What is the Mechanism of the Calcium Influx to Pancreatic Acinar Cells Evoked by Secretagogues?

O. H. Petersen and Y. Maruyama

The Physiological Laboratory, University of Liverpool, P.O. Box 147, Brownlow Hill, Liverpool, L69 3BX, Great Britain

Introduction. In a recent paper, O'Doherty and Stark (1982) reported that the acetylcholine-evoked increase in the free ionized calcium concentration \([\text{Ca}^{++}]_i\) in mouse pancreatic acinar cells is acutely dependent on the presence of extracellular Ca. They concluded that acetylcholine (ACh) increases the membrane Ca permeability in addition to increasing Na permeability. This finding of a secretagogue-evoked increase in membrane Ca permeability is in agreement with earlier tracer flux studies (Kondo and Schulz 1976) and with the known dependence of pancreatic acinar fluid and enzyme secretion on extracellular Ca during sustained stimulation (Petersen and Ueda 1976; Ueda and Petersen 1977).

However, electrophysiological studies have failed to identify a Ca component in the ACh-evoked membrane potential and conductance change although sustained ACh-evoked acinar membrane depolarization is acutely dependent on extracellular Ca (Petersen 1980).

The purpose of this brief note is to describe a very simple hypothesis that seems to resolve the apparent discrepancy and is able to account satisfactorily for stimulus-secretion coupling.

The Patch Clamp Results

It has now become possible to investigate mechanisms of membrane ion permeation very directly with the improved patch-clamp technique for high-resolution current recording (Hamill et al. 1981). Recent studies on mouse and rat pancreatic plasma membrane patches excised from the baso-lateral aspect of collagenase-isolated acini have demonstrated the existence of a 30-35 pS cation channel activated by micromolar concentration of Ca from the internal side of the membrane. This channel, which discriminates poorly between Na and K has a mean open time in the 100 ms to 1 s range and is not voltage sensitive (Maruyama and Petersen 1982a). The internal Ca control of this channel probably provides the explanation for the previously demonstrated internal Ca control of radioactive Na efflux from isolated pancreatic plasma membrane vesicles (Schulz and Heil 1979). It has very recently been demonstrated that this 30-35 pS cation channel can be activated by cholecystokinin (CCK) and ACh in intact cells when the stimulants are applied outside the patch area; i.e. the secretagogues activate the channels indirectly via a messenger (Maruyama and Petersen 1982b, Petersen and Maruyama 1983). A simple scheme thus presents itself as shown in Fig. 1. ACh or CCK releases intracellular Ca from the same pool increasing \([\text{Ca}^{++}]_i\). This in turn opens up the cation channel resulting in Na influx. Removal of agonist (and/or inclusion of antagonist) causes rapid reuptake of Ca into the trigger pool (Schulz and Stolze 1980, Stolze and Schulz 1980) reduces \([\text{Ca}^{++}]_i\) and thus closes the channel.

![Fig. 1. Simplified scheme of the control of membrane conductance in pancreatic acini. The most important agonists controlling this pathway are acetylcholine (ACh) and cholecystokinin (CCK). The antagonists are atropine and dibutyryl cyclic guanosine 3,5-monophosphate (8Br cGMP) respectively. It is proposed that the Ca-activated cation channel is not perfectly selective for monovalent ions, but allows a small leak of Ca. Under normal ionic conditions there exists a very large electrochemical gradient favouring Ca influx and opening of the channel will thus lead not only to Na influx but also to a small Ca influx.](image-url)}
CALCIUM LEAK THROUGH CATION CHANNEL

The crucial question is now whether the Ca-activated cation channel is also permeable to Ca. In experiments on excised membrane patches using symmetrical Na sulphate solutions with or without the presence of a Ca gradient we have been unable to observe a difference in the reversal potential for the unitary current steps through the channel (Maruyama and Petersen 1982a). This might seem to argue against Ca permeability of the channel, however, calculations of the reversal potential (using the extended form of the constant field equation taking into account both mono and divalent ions - Jan and Jan 1976), even assuming a Ca/Na permeability ratio as high as 1, indicates that an imposed Ca gradient of 1.28 mM outside against 0.001 mM inside would change the reversal potential by less than 0.5 mV from the 0 mV obtained with completely symmetrical solutions (e.g. with a 1.28 mM Ca concentration on both sides). A deviation of this magnitude is far too small to have been observable in these experiments (Maruyama and Petersen 1982a). An evaluation of the possible Ca permeability of this type of cation channel, which is also found in excitable tissues such as cardiac cells and neuroblastoma cells (Colquhoun, Neher, Reuter and Stevens 1981; Yellen 1982) has been undertaken by Yellen (1982). He was unable to detect inward current through the channel when all Na in the external solution was replaced by Ca. Yellen (1982) concluded that these Ca-activated cation channels were essentially impermeable to Ca. We have made similar observations on mouse pancreatic acini (Petersen and Maruyama 1983). However, the Ca influx required to raise $[Ca^{++}]_i$ sufficiently in the pancreatic acinar cells may be small and it is worth considering whether a slight leak of Ca through the Ca-activated cation channel could account for the findings so far made.

ESTIMATION OF Ca/Na CONDUCTANCE RATIO

Considering the effects of ACh on the pancreatic acinar cell ionized Na and Ca concentrations reported by O'Doherty and Stark (1982) it is clear that the absolute increase in $[Na^+]_i$ evoked by maximal stimulation (5 mM) is much larger than the corresponding increase in $[Ca^{++}]_i$ (0.0005 mM). Even allowing for the likely intracellular binding of the vast majority of extracellular Ca flowing into the acinar cells it appears not unreasonable to assume that the Ca influx is at least about 100 times smaller than the Na influx.

We can use the following simple quantitative treatment:

$$I_i = g_i (V - E_i)$$ \hspace{1cm} (1)

and

$$I_1 = z F J_1$$ \hspace{1cm} (2)

where $I_i$ is the current carried by the ion $i$, $g_i$ the conductance for the ion $i$, $V$ the membrane potential, $z$ the valency, $F$ the Faraday number and $J_1$ the flux of ion $i$.

With the help of (1) and (2) it can be calculated using reasonable values for membrane potential (Petersen 1980) and intracellular ionic concentrations (O’Doherty and Stark, 1982) that a $J_1 / J_{Ca}$ ratio of 100 can be obtained with a $g_{Na}/g_{Ca}$ ratio of 68. The Ca-activated cation channels that were studied in the pancreatic acinar plasma membrane have a single channel conductance of 30-35 pS in symmetrical Na solutions or in the presence of normal Na and K gradients. In the presence of symmetrical high Ca solutions (without Na) the single-channel conductance would then be expected to be about 0.5 pS. At a membrane potential of 50 mV unitary current steps would only be about 0.02 pA instead of the 1.6 pA actually observed in the presence of Na solutions. Such small current steps could not possibly be resolved. It is thus clear that a leak of Ca through the Ca-activated cation channel, too small to be noticeable even in high resolution current recording experiments under optimal conditions, could account for a very appreciable Ca influx.

ARE THERE LIKELY TO BE CA-SELECTIVE CHANNELS?

Ca channels have recently been studied with the patch clamp technique in neurons (Lux and Nagy 1981). These channels have conductances of 5 to 15 pS with a mean open time of only about 3 ms. Such channels, if they were present in the pancreatic acinar plasma membrane could easily have been missed in the patch clamp experiments so far carried out (Maruyama and Petersen 1982a). It is therefore by no means possible to rule out the existence of Ca selective channels in the pancreatic acinar cells. However, the Ca-channels described by Lux and Nagy (1981) were voltage-sensitive, since the frequency of occurrence of the unitary events increased strongly with depolarization. Regulation of Ca channels by neurotransmitters has been described, but also in such cases the Ca channels are gated by membrane potential (Reuter, Stevens, Tsien and Yellen 1982). In the pancreatic acinar cells there are no indications of electrical excitability (Petersen 1980) and secretion cannot be evoked by membrane depolarization per se (Schulz and Stolze 1980; Pearson, Davison, Collins and Petersen 1981). There is no reason, therefore, to expect that Ca-channels similar to those described in electrically excitable tissues will eventually be found in this tissue.

CONCLUSION

The simple model shown in Fig. 1 can be used to explain pancreatic acinar stimulus-secretion coupling by assuming that the Ca-activated cation channel is not perfectly selective for mono-valent cations, but enables a very slight...