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Characterization of the G Protein Coupling of a Glucagon Receptor to the K_{ATP} Channel in Insulin-secreting Cells

B. Ribalet, S. Ciani

Department of Physiology, Ahmanson Laboratory of Neurobiology, University of California, Los Angeles, Los Angeles, California 90024

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Abstract. The G-protein-mediated coupling of a glucagon receptor to ATP-dependent K channels—K_{ATP}—has been studied in insulin-secreting cells using the patch clamp technique. In excised outside-out patches, K_{ATP} channel activity was inhibited by low concentrations of glucagon (IC_{50} = 2.4 nM); the inhibitory effect vanished at concentrations greater than 50 nM. In cell-attached patches, inhibition by bath-applied glucagon was seen most often, although stimulation was observed in a few cases. A dual action of the hormone is proposed to resolve these apparently divergent results. In excised inside-out patches, K_{ATP} channel activity was inhibited by addition of $\beta\gamma$ subunits purified from either erythrocyte or retina (IC_{50} = 50 pM and 1 nM, respectively). Subsequent exposure of the patch to α_i or α_o reversed this effect. In excised inside-out patches, increasing Mg^{2+} in the bath stimulated the channel activity between 0 and 0.5 mM, but blocked it at higher concentrations (IC_{50} = 2.55 mM). In most cases (70%), GTP had a stimulatory effect at concentrations up to 100 μM. However, in three cases, similar GTP levels had clear inhibitory effects. In excised inside-out patches, cholera toxin (CTX) caused channel inhibition. Although the effect could not be reversed by removal of the toxin, the activity was restored by subsequent addition of purified α_i or α_o. These results are compatible with a model whereby channel inhibition by activated G_s-coupled receptors occurs, at least in part, via association of the $\beta\gamma$ subunits of G_s with α_i/α_o subunits and deactivation of the α_i/α_o-dependent stimulatory pathway. On the basis of this hypothesis, a model is developed to describe the effects of G proteins on the K_{ATP} channel, as well as to account for the concentration-dependent stimulation and inhibition of K_{ATP} channel by Mg^{2+}. An interpretation of the ability of glucagon to potentiate, but not initiate, insulin release is also given in terms of this model and the effects of ATP on K_{ATP} channels.

Key words: K_{ATP} channel — Patch clamp — G protein — Mg^{2+} — Theoretical model

Introduction

Glucagon potentiates the secretion of insulin induced by glucose in pancreatic β cells (Kofod et al., 1988*a*), but does not initiate secretion in the absence of glucose; the mechanisms underlying these hormonal effects have yet to be identified. Although the increase in cAMP production due to the activation of "stimulatory" guanosine nucleotide-binding G protein, G_s, by glucagon (Goldfine, Roth & Birnbaumer, 1972) is probably involved in potentiation of insulin release (Pipeleers et al., 1985), evidence has also been presented in favor of a cAMP-independent regulatory mechanism (Kofod et al., 1988*a*; Ullrich & Wollheim, 1988).

In a recent study, it was suggested that potentiation of insulin release by the glucagon-like peptide, GLP-1, is due to elevation of cAMP production causing inhibition of the K_{ATP} channel (Holz, Kuhtreiber & Habener, 1993). Our observation that raising the cAMP level increases K_{ATP} channel activity via stimulation of cAMP-dependent protein kinase (PKA) (Ribalet, Ciani & Eddlestone, 1989*a*) does not support this hypothesis. To resolve these conflicting results, we postulate that K_{ATP} channel inhibition by glucagon occurs via a mechanism that is cAMP independent, and which involves a direct (membrane-delimited) effect of one or more of the subunits of the G protein, G_s.

Upon activation of a trimeric G protein, the α subunit and the $\beta\gamma$ heterodimer are free to act independently, thus, in principle, G-protein-mediated modulation of the

Correspondence to: B. Ribalet

K_{ATP} channel may be due to either or both of these components. Recent experiments with cardiac cells have suggested that βγ heterodimers have a "membrane-delimited" effect both on the K_{ACh} and the K_{ATP} channels (Okabe et al., 1990; Ito et al., 1992). The present results confirm these observations and indicate that βγ dimers originating from activation of G_s mediate the inhibitory effect of glucagon on the channel by favoring the conversion of stimulatory α_i subunits (Ribalet et al., 1991) into inactive, trimeric G_i.

Increasing the concentrations of Mg^{2+} between 1 and 10 mM has been shown to cause K_{ATP} channel inhibition in pancreatic β cells (Findlay, 1987; Ashcroft & Kakei, 1989), while raising the Mg^{2+} level between 0 and 0.5 mM has the opposite effect (Ashcroft & Kakei, 1989). Since Mg^{2+} blocks channel activity in the absence of ATP, while having no effect on the dose-dependent channel inhibition by ATP, it has been suggested that this inhibitory effect of Mg^{2+} is independent of that of ATP (Ashcroft & Kakei, 1989). On the basis of the present results and the observation that maximal activation of G_i and G_s requires different concentrations of Mg^{2+}, a model is developed to account for the ATP-independent effect of Mg^{2+} on the channel activity.

An implication of this model suggests a mechanism for the ability of glucagon to potentiate insulin secretion in the presence of glucose, as well as for its inability to initiate insulin release in the absence of glucose.

Materials and Methods

Cell Cultures and Experimental Media

The K_{ATP} channel studies were performed using cells from the insulin-secreting cell lines RINm5F (passage nos. 42–56) and HIT (passage nos. 70–76). Both cell lines were kindly provided by A.E. Boyd, III. The cells were incubated at 37°C in RPMI 1640 medium, supplemented with 10% (vol/vol) fetal calf serum, penicillin (100 U/ml), streptomycin (100 U/ml) 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 with 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 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, the pH was adjusted to 7.2 with KOH. For experiments with excised inside-out patches, the bath solution was similar, except that no calcium was added. For experiments with inside-out patches, both ATP (10 μM) (Sigma) and GTP (50–100 μM) (Boehringer Mannheim Diagnostics, Houston, TX) were present in the bath solution to prevent loss of channel activity (Ribalet, Ciani & Eddlestone, 1989*b*). Glucagon and GTPγS were obtained from Sigma. The βγ dimer from erythrocytes, provided by Drs. C. Codina and L. Birnbaumer (Baylor College of Medicine), was kept in a 1 μM buffered stock solution containing 10 mM HEPES, 1 mM EDTA, 0.1% lubrol, and 200 mM NaCl. The βγ dimer from retina, provided by Dr. R. Cerione (Cornell University, Ithaca, NY), was kept in glycerol as a 11.5 μM stock solution. Stock solution of the A subunit of cholera toxin, (List Biological Laboratories, Campbell, CA) was prepared with 25 μg of the protein diluted in 1 ml of solution containing (mM): 50 TRIS, 200 NaCl, 1 Na_2 EDTA and 3 NaN_3 at pH 7.5. The α subunits of the G proteins G_i and G_o, kindly provided by Drs. J. Codina and L. Birnbaumer (Baylor College of Medicine), were kept at a concentration of 2 μM in a buffered solution containing (mM) 10 TRIS, 1 EDTA, 1 dithiothreitol, 7.5 $MgCl_2$, 20 KCl. Prior to the experiment, this concentrated solution was diluted 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 being 0.8.

Data Recording and Analysis

The techniques to prepare patch electrodes and record single channel events were similar to those described previously (Ribalet et al., 1988; *see also* Hamill et al., 1981, for details). The data, filtered at 2 kHz with an 8-pole Bessel filter, were recorded either with an EPC 7 List (Darmstadt, FRG) or an Axopatch 1A (Axon Instruments, Burlingame, CA) 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 (Bezanilla, 1985). Prior to measurement of the channel mean open and closed times, 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 (Colquhoun & Sigworth, 1983). This schematized record was utilized for the subsequent analysis. The percent open time, which is the parameter used to assess the level of channel activity, was determined from data samples of 30 sec duration. All data used for analysis and illustration purposes are normalized or compared to control samples obtained from the same patch.

Results

K_{ATP} Channel Modulation by Glucagon

It has been recently proposed that potentiation of insulin release by glucagon in the presence of glucose is due to inhibition of K_{ATP} channels (Holz et al., 1993). Patch clamp experiments were carried out to identify the mechanism(s) responsible for K_{ATP} channel inhibition by glucagon.

K_{ATP} Channel Modulation by Glucagon in Excised Outside-Out Patches

Experiments with excised outside-out patches were performed to investigate whether glucagon regulates channel activity via a membrane-delimited mechanism. (These mechanisms will be referred to as "direct.") The data in the two upper traces of Fig. 1 indicate that 15 nM glucagon blocks K_{ATP} channel activity. A quantitation of this effect is shown in *D*, where the channel activities from six experiments were normalized, averaged and

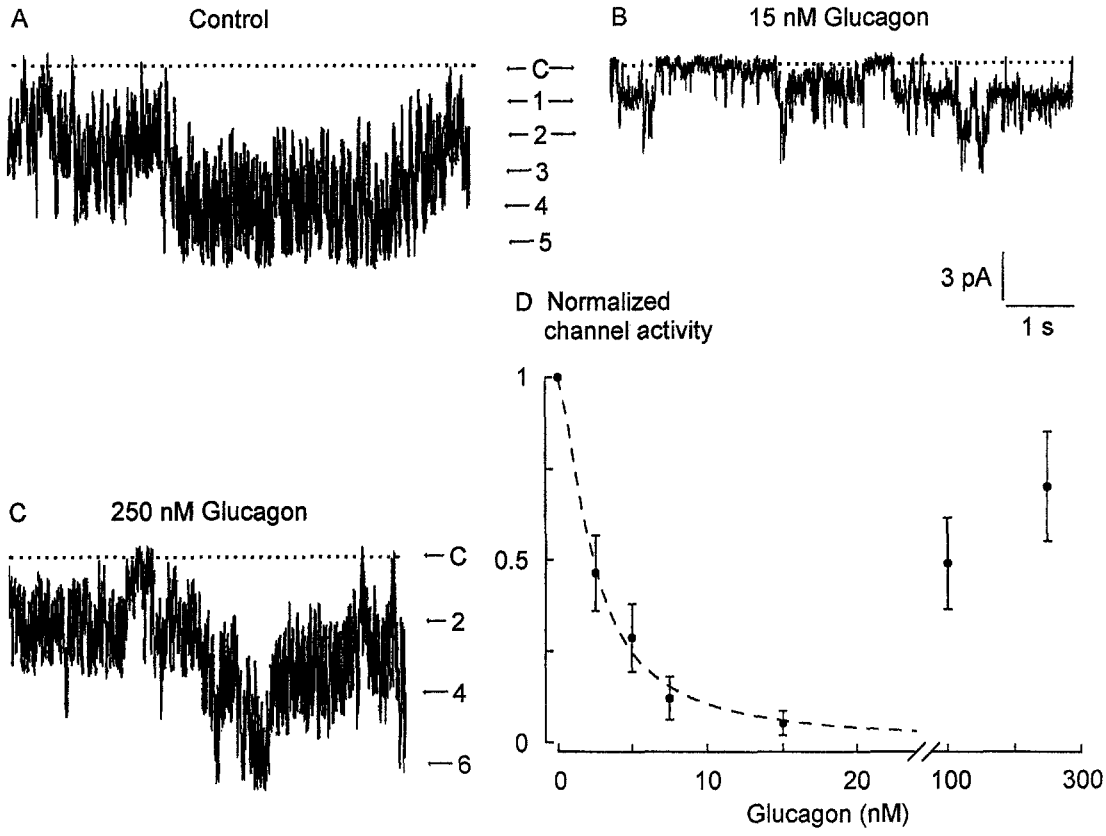

Fig. 1. Dose-dependent effects of glucagon on K_{ATP} channel activity in outside-out patch experiments. Recordings in *A* through *B* are from the same patch and are representative of five other experiments in which glucagon was increased in the range 2.5 to 250 nM. The data shown in *A* and *B* depict the inhibitory effect of bath-applied glucagon (15 nM). Downward deflections represent inward current in symmetrical 140 mM K^+ for a holding pipette potential of −70 mV. The pipette solution contained 50 μM GTP and 20 μM ATP. The data in *B* were taken 2 min after the addition of glucagon. The data in *C*, taken 3 min after addition of 250 nM glucagon, illustrate the recovery of channel activity. In *D*, data points in the absence of glucagon and up to 15 nM are averages of five measurements obtained from six patches, those at 100 and 250 nM are averages of three originating from the same set of experiments. To permit comparison between patches, the channel activity measured at various glucagon concentrations was normalized to the activity measured in the same patch in the absence of agonist. The individual values were then averaged, plotted as a function of glucagon concentration, and fitted with the following equation:

$$I/Io = 1/(1 + (C/K)^n)$$

C is the glucagon concentration, *K* is the IC_{50} constant and *n* is a Hill coefficient. The fit of the data yielded $K = 2.4$ nM and $n = 1.5$. The horizontal axis is interrupted between 25 and 75 nM, and the scale is expanded after the interruption.

plotted as a function of the concentration of glucagon. Inhibition of channel activity was half-maximal at 2.4 nM glucagon, a value very close to that found for glucagon-mediated regulation of cAMP production (Goldfine et al., 1972) and insulin release (Kofod et al., 1988*a*).

The data in *C* indicate that the inhibitory effect of glucagon is virtually abolished at higher concentrations (250 nM). This decrease of inhibition is also illustrated in the right side of *D*, which shows data averaged from three experiments at glucagon concentrations of 100 and 250 nM, respectively. This reversal of glucagon-induced K_{ATP} channel inhibition, which begins at about 20 nM, and is almost complete at 250 nM, is consistent with the previously proposed hypothesis of a concentration-dependent desensitization of glucagon and glucagon-like peptide receptors (Hoosein & Gurd, 1984; Fehmann & Habener, 1992).

The data in Fig. 1 strongly suggest that glucagon modulates K_{ATP} channel activity via "direct" mechanisms.

K_{ATP} Channel Modulation by Glucagon in Cell-Attached Patches

To investigate whether channel modulation by glucagon may be also mediated by diffusible second messengers, experiments were performed in cell-attached patches. In this patch configuration, the effects of bath-applied glucagon on the K_{ATP} channel were variable. In four out

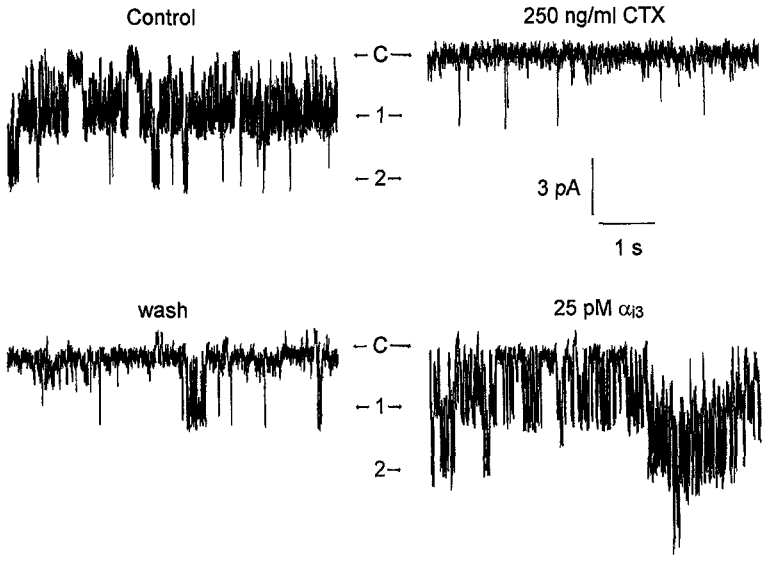

Fig. 2. Effects of cholera toxin (CTX) on K_{ATP} channel activity in an excised inside-out patch. The patch of membrane was held at −70 mV, the bath solution contained 10 μM ATP and 50 μM GTP. All the data shown in this figure originate from the same experiment. The two upper traces illustrate the effect of 250 ng/ml CTX on channel activity. Control activity shown in the upper left panel was recorded in the presence of 10 μM NAD^+. The upper right trace shows the blocking effect of CTX, 8 min after addition of the toxin to the bath. The lower left trace, taken 10 min after toxin removal, illustrates the lack of reversibility of the effect of CTX. The data in the bottom right corner show recurrence of channel activity 8 min after addition of 25 pM α_{i3} to the bath.

of six experiments where addition of 15 nM glucagon to the bath had a significant effect on K_{ATP} channel activity, there was an 81+/−8.9% reduction in channel activity compared to control; in the two other experiments the channel activity increased by 3.9-fold.

In two experiments in which application of glucagon to the bath caused a reduction of channel activity, and in one experiment in which glucagon caused an increase of channel activity, the changes in activity were accompanied by a 19.5+/−3.2% decrease in single channel current amplitude. Considering the conditions of the experiments (140 mM KCl in the electrode and 0 mV pipette potential), a decrease in the (inward) current amplitude is consistent with cell membrane depolarization, presumably resulting from closure of a high percentage of K_{ATP} channels in the membrane. Thus, addition of glucagon to the bath may cause either stimulation or inhibition of the channels in the patch, but consistently inhibits those outside the patch.

These results suggest that glucagon has a dual effect on the K_{ATP} channel, one inhibitory via membrane-delimited pathways, and one stimulatory, probably via diffusible messenger (e.g., cAMP).

K_{ATP} Channel Modulation by the Subunits of G_s

Channel Modulation by CTX; Role of Endogenous Subunits of G_s

Since glucagon-mediated modulation of cell functions involves activation of G_s, we investigated whether endogenous, membrane-bound G_s may be involved in linking glucagon receptors to K_{ATP} channels in excised patches. To test this hypothesis, we studied the effect of cholera toxin (CTX) on K_{ATP} channel activity in excised inside-out patches. CTX, which irreversibly activates G_s by ADP-ribosylating its alpha subunit α_s (Birnbaumer, Abramowitz & Brown, 1990), caused a 78.8+/−8.1% channel block in the presence of 10 μM NAD^+. At this concentration NAD^+ had no effect on channel activity while being inhibitory at higher concentrations (*results not shown*). The data shown in the upper traces of Fig. 2 are representative of six experiments in which CTX inhibited channel activity.

We deduce from these data that blocking of K_{ATP} channel activity by activated, membrane-bound G_s may account for the inhibitory effect of glucagon.

K_{ATP} Channel Modulation by the α and βγ Subunits of G_s

Since activation by CTX causes trimeric G_s proteins to dissociate into their α_s and βγ subunits, it follows that K_{ATP} channel inhibition by CTX may involve either or both of these components.

Purified α_s subunits, which are much less abundant than α_o or α_i in cell membranes (Codina et al., 1984), were available in a very limited amount; only few results could therefore be obtained with these subunits. In three experiments, addition of α_s up to 100 pM had almost no effect on K_{ATP} channel activity. However, in one case the channel activity was reduced by 35% after prolonged exposure (10 min). It is unclear whether this irreversible slow decay was due to the presence of α_s or to "channel rundown." More experiments need to be performed to clarify this point, although it should be noted that muscarinic K channels (K_{ACh}), which are activated by α_i and α_o, have also been shown to be insensitive to α_s (Yatani et al., 1987).

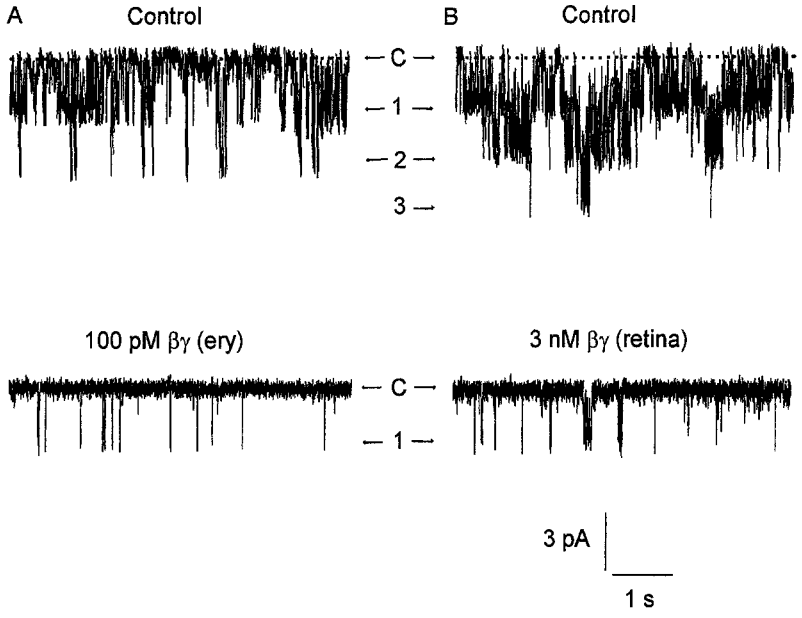

Fig. 3. Inhibitory effect of βγ on K_{ATP} channel activity in inside-out patch experiments. The upper and lower traces in *A* are from a different patch than those in *B*. The recording in the two upper traces was obtained in the absence of exogenous βγ subunits, that in the lower trace in *A* was obtained 3 min after the addition of 100 pM βγ subunit from erythrocyte, that in the lower trace of *B* was acquired 5 min after addition of 3 nM βγ subunit from retina. Downward deflections represent inward current in symmetrical K^+ for a holding pipette potential of −70 mV. The bath solution contained 10 μM ATP and 50 μM GTP. The numbers between *A* and *B* relate the current amplitudes to the number of open channels.

K_{ATP} Channel Activity is Inhibited by βγ. The data in Fig. 3 demonstrate that, using excised inside-out patches, addition of βγ subunits, purified from either erythrocyte ($\beta\gamma_E$) (*A*) or retina ($\beta\gamma_T$) (*B*), blocked channel activity almost completely. βγ dimers from erythrocyte were kept in a stock solution with 0.1% lubrol (wt/vol), those from retina were kept in glycerol. At 0.1%, both solvents substantially blocked K_{ATP} channel activity. However, in three control experiments, lubrol had no effect on channel activity at concentrations similar to those coapplied with βγ dimers (10^{-3}% lubrol for 100 pM $\beta\gamma_E$). A similar lack of effect of these low concentrations of solvent has been previously reported in the case of the K_{ACh} channel (Okabe et al., 1990; Ito et al., 1992). When $\beta\gamma_T$ dimers (10 nM) were coapplied with 10^{-2}% glycerol, K_{ATP} channel activity was blocked by 85%. In three control studies, this concentration of glycerol inhibited channel activity by less than 15%. Thus, both types of βγ dimer block K_{ATP} channel activity. However, as shown in Fig. 4*B*, the concentration ranges over which the two types of βγ dimer exert their inhibitory effects are quite different: approximately 20 times more $\beta\gamma_T$ is required to obtain an effect comparable to that of $\beta\gamma_E$. The IC_{50}s deduced from the fit of these data were 50 pM and 1 nM for $\beta\gamma_E$ and $\beta\gamma_T$, respectively.

These data support the hypothesis that K_{ATP} channel inhibition by activated G_s may be due to the βγ heterodimers.

α_i Reverses CTX-induced K_{ATP} Channel Inhibition. Although the results described above clearly indicate that βγ dimers inhibit K_{ATP} channel activity, it remains to be established whether this effect is due to suppression of α_i/α_o-induced K_{ATP} channel stimulation or to a direct effect of βγ on the channel. The left trace at the bottom of Fig. 2 shows that the inhibitory effect of CTX on K_{ATP} channel activity was not reversed by removal of the toxin; however, the right trace demonstrates that, under these conditions, addition of exogenous α_i fully restored channel activity.

Taken together, these results favor the hypothesis that K_{ATP} channel inhibition by endogenous G_s may be mediated via βγ, and that the effect of βγ is to suppress the previously described stimulatory effect of α_i (Ribalet et al., 1991; Ito et al., 1992), rather than that of directly inhibiting the channel. Inhibition of channel activity would thus result from the reassociation of $\beta\gamma_{Gs}$ with α_i/α_o.

Model for K_{ATP} Channel Modulation by α_i and βγ G_s

A model has been formulated, which accounts for our results and also provides some estimates of parameters related to the mechanisms of K_{ATP} channel regulation by G protein subunits. The main assumptions are based on established G protein properties, while the theory assumes that standard thermodynamical and kinetic principles apply to phenomena occurring in the "microenvironment" of the membrane patch.

K_{ATP} Channel Stimulation by α_i

The basic assumption of the model is that stimulatory interactions occur between the α subunits of either G_i or G_o and the K_{ATP} channels. Evidence in favor of this hypothesis has already been given by experiments show-

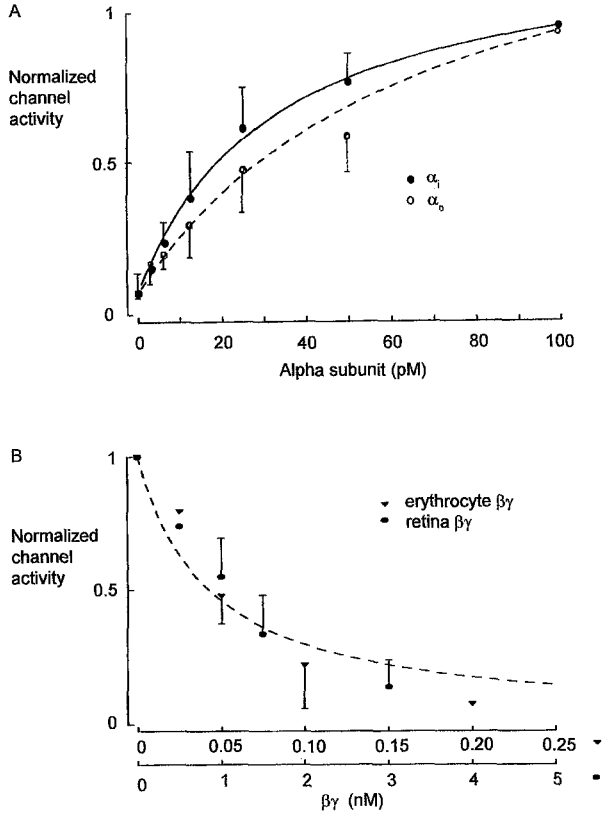

Fig. 4. Dose-dependent modulation of K_{ATP} channel activity by α and βγ subunits in inside-out patches. (*A*) Stimulatory effect of α_o and α_i subunits. The open and filled circles are averaged channel activity obtained from six and eight experiments for α_o and α_i, respectively. To permit comparison of data obtained in different patches, the channel activity measured at various α subunit concentrations was normalized to the activity measured in the same patch in the absence of exogenous G protein. These data points were then averaged for each concentration of α subunit and normalized to their asymptotically highest value. The continuous and dotted lines were generated by fitting the data for α_i and α_o with Eq. (5) of the model. The graph in *B* illustrates the inhibitory effect of βγ subunits. The channel activity, normalized to the activity measured in the same patch in the absence of βγ subunit, was plotted as a function of βγ subunit concentration, and fitted using Eq. (13) of the model (dashed line). Symbols without error bars represent single measurements. The other data points are an average of three (inverted triangles) and four (circles) measurements. In the horizontal axis, the upper scale refers to the concentration of βγ subunits from erythrocyte, the lower one to the concentration of βγ subunits from retina. The dotted line represents a fit to the data using Eq. (13). The estimated apparent K_D, which is the reciprocal of the coefficient of [βγ*(sol.)] in Eq. (13), is 52 pm for βγ from erythrocytes and 1 nm for βγ from retina.

ing that K_{ATP} channels are stimulated by purified α subunits and inhibited by pertussis toxin (PTX) (Ribalet et al., 1989*b* and 1991). We describe this interaction by the complexation reaction in Eq. (2), although intervention of intermediate enzymes may occur (B. Ribalet and G.T. Eddlestone, *submitted for publication*).

Thus, postulating that K_{ATP} channel activity, $[K_{ATP}]_{act}$, is proportional to the number of channels per unit area that interact with either α_i or α_o, $[K_{ATP} \cdot \alpha_i]$, and using α_i as a common symbol for both α_i and α_o, we have

$$[K_{ATP}]_{act.} = \Omega[K_{ATP} \cdot \alpha_i] \tag{1}$$

where Ω is a quantity that incorporates the channel kinetic characteristics[1]. Assuming that the surface density of $K_{ATP} \cdot \alpha_i$ is determined by the equilibrium

$$K_{ATP} + \alpha_i \overset{K_{ch}}{\leftrightarrow} K_{ATP} \cdot \alpha_i \tag{2}$$

and considering that the total number of channels in the membrane is

$$[K_{ATP}]^T = [K_{ATP}] + [K_{ATP} \cdot \alpha_i] \tag{3}$$

Eq. (1) can be expressed in terms of $[K_{ATP}]^T$, $[\alpha_i]$ and K_{ch} (*see* Eq. (A4) for the case in which there are no extrinsic α_i, $[\alpha_i*(sol)] = 0$).

To account for the effect of exogenous α_i, we postulated that the subunits first partition into the membrane according to

$$\alpha_i*(sol) \overset{k_{\alpha i}}{\leftrightarrow} \alpha_i*(memb) \tag{4}$$

where $K_{\alpha i}$ is a partition coefficient and the asterisk denotes exogenous components. Then, once in the membrane, α_i* subunits interact with K_{ATP} channels according to a reaction similar to (2)

$$K_{ATP} + \alpha_i*(memb) \overset{K^*_{ch}}{\leftrightarrow} K_{ATP} \cdot \alpha_i* \tag{2'}$$

The binding constants, K_{ch}* and K_{ch}, referring to exogenous and endogenous α_i, respectively, are not assumed to be identical, since the added α subunits may be of a different type than the endogenous ones, and the degree of channel activation may depend on the type of α subunit, as was found to be the case for the muscarinic K channel (Yatani et al., 1987). However, it has been shown elsewhere (B. Ribalet and G.T. Eddlestone, *submitted for publication*) that α_i and α_o stimulate K_{ATP} channels with similar potencies, indicating that in our

[1] In a more comprehensive model, Eq. (1) should include a constant term, allowing for some residual channel activity in the absence of activated G proteins. However, the effect of pertussis toxin indicates that such residual activity ought to be low. Thus, since this constant term would not have significantly altered the fitting parameters, it has not been included in Eq. (1) for simplicity.

system the distinction between K_{ch} and K_{ch}* may not be critical. From the equilibria of reactions (2′) and (4), an expression was obtained that takes into account the contribution of both endogenous and exogenous α_i subunits (*see* Eq. (A4) in the Appendix). Normalizing this equation to its upper limiting value, which is practically obtained for $[\alpha_i^*(\text{sol})] = 250$ pM, we find

$$[K_{ATP}]_{\text{norm.act.}} = \frac{P_1 + [\alpha_i^*(\text{sol})]}{P_2 + [\alpha_i^*(\text{sol})]} \tag{5}$$

where P_1 and P_2 are related to the equilibrium constants of reactions (2), (2′) and (4), and to the surface density of endogenous α_i, by

$$P_1 = K_{ch}[\alpha_i]/k_{\alpha i}K_{ch}^* \tag{6}$$

and

$$P_2 = (1 + K_{ch}[\alpha_i])/k_{\alpha i}K_{ch}^* \tag{7}$$

Since the definitions (6) and (7) imply that $P_1 < P_2$, Eq. (5) predicts an increase of the channel activity as a function of exogenous α_i^*. The ratio P_1/P_2, which is determined by fitting the data, represents the fraction of channels that are initially bound to endogenous α_i, prior to addition of exogenous α_i^*. As shown in Fig. 4*A*, the fit to the data obtained with Eq. (5) is quite satisfactory.

K_{ATP} Channel Modulation by βγ

According to our model, βγ-induced K_{ATP} channel inhibition is due to the high affinity binding of the heterodimer to α_i. Elevation of βγ, due to G protein activation or to addition of the subunits to the bath, causes α_i to revert to its trimeric form, thus lowering the level of α_i and, consequently, reducing the channel activity. Binding of α_i to βγ is then described by the reaction

$$\alpha_i + \beta\gamma \overset{K_i}{\leftrightarrow} \alpha_i\beta\gamma + P_i^- \tag{8}$$

where βγ may originate from the activation of any trimeric G proteins, or from the insertion of purified exogenous heterodimers in the membrane. In reaction (8), P_i^- ions are generated by the GTPase activity of α_i. A reaction similar to (8), involving α_s instead of α_i, will be considered later when discussing the effects of Mg^{2+}.

As shown in the Appendix, the equilibria of reactions (2) and (8) may be used, together with the conservation equation for α_i, to express the channel activity as a function of the membrane surface density of the βγ dimers, [βγ]. We find:

$$[K_{ATP}]_{\text{act.}} = \Omega\, K_{ch}[K_{ATP}]^T W/(1 + Z[\beta\gamma]) \tag{9}$$

where the constants W and Z are defined by

$$W = [\alpha_i]^T/D \text{ and } Z = K_i'/D \tag{10}$$

with

$$D = 1 + K_{ch}[\alpha_i]^T \text{ and } K_i' = K_i/[P_i^-] \tag{11}$$

Ω in Eq. (9) is the same as in Eq. (1), K_{ch} and K_i are the equilibrium constants of reactions (2) and (8), and $[K_{ATP}]^T$ is defined in Eq. (3).

In the experiments with exogenous βγ subunits (denoted βγ*), it is assumed that the heterodimers partition in the membrane according to the reaction

$$\beta\gamma^*(\text{sol}) \overset{k_{\beta\gamma}}{\leftrightarrow} \beta\gamma^*(\text{memb}) \tag{12}$$

and subsequently combine with α_i according to a reaction similar to (8)

$$\alpha_i + \beta\gamma^*(\text{memb}) \overset{K_i}{\leftrightarrow} \alpha_i\beta\gamma^* + P_i^- \tag{8′}$$

Assuming that only limited activation of G_s occurs at the Mg^{2+} concentration used in the experiments with exogenous βγ*, interaction between the latter and α_s is presently neglected. However, activation of G_s will be considered later to account for the effects of elevated Mg^{2+} concentrations on channel activity.

Combining the chemical equilibrium equation for reactions (12), (8) and (8′) with Eq. (9) yields an expression for channel activity as a function of exogenous βγ* subunit concentration, [βγ*(sol)] (Eq. (A10) in the Appendix). Normalizing that equation with respect to its reference value for [βγ*(sol)] = 0, finally gives

$$[K_{ATP}]_{\text{norm.act.}} = 1\Big/\left(1 + \frac{k_{\beta\gamma}Z[\beta\gamma^*(\text{sol.})]}{1 + Z[\beta\gamma]}\right) \tag{13}$$

Assuming that the surface density of intrinsic βγ, [βγ], which appears in the denominator of Eq. (13), is constant, or negligibly small, the only parameter in Eq. (13) is the coefficient of [βγ*(sol.)], which is a complex combination of partition coefficients and equilibrium constants, (*see* Eqs. (10), (11) and (12)). Equation (13) was used to fit the data in Fig. 4*B*.

K_{ATP} Channel Modulation by Mg^{2+}

In agreement with previous findings (Findlay, 1987; Ashcroft & Kakei, 1989), the data of *A* and *B* in Fig. 5 show that in β cell excised inside-out patches, increasing the bath concentration of Mg^{2+} from 1 to 10 mM caused

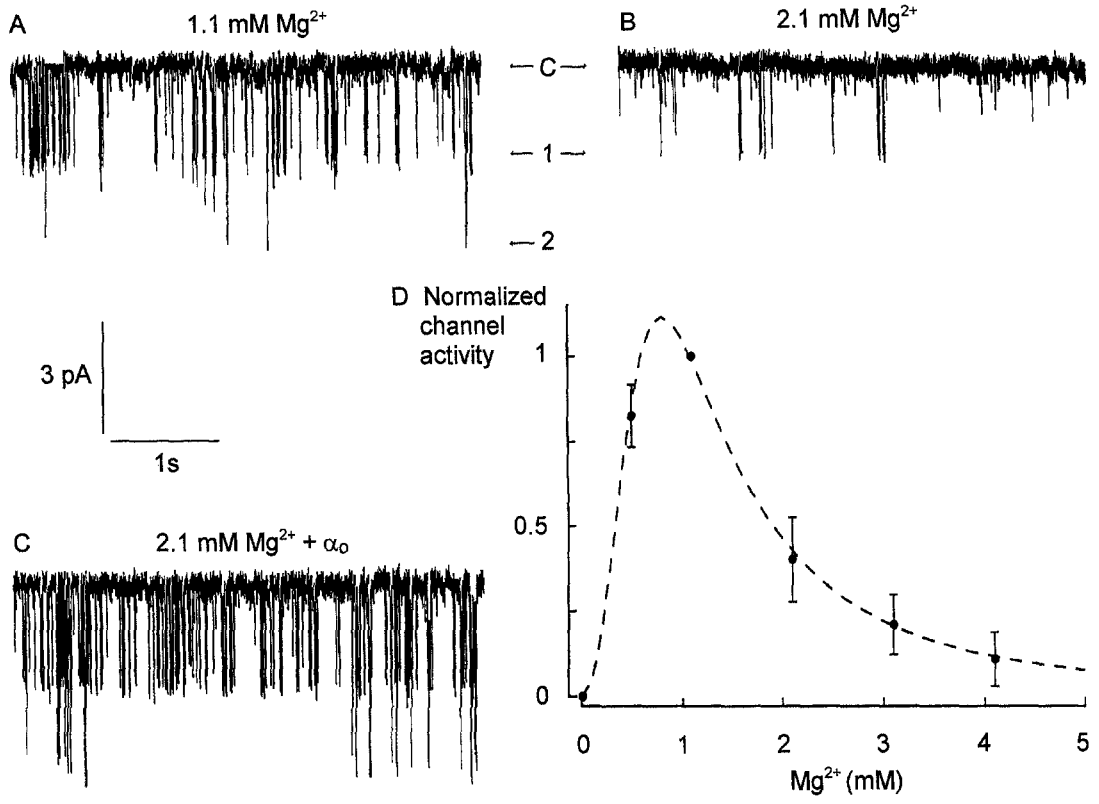

Fig. 5. Effects of Mg^{2+} on K_{ATP} channel activity in inside-out patch experiments. The data shown in *A* and *B* are from the same patch and depict the inhibitory effect of Mg^{2+}. Downward deflections represent inward current in symmetrical K^+ for a holding pipette potential of −70 mV. The data in *B* were taken 2 min after elevating Mg^{2+} from 1.1 up to 2.1 mM. (*C*) Recurrence of channel activity 4 min after the addition of 25 pM α_o. In *D*, the channel activity, normalized to its highest experimental value (corresponding to 1.1 mM Mg^{2+} in the same patch), is plotted as a function of the Mg^{2+} concentration, and fitted with Eq. (14) of the model. All data points are averages of five measurements, except for that assigned at 0 Mg^{2+}, which is arbitrarily set to zero on the basis of our assumption that Mg^{2+} is necessary for channel activation by stimulated G_i. This assumption is supported by the data shown in Fig. 6, where lowering Mg^{2+} concentration from 1.1 to 0.1 mM results in almost complete channel block (*see also* footnote[1] after Eq. (1)). The data in *D* were fitted with Eq. (9), using Eqs. (14) and (15) to substitute $Z[\beta\gamma]$ with its explicit dependence on $[Mg^{2+}]$. Apart from a proportionality coefficient, (equal to 26,021), which represents the numerator of Eq. (9) multiplied by a scaling factor, the final function depends on six independent parameters. The values and dimensions of these parameters are: $l_s/l_i = 6.32 \times 10^5$, $[\alpha_i]^T/[\alpha_s]^T = 4$, $[\alpha_s]^T/l_i = 4.78 \times 10^{-3}$, $n = 4$, $m_i = 1.633\ \text{mM}^{-1}$, $m_s = 4.7 \times 10^{-2}\ \text{mM}^{-1}$, where $[\alpha_i]^T$ and $[\alpha_s]^T$ represent the total number of α_i and α_s subunits per unit area, while l_s, l_i, m_s, m_i and n are defined in Eq. (15) of the model.

gradual inhibition of K_{ATP} channel activity. However, Fig. 5*D* also shows that increasing the Mg^{2+} concentration from 0.1 to 0.5 mM stimulated the channel, a behavior similar to that reported by Ashcroft and Kakei (1989). Thus, the overall relationship between K_{ATP} channel activity and Mg^{2+} concentration, shown in Fig. 5*D*, is bell-shaped with a maximum for the channel activity at around 0.5 mM Mg^{2+} and almost complete channel closure at 5 mM Mg^{2+}.

To account for these effects of Mg^{2+}, we propose a mechanism based on the results presented in this paper, as well as those of biochemical studies describing different Mg^{2+} requirements for agonist-independent activation of G_i and G_s. Agonist-independent activation of G_i occurs at Mg^{2+} concentrations below 1 to 2 mM, while agonist-dependent activation of G_s requires Mg^{2+} concentrations between 2 and 20 mM (Katada et al., 1984; Cerione et al., 1985*a*). Consistent with the hypothesis that K_{ATP} channels are activated by α_i, the Mg^{2+}-dependent activation of G_i would be responsible for channel stimulation between 0 and 0.5 mM Mg^{2+}, while the Mg^{2+}-dependent activation of G_s, with consequent liberation of $\beta\gamma$ and their association with α_i/α_o, would account for channel inhibition between 0.5 and 5 mM Mg^{2+}. Thus, at sufficiently high Mg^{2+} concentration a reaction similar to (8) occurs also for G_s:

$$\alpha_s + \beta\gamma \overset{K_s}{\leftrightarrow} \alpha_s\beta\gamma + P_i^- \qquad (8'')$$

To quantify the effects of Mg^{2+}, channel activity was expressed as a function of Mg^{2+} concentration. The first step in this approach consisted in expressing $Z[\beta\gamma]$,

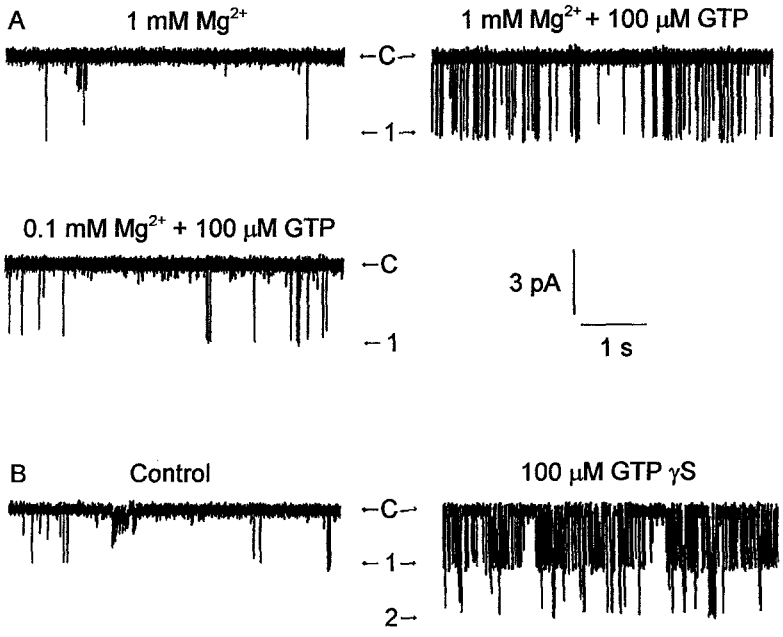

Fig. 6. Mg^{2+} dependence of K_{ATP} channel stimulation by guanosine triphosphate in excised inside-out patches. The traces shown in *A* were obtained at a holding potential of −70 mV, those in *B*, which are from a different patch were recorded at −60 mV. K^+ (140 mM) was present on both sides of the membrane, and 10 μM ATP was added to the bath solution in both cases. (*A*) Stimulatory effect of GTP. The control activity illustrated in the left upper trace was recorded in the presence of 1 mM Mg^{2+}. The upper right trace shows the effect of 100 μM GTP on channel activity, two minutes after addition of the nucleotide. The lower trace of *A* was recorded in the presence of GTP, 5 min after lowering Mg^{2+} to 0.1 mM. (*B*) Stimulatory effect of GTPγS. The control activity shown in the left trace was recorded in the presence of 1 mM Mg^{2+}. In this panel, the right trace showing the effects of GTPγS was recorded 3 min after bath application of the nonhydrolyzable analogue.

which appears in Eq. (9), in terms of the total membrane-surface densities of the α subunits, $[\alpha_i]$ and $[\alpha_s]$, and of the equilibrium constants of reactions (8′) and (8″). Following the procedure outlined in the Appendix, and approximating *D*, defined in Eq. (11), with 1, one finds

$$Z[\beta\gamma] = K_i'[\beta\gamma] = \frac{1}{2}\sqrt{4K_i'[\alpha_s]^T\left(\frac{K_i'}{K_s'} + \frac{[\alpha_i]^T}{[\alpha_s]^T}\right) + \left(1 + \frac{K_i'}{K_s'}\right)^2} - \frac{1}{2}\left(1 + \frac{K_i'}{K_s'}\right) \tag{14}$$

where $[\alpha_i]^T$ and $[\alpha_s]^T$ denote the total amount of α_i and α_s per unit area complexed and uncomplexed, and where K_s' is related to the equilibrium constant of reaction (8″), K_s, by

$$K_s' = K_s/[P_i^-] \tag{11″}$$

which is similar to the definition of K_i' in Eq. (11).

Finally, to express $Z[\beta\gamma]$, and thus the channel activity in Eq. (9), as a function of the Mg^{2+} concentration, the dependence of K_i' and K_s' upon Mg^{2+} must be specified. The present results, together with the biochemical data obtained with adenylate cyclase (Katada et al., 1984; Cerione et al., 1985), suggest that the dissociation constants for G_s and G_i are both increasing functions of Mg^{2+} which saturate at a sufficiently high Mg^{2+} concentration. Defining these dissociation constants as the reciprocal of K_i' and K_s', the simplest functions that meet the above requirements are expressions of the type

$$\frac{1}{K_i'} = l_i\frac{(m_i[Mg^{2+}])^n}{1 + (m_i[Mg^{2+}])^n} \text{ and } \frac{1}{K_s'} = l_s\frac{(m_s[Mg^{2+}])^n}{1 + (m_s[Mg^{2+}])^n} \tag{15}$$

where *l* and *m* are unknown parameters and where the exponent *n* is an index of cooperativity for the effect of Mg^{2+}, similar to a Hill coefficient. Channel activity as a function of Mg^{2+} was obtained by substituting Eq. (15) into Eq. (14) and finally into Eq. (9). It is not surprising that the fit to the data in Fig. 5 is very good, considering that the final equation for channel activity contains several parameters. However, as emphasized in the Discussion, the values for some of these parameters are very similar to those deduced from totally independent measurements for the G protein modulation of adenylate cyclase (Codina et al., 1984; Katada et al., 1984).

K_{ATP} Channel Modulation by GTP and GTPγS

It has been previously shown, using excised inside-out patches, that GTP stimulates and maintains the K_{ATP} channel in an active state (Dunne & Petersen, 1986; Ribalet et al., 1989*b*). The data presented in Fig. 6*A* illustrate the Mg^{2+} dependence of GTP-induced stimulation of channel activity in the absence of agonist. The upper traces show K_{ATP} channel stimulation by GTP in the presence of 1 mM Mg^{2+}, and suppression of this stimulatory effect when Mg^{2+} is reduced to 0.1 mM (lower trace Fig. 6*A*). Figure 6*B* demonstrates that the nonhy-

drolyzable analogue GTPγS has stimulatory effects which are similar to those of GTP. The K_{ATP} channel responses to GTP and GTPγS were characteristic of the G protein mode of action, occurring often with a 1 to 2 min delay, and reached a steady-state level within 5 to 6 min. Results similar to those shown in Fig. 6 were obtained in eight other experiments.

However, K_{ATP} channel stimulation by GTP occurred in only 70% of the cases in which the nucleotide had an effect. In three patches, addition of 100 μM GTP caused reduction of channel activity to less than 35% of control, an effect that occurred after a delay of 2 min and could be fully reversed by removing GTP.

To account for the variable effects of GTP, it is assumed that the GTP-dependent activations of G_i and G_s, which have opposite effects on the channel, vary from patch to patch due to differences in the amounts of the two types of G protein, or differences in the effectiveness of the two regulatory systems.

Discussion

Glucagon potentiates but does not initiate insulin release by pancreatic β cells (Kofod et al., 1988*a*). Activation of the glucagon receptor, which is coupled to the "stimulatory" guanosine nucleotide-binding G protein, G_s, causes an increase in cAMP production (Goldfine et al., 1972) which may play a role in potentiation of insulin release by glucagon (Pipeleers et al., 1985). However, the stimulatory effect of glucagon also involves a cAMP-independent mechanism which has not yet been identified (Kofod, Unson & Merrifield, 1988*b*) but that could be related to K_{ATP} channel inhibition (Holz et al., 1993). We used the excised patch to test this hypothesis and determine whether K_{ATP} channel regulation by glucagon is mediated via a direct "membrane-delimited" effect of activated G_s.

Activation of the G Protein, G_s, Couples Directly Glucagon Receptors to K_{ATP} Channels

The data presented in Fig. 1 show that glucagon, at concentrations up to 15 nM, blocks K_{ATP} channel activity in excised patches, supporting the hypothesis of a membrane-associated regulatory mechanism. The IC_{50} for channel inhibition by glucagon (2.4 nM) agrees quite well with the EC_{50} (close to 3 nM) reported for stimulation of cAMP production (Goldfine et al., 1972), thus suggesting the involvement of activated G_s in K_{ATP} channel modulation by glucagon. In addition, the observation that cholera toxin (CTX), which activates G_s irreversibly, inhibits K_{ATP} channel activity, supports the hypothesis that activation of native G_s causes channel closure. Taken together, these results indicate that K_{ATP} channel inhibition by glucagon is mediated, at least in part, via activation of membrane-associated G_s.

At concentrations greater than 25 nM, the inhibitory effect of glucagon vanishes and the channel activity is almost unaffected at 250 nM. The reversal of glucagon-induced K_{ATP} channel inhibition is compatible with the hypothesis of a concentration-dependent desensitization of glucagon and glucagon-like peptide receptors (Hoosein & Gurd, 1984; Fehmann & Habener, 1992). Glucagon receptors desensitization occurs in excised patches where the effects of diffusible second messengers should no longer be present. This observation suggests that if phosphorylation is involved in receptor desensitization, membrane-bound enzymes, such as protein kinase C are much more likely to be involved than the cAMP-dependent protein kinase A (Murphy & Houslay, 1988). Experiments are in progress to determine whether desensitization indeed involves receptor phosphorylation in the excised patch.

K_{ATP} Channel Modulation by G_s Subunits

Upon activation, the trimeric G protein G_s dissociates into α_s and βγ dimers, implying that either or both of these components could "directly" inhibit the channel. Our data indicate that βγ dimers have a potent inhibitory effect, which may account, at least in part, for the inhibition of K_{ATP} channels by activated G_s. Similar effects of βγ on K_{ATP} channels have previously been reported (Ito et al., 1992).

K_{ATP} channel inhibition by βγ may result from a direct effect on the channel or from deactivation of the α_i-mediated stimulatory pathway described elsewhere (Ribalet et al., 1991; B. Ribalet and G.T. Eddlestone, *submitted for publication*), following reassociation of βγ with α_i or α_o subunits. It has been suggested that the latter mechanism is responsible for muscarinic K channel regulation by G protein subunits (Okabe et al., 1990), and our data indicate that a similar mechanism is likely to apply to the K_{ATP} channel. Consistent with this hypothesis is the observation that K_{ATP} channel inhibition by βγ and CTX is reversed by addition of purified, GTPγS-activated α_i or α_o. Due to their low affinity for βγ, these preactivated subunits should not form inactive trimers, and should thus be insensitive to changes in the level of βγ. Comparable observations have been made for the muscarinic K channel, where the inhibitory effect of βγ is reduced in the presence of preactivated α_i (Okabe et al., 1990).

For the "reassociation hypothesis" to be valid there must be a basal, agonist-independent activity of G_i or G_o. Blocking of K_{ATP} channel activity with PTX in excised patches (Ribalet et al., 1989 and 1991; B. Ribalet and G.T. Eddlestone, *submitted for publication*) constitutes compelling evidence that G_i and or G_o are activated in

the absence of agonist in the β cell membrane. K_{ATP} channel inhibition by reassociation of βγ with stimulatory α_i/α_o is therefore a plausible hypothesis.

K_{ATP} Channel Modulation by Mg^{2+} Involves Activation of the G Proteins G_i and G_s

Mg^{2+} plays an important role in G protein activation. Two characteristic features of this effect are particularly relevant to our discussion: the shift in Mg^{2+} requirement for G protein activation from millimolar to micromolar concentrations after addition of agonist, and the lower Mg^{2+} requirement for activation of G_i than for activation of G_s in the absence of agonists (half-maximum activation of G_i occurs at 1–2 mM, while that of G_s is observed at 10–15 mM) (*see review by* Birnbaumer et al., 1990). Differential activation of G_i and G_s by Mg^{2+} has been proposed to account for the observation that adenylate cyclase inhibition by Mg^{2+} is much greater at 2 than at 50 mM (82 and 18%, respectively) (Northup, Smigel & Gilman, 1982; Bokoch et al., 1984; Katada et al., 1984; Cerione et al., 1985*a*). More specifically, below 2 mM the effect of G_s is small and the inhibition by G_i maximal, while at 50 mM, the activation of G_s overrides the inhibitory effect of G_i.

This differential activation of G_i and G_s by Mg^{2+} may account for the data in Fig. 5, showing that in excised inside-out patches increasing the Mg^{2+} concentration in the bath between 1 and 10 mM causes progressive K_{ATP} channel inhibition, while increasing Mg^{2+} between 0 and 0.5 mM causes channel activation. The concentration-dependent modulation of the K_{ATP} channel by Mg^{2+} is therefore bell-shaped as shown in Fig. 5*D*. We have developed a model to derive testable expressions for the channel activity as a function of controllable variables, such as the concentrations on exogenous α_i/α_o subunits, βγ dimers, as well as Mg^{2+}. Of the parameters deduced by fitting the data (*see* legend of Fig. 5), two are related to the Mg^{2+}-dependent activation of G_i and G_s, yielding values of 0.65 and 17.5 mM for the half-maximum dissociation of G_i and G_s, respectively. A third one, which represents the ratio of the total amount of G_i and G_s proteins, was found to be approximately equal to 4. The similarity between the values of these three parameters and those reported for the Mg^{2+}-dependent regulation of adenylate cyclase (Cerione et al., 1985*a*) strengthens our hypothesis. An important quantity deduced from the model is the ratio of the dissociation constant for G_s to that of G_i. While being smaller than 1 at very low Mg^{2+}, it increases very steeply at higher Mg^{2+} concentrations, such that the dissociation constant for G_s becomes greater than that for G_i when the Mg^{2+} level is above 1 mM (a maximum ratio of $6 \cdot 10^5$ was obtained at $[Mg^{2+}] > 20$ mM). These numerical estimates suggest a very potent agonist-independent activation of G_s for $[Mg^{2+}] > 1$ mM, and are consistent with the observation that in the absence of agonist and of βγ derived from G_i, the stimulation of cAMP production by G_s is as pronounced as in the presence of agonist (Cerione et al., 1985*b*). By contrast, the agonist-independent activation of G_i deduced from the parameters of our model is low compared to that of G_s at $[Mg^{2+}] > 1$ mM. These inferences suggest that in β cells at physiological Mg^{2+} concentrations (>1 mM), activation of the G_i and/or G_o proteins, which are coupled to the K_{ATP} channel, occurs primarily in the presence of agonists. However, the observation that PTX blocks K_{ATP} channel activity in excised patches strongly supports the hypothesis that agonist-independent activation of these G proteins plays an important role in modulation of the K_{ATP} channel in our system.

K_{ATP} Channel Modulation by GTP

To further characterize the involvement of G proteins in K_{ATP} channel modulation, we also tested for the effect of GTP. The results obtained with the addition of 50 or 100 μM GTP in excised inside-out patch were quite variable. Out of the 13 patches in which GTP had an effect, 9 showed stimulation of K_{ATP} channel activity while the others showed substantial channel inhibition. Both of these effects were reversible and occurred with a lag which is characteristic of G protein activation. According to our model, the effect of GTP on K_{ATP} channel activity is determined by the relative degree of activation of the G_i-dependent stimulatory pathway and of the G_s-dependent inhibitory pathway. The effect of GTP was investigated in the presence of 1 mM Mg^{2+}, a concentration at which, according to our model, the dissociation of G_s and G_i may both be significant so that some variability from patch to patch in the predominance of either pathway may be expected.

Is Mg^{2+}- and G-protein-dependent Inhibition of K_{ATP} Channels Involved in the Control of Insulin Release by Glucagon?

Our data suggest that K_{ATP} channel inhibition by glucagon occurs via activation of G_s and liberation of βγ. This hypothesis, together with the conditions for membrane depolarization by K_{ATP} channel inhibition, suggests a mechanism which accounts for some of the effects of glucagon on membrane potential and insulin release. When glucose is absent, at least two factors contribute to the high K_{ATP} channel activity: the low level of ATP and also, according to our model, the level of free Mg^{2+}, which is estimated to be close to 1 mM (Ashcroft, Weerasinghe & Randhall, 1973). Since the dissociation of G_i and the channel activity are near maximum at this Mg^{2+} concentration, the degree of channel inhibition that

results from liberation of βγ dimers following application of glucagon may not be sufficient to alter the membrane potential. We have previously shown that a 75% reduction of channel activity is necessary for glucose to induce membrane depolarization (Ribalet et al., 1988). By contrast, in the presence of glucose, higher levels of ATP will set the channel activity to a low basal level so that glucagon-mediated inhibition via βγ may be sufficient to block the residual K^+ permeability to the point of affecting the membrane potential. Moreover, higher ATP levels will also cause $[Mg^{2+}]$ to decrease due to buffering, thus reducing the activation of G_i and consequently enhancing the effectiveness by which βγ-mediated channel inhibition alters the membrane potential.

Does cAMP Play a Role in the Modulation of Insulin Release by Glucagon?

It has been postulated that increased cAMP mediates GLP-1-induced K_{ATP} channel inhibition (Holz et al., 1993). However, the present results demonstrate that glucagon inhibits K_{ATP} channel activity in excised patches, suggesting that this inhibitory effect does not require activation of a diffusible second messenger. In addition, the cell-attached patch clamp data also argue against a role for cAMP in reducing K_{ATP} channel activity. In four out of six cell-attached patches in which bath-applied glucagon had an effect, the channel activity decreased, in the two others the channel activity increased. Inhibition of K_{ATP} channels by $\beta\gamma_{Gs}$ heterodimers and stimulation by PKA via cAMP (Ribalet et al., 1989*a*) may explain this dual effect of glucagon. More specifically, cAMP may diffuse more readily than the βγ dimers that are anchored to the membrane and thus have easier access to targets relatively far from the site of stimulation. Thus, for the channels in the patch, which are distant from the agonist-activated receptors, the inhibitory effect of βγ dimers may sometimes be weaker and may be overridden by the stimulatory effect of cAMP. By contrast, the channels outside the patch, which are associated with the site of action of the agonist, will be exposed to consistent modulations both by cAMP and the βγ dimers. Accordingly, the variable effect of glucagon on the activity of channels present in the patch, as opposed to the consistent inhibition of the channels outside the patch, may be accounted for assuming that K_{ATP} channel stimulation by cAMP is subordinate to the "direct" inhibitory effect of the βγ dimers derived from G_s.

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References

Ashcroft, F.M., Kakei, M. 1989. ATP-sensitive K^+ channels in rat pancreatic β-cells: Modulation by ATP and Mg^{2+} ions. *J. Physiology* **416:**349–367

Ashcroft, S.J.H., Weerasinghe, L.C.C., Randhall, P.J. 1973. Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. *Biochem. J.* **132:**223–231

Bezanilla, F. 1985. A high capacity data recording device based on a digital audio processor and a video cassette recorder. *Biophys. J.* **47:**437–441

Birnbaumer, L., Abramowitz, J., Brown, A.M. 1990. Receptor-effector coupling by G proteins. *Biochim. Biophys. Acta* **1031:**163–224

Bokoch, G.M., Katada, T., Northup, J.K., Ui, M., Gilman, A.G. 1984. Purification and properties of the inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. *J. Biol. Chem.* **259(6):**3560–3567

Cerione, R.A., Codina, J., Kilpatrick, B.F., Staniszewski, C., Gierschik, P., Somers, R.L., Spiegel, A.M., Birnbaumer, L., Caron, M., Lefkowitz, R.J. 1985*a*. Transducin and the inhibitory nucleotide regulatory protein inhibit the stimulatory nucleotide regulatory protein mediated stimulation of adenylate cyclase in phospholipid vesicle systems. *Biochemistry* **24:**4499–4503

Cerione, R.A., Staniszewski, C., Caron, M.G., Lefkowitz, R.J., Codina, J., Birnbaumer, L. 1985*b*. A role for N_i in the hormonal stimulation of adenylate cyclase. *Nature* **318:**293–295

Codina, J., Hildebrandt, J.D., Sekura, R.D., Birnbaumer, M., Bryan, J., Manclark, C.R., Iyengar, R., Birnbaumer, L. 1984. N_s and N_i, the stimulatory and inhibitory regulatory components of adenylate cyclases. *J. Biol. Chem.* **259(9):**5871–5886

Colquhoun, D., Sigworth, F.J. 1983. Fitting and statistical analysis of single channel records. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 191–263. Plenum, New York-London

Dunne, M.J., Petersen, O.H. 1986. GTP and GDP activation of K^+ channels that can be inhibited by ATP. *Pfluegers Arch.* **407:**564–565

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 βTC-1 cells. *Endocrinology* **130(1):**159–166

Findlay, I. 1987. The effect of magnesium upon adenosine triphosphate-sensitive potassium channels in a rat insulin-secreting cell line. *J. Physiol.* **391:**611–629

Goldfine, I.D., Roth, J., Birnbaumer, L. 1972. Glucagon receptor in β-cells. *J. Biol. Chem.* **247(4):**1211–1218

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Holz, G.G., IV, Kuhtreiber, W.M., Habener, J.F. 1993. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1 (7-37). *Nature* **361:**362–365

Hoosein, N.M., Gurd, R.S. 1984. Human glucagon-like peptides 1 and 2 activate rate brain adenylate cyclase. *FEBS Lett.* **178(1):**83–86

Ito, H., Tung, R.T., Sugimoto, T., Kobayashi, I., Takahashi, K., Katada, T., Ui, M., Kurachi, Y. 1992. On the mechanism of G protein βγ subunit activation of the muscarinic K^+ channel in guinea pig atrial cell membrane. *J. Gen. Physiol.* **99:**961–983

Katada, T., Bokoch, G.M., Northup, J.K., Ui, M., Gilman, A.G. 1984.

The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. *J. Biol. Chem.* **259(6):**3568–3577

Kofod, H., Andreu, D., Thams, P., Merrifield, R.B., Hedeskov, C.J., Hansen, B., Lernmark, A. 1988*a*. Insulin release by glucagon and secretin: studies with secretin-glucagon hybrids. *Am. J. Physiol.* **254:**E454–E458

Kofod, H., Unson, C.G., Merrifield, R.B. 1988*b*. Potentiation of glucose-induced insulin release in islets by desHis^1[Glu^9]glucagon amide. *Int. J. Prot. Res.* **32:**436–440

Murphy, G.J., Houslay, M.D. 1988. Resensitization of hepatocyte glucagon-stimulated adenylate cyclase can be inhibited when cyclic AMP phosphodiesterase inhibitors are used to elevate intracellular cyclic AMP concentrations to supraphysiological values. *Biochem. J.* **249:**543–547

Northup, J.K., Smigel, M.D., Gilman, A.G. 1982. The guanine nucleotide activating site of the regulatory component of adenylate cyclase. *J. Biol. Chem.* **257:**11416–11423

Okabe, K., Yatani, A., Evans, T., Ho, Y-K., Codina, J., Birnbaumer, L., Brown, A.M. 1990. βγ dimers of G proteins inhibit atrial muscarinic K^+ channels. *J. Biol. Chem.* **265(22):**12854–12858

Pipeleers, D.G., Schuit, F.C., In'T Veld, P.A., Maes, E., Hooghepeters, M., Van De Winkel, M., Gepts, W. 1985. Interplay of nutrients and hormones in the regulation of insulin release. *Endocrinology* **117(3):**824–833

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. *J. Gen. Physiol.* **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. *Biophys. J.* **55:**587*a*

Ribalet, B., Ciani, S., Hales, T.G., Eddlestone, G.T. 1991. Role of G proteins in K(ATP) channel modulation by the neuropeptide somatostatin. *Biomed. Res.* **12(2):**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. *J. Gen. Physiol.* **92:**219–237

Ullrich, S., Wollheim, C.B. 1988. GTP-dependent inhibition if insulin secretion by epinephrine in permeabilized RINm5F cells. *J. Biol. Chem.* **263:**8615–8620

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

Appendix

The purpose of this Appendix is to give the derivation of equations used in Results: Eq. (5) for the normalized channel activity in the presence of exogenous α_i subunits, Eqs. (9) and (13) for the channel activity as a function of the βγ subunits, and Eq. (14) for the effect of Mg^{2+}.

Derivation of Eq. (5) for the Normalized Activity in Experiments with Exogenous α_i/α_o Subunits

In the presence of exogenous α_i subunits, Eq. (1) for channel activity must be generalized to include the contribution of the channels that are bound to those subunits:

$$[K_{ATP}]_{act.} = \Omega \{[K_{ATP} \cdot \alpha_i] + [K_{ATP} \cdot \alpha_i^*]\} \quad (A1)$$

Ω is the same quantity as in Eq. (1), and the asterisk is used to distinguish the exogenous subunits from the endogenous ones.

Considering the equilibria of reactions (2), (4) and (2′), the right-hand side of Eq. (A1), divided by Ω, can be written

$$[K_{ATP} \cdot \alpha_i] + [K_{ATP} \cdot \alpha_i^*] = \{K_{ch}[\alpha_i] + K_{ch}^* k_{\alpha i}[\alpha_i^*(sol)]\} [K_{ATP}] \quad (A2)$$

where the constants, K_{ch}, K_{ch}^* and the partition coefficient $k_{\alpha i}$ are defined in the equilibria referred to above.

The total number of channels per unit area, $[K_{ATP}]^T$, can be expressed as

$$[K_{ATP}]^T = [K_{ATP}] + [K_{ATP} \cdot \alpha_i] + [K_{ATP} \cdot \alpha_i^*] \quad (A3)$$

Eliminating $[K_{ATP}]$ from (A2) and (A3), solving for $([K_{ATP} \cdot \alpha_i] + [K_{ATP} \cdot \alpha_i^*])$ and substituting in Eq. (A1) yields

$$[K_{ATP}]_{act.} = \Omega \frac{K_{ch}[\alpha_i] + K_{ch}^* k_{\alpha i}[\alpha_i^*(sol)]}{1 + K_{ch}[\alpha_i] + K_{ch}^* k_{\alpha i}[\alpha_i^*(sol)]} [K_{ATP}]^T \quad (A4)$$

Equation (5) of the Results is obtained by dividing both sides of Eq. (A4) by its maximum value, $\Omega[K_{ATP}]^T$, and then dividing both the numerator and the denominator of the right-hand side by $K_{ch}^* k_{\alpha i}$.

Derivation of Eqs. (9) and (13)

Assuming that activation and deactivation of G proteins is described by the equilibrium of the reaction

$$\alpha_{i\text{-GTP}} + \beta\gamma \overset{K_i}{\leftrightarrow} \alpha_{i\text{-GDP}}\, \beta\gamma + P_i^-$$

or more simply

$$\alpha_i + \beta\gamma \overset{K_i}{\leftrightarrow} \alpha_i\beta\gamma + P_i^- \quad (A5)$$

we have

$$[\alpha_i\beta\gamma] = K_i'[\alpha_i][\beta\gamma] \text{ with } K_i' = K_i/[P_i^-] \quad (A6)$$

K_i being the equilibrium constant of reaction (A5) and $[P_i^-]$ the concentration of phosphate in the solution. It is assumed that K_i is the same for the two types of βγ dimer studied; the different effects of these two types of dimer are then attributed to differences in their membrane-solution partition coefficients defined in Eq. 12.

If exogenously added βγ subunits partition into the membrane according to reaction (12), Eq. (A6) becomes

$$[\alpha_i\beta\gamma] = (K_i'[\alpha_i])\,([\beta\gamma] + k_{\beta\gamma}\,[\beta\gamma^*(sol)]) \quad (A7)$$

Combining Eq. (A7) with the conservation equation for α_i subunits,

$$[\alpha_i]^T = [\alpha_i] + [\alpha_i\beta\gamma] + [K_{ATP} \cdot \alpha_i] \quad (A8)$$

and recalling that the surface density of channels is expected to be much lower than that of the G proteins, so that the term $[K_{ATP} \cdot \alpha_i]$ in Eq. (A8) is neglibly small, we find, combining Eqs. (A7) and (A8)

$$[\alpha_i] = \frac{[\alpha_i]^T}{1 + (K_i')([\beta\gamma] + k_{\beta\gamma}[\beta\gamma^*[sol)])} \quad (A9)$$

Finally, substitution of the right-hand side of Eq. (A9) in Eq. (A4) for the case in which $[\alpha_i^*(sol)] = 0$ gives

$$[K_{ATP}]_{act.} = \frac{\Omega K_{ch}[K_{ATP}]^T W}{1 + Z([\beta\gamma] + k_{\beta\gamma}[\beta\gamma^*(sol)])} \quad (A10)$$

where W and Z are defined in Eqs. (10) and (11). Equation (9) is obtained from (A10) when $[\beta\gamma^*(sol)] = 0$, while Eq. (13) is obtained by dividing both sides of Eq. (A10) by $\Omega K_{ch}[K_{ATP}]^T W/(1 + Z([\beta\gamma])$

Derivation of Eq. (14)

If α_i and α_s are the two main types of α subunits present in the patch, the total surface density of $\beta\gamma$ subunits is given by

$$[\beta\gamma]^T = [\beta\gamma] + [\alpha_i\beta\gamma] + [\alpha_s\beta\gamma] \quad (A11)$$

Moreover, if Mg^{2+} is sufficiently high for $\alpha_s\beta\gamma$ to be partially dissociated, an equation formally identical to Eq. (A6) can be written for G_s, the binding constants, K_i and K_i', being substituted by K_s and K_s'. Substituting such equation, along with Eq. (A6), in Eq. (A11) gives after rearrangements

$$[\beta\gamma] = \frac{[\beta\gamma]^T}{1 + K_i'[\alpha_i] + K_s'[\alpha_s]} \quad (A12)$$

where K_i' is defined in (A6) and K_s' has the equivalent meaning for G_s.

Substituting the right-hand side of Eq. (A9) for $[\alpha_i]$ (with $[\beta\gamma^*(sol)] = 0$), and the right-hand side of the companion equation for $[\alpha_s]$, into Eq. (A12), yields a cubic equation in $[\beta\gamma]$. However, this equation can be reduced to a quadratic equation by assuming that the level of inactivated G proteins exceeds the level of the G proteins which are activated. Equation (14) in the paper is the solution of this quadratic equation.