Characterization of the G protein coupling of a somatostatin receptor to the K^+_{ATP} channel in insulin-secreting mammalian HIT and RIN cell lines

B. Ribalet and G. T. Eddlestone*

*Department of Physiology and Ahmanson Laboratory of Neurobiology, University of California, Los Angeles, Los Angeles, CA 90024 and *Department of Pharmacology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

1. The G protein-mediated coupling of a somatostatin (somatotropin-releasing inhibitory factor; SRIF) receptor to the ATP-dependent K^+ channel (K^+_{ATP} channel) has been studied in insulin-secreting cells using the patch clamp technique.

2. In excised outside-out patches, the concentration-dependent stimulation of the K^+_{ATP} channel by SRIF was biphasic. Stimulation reached a maximum at 15 nM (EC_{50} = 5·5 nM), then decayed to a minimum at 50 nM and returned to maximum stimulation at 500 nM.

3. In cell-attached patches, bath-applied SRIF caused K^+_{ATP} channel stimulation in most experiments. In a few cases, however, SRIF suppressed channel activity, a response that was reversed by addition of dibutyryl cyclic AMP (DBcAMP). Channel stimulation by SRIF or by DBcAMP did not occur in the presence of glucose.

4. In excised inside-out patches, the α-subunits of G_i- or G_o-type G proteins stimulated the K^+_{ATP} channel (EC_{50} = 29 and 42 pM, respectively). The K^+_{ATP} channel stimulation by α_i- or α_o-subunits had no effect on the concentration-dependent inhibition by ATP.

5. In excised inside-out patches, K^+_{ATP} channel activity was reduced by inhibitors of protein kinase C (PKC) and stimulated by a PKC activator. The stimulatory effect of PKC was unaffected by the presence of pertussis toxin, but stimulation by exogenous α-subunits of the G protein G_i or G_o was prevented by PKC inhibitors.

6. From these data we deduce that SRIF can affect K^+_{ATP} channel activity directly via a membrane-delimited pathway or indirectly via a pathway requiring diffusible messengers. In the former case, α_i/α_o may either enhance PLC activity, stimulating PKC and thus inducing K^+_{ATP} channel phosphorylation with consequent increase of activity, or channel phosphorylation by PKC may facilitate a direct stimulation of the channel by α_i/α_o. In the latter case, an α_i/α_o-induced fall in cAMP contributes to reduced PKA-mediated phosphorylation and suppression of channel activity.

Insulin secretion may be enhanced or suppressed by ligands that act through heterotrimeric guanosine nucleotide-binding proteins (G proteins). Receptors for inhibitory ligands, which include somatostatin (somatotropin-releasing inhibitory factor; SRIF), galanin and α_2-adrenergic receptors are coupled to effector systems by G_i- or G_o-type G proteins; stimulatory ligand receptors (including those for glucagon, glucagon-like peptide (GLP-1) and β-adrenergic receptors) are coupled via G_s-type G proteins. Initial studies of both stimulatory and inhibitory ligands were interpreted in terms of actions mediated by increased or decreased intracellular cAMP concentrations, resulting from effects of the G proteins on adenylate cyclase activity. While cAMP certainly has a role in the regulation of the secretory response, it is now clear that other pathways, controlled by G protein-linked receptors, contribute to modulation of secretion via cAMP-independent mechanisms.

In addition to adenylate cyclase, other cellular targets for G proteins include potassium channels, ATP-sensitive K^+ channels (K^+_{ATP} channels) (De Weille, Schmid-Antomarchi, Fosset & Lazdunski, 1989; Ribalet, Ciani, Hales & Eddlestone, 1991) and a small inward-rectifying K^+ channel (Rorsman *et al.* 1991), voltage-dependent calcium channels (Hsu, Xiang, Rajan, Kunze & Boyd, 1991; Piros, Evans, Ribalet & Hales, 1993) and a 'late' locus, close to the exocytotic event (Ullrich & Wollheim, 1988). The changes in secretion resulting from modulation of ion channels are associated with changes of intracellular free calcium ($[Ca^{2+}]_i$), while changes in cAMP and the

presently unidentified 'late' locus may modify the secretory process at a step which is independent of $[Ca^{2+}]_i$ (Bhathena *et al.* 1976; Curry & Bennett, 1976). The present study focuses on the effect of G protein-coupled receptors on K^+_{ATP} channels and investigates the properties of a mechanism that may regulate $[Ca^{2+}]_i$.

Microelectrode studies of β-cell membrane potential initially suggested that diminution of Ca^{2+} entry due to increase in potassium permeability was responsible for the inhibitory effects of SRIF and adrenaline (Pace & Tarvin, 1981; Cook & Perara, 1982; Drews, Debuyser, Nenquin & Henquin, 1990). The observation that galanin and SRIF activate K^+_{ATP} channels in both cell-attached and excised outside-out patches (De Weille *et al.* 1989; Ribalet *et al.* 1991) supported such a view. However, $^{86}Rb^+$ efflux data could not be reconciled with this hypothesis, since adrenaline, galanin and SRIF inhibit $^{86}Rb^+$ efflux from glucose-stimulated islets (Drews *et al.* 1990; Debuyser, Drews & Henquin, 1991; Hsu *et al.* 1991), which is the converse of what would be predicted for a mechanism mediated via enhanced K^+_{ATP} channel activity. The view that K^+_{ATP} channels are not involved in G protein-mediated inhibition of insulin release was reinforced by the demonstration that adrenaline inhibits the secretion evoked by the K^+_{ATP} channel blocker tolbutamide (Debuyser *et al.* 1991).

While it is difficult to ascribe a role to K^+_{ATP} channel stimulation in the process of secretory inhibition by G_i/G_o-type G proteins, it is clear that there exists a G protein-dependent modulatory pathway which stimulates this channel. Although inhibitory ligands, such as SRIF, reduce adenylate cyclase activity and thereby lower cellular cAMP, this process is unlikely to mediate the effect on the K^+_{ATP} channel, since elevation of intracellular cAMP with the membrane-permeant analogue dibutyryl cAMP (DBcAMP) causes K^+_{ATP} channel stimulation via activation of the cAMP-dependent protein kinase (PKA; Ribalet, Ciani & Eddlestone, 1989*a*). Similar conclusions have been reached by others (De Weille *et al.* 1989). The block of SRIF-induced K^+_{ATP} channel stimulation by pretreatment with pertussis toxin in the excised patch (Ribalet *et al.* 1991) suggests, instead, that G_i and/or G_o are directly involved in the stimulation of K^+_{ATP} channels. Such a proposition is supported by evidence obtained in cardiac cells which indicates that there is a direct (membrane-delimited) stimulatory effect of G protein α_i-subunits on both muscarinic K^+ channels (K^+_{ACh} channels) and K^+_{ATP} channels (Yatani, Codina, Brown & Birnbaumer, 1987; Ito *et al.* 1992).

In brain tissue, α_o-subunits are closely associated with protein kinase C (PKC) in the cell membrane (Worley, Baraban, Van Dop, Neer & Snyder, 1986), and the two proteins have been reported to have functional interactions. G proteins regulate phosphoinositide metabolism, activating phospholipase C (PLC), which stimulates PKC via formation of diacylglycerol (DAG) (for review see Gilman, 1987). In addition, PKC phosphorylates α_i-subunits, which lose their ability to modulate the activity of adenylate cyclase when phosphorylated (Jakobs, Bauer & Watanabe, 1985; Bushfield *et al.* 1990). Comparison of the results presented here with those obtained using 12-*O*-tetradecanoylphorbol-13-acetate (TPA; Ribalet, Eddlestone & Ciani, 1988; De Weille *et al.* 1989) demonstrates that PKC and the G protein α_o- and α_i-subunits modulate K^+ channel activity in a similar manner. Based on these findings, a model is proposed whereby α_i/α_o-subunits and PKC interact with each other in a membrane-delimited pathway to regulate K^+_{ATP} channel activity.

The present study investigates several aspects of the relationship between the K^+_{ATP} channel, SRIF, G proteins and PKC. Evidence is presented which supports the hypothesis that the effect of SRIF involves PTX-sensitive G proteins, which interact with PKC to stimulate the K^+_{ATP} channel, and that K^+_{ATP} channel regulation by this stimulatory pathway is subordinate to the glucose- and ATP-dependent inhibitory pathway.

METHODS

Cell cultures and experimental media

The K^+_{ATP} channel studies were performed using cells from the insulin-secreting cell lines RINm5F from rat (passage no. 42–56) and HIT-T15 (Simian virus 40-transformed Syrian hamster islet cells; passage no. 70–76). Both cell lines were kindly provided by the late A. E. Boyd III. The cells were incubated at 37 °C in RPMI 1640 medium, supplemented with 10% (v/v) fetal calf serum, penicillin (100 units ml^{-1}), streptomycin (100 μg ml^{-1}) and 2 mM glutamine. The cells were divided once a week by treatment with trypsin and the medium was changed twice between divisions. One hour before starting the experiment, the culture medium was exchanged for a glucose-free solution containing (mM): 135 NaCl, 5 KCl, 2·5 $CaCl_2$, 1·1 $MgCl_2$ and 10 Hepes, the pH being adjusted to 7·2 with NaOH. This solution was also used in the experimental chamber when an extracellular-type solution was needed, for cell-attached and outside-out patch experiments. For cell-attached and inside-out patch experiments, the composition of the solution in the patch pipette was (mM): 140 KCl, 2·5 $CaCl_2$, 1·1 $MgCl_2$ and 10 Hepes, with the pH adjusted to 7·2 using KOH; that of the bath solution, for experiments with excised inside-out patches, was similar, except that no calcium was added. For experiments with inside-out patches, both ATP (10 μM; Sigma) and GTP (5–100 μM; Boehringer Mannheim Diagnostics, Inc., Houston, TX, USA) were present in the bath solution to prevent loss of channel activity (Ribalet, Ciani & Eddlestone, 1989*b*). The α-subunit of the G proteins G_i and G_o, kindly provided by Drs J. Codina and L. Birnhaumer (UCLA, School of Medicine), were kept at a concentration of 2 μM in a buffered solution containing (mM): 10 Tris, 1 EDTA, 1 dithiothreitol, 7·5 $MgCl_2$ and 20 KCl. This concentrated solution was diluted before the experiment in glucose-free extracellular medium to obtain an α-subunit concentration of 10 nM. The purified G protein α-subunits were maintained in their active state by treatment with GTPγS; the ratio of the nucleotide to G protein was 0·8.

Somatostatin was ohtained from Bachem Inc., Torrance, CA, USA. Stock solution of the A protomer of pertussis toxin (List Biological Laboratories, Campbell, CA, USA) was prepared with 10 μg protein diluted in 500 μl sterile water containing 10 mM Tris, 0·1 mM Na_2EDTA and 0·04% CHAPS (3-((3-cholamidopropyl)-dimethylammonio)-1-propanesulphonate) at pH 8·0. Aliquots of these stock solutions were added directly to the experimental chamber to obtain a final concentration of 100 ng ml^{-1}. The peptide inhibitor of protein kinase C, peptide 19–36, was obtained from Peninsula Laboratories, Inc., Belmont, CA, USA; SC-9, [*N*-(6-phenylhexyl)-5-chloro-1-naphthalenesulphonamide], the PKC activator, from Seikagaku America, Inc., St Petersburg, FL, USA; and H-7 (1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride) from Molecular Probes, Inc., Eugene, OR, USA. H-7 was dissolved directly in the experimental solution, while SC-9 was stored in dimethyl sulphoxide (DMSO). After dilution of the SC-9 stock solution in the experimental medium, the final concentration of DMSO was less than 0·05%, a concentration which does not affect K^+ channel activity.

Data recording and analysis

The techniques to prepare patch electrodes and record single channel events were similar to those described previously (see Ribalet *et al.* 1988, for details). The data, filtered at 2 kHz with an 8-pole Bessel filter, were recorded either with an EPC 7 List (Darmstadt, Germany) or an Axopatch 1A (Axon Instruments, Burlingame, CA, USA) patch amplifier and stored on video cassette at a fixed frequency of 44 kHz after digitization with a digital audio processor. For analysis, the data were acquired on a computer hard disk at a rate of 5·5 kHz; the transfer was carried out with a two-buffer interface, allowing continuous acquisition. Before measurement of the channel mean open and closed time, amplitude histograms of the current steps were built to determine the half-amplitude threshold, and this threshold was used to form an idealized record of the original data. This schematized record was used for the subsequent analysis. The percentage open time (NP_o), which is the parameter used to assess the level of channel activity, was determined from data samples of 30 s duration. Under control conditions, NP_o values varied widely from patch to patch: for instance, the experiments performed to test the effect of pertussis toxin showed control values ranging from 0·028 to 0·82. For this reason, the changes in channel activity are quantified using ratios of experimental values of NP_o *vs.* controls.

RESULTS

The G protein coupling of SRIF receptors to K^+_{ATP} channels

K^+_{ATP} channel modulation by SRIF in outside-out patches

The action of SRIF is mediated via stimulation of G proteins, which influence the effector function either directly via a process limited to the membrane, or indirectly via diffusible second messengers. While direct action involves the G protein α- and/or βγ-subunits, indirect action may be mediated by inhibition of adenylate cyclase, reduction of cAMP and consequent suppression of PKA activity.

Excised outside-out patch clamp experiments were

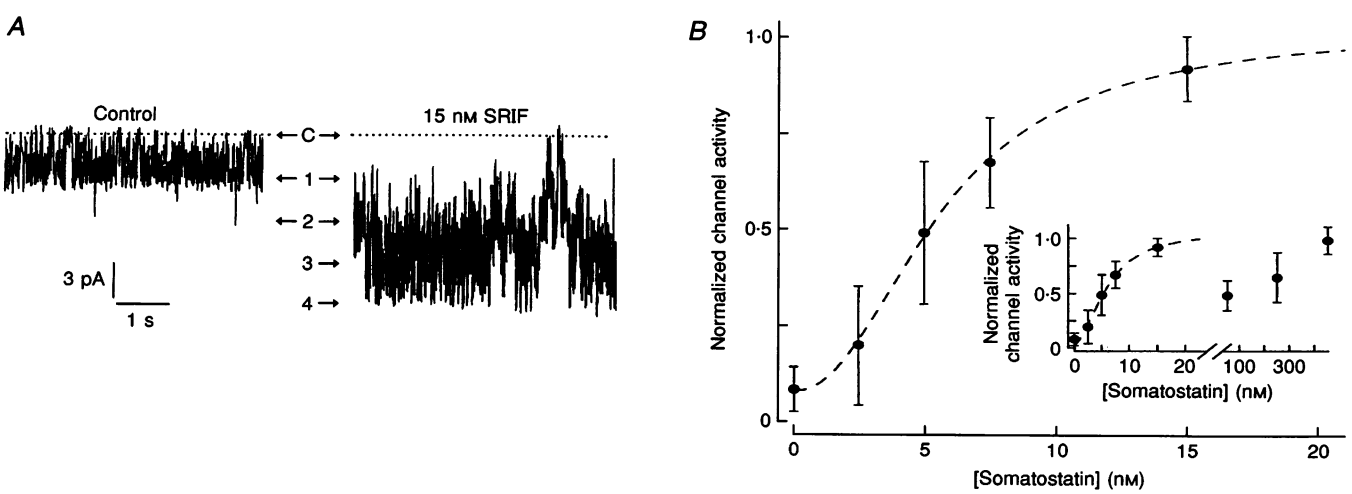


Figure 1. Concentration-dependent effects of SRIF on K^+_{ATP} channel activity in outside-out patch experiments

The data shown in *A* depict the effect of bath-applied SRIF (15 nM). The downward current steps represent inward current with 140 mM KCl on both sides of the membrane, for a holding pipette potential of −70 mV. The pipette solution contained 50 μM GTP and 20 μM ATP. The numbers beside the current traces represent the number of channels open with C being the closed state. In *B*, the channel activity normalized to the activity measured in the absence of agonist (I/I_o) was fitted with the equation: $I/I_o = A/(1 + (K/C)^n)$, where C is the concentration of SRIF, n is the Hill coefficient and K is the EC_{50}. The fit of the data yielded $n = 1·8$ and $K = 5·5$ nM. The inset to *B* has the same axes as the main graph, but channel activity measured at higher SRIF concentrations (50, 250 and 450 nM) is also included. The horizontal axis is interrupted between 25 and 50 nM and the scale is expanded following this interruption.

carried out to determine whether, in β-cells, modulation of K^+_{ATP} channels by SRIF receptors is 'direct' or 'indirect'. The data presented in Fig. 1*A* and *B* show that SRIF (3–15 nM) stimulated K^+_{ATP} channel activity, an effect which was consistently observed in outside-out patches. In Fig. 1*B*, averaged data obtained from eight experiments are plotted as a function of SRIF concentration. The fit to the data in Fig. 1*B* yields an EC_{50} of 5·5 nM for K^+_{ATP} channel stimulation by SRIF.

When concentrations of SRIF greater than 50 nM were applied, a complex response was observed. This response is depicted in the inset of Fig. 1*B*, where the data from three experiments are averaged for concentrations of SRIF greater than 50 nM. At 50 nM, the stimulatory effect of SRIF diminished and K^+_{ATP} channel activity was reduced compared with that observed at 15 nM. However, as SRIF was increased further, the stimulatory effect recovered and the level of activity reached at concentrations higher than 400 nM was close to that measured at 15 nM.

These data indicate that K^+_{ATP} channel modulation by SRIF can occur 'directly', without intervention of a diffusable second messenger. The biphasic concentration-dependent response to SRIF indicates possible receptor desensitization and is examined further in the Discussion.

Effect of PTX on K^+_{ATP} channel activity: role of endogenous α_i/α_o subunits

It has been proposed that PTX-sensitive G proteins (G_i or G_o) couple the SRIF receptor to the K^+_{ATP} channel (Ribalet *et al.* 1991). To confirm that endogenous membrane-associated G_i- or G_o-like G proteins control K^+_{ATP} channel activity, we tested the effect of the active A protomer of pertussis toxin (PTX_A) in inside-out patch clamp experiments.

The presence of NAD^+ is required for pertussis toxin to catalyse the ADP ribosylation and block the function of the α-subunit of G_i or G_o (Katada & Ui, 1982). While higher concentrations of NAD^+ inhibited K^+_{ATP} channel activity (results not shown), 10 μM NAD^+, the concentration used in this study, had little or no effect on the channel. In these conditions, addition of 100 ng ml^{-1} PTX_A had a potent inhibitory effect (upper traces of Fig. 2), with similar results obtained in eight other experiments. On average, channel activity reached a level corresponding to 26 ± 6% of control values, 2–7 min after addition of PTX_A. As illustrated in the bottom left-hand trace of Fig. 2, there was almost no recovery of channel activity after removal of the toxin. The lack of reversibility of this inhibitory effect is also evidenced by the average level of channel activity, which did not exceed 48% of its control value ($n = 6$) 1–3 h after toxin removal. However, as shown in the bottom right-hand trace, channel activity could be restored by adding activated α-subunits of G_{i3}. In two out of four trials, addition of purified α_{i3} in the presence of PTX was accompanied by a 1·68-fold increase in NP_o.

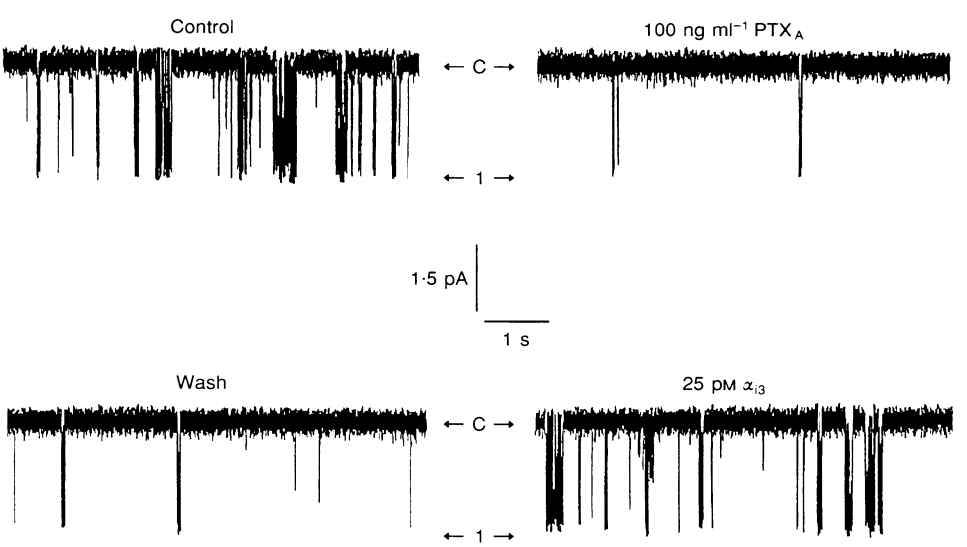


Figure 2. Effects of pertussis toxin on K^+_{ATP} channel activity in the excised inside-out patch

The holding potential was −60 mV, and 140 mM KCl was present on both sides of the membrane; the bath solution contained 10 μM ATP. All the data shown in this figure originate from the same experiment. The two upper traces illustrate the effect of 100 ng ml^{-1} of the active protomer of PTX (PTX_A) on channel activity. Control activity shown in the top left-hand trace was recorded in the presence of NAD^+. The top right-hand trace, showing the blocking effect of PTX_A, was taken 6 min after addition of the toxin. The bottom left-hand trace was acquired 15 min after toxin removal and illustrates the lack of reversibility of the PTX effect. The bottom right-hand trace shows recovery of channel activity 3 min after addition of 25 pM α_{i3} to the bath.

From these results we can deduce that: (1) PTX-sensitive G protein α-subunits are present in the excised patch of membrane and link the SRIF receptor to the K^+_{ATP} channel, as previously suggested (Ribalet *et al.* 1991); and (2) there is 'background' (agonist-independent) activity of PTX-sensitive G protein(s) in isolated membranes of insulin-secreting cells.

K^+_{ATP} channel modulation by exogenous α-subunit of G_i and G_o

The involvement of endogenous G_i and/or G_o in K^+_{ATP} channel modulation is suggested by the inhibitory effect of PTX. To determine whether modulation of the K^+_{ATP} channel is restricted to an α-subunit of either the G_i or the G_o family, we investigated the effect of purified G protein α_{i3}- and α_o-subunits applied exogenously to inside-out patches. The subunits α_{i3} (from erythrocytes) and α_o (from brain) were selected because they modulate K^+ channel function in other systems (Yatani *et al.* 1987). Addition of either type of α-subunit was consistently followed by strong and sustained enhancement of K^+_{ATP} channel activity, beginning within a few seconds to 2 min after α-subunit addition. The stimulatory effect of 100 pM α_o is illustrated in Fig. 3*A* and that of 12·5 pM α_{i3} in Fig. 3*B*. For both α_{i3} and α_o, maximum K^+_{ATP} channel stimulation was obtained at α-subunit concentrations between 100 and 250 pM. The maximum effect could be as great as a 15-fold increase in channel activity. Figure 3*C* is a plot of the concentration-dependent stimulation of channel activity by α_i and α_o; the data points are averaged from eight and six experiments for α_{i3} and α_o, respectively. There was essentially no difference between the potency of the two types of α-subunits, with half-maximal stimulatory values of 30 pM for α_{i3} and 42 pM for α_o, not being statistically significant. When reversibility was tested, in five of eight experiments there was almost no decay of channel activity after G protein withdrawal. In three of eight experiments there was a partial reversal of the effect after 10 min. These properties closely resemble those reported for regulation by α_i-subunits of cardiac muscarinic K^+ channel and K^+_{ATP} channel (Yatani *et al.* 1987; Ito *et al.* 1992).

From these results, we deduce that K^+_{ATP} channel modulation by SRIF may be mediated by α-subunits of either G_i or G_o.

Role of PKC in α_i-mediated modulation of the K^+_{ATP} channel

'Direct' modulation of the K^+_{ATP} channel by the α-subunits of the G proteins G_i and G_o can be due to a direct interaction of the α-subunit with the channel, as suggested for the atrial muscarinic K^+ channel (K^+_{ACh} channel; Yatani *et al.* 1987), or alternatively to a 'membrane-delimited' process involving PKC. The latter proposal is based on the finding that, like α_i and α_o, the PKC activator TPA stimulates K^+_{ATP} channel activity (Ribalet *et al.* 1988; De Weille *et al.* 1989), and that α_i/α_o-subunits and PKC have functional interactions (Jakobs *et al.* 1985; Gilman, 1987). To test for such interactions, we investigated whether PKC is associated with the membrane and modulates K^+_{ATP} channel activity in excised patches of membrane.

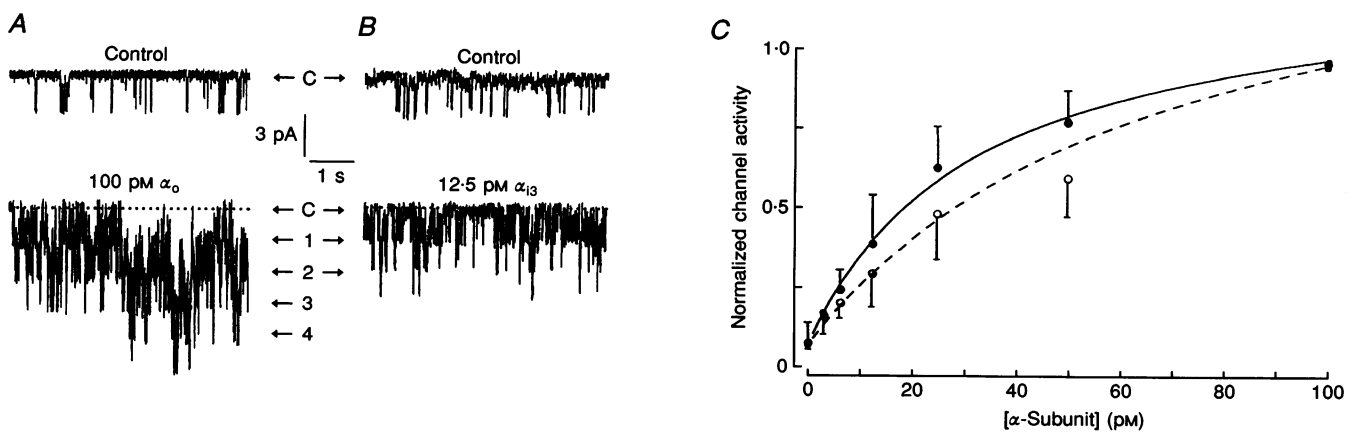


Figure 3. Effects of α-subunits on K^+_{ATP} channel activity in excised inside-out patches

The membrane potential was held at −70 mV, and 140 mM KCl was present on both sides of the membrane; the bath solution contained 10 μM ATP. The top traces of *A* and *B* illustrate the channel activity before addition of α-subunit. The bottom trace in *A* illustrates the level of activity reached 15 min after addition of 100 pM α_o; that in *B* was recorded 8 min after addtion of 12·5 pM α_i. In these two panels, downward deflections represent inward current; the numbers beside the trace indicate the number of channels open. In *C* are shown averaged data points of channel activity measured in the presence of increasing concentration of α_o (○) and α_{i3} (●). The data were normalized to the level of activity measured in the absence of exogenous α-subunit and fitted using the equation: $I/I_o = A/(1 + (K/C))$, where C is the concentration of α-subunit added to the bath. Fitting of the data yielded an EC_{50} (K in the equation) of 29 pM for α_i (continuous line) and 42 pM for α_o (dashed line).

K^+_{ATP} channel modulation by protein kinase C

Activation of the K^+_{ATP} channel in the cell-attached patch by the phorbol ester TPA, which substitutes for diacylglycerol in the stimulation of PKC, suggests that stimulation of PKC causes channel activation. The results presented here, which were obtained using excised inside-out patches, support this hypothesis.

The isoquinolinesulphonamide H-7, a potent inhibitor of PKC and cyclic nucleotide-dependent protein kinases (Hidaka, Inagaki, Kawamoto & Sasaki, 1984), rapidly blocked K^+_{ATP} channel activity in excised inside-out patches (Fig. 4*A*). Channel inhibition began within 1 min following the addition of H-7 and was almost complete after 2–3 min, reaching a level corresponding to 13·7 ± 3·4% of control values. In ten of twelve experiments there was a rapid recovery from inhibition, NP_o reaching a level 1·35 ± 0·2 times that of control values 2–3 min after removal of H-7. However, since H-7 also blocks the activity of PKA, which stimulates the K^+_{ATP} channel (Ribalet *et al.* 1988), the effect of a synthetic peptide inhibitor of PKC, peptide 19–36, was also investigated. This inhibitor, which has an amino acid sequence corresponding to that of a pseudosubstrate for PKC, is 100 times more specific for PKC than for PKA (House & Kemp, 1987). In excised inside-out patches, addition of peptide 19–36 caused pronounced and reversible K^+_{ATP} channel inhibition (Fig. 4*B*). The activity

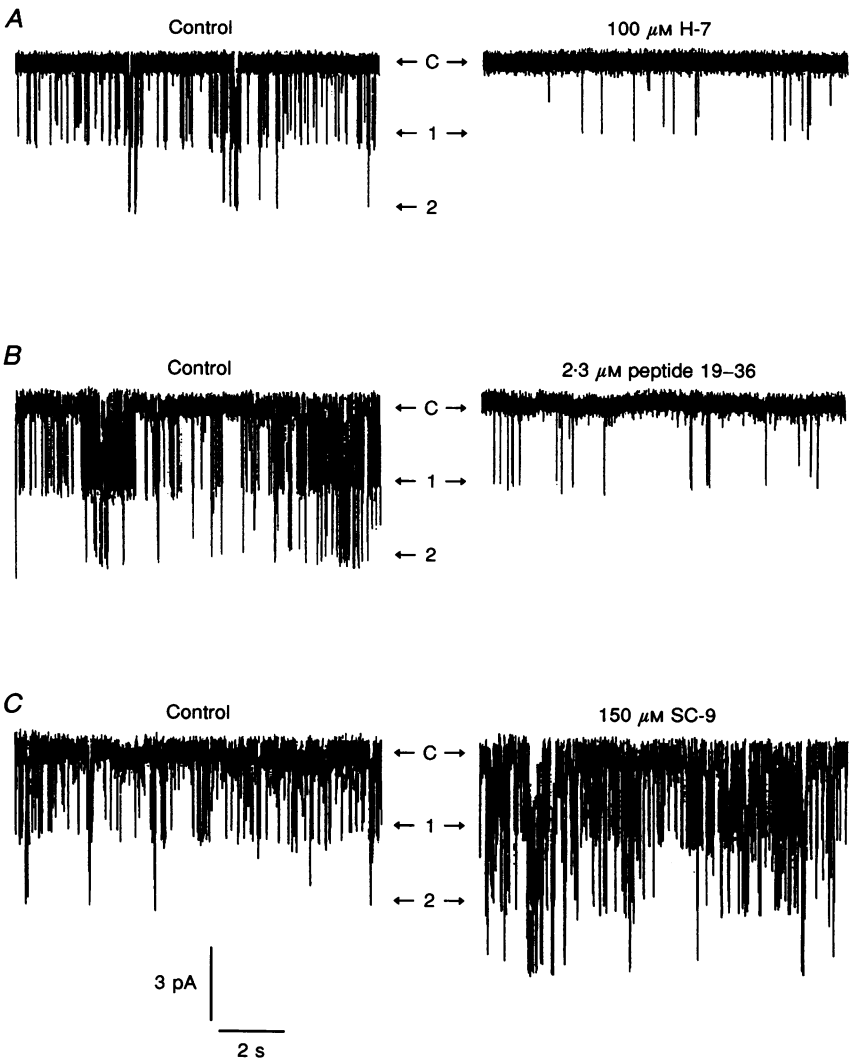


Figure 4. Effects of protein kinase C (PKC) modulators on K^+_{ATP} channel activity in excised inside-out patches

In these experiments, there was 140 mM KCl on both sides of the membrane; the bath solution contained 20 μM ATP and 50 μM GTP. The membrane was held at −70 mV in *A* and *B* and at −60 mV in *C*. *A* shows the effect of the PKC inhibitor H-7; the right-hand trace was acquired 1 min after addition of 100 μM H-7. *B* depicts the effect of the inhibitor peptide (peptide 19–36), 6 min after its addition (right-hand trace). *C* shows the effect of the activator SC-9 (150 μM), 2 min after its addition. The recordings presented in this figure were obtained from 3 different patches.

was reduced on average to $9 \pm 1{\cdot}4\%$ of control values ($n = 3$) by the peptide, and returned to within $12 \pm 8\%$ of its original value upon removal of the inhibitor. The onset of the inhibitory effect of the synthetic peptide was slow compared with that of H-7. This may be due to a slower diffusion of the peptide or, alternatively, to the competitive type of inhibition that takes place between the synthetic peptide and PKC substrates (House & Kemp, 1987).

To investigate the role of PKC in K^+_{ATP} channel regulation further, SC-9, a phospholipid substitute for PKC activation (Nishino, Kitagawa, Iwashima, Ito, Tanaka & Hidaka, 1986), was applied to the excised inside-out patch. Stimulation of PKC by SC-9 activated the K^+_{ATP} channel. The result shown in Fig. 4*C* is typical of five experiments. The channel stimulation began 1–2 min following SC-9 addition, and reached a steady state $2{\cdot}54 \pm 0{\cdot}26$ times greater than control values, 3 min later. This stimulatory effect was reversible and NP_o was only $1{\cdot}04 \pm 0{\cdot}17$ times the control values 10 min after removal of SC-9.

These data demonstrate that PKC is associated with the membrane and tonically stimulates the K^+_{ATP} channel in excised patches.

Is K^+_{ATP} channel modulation by α_i mediated via PKC?

To investigate whether K^+_{ATP} channel modulation by G protein α_i- and α_o-subunits is mediated via PKC, we

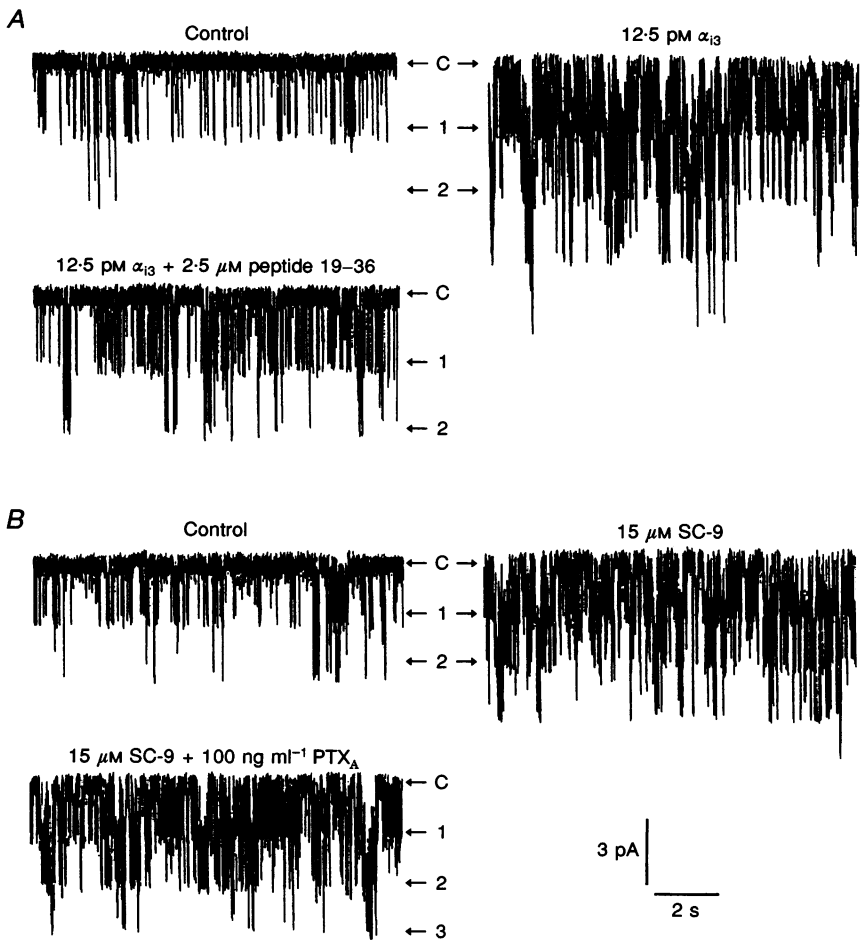


Figure 5. Effects of PKC modulators on α_i-mediated K^+_{ATP} channel activation in inside-out patches

A shows the effect of the peptide inhibitor of protein kinase C (peptide 19–36) on α_i-activated K^+_{ATP} channels. The top left-hand trace illustrates control activity before addition of 12·5 pM α_i-subunit to the bath (top right-hand trace). There was a 2 min interval between the two top traces. The bottom trace shows the block of α_i-stimulated K^+_{ATP} channels 4 min after addition to the bath of the peptide inhibitor of PKC. *B* illustrates the effect of SC-9 on K^+_{ATP} channel activity in an excised inside-out patch exposed to the A protomer of pertussis toxin (PTX_A). In this experiment, 10 μM NAD^+ was added to the control solution and the patch was then exposed to 150 μM SC-9 (top right-hand trace) and 100 ng ml^{-1} PTX_A was added (bottom trace of *B*). There is a 6 min interval between the upper right-hand trace and the lower one in *B*.

studied the effect of α_i and α_o in the presence of the PKC inhibitors H-7 and peptide 19–36. The data presented in Fig. 5*A* show that inhibition of PKC prevented K^+_{ATP} channel stimulation by α_{i3}. The two upper traces of Fig. 5*A* illustrate the effect of α_{i3} on channel activity in the absence of the protein kinase inhibitor; as already shown in Fig. 3, this addition caused a substantial increase of channel activity. In four experiments the steady-state activity reached in the presence of 12·5 pM exogenous α-subunits and before the addition of PKC inhibitor was 6·62 ± 1·01 times greater than that of controls. Subsequent addition of peptide 19–36 or H-7 reversed the stimulatory effect of the G protein α-subunit (lower trace of Fig. 5*A*). Similar results were obtained when addition of 12·5 pM α_o failed to reactivate K^+_{ATP} channel activity previously blocked by the inhibitor of PKC. On average, the probability of channel opening was 1·11 ± 0·54 times that of controls ($n = 5$) when the inhibitor and the α-subunit were present together. It should be noted that in three of five experiments NP_o was reduced to 25% of control values on average, following the addition of PKC inhibitor in the presence of G protein α-subunits. Comparison of these data with those presented above for H-7 and peptide 19–36 in the absence of exogenous G protein, indicate that added G protein α-subunits have little effect on the potency of PKC inhibitors to block the channel.

These results are consistent with a PKC-mediated activation of the K^+_{ATP} channel by α_i/α_o.

Is K^+_{ATP} channel modulation by PKC mediated via α_i?

That both stimulation of PKC and addition of α_i and α_o enhance K^+_{ATP} channel activity may be interpreted, as proposed above, as the result of channel modulation by α_i/α_o via PKC, or alternatively as the consequence of channel modulation by PKC via α_i/α_o. The latter hypothesis stems from the observation that PKC phosphorylates and alters the function of G protein α-subunits (Jakobs *et al.* 1985; Bushfield *et al.* 1990).

To test whether PKC modulates K^+_{ATP} channel activity via α_i or α_o, we compared the effect of the PKC activator SC-9 in the presence and in the absence of PTX_A. We have already shown in Fig. 2 that ADP ribosylation of endogenous α_i and/or α_o by PTX_A caused K^+_{ATP} channel inhibition. The data in Fig. 5*B* show that addition of PTX_A (in the presence of NAD^+) (lower trace) had almost no effect on the activity of the K^+_{ATP} channel previously stimulated by SC-9 (upper traces of Fig. 5*B*). Similar results were obtained when the effect of SC-9 was tested after inhibition of K^+_{ATP} channels by PTX_A. In fact, we found that the 2 ± 0·3-fold increase in channel activity evoked by SC-9, in the presence of PTX_A, was not significantly different from the 2·54 ± 0·26-fold increase obtained in the absence of PTX_A.

These results suggest that the effect of PKC on the K^+_{ATP} channel is not mediated via α_i and is most likely to be 'direct'.

The physiological role of K^+_{ATP} channel modulation by SRIF

We have demonstrated that SRIF stimulates K^+_{ATP} channel activity in outside-out patches. It may be postulated that this effect accounts for β-cell membrane hyperpolarization and inhibition of the insulin release induced by glucose (Pace & Tarvin, 1981; De Weille *et al.* 1989). However, glucose is a potent inhibitor of the K^+_{ATP} channel and may suppress the stimulatory effect of SRIF. To test this hypothesis, we investigated the effect of glucose on SRIF-activated K^+_{ATP} channels in cell-attached patches. The choice of this patch configuration was dictated by the mode of action of glucose on the K^+_{ATP} channel, which requires ATP production by the cell metabolism.

K^+_{ATP} channel modulation by SRIF in cell-attached patches

In the absence of glucose, addition of SRIF to the bath caused K^+_{ATP} channel stimulation in the cell-attached patch (Fig. 6*A*) in a manner comparable with that observed in excised patches (Fig. 1). In Fig. 6*A*, the upper trace represents the channel activity under control conditions, in the absence of glucose; the lower trace shows the effect of 5×10^{-7} M SRIF, 4 min after its addition. In six experiments the maximum stimulation by SRIF represented as much as a 14·3 ± 2·6-fold increase in K^+_{ATP} channel activity. The reversibility of the effect of SRIF was variable, with channel activity returning to within 10% of control values in three experiments, 10 min after removal of the agonist, while there was no diminution of channel activity in one experiment and a continuous increase of activity in two others.

In addition, as previously reported (Ribalet *et al.* 1989*a*), 50 μM DBcAMP has a stimulatory effect; this effect was additive with that of SRIF, the channel activity increasing as much as 28·34 ± 7·17 times compared with controls under these conditions (data not shown).

Although stimulation of K^+_{ATP} channels by SRIF has been reported by others, in cell-attached patches (De Weille *et al.* 1989), increased channel activity was not consistently observed in the present study. In three experiments, channel activity was reduced to 19 ± 7·6% of control values by SRIF. In two of these experiments, addition of DBcAMP to the bath reversed the inhibitory effect of SRIF and resulted in a 2-fold increase in NP_o compared with controls. These results indicate that SRIF-induced K^+_{ATP} channel inhibition may be due to reduction of cAMP production.

Based on these observations, it is postulated that G protein-mediated modulation of the K^+_{ATP} channel by

SRIF involves 'direct' stimulation by the G protein α-subunits and 'indirect' inhibition due to decreased cAMP concentrations.

Inhibition of SRIF-induced K^+_{ATP} channel activation by glucose

To investigate whether inhibition of glucose-induced insulin release by SRIF involves stimulation of the K^+_{ATP} channel, we studied the effect of SRIF on K^+_{ATP} channel activity in the presence of increasing concentrations of glucose.

In contrast to the robust stimulation of K^+_{ATP} channel activity by 5×10^{-7} M SRIF in the absence of glucose (Fig. 6*A*), the stimulatory effect of SRIF progressively decreased as glucose was increased; there was no discernible effect of SRIF at glucose concentrations of 5 mM and greater (Fig. 6*B* and *C*). The data from Fig. 6*A–C*, together with those of four other experiments, are summarized in Fig. 6*D*. It is apparent from the plot of the data that the glucose concentration-dependent inhibition of K^+_{ATP} channel activity is unaffected by SRIF. The glucose dependence obtained in the presence of SRIF in this series of experiments (half-maximal block of channel activity at about 0·5 mM glucose and almost complete block at 5 mM glucose) closely resembles that previously reported for K^+_{ATP} channels in normal β-cells, HIT cells and RINm5F cells, in the absence of SRIF (Misler, Falke, Gillis & McDaniel, 1986; Ribalet *et al.* 1988; Eddlestone, Ribalet & Ciani, 1989). It was also found that glucose blocked with a similar efficacy the combined effect of SRIF and DBcAMP (result not shown).

These data suggest that activation of the K^+_{ATP} channel does not play a role in the inhibition of glucose-induced insulin release by SRIF. This hypothesis is strengthened by the results presented in the following section.

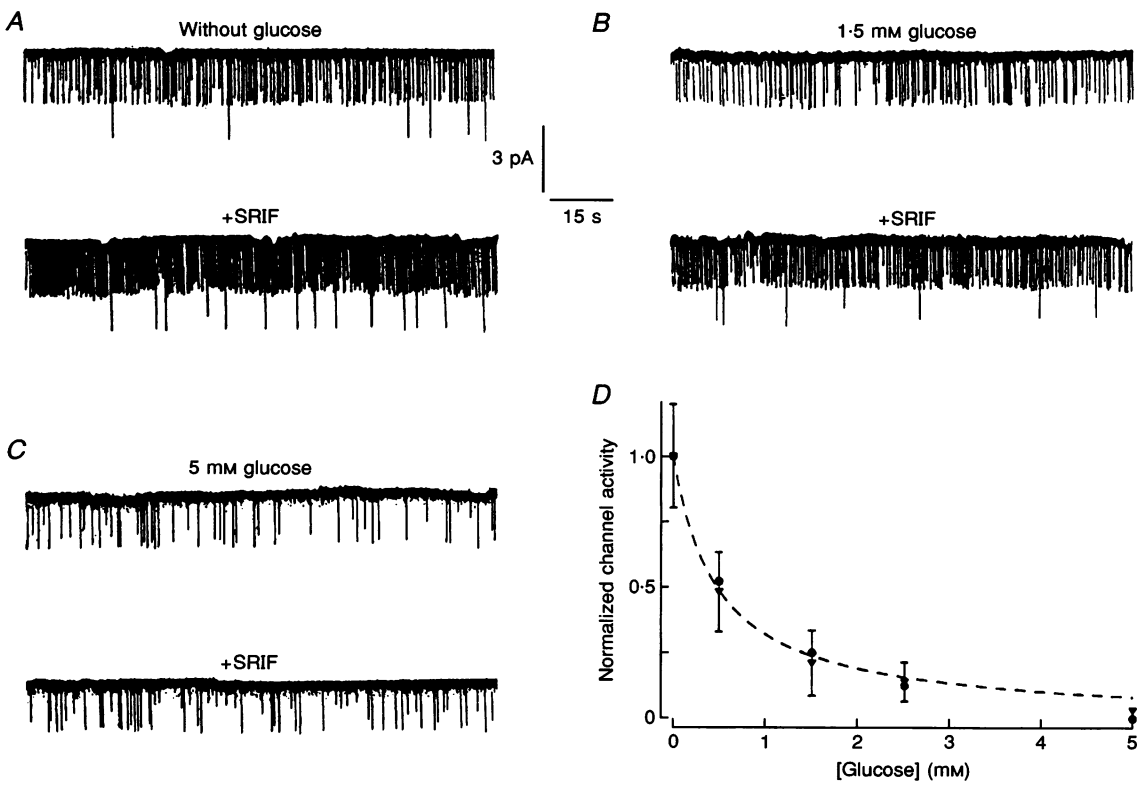


Figure 6. Effects of SRIF on K^+_{ATP} channel activity in the cell-attached patch in the presence of different glucose concentrations

For the 3 pairs of records (*A*, *B* and *C*), the data in the top trace were obtained in the absence of SRIF, while those in the bottom trace were obtained in the presence of 5×10^{-7} M SRIF. Glucose was present at the concentration indicated above each pair of records, both in the presence and in the absence of SRIF. Downward deflections represent inward current flowing through the open channels. The pipette potential was 0 mV and the pipette solution contained 140 mM KCl. In *D*, the normalized channel activity calculated as the ratio of channel activity measured in the presence to that in the absence of glucose is plotted as a function of glucose concentration. Data were obtained in the presence (▼) and in the absence (●), respectively, of 500 nM SRIF. The fit to the inverted triangles, shown as a dashed line, was obtained using a non-linear curve-fitting program. The error bars show the S.E.M. values; in each case $n = 3$.

Inhibition of α_o/α_i-induced K^+_{ATP} channel activation by ATP

The K^+_{ATP} channel is stimulated by SRIF via activation of G_i or G_o and inhibited by glucose via elevation of ATP. The observation that glucose prevents K^+_{ATP} channel activation by SRIF suggests that the ATP-dependent inhibitory pathway prevails over the α_i/α_o-dependent stimulatory pathway in regulating the K^+_{ATP} channel activity. To test this hypothesis, inside-out patch clamp experiments were performed to examine the stimulatory effect of α_i or α_o in the presence of increasing concentrations of ATP. The data presented in Fig. 7 show that, in the presence of G protein α_o-subunit, ATP blocks K^+_{ATP} channel activity in a manner similar to that observed in the absence of the subunit (Ribalet *et al.* 1989*a*). Data in Fig. 7*A* and *B* illustrate the stimulatory effect of α_o in the presence of 15 μM ATP; Fig. 7*C* shows that increasing the ATP concentration to 250 μM blocked channel activity. Channel activity data from five experiments were normalized to the maximum activity measured in the presence of 10 μM ATP, averaged and plotted as a function of ATP concentration (● in Fig. 7*D*). The shaded area delimited by the two dashed lines in Fig. 7*D* shows the variation of the ATP concentration dependence of channel block obtained with no exogenously added α-subunit (Ribalet *et al.* 1989*a*). The mean data points obtained with either 100 pM α_o or α_i (●) fall within the shaded area, suggesting that G_i or G_o α-subunits do not affect the channel sensitivity to ATP.

The observation that K^+_{ATP} channel modulation by α_i- or α_o-subunit is subordinate to the regulation by ATP, corroborates the previous finding that K^+_{ATP} channel stimulation by SRIF is prevented by glucose.

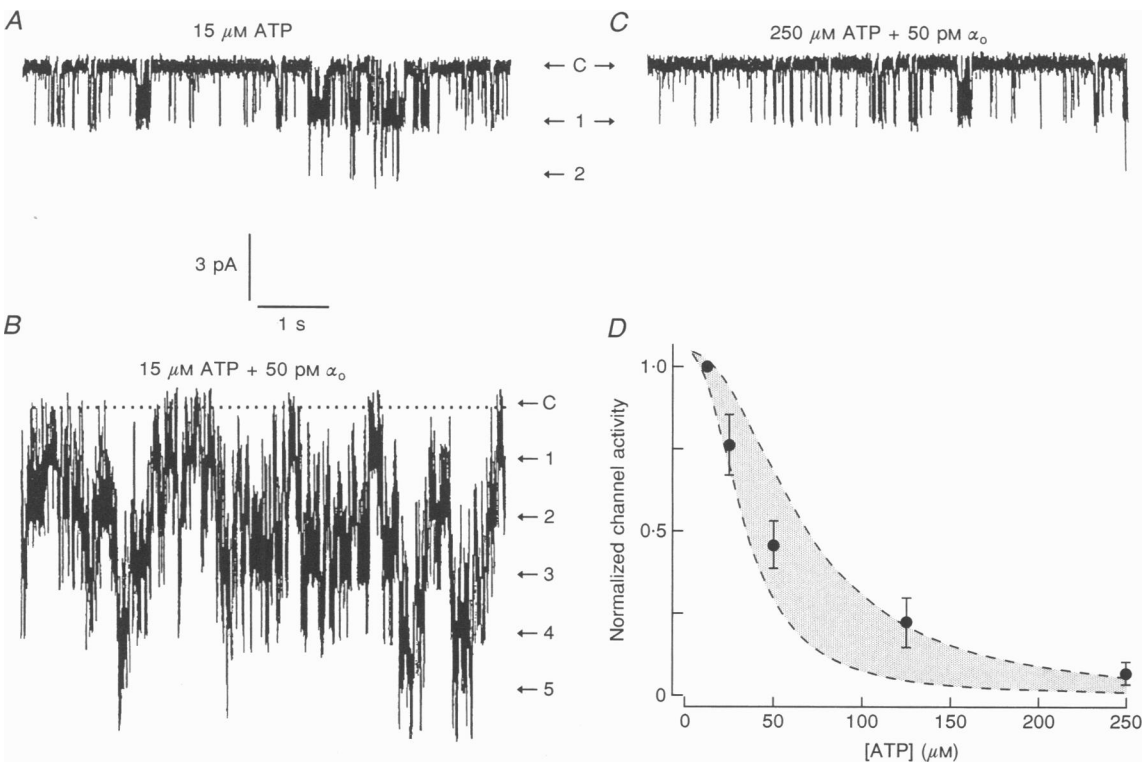


Figure 7. ATP-dependent inhibition of K^+_{ATP} channel activity by G protein α-subunits in excised inside-out patch experiments

A, *B* and *C* depict single-channel inward currents obtained at a holding potential of −70 mV with 140 mM K^+ on both sides of the membrane. The current trace in *A* was recorded under control conditions in the presence of 15 μM ATP. The data shown in *B* were acquired 8 min after addition of 50 pM α_o to the bath (the numbers beside the trace represent numbers of open channels). In *C*, the current trace demonstrates the effect of 250 μM ATP less than 1 min after its addition. In *D*, data obtained in the presence of α_o and α_i are averaged and plotted as a function of ATP concentration (●). The shaded area within the dashed lines was obtained from previous data analysis and indicates the variation of K^+_{ATP} channel sensitivity to ATP in the absence of exogenous α_o or α_i (Ribalet *et al.* 1989). Fitting of the data in *D* (fit not shown) yielded an IC_{50} of 35 μM and a Hill coefficient of 1·65. These values are not significantly different from those obtained previously (Ribalet *et al.* 1989).

DISCUSSION

The reversal of SRIF-induced inhibition of insulin release by elevated extracellular [Ca^{2+}] led to the proposal that a decrease in Ca^{2+} influx underlies the inhibitory effect of SRIF (Curry & Bennett, 1976; Bhathena *et al.* 1976). Reduction of Ca^{2+} entry by SRIF results from Ca^{2+} channel inhibition, either directly or by stimulation of K^+ channels, which causes cell membrane hyperpolarization. As outlined in the Introduction, a role for enhanced K^+ current activity has been postulated, but a significant body of evidence favours direct suppression of Ca^{2+} current as the mechanism whereby SRIF inhibits insulin release. Data obtained in the present study provide further support for the argument that K^+_{ATP} channel modulation does not contribute to the SRIF-induced suppression of insulin release, while also offering insight into the nature of the link between the SRIF receptor and the K^+_{ATP} channel.

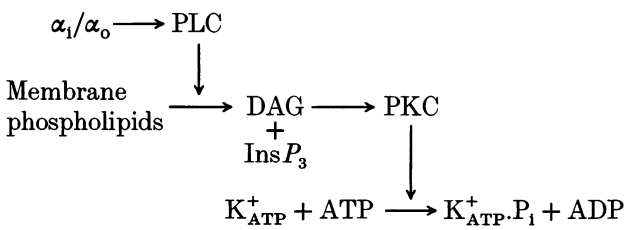
Investigation of SRIF receptor coupling to G proteins and PKC using K^+_{ATP} channels as a probe

PTX-sensitive G protein mediated stimulation of K^+_{ATP} channels by SRIF

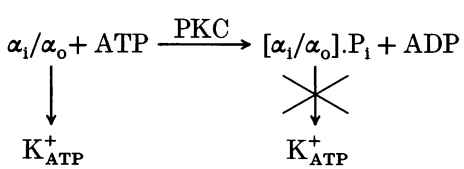
The first series of experiments was carried out on excised inside-out patches to differentiate 'direct' or membrane-delimited actions of SRIF on the K^+_{ATP} channel from 'indirect' action involving diffusible second messengers. Previously published data had shown that all the components of a stimulatory pathway linking the SRIF receptor to the K^+_{ATP} channel, including SRIF-activated PTX-sensitive G proteins, are present in the excised patch (Ribalet *et al.* 1991). The present data confirm and extend our previous observation, indicating three fundamental aspects of G protein–K^+_{ATP} channel relations. (1) Treatment with PTX_A in the absence of ligand reduces channel activity (Fig. 2), indicating that, in addition to SRIF-induced G protein activation, there is agonist-independent activation of PTX-sensitive G proteins that tonically stimulates K^+_{ATP} channels. (2) Channel activity is restored in a PTX_A-treated patch by addition of pre-activated α-subunit (Fig. 2), indicating that the inhibitory effect of PTX is due to ADP ribosylation of α_i/α_o-subunits rather than the channel itself. (3) The α-subunits of both G_i and G_o stimulate K^+_{ATP} channel activity. This observation raises the question of whether the modulatory effect of SRIF involves receptor coupling to G_i and/or G_o. We postulate that these three features are key elements in the modulation of β-cell function by G_i- or G_o-coupled receptors.

Is protein kinase C involved in K^+_{ATP} channel modulation by G proteins?

The data presented in Fig. 4 show that K^+_{ATP} channels are stimulated by a PKC activator and blocked by PKC inhibitors; these observations are good evidence that PKC is present in excised patches and that its activation stimulates the K^+_{ATP} channel. Thus, both PKC and G protein α_i/α_o-subunits stimulate the K^+_{ATP} channel; a parallel is also found in the Ca^{2+}-dependent K^+ channel (K^+_{Ca} channel), where α_i- or α_o-subunits and PKC are inhibitory (B. Ribalet, unpublished observations). These observations, together with the finding that α_o and PKC are closely associated in brain cell membranes (Worley *et al.* 1986), led us to hypothesize that the two moieties regulate K^+ channel activity in a concerted fashion rather than independently. Two types of interactions between α_i/α_o and PKC have been considered that may underlie this behaviour. One is the modulation of PKC by α_i and α_o via stimulation of phospholipase C and production of DAG (for review see Gilman, 1987); control of K^+_{ATP} channel activity by this process would occur as follows:



In this case, PKC acts directly on the channel and the α-subunit acts via PKC. The other possible mechanism involves modulation of α-subunit activity by PKC (Jakobs *et al.* 1985; Bushfield *et al.* 1990); in this case, the α-subunit directly interacts with the channel and phosphorylation of α_i/α_o by PKC inhibits the G protein function. Channel regulation will then be as follows:



Evidence presented in Fig. 5 supports the former scheme and refutes the latter; the α-subunit has no stimulatory effect on the channel in the presence of PKC inhibitor (Fig. 5*A*), and PTX fails to block the stimulatory effect of SC-9 (Fig. 5*B*). These data are consistent with a channel regulation by α_i/α_o-subunits, which is mediated via PKC-catalysed phosphorylation. However, they do not rule out the possibility that PKC-induced channel phosphorylation facilitates direct channel stimulation by α_i/α_o-subunits.

Is the modulation of K^+_{ATP} channels and β-cell function by SRIF mediated by G_i and/or G_o?

The data presented in the inset to Fig. 1*B* indicate that the concentration-dependent K^+_{ATP} channel stimulation by SRIF is biphasic. At nanomolar concentrations SRIF was stimulatory, but above 15 nM this effect vanished and subsequently recovered as the agonist concentration was

increased further from 50 to 500 nM. It is interesting to compare the concentration-dependent effects of SRIF on K^+_{ATP} channel activity with those obtained on insulin release and cAMP production in the insulin-secreting HIT cell. Inhibition of insulin release requires only low concentrations of SRIF (IC_{50} = 0·3 nM), while inhibition of adenylate cyclase occurs at concentrations close to 1 μM (Hurst & Morgan, 1989; Hsu *et al.* 1991). These observations suggest that inhibition of insulin release by low concentrations of SRIF does not involve activation of a G_i protein, the adenylate cyclase inhibitory G protein, but rather activation of a G protein of the G_o family. This conclusion stems from the observations that: (a) inhibition of insulin release by SRIF is mediated via a PTX-sensitive G protein (Katada & Ui, 1982); (b) PTX catalyses the ADP-ribosylation of two types of G protein, G_i and G_o (Sternweis & Robishaw, 1984; Neer, Lok & Wolf, 1984; Katada, Oinuma & Ui, 1986); and (c) both G_o and G_i are present in the β-cell membrane (Katada & Ui, 1982; Terashima, Katada, Oinuma, Inoue & Ui, 1987; Hsu *et al.* 1991; Cormont, Le Marchand-Brustel, Van Obberghen, Spiegel & Sharp, 1991). Assuming that a G protein of the G_o family mediates the effects of low levels of SRIF, while G_i proteins are involved at concentrations of SRIF greater than 100 nM, it may be concluded that 'direct' stimulation of K^+_{ATP} channels by low nanomolar concentrations of SRIF involves α_o-subunits, with α_i being effective at SRIF concentrations greater than 100 nM.

That a G_o-type G protein is associated with the high-affinity response of SRIF and a G_i-type G protein with the lower affinity one implies either that two receptor types are present, or that a single receptor type interacts with the two G protein species. There is evidence in favour of high- and low-affinity receptor sites for the somatostatin analogue SRIF-28 in β-cells (Maletti, Andersson, Marie, Rosselin & Mutt, 1992), and coupling of both G_{i3} and G_o to a single SRIF receptor subtype (Law, Yasuda, Bell & Reisine, 1993). Since a change in β_2-adrenergic, α_2-adrenergic and muscarinic receptor affinity due to desensitization has been associated with a change in specificity for the G protein to which they couple (Ashkenazi *et al.* 1987; Okamoto, Murayama, Hayashi, Inagaki, Ogata & Nishimoto, 1991), the disappearance of the effect of SRIF between 20 and 100 nM renders the single receptor hypothesis very attractive. The reversal of inhibition above 20 nM is compatible with desensitization of the SRIF receptor (Mayor, Benovic, Caron & Lefkowitz, 1987), and so such a phenomenon may account for a change in receptor affinity and receptor coupling to G_o, at low nanomolar concentrations, and to G_i, at higher concentrations.

Are K^+_{ATP} channels involved in the inhibition of insulin release by activated G_i/G_o-coupled receptors?

In the first series of experiments, we investigated the 'direct' modulation of the K^+_{ATP} channel by SRIF. To determine whether this mechanism has a role in SRIF-induced suppression of insulin release, we also tested the effect of SRIF in the cell-attached patch configuration in which the cell metabolism and the 'indirect' pathways are involved. As previously shown, in this patch configuration SRIF enhances K^+_{ATP} channel activity; by doing so, it may assist in the hyperpolarization of the membrane and closure of voltage-gated calcium channels (De Weille *et al.* 1989). While such observations could be, and were, interpreted in terms of enhanced K^+ permeability in SRIF-induced suppression of insulin release, it must be noted that these experiments were performed in the absence of glucose. Experiments carried out in the presence of glucose showed that under these conditions, SRIF did not stimulate K^+_{ATP} channel activity (Fig. 6*A–C*). The plot of glucose concentration dependence of channel block in the absence and presence of SRIF (Fig. 6*D*) confirms that the stimulatory effect of SRIF is refractory to the inhibition by the nutrient.

The data presented in Fig. 7 show that the ability of ATP to inhibit K^+_{ATP} channel activity is not affected by G protein α-subunits. This result corroborates the cell-attached patch data described above, since the K^+_{ATP} channel is blocked by glucose via ATP production and stimulated by SRIF via activation of G_i/G_o, and suggests that the interaction which underlies this hierarchical signalling process occurs at the level of the cell membrane.

These observations lend further support to the hypothesis that SRIF-induced inhibition of insulin release is predominantly mediated via inhibition of calcium channels rather than stimulation of K^+ permeability.

Role of cAMP modulation by G protein-coupled receptors

While previous studies (De Weille *et al.* 1989), in addition to this one, have described stimulation of the K^+_{ATP} channel by SRIF in cell-attached patches, it was also noted in the present work that a significant number of patches responded to SRIF with varying degrees of channel inhibition (3 of 11). Since only channel stimulation was observed in excised patches in response to SRIF, the simplest explanation for channel inhibition in the cell-attached patch is that it involves a diffusible component that is lost in excised patches. Evidence in favour of cAMP being this component is derived from the findings that SRIF inhibits cAMP production at the concentration

used in our cell-attached patch experiments and that increasing the cAMP level reversed the inhibitory effect of SRIF on K^+_{ATP} channel activity. These data suggest that SRIF modulates K^+_{ATP} channel activity via two separate G protein-dependent mechanisms acting in opposite manners; one is stimulatory and involves 'direct' regulation by G proteins, while the other is inhibitory and acts 'indirectly' via lowering the cAMP production. The channel stimulation by SRIF, in most cell-attached patches, argues that modulation of the cAMP level by G protein-coupled receptors is unlikely to control the K^+_{ATP} channel activity in intact cells, since the inhibition by reduced cAMP levels may be subordinate to the 'direct' stimulation by G proteins.

Thus the question arises as to whether activation of G_i by SRIF plays a physiological role in modulating insulin release. For such a role, it would be necessary for SRIF to reach a concentration of the order of 1 μM, a condition which may occur at the periphery of the islets, where δ-cells and β-cells are closely associated. In this case, decreased PKA activity due to lowering of cAMP may have short- and long-term inhibitory effects on Ca^{2+} currents (Yue, Hergiz & Marban, 1990) and insulin synthesis (Fehmann & Habener, 1992), respectively.

Summary

In pancreatic β-cells, the SRIF receptor may be coupled to the K^+_{ATP} channel via both G_o and G_i. Taking into account the involvement of PKC, the channel regulation by G proteins may include the following steps: SRIF $\rightarrow \alpha_i/\alpha_o \rightarrow$ PKC $\rightarrow K^+_{ATP}$ channel, or alternatively PKC may facilitate the effect of α-subunits on the channel. This regulation is subordinate to the channel inhibition by ATP, so it is argued that K^+_{ATP} channels do not play a role in the inhibition of insulin release by SRIF.

Ashkenazi, A., Winslow, J. W., Peralta, E. G., Peterson, G. L., Schimerlik, M. I., Capon, D. J. & Ramachandran, J. (1987). An M2 muscarinic receptor subtype coupled to both adenylate cyclase and phosphoinositide turnover. *Science* **238**, 672–675.

Bhathena, S. J., Perrino, P. V., Voyles, N. R., Smith, S. S., Wilkins, S. D., Coy, D. H., Schally, A. V. & Recant, L. (1976). Reversal of somatostatin inhibition of insulin and glucagon secretion. *Diabetes* **25**, 1031–1040.

Bushfield, M., Murphy, G. J., Lavan, B. E., Parker, P. J., Hruby, V. J., Milligan, G. & Houslay, M. D. (1990). Hormonal regulation of G_{i2} α-subunit phosphorylation in intact hepatocytes. *Biochemical Journal* **268**, 449–457.

Cook, D. L. & Perara, E. (1982). Islet electrical pacemaker response to alpha-adrenergic stimulation. *Diabetes* **31**, 985–990.

Cormont, M., Le Marchand-Brustel, Y., Van Obberghen, E., Spiegel, A. M. & Sharp, G. W. G. (1991). Identification of G protein α-subunits in RINm5F cells and their selective interaction with galanin receptor. *Diabetes* **40**, 1170–1176.

Curry, D. L. & Bennett, L. L. (1976). Does somatostatin inhibition of insulin secretion involve two mechanisms of action? *Proceedings of the National Academy of Sciences of the USA* **73**, 248–251.

Debuyser, A., Drews, G. & Henquin, J.-C. (1991). Adrenaline inhibition of insulin release: role of the repolarization of the B cell membrane. *Pflügers Archiv* **419**, 131–137.

De Weille, J. R., Schmid-Antomarchi, H., Fosset, M. & Lazdunski, M. (1989). Regulation of ATP-sensitive K^+ channels in insulinoma cells: Activation by somatostatin and protein kinase C and the role of cAMP. *Proceedings of the National Academy of Sciences of the USA* **86**, 2971–2975.

Drews, G., Debuyser, A., Nenquin, M. & Henquin, J.-C. (1990). Galanin and epinephrine act on distinct receptors to inhibit insulin release by the same mechanisms including an increase in K^+ permeability of the B-cell membrane. *Endocrinology* **126**, 1646–1653.

Eddlestone, G. T., Ribalet, B. & Ciani, S. (1989). A comparative study of K channel behavior in β cell lines with different secretory responses to glucose. *Journal of Membrane Biology* **109**, 123–134.

Fehmann, H.-C. & Habener, J. F. (1992). Insulinotropic hormone glucagon-like peptide-I (7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma β TC-1 cells. *Endocrinology* **130**, 159–166.

Gilman, A. G. (1987). G proteins: transducers of receptor-generated signals. *Annual Review of Biochemistry* **56**, 615–649.

Hidaka, H., Inagaki, M., Kawamoto, S. & Sasaki, Y. (1984). Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry* **23**, 5036–5041.

House, C. & Kemp, B. E. (1987). Protein kinase C contains a pseudosubstrate prototope in its regulatory domain. *Science* **238**, 1726–1728.

Hsu, W. H., Xiang, H., Rajan, A. S., Kunze, D. L. & Boyd, A. E. III (1991). Somatostatin inhibits insulin secretion by a G protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta cell. *Journal of Biological Chemistry* **266**, 837–843.

Hurst, R. D. & Morgan, N. G. (1989). Intracellular events responsible for the inhibition of insulin secretion by somatostatin. *Biochemical Society Transactions* **17**, 1085–1086.

Ito, H., Tung, R. T., Sugimoto, T., Kobayashi, I., Takahashi, K., Katada, T., Ui, M. & Kurachi, Y. (1992). On the mechanism of G protein $\beta\gamma$ subunit activation of the muscarinic K^+ channel in guinea pig atrial cell membrane. *Journal of General Physiology* **99**, 961–983.

Jakobs, K. H., Bauer, S. & Watanabe, Y. (1985). Modulation of adenylate cyclase of human platelets by phorbol ester. Impairment of the hormone-sensitive inhibitory pathway. *European Journal of Biochemistry* **151**, 425–430.

Katada, T., Oinuma, M. & Ui, M. (1986). Two guanine nucleotide-binding proteins in rat brain serving as the specific substrate of islet-activating protein, pertussis toxin. *Journal of Biological Chemistry* **261**, 8182–8191.

KATADA, T. & UI, M. (1982). Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. *Proceedings of the National Academy of Sciences of the USA* **79**, 3129–3133.

LAW, S. F., YASUDA, K., BELL, G. I. & REISINE, T. (1993). $G_{i\alpha 3}$ and $G_{o\alpha}$ selectively associate with the cloned somatostatin receptor subtype SSTR2. *Journal of Biological Chemistry* **268**, 10721–10727.

MALETTI, M., ANDERSSON, M., MARIE, J.-C., ROSSELIN, G. & MUTT, V. (1992). Solubilization and partial purification of somatostatin-28 preferring receptors from hamster pancreatic β cells. *Journal of Biological Chemistry* **267**, 15620–15625.

MAYOR, F., BENOVIC, J. L., CARON, M. G. & LEFKOWITZ, R. J. (1987). Somatostatin induces translocation of the β-adrenergic receptor kinase and desensitizes somatostatin receptors in S49 lymphoma cells. *Journal of Biological Chemistry* **262**, 6468–6471.

MISLER, S., FALKE, L. C., GILLIS, K. & MCDANIEL, M. L. (1986). A metabolite-regulated potassium channel in rat pancreatic β-cell. *Proceedings of the National Academy of Sciences of the USA* **83**, 7119–7123.

NEER, E. J., LOK, J. M. & WOLF, L. G. (1984). Purification and properties of the inhibitory guanine nucleotide regulatory unit of brain adenylate cyclase. *Journal of Biological Chemistry* **259**, 14222–14229.

NISHINO, H., KITAGAWA, K., IWASHIMA, A., ITO, M., TANAKA, T. & HIDAKA, H. (1986). *N*-(6-Phenylhexyl)-5-chloro-1-naphthalene-sulfonamide is one of a new class of activators for Ca^{2+} activated, phospholipid-dependent protein kinase. *Biochimica et Biophysica Acta* **889**, 236–239.

OKAMOTO, T., MURAYAMA, Y., HAYASHI, Y., INAGAKI, M., OGATA, E. & NISHIMOTO, I. (1991). Identification of a G_s activator region of the β2-adrenergic receptor that is autoregulated via protein kinase A-dependent phosphorylation. *Cell* **67**, 723–730.

PACE, C. & TARVIN, J. T. (1981). Somatostatin: mechanism of action in pancreatic islet B-cells. *Diabetes* **30**, 836–842.

PIROS, E., EVANS, C. J., RIBALET, B. & HALES, T. (1993). Calcium channel inhibition by somatostatin and an opioid in HIT and NG108-15 cells. *Society for Neuroscience Abstracts* **19**, 1551a.

RIBALET, B., CIANI, S. & EDDLESTONE, G. T. (1989*a*). ATP mediates both activation and inhibition of K(ATP) channel activity via cAMP dependent protein kinase in insulin secreting cell lines. *Journal of General Physiology* **94**, 693–717.

RIBALET, B., CIANI, S. & EDDLESTONE, G. T. (1989*b*). Modulation of ATP-sensitive K channels in RINm5F cells by phosphorylation and G proteins. *Biophysical Journal* **55**, 587a.

RIBALET, B., CIANI, S., HALES, T. G. & EDDLESTONE, G. T. (1991). Role of G proteins in K(ATP) channel modulation by the neuropeptide somatostatin. *Biomedical Research* **12**, 37–40.

RIBALET, B., EDDLESTONE, G. T. & CIANI, S. (1988). Metabolic regulation of the K(ATP) and a Maxi-K(V) channel in the insulin secreting RINm5F cell. *Journal of General Physiology* **92**, 219–237.

RORSMAN, P., BOKVIST, K., AMMALA, C., ARKHAMMAR, P., BERGGREN, P.-O., LARSSON, O. & WAHLANDER, K. (1991). Activation of a low-conductance G protein-dependent K^+ channel in mouse pancreatic B cells. *Nature* **349**, 77–79.

STERNWEIS, P. C. & ROBISHAW, J. D. (1984). Isolation of two proteins with high affinity for guanosine nucleotides from membranes of bovine brain. *Journal of Biological Chemistry* **259**, 13806–13813.

TERASHIMA, T., KATADA, T., OINUMA, M., INOUE, Y. & UI, M. (1987). Endocrine cells in pancreatic islets of Langerhans are immunoreactive to antibody against guanine nucleotide-binding protein (G_o) purified from brain. *Brain Research* **417**, 190–194.

ULLRICH, S. & WOLLHEIM, C. B. (1988). GTP-dependent inhibition of insulin secretion by epinephrine in permeabilized RINm5F cells. *Journal of Biological Chemistry* **263**, 8615–8620.

WORLEY, P. F., BARABAN, J. M., VAN DOP, C., NEER, E. J. & SNYDER, S. H. (1986). G_o, a guanine nucleotide-binding protein: Immunohistochemical localization in rat brain resembles distribution of second messenger systems. *Proceedings of the National Academy of Sciences of the USA* **83**, 4561–4565.

YATANI, A., CODINA, J., BROWN, A. M. & BIRNBAUMER, L. (1987). Direct activation of mammalian atrial muscarinic potassium channels by GTP regulatory protein G_k. *Science* **235**, 207–211.

YUE, D. T., HERGIZ, S. & MARBAN, E. (1990). β-Adrenergic stimulation of calcium channels occurs by potentiation of high-activity gating modes. *Proceedings of the National Academy of Sciences of the USA* **87**, 753–757.

Acknowledgements

The authors would like to thank Dr A. E. Boyd III for supplying the RINm5F and HIT cells, Drs J. Codina and L. Birnbaumer for supplying the α-subunits of the G proteins G_i and G_o, and Mrs Satoko Hagiwara for preparing and maintaining the cell cultures. This work was supported by grant DCB-8919368 from the National Science Foundation and a research grant (W-P 880513) from the American Diabetes Association to B. R., and by grant RO1-DK39652 from the National Institutes of Health to G. T. E.

Received 8 March 1994; accepted 15 November 1994.