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Investigation of the Relationship between Cell-Surface Calcium-Ion Gating and Phosphatidylinositol Turnover by Comparison of the Effects of Elevated Extracellular Potassium Ion Concentration on Ileum Smooth Muscle and Pancreas

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Incubation of fragments of guinea-pig ileum smooth muscle in the presence of an elevated extracellular K^+ concentration, which causes an increase in cell-surface Ca^{2+} permeability and thus leads to contraction, caused a marked increase in phosphatidylinositol turnover, as assessed by incorporation of $^{32}P_i$. This response was not diminished by atropine or propylbenzylcholine mustard, two muscarinic cholinergic antagonists, and was therefore not caused by the release of endogenous acetylcholine within the tissue. In contrast, exposure of guinea-pig pancreas fragments to high extracellular $[K^+]$, which does not increase cell-surface Ca^{2+} permeability or evoke secretion, did not cause an increase in phosphatidylinositol turnover, even though such an increase was triggered by carbamoylcholine, which is a secretagogue. These observations are consistent with a suggested function for phosphatidylinositol breakdown in the mechanisms of cell-surface Ca^{2+} gates.

Stimulation with appropriate hormones and neurotransmitters provokes increased turnover of phosphatidylinositol in many tissues (Michell, 1975). Muscarinic cholinergic, α -adrenergic and H_1 histaminergic stimuli and 5-hydroxytryptamine produce this effect in ileum smooth muscle (Jafferji & Michell, 1976*a,b*), and in pancreas this response is elicited by muscarinic cholinergic stimuli and by pancreozymin or caerulein (Hokin, 1968*a,b*, 1974; Baudhuin *et al.*, 1971). Thus the stimuli that produce the phosphatidylinositol response are the same as those that elicit the 'physiological' (contractile and exocytotic secretory) responses of these two tissues. The physiological responses are brought about by an increase in the intracellular concentration of Ca^{2+} that is, in turn, caused wholly or in part by an increase in cell-surface Ca^{2+} permeability in the stimulated cells (Hurwitz & Suria, 1971; Rubin, 1974; Douglas, 1974). However, several lines of evidence have indicated that this rise in intracellular Ca^{2+} concentration is not the trigger for phosphatidylinositol breakdown, the reaction that initiates increased phosphatidylinositol turnover (Trifaró, 1969; Jones & Michell, 1975, 1976; Oron *et al.*, 1975; Jafferji & Michell, 1976*c*). The simplest interpretation of the function of the phosphatidylinositol response that is available at present is that it is involved in the operation of the cell-surface Ca^{2+} gates (Michell, 1975; Michell *et al.*, 1976*a,b*; Jafferji & Michell, 1976*c*).

Contraction of ileum smooth muscle can also be brought about by depolarizing the plasma membrane with a high concentration of extracellular K^+ , this

effect being mediated by an increased influx of Ca^{2+} ions through potential-sensitive Ca^{2+} gates; this response thus involves the operation of Ca^{2+} gates but by-passes interactions between receptors and their agonists (Hurwitz & Suria, 1971; Triggle, 1971; Berridge, 1975). In contrast, the pancreas is not sensitive to K^+ -induced depolarization and therefore it does not secrete when exposed to high concentrations of extracellular K^+ (Matthews, 1974). If phosphatidylinositol breakdown is essential to the mechanisms that operate cell-surface Ca^{2+} gates, rather than being simply an accompaniment of receptor-agonist interactions, then it should be observed in ileum smooth muscle when it is exposed to depolarization by high $[K^+]$, but not in the pancreas under similar conditions. The purpose of the present paper is to report experiments that demonstrate that this is the observed pattern of response. Previous work has shown that elevated extracellular $[K^+]$ provokes an increased phosphatidylinositol turnover in cerebral cortex (Yoshida & Quastel, 1962; Brossard & Quastel, 1963), sympathetic ganglia (Nagata *et al.*, 1973) and synaptosomes (Hawthorne & Bleasdale, 1975), but did not unequivocally demonstrate that the effects observed were not due to endogenously released neurotransmitters.

Materials and Methods

The sources of materials were those described previously (Jafferji & Michell, 1976*a*). The ileum longitudinal smooth-muscle fragments or pancreas

fragments were obtained and incubated as described previously (Jafferji & Michell, 1976a), except that for the high-[K⁺] incubations a modified Krebs-Ringer medium was used in which Na⁺ was replaced by K⁺ to a concentration of 95 mM. The tissue fragments were incubated with ³²P_i in normal Krebs-Ringer medium for 30 min, recovered by filtration, then incubated for a further 30 min in either the same medium or ³²P-labelled high-K⁺ medium. Whenever antagonists were included, they were present during both periods of incubation. Reactions were terminated and phospholipids were extracted, separated and analysed as described previously (Jafferji & Michell, 1976a).

Results

Smooth muscle

When the muscle fragments were transferred to high-K⁺ medium the labelling of phosphatidylinositol rose 1.5–3-fold in each of seven experiments, two of which are shown in Table 1; this reflects an approximately 2–5-fold increase in the rate of ³²P_i incorporation into phosphatidylinositol in the second period

Table 1. Stimulation of phosphatidylinositol turnover in ileum smooth muscle by an elevated extracellular K⁺ concentration

Tissue fragments were incubated in ³²P-labelled Krebs-Ringer medium for 30 min, followed by a further 30 min in either the same medium or a medium containing 95 mM-K⁺. Inhibitors were present during both periods of incubation. The stimulatory effect of 95 mM-K⁺ was seen in seven experiments and the results shown are from two of these experiments, which also included an analysis of the effects of cholinergic antagonists. The values given are means ± S.E.M. for the numbers of incubations specified in parentheses.

	Specific radioactivity of phosphatidylinositol (d.p.m./nmol)	
	Expt. 1	Expt. 2
Control	324 ± 18 (4)	190 ± 17 (3)
95 mM-K ⁺	924 ± 123 (5)*	300 ± 23 (4)†
95 mM-K ⁺ and 4.25 μM-atropine	1055 ± 133 (4)*‡	295 ± 15 (4)†‡
95 mM-K ⁺ and 1.25 μM-propylbenzylcholine mustard	1022 ± 152 (5)*‡	344 ± 39 (3)†‡

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

† Significantly different from control values ($P < 0.02$).

‡ Not significantly different from values with 95 mM-K⁺ alone ($P > 0.5$).

of incubation. The labelling of the major phospholipids was unaffected by high [K⁺] (results not shown).

The smooth-muscle fragments that we were studying retained both remnants of their autonomic innervation and also Auerbach's plexus, and therefore they probably contained about 28 μg of acetylcholine/mg wet weight of tissue (Paton & Aboozar, 1968). To ensure that the phosphatidylinositol response to high [K⁺] was not simply due to the release of this acetylcholine within the tissue, we tested the effects of atropine and propylbenzylcholine mustard, two muscarinic cholinergic antagonists, on the response. Neither prevented the response to high [K⁺] (Table 1), even though both abolished the cholinergically stimulated phosphatidylinositol response (Jafferji & Michell, 1976a). Neither drug had any effect on the labelling of phosphatidylinositol in unstimulated tissue fragments (Jafferji & Michell, 1976a).

Pancreas

Pancreas does not respond to high [K⁺] with either secretion or Ca²⁺ fluxes across the cell surface, provided that appropriate controls are included to exclude effects of endogenously released acetylcholine (Matthews, 1974). Similarly, pancreas fragments did not show any change in phosphatidylinositol labelling when exposed to high [K⁺] (Table 2), even though the same tissue showed a dramatic phosphatidylinositol response to muscarinic cholinergic stimulation (Table 2 and Hokin, 1968a,b, 1974).

Table 2. Stimulation of phosphatidylinositol turnover in pancreas by carbamoylcholine, but not by elevated extracellular K⁺ concentration

Experimental details were as in Table 1, except that pancreas fragments were used. Results are from two separate experiments and are means ± S.E.M. for four incubations.

	Specific radioactivity of phosphatidylinositol (d.p.m./nmol)	
	Expt. 1	Expt. 2
Control	59 ± 3	21 ± 2
95 mM-K ⁺	56 ± 3†	
95 mM-K ⁺ and 2.5 μM-atropine	54 ± 2†	25 ± 3†
2.5 μM-Atropine	44 ± 2†	20 ± 1†
100 μM-Carbamoylcholine	307 ± 22*	
100 μM-Carbamoylcholine and 2.5 μM-atropine	52 ± 2†	

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

† Not significantly different from control ($P > 0.5$).

Discussion

It was realized recently that those hormones and neurotransmitters that bring about an increase in phosphatidylinositol turnover in their target cells are the same ones that cause an increase in cell-surface Ca^{2+} permeability (Michell, 1975). However, the phosphatidylinositol response differs from other cell responses to these stimuli in that it does not appear to be controlled by the resulting increase in intracellular Ca^{2+} concentration (see the introduction). It was this difference that first led to the suggestion that phosphatidylinositol breakdown, the reaction that initiates the increase in turnover, might be involved in some way in the operation of receptor-sensitive cell-surface Ca^{2+} gates (Michell, 1975; Michell *et al.*, 1976a,b).

In some tissues, including the longitudinal smooth muscle of guinea-pig ileum, there are potential-sensitive Ca^{2+} gates at the cell surface, which can be opened by exposure of the tissue to elevated concentrations of extracellular K^+ (Hurwitz & Suria, 1971; Triggle, 1971; Berridge, 1975). Other tissues, including the pancreas, do not possess such potential-sensitive Ca^{2+} gates (Matthews, 1974). This distinction offered an opportunity to extend our previous investigations of the association between the stimulation of phosphatidylinositol metabolism and the occurrence of Ca^{2+} gating. The results give further corroboration to the previously recognized association between these two processes, in that high $[\text{K}^+]$ stimulated phosphatidylinositol turnover in the smooth muscle, but had no effect on this process in the pancreas. This distinction must be a result of differences in the effects of high $[\text{K}^+]$ on the two tissues, since previous work has established that both of these tissues possess the necessary biochemical mechanisms with which to display increased phosphatidylinositol metabolism when appropriately stimulated (Jafferji & Michell, 1976a,b,c; Hokin, 1968a,b, 1974; and Table 2 of this paper). The experiments with smooth muscle also suggest that the functional association is between phosphatidylinositol metabolism and Ca^{2+} gating rather than between phosphatidylinositol metabolism and a receptor-agonist interaction, since high- $[\text{K}^+]$ depolarization is a route to opening of Ca^{2+} gates which by-passes such interactions.

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