The important distinction between this model and Dobzhansky's (*3*) is that, because of the sexual selection mechanism, the spatial pattern of reproductive character displacement between sympatric and allopatric populations is not predicted. Rather, the new male character and associated female preference would start in the area of reproductive overlap, but on account of the runaway Fisherian process, would continue into the areas of allopatry, and thus be found throughout the population, a process that has been mathematically supported for three types of polygamous mating systems (*15*).

In addition to avoiding the objections to an adaptive response through natural selection, the evolution of lepidopteran male pheromones through sexual selection is also supported by other lines of evidence. First, male scent–emitting structures exhibit striking morphological diversity across the Lepidoptera, especially compared to female scent structures; they typically appear sporadically within a taxa and their relative complexity bears little relation to lepidopteran phylogeny (*16*). Second, male pheromones usually (although not universally) are active over only a short range (*7*), making them an energy-inefficient mechanism for reproductive isolation. If natural selection were the driving force behind the species-specific nature of mating systems, one would expect selection for a more energy-conservative long-distance mechanism. Furthermore, as in other groups, there is no evidence for reproductive character displacement, as no intraspecific variance in male structures for scent emission has been reported; throughout the range of a species, these structures appear to be either present or absent. Finally, that sexual selection can operate in moth mating systems has been demonstrated in *Ephestia elutella*. Females of this species not only show a mating preference for larger males, but this preference is apparently based on the fact that large males produce significantly more pheromone (*17*).

In summary, evidence from surveys of host-plant associations and geographical distributions provides independent measures of interspecific contact. Both suggest the evolution of male pheromones in Lepidoptera as reproductive isolating mechanisms due to an adaptive response to interpopulational mating mistakes. The survey format encompassing a large number of species suggests that our conclusions are robust for the Lepidoptera and avoids the difficulty of sampling bias possible in studies of individual species pairs. Although these data do not distinguish between the two adaptive response models presented, the sexual selection model is more consistent with other lines of evidence.

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Direct Activation of Mammalian Atrial Muscarinic Potassium Channels by GTP Regulatory Protein G_k

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The mammalian heart rate is regulated by the vagus nerve, which acts via muscarinic acetylcholine receptors to cause hyperpolarization of atrial pacemaker cells. The hyperpolarization is produced by the opening of potassium channels and involves an intermediary guanosine triphosphate–binding regulatory (G) protein. Potassium channels in isolated, inside-out patches of membranes from atrial cells now are shown to be activated by a purified pertussis toxin–sensitive G protein of subunit composition αβγ, with an α subunit of 40,000 daltons. Thus, mammalian atrial muscarinic potassium channels are activated directly by a G protein, not indirectly through a cascade of intermediary events. The G protein regulating these channels is identified as a potent G_k; it is active at 0.2 to 1 p*M*. Thus, proteins other than enzymes can be under control of receptor coupling G proteins.

MUSCARINIC AGONISTS, SUCH AS acetylcholine (ACh) or carbachol, attenuate adenylyl cyclase activity in heart membranes (*1, 2*) and hyperpolarize atrial cells by opening potassium channels (*3, 4*). Both effects depend on a guanine nucleotide–binding coupling (G) protein (*1, 5*) and are blocked by pertussis toxin (PTX) (*6, 5*). However, the two effects do not appear to be causally related, because adenylyl cyclase activity can be inhibited in isolated depolarized membranes (*1, 2*) and cyclic nucleotides do not affect opening of K^+ channels (*7*). Indeed, two types of electrophysiological experiments suggest that ACh-mediated opening of K^+ channels does not require generation of a soluble second messenger: (i) K^+ channels in a cell-attached "gigaseal" patch cannot be activated by ACh applied to the cell surface outside the patch but open readily upon addition of the neurotransmitter through the patch pipette (*8*); and (ii) addition of a nonhydrolyzable guanosine triphosphate (GTP) analog, guanosine 5′-(γ-thio)triphosphate (GTPγS), to the cytoplasmic face (bath side) of an inside-out patch activates the K^+ channel (*9*). Although the fact that PTX uncouples the muscarinic response suggests the involvement of a G protein of the αβγ type, neither

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the direct coupling of this protein to the K^+ channel, nor the lack of involvement of an intramembrane second messenger (such as diacylglycerol activating protein kinase C) has been critically tested. We now report that a pure PTX substrate from human erythrocytes ("G_i"), activated with GTPγS, binds and stimulates selectively K^+ channels with properties similar to those of the K^+ channel activated in a GTP-dependent manner by ACh. This effect is not mimicked by an activator of protein kinase C (phorbol ester) and occurs in the absence of adenosine triphosphate (ATP). Thus other proteins, in addition to enzymes such as adenylyl cyclase and phosphodiesterases, can be regulated by a signal transducing G protein with the structure αβγ; the mammalian atrial muscarinic K^+ channel is one of these proteins. Regulation of this type may also underlie K^+ channel–mediated hyperpolarization in other cells, as occurs in certain nerve cells (for example, pyramidal cells of the hippocampus) in response to γ-aminobutyric acid (GABA) and serotonin (*10*) and in secretory cells (for example, pituitary cells) in response to somatostatin (*11*).

In 25 experiments atrial cells were dispersed from adult guinea pig hearts by collagenase digestion (*12*) and stored at room temperature as described (*13*). Single-channel currents were recorded with a gigaseal patch clamp by the method of Hamill *et al.* (*14*) with a List EPC-7 amplifier. The currents were analyzed by methods described by Lux and Brown (*15*). For whole-cell current recordings by the broken-patch technique (*14*), the bathing medium was a Tyrode solution with 135 m*M* NaCl, 5.4 m*M* KCl, 1.8 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 10 m*M* glucose, and 5 m*M* Hepes, *p*H adjusted to 7.4 with NaOH, and the patch pipettes contained 140 m*M* KCl, 2 m*M* $MgCl_2$, 1 m*M* EGTA, 1 m*M* ATP, 0.1 m*M* adenosine 3′,5′-monophosphate (cAMP), and 5 m*M* Hepes, *p*H adjusted to 7.3 with NaOH. For recording of currents from cell-attached or inside-out patches, the bathing medium was 140 m*M* KCl, 2 m*M* $MgCl_2$, 1 m*M* EGTA, 2 m*M* ATP, 0.1 m*M* cAMP, and 5 m*M* Hepes, *p*H 7.3 with NaOH, and patch pipettes contained 140 m*M* KCl, 1.8 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, and 5 m*M* Hepes, adjusted to *p*H 7.3 with NaOH. G proteins and nucleotides were added to either the patch pipettes or bathing media as described below. All experiments were performed at room temperature in an experimental chamber (0.1-ml volume) on a microscope stage. External solutions were either added (G proteins) or perfused through the chamber at 2 ml/min by gravity. G proteins were purified (*15, 16*) and concentrated to 40 μg/ml (500 n*M*) in protein buffer (PB) containing Lubrol PX (0.1%), 50 m*M* $MgCl_2$, 20 m*M* β-mercaptoethanol, 30% (volume to volume) ethylene glycol, 10 m*M* Hepes, *p*H 8.0 with NaOH, and, if unactivated, 100 μ*M* GTP. G proteins were activated (*17*) by incubation in PB with 1 m*M* GTPγS in the presence of 100 μ*M* GTP and 50 m*M* $MgCl_2$ for 30 minutes at 32°C. Unbound nucleotides were removed by extensive dialysis against PB without GTP or GTPγS. G proteins were diluted to 25 times the final desired concentrations in PB and tested for activity after a 25-fold dilution into the pipette (whole-cell current measurements) or bath (inside-out patch current measurements) solutions. Protein buffer or G proteins that had been treated with GTPγS in PB and then boiled were without effects.

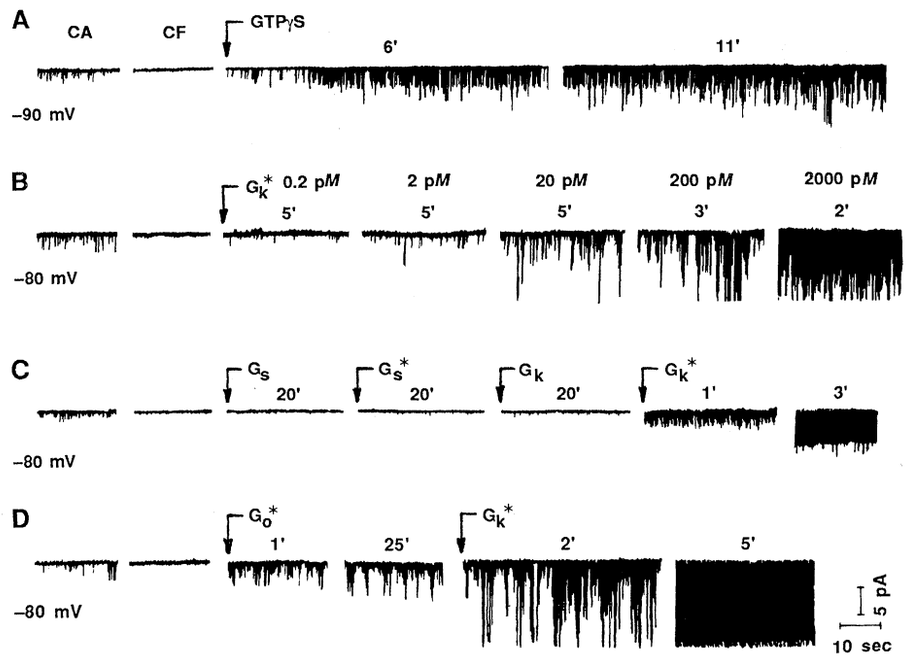

Fig. 1. Activation of atrial K^+ channels in inside-out patches by GTPγS and GTPγS-treated, PTX-sensitive G protein from human erythrocytes (G_k*). Atrial cell K^+ currents in membrane patches were recorded in symmetrical isotonic K^+ solutions at the indicated holding potentials, first in the cell-attached (CA) mode and then after patch excision under cell-free (CF) conditions both before and after addition to the bathing media of (**A**) 100 μ*M* GTPγS, (**B**) increasing concentrations of activated G_k*, (**C**) 2 n*M* each of G_s, G_s*, G_k, and G_k*, or (**D**) 2 n*M* GTPγS-activated bovine brain G_o (G_o*) followed by 2 n*M* G_k*. Times on top of each trace are minutes elapsed between the last addition and the recording of the trace shown. The first additions were made between 7 and 10 minutes after patch excision. In (A), GTPγS was added 7 minutes after patch excision and the records were obtained 6 and 11 minutes after this addition. In (B), G_k* additions were made at 25-minute intervals, the first 7 minutes after the excision of the patch. The effects of each concentration of G_k* appeared rapidly with typical records of bursts of channel openings obtained within 5 minutes or less. In (C), the bath solution contained 100 μ*M* GTP and the G proteins were added to concentrations of 2 n*M* at 25-minute intervals. The records were obtained 20 minutes after addition of G_s, G_s*, and G_k, and 1 and 3 minutes after addition of the activated G_k*. In (D), records were obtained 1 and 25 minutes after addition of 2 n*M* bovine brain G_o* (90 to 95% αβγ with α of M_r 39,000 and 1 to 5% αβγ with α of M_r 40,000 to 41,000) and 2 and 5 minutes after addition to the same bath solution of 2 n*M* G_k*. Results for the different test compounds are typical of four experiments with GTPγS, six experiments with G_k*, three experiments with G_s*, and four experiments with G_o*. Calibration bars refer to all records.

We next used excised patches to test the hypothesis that the above described regulation of K^+ channels is due to direct interaction between a G protein and the K^+ channel protein or proteins. Three types of experiments were performed: (i) addition of G proteins that had been previously activated or not activated by treatment with GTPγS and Mg^{2+} (*17*); (ii) addition of the potent protein kinase C activator tetradecanoyl phorbol acetate (TPA); and (iii) substitution of adenyl-5′-yl imidodiphosphate

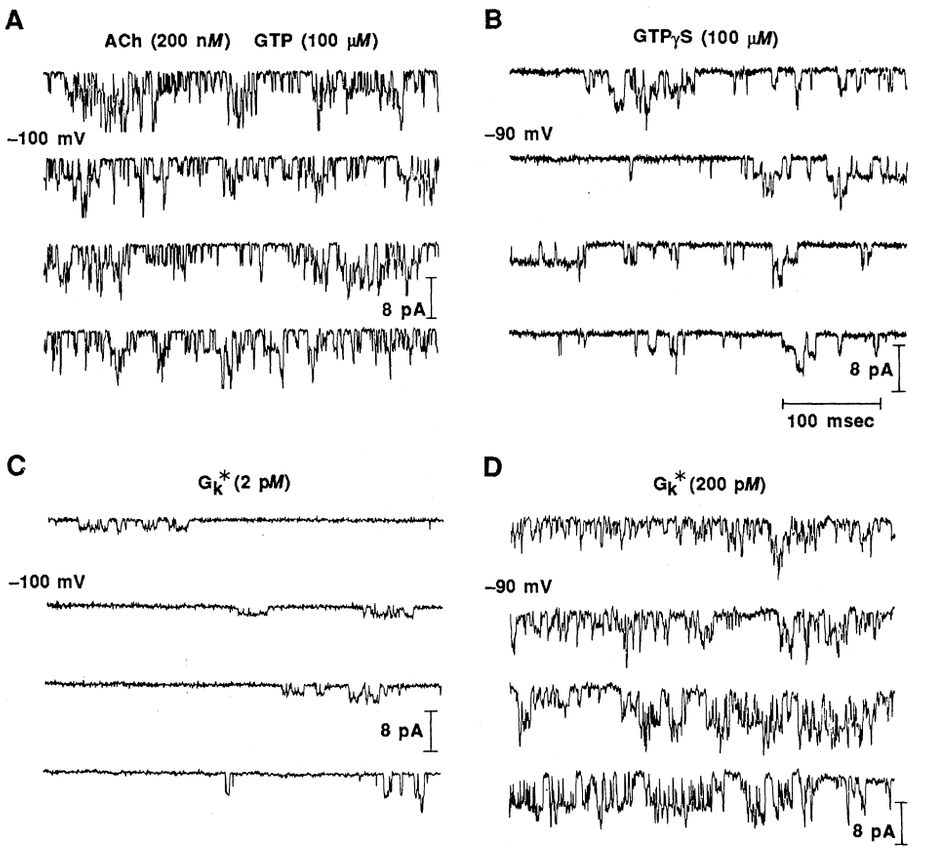

Fig. 2. Effects of (**A**) ACh (200 n*M* in the patch pipette) in the presence of 100 μ*M* GTP in the bath solution, of (**B**) 100 μ*M* GTPγS in the bath solution, and of (**C**) 2 p*M* and (**D**) 200 p*M* G_k* on K^+ channel activity in excised inside-out patches held at the indicated membrane potentials. ACh was present only for the experiment in (A). For each experimental condition, four typical 400-msec records (sampled at 5 to 10 kHz and filtered at 2 kHz with a four-pole Bessel filter) are shown. Results are typical of (A) 16 experiments, (B) 4 experiments, and (C and D) 6 experiments.

[AMP-P(NH)P] for ATP in the perfusion bath in which the excised patch was incubated during measurement of K^+ currents.

The first series of experiments (Fig. 1, B and D, and Fig. 2, C and D) showed that the purified "G_i" of human erythrocytes (*16, 17*) is a potent activator of K^+ channels in excised patches. In the absence of added G protein, we observed essentially no K^+ channel openings. Concentrations as low as 1 to 2 p*M* of activated "G_i" (G_k* in Figs. 1 and 2) led to the opening of K^+ channels. Even 0.2 p*M* "G_i" caused some opening of K^+ channels in two of four experiments (Fig. 1B). Increasing the concentration of activated "G_i" led to marked increases in the frequency of openings and, in all experiments, opening became maximal and essentially continuous upon addition of 1000 to 2000 p*M* activated "G_i" (Fig. 1, B to D).

The effect of "G_i" required that the protein be activated with guanine nucleotide before its addition to the bath. Unactivated human erythrocyte G_s (GTP was present throughout these experiments to stabilize the unactivated G proteins), activated G_s (G_s*), and unactivated human "G_i," at final concentrations of 2000 p*M* elicited no openings of K^+ channels and did not interfere with the effects of 2000 p*M* GTPγS-treated "G_i" (Fig. 1C). We also tested the effect of a GTPγS-treated preparation of bovine brain G_o (G_o*) (*19*). This preparation was much less effective in opening K^+ channels than GTPγS-treated human erythrocyte "G_i" (Fig. 1D). In fact, on a molar basis brain G_o* was only about 1 to 5% as active as the activated "G_i"; this could have been due to contamination with G_i-type protein in the G_o preparation (Fig. 3). On the basis of the above results, we call the G protein responsible for increasing the probability of opening of the atrial ACh-regulated K^+ channel, G_k.

Addition of TPA (10 μ*M*) to the bath did not cause K^+ channel opening. Substitution of AMP-P(NH)P for ATP in the bath did not interfere with the effect of 200 p*M* G_k* and, over a period of 30 to 40 minutes, failed to reverse the action of G_k*.

In additional studies the activity of K^+ channels in inside-out patches held by pipettes containing 200 n*M* ACh was lost after treatment with nicotinamide adenine dinucleotide (NAD^+) and PTX that had been activated by incubation with dithiothreitol, confirming the results of Kurachi *et al.* (*9*). In one preliminary experiment, we washed this treated patch and then could recover K^+ channel activity by adding native G_k plus GTP to the bathing medium. Thus it appears that exogenous G_k can substitute for endogenous G_k with respect to G_k receptor interaction, as well as G_k-K^+ channel interaction.

Our studies confirmed reports that single atrial muscarinic K^+ channels show inward rectification, and, compared to voltage-gated inwardly rectifying K^+ channels (*20*), have much briefer durations—less than 2 msec on the average (*4*). The channels usually opened in bursts and only occasionally opened in the absence of ACh. Thus, when ACh was added, the burst durations and opening probability increased. In excised patches the single-channel records (Fig. 2) showed multiple amplitudes when activation was produced by ACh and GTP, GTPγS, or G_k*. At high concentrations of activator, the different current levels were multiples of the most frequently observed single-channel current amplitude, the conductance of which was about 45 pS between 0 and −100 mV. These multiples of the single-channel amplitudes were attributed to simultaneous openings because most patches contained several channels. At low (1 to 2 p*M*) concentrations of G_k*, the predominant conductance had a similar mean open time but was smaller, about 22 pS (Fig. 2C). Transitions between the two conductances were observed and occurred more frequently than predicted for independent channels. The distribution of intervals between openings was complex and included gaps within a burst, gaps between bursts, and clustering of bursts, the latter possibly due to transient desensitizations.

A G protein has been shown to be involved in the regulation of heart K^+ channels by muscarinic ACh receptors; this step is PTX-sensitive (*5*). Yet it was not known whether the coupling protein had to be a member of the family of proteins involved in regulation of adenylyl cyclase. For example, K^+ channel regulation could have been under control of a G protein similar to *ras* or an elongation factor protein of the EF-2 type. Both of these bind and hydrolyze GTP and share sequence homologies with α subunits of the adenylyl cyclase–related G proteins, but neither associates with βγ complexes or undergoes a subunit dissociation reaction as part of their mode of action.

Our studies identify the PTX substrate in the complex αβγ (Fig. 3A), which was

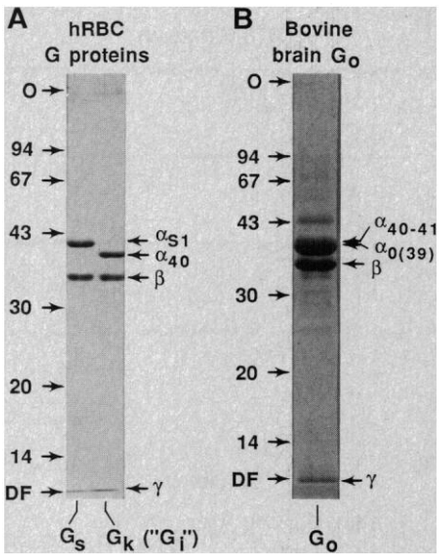

Fig. 3. SDS–polyacrylamide gel electrophoresis (12.5% acrylamide) of G proteins used in this study. (**A**) Two micrograms each of human red blood cell (hRBC) G proteins. G_s, the stimulatory regulatory component of adenylyl cyclase; G_k, the protein defined as the regulatory component of receptor-gated K^+ channels, also referred to as "G_i," the putative inhibitory regulatory component of adenylyl cyclase. (**B**) Ten micrograms of DEAE-Toyopearl–purified bovine brain G_o. Migration of α_{40-41} is indicated. Subunit composition of the proteins: G_s, $\alpha_{s1}\beta\gamma$; G_k, $\alpha_{40}\beta\gamma$; G_o, $\alpha_{39}\beta\gamma$. Photographs are of Coomassie blue–stained gel slabs. O, origin; DF, dye front. Electrophoresis and purification were as described in Codina *et al.* (*16*), and chromatography over DEAE-Toyopearl (DEAE-Fractogel; Pierce Chemical Co.) was according to Katada *et al.* (*25*).

isolated by us (*16*), as a regulatory protein able to activate a class of K^+ channels, that is, as a G_k. The same G protein (α subunit of 40,000 daltons) has been shown to interact with and—as seen by stimulation of GTP hydrolysis—be activated by human platelet α_2-adrenoceptors (*21*). When we isolated αβγ, no pathways other than hormonal inhibition of adenylyl cyclase were known to be affected by PTX and this PTX substrate was therefore tentatively classified as "G_i" (or "N_i"), the inhibitory regulatory protein of adenylyl cyclase (*16*). More recent studies lead us to be cautious about the role or roles of this protein. For instance, we now show it to activate K^+ channels. In addition, α_2-adrenoceptors in platelets not only attenuate adenylyl cyclase activity, but also affect phosphoinositide turnover (*22*). Although it is possible that the effect of α_2-adrenoceptors on phosphoinositide turnover may be an indirect one that occurs via arachidonic acid metabolites (*22*), a direct action on a phospholipase cannot be excluded. Hence, coupling of the human erythrocyte PTX-sensitive G protein to α_2-adrenoceptors no longer classifies it unequivocally as a G_i. Moreover, attempts to demonstrate a direct inhibitory effect of the human PTX-sensitive G protein on adenylyl cyclase activity in reconstitution assays have failed (*23*), calling for further caution.

The concentrations at which the human erythrocyte G_k stimulated opening of K^+ channels were extremely low (0.1 to 1 p*M*), and effects resembling those of saturating concentrations of ACh in the presence of GTP were obtained with 100 to 200 p*M* (for example, Fig. 2). These data, the lack of effect of 2 n*M* activated G_s, and the marginal effects of a bovine brain PTX-sensitive G_o preparation indicate that the effects of PTX-sensitive G protein from the human erythrocyte are specific. In fact, activation of atrial K^+ channels is the most potent in vitro activity of the purified protein and is the reason for calling it G_k, symbolized as G_k* in its activated form.

The relation of atrial G_k—or our human erythrocyte G_k—to heart G_i is not clear at this time. G_i, defined as the mediator of inhibitory regulation by receptors that attenuate adenylyl cyclase activity, exists in ventricular heart cells, as indicated by actual measurements of muscarinic receptor–mediated inhibition of adenylyl cyclase activity in membranes isolated from ventricular tissue (*1, 2*). It is also present in murine atria, in which ACh reduces cAMP levels (*24*). Thus, G_k could be G_i. On the other hand, in murine atria ACh also stimulates phosphoinositide breakdown, presumably by stimulating phospholipase C–dependent hydrolysis of phosphatidylinositol bisphosphate to inositol trisphosphate plus diacylglycerol (*22*). This opens the possibility that G_k might be a G_p, that is, a phospholipase C–stimulating G protein. The phorbol ester TPA did not mimic the action of G_k, and G_k acted equally well when AMP-P(NH)P was substituted for ATP. This ruled out that opening of K^+ channels was due to activation of phospholipase C by locally produced diacylglycerol and subsequent phosphorylation by protein kinase C of the channel protein or proteins. Yet these experiments do not rule out the possibility that G_k may be a G_p. It is still unknown whether G_k is a novel G protein or a known G protein.

Although our experiments are not conclusive in this matter, brain G_o does not appear to be a G_k. The protein we used was prepared by DEAE-Toyopearl chromatography (*25*) of partially purified mixture of two G proteins commonly referred to as G_o and G_i (or N_o and N_i). However, even though we were able to remove most of the G_i-like material (that is, αβγ with a PTX-sensitive α subunit slightly larger than the 39,000 dalton α_o), we cannot be assured that the ratio of α_o to "α_i" was greater than 20 to 1, and the responses seen could very likely be due to contaminating brain G_k, rather than to an intrinsic activity of G_o.

The very low concentration of exogenous G_k* required for opening of the atrial K^+ channel indicates that the presence of endogenous G_k, through which ACh regulates K^+ channels, did not prevent the effects of the exogenously added protein. Moreover, although the action of saturating concentrations of GTPγS appeared with an average lag of 5 to 6 minutes ($n = 4$), saturating concentrations of G_k* acted more rapidly—within 2 to 3 minutes ($n = 6$) (Fig. 1, B to D), suggesting that the exchange of G_k* for G_k at the inner surface of the membrane occurred readily. Indeed, the time course of action of G_k* in atrial membranes containing endogenous G_k resembles that during reconstitution of *cyc*$^-$ adenylyl cyclase with similarly low concentrations of G_s*, where, because of the genetic absence of G_s, only the formation of the adenylyl cyclase–G_s adduct is necessary (*26*).

Thus, the data are consistent with the notion that atrial K^+ channels and atrial G_k do not exist as tightly associated, "precoupled" complexes, but rather that coupling ensues as a consequence of activation of the G protein by GTP under the influence of occupied muscarinic ACh receptor. On the other hand, a regulatory mechanism localized within the membrane would respond more quickly than one that used a cytoplasmic second messenger. This may be relevant to the observation that vagal stimulation produces its effects on heart rate much more quickly than sympathetic stimulation.

Other channels might also be affected by G proteins, in the manner shown here for G_k. Notable among these is a receptor-operated Ca^{2+} channel that, although referred to (*27*), has been experimentally elusive. The methods we have used—inside-out patches of plasma membranes tested for effects of either nonhydrolyzable GTP analogs or of purified G proteins of as yet undefined function—should serve to further investigate the role of G proteins in regulating heretofore unrecognized membrane functions.

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Metalloregulatory DNA-Binding Protein Encoded by the *merR* Gene: Isolation and Characterization

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The MerR protein mediates the induction of the mercury resistance phenotype in bacteria; it has been isolated in order to study the effects of metal-ion induced changes in the metabolism of prokaryotic cells at the molecular level. After DNA sequences responsible for negative autoregulation were removed, the 16-kilodalton protein was overproduced and purified to more than 90 percent homogeneity by a salt extraction procedure that yields about 5 milligrams of protein per gram of cells. Complementation data, amino terminal analysis, gel filtration, and deoxyribonuclease I protection studies demonstrate that the purified *merR* gene product is a dimer under nondenaturing conditions and that it binds specifically to DNA, in the presence and absence of mercury, at a palindromic site which is directly between the −10 and −35 regions of the structural genes and adjacent to its own promoter. These initial results indicate that MerR is a DNA-binding metalloregulatory protein that plays a central role in this heavy metal responsive system and they delineate an operator site in the *mer* operon.

THE TRANSCRIPTION OF A VARIETY of genes, including those of the eukaryotic heat shock system (*1*), the metallothioneins (*2*), the prokaryotic mercury resistance (*3*), and iron uptake systems (*4*), responds dramatically to changes in the concentrations of specific heavy metals. With the exception of those iron uptake systems, none of the factors regulating metal-responsive gene expression, which could be called metalloregulatory proteins, has been identified. The *merR* gene product, a positive and negative effector of mercury resistance genes in bacteria, was studied as a prototype for the metal-responsive switches that can sense and translate inorganic signals into changes in metabolism. Both the *merR* gene product and subtoxic Hg(II) concentrations (10^{-6} to $10^{-8}M$) are required for transcriptional activation of the plasmid-based mercury resistance *(mer)* operon (*5*). The Tn501 *mer* operon (Fig. 1A) encodes transport proteins (*merT* and *merP* gene products) and mercuric ion reductase (*merA* gene product), a flavoenzyme that reduces mercuric ion to the volatile Hg(0) state (*3, 6*). Genetic evidence indicates that the trans-acting *merR* gene product exerts negative control of the structural genes in the absence of Hg(II), and positive control in the presence of Hg(II) (*5*). In addition, the *merR* gene is apparently autoregulated in a negative manner whether or not the effector, Hg(II) (*5, 7*), is present.

To date, the corresponding MerR protein has not been isolated or characterized. The DNA sequence of the *merR* region of both the Tn501 and R100 *mer* systems reveals three open reading frames, ORF1, ORF2, and ORF3 with possible encoded proteins of 6.5, 12.4, and 15.9 kD, respectively (*8*). ORF1 and ORF2 are in the same orientation as the *merTPAD* structural genes, but ORF3 runs on the opposite DNA strand. Transcriptional and translational fusions of the *merR* gene from either R100 or Tn501 with the *lacZ* gene suggest that ORF3, transcribed in the opposite direction of *merTPAD*, encodes the functional *merR* gene product (*7, 9*). Transcription in vivo is observed in both orientations, but only in the orientation including ORF3 can transcription be repressed by a complementing $merR^+$ plasmid (*9*). Furthermore, S1 mapping experiments reveal a single transcript corresponding to ORF3, but no transcripts for ORF1 or ORF2 (*7*).

Isolation and characterization of the protein encoded by the *merR* gene has been difficult because of negative regulation by MerR protein of its own synthesis. To overcome this problem and achieve reasonable overproduction, we constructed a series of plasmids with deletions in the DNA sequence 5′ to ORF3 *merR* gene. Fragments from a controlled Bal-31 digest were ligated directly downstream of the inducible tac promoter in plasmid pKK223-3. Screening of these mutants revealed a single construction, pTO90-16 (Fig. 1B), which directed the inducible expression of a peptide with a 16-kD subunit corresponding in molecular size to that of the peptide predicted from the DNA sequence of ORF3. Three lines of evidence indicate that pTO90-16 contains an intact and functional *merR* gene. (i) pTO90-16 complements an $Hg^s/merR^-$ plasmid, pUB986, in cells grown on minimal media plates containing 50 μM $HgCl_2$ or LB culture containing 100 μM $HgCl_2$, whereas the original vector, pKK223-3, will not (*10*). (ii) Sequencing of the first 100 bp of the Eco RI insert of pTO90-16 indicates that the Bal-31 exonuclease cutting was terminated 18 bp upstream of the start codon for the *merR* gene ORF3 (Fig. 1B), demonstrating that the coding regions of ORF3 *merR* gene, including the Shine-Delgarno sequence, were left intact. (iii) The overproduced 16-kD polypeptide was removed by electroelution from an SDS-polyacrylamide gel slice, and the amino acid sequence of the first ten residues (MENNLENLTI) (*11*) was determined (*12*). This sequence is identical to that predicted from the known DNA sequence of ORF3 (see

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