

# Reconstitution of Somatostatin and Muscarinic Receptor Mediated Stimulation of K<sup>+</sup> Channels by Isolated G<sub>K</sub> Protein in Clonal Rat Anterior Pituitary Cell Membranes\*

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Somatostatin (SS) inhibits secretion from many cells, including clonal GH<sub>3</sub> pituitary cells, by a complex mechanism that involves a pertussis toxin (PTX)-sensitive step and is not limited to its cAMP lowering effect, since secretion induced by cAMP analogs and K<sup>+</sup> depolarization are also inhibited. SS also causes membrane hyperpolarization which may lead to decreases in intracellular Ca<sup>2+</sup> need for secretion. Using patch clamp techniques we now demonstrate: 1) that both (SS) and acetylcholine applied through the patch pipette to the extracellular face of a patch activate a 55-picosiemens K<sup>+</sup> channel without using a soluble second messenger; 2) that, after patch excision, the active state of the ligand-stimulated channel is dependent on GTP in the bath, is abolished by treatment of the cytoplasmic face of the patch with activated PTX and NAD<sup>+</sup>, and after inactivation by PTX, is restored in a GTP-dependent manner by addition of a nonactivated human erythrocyte PTX-sensitive G protein, and 3) that the 55-picosiemens K<sup>+</sup> channel can also be activated in a ligand-independent manner with guanosine [ $\gamma$ -thio] triphosphate (GTP $\gamma$ S) or with Mg<sup>2+</sup>/GTP $\gamma$ S-activated erythrocyte G protein. We call this protein G<sub>K</sub>. It is an  $\alpha$ - $\beta$ - $\gamma$  trimer of which we have previously shown that the  $\alpha$ -subunit is the substrate for PTX and that it dissociates on activation with Mg<sup>2+</sup>/GTP $\gamma$ S into  $\alpha$ -GTP $\gamma$ S plus  $\beta$ - $\gamma$ . A similarly activated and dissociated preparation of G<sub>s</sub>, the stimulatory regulatory component of adenylyl cyclase, having a different  $\alpha$ -subunit but the same  $\beta$ - $\gamma$ -dimer, was unable to cause K<sup>+</sup> opening. These experiments establish that a K<sup>+</sup> channel-mediated membrane hyperpolarization

is a primary response of a secretory cell to an inhibitory hormone, that a PTX-sensitive G protein directly couples hormone receptors to K<sup>+</sup> channels in endocrine cells, and that the effect of G<sub>K</sub> on the effector is mediated by its  $\alpha$ -subunit. (*Molecular Endocrinology* 1: 283-289, 1987)

## INTRODUCTION

Somatostatin (SS) inhibits hormone secretion in normal (1) and clonal pituitary cells (2, 3) as well as in other hormone-secreting cells (4). The mechanism by which SS inhibits hormone secretion is complex and has been suggested to be linked to its ability to reduce cAMP levels (5, 6), due to a GTP-dependent inhibition of adenylyl cyclase (7) which involves the pertussis toxin (PTX)<sup>1</sup> sensitive inhibitory regulatory protein G<sub>i</sub> (8). In PRL-secreting clonal rat pituitary cells of the GH<sub>3</sub>/GH<sub>4</sub>C<sub>1</sub> type, SS also causes membrane hyperpolarization (9) and, possibly secondary to this (10), decreases in intracellular free Ca<sup>2+</sup> (11). Acetylcholine (ACh), acting through a muscarinic receptor (mAChR) has the same effects as SS (12-14). All effects of SS and ACh are inhibited by PTX, suggesting the involvement of a common signal transduction mechanism (11, 15). Although reduction of cAMP levels and consequent impairment of phosphorylation events (16) could be the mode of action of SS and ACh in these cells, there is evidence that decreased cAMP cannot solely account for their inhibitory actions. Notably, SS also inhibits hormone secretion induced by agents that bypass the adenylyl

<sup>1</sup> PTX, exotoxin of *Bordetella pertussis* that ADP-ribosylates  $\alpha$ -subunits of certain G proteins.

cyclase system, such as 8-bromo-cAMP (17), or which act in a cAMP-independent manner, such as TRH (18, 19). Moreover, SS also impairs secretion induced by depolarization with  $K^+$  (3, 17). We recently showed that  $K^+$  channels in membranes from guinea pig atria can be activated directly by addition of a guanosine [ $\gamma$ -thio] triphosphate (GTP $\gamma$ S)-activated G protein, which we had previously purified to better than 95% homogeneity and now call  $G_k$  (20). This protein is a PTX substrate which on treatment with GTP $\gamma$ S and  $Mg^{2+}$  dissociates into an  $\alpha$ -GTP $\gamma$ S complex and a  $\beta$ - $\gamma$ -dimer.<sup>2</sup> Using  $GH_3$  cells and patch clamp techniques, we now tested whether these cells also contain a  $K^+$  channel stimulated by GTP $\gamma$ S-activated  $G_k$  ( $G_k^*$ ), whether SS and ACh activate such a channel, whether this receptor-mediated activation is sensitive to PTX, and, if so, whether a PTX-inactivated system could be reconstituted with native, unactivated exogenously added  $G_k$ .

## RESULTS AND DISCUSSION

To characterize receptor- and/or  $G_k$ -regulated  $K^+$  channels in membranes of pituitary  $GH_3$  cells  $K^+$  currents in inside-out membrane patches were studied using patch clamp techniques (21). In preliminary experiments carried out at negative holding potentials we occasionally observed the presence of  $Cl^-$  channels with a conductance of about 100 picosiemens (pS) which were insensitive to either SS or ACh. At positive holding potentials we identified Ca-activated and voltage-dependent  $K^+$  currents (22), which made it impossible to characterize reliably effects of SS or ACh. Thus, for all experiments, recordings were made at negative membrane potentials using solutions which were low in  $Cl^-$  as described in *Materials and Methods*.

Single channel currents from membrane patches were analyzed before (cell-attached) and after (inside-out) excision from the cultured cells (21). In the absence of hormonal ligands in the pipette we observed before as well as after patch excision low amplitude background openings ( $\ll 2$  pA at  $-80$  mV; Fig. 1, a and b), and depending on the patch occasional brief larger unitary openings. In contrast, in patches from SS- or ACh-responsive cells, selected by adding to the pipette saturating concentrations of SS or the muscarinic ligands ACh or carbachol (CCh) we observed frequent openings occurring in bursts and clusters of bursts with unitary openings at  $-80$  mV of ca 4.4 pA. There were usually several channels in a patch and simultaneous openings were prominent when strong activation occurred. The ligand-induced activities persisted after excision and placement into GTP-containing solutions.

Figure 1a illustrates typical single channel recordings from a patch in the inside-out configuration exposed to

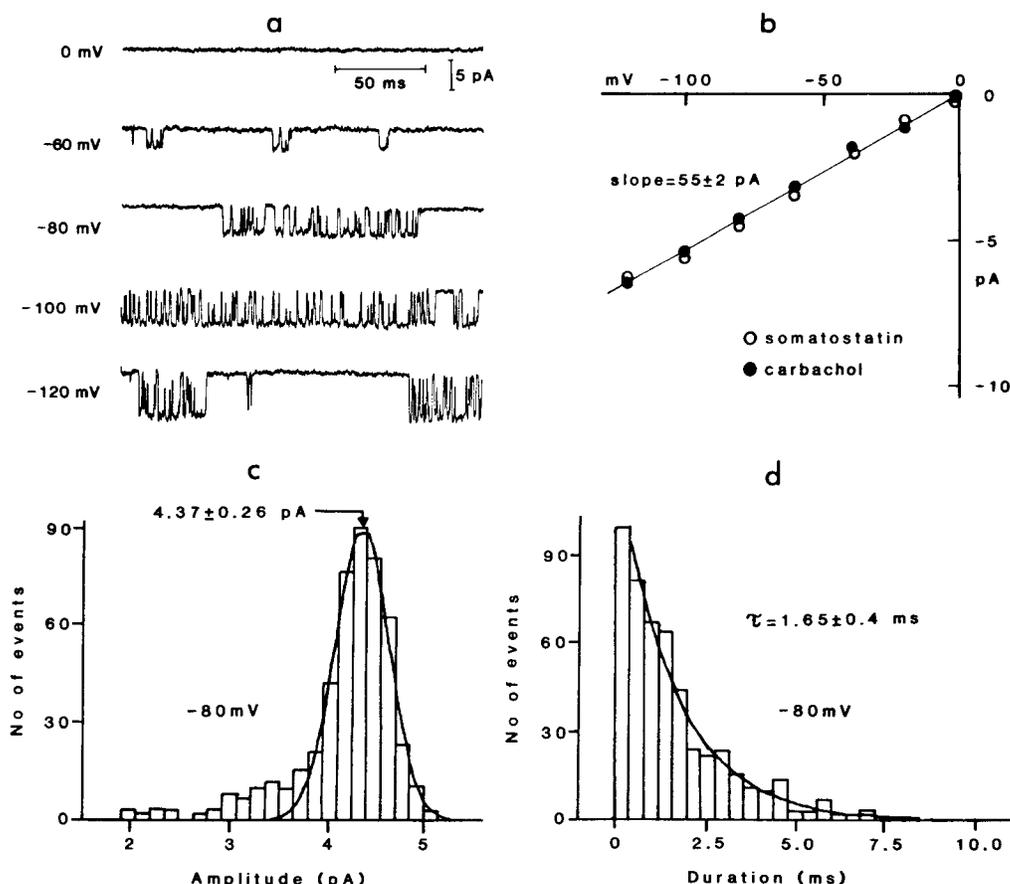
10  $\mu$ M CCh (in the pipette) and 100  $\mu$ M GTP (in the bath) obtained at holding potentials between 0 and  $-120$  mV. An amplitude histogram of openings obtained at  $-80$  mV in such receptor-stimulated patches (Fig. 1c) showed the presence of one major group of unitary openings having a Gaussian amplitude distribution with a mean  $\pm$  SD of  $4.35 \pm 0.26$  pA. A significant number (ca 14% of the total) of what appear to be incomplete openings with smaller amplitudes were also observed. In excised patches the 4.4 pA amplitude openings were only seen in stimulated patches, *i.e.* in patches held by pipettes containing a receptor ligand and when the bathing media had GTP. Analysis of the current-voltage relations of the ligand-induced openings showed them to have a slope conductance of  $55 \pm 3$  pS (Fig. 1b), a null potential of zero mV and a distribution of single channel open times at  $-80$  mV that fitted well a first order decay curve with a time constant of  $1.6 \pm 0.4$  msec. No differences between SS-induced and CCh-induced currents could be detected, either in terms of conductance (Fig. 1b), amplitude histogram, or mean open time (not shown).

No currents induced by hormonal ligands were found when the pipette solutions contained  $K^+$  channel blockers such as 20 mM tetraethylammonium (25 trials) or 5 mM CsCl (25 trials). In the absence of these blockers, hormone-induced currents were observed in an average of 30% of the trials. The currents were  $K^+$ -selective: changing the  $K^+/Na^+$  ratio in the bath from 130:0 to 20:110 and 5.4:125 caused, a shift in reversal potentials to  $-47$  mV and  $-80$  mV, respectively, as predicted by the Nernst equation. The currents were not affected by the Ca-activated  $K^+$  channel blocker apamin and were  $Ca^{2+}$ -independent (all solutions contained less than  $10^{-9}$  M  $Ca^{2+}$  due to presence of 5 mM EGTA). The currents were also observed in the presence of the  $Na^+$  channel blocker tetrodotoxin ( $10^{-6}$  M) added to the pipette solution. From these results we conclude that  $GH_3$  cells have a set of  $K^+$  channels with unit conductance of 55 pS in isotonic  $K^+$ , which are activated indiscriminately by either SS or mAChR.

The muscarinic mammalian heart  $K^+$  channel activated by  $G_k$  rectified inwardly and had little or no conductance of outward current (20). We do not know if the  $K^+$  channel of pituitary cells studied presently shares this property since its behavior at positive potentials was masked by the appearance of numerous outwardly conducting  $K^+$  channels. The conductance of the  $GH_3$  cell  $K^+$  channel is larger than that of the atrial  $K^+$  channel (55 pS vs. 45 pS in isotonic  $K^+$ ) but the brief open times of these ligand-gated channels were similar and much shorter than those found in voltage-gated inwardly rectifying  $K^+$  channels (23, 24).

The involvement of a GTP-binding protein coupling occupancy of receptors by SS and ACh (or CCh) to activation of  $K^+$  channels was demonstrated by three types of experiments: 1)  $K^+$  channels activated in the cell-attached mode did not remain active if, after excision, they were placed into solutions without GTP (Fig. 2c); or if in the presence of GTP, excess of the inhibitor

<sup>2</sup> G proteins, heterotrimeric guanine nucleotide-binding proteins of forms of  $\alpha$ - $\beta$ - $\gamma$ -subunits are responsible for coupling certain type of receptors to specific effector functions.



**Fig. 1.** Single-Channel Activity and Current Voltage Relations of Receptor-Stimulated K<sup>+</sup> Channels

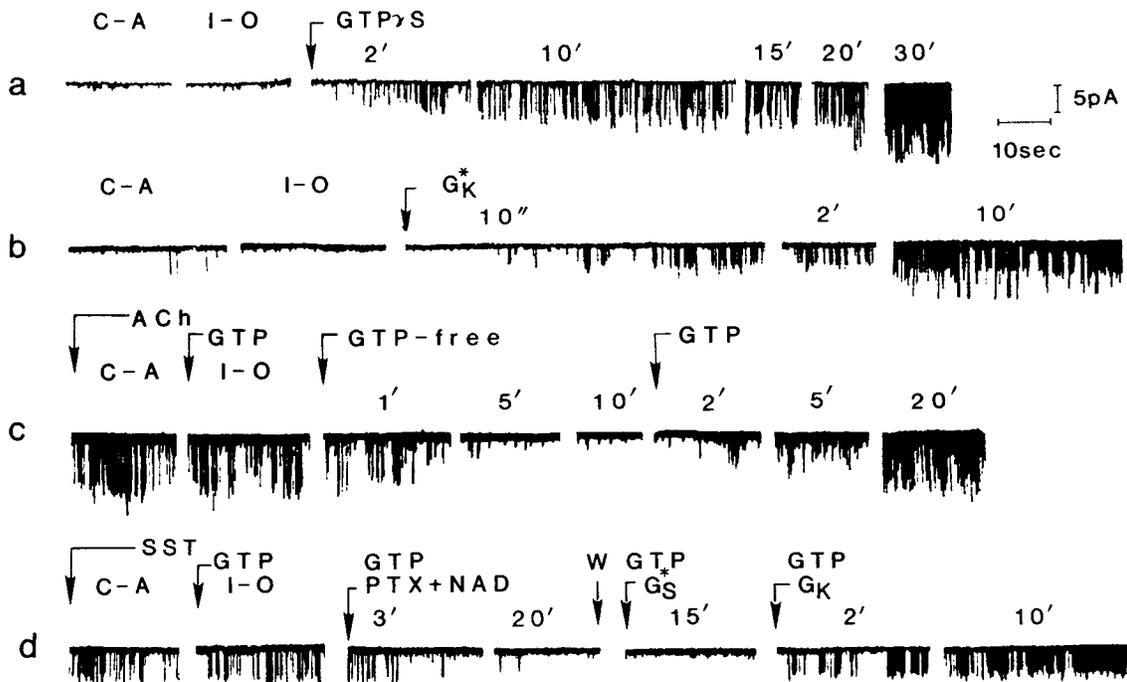
a, Representative records of 100-msec duration of activity in a patch with 10  $\mu\text{M}$  CCh in the pipette side and 100  $\mu\text{M}$  GTP on the bath side of the membrane, obtained at the indicated holding potentials. b, Current-voltage relations for K<sup>+</sup> channels activated by 10  $\mu\text{M}$  CCh (●) or 0.5  $\mu\text{M}$  SS (○). Each point is the mean value from four independent experiments with CCh and five independent experiments with SS; line, best least squares fit of the experimental data to a straight line. c, Amplitude histogram of channel openings obtained in the presence of CCh in the pipette and GTP in the bath at a holding potential of -80 mV; line, best fit of experimental data to a Gaussian distribution, obtained using a nonlinear least squares algorithm (32). d, Open-time duration histogram of the 55 pS channel openings at -80 mV holding potential; line, best fit of data to single exponential decay function.

guanosine[ $\beta$ -thio]diphosphate was added (not shown); 2) addition of GTP $\gamma$ S (100  $\mu\text{M}$ ) to patches excised without prior activation by ligands led, after lag times that varied between 2 to 10 min ( $n = 5$ ), to activation of K<sup>+</sup> channels (Fig. 2a) with the same unit conductance as seen with receptor ligands plus GTP (Fig. 3), and 3) addition to excised patches of purified human red blood cell PTX-substrate G<sub>k</sub>, commonly referred to as G<sub>i</sub> (or N<sub>i</sub>) (25), rapidly, within seconds, activated K<sup>+</sup> channels (Fig. 2d), which again had the same unit conductance as those seen after activation by ligands (plus GTP) or by GTP $\gamma$ S (Fig. 3)

Because of its effect on K<sup>+</sup> channels, we refer to this protein as G<sub>k</sub> (20). However, G<sub>k</sub> to be active by itself, *i.e.* on patches held by pipettes without SS or muscarinic agonist, required an activation (G<sub>k</sub><sup>\*</sup>) with GTP $\gamma$ S and Mg<sup>2+</sup> before addition to the patch. The treatment used (see *Materials and Methods*) is known to lead to tight binding of the nucleotide and concordant subunit dissociation (26) which are both thought to be the basis

for activation of the effector-stimulating function of this protein (27). Heated G<sub>k</sub><sup>\*</sup> (30 min, 100 C) resulted in loss of G<sub>k</sub> activity.

The experiments described above defined the existence in GH<sub>3</sub> cell membrane patches of a GTP-dependent step in the receptor mediated activation of K<sup>+</sup> channels; they also indicated that these K<sup>+</sup> channels can be activated by a purified human erythrocyte (hRBC) G protein, G<sub>k</sub>, that is a substrate for PTX (25). They did not, however, test whether the G protein endogenous to the GH<sub>3</sub> cell is a substrate for PTX or whether the human erythrocyte G<sub>k</sub> in addition to interacting with the GH<sub>3</sub> K<sup>+</sup> channel can also interact with the SS or ACh receptors of GH<sub>3</sub> cells, and indeed replace the natural coupling protein. To answer these questions, K<sup>+</sup> channels in the patches were first activated under the cell-attached configuration by either SS ( $n = 3$ ) or CCh ( $n = 5$ ) and then pulled off, so as to allow access to the inside surface of the membrane and treatment with activated PTX in the presence of NAD<sup>+</sup>.



**Fig. 2.** Characteristics of the Coupling System Mediating  $K^+$  Channel Activation by Receptors in  $GH_3$  Cell Membranes  
 $GH_3$  cell  $K^+$  currents in membrane patches were recorded in symmetrical isotonic  $K^+$  solutions at a holding potential of  $-80$  mV, first in the cell-attached mode (C-A) and then, after excision, in the inside-out configuration (I-O). Downward deflections denote channel openings and represent the current required to maintain the transmembrane potential at the holding value. Times on top of the traces refer to minutes (or seconds) elapsed between the last addition of test material and the recording of the trace shown. The first additions were made between 5 and 7 min after patch excision. *a*, Basal channel activities in cell-attached and inside-out patches and activation of  $K^+$  currents by addition of  $100 \mu M$   $GTP\gamma S$ ; records were obtained 1, 10, 15, 20, and 30 min after addition of nucleotide. *b*, Effect of hRBC  $G_k^*$  to activate  $GH_3$   $K^+$  channels in the absence of either receptor ligands (in the pipette) or GTP (in the bath). Records of  $K^+$  current activity in response to  $G_k^*$  were obtained 10 sec, 2 min, and 10 min after addition of  $200 \mu M$   $G_k^*$  to the bath surrounding the inside-out patch. *c*, Dependence of receptor-induced  $K^+$  currents in isolated patches on GTP. GTP-containing ( $100 \mu M$ ) and GTP-free media were exchanged by perfusion; the patch pipette contained  $10 \mu M$  ACh throughout. *d*, Dependence of SS-stimulated  $K^+$  currents on a PTX-sensitive step and reconstitution of receptor- $K^+$  channel coupling by readdition  $G_k$ , but not  $G_s$ . Throughout, the pipette contained  $0.5 \mu M$  SS and the bath solutions  $100 \mu M$  GTP. Preactivated PTX and  $NAD^+$  were added to give  $10 \mu g/ml$  and  $1$  mM, respectively; washing after PTX treatment (W) was for 5 min by perfusion of the chamber at 2 ml/min with the same GTP-containing medium but without PTX and  $NAD^+$ . Preactivated  $G_s^*$  and, 25 min afterwards,  $G_k$  were added to give final concentrations of  $2000 \mu M$  each. Records were obtained 3 and 20 min after PTX plus  $NAD^+$  addition, 15 min after  $G_s^*$  addition and 2 and 10 min after  $G_k$  addition.

As shown for SS in Fig. 2, but also observed with CCh ( $n = 2$ , not shown), PTX treatment caused, after 5–20 min of incubation the total loss of  $K^+$  channel activity in spite of the continued presence of stimulatory receptor ligand in the pipette and of GTP in the bath.

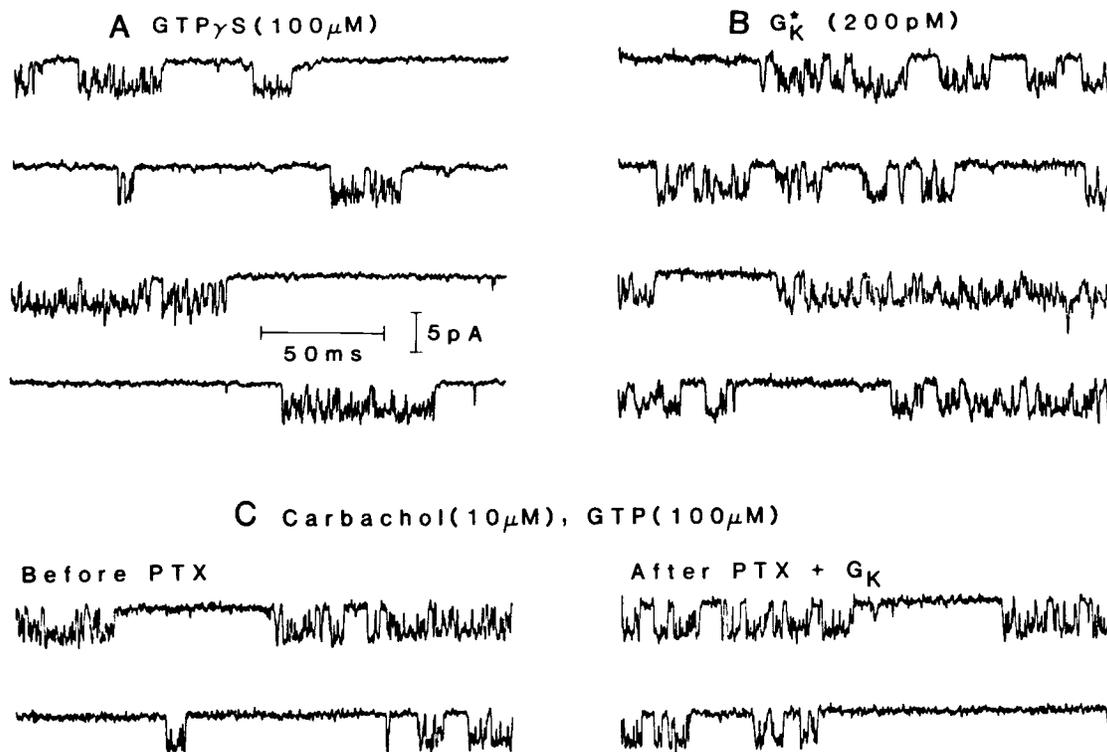
Activation of  $K^+$  channels by occupied receptors could be reconstituted in PTX-treated patches on addition of a native, nonactivated preparation of hRBC  $G_k$  plus GTP. This effect was dependent on simultaneous presence of GTP. No such activation of  $K^+$  channels could be elicited in either control or PTX-treated membranes by unactivated hRBC  $G_k$  plus GTP, if the patches were held by pipettes not containing a receptor agonist (not shown). This indicates that reappearance of channel activity such as shown in Fig. 2d was due to receptor-mediated activation of  $G_k$  by GTP, followed by interaction of activated  $G_k$ -GTP with the  $K^+$  channel.

At the single channel level, the unit conductance of the  $GH_3$   $K^+$  channel activity restored after PTX treat-

ment by the hRBC  $G_k$  was the same as seen in the same patch before inactivation of the endogenous  $G_k$  by PTX (see left two vs. right two tracings in Fig. 3b).

During these studies we noticed that the frequency of openings obtained in patches under the influence of ligands and endogenous  $G_k$  was lower than could be elicited by addition of excess exogenous  $G_k$  to reconstitute ligand-mediated openings (Fig. 2c) or of either  $GTP\gamma S$  or  $G_k^*$  (Fig. 2 a and b). Further, the simultaneous openings in the multichannel patches were also less frequent with receptor ligands and endogenous  $G_k$  than with  $G_k^*$  or  $GTP\gamma S$  (Fig. 2). Further, appearance of simultaneous openings was time dependent, being faster with  $G_k$  or  $G_k^*$  than with  $GTP\gamma S$ .

The specificity of the  $K^+$  channels for  $G_k$  was tested. Neither unactivated  $G_s$ , the stimulatory G protein of adenylyl cyclase, nor  $GTP\gamma S$ -activated  $G_s$  ( $G_s^*$ ) caused opening of  $K^+$  channels when added at concentrations up to  $2000 \mu M$ , indicating that the effect of  $G_k$  is of a



**Fig. 3.** Representative Records of 100-msec duration of Single Channel Openings Observed in Inside-Out Patches  
 A, Effect of  $100\ \mu\text{M}$   $\text{GTP}\gamma\text{S}$ ; B, effect of  $200\ \text{pM}$   $\text{G}_k^*$ ; C, effect of  $10\ \mu\text{M}$  CCh (in the pipette) and  $100\ \mu\text{M}$  GTP (in the bath) before (left two tracings) and 5 min after (right two tracings) reconstitution of receptor-mediated activation of  $\text{K}^+$  channels in a PTX-treated patch by addition of  $2000\ \text{pM}$  hRBC nonactivated  $\text{G}_k$ . PTX treatment was for 30 min and had produced total loss of receptor-stimulated  $\text{K}^+$  currents (not shown). For experimental details see legend to Fig. 1.

specific nature (e.g. Fig. 2d). The reconstitution of the PTX-treated system was obtained adding  $2000\ \text{pM}$   $\text{G}_k$ . In experiments without hormonal ligand,  $\text{GTP}\gamma\text{S}$ -activated hRBC  $\text{G}_k$  ( $\text{G}_k^*$ ) was used at  $200\ \text{pM}$ , but significant  $\text{K}^+$  channel stimulating activity was readily obtained also at  $2\ \text{pM}$  added  $\text{G}_k^*$  (not shown), making this a very potent  $\text{K}^+$  channel activator, and showing that on a molar basis  $\text{G}_s$  is contaminated with less than 0.1%  $\text{G}_k$ . Our previous studies with atrial membrane patches (20) had also compared effects of  $\text{GTP}\gamma\text{S}$ -activated  $\text{G}_k$  ( $\text{G}_k^*$ ) to those of  $\text{GTP}\gamma\text{S}$ -activated  $\text{G}_s$  ( $\text{G}_s^*$ ) and detected no activity of  $2000\ \text{pM}$   $\text{G}_s^*$ , while openings were readily induced by  $2\ \text{pM}$   $\text{G}_k^*$ .

$\text{G}_s$  and  $\text{G}_k$  are heterotrimers of the  $\alpha$ - $\beta$ - $\gamma$ -type, that differ only in their  $\alpha$ -subunits. We (26) as well as others (28, 29) have shown previously that the activation treatment leading to formation of  $\text{G}_k^*$  and  $\text{G}_s^*$  involves, at the molecular level, the formation of  $\alpha$ - $\text{GTP}\gamma\text{S}$  complexes plus free  $\beta$ - $\gamma$ -dimers. These do not reassociate provided they are kept at temperatures above  $15\ \text{C}$  and  $\text{GTP}\gamma\text{S}$  does not dissociate from either of the  $\alpha$ -subunits unless they are incubated for 10 h or more in  $\text{Mg}^{2+}$ -free media (26). It follows from these previous studies that under the conditions of assay (room temperature in the presence of  $ca\ 1\ \text{mM}$   $\text{Mg}^{2+}$ ) membranes exposed to  $\text{G}_k^*$  are actually exposed to an equimolar mixture of  $\alpha$ - $\text{GTP}\gamma\text{S}$  plus free  $\beta$ - $\gamma$ -dimers, and membranes exposed to  $\text{G}_s^*$  are actually exposed to an

equimolar mixture  $\alpha$ - $\text{GTP}\gamma\text{S}$  plus free  $\beta$ - $\gamma$  dimers which are the same as those present in  $\text{G}_k^*$  (30). Since only  $\text{G}_k^*$  activates  $\text{K}^+$  channels (Fig. 2d) of the present manuscript and Fig. 1 of Ref. 20), we conclude that receptor-stimulated activation of  $\text{K}^+$  channels is mediated by the  $\alpha$ -subunits of G proteins and not their  $\beta$ - $\gamma$ -dimers. It is not clear how this conclusion relates to that of Clapham and co-workers (31) proposing that  $\beta$ - $\gamma$ -dimers, added at concentrations higher than  $20,000\ \text{pM}$ , cause activation of atrial  $\text{K}^+$  channels. Due to the concentrations used, the results obtained by these authors either represent a phenomenon unrelated to the problem of receptor-mediated regulation of  $\text{K}^+$  channels addressed here, or are merely due to presence of contaminating ( $ca\ 0.1\%$ )  $\text{G}_k$  in their  $\beta$ - $\gamma$ -preparations. Alternatively, it could also be that the effects of such high concentrations of  $\beta$ - $\gamma$  relate specifically to the system analyzed by them. These authors analyzed the behavior of  $\text{K}^+$  channels in embryonic avian atrial cells after about 1 day of culture *in vitro*. But still, the relation of their observations to the mechanism by which  $\text{G}_k$  transduces the receptor signal remains unclear.

Our experiments demonstrate that PTX uncouples  $\text{GH}_3$  cell  $\text{G}_k$  and that exogenously added  $\text{G}_k$  from human erythrocytes easily takes the place of the endogenous  $\text{G}_k$ . This could not be if the system would have been precoupled, i.e. if  $\text{G}_k$  and  $\text{K}^+$  channel molecules existed as tight complexes regulated by receptors or if all

receptor and  $G_k$  molecules would exist in constant tight association. In either of these cases, ADP-ribosylation of  $G_k$  with PTX should have led to permanent loss of receptor- $K^+$  channel coupling. Thus, the data indicate that all three types of components, receptors, G coupling proteins, and the effector system— $K^+$  channels—must exist, at most, in loose association. In view of the extremely low concentrations required for  $G_k$  action in the patches, activation of components—receptor by ligands and  $G_k$  by GTP—must involve not only conformational changes but also increases in the relative affinities of one for another.

Activation of  $K^+$  channels by  $G_k$  (activated either by  $GTP\gamma S$  or by GTP under the influence of hormone receptor) is shown here in a secretory endocrine cell. We previously demonstrated the same for mammalian atrial  $K^+$  channels that were known to be regulated by muscarinic ligands in a GTP-dependent manner (32) and to be activated in inside-out patches by  $GTP\gamma S$  (33). These findings suggest that receptor-operated G protein-dependent  $K^+$  channels may have a quite wide distribution. Indeed, a recent report by Andrade *et al.* (34), strongly suggests that the same regulatory mechanism is operating also in the central nervous system. The direct coupling of SS and ACh receptors to  $K^+$  channel by a G protein without involvement of a soluble second messenger, establishes hyperpolarization as a primary response of an endocrine secretory cell to an inhibitory hormone. This response operates in parallel to inhibition of adenylyl cyclase activity. In addition, our experiments provide evidence that exogenously added  $G_k$  protein interacts readily not only with  $K^+$  channels but also with membrane receptors. Clearly, the signal transduction process involved in hormonal stimulation of  $K^+$  channel activity—receptors promoting activation of a G protein by GTP and activated G protein stimulating the effector  $K^+$  channel—is essentially identical, except for the nature of the G protein involved, to that operating hormonal stimulation of adenylyl cyclases.

It is interesting to note that in each of the two types of cells thus far examined by us, the ligands that activate  $K^+$  channels also cause inhibition of adenylyl cyclase. Both responses are PTX sensitive. It remains for further studies to determine whether the G protein that couples receptors to  $K^+$  channels is the same as the one involved in inhibition of adenylyl cyclase, *i.e.* whether  $G_k$  and  $G_i$  are one or two proteins.

## MATERIALS AND METHODS

GH<sub>3</sub> cells, obtained from the American Type Culture Association (Rockville, MD) and from Dr. Marvin Gershengorn, Cornell University Medical School (New York, NY) were grown in monolayers on glass coverslips in Ham's F 10 medium supplemented with 15% horse serum in a H<sub>2</sub>O-saturated atmosphere of 5% CO<sub>2</sub>-95% O<sub>2</sub> at 37 C.

Human RBC G proteins ( $G_s$  and  $G_k$ ) were purified according to Codina *et al.* (35, 36) with the exception that the last diethylaminoethyl-cellulose chromatography was replaced by chromatography over diethylaminoethyl-Toyopearl (37). As documented visually elsewhere (20),  $G_s$  and  $G_i$  prepared in this manner were better than 95% pure and essentially pure of each other (see *Results*).

Purified proteins were concentrated by free-flow electrophoresis (38) at 4 C into a buffer containing 0.1% Lubrol PX, 0.1 mM GTP, 1 mM EDTA, 20 mM  $\beta$ -mercaptoethanol, 30% (vol/vol) ethyleneglycol and 10 mM Na-HEPES, pH 8.0 (buffer A). Activation of G proteins was at 32 C for 30 min in buffer A plus 1 mM  $GTP\gamma S$  and 50 mM MgCl<sub>2</sub>, followed by extensive dialysis against buffer A with 50 mM MgCl<sub>2</sub> to remove free  $GTP\gamma S$ . G proteins activated in this manner ( $G_k^*$  and  $G_s^*$ ) were adjusted to 40  $\mu g/ml$  protein, *i.e.* 500 nM, and stored at -70 C until used. For use, G proteins were diluted with buffer A to 250 times the desired final concentration and then diluted a further 250-fold with bath solution.

Dialyzed PTX was activated (10 min, 32 C) at 250  $\mu g/ml$  in a final volume of 20  $\mu l$  50 mM DTT and 1 mM [adenyl-5'-yl imidodiphosphate] (pH 7.6), cooled, diluted to 50  $\mu l$  with 16.7 mM NAD<sup>+</sup>.

For recording of  $K^+$  currents in isolated membrane patches, gigohm seals were established according to Hamill *et al.* (21) and single channel currents were recorded on FM tape using a List EPC-7 amplifier. The tapes were low-pass filtered at 1–3 kHz, sampled at 10 kHz, and analyzed as described by Lux and Brown (39) using a PDP 11/23 computer. All experiments were carried out at room temperature in an experimental chamber of 100- $\mu l$  capacity placed on a microscope stage.

For all the experiments reported the internal pipette solutions in contact with the external surface of membrane patches contained (in millimolars): KOH 130, methane-sulfonic acid 130, MgCl<sub>2</sub> 1, EGTA 5, and HEPES 5 (adjusted to pH 7.3 with Tris). The bathing media in which the pipettes with the membrane patches were held contained in addition 100  $\mu M$  cAMP and 1 mM adenylyl-5'-yl imidodiphosphate, as well as various test compounds (*e.g.* GTP,  $GTP\gamma S$  and/or G proteins). Bathing solutions were either added (G proteins, PTX plus NAD<sup>+</sup>) or perfused (GTP,  $GTP\gamma S$ ) at a rate of 2 ml/min by gravity flow.

To test effects of SS and muscarinic ligands on  $K^+$  currents, they were added to the internal pipette solutions at concentrations known to cause inhibition of PRL secretion: 100–500  $\mu M$  SS and 1–100  $\mu M$  ACh or CCh. These concentrations are between 20 and 50 times the EC<sub>50</sub> with which they affect GH cell functions including hormone secretion, hyperpolarization, decrease in cAMP levels, and decrease in intracellular free Ca<sup>2+</sup> (2, 3, 5, 6, 9, 11–13, 16, 18).

## Materials

SS, ACh, and CCh were from Sigma (St. Louis, MO); guanine nucleotides were from Boehringer Mannheim (Indianapolis, IN). The sources of all other reagents were described previously (26).

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