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# Calcium signalling and secretory epithelia

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ABSTRACT

$Ca^{2+}$ is now firmly established as the most important intracellular regulator of physiological and pathological events in a vast number of different cell types, including secretory epithelia. In these tissues, $Ca^{2+}$ signalling is crucially important for the control of both fluid secretion and electrolyte secretion as well as the regulation of macromolecule secretion. In this overview article, I shall attempt to give some general background to the concepts underlying our current thinking about $Ca^{2+}$ signalling in epithelia and its roles in regulating secretion. It is outside the scope of this review to provide a comprehensive account of $Ca^{2+}$ signalling and the many different processes in the many different secretory epithelia that are controlled by $Ca^{2+}$ signals. It is my aim to draw attention to some general features of $Ca^{2+}$ signalling processes in secretory epithelia, which are rather different from those in, for example, endocrine glands. The principal examples will be taken from studies of exocrine cells and, in particular, pancreatic acinar cells, as they are the pioneer cells with regard to investigations of $Ca^{2+}$ signalling due to primary intracellular $Ca^{2+}$ release. They also represent the cell type which has been characterized in most detail with regard to $Ca^{2+}$ transport events and mechanisms.



## 1. Introduction: fundamentally different $Ca^{2+}$ signalling mechanisms in endocrine and exocrine secretory cells

The history of $Ca^{2+}$ and secretion started with the realization that neurotransmitter secretion is $Ca^{2+}$-dependent [1]. In gland cells, the original studies by Douglas and Rubin [2] – of the effects of cholinergic stimulation on catecholamine secretion from the adrenal medulla – established that endocrine secretion was absolutely and acutely dependent on the presence of external $Ca^{2+}$, whereas later work on salivary glands and the pancreas showed that exocrine secretion could be evoked in the absence of external $Ca^{2+}$, but could not be sustained unless external $Ca^{2+}$ was present [3–5].

It is now clear that in all secretory cells – nerve cells, endocrine and exocrine cells – exocytosis requires ATP, $Ca^{2+}$ and $Mg^{2+}$ [6]. Comparison of stimulus-secretion coupling in exocrine and endocrine cells, exemplified by the neighbouring pancreatic acinar and insulin-secreting beta-cells, show that these two cell types go about supplying $Ca^{2+}$ and ATP for the secretion process in radically different ways. In the insulin-secreting beta-cells, the initial step after cellular glucose uptake is ATP production due to metabolism of glucose, which results in an increase in the intracellular ATP/ADP ratio [7,8]. This, in turn, reduces the open state probability of the ATP/ADP-sensitive $K^+$ channels [9,10], which are crucial for setting the membrane potential in these cells, so that membrane depolarization occurs [11]. The depolarization opens voltage-gated $Ca^{2+}$ channels causing $Ca^{2+}$ influx [12–14] and the resulting rise in the cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) activates exocytotic insulin secretion [7,8] (Fig. 1). The sequence of events is thus: (1) glucose uptake, (2) glucose metabolism, (3) ATP generation, (4) closure of $K^+$ channels resulting in membrane depolarization, (5) opening of voltage-gated $Ca^{2+}$ channels and finally $Ca^{2+}$-dependent exocytotic secretion [7,8,14,15]. The stimulant-evoked membrane conductance changes thus precede and cause the rise in $[Ca^{2+}]_i$.

The sequence of events is completely different in the pancreatic acinar cells. Here the primary event is stimulant-evoked release of $Ca^{2+}$ from intracellular stores (Fig. 2) causing a rise in $[Ca^{2+}]_i$, which in turn results in rapid uptake of $Ca^{2+}$ into strategically placed mitochondria in the secretory pole [21] (Fig. 2A). The rise in the intra-mitochondrial $[Ca^{2+}]$ then activates three $Ca^{2+}$-sensitive dehydrogenases in the Krebs cycle resulting in increased ATP production [22,23] (Fig. 2A). There is no primary membrane conductance change in these cells, but the rise in $[Ca^{2+}]_i$ due to the primary $Ca^{2+}$ release from the ER activates $Cl^-$ channels in the secretory apical membrane (Fig. 2B), which are important for the fluid secretion that needs to accompany the exocytotic secretion of digestive (pro)enzymes. Whereas in the insulin-secreting beta-cells the crucial cytosolic $[Ca^{2+}]$ rise is a consequence of primary

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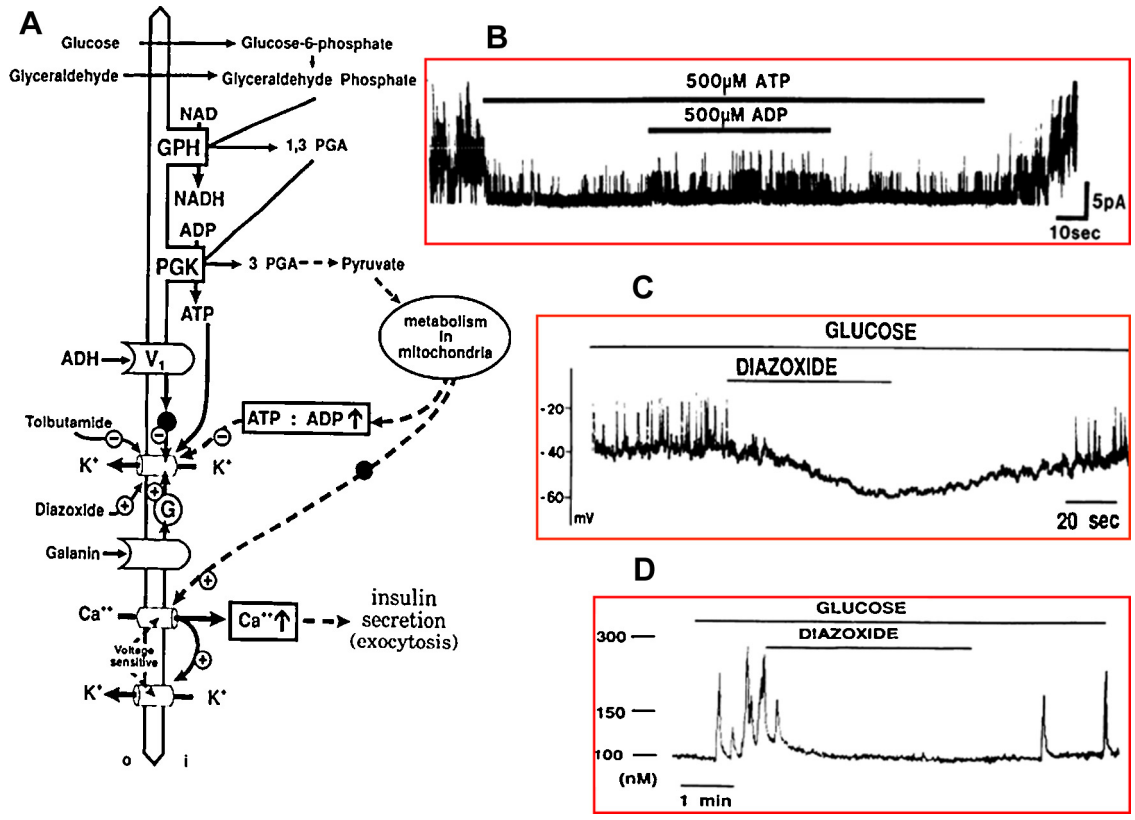


**Fig. 1.** Stimulus-secretion coupling in the insulin-secreting pancreatic beta-cells. (A) Simplified scheme illustrating how the metabolism of glucose influences ion channel opening in the plasma membrane and thereby activates $Ca^{2+}$ influx stimulating exocytotic insulin secretion. GPH, glyceraldehyde phosphate dehydrogenase; 1,3 PGA, 1,3-diphosphoglycerate; 3 PGA, 3-phosphoglycerate; PGK, phosphoglycerate kinase; ADH, antidiuretic hormone (vasopressin). (B) Continuous cell-attached membrane patch current recording obtained from an insulin-secreting cell line (RINm5F) after permeabilization of the plasma membrane outside the isolated patch area (functionally equivalent to isolated inside-out membrane patch). The recording starts during a period in which there is no ATP in the external solution (in contact with the functional inside of the membrane area from which recording is made) and then shows that addition of ATP evokes closure of most of the $K^+$ channels. Subsequent addition of ADP, on top of ATP, increases – reversibly – the probability of $K^+$ channel opening. (C) Whole-cell voltage recording from insulin-secreting cell. In the presence of glucose there is continuous action potential firing. Diazoxide (an agent opening ATP/ADP-sensitive $K^+$ channels) hyperpolarizes the plasma membrane and stops the firing of action potentials. The effect is reversible. (D) Continuous $[Ca^{2+}]_i$ measurement in insulin-secreting cell. Glucose evokes cytosolic $Ca^{2+}$ spiking, which is then abolished by application of diazoxide, demonstrating the voltage-sensitive nature of the glucose-elicited $Ca^{2+}$ spiking.

Compiled and modified from Petersen [15], Dunne and Petersen [9], and Martin et al. [16].

membrane conductance changes, the sequence is reversed in the acinar cells, because here it is the cytosolic $[Ca^{2+}]$ rise that elicits the membrane conductance change that allows fluid secretion to occur. It may seem puzzling why two secretory cell types in the same organ should handle $Ca^{2+}$-dependent secretion in such fundamentally different ways, but the acinar cells could not function properly if they had to depend on voltage-gated $Ca^{2+}$ channels. This is because opening of such channels requires large amplitude membrane depolarization, which would make fluid secretion very difficult due to the major changes in transmembrane driving forces. The beta-cell needs small conductance changes to evoke large potential changes, whereas the acinar cells require large ion fluxes for salt and fluid secretion, but only very small potential changes in order not to destroy the crucial transmembrane ionic gradients that drive ion flow. Furthermore, the $Ca^{2+}$ signals that are needed to activate pancreatic acinar secretion should not occur at the basal membrane, but at the apical membrane and this is best achieved by localizing $Ca^{2+}$ release channels in the apical part of the ER, far away from the base of the cell [24].

## 2. $Ca^{2+}$ and exocrine secretion

Work on the role of $Ca^{2+}$ in epithelial secretion only began in earnest with the studies on salivary secretion carried out by Douglas and Poisner [3]. In contrast to what had been found in experiments on the perfused adrenal gland [2], the initial secretion of saliva in response to ACh stimulation was not abolished by removal of external $Ca^{2+}$ and the secretory response to stimulation only gradually diminished during prolonged exposure to a $Ca^{2+}$-free solution. This made it difficult to come to a conclusion about the exact step in the secretion process that was $Ca^{2+}$-dependent [3].

Important findings establishing the existence of an ATP-dependent $Ca^{2+}$ uptake mechanism in microsomal preparations from salivary glands were made by Selinger et al. [25] and Alonso et al. [26], but this still did not clarify the mechanism by which $Ca^{2+}$ could control secretion. Shortly thereafter, Nielsen and I [27] carried out experiments on perfused cat submandibular glands investigating the movements of $^{45}Ca^{2+}$, in which we established that ACh and adrenaline primarily evoked release of $Ca^{2+}$ from an intracellular store and only secondarily stimulated $Ca^{2+}$ uptake into the cells from the extracellular solution. Similar data were shortly thereafter generated in studies on the exocrine pancreas [28,29] (Fig. 3). It was also shown that enzyme secretion followed rapidly after the initial intracellular $Ca^{2+}$ release (Fig. 3A). The main conclusion of the early mammalian exocrine gland $Ca^{2+}$ transport studies, namely that ACh evokes release of $Ca^{2+}$ stored in the endoplasmic reticulum (ER) causing a rise in the cytosolic $[Ca^{2+}]$, has turned out to be correct, although it was only several years later that the predicted ACh-evoked rise in $[Ca^{2+}]_i$ could be demonstrated by exploiting the