acetylcholine and noradrenaline in iris smooth muscle (Abdel-Latif, 1976). This Ca\(^{2+}\)-independent behaviour of the phosphatidylinositol response, in contrast with the Ca\(^{2+}\) dependence of the normal physiological responses of these cells (glycogenolysis, K\(^+\) efflux, catecholamine secretion and contraction respectively), has been the most important of the experimental observations that have led to the suggestion that phosphatidylinositol breakdown might be implicated directly in receptor mechanisms.

The question of whether phosphatidylinositol breakdown is the initial event provoked by stimulation of hepatocytes has initially been approached by using vasopressin, the hormone that gives the largest labelling response. When \(^{32}\)P-labelled hepatocytes isolated from animals that had been given an injection of \([^{32}\)P]P, 24 h previously were treated with 10 units of vasopressin/ml for 5 min or for 15 min there was a small, but statistically significant, loss of \(^{32}\)P from their phosphatidylinositol, but not from other lipids. During the first few minutes of stimulation, this loss appeared to involve at least 0.5% of the labelled phosphatidylinositol (and presumably of the total cell phosphatidylinositol) per minute.

It therefore seems that the phosphatidylinositol turnover induced by angiotensin, vasopressin or adrenaline in rat hepatocytes has characteristics similar to those of phosphatidylinositol responses in other tissues. Once again, these characteristics are compatible with the idea that phosphatidylinositol breakdown may be a reaction essential to the mechanisms by which certain receptors provoke an increase in cell surface Ca\(^{2+}\) permeability.

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Enhanced Phosphatidylinositol Breakdown as a Calcium-Independent Response of Rat Parotid Fragments to Substance P

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Secretion of amylase in rat parotid gland is mediated mainly through a raised cyclic AMP concentration provoked by activation of β-adrenergic receptors (Batzri & Selinger, 1973). However, both amylase secretion and an efflux of K+ ions can be provoked, independently of cyclic AMP, through stimulation of three other types of receptor: these respond to α-adrenergic stimuli (Batzri et al., 1973; Butcher et al., 1975; Leslie et al., 1976; see also Jones & Michell, 1978), to muscarinic cholinergic stimuli (Monnard & Shorderet, 1973; Leslie et al., 1976; Petersen, 1976) and to substance P and related peptides (Rudich & Butcher, 1976). Although it is not known exactly how activation of these receptors leads to control of cell responses, there is considerable evidence to indicate that Ca2+ is their intracellular second messenger. In particular, the amylase secretion and K+ efflux triggered by these three receptors are decreased or abolished when Ca2+ is omitted from the incubation medium (Selinger et al., 1973; Leslie et al., 1976; Putney, 1976; Rudich & Butcher, 1976) and these responses can be triggered, in the presence of extracellular Ca2+, by the ionophore A23187 (Selinger et al., 1974; Putney, 1976).

In the parotid gland, as in many other tissues, activation of muscarinic cholinergic or α-adrenergic receptors stimulates phosphatidylinositol metabolism (Oron et al., 1973, 1975; Michell & Jones, 1974; Jones & Michell, 1974, 1975, 1976; Rossignol et al., 1974). It now seems clear that this response, both in the parotid gland and elsewhere, consists of receptor-controlled breakdown of phosphatidylinositol followed by a compensatory resynthesis of the lipid (see Michell et al., 1977a, for review; Billah & Michell, 1978). Phosphatidylinositol breakdown differs from other responses by its apparent insensitivity to changes in the extracellular Ca2+ concentration, and this has led to the suggestion that it may have a role in the coupling of Ca2+-mobilizing receptors to the opening of a hypothetical Ca2+ gate in the plasma membrane (Michell, 1975; Michell et al., 1977a,b; Jones & Michell, 1978).

In the parotid gland, it seems that substance-P receptors have a mode of action similar to that of α-adrenergic and muscarinic cholinergic receptors (Putney, 1977).