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MESSENGER-MEDIATED CONTROL OF POTASSIUM CHANNELS IN SECRETORY CELLS

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SUMMARY

In exocrine acinar cells (pancreas, salivary gland, lacrimal gland) stimulation with hormones or neurotransmitters evokes K^+ loss due to opening of K^+ channels in the plasma membrane whereas in the insulin-secreting pancreatic β-cells, stimulation with glucose or glyceraldehyde evokes membrane depolarization due to closure of K^+ channels. By measuring directly the small K^+ currents flowing through single channels, in electrically isolated patches of plasma membrane of intact cells, it can be shown that stimulants having no direct access to the small membrane area from which recording is made can influence the pattern of channel opening. In the case of hormonal activation of exocrine acinar cells, Ca^{2+} is the final messenger and the K^+-selective channel involved in the response has a high unit conductance, is very voltage sensitive and can be blocked by external tetraethylammonium. In the case of the insulin-secreting cells, the K^+ channel which is inhibited by metabolic stimulation is a voltage-insensitive, inward rectifier which can be blocked by quinine. In experiments on permeabilized cells or cell-free excised, inside-out, membrane patches it can be shown that ATP evokes channel closure and ATP produced by glycolysis may therefore function as the internal messenger.

INTRODUCTION

Channels (pores) permeable to K^+ are of enormous general significance since these transport proteins are responsible for the resting membrane potential in animal cells and also play a crucial role in many aspects of cellular regulation. This short review deals only with channels selectively permeable to K^+ and will focus exclusively on Ca^{2+}-activated and ATP-sensitive channels. Both channels are found in many different types of cells and probably play different roles according to the particular cell function. Direct evidence for Ca^{2+}-activated K^+ channels was first provided by single-channel current recordings from adrenal chromaffin cells (Marty, 1981), and soon thereafter from muscle (Barrett, Magleby & Pallotta, 1982) as well as epithelial cells (Maruyama, Gallacher & Petersen, 1983*a*). Direct evidence for ATP-sensitive K^+ channels was first obtained in cardiac cells (Noma, 1983) and soon thereafter in insulin-secreting pancreatic β-cells (Cook & Hales, 1984). We shall here be concerned only with two types of secretory cells, exocrine acinar cells (salivary

Key words: potassium channel, secretory cell, messenger-mediated control.

glands, pancreas and lacrimal gland), which are controlled by neurotransmitters (for example acetylcholine) or hormones (for example cholecystokinin), and endocrine pancreatic insulin-secreting cells, controlled by glucose metabolism. In both cases, regulation of K^+ conductance pathways turns out to be crucial for the particular cellular function (fluid secretion, insulin secretion), but the mechanisms involved are rather different. Patch-clamp, single-channel current recording is the most direct method for investigating regulation of membrane conductance pathways and the different patch-clamp recording configurations used will therefore first be briefly described (see Fig. 1).

RECORDING CONFIGURATIONS IN PATCH-CLAMP EXPERIMENTS

Conventional intracellular microelectrode methods for current measurement are associated with a background noise of at least 100 pA. The current flowing when a single channel opens is, however, only a small fraction of this background noise. Neher & Sakmann (1976) solved this problem by the patch-clamp method. Instead of inserting a microelectrode into a cell, they pressed a microelectrode tip onto its surface, effectively isolating a patch of membrane. The intrinsic noise increases with the area of the membrane under study and by isolating a small area ($1-10\,\mu m^2$) such low extraneous noise levels are attained that the picoampere currents flowing through single channels can be measured directly.

The seal between the tip of the microelectrode and the outer surface of the cell membrane has under suitable conditions (fire-polished and clean micropipette tip and clean membrane surface) a high electrical resistance [of the order of giga (10^9) ohms] and is mechanically surprisingly stable. The discovery in 1980 of this high-resistance seal by E. Neher, B. Sakmann and their coworkers as well as by R. Horn & J. B. Patlak turned out to be very important as it made entirely new types of experiments possible (Fig. 1) (Hamill *et al.* 1981). The electrically isolated membrane patch can be pulled off the cell (excised) in such a way that the inside of the plasma membrane faces the bath solution (inside-out) or alternatively so that the outside faces the solution in the micropipette (outside-out). By breaking the patch membrane in the cell-attached recording conformation, the solution in the pipette interior gains direct access to the cell interior and cell dialysis is carried out under conditions where the currents across the whole cell membrane can be measured. Equilibration of the cell interior with the bath solution can be done while single-channel currents are recorded by making holes in the plasma membrane outside the isolated patch area with the help of detergents like saponin.

Ca^{2+}- AND VOLTAGE-ACTIVATED K^+ CHANNELS

There are several types of Ca^{2+}-activated pores, but the best characterized one is the high-conductance K^+ channel (Latorre & Miller, 1983; Petersen & Maruyama, 1984). The maximal unit conductance is about 200–300 pS and the probability of channel opening is markedly increased by membrane depolarization or reversal of the

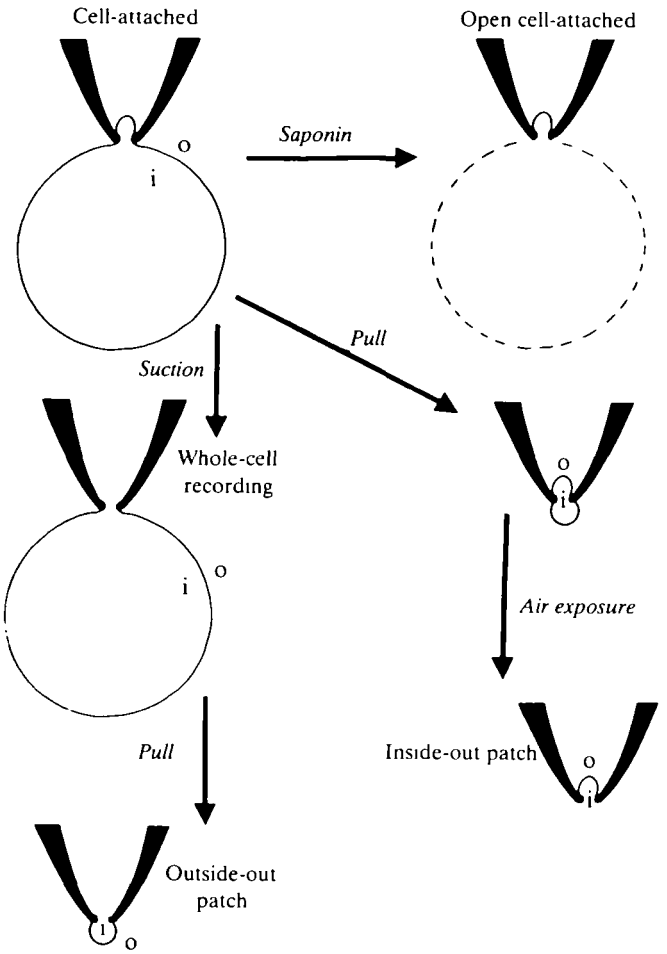

Fig. 1. Schematic representation of the different patch-clamp recording configurations and the procedures used to establish them. A patch-clamp experiment starts in the cell-attached configuration (top left). The tip of the fire-polished recording pipette is gently brought into contact with the cell surface and slight suction is applied to the pipette resulting in a high-resistance seal between the tip of the glass pipette and the cell membrane. The detergent saponin can be introduced briefly into the bath to permeabilize the cell membrane (outside the isolated patch area) in this way allowing equilibration beween cell interior and exterior (open cell-attached configuration). Starting from the original cell-attached situation the patch membrane can be mechanically pulled off leading to the formation of a closed membrane vesicle in the pipette tip. The outer surface of this vesicle can be disrupted by passing the pipette tip briefly through the air–water interface of the bath leading to the excised inside-out membrane configuration. If a short pulse of suction is applied to the pipette in the cell-attached mode the membrane patch can be broken and direct continuity between pipette and cell interior established. The currents flowing across the entire cell membrane can now be recorded (whole-cell recording). If the pipette is thereafter pulled away from the cell the excised membrane fragments will reseal so that an excised outside-out membrane patch is obtained. (From Petersen & Petersen, 1986.)

normal potential. The basic properties of the channel have been worked out in single-channel current recording experiments on excised membrane patches (Fig. 1). When the inside of the membrane is exposed to a Ca^{2+}-free solution containing the Ca^{2+} chelator EGTA, there are virtually no channel openings at negative (normal) membrane potentials. However, when the membrane potential is made positive large unitary current steps can be observed. Fig. 2 shows results recorded from an excised outside-out patch of plasma membrane taken from the insulin-secreting pancreatic cell line RINm5F. At a membrane potential of 0 mV there are no high-conductance channel openings (upper traces) although openings of a much smaller K^+ channel can be observed at a higher gain (lower traces). A voltage jump to +80 mV activates at least three similar high-conductance channels in the patch membrane. In this particular cell type the voltage activation is largely transient (Findlay *et al.* 1985*c*) as is the case in skeletal muscle (Pallotta, 1985*a*) whereas in many other preparations sustained activation is observed (Maruyama, Petersen, Flanagan & Pearson, 1983*b*).

Channel activation can also be evoked by Ca^{2+} but only from the inside of the membrane. Fig. 3 shows single-channel current recordings from an excised inside-out membrane patch taken from a rat pancreatic islet cell. At 0 mV membrane potential and Ca^{2+}-free, EGTA-containing solution in contact with the inside of the membrane there are no openings of the high-conductance channel but, again, a small K^+ channel is in evidence. When the free Ca^{2+} concentration in the bath solution

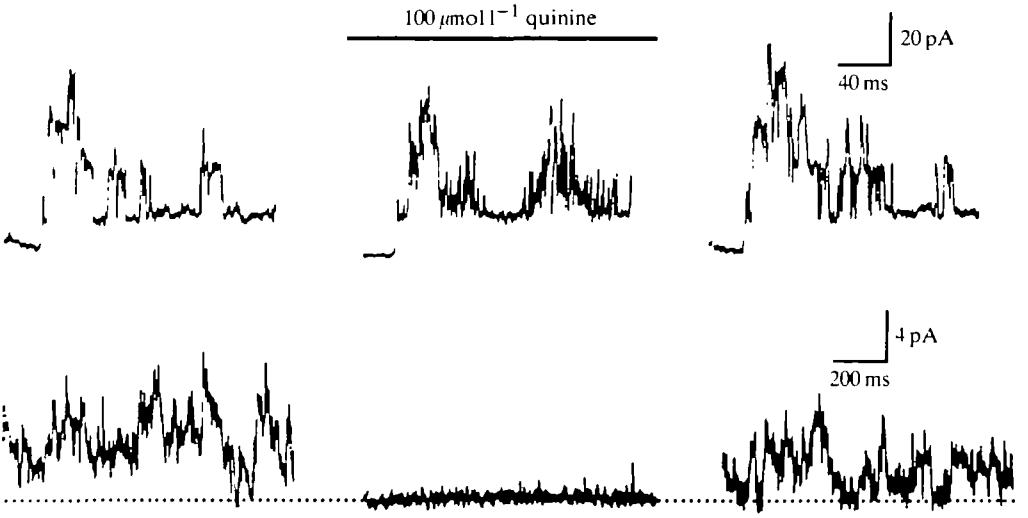

Fig. 2. Rat insulinoma cell line m5F. The effect of quinine on currents through Ca^{2+}- and voltage-activated K^+ channels and inward rectifying K^+ channels. Single-channel current recording from an excised outside-out membrane patch. The upper three traces illustrate voltage-activation (voltage jump from 0 to 80 mV membrane potential) of the Ca^{2+}-activated, high-conductance K^+ channels in the absence and presence of 100 μmol l^{-1} quinine and after return to the quinine-free solution. The lower three traces from the same patch, recorded at a higher gain at a membrane potential of 0 mV, demonstrate that 100 μmol l^{-1} quinine abolishes unitary currents through the inward rectifier K^+ channels. This effect is reversible. (From Findlay *et al.* 1985*c*.)

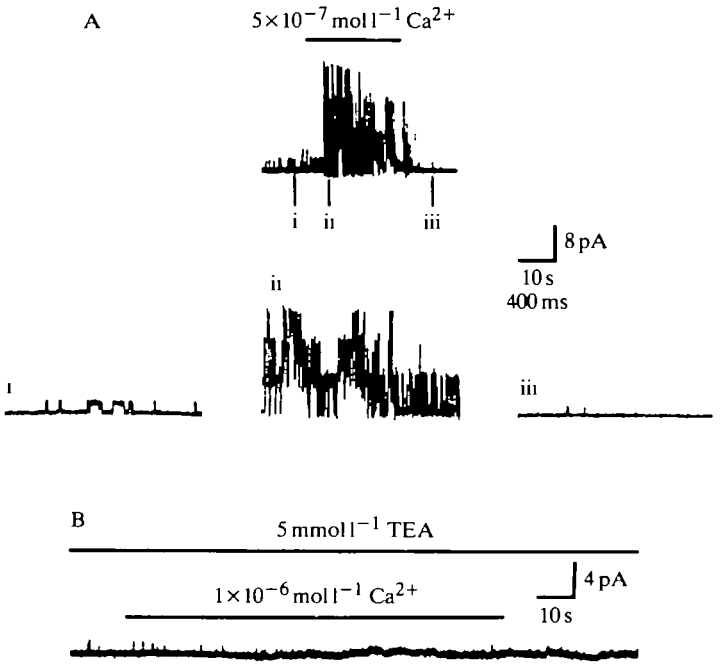

Fig. 3. Rat pancreatic islet cell. Single-channel current records obtained from excised inside-out membrane patches exposed to quasi-physiological Na^+/K^+ gradients. In both cases the patches were voltage-clamped at 0 mV throughout. (A) The upper trace represents a continuous record. The K^+-rich solution which was superfused past the patch pipette contained 1 mmol l^{-1} EGTA and no added Ca. For the period indicated by the bar the K^+-rich solution contained 5×10^{-7} mol l^{-1} Ca^{2+}. The lower traces (i–iii) represent records obtained on a faster time base from the indicated portions of the upper trace. (B) The Na^+-rich solution in the pipette also contained 5 mmol l^{-1} TEA (tetraethylammonium). The K^+-rich solution which was superfused past the pipette contained 1 mmol l^{-1} EGTA and no added Ca. For the period indicated by the bar this was exchanged for K^+-rich solution containing 10^{-6} mol l^{-1} Ca^{2+}. (From Findlay, Dunne & Petersen, 1985*b*.)

(in contact with membrane inside) ($[Ca^{2+}]_i$) is raised to 5×10^{-7} mol l^{-1} there is marked activation of at least three similar large K^+ channels in the membrane patch.

The activation of the high-conductance K^+ channel induced by changing the membrane potential or $[Ca^{2+}]_i$ is graded. The relationship between the open-state probability (p) and the membrane potential can be described graphically by a sigmoid saturating curve. This curve is shifted towards less positive or more negative membrane potentials by increasing $[Ca^{2+}]_i$ (Barrett, Magleby & Pallotta, 1982; Maruyama *et al.* 1983*b*; Findlay, Dunne & Petersen, 1985*a*). The sensitivity of high-conductance K^+ channels to $[Ca^{2+}]_i$ seems to vary considerably from one cell type to another (Petersen & Maruyama, 1984). The mammalian exocrine acinar cells have K^+ channels that can be activated by very low Ca^{2+} concentrations (Maruyama *et al.* 1983*b*; Findlay, 1984; Petersen *et al.* 1985; Gallacher & Morris, 1986) whereas, at the other end of the spectrum, the skeletal muscle channel requires relatively high Ca^{2+} concentrations for channel opening at negative membrane potentials

(Barrett *et al.* 1982). Pallotta (1985*b*) has recently shown that it is possible to remove the Ca^{2+}-dependent component of channel opening in rat skeletal muscle. Ca^{2+}-activated, high-conductance channels in excised inside-out patches from cultured rat skeletal muscle were treated with the protein-modifying reagent *N*-bromoacetamide (NBA). After the NBA treatment, the open-state probability was no longer sensitive to changes in $[Ca^{2+}]_i$, but channel activity was still voltage-dependent in the manner expected from a channel exposed to Ca^{2+}-free solution. These interesting experiments (Pallotta, 1985*b*) confirm the view that this channel type is Ca^{2+}-activated rather than Ca^{2+}-dependent (Petersen & Maruyama, 1984) and show that it is possible to remove the Ca^{2+}-sensitivity of the channel by chemical treatment.

The high-conductance K^+ channel can be blocked specifically by tetraethylammonium (TEA) acting selectively from outside the membrane in relatively low concentrations ($<5\ mmol\ l^{-1}$). Fig. 3 shows that $5\ mmol\ l^{-1}$ TEA on the membrane outside blocks the internal Ca^{2+} activation of the channel and similar results have been obtained with regard to voltage activation (Iwatsuki & Petersen, 1985*a*; Findlay *et al.* 1985*b*,*c*). Ba^{2+} is a useful blocker of many K^+ channels including the high conductance Ca^{2+}-activated type (Iwatsuki & Petersen, 1985*b*) whereas charybdotoxin appears, like TEA, to be a relatively specific inhibitor of the large Ca^{2+}-activated channel acting from outside the membrane (Miller, Moczydlowski, Latorre & Phillips, 1985). Quinine, a substance which has been claimed to be a selective blocker of Ca^{2+}-activated channels (Armando-Hardy *et al.* 1975) is in fact a better blocker of the smaller Ca^{2+}-insensitive and ATP-sensitive inward rectifying K^+ channel (Fig. 2) (Findlay *et al.* 1985*c*).

THE ATP-REGULATED INWARD RECTIFYING CHANNEL

One class of K^+ channel can be closed by ATP acting on the internal surface of the membrane. Fig. 4 shows single-channel current recordings from an excised inside-out membrane patch obtained from a cultured rat pancreatic islet cell. At 0 mV membrane potential and with Ca^{2+}-free solution (containing EGTA) in contact with the inside of the membrane, openings of several K^+ channels are seen. ATP ($1\ mmol\ l^{-1}$) added to the solution in contact with the inside of the membrane immediately closes all the channels and this effect is quickly reversible. The inhibitory effect does not require metabolism of ATP as Mg^{2+}-free ATP (Fig. 4) or non-metabolizable ATP analogues, such as adenylylimidodiphosphate (Cook & Hales, 1984) or β-γ-methylene ATP (Ashcroft, Ashcroft & Harrison, 1985) have the same effect as Mg^{2+}-ATP. The relative potency of adenosine nucleotides was ATP > ADP ≫ AMP and ATP analogues with altered sugar residues showed a reduced effectiveness. Base modifications using other purines (GTP or ITP) or pyrimidines (CTP or UTP) also reduce the effectiveness considerably (Cook & Hales, 1984; Spruce, Standen & Stanfield, 1986). ATP analogues show a rather similar order of potency in blocking the ATP-sensitive K^+ channel to that of cell activation *via* P_2 purinergic receptors (Gallacher, 1982). In this context it is interesting to note that quinine is a rather specific inhibitor of the ATP-sensitive K^+

channel (Fig. 2) (Findlay *et al.* 1985*c*) while the stereoisomer of quinine, quinidine, has been used as a relatively specific antagonist for the purinergic P_2 receptor (Gallacher, 1982).

Unlike the high-conductance Ca^{2+} and voltage-activated K^+ channel the ATP-sensitive K^+ channel has rectifying properties. In excised membrane patches exposed to symmetrical K^+-rich solutions, the single-channel, current–voltage relationship is nonlinear so that the conductance for outward current is considerably smaller than that for inward current (Cook & Hales, 1984; Findlay *et al.* 1985*b*).

In the pancreatic β-cells the pattern of opening and closing of the ATP-sensitive channel is relatively insensitive to changes in the membrane potential (Cook & Hales, 1984; Findlay *et al.* 1985*b*). In contrast, the ATP-sensitive K^+ channel in the skeletal muscle membrane is voltage-sensitive in such a way that depolarization favours opening (Spruce, Standen & Stanfield, 1985).

ACTIVATION OF K^+ CHANNELS IN EXOCRINE ACINAR CELLS BY SECRETAGOGUES

Microelectrode studies carried out on different glands in many species have shown that one important action of secretagogues is to evoke an increase in membrane K^+

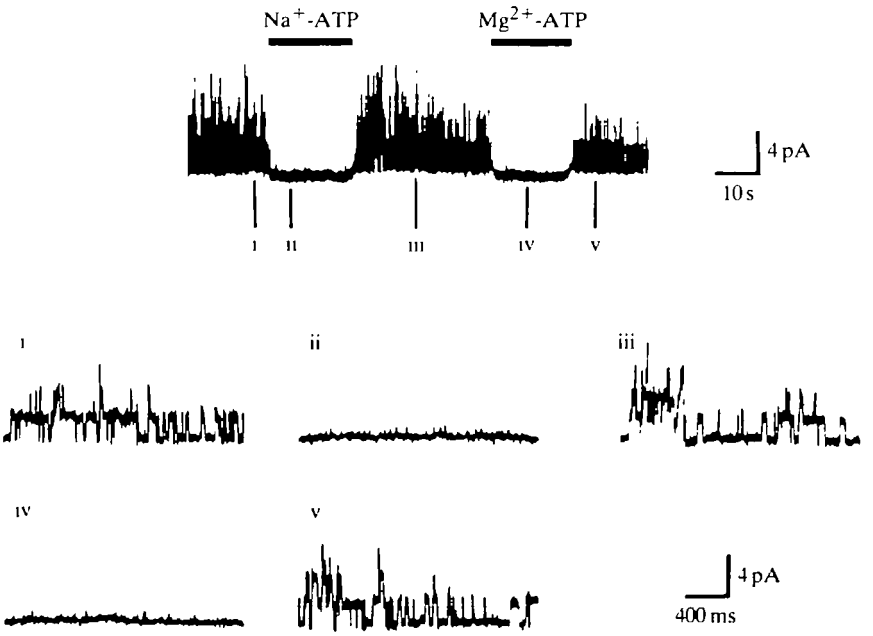

Fig. 4. Rat pancreatic islet cell. Single-channel current record from inward rectifying K^+ channels in an excised, inside-out membrane patch exposed to quasi-physiological Na^+/K^+ gradients. The membrane patch was voltage-clamped at 0 mV throughout. The upper trace represents a continuous current record. For the periods indicated by the bars the K^+-rich solution superfused past the pipette also contained 1 mmol l^{-1} Na^+-ATP and 1 mmol l^{-1} Mg^{2+}-ATP, respectively. The lower traces (i–v) represent current records obtained on a faster time base from the indicated portions of the upper trace.

conductance (Petersen, 1976, 1980). The long latency (about 300 ms) for nerve stimulation-evoked changes in acinar cell membrane potential, which had been observed even in the earliest electrophysiological study on salivary glands (Lundberg, 1955), could be explained by assuming that the increased K^+ conductance is not a direct result of secretagogue–receptor interaction, but is mediated by an intracellular messenger. The finding that intracellular injection of Ca^{2+} into lacrimal acinar cells mimics the hyperpolarization evoked by extracellular acetylcholine (ACh) or adrenaline application (Iwatsuki & Petersen, 1978) was a strong argument in favour of the 'messenger hypothesis'. The direct demonstration of Ca^{2+}-activated K^+ channels in acinar cells from mouse and rat salivary glands (Maruyama *et al.* 1983*a*), pig pancreas (Maruyama *et al.* 1983*b*) and rat and mouse lacrimal glands (Trautman & Marty, 1984; Findlay, 1984) further strengthened the hypothesis that intracellular Ca^{2+} is the messenger for the hormonal activation of membrane K^+ channels in exocrine gland cells (Petersen & Maruyama, 1984).

The first direct evidence for the Ca^{2+} messenger hypothesis was provided by Maruyama & Petersen (1984), Trautmann & Marty (1984) and Findlay (1984). In all three cases the patch-clamp, whole-cell current recording configuration (Fig. 1) was employed. The basic finding that stimulation with acinar secretagogues enhances the voltage-activated outward K^+ current is illustrated in Fig. 5. The acinar cell is equilibrated with the intracellular K^+-rich pipette solution containing $0{\cdot}5\ mmol\ l^{-1}$ EGTA and no added Ca^{2+}. In this situation there is a stable resting situation in which depolarizing voltage pulses from the -40 mV holding potential evoke large outward K^+ currents, whereas hyperpolarizing voltage pulses are associated with very little inward current. Stimulation with $10^{-6}\ mol\ l^{-1}$ CCK5 (cholecystokinin pentapeptide) causes a sustained increase in the voltage-activated outward currents and a small increase in the inward current. Application of TEA ($5\ mmol\ l^{-1}$) to the bath reduces the outward currents drastically (below the control level) but has no effect on the inward current. Subsequent removal of the stimulant hormone (CCK5) reduces the inward current.

That the outward K^+ currents represented by the traces shown in Fig. 5 are due to high-conductance Ca^{2+}- and voltage-activated K^+ channels is shown by the following evidence.

(1) The whole-cell K^+ current is Ca^{2+} activated and the Ca^{2+} sensitivity is similar to that described for the single-channel currents (Maruyama & Petersen, 1984).

(2) The activation of the whole-cell currents occurs over the same voltage range as described for the single-channel currents (Maruyama *et al.* 1983*b*; Maruyama & Petersen, 1984; Trautmann & Marty, 1984; Findlay, 1984).

(3) Ca^{2+}-activated, single-channel K^+ currents can be relatively specifically blocked by low concentrations ($< 5\ mmol\ l^{-1}$) of TEA from the outside of the plasma membrane and the whole-cell currents can be blocked in exactly the same way (Iwatsuki & Petersen, 1985*a*; Findlay *et al.* 1985*b*; Suzuki, Petersen & Petersen, 1985).

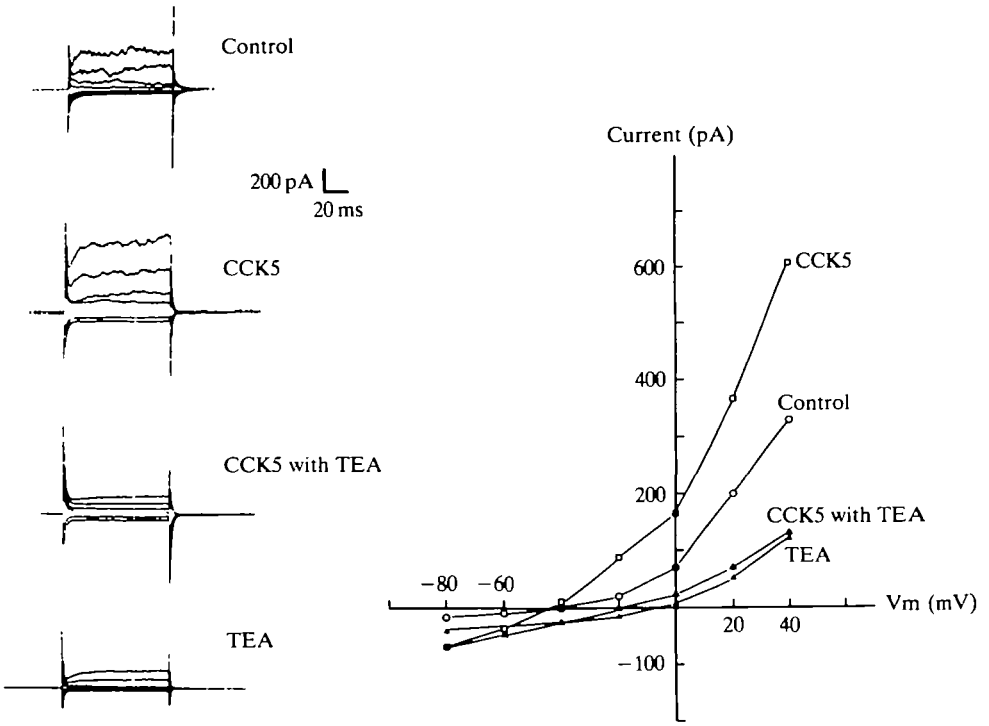

Fig. 5. Pig pancreatic acinar cell. Whole-cell, voltage-clamp current recordings from a single acinar cell. The bath contained the extracellular Na^+-rich solution and the pipette was filled with the intracellular K^+-rich solution without Ca^{2+} (containing 0·5 mmol l^{-1} EGTA). Currents associated with depolarizing voltage steps are shown as upward deflections (outward current) and with hyperpolarizing steps as downward deflections. The holding potential was −40 mV and 90 ms voltage steps to −20, 0, +20 and +40 as well as −60, −80 and in one case −100 mV were applied. The currents recorded before stimulation (control), 3 min after start of continued exposure to 10^{-6} mol l^{-1} CCK5 (CCK5, cholecystokinin pentapeptide), 3 min after addition of 5 mmol l^{-1} TEA still in the presence of CCK5 (CCK5 with TEA) and 3 min after discontinuation of CCK5 stimulation but still in the presence of TEA (TEA) are shown. The relationship between the steady-state currents and the membrane potential (Vm) in the different experimental situations obtained from the displayed current traces are shown in the graph. (From Suzuki, Petersen & Petersen, 1985).

(4) Ensemble fluctuation analysis of the whole-cell currents indicates that the outward current can be entirely accounted for by a relatively small number (50–150) of high-conductance channels (Trautmann & Marty, 1984; Maruyama *et al.* 1986). (The small number of channels per cell would have been a problem if extensive cell–cell communication did not occur – see Petersen, 1985.)

The effect of secretagogues (CCK5 or ACh), which is to enhance the outward K^+ current, is mediated by intracellular Ca^{2+} activating the high-conductance channels and the evidence for this can be summarized as follows.

(1) The CCK-evoked increase in outward K^+ current in pig pancreatic acinar cells and the similar ACh-evoked effect in lacrimal acinar cells is blocked by equilibrating the cell interior with a high concentration of the Ca^{2+} chelator EGTA

(10 mmol l^{-1}) (Maruyama & Petersen, 1984; Trautmann & Marty, 1984; Findlay, 1984).

(2) The sustained CCK5-evoked increase in outward K^+ current is abolished by removal of external Ca^{2+} (Maruyama & Petersen, 1984).

(3) Ensemble fluctuation analysis suggests that the additional outward K^+ current evoked by activation of muscarinic receptors is due to opening of high-conductance K^+ channels (Trautmann & Marty, 1984).

(4) The CCK5-evoked increase in whole-cell outward K^+ current is blocked by 5 mmol l^{-1} external TEA (Suzuki *et al.* 1985).

The most direct evidence for secretagogue activation of Ca^{2+}- and voltage-activated, high-conductance K^+ channels has recently been obtained from single-channel current recording experiments carried out using the cell-attached configuration. Fig. 6 shows an example of such an experiment on an isolated pig pancreatic acinar cell cluster. In the control period before stimulation there were only infrequent and short-lasting channel openings. 10–20s after the start of stimulation with CCK5 (hormone is added to the bath and there is therefore no direct contact between the hormone and the channel from which recording is made) a marked increase in the frequency of channel openings was observed and this higher frequency of channel opening (activation) was maintained as long as the hormonal stimulation continued. Fig. 6 also shows the relationship between the channel open-state probability and the membrane potential in the control period and in the presence of two different CCK5 concentrations. The marked CCK5-evoked dose-dependent increase in open-state probability is clear and it can also be seen that there is a marked voltage-sensitivity. CCK5 has clearly acted to increase the open-state probability of the Ca^{2+}- and voltage-activated, high-conductance K^+ channel. The evidence for the channel classification is as follows (Suzuki *et al.* 1985).

(1) The relationship between single-channel current amplitude and change in membrane potential is identical before and after CCK5 stimulation with a slope corresponding to a unit conductance of 200–250 pS.

(2) The channel is voltage dependent with depolarization increasing the open-state probability in the absence as well as presence of CCK5.

(3) TEA at a low concentration (5 mmol l^{-1}) acting from outside the membrane blocks channel opening both before and after stimulation.

(4) The CCK5-evoked increase in channel open-state probability is only sustained in the presence of Ca^{2+} on the outside of the membrane patch from which channel recording was made.

Activation of K^+ channels evoked by ACh (10^{-5} mol l^{-1}) has been demonstrated in submandibular acinar cells (Gallacher & Morris, 1986) and in lacrimal acinar cells Marty, Tan & Trautmann (1984) have shown activation of K^+ channels evoked by bath application of the Ca^{2+} ionophore A23187 (0·2 μmol l^{-1}). Messenger-mediated stimulation of Ca^{2+}-activated K^+ channels by hormones and neurotransmitters is not only observed in exocrine acinar cells but has also recently been reported to occur in clonal pituitary cells. Dubinsky & Oxford (1985), in experiments of a type similar to the one illustrated in Fig. 6, showed that thyrotropin-releasing hormone (TRH)

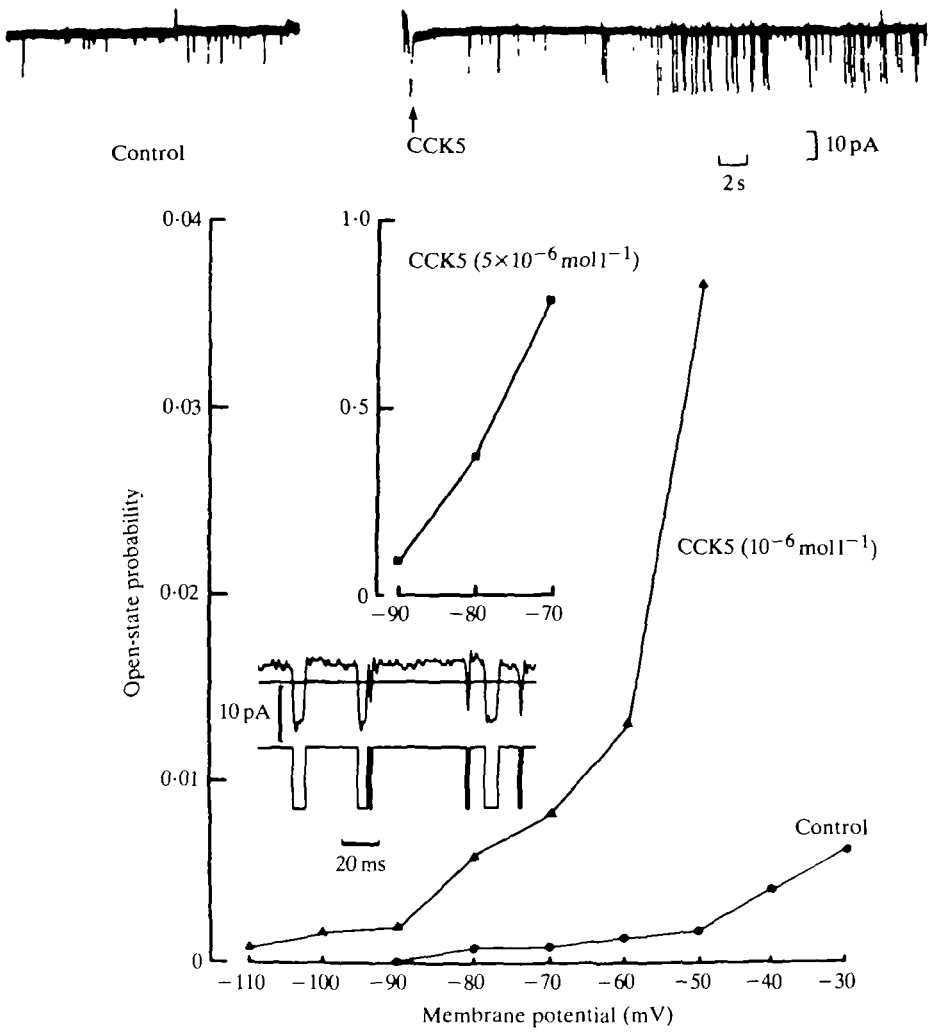

Fig. 6. Pig pancreatic acinar cell. Single-channel current recording from basolateral membrane patch. All records were obtained in the cell-attached configuration. The recording pipette was filled with a K^+-rich solution containing 2·5 mmol l^{-1} Ca^{2+}, whereas the bath was filled with Na^+-rich (extracellular) solution. The resting membrane potential was about −60 mV. The upper traces show a recording made on a slow time base. In the control situation there are only a few inward current steps. Application of CCK5 (cholecystokinin pentapeptide, 10^{-6} mol l^{-1}) to the bath evokes, after a latency of about 18 s, a clear and sustained increase in the frequency of channel openings (inward current steps). The single-channel current amplitude is also enhanced because of membrane hyperpolarization (to about −80 mV). The graphs below show the plots of channel open-state probability as a function of membrane potential in the control situation and during stimulation with 10^{-6} and 5×10^{-6} mol l^{-1} CCK5. The inset shows the single-channel current recorded on a fast time base together with the idealized current trace obtained from computerized threshold analysis of the digitized data (bottom trace). The inset trace was obtained in the presence of CCK5 (10^{-6} mol l^{-1}) at a membrane potential of −50 mV. (From Suzuki, Petersen & Petersen, 1985.)

($0{\cdot}1\ \mu mol\ l^{-1}$) markedly increased the open-state probability of the high-conductance K^+ channel.

There are at least two intriguing points concerning the hormone- or neurotransmitter-evoked increase in open-state probability of Ca^{2+}-activated K^+ channels (Suzuki *et al.* 1985; Dubinsky & Oxford, 1985; Gallacher & Morris, 1986) that deserve further investigation.

(1) The latency is relatively long: about 5 s in the study of Dubinsky & Oxford (1985), about 10–20 s in the study of Suzuki *et al.* (1985) and about 20–30 s in the experiments of Gallacher & Morris (1986). In the exocrine acinar cells the latency for hormone- or neurotransmitter-evoked electrical changes in conventional microelectrode experiments is less than 1 s (Petersen, 1980) and in the cell-attached, single-channel current recording experiments of Suzuki *et al.* (1985) it could be clearly observed that there was an increase in the amplitude of the inward single-channel K^+ currents due to membrane hyperpolarization (the patch pipette was filled with a high 'intracellular' K^+ concentration) before an increase in the frequency of channel openings could be observed. This indicates most likely some delay in penetration of the intracellular messenger into the cytosolic compartment inside the electrically isolated patch membrane. Intracellular Ca^{2+} diffusion is known to be severely restricted (Baker, 1978) and it is therefore possible that $[Ca^{2+}]_i$ in the isolated membrane patch area does not correspond exactly to $[Ca^{2+}]_i$ in the cytosol at large.

(2) In the pig pancreatic acinar cells, sustained CCK5 activation of single K^+ channels was dependent on the presence of Ca^{2+} in the patch pipette solution (Suzuki *et al.* 1985). This may not be the case in the mouse submandibular acinar cells, although the effects of $10^{-5}\ mol\ l^{-1}$ ACh on single-channel currents with Ca^{2+}-free EGTA solution in the patch pipette solution were largely transient with only a smaller sustained component (Gallacher & Morris, 1986). It is not known whether the ACh response in the salivary gland would have been better sustained with Ca^{2+} present in the pipette solution. In the clonal pituitary cells only brief pulses of stimulation were used (Dubinsky & Oxford, 1985) and it is therefore not possible to comment on the possible external Ca^{2+} requirement for sustained activation.

In the exocrine acinar cells there is clear evidence from classical microelectrode studies that there are two pools of Ca^{2+} involved in K^+ channel activation. In the absence of extracellular Ca^{2+}, transient stimulant-evoked hyperpolarizations can easily be observed, but to sustain the stimulant-evoked electrical responses extracellular Ca^{2+} is required (Maruyama *et al.* 1983*b*; Pearson, Flanagan & Petersen, 1984). The first phase of stimulation which is independent of external Ca^{2+}, therefore, relies on the release of intracellular Ca^{2+}. The mechanism by which this occurs is now relatively clear (Fig. 7). Hormone-receptor activation of phospholipase C splits phosphatidylinositol bisphosphate into inositol trisphosphate (IP_3) and diacylglycerol (Berridge & Irvine, 1984; Hokin, 1985). IP_3 acts on the endoplasmic reticulum membrane by activating a Ca^{2+} pathway allowing release of these ions into the cytosol (Streb, Irvine, Berridge & Schulz, 1983; Streb *et al.* 1984; Muallem, Schoeffield, Pandol & Sachs, 1985). The sustained phase of stimulation depends on

extracellular Ca^{2+} and it has previously been shown that pancreatic secretagogues increase unidirectional Ca^{2+} flux into acinar cells (Kondo & Schulz, 1976). The mechanism underlying this Ca^{2+} uptake remains obscure, but the recent results of Suzuki *et al.* (1985) may indicate that we are dealing with a messenger-mediated Ca^{2+} uptake rather than with receptor-operated Ca^{2+} channels.

SECRETAGOGUE INHIBITION OF K^+ CHANNELS IN INSULIN-SECRETING CELLS

The major physiological stimulus for insulin secretion from pancreatic β-cells is an elevation of the plasma glucose concentration. The initial effect is to evoke membrane depolarization (Dean & Matthews, 1968) followed by a cyclical pattern of electrical activity consisting of slow waves of depolarization with action-potential-like spikes (Petersen, 1980; Henquin & Meissner, 1984). Tracer flux studies have

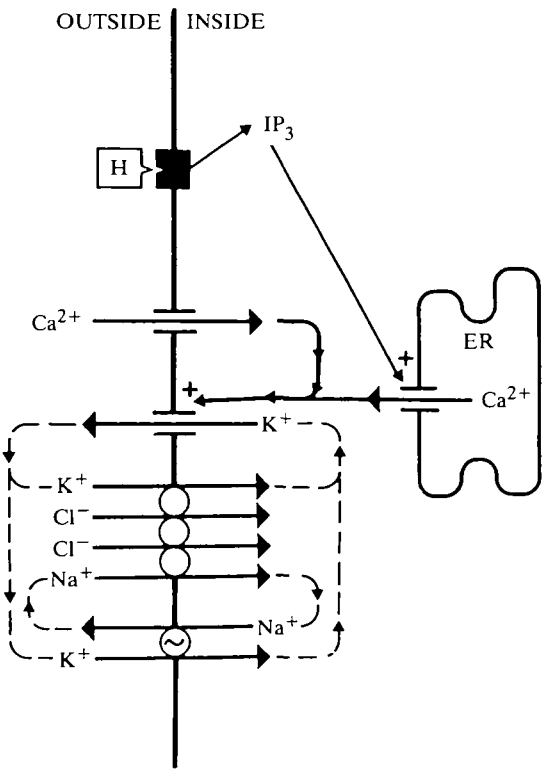

Fig. 7. Schematic diagram indicating the mechanism by which hormone–receptor interaction opens selective K^+ channels in the plasma membrane and thereby stimulates uphill Cl^- uptake into the cell. Hormone-receptor interaction (H) results in the release of inositol-1,4,5-trisphosphate (IP_3) which acts on the endoplasmic reticulum membrane (ER) to open a Ca^{2+} channel allowing release of the ion into the cytosol. Ca^{2+} activates the K^+ channel. In the steady state the three transport proteins, K^+ channel, Na^+-$2Cl^-$-K^+ cotransporter and Na^+-K^+ pump function together as an electrogenic Cl^- pump. The mechanism by which Ca^{2+} uptake from the extracellular solution is mediated is unclear, but hormone–receptor interaction evokes directly or indirectly an increased Ca^{2+} influx.

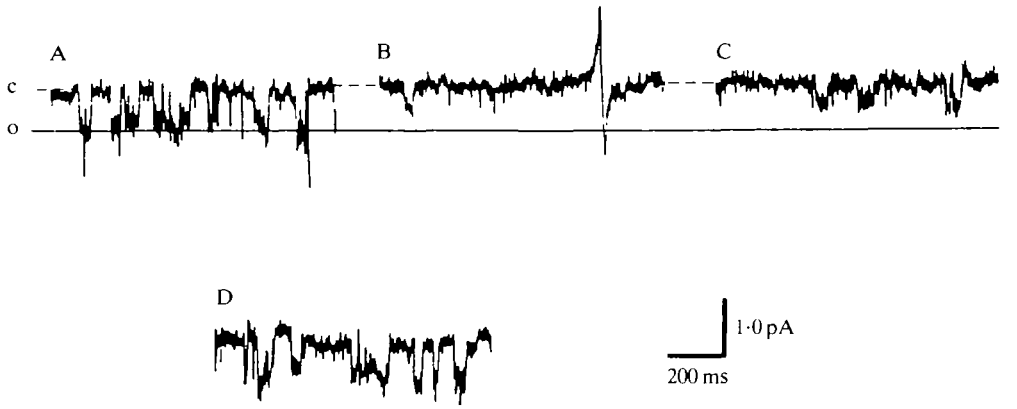

Fig. 8. Rat insulinoma cell line m5F. Single-channel current recording obtained in cell-attached configuration. The recording pipette was filled with K^+-rich intracellular solution whereas the bath contained the Na^+-rich extracellular solution. (A)–(D) are consecutive traces from the same membrane patch. (A) Control; (B) 30 s after start of stimulation with 10 mmol l^{-1}, D-glyceraldehyde (an action potential is seen); (C) 1½ min after start of glyceraldehyde stimulation; (D) 1½ min after return to control solution. c represents current level when all channels are closed and o the current with one channel open. The pipette voltage was kept constant at 0 mV.

indicated that glucose metabolism is associated with a decrease in membrane K^+ permeability (Sehlin & Taljedal, 1975) and Ashcroft, Harrison & Ashcroft (1984) have demonstrated glucose-induced closure of single K^+ channels in isolated rat pancreatic β-cells. Fig. 8 shows an example of secretagogue-evoked inhibition of single K^+ channels in an insulin-secreting cell. This experiment was carried out on the rat insulinoma cell line m5F which does not respond to glucose stimulation, but secretes insulin when challenged with glyceraldehyde (Praz *et al.* 1983). Before stimulation repeated opening of K^+ channels were observed (Fig. 8A) whereas about 30 s after the start of stimulation with 10 mmol l^{-1} D-glyceraldehyde channel openings were rare, the single-channel current amplitude was markedly reduced and action potentials were observed (Fig. 8B). The effect of glyceraldehyde stimulation was partially transient since 1½ min after the start of stimulation the channel open-state probability had increased somewhat and action potential firing had ceased (Fig. 8C). 1½ min after returning to the control solution the single-channel current amplitude was back to full size and the open-state probability was as high as in the first control period (Fig. 8D). The shape of the single-channel, current–voltage relationship before and after stimulation was similar but shifted along the x-axis corresponding to a depolarization of about 40 mV (from about −60 mV to −20 mV). The decrease in single-channel current amplitude during glyceraldehyde stimulation (Fig. 8) is therefore simply due to the membrane depolarization (inward currents through the K^+ channel were recorded since the patch pipette was filled with K^+-rich 'intracellular' solution whereas the bath was filled with Na^+-rich extracellular solution).

The channels shown in Fig. 8 are the dominant ones in intact resting pancreatic islet cells and have the same conductance properties as the ATP-sensitive K^+

channels studied in excised patches (Ashcroft *et al.* 1984; Findlay *et al.* 1985*b*). Rorsman & Trube (1985) have compared the kinetics of the glucose-sensitive resting K^+ channels in intact β-cells with the ATP-sensitive K^+ channel in excised inside-out membrane patches from the same cells and concluded that the channels are identical. Evidence that the ATP-sensitive K^+ channels dominate the overall K^+ conductance comes from whole-cell recording experiments in which a very high input resistance was found if the medium dialysing the cell interior contained $3\,mmol\,l^{-1}$ ATP. The absence of ATP evoked a large additional K^+ conductance (Rorsman & Trube, 1985). It has been known for some time that quinine is able markedly to depolarize the pancreatic β-cell membrane (Henquin, 1982) and since it is now clear that quinine in low concentrations (about $100\,\mu mol\,l^{-1}$) is a relatively specific inhibitor of the ATP-sensitive K^+ channel (Findlay *et al.* 1985*c*) this result is consistent with the view that the resting potential is mainly due to the presence of ATP-sensitive K^+ channels.

In cardiac cells the ATP-sensitive K^+ channel is not operational under resting conditions. This is consistent with the knowledge that the intracellular ATP concentration is about $3–4\,mmol\,l^{-1}$ and that in excised, inside-out or open, cell-attached membrane patches ATP at a concentration of $2\,mmol\,l^{-1}$ totally inhibits channel opening (Kakei, Noma & Shibasaki, 1985). With regard to pancreatic islet cells, even lower ATP concentrations have been reported to abolish K^+ channel openings in excised membrane patches (Cook & Hales, 1984) yet these channels are nevertheless operational in the intact cells. It turns out that immediately after excision of a membrane patch from an islet cell into the inside-out configuration there is a dramatic increase in the number of active channels from about 1–2 to 10–20 (Findlay *et al.* 1985*b*,*c*). This is consistent with the hypothesis that these are ATP-sensitive channels since excision exposes the membrane inside to the ATP-free bathing solution. Subsequent ATP addition to the bath closes all the channels. Similar results can be obtained by opening up intact cells from which single-channel recordings are being made in the cell-attached configuration by saponin or digitonin treatment (see Fig. 1). The typical pattern of activity seen in intact resting cells (Fig. 8) must therefore be interpreted as a state in which the vast majority of the many ATP-sensitive K^+ channels present are closed due to the action of intracellular ATP, i.e. the open-state probability of the channels is very low. In excised patches there is a marked run-down of ATP-sensitive K^+ channels (Findlay *et al.* 1985*b*,*c*) and therefore many single-channel recordings obtained from excised patches after extensive run-down of channel activity look not dissimilar to those obtained in the cell-attached configuration from intact, resting cells. This has caused considerable confusion as it has been suggested that the degree of channel activation in the intact cell is far too high in relation to the known ATP-sensitivity in excised patches and the known ATP concentration in intact resting cells (Rorsman & Trube, 1985; Ashcroft *et al.* 1985). The discrepancy is, however, much smaller than generally assumed since the channel open-state probability is in fact extremely low in the intact cell and it is only because of the high channel density that openings can be observed (Findlay *et al.* 1985*b*).

It is known that the intracellular ATP concentration increases after glucose stimulation (Malaisse & Herchuelz, 1982; Ashcroft *et al.* 1985) and it is therefore attractive to suggest that the ATP generated during glycolysis (Fig. 9) is responsible for the closure of K^+ channels. As pointed out by Dean, Matthews & Sakamoto (1975), one of the crucial metabolic steps in the glycolytic pathway appears to be the one catalysed by phosphoglycerate kinase (PGK) which is an enzyme bound to the plasma membrane (Fig. 9). ATP generated in this reaction step could therefore act locally to decrease the open-state probability of the resting K^+ channel and thereby depolarize the cell.

IMPORTANCE OF MESSENGER-MEDIATED CHANGES IN K^+ CHANNEL OPENING FOR SECRETION

The activation of K^+ channels in exocrine acinar cells by hormones and neurotransmitters appears to be important mainly for the process of fluid secretion. The acinar cells secrete a NaCl-rich primary fluid under the influence of nervous or hormonal stimulation. The original theory of Lundberg (1958) suggested that this was due to activation of an electrogenic Cl^- pump in the basolateral plasma membrane responsible for both the transepithelial Cl^- movement and the membrane

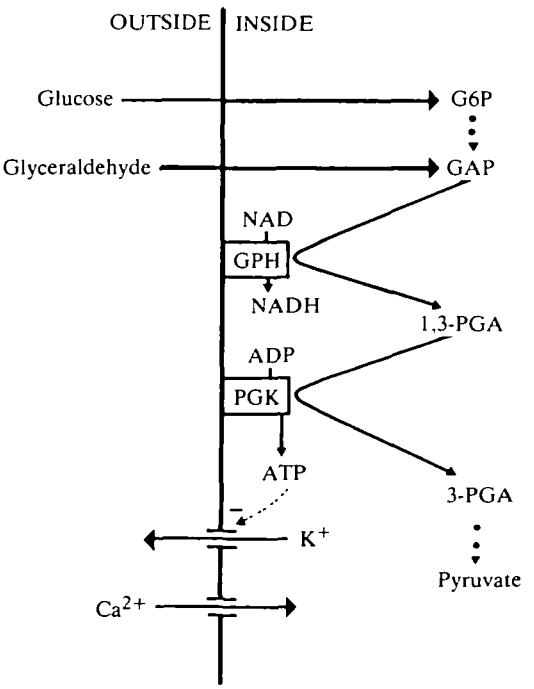

Fig. 9. Schematic diagram indicating the mechanism by which glucose or glyceraldehyde closes K^+ channels in insulin-secreting cells. Abbreviations: G6P, glucose-6-phosphate; GAP, glyceraldehyde phosphate; GPH, glyceraldehyde phosphate dehydrogenase; 1,3-PGA, 1,3-diphosphoglycerate; 3-PGA, 3-phosphoglycerate. The closure of K^+ channels evokes depolarization and this activates the voltage-gated Ca^{2+} channels.

hyperpolarization (secretory potential) observed. We now know that the hyperpolarization is due to activation of K^+ channels in the basolateral plasma membrane (Petersen & Maruyama, 1984) but the electrogenic Cl^- pump postulated by Lundberg (1958) does in a way exist although, as shown in Fig. 7, it has three distinct molecular components: the Ca^{2+}-activated K^+ channel, the Na^+-K^+ pump and the Na^+-$2Cl^-$-K^+ cotransporter (Suzuki & Petersen, 1985). In the steady-state stimulated situation these three transport proteins function as an electrogenic Cl^- pump (Fig. 7) and undoubtedly play a major role in acinar fluid secretion (Petersen & Maruyama, 1984). So far the only point of regulation appears to be the K^+ channel activated by intracellular Ca^{2+}. The Cl^- pumped into the cell has to leave *via* the luminal membrane through Cl^- channels and evidence for a Ca^{2+}-regulated Cl^- conductance has recently been provided (Marty, Tan & Trautmann, 1984; Findlay & Petersen, 1985). It is assumed that Na^+ moves into the lumen *via* the paracellular pathway through leaky 'tight junctions' due to the lumen negativity generated by the Cl^- current and in this way it is possible to postulate regulation of transepithelial fluid movement by control of K^+ channels in the basolateral membrane and of Cl^- channels in the luminal membrane (Suzuki & Petersen, 1985).

The importance of secretagogue-evoked inhibition of K^+ channels in islet cells lies in the resulting depolarization. The insulin-secreting cells possess Ca^{2+} channels activated by membrane depolarization. Voltage-activated Ca^{2+} currents have recently been studied with the help of patch-clamp, whole-cell current recording in mouse pancreatic β-cells (Satin & Cook, 1985; Rorsman & Trube, 1986) and the rat insulinoma cell line m5F (Findlay & Dunne, 1985). The voltage-activated Ca^{2+} current in the insulinoma cell line has properties adequately explaining the action potential (Findlay & Dunne, 1985) and therefore the increase in $[Ca^{2+}]_i$ observed after glyceraldehyde stimulation (Wollheim, Ullrich & Pozzan, 1984). The stimulant-evoked increase in $[Ca^{2+}]_i$ is an important signal for insulin secretion by exocytosis (Wollheim & Sharp, 1981) although the mechanisms by which Ca^{2+} acts are still obscure.

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