Calcium Ion Fluxes and Insulin Release in Pancreatic Islets

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Various techniques are currently used to investigate the handling of calcium by endocrine pancreatic cells. They include the measurement of the total calcium content of isolated islets of Langerhans (W. J. Malaisse, unpublished work), the measurement of $^{45}\text{Ca}^{2+}$ net uptake, efflux and subcellular distribution in the islets (Malaise-Lagae & Malaisse, 1971; Malaisse et al., 1973), the ultrastructural localization and quantification of calcium in the B-cell (Ravazzola et al., 1976), and the characterization of the B-cell bioelectrical activity (Dean & Matthews, 1970a,b).

On the basis of the results obtained by some of these methods, a model was designed to depict the movements of $\text{Ca}^{2+}$ in the pancreatic B-cell (Malaise & Pipeleers, 1974) and their regulation by environmental factors.

The entry of $\text{Ca}^{2+}$ into the B-cell across the plasma membrane represents the first of such movements. Apparently, a major fraction of this $\text{Ca}^{2+}$ influx occurs through $\text{Ca}^{2+}$ channels characterized by their sensitivity to organic $\text{Ca}^{2+}$ antagonists (Devis et al., 1975; Somers et al., 1976b; Malaisse et al., 1976a,c, 1977a), competition between $\text{Ca}^{2+}$, $\text{Mg}^{2+}$ (Malaisse et al., 1976a) and $\text{Ba}^{2+}$ (Somers et al., 1976c), and inhibition by $\text{Co}^{2+}$ (Henquin & Lambert, 1975). A lesser fraction of $\text{Ca}^{2+}$ entry into the B-cell may occur through a veratridine-sensitive $\text{Na}^{+}$ channel (Donatsch et al., 1976).

In artificial systems, the modulation of ionophore-mediated $\text{Ca}^{2+}$ translocation displays similarities with the regulation of $\text{Ca}^{2+}$ influx in the B-cell. For instance, it was observed that organic $\text{Ca}^{2+}$ antagonists inhibit the ionophore A23187-mediated translocation of $\text{Ca}^{2+}$ from an aqueous Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid] buffer into an organic immiscible phase (Malaise, 1977), the inhibitory action of verapamil being, as in the B-cell, competitively antagonized by $\text{Ca}^{2+}$. The hypoglycaemic sulphonylurea, thought to bind to the B-cell membrane (Hellman et al., 1973), may also modify $\text{Ca}^{2+}$ and/or $\text{Na}^{+}$ influx by interfering with the B-cell native ionophoretic systems (Malaisse et al., 1977b).

A second type of $\text{Ca}^{2+}$ movement consists of the uptake by, and release from, the organelles of the vacuolar system. Methylxanthines, possibly by raising the concentration of cyclic AMP in the B-cell, are said to provoke a cytosolic accumulation of $\text{Ca}^{2+}$ by increasing the net release of $\text{Ca}^{2+}$ from such a vacuolar pool (Brisson et al., 1972; Brisson & Malaisse, 1973; Howell & Montague, 1975). An increase in the cytosolic concentration of $\text{Na}^{+}$ may mimic the effect of theophylline (Lowe et al., 1976).

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Fig. 1. Sequential events in the process of glucose-induced insulin release

(a) Step increase in glucose concentration of incubation media (Ⅲ) and progressive increase in glucose concentration of the effluent in a system of perifused islets (Ⅲ). (b) Net rate of glucose entry on raising the extracellular glucose concentration from 0 to 16.7 mm (see Malaisse et al., 1976e). (c) Changes in NADH content of the islets [----, control data (no glucose present)]. (d) Efflux of $^{45}\text{Ca}^{2+}$ from islets perifused with a $\text{Ca}^{2+}$-deprived media is expressed as a percentage of the mean control value found over the 5 min preceding glucose administration. (e) Pattern of insulin release obtained after depriving the islets of glucose for 45 min. | Mean ± 1 S.E.M. for insulin output. 1977
The third major calcium movement is its uphill efflux from the cytosol into the extracellular milieu. Little is known about the nature of the system(s) mediating such an efflux: membrane-bound adenosine triphosphatase, passive transport coupled with the downhill movement of another ion or molecule? Glucose, which represents the major regulator of insulin release under physiological conditions, seems to decrease the transfer rate constant for Ca\(^{2+}\) efflux from the B-cell (Malaise et al., 1973). Thus under all conditions so far investigated a rise in the extracellular calcium concentration invariably resulted in an initial fall in \(\text{^{45}Ca}^{2+}\) efflux from prelabelled perfused islets.

The effect of glucose on \(\text{^{44}Ca}^{2+}\) efflux is apparently secondary to its intracellular metabolism. Indeed, under a variety of experimental situations, including exposure of the islets to the anomers of D-glucose (Malaise et al., 1976d), the net uptake of \(\text{^{45}Ca}^{2+}\) is proportional to the rate of glycolysis (Sener et al., 1976). The intimate nature of the link between glycolytic and Ca\(^{2+}\) fluxes remains to be elucidated. Our present working hypothesis is that the cytosolic concentration of NADH plays a crucial role in such a coupling.

The data so far available on the movements of Ca\(^{2+}\) were used to compute flux rates and pool sizes for this cation in the B-cell. The calculated concentration of Ca\(^{2+}\) in the cytosol in such a model allows one to predict the rate of insulin release under steady-state conditions (W. J. Malaise, unpublished work). Heterogeneity of the cytosolic Ca\(^{2+}\) pool (e.g. due to the existence of an ectoplasmic subcompartment) is postulated to account for rapid changes in hormone-secretion rate.

Incidentally, there exists a chronological hierarchy between the successive events in the secretory sequence. The rise in extracellular glucose concentration (Fig. 1a) is followed by an almost immediate increase in the concentration of intracellular substrates (e.g. glucose; Fig. 1b) and cofactors (e.g. NADH; Fig. 1c), itself leading, during the second minute of exposure to glucose, to a subsequent change in Ca\(^{2+}\) efflux (Fig. 1d). The latter phenomenon causes insulin release after 2 min of stimulation by glucose (Fig. 1e).

A last aspect of Ca\(^{2+}\) handling by the B-cell corresponds to the release of \(\text{^{45}Ca}^{2+}\) usually occurring at the time of insulin release (Malaise et al., 1973). The relatively high calcium content of the secretory granules, as judged from both radioisotopic and ultrastructural criteria, suggests that such a phenomenon could correspond, in part at least, to a release of Ca\(^{2+}\) concomitant with the exocytotic process. However, under suitable conditions, glucose-induced insulin release can be abolished without complete suppression of the increase in \(\text{^{45}Ca}^{2+}\) efflux which occurs, in response to glucose administration, a few minutes after the initial fall in effluent radioactivity (W. J. Malaise, unpublished work).

The remodelling of Ca\(^{2+}\) fluxes evoked by different insulinotropic agents may in turn affect several parameters of islet function. For instance, the cytosolic accumulation of bivalent cations evoked by glucose, theophylline, Ba\(^{2+}\) or a high extracellular Ca\(^{2+}\) concentration seems to be associated with a decrease in plasma-membrane permeability to effluent \(^{86}\text{Rb}\) (Boschero & Malaise, 1977) and, hence, could contribute to the membrane depolarization which precedes the bioelectrical spike activity in the B-cell (Meissner & Atwater, 1976). And last, the accumulation of Ca\(^{2+}\) at the ectoplasmic site of the B-cell may trigger insulin release both by removing an electrostatic energy barrier to membrane–membrane interaction (Matthews, 1970) and activating the microtubular–microfilamentous system thought to provide the motive force for the translocation and release of secretory granules (Malaise et al., 1975). In support of such a view, glucose and the ionophore A23187 were found to augment the contractile activity of the cell boundary in monolayer cultures of endocrine pancreatic cells examined by time-lapse cinematography (Orci et al., 1974; Somers et al., 1976a).

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Intracellular Localization of Calcium in Pancreatic B-Cells

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The first indications that Ca²⁺ is an essential requirement for secretion of insulin were obtained in perfused pancreas preparations by Grodsky & Bennett (1966), and it has subsequently been shown that an adequate extracellular Ca²⁺ concentration is essential for sustained secretion of insulin in vitro by any of the pancreas or isolated islet preparations that are now in common use. More recent work, especially by Malaisse and his collaborators, has explored the fluxes of ⁴⁵Ca²⁺ into and out of intact cells in isolated islets in a wide variety of situations, and these studies are discussed elsewhere in this colloquium (Malaisse, 1977). The present paper reviews some investigations of the localization of calcium within B-cells, and of factors which may alter Ca²⁺ distribution between individual organelles during various phases of insulin secretion.

Calcium distribution in B-cell organelles

It is obviously of critical importance to the interpretation of data on Ca²⁺ fluxes across plasma membranes to obtain some knowledge of the compartmentation and sites of accumulation of Ca²⁺ in various organelles. Two approaches have been made to this problem: firstly, to attempt indirect localization of Ca²⁺ by the use of pyroantimonate procedures, and secondly, to attempt direct analysis of the Ca²⁺ content of various organelles by means of X-ray micro-analysis.

Pyroantimonate procedures. Treatment of tissues with potassium pyroantimonate during fixation with osmium tetroxide was originally suggested as a procedure for