A Possible Role for Phosphatidylinositol Breakdown in Muscarinic Cholinergic Stimulus–Response Coupling

ROBERT H. MICHELL, LYNNE M. JONES and SHAMSHAD S. JAFFERJI
Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

A substantial body of evidence now favours the view that an increase in intracellular Ca\(^{2+}\) concentration, caused largely by a downhill flow of Ca\(^{2+}\) ions through a receptor-controlled Ca\(^{2+}\)-gating system at the plasma membrane, is the factor that couples activation of muscarinic cholinergic receptors to the initiation of a physiological response in target cells (Rubin, 1970, 1974; Hurwitz & Suria, 1971; Triggle, 1972; Chang & Triggle, 1973a; Douglas, 1974). Much of this evidence has come from studies of exocrine (e.g. pancreas and parotid gland) and endocrine (adrenal medulla) exocytotic secretory tissues and from analysis of the contractile responses of gastrointestinal smooth muscles, but even in tissues where the evidence is less complete there is at present no very strong indication of any other form of coupling between activation of this type of receptor and cell responses. The only muscarinic response that is particularly difficult to understand in these terms is the muscarinic cholinergic feedback-inhibition of acetylcholine release from nerve terminals (Muscholl, 1973; Szerb & Somogyi, 1973).

In addition, there is evidence which indicates that some of the Ca\(^{2+}\) ions responsible for the triggering of the initial 'phasic' response of stimulated tissues are released from a membrane-bound pool at the plasma membrane (Chang & Triggle, 1973a; Triggle & Triggle, 1976; Petersen & Ueda, 1976). Studies with Ca\(^{2+}\)-antagonistic cations, both inorganic (e.g. Mn\(^{2+}\), La\(^{3+}\) and Tm\(^{3+}\)) and organic [e.g. nifedipine or methoxyverapamil (D600); Fleckenstein et al., 1975], have indicated that the sensitivities to inhibition of the phasic response and of the sustained tonic response are often similar, despite the fact that the phasic response is triggered by Ca\(^{2+}\) release from a membrane-bound pool and the tonic response by an influx of Ca\(^{2+}\) ions from the exterior (Triggle & Triggle, 1976; Ticku & Triggle, 1976). It has also been noted that the same Ca\(^{2+}\) antagonists are inhibitory to the entry of Ca\(^{2+}\) ions both through receptor-controlled gates and through the potential-sensitive slow-responding Ca\(^{2+}\) gates of tissues such as squid axon, heart muscle and ileum muscle (Fleckenstein et al., 1975; Godfraind & Kaba, 1972; Ticku & Triggle, 1976).

Electrophysiological evidence indicates that ion movements are not detected in muscarinically stimulated cells until about 100–200 ms after the application of a stimulus and that the effect of receptor activation may persist for many seconds after removal of the stimulus (Bolton, 1975; Purves, 1976). It therefore seems probable that the initial rise in Ca\(^{2+}\) concentration within a muscarinically controlled cell is a relatively slow process, with kinetics more reminiscent of the enzyme-catalysed production of a signal such as cAMP than of a dramatic event of the type produced by the rapid opening of ion channels through conformational re-arrangement of a pore-forming protein (e.g. the nicotinic cholinergic receptor), and that closure of the Ca\(^{2+}\) gates is also a relatively slow process.

Scheme 1 shows a speculative attempt to synthesize this information into a working model of a muscarinic receptor-sensitive Ca\(^{2+}\)-gating system: this scheme is partially based on those considered before by Triggle (1972), Hurwitz & Suria (1971), Matthews (1974) and others. A cell surface Ca\(^{2+}\)-binding site, which is in relatively slow equilibrium with the extracellular Ca\(^{2+}\) pool and which lies in or adjacent to a closed Ca\(^{2+}\) gate, is occupied by Ca\(^{2+}\) ion in the unstimulated cell. When a receptor is activated this triggers an enzyme reaction that somehow activates the gate mechanism and this bound Ca\(^{2+}\) ion is released and passes into the cell; the channel then remains open for the subsequent passage of a considerable number of additional Ca\(^{2+}\) ions. The opening of the gate and the release of the Ca\(^{2+}\) ion from its binding site are probably consequences
Scheme 1. Proposed scheme of events involved in stimulus–response coupling at the muscarinic cholinergic receptor.
of the same molecular event (Chang & Triggle, 1973a). After each channel has been open for a considerable period, probably a few seconds, it is closed by an inactivating mechanism (Purves, 1976). Subsequently it is resistant to reopening (i.e. it is desensitized) for some time before it returns to the state that is responsive to receptor activation (Chang & Triggle, 1973b).

The ultimate aim of work on this controlled Ca\(^{2+}\)-gating system must be to delineate the molecular mechanisms both of the gating mechanism itself and of the controls, quite probably enzymic, which open, close, desensitize and resensitize the system. At present the details of these mechanisms are completely unknown, but some evidence points strongly to the possibility that they may involve the removal of the phosphorylinositol headgroup from phosphatidylinositol, a quantitatively minor membrane phospholipid. This idea arose some time ago when it was realized that phosphatidylinositol turnover could be activated by a wide variety of ligands which interact with cell-surface receptors, but that the physiological responses of these tissues were diverse (Hokin, 1968, 1969; Durell et al., 1969; Lapetina & Michell, 1973). However, no coherent synthesis of the information from the many systems in which stimuli trigger increased phosphatidylinositol breakdown was possible until it was realized that a common factor which links all of the effective stimuli is that they bring about their target-cell responses through an increase in intracellular Ca\(^{2+}\) concentration (Michell, 1975). The muscarinic cholinergic receptor is at present the best characterized of these Ca\(^{2+}\)-mobilizing systems at the plasma membrane, which also include \(\alpha\)-adrenergic, \(\mathrm{H}_1\)-histamine, 5-hydroxytryptamine, pancreozymin, phytohaemagglutinin, thyrotropin and other receptors, and potential-sensitive slow Ca\(^{2+}\) channels (see Michell, 1975; Jafferji & Michell, 1976b,d).

Since we first noticed this correlation, we have directed our efforts at determining how close is the association between Ca\(^{2+}\) gating and the stimulation of phosphatidylinositol metabolism. As a result of these studies, the arguments which now support the idea that phosphatidylinositol breakdown is a reaction integral to stimulus–response coupling are as follows.

1. Increased phosphatidylinositol breakdown occurs only as a consequence of stimuli that cause an increased influx of Ca\(^{2+}\) ions across the cell surface and in response to all such Ca\(^{2+}\)-mobilizing receptors that have been tested (Michell, 1975; Jafferji & Michell, 1976b,d).

2. Present evidence is consistent with the view that increased phosphatidylinositol metabolism always accompanies the activity of muscarinic receptors, as it must if it is central to the mechanisms whereby the activation of these receptors brings about the opening of Ca\(^{2+}\) gates (Michell et al., 1976b). We have studied the effects of cholinergic stimulation on phosphatidylinositol metabolism in ileum smooth muscle, where the phosphatidylinositol response had not previously been sought, and in isolated superior cervical sympathetic ganglia, where a previous study had suggested that it might be controlled by nicotinic stimulation. Enhanced phosphatidylinositol turnover occurred in response to muscarinic cholinergic stimulation in both tissues (Jafferji & Michell, 1976a; Lapetina et al., 1976), as expected if it is intrinsic to the muscarinic stimulus–response-coupling mechanism.

3. Unlike the majority of cell responses to muscarinic cholinergic stimulation, the phosphatidylinositol response is not triggered by a rise in intracellular Ca\(^{2+}\) concentrations. The evidence for this statement is of three types. First, phosphatidylinositol turnover is stimulated by muscarinic agonists even in cells that have been deprived of Ca\(^{2+}\) ions and do not exhibit normal physiological functions (Trifaró, 1969; Jones & Michell, 1975; Oron et al., 1975). Secondly, increase of the intracellular Ca\(^{2+}\) concentration by using an ionophore, a method which by-passes the operation of the receptor-controlled Ca\(^{2+}\)-gating system, does not reproduce the effects of receptor activation on phosphatidylinositol metabolism (Rossignol et al., 1974; Jones & Michell, 1975; Oron et al., 1975). Thirdly, the muscarinic cholinergic stimulation of phosphatidylinositol turnover is not diminished by exposure of cells to several Ca\(^{2+}\) antagonistic drugs, which prevent the receptor-controlled influx of Ca\(^{2+}\) ions into
stimulated cells (Jafferji & Michell, 1976c). It is possible that the phosphatidylinositol response is the first example of a muscarinic response whose control involves a stimulus–response mechanism other than that which increases cell-surface Ca2+ permeability and leads to an increase in intracellular Ca2+ concentrations, but it is simpler to envisage that phosphatidylinositol breakdown is a reaction early in the sequence of events which leads to increased cell-surface permeability to Ca2+ ions. There is also evidence which appears to eliminate changes in concentrations of cAMP, cGMP, K+ or Na+ as possible candidates for the muscarinic receptor–phosphatidylinositol-breakdown coupling mechanism (Michell, 1975; Jones & Michell, 1976).

(4) Activation of phosphatidylinositol breakdown shows a dose–response curve for agonists which is quite different from that displayed by ‘physiological’ responses such as contraction or secretion. This curve is displaced to high agonist concentrations and markedly flattened, and it is essentially identical to the receptor-occupation curve. The close similarity between these two curves would be simply explained if it was a result of a tight and fairly direct coupling between the activation of the muscarinic receptor by an agonist and the subsequent control by the activated receptor of the enzyme that catalyses phosphatidylinositol breakdown (Michell et al., 1976a,b).

(5) We have compared the effects of high K+ concentration depolarization on ileum smooth muscle, a tissue with potential-sensitive Ca2+ gates, and on pancreas, in which changes in membrane potential occur during exposure to high K+ concentrations, but which does not possess potential-sensitive Ca2+ gates (Matthews, 1974). High K+ concentrations only stimulated phosphatidylinositol turnover in the smooth muscle (Jafferji & Michell, 1976d). This result again emphasizes the association between Ca2+ gating and phosphatidylinositol breakdown and is consistent with the idea that there are probably mechanistic similarities between receptor-sensitive and potential-sensitive Ca2+ gates.

We believe that the evidence summarized above constitutes a strong case in favour of a functional relationship between phosphatidylinositol breakdown and the control of cell-surface Ca2+ gates, including those which are opened by the activation of muscarinic cholinergic receptors. It does not, however, give any clear indication of the molecular mechanisms involved. Indeed, we have so far discussed this reaction only as a candidate for a role in controlling the opening of cell-surface Ca2+ gates (i.e. as the enzyme-mediated step in Scheme 1). This seems reasonable, since the Ca2+ insensitivity of this reaction strongly suggests that it precedes Ca2+ influx, but we certainly cannot rule out the participation of phosphatidylinositol breakdown (and subsequent events in lipid metabolism) in the opening, closing or desensitization of the gating system, since these are all processes which would occur to a considerable extent within the period needed for any of our biochemical experiments.

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

Chang, K.-J. & Triggle, D. J. (1973a) J. Theor. Biol. 40, 125-154
Chang, K.-J. & Triggle, D. J. (1973b) J. Theor. Biol. 40, 155-172
Godfraind, T. & Kaba, A. (1972) Arch. Int. Pharmacodynam. 196, 35–49

1977
Lapetina, E. G. & Michell, R. H. (1973) FEBS Lett. 31, 1-10
Michell, R. H. (1975) Biochim. Biophys. Acta 415, 81-147
Muscholl, E. (1973) Proc. 5th Int. Cong. Pharmacol. 4, 440-457

ETIOPLASTS: a Colloquium organized on behalf of the Bioenergetic Organelle Group by J. W. Bradbeer (London)

Etioplast Structure and its Relevance to Chloroplast Development

RACHEL M. LEECH

Department of Biology, University of York, York YO1 5DD, U.K.

The term 'etioplast' was coined by Kirk & Tilney-Bassett (1967) to describe the plastids found in the leaf cells of higher-plants after growth entirely in the dark. Etioplasts are characterized by their complete lack of chlorophyll and by the complexity of their central internal membrane system, which is in the form of a modified strutted dodecahedron known as a prolamellar body (Gunning & Jagoe, 1967; Weier & Brown, 1970). Etioplast formation is virtually unknown in normally grown plants, but the membrane changes initiated by the illumination of etiolated plastids lead to the assembly of fully functional photosynthetic reaction centres and electron-transport systems in membrane associations resembling those in normal chloroplasts. Largely because of the ease with which the differentiation of the etioplast can be controlled by light, the structural and functional changes occurring in illuminated etioplasts (i.e. 'etiocloroplasts') have been widely studied. It has been generally assumed that etioplasts are chloroplasts whose normal development has been arrested in the absence of light and that the effect of illumination is to trigger the continuation of their development according to its normal pattern. However, a considerable body of information from both the ultrastructural and biochemical analysis of etiolated greening tissues from a variety of plants shows that although completely lacking chlorophyll, etioplasts have nevertheless reached a sophisticated level of development far beyond that encountered in the proplastids from which they originate. Proplastids, which are spherical (1 μm in diameter) undifferentiated double-membrane-bound organelles with few internal