Protein kinase C and the modulation of ATP-sensitive potassium channels in insulin-secreting cells.

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In the insulin-secreting cells of the pancreatic islets of Langerhans, ATP-sensitive potassium (K+ATP) channels play an important role in the regulation of the cell membrane potential. In brief, glucose-induced closure of the K+ATP channels initiates a depolarisation of the membrane, which is responsible for opening voltage-gated Ca2+ channels, leading to a rise in the cytoplasmic calcium ion concentration ([Ca2+]i) (for reviews see [1,2,3]). An increase in [Ca2+]i is a prerequisite for glucose-induced insulin secretion [4]. In addition, there is evidence to indicate that the K+ATP channels are also the target site for a number of hormones and neurotransmitters that are able to promote or inhibit secretion by mechanisms, that are in part dependent upon changes in the cell membrane potential [2,3].

The cellular events that govern opening and closure of these channels have been addressed by several laboratories, and whilst there is a great many reports for interactions of different cytoplasmic nucleotides with the channel, the molecular events that underlie these effects are only partially understood. This is particularly relevant to the adenine nucleotides, since both ATP and ADP have multifarious effects on the channels: promoting both inhibition and activation under different conditions [1-3]. One mechanism through which these effects could be mediated, is by the actions of protein kinases associated with the ion channel. With this in mind our study was undertaken to investigate the actions of protein kinase inhibitors on K+ATP channels in both the presence and the absence of intracellular ATP and ADP.

The experiments were performed using RINm5F insulinoma cells by the technique of patch-clamp electrophysiology, using the cell-free inside-out patch, and the permeabilized (or 'open') cell recording configurations under quasi-physiological cation gradients. By these approaches we were able to gain access to the internal face of the membrane through changes made to the composition of the bathing solution. Experiments were also made using the outside-out configuration in order to examine the effects of externally applied polymyxin B on single-channel current events.

When applied either to the inside or the outside face of the membrane we consistently found that polymyxin B induced blockade of ATP-regulated K+ channels: reducing the channel open-state probability to approximately 10% of that seen in the control situation (n=10 separate patches). There were, however, apparent differences in the mechanisms of block. From the outside channel inhibition was associated with a reduction in the amplitude of single-channel current events, and the appearance of sub-conductance states. These effects were not seen when polymyxin B was directly added to the inside of the plasma membrane; under these conditions changes in the frequency of channel opening were the predominant mechanisms of channel block. Polymyxin B-induced channel inhibition was also seen in patches of membrane excised from cells pre-treated overnight with 1 μM of the phorbol ester, phorbol myristate acetate (PMA) in order to down-regulate C-kinase (n=15). These data would therefore tend to suggest that polymyxin B has effects directly on the channel, and not associated with protein kinase C-mediated events.

In a separate series of experiments we examined the action of polymyxin B in the presence of cytosolic ATP and ADP. With 0.5mM ATP: 0.5mM ADP available, polymyxin B once again induced channel inhibition [with the same potency as that seen in the absence of the nucleotides] but under these conditions we found that the actions of the peptide were not reversible. Recovery from block was only seen following the removal of the externally applied nucleotides. This novel effect of polymyxin B was seen on 18/20 occasions in 16 separate patches. However, when exactly the same protocol was carried out on patches of membrane excised from cells pre-treated overnight with 1 μM PMA: polymyxin-B induced block was readily reversible (n=8).

There is therefore a marked difference in the mechanism of action of polymyxin B on K+ATP channels in the presence of a ATP-ADP depending upon whether the cells have undergone PKC down-regulation or not.

In conclusion we believe that this data indicated that polymyxin B has multiple effects on K+ATP channels in insulin-secreting pancreatic B-cells. There are direct actions of the compound upon the ion channels; and difference in the mechanism of block whether the peptide is applied internally or externally, and we also have evidence for indirect effects possible involving endogenous protein kinases associated with the channel, see figure 1.

Figure 1. Possible mechanisms for polymyxin B-induced block of K+ATP channels in insulin-secreting cells. (i) polymyxin B has direct effects upon the channel characterised as a 'gating' modifier from the inside of the membrane and a 'conductance' modifier from the outside, and (ii) polymyxin B appears to have indirect effects on the channels, possible mediated through inhibition of endogenous protein kinase(s) associated with the channel.

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