The ATP-sensitive potassium (K$_{ATP}$) channel, a particular class of potassium channel inhibited by intracellular ATP, was first described in cardiac muscle [1] and has subsequently been found in pancreatic B-cells [2], skeletal muscle [3], smooth muscle [4] and central neurons [5]. There is considerable evidence that this channel plays an essential role in the regulation of insulin secretion from the pancreatic B-cell [6].

Glucose-stimulated insulin release is dependent on both the depolarization of the B-cell membrane which reaches the threshold for initiation of electrical activity at concentrations between 5 and 7 mM. This electrical activity consists of slow oscillations of membrane potential (bursts or slow waves) between a depolarized plateau potential on which Ca$^{2+}$-dependent action potentials are superimposed and a hyperpolarized silent phase. Further increases in glucose concentration augment the frequency of action potentials by increasing the duration of the plateau and decreasing the intervals between them. Finally, at glucose concentrations above 16 mM electrical activity is continuous. Glucose modulation of B-cell electrical activity serves to regulate Ca$^{2+}$ entry across the plasma membrane and thereby insulin secretion.

The resting potential of the unstimulated B-cell is predominantly determined by the K$_{ATP}$ channel. All primary secretagogues, including glucose and sulphophyrene, depolarize the B-cell, and initiate electrical activity by closing this channel. The properties and regulation of the K$_{ATP}$ channel have been previously reviewed [6]. Here we concentrate on three questions which have been the focus of recent studies: (1) Is ATP the main physiological regulator of K$_{ATP}$ channel activity in the B-cell? (2) Can the modulation of K$_{ATP}$ channel activity account for the concentration-dependent effects of glucose on B-cell electrical activity and insulin secretion? [8] Is the inhibitory action of hormones, such as galanin, on B-cell electrical activity mediated through changes in K$_{ATP}$ channel activity?

Is ATP the physiological modulator of K$_{ATP}$ channel activity?

The modulation of K$_{ATP}$ channel activity by cytosolic constituents is extremely complex and still not completely understood. However, there is considerable evidence that the primary determinant of channel activity in the intact B-cell is the intracellular ratio of ATP to ADP. Local changes in this ratio constitute the mechanism by which channel activity is linked to glucose metabolism.

Several observations support a physiological role for ATP as a regulator of K$_{ATP}$ channel activity. First, channel activity is blocked by micromolar ATP concentrations in excised patches [2]. Secondly, the activity of the channel in cell-attached patches is increased by metabolic inhibitors which lower intracellular [ATP] and decreased by secretagogues which raise cytosolic [ATP] [10-13]. Thirdly, there is a good correlation between the glucose dependence of channel inhibition and that of the increase in cytosolic ATP. Most of the change in both channel activity and ATP concentration occurs between 0 and 7 mM-glucose [10, 14]. Finally, the time course of changes in intracellular ATP and in K$_{ATP}$ channel activity are similar [15, 16].

The major argument against ATP as the physiological link between B-cell metabolism and channel activity has always been that in the inside-out patch the channel is half-maximally inhibited by 10 $\mu$M- and completely blocked by 1 mM-ATP [2]. This does not accord with the level of channel activity observed in cell-attached patches on intact cells where measured intracellular ATP concentrations are around 5 mM [17]. Recent experiments suggest, however, that the ATP sensitivity in the intact B-cell may be much lower. Simultaneous measurements of ATP concentration and sulphophyrene-sensitive Rb$^{+}$-efflux in intact tumour cells suggest that the channels are half-maximally inhibited by 1.5-2 mM-ATP and about 90% blocked by 5 mM-ATP [18, 19]. A similar ATP sensitivity is observed in whole-cell recordings using pipettes containing different ATP concentrations (P. Rorsman, unpublished work). The channel open probability in the intact cell is in good agreement with the ATP sensitivity measured in flux studies. In the absence of glucose, the input conductance of the intact B-cell is about 1-2 nS (F. M. Ashcroft & P. Rorsman, unpublished work using the perforated patch whole-cell recording method; [20]). The maximum input conductance, obtained in standard whole-cell recordings after washout of intracellular ATP, is between 10 and 20 nS. Thus more than 90% of K$_{ATP}$ channels are closed in the resting B-cell, as expected from the ATP sensitivity measured in intact cells for a cytosolic ATP concentration of 5 mM.

One reason which may account for the difference between the ATP sensitivity measured in the intact cell and in the inside-out patch is that the ATP sensitivity in the inside-out patch is altered due to loss of additional channel modulators. For example, ADP is known to reduce the ATP sensitivity of the channel [21, 22]. Another possibility is that the ATP concentration just below the membrane may be lower than the measured total ATP [18]. This might occur as a consequence of the activity of membrane ATPases. At first sight it might seem surprising that only a small fraction (<10%) of the K$_{ATP}$ channels are subject to glucose modulation. Indeed, it has been suggested that this finding might be an artefact resulting from the low temperature (20-22°C) at which patch-clamp recordings are normally made. This does not appear to be the case, however, since both the flux studies and our perforated patch recordings were carried out at higher temperatures (37°C [19] and 30°C, respectively). A large number of channels operating near their fully inhibited state may have physiological advantages, such as a stable membrane potential, minimal energy expenditure and a rapid response time [23].

It has recently been suggested that activation of protein kinase C (PKC) may mediate channel inhibition induced by secretagogues [24]. Support for this hypothesis comes from the work of Wolheim et al. [24] on the insulin-secreting B-cell line, RINm5F. They demonstrated that t-glycerol-aldehyde, which closes ATP channels and stimulates secretion in this cell line, increases the intracellular concentration of 1,2-diacylglycerol, an endogenous activator of PKC.
Do hormones influence $K_{\text{ATP}}$ channel activity?

A number of hormones and neurotransmitters are capable of inhibiting glucose-stimulated insulin release [29, 30]. Among these, the most widely studied have been adrenaline (or the $\alpha_2$-adrenergic agonist clonidine), somatostatin and galanin. Although it is clear that these inhibitors act at a number of different levels in the secretory process, all have been shown to hyperpolarize the B-cell membrane, inhibit electrical activity and reduce cytosolic calcium concentrations [29-34]. In addition, they appear to exert a direct inhibitory action on the secretory machinery [28, 29]. All of these effects can be blocked by pertussis toxin, suggesting that they involve activation of a G-protein or G-proteins [29, 31]. The similarity between the actions of galanin, somatostatin and clonidine suggest that subsequent to activation of the receptor they may act by a common mechanism. Here we focus on galanin, the best studied of the three to date.

It was early noted that the effects of galanin were similar to those produced by diazoxide, an activator of $K_{\text{ATP}}$ channels, and it was therefore postulated that galanin also promoted $K_{\text{ATP}}$ channel activity [35]. This hypothesis has since been confirmed by patch-clamp studies and $\text{^{86}Rb^{+}}$ efflux measurements on RINm5F cells [31, 34]. The most detailed of these studies has shown that galanin-stimulated $K_{\text{ATP}}$ channel activity involves G-protein activation [31]. The results suggested that a membrane-associated G-protein directly couples receptor activation to channel opening and that a diffusible second messenger is not involved in mediating the action of galanin on electrical activity.

It has been much more difficult to demonstrate consistently that either galanin, somatostatin or clonidine influence $K_{\text{ATP}}$ channel activity in normal B-cells. Studies in our own laboratories have failed to observe consistent increases in

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**Fig. 1. Current-clamp recording of membrane potential (MP) from a mouse B-cell isolated as in [8]**

Perforated patch recording was performed as in [20]. Pipette solution (mm): 20 NaCl, 10 KCl, 70 K$_2$SO$_4$, 7 MgCl$_2$, 10 Hepes-KOH (pH 7.4), 50 sucrose, 50 $\mu$g of nystatin/ml in 0.2% dimethylsulphoxide. Bath solution (mm): 138 NaCl, 5.6 KCl, 2.6 CaCl$_2$, 1.2 MgCl$_2$, 10 Hepes-NaOH (pH 7.4). Tolbutamide (900 $\mu$g in 0.3% dimethylsulphoxide) was added to the bath solution (arrow) and was present for the rest of the recording. Galanin (100 nm; CalBiochem) was added to the bath solution for the period indicated by the bar. Temperature: 30°C. The Figure shows the chart recorder trace.

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before there is any change in the intracellular ATP concentration. In addition, they found that two exogenous agonists of PKC, a membrane-permeable diacylglycerol (DC$_{18:1}$) and the phorbol ester phorbol 1,2-myristate 13-acetate (PMA) mimicked the effect of glyceraldehyde on $K_{\text{ATP}}$ channel activity. These results constitute good evidence in favour of PKC activation being the major pathway for glyceraldehyde-stimulated insulin release in RINm5F cells. However, studies from two other laboratories have shown opposite effects of PMA in the same cell line [13, 25]. This discrepancy may be a consequence of the well-known ability of cell lines to change their properties in culture. It is questionable whether PKC activation plays any role in glucose-stimulated insulin secretion in normal B-cells, since 12-tetradecanoylphorbol 13-acetate has little effect on B-cell electrical activity [26]. This emphasizes that the normal B-cell in primary culture may provide a better model for the mechanism of insulin release in vivo than a cell line. Cell lines, however, are useful for studying the biophysical properties of the $K_{\text{ATP}}$ channel or as a source material for channel isolation.

Does glucose regulate spike frequency through modulation of $K_{\text{ATP}}$ channels?

Recent microelectrode measurements of membrane potential have demonstrated that low concentrations of tolbutamide produce the same effects on electrical activity as increasing the glucose concentration [27]. Since tolbutamide specifically blocks the $K_{\text{ATP}}$ channel in B-cells [7], the possibility arises that inhibition of the $K_{\text{ATP}}$ channel is important in both the initiation and the modulation of glucose-stimulated electrical activity. This conclusion is not at variance with the finding that in cell-attached patches the $K_{\text{ATP}}$ channel is almost completely blocked by glucose concentrations above 5 mM [9-13]. The B-cell contains more than 1000 $K_{\text{ATP}}$ channels [28], so that even when channel activity is reduced to 1%, at least 10 channels remain active in the cell. This level of activity is not readily resolved in a cell-attached patch but may nevertheless contribute a significant $K^+$ permeability. Consequently, analysis of channel activity at high glucose concentrations requires the use of the whole-cell configuration.

We have recently used the perforated patch method [20] to record whole-cell $K^+$ currents in intact B-cells which are capable of glucose metabolism and in which intracellular second messenger systems are retained. Our results support the idea that some $K_{\text{ATP}}$ channels remain open at glucose concentrations above 7 mM. Even in the presence of 20 mM glucose it is possible to reduce the whole-cell input conductance with 25 $\mu$M tolbutamide.

We emphasize, however, that although $K_{\text{ATP}}$ channels may modulate the duration and the frequency of the slow waves, they do not appear to be directly involved in their generation. In the perforated patch recordings it is possible to record whole-cell $K^+$ currents and changes in membrane potential from the same cell. We have found that the silent phases between the bursts do not appear to be associated with an increased $K^+$ conductance. This suggest that oscillations in $K_{\text{ATP}}$ channel activity do not produce the bursts and also excludes the involvement of other $K^+$ channels (for example, Ca$^{2+}$-activated $K^+$ channels).

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K<sub>KATP</sub> channel activity in either whole-cell recordings or in cell-attached patches under the same conditions as those reported for RINmSF cells. In a few cases, galanin inhibited spiking produced by 50 mM-glucose and led to a slight, transient increase in channel openings in cell-attached patches when added to the bath solution [36]. In most cells there was no effect. Similar difficulties were found with somatostatin and clonidine.

Recently, we have utilized the perforated patch method of whole-cell recording to investigate the effects of galanin on B-cell electrical activity and whole-cell patch-clamp recordings under the same conditions as those reported for RINmSF cells. In a few cases, galanin inhibited cell-attached patches under the same conditions as those produced by glucose or by tolbutamide (Fig. 1).

B-cell electrical activity and whole-cell patch-clamp recordings under the same conditions as those produced by glucose or by tolbutamide (Fig. 1).

In contrast, hormones and neurotransmitters (acetylcholine or its analogue carbachol, and cholecystokinin) whose plasma membrane receptors are coupled to phospholipase C; agonists that activate adenylate cyclase via their specific membrane receptors [I, 21.

Insulin secretion from the pancreatic B-cell is under the influence of a large number of modulators. The main secretagogues include nutrients, gastrointestinal hormones, neurotransmitters and neurohormones. For simplicity, the secretagogues can be subdivided into three groups according to their mode of action: (1) nutrient stimuli (carbohydrates and amino acids) which enter the B-cell and exert their main action by increasing the flux through specific pathways; (2) Ca<sup>2+</sup>-mobilizing hormones and neurotransmitters (acetylcholine or its analogue carbachol, and cholecystokinin) whose plasma membrane receptors are coupled to phospholipase C; (3) hormones, such as glucagon, glucagon-like peptide 1, gastric inhibitory polypeptide and δ-neuropeptide agonists that activate adenylate cyclase via their specific membrane receptors [1, 2]. In vitro, only the compounds belonging to group 1 are capable of initiating and sustaining insulin release [3]. In contrast, hormones and neurotransmitters

Abbreviations used: [Ca<sup>2+</sup>]<sub>i</sub>, cytosolic Ca<sup>2+</sup> concentration; KIC, α-ketoisocaproic acid.

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Cytoplasmic calcium ions and other signalling events in insulin secretion

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Insulin secretion from the pancreatic B-cell is under the influence of a large number of modulators. The main secretagogues include nutrients, gastrointestinal hormones, neurotransmitters and neurohormones. For simplicity, the secretagogues can be subdivided into three groups according to their mode of action: (1) nutrient stimuli (carbohydrates and amino acids) which enter the B-cell and exert their main action by increasing the flux through specific pathways; (2) Ca<sup>2+</sup>-mobilizing hormones and neurotransmitters (acetylcholine or its analogue carbachol, and cholecystokinin) whose plasma membrane receptors are coupled to phospholipase C; (3) hormones, such as glucagon, glucagon-like peptide 1, gastric inhibitory polypeptide and δ-neuropeptide agonists that activate adenylate cyclase via their specific membrane receptors [1, 2]. In vitro, only the compounds belonging to group 1 are capable of initiating and sustaining insulin release [3]. In contrast, hormones and neurotransmitters