lution (*13*) before GS-MS analysis (Hewlett-Packard 5890, electron-impact ionization at 70 eV). The carrier gas was He, and a 12 m by 0.2 mm HL-1 (crosslinked methylsilicone gum) capillary column was used. The following products were identified as methyl derivatives: chloromaleic acid **1**, dimethyl ester, MS data (fragments and relative intensity): 180 [$(M+2)^+$, 2.0], 178 (M^+, 5.8), 149 [$(M+2-OCH_3)^+$, 33.5], 147 [$(M-OCH_3)^+$, 100], 121 [$(M+2-COOCH_3)^+$, 1.1], 119 [$(M-COOCH_3)^+$, 2.5], 111 [$(M-OCH_3-HCl)^+$, 2.1], 59 ($COOCH_3^+$, 17.6). Chlorofumaric acid **2**, dimethyl ester, MS: 180 [$(M+2)^+$, 3.0], 178 (M^+, 10.0), 149 [$(M+2-OCH_3)^+$, 33.1], 147 [$(M-OCH_3)^+$, 100], 121 [$(M+2-COOCH_3)^+$, 3.0], 119 [$(M-COOCH_3)^+$, 7.0], 111 [$(M-OCH_3-HCl)^+$, 4.0], 59 ($COOCH_3^+$, 31.0). Maleic acid **3**, dimethyl ester, MS: 144 (M^+, 1.1), 113 [$(M-OCH_3)^+$, 100], 85 [$(M-COOCH_3)^+$, 18.1], 82 [$(M-2OCH_3)^+$, 2.8], 59 ($COOCH_3^+$, 23.8). Fumaric acid **4**, dimethyl ester, MS: 144 (M^+, 1.7), 113 [$(M-OCH_3)^+$, 100], 85 [$(M-COOCH_3)^+$, 71.0], 82 [$(M-2OCH_3)^+$, 7.1], 59 ($COOCH_3^+$, 41.0). Compound **5**, 2,6-dichloro-4-(2,4,6-trichlorophenoxy)phenol, methyl ether, MS: 374 [$(M+4)^+$, 36], 372 [$(M+2)^+$, 58], 370 (M^+, 34), 359 [$(M+4-CH_3)^+$, 65], 357 [$(M+2-CH_3)^+$, 100], 355 [$(M-CH_3)^+$, 61]. The distribution pattern of cluster peaks is characteristic of a molecule having five Cl atoms. Compound **6**, monoester-dimethyl ether derivative, MS: 342 [$(M+4)^+$, 2.0], 340 [$(M+2)^+$, 5.8], 338 (M^+, 5.9), 311 [$(M+4-OCH_3)^+$, 5], 309 [$(M+2-OCH_3)^+$, 16], 307 [$(M-OCH_3)^+$, 18], 283 [$(M+4-COOCH_3)^+$, 32.0], 281 [$(M+2-COOCH_3)^+$, 93], 279 [$(M-COOCH_3)^+$, 100], 175 [$C_6H_2Cl_2(OCH_3)^+$, 6.5]. The distribution of peaks in the molecular ion cluster region and in clusters after OCH_3 and $COOCH_3$ losses are typical of a molecule with three Cl atoms.

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14. Maleic and fumaric acids were purchased from Aldrich. Chlorofumaric acid was prepared according to the methods of W. H. Perkin [*J. Chem. Soc.* **53**, 695 (1888)] and M. Akhtar, N. P. Botting, M. A. Cohen, and D. Gani [*Tetrahedron* **43**, 5899 (1987)]. Chloromaleic acid was prepared according to the method of V. G. Gruzdev and G. V. Gruzdev [*J. Gen. Chem. USSR* **56**, 1872 (1986)].
15. The NMR analysis of products of oxidative dechlorination and aromatic cycle cleavage of TCP was performed as for run 2 of Table 2. The reaction mixture (100 ml) was dried in vacuum, and 7 ml of 1 M HCl saturated with NaCl was added to the dry residue. The products were extracted with diethyl ether (three times with 60 ml). After evaporation of the ether extracts, the solid residue was dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) for NMR analysis. We added 6 μl of $CHCl_3$ (0.075 mmol) as an internal standard to quantify the oxidation products. The total yield of coupling products was based on two protons per aromatic ring.
16. Coupling products that have a quinone function cannot be analyzed by GS-MS directly or after treatment with $Me_3S^+OH^-$.
17. Coupling products of TCP oxidation were also analyzed by EI-MS after separation of organic-soluble material as follows. The reaction conditions were those as for run 13, Table 1. After the reaction mixture had been stirred for 4 min, 40 ml of the violet reaction mixture was treated with CH_2Cl_2 (four times with 20 ml) and the organic phase was dried. The crude product was extracted with 5 ml of CH_2Cl_2, and the insoluble material was isolated by filtration. This latter solid residue was soluble in acetonitrile and was identified by EI-MS as 2,6-dichloro-4-(2,4,6-trichlorophenoxy)phenol **5** on the basis of a molecular ion peak cluster typical for a molecule containing five Cl atoms. MS: 360 [$(M+4)^+$, 1.4], 358 [$(M+2)^+$, 3.6], 356 $(M)^+$, 1.3), 290 [$(M+4-2Cl)^+$, 0.7], 288 [$(M+2-2Cl)^+$, 2.8], 286 [$(M-2Cl)^+$, 3.6], 211 (2.1), 209 (6.4), 207 (7.2), 196 (100) (see Fig. 4). After loss of two Cl atoms, the peak distribution indicates a fragment having three Cl atoms. This loss of two Cl atoms suggests that the OH group is in the *para* position to the diphenyl ether bond. The phenoxyphenol with an OH group *ortho* to the diphenyl ether bond should show (M-HCl) fragmentation accompanying ring closure (*19*). We added 12 ml of hexane to the remaining red-brown CH_2Cl_2 solution; this produced a red-brown precipitate containing 2-hydroxy-6-(2,4,6-trichlorophenoxy)-1,4-benzoquinone **7**, MS: 321 [$(M+4-H)^+$, 2.1], 319 [$(M+2-H)^+$, 8.6], 317 [$(M-H)^+$, 8.8], 301 [$(M+2-H_2O)^+$, 4.3], 299 [$(M-H_2O)^+$, 3.2], 291 [$(M+2-CO)^+$, 3.2], 289 [$(M-CO)^+$, 4.3], 257 (4.3), 198 (72), 196 (79), 97 (100), a compound containing three Cl atoms. The remaining yellow solution was dried, and succeeding EI-MS analysis showed the presence of 2,6-bis(2,4,6-trichlorophenoxy)-1,4-benzoquinone **8**, 500 [$(M+4)^+$, 1.9], 498 [$(M+2)^+$, 3.4], 496 (M^+, 1.0), 465 [$(M+4-Cl)^+$, 5.2], 463 [$(M+2-Cl)^+$, 9.0], 461 [$(M-Cl)^+$, 4.8], 319 (53.2), 317 (51.4), 303 (62.8), 301 (60.0), 97 (100), a molecule containing six Cl atoms (Fig. 4).

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A Region of Adenylyl Cyclase 2 Critical for Regulation by G Protein βγ Subunits

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Receptor-mediated activation of heterotrimeric guanine nucleotide–binding proteins (G proteins) results in the dissociation of α from βγ subunits, thereby allowing both to regulate effectors. Little is known about the regions of effectors required for recognition of Gβγ. A peptide encoding residues 956 to 982 of adenylyl cyclase 2 specifically blocked Gβγ stimulation of adenylyl cyclase 2, phospholipase C–β3, potassium channels, and β-adrenergic receptor kinase as well as inhibition of calmodulin-stimulated adenylyl cyclases, but had no effect on interactions between Gβγ and $G\alpha_o$. Substitutions in this peptide identified a functionally important motif, Gln-X-X-Glu-Arg, that is also conserved in regions of potassium channels and β-adrenergic receptor kinases that participate in Gβγ interactions. Thus, the region defined by residues 956 to 982 of adenylyl cyclase 2 may contain determinants important for receiving signals from Gβγ.

G protein signaling is mediated by both Gα (*1*) and Gβγ (*2*) subunits. Effectors regulated by Gβγ include K^+ channels, phospholipase C–β, and adenylyl cyclases. Adenylyl cyclases 2 (AC2) and 4 (AC4) are conditionally stimulated by Gβγ (*2*). Purified AC2 directly interacts with Gβγ (*3*). Here, we sought to identify regions of AC2 that participate in interactions with Gβγ. Of the eight ACs that have been cloned and expressed (*4*), two (AC2 and AC4) are stimulated by Gβγ in the presence of activated $G\alpha_s$, one (AC1) is inhibited by Gβγ, and three (AC3, AC5, and AC6) do not appear to be directly regulated by Gβγ (*2*, *5*). We identified three regions of AC2 that are conserved only between AC2 and AC4 and tested whether peptides corresponding to these regions affected Gβγ regulation of AC2 and other Gβγ-regulated effectors, including K^+ channels, phospholipase C–β3 (PLC-β3), β-adrenergic receptor kinase (β-ARK), and AC1.

We synthesized three synthetic peptides corresponding to portions of AC2 (QAID peptide, residues 558 to 569; QEHA peptide, residues 956 to 982; and TEMS peptide, residues 1077 to 1090) and tested their effects on Gβγ stimulation of AC2 (*6*). AC2 expressed in Sf9 cells was stimulated by Gβγ in the presence of activated $G\alpha_s$ (*2*). The QEHA peptide inhibited Gβγ stimulation of AC2 expressed in Sf9 cell membranes; the inhibition of Gβγ stimulation appeared specific because stimulation by mutant (Q227L) activated $G\alpha_s$ was not affected (Fig. 1A). At all concentrations tested, the QEHA peptide did not affect basal AC2 activity or activity stimulated by Q227L-$G\alpha_s$ or forskolin (Fig. 1B). Because forskolin still stimulated AC2

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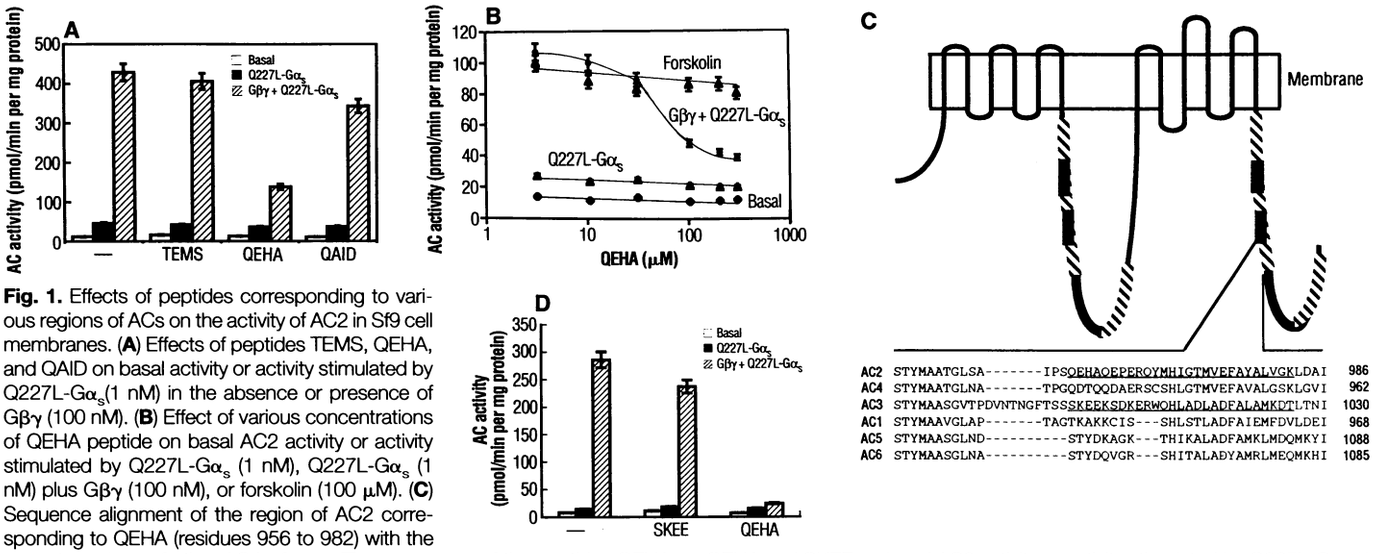

Fig. 1. Effects of peptides corresponding to various regions of ACs on the activity of AC2 in Sf9 cell membranes. (**A**) Effects of peptides TEMS, QEHA, and QAID on basal activity or activity stimulated by Q227L-$G\alpha_s$(1 nM) in the absence or presence of $G\beta\gamma$ (100 nM). (**B**) Effect of various concentrations of QEHA peptide on basal AC2 activity or activity stimulated by Q227L-$G\alpha_s$ (1 nM), Q227L-$G\alpha_s$ (1 nM) plus $G\beta\gamma$ (100 nM), or forskolin (100 μM). (**C**) Sequence alignment of the region of AC2 corresponding to QEHA (residues 956 to 982) with the cognate regions of other AC isoforms. The sequences of the peptides QEHA (AC2) and SKEE (AC3) are underlined. The highly conserved (80% similarity over eight amino acids) and the moderately conserved (60 to 80% similarity over eight amino acids) regions of the proteins are shown as solid boxes and shaded boxes, respectively, in the schematic representation. (**D**) Effects of peptides QEHA and SKEE on basal AC2 activity or activity stimulated by Q227L-$G\alpha_s$ (1 nM) in the absence or presence of $G\beta\gamma$ (100 nM). All values are means ± SD of triplicate determinations. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

activity in the presence of the QEHA peptide, the inhibition of $G\beta\gamma$ stimulation of AC2 by this peptide was apparently not the result of simple inhibition of enzyme activity. The location of the QEHA peptide within the presumed structure of mammalian ACs is shown in Fig. 1C. To explore further the specificity of the effect of the QEHA peptide, we synthesized and tested a peptide (SKEE) corresponding to the cognate region of AC3. The SKEE peptide inhibited $G\beta\gamma$ stimulation of AC2 by $G\beta\gamma$ only slightly (Fig. 1D). Several peptides corresponding to regions of β-ARK with unrelated sequences also had no effect on $G\beta\gamma$ regulation of AC2 (*7*).

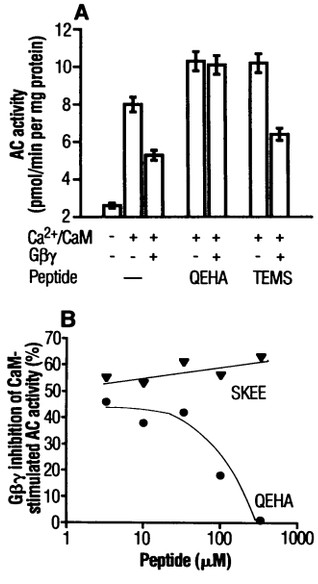

Fig. 2. Effects of the peptides TEMS and QEHA (**A**) and various concentrations of peptides QEHA and SKEE (**B**) on $G\beta\gamma$ inhibition of rat brain membrane AC activity stimulated by Ca^{2+} and calmodulin (CaM). Values for (A) are means ± SD of triplicate determinations. In (B), the percentage inhibition of Ca^{2+}-CaM–stimulated AC activity by $G\beta\gamma$ subunits in the presence of various concentrations of peptides was calculated by subtracting the AC activity in the presence of Ca^{2+}, CaM, and $G\beta\gamma$ from that in the presence of Ca^{2+} and CaM, and dividing by the activity in the presence of Ca^{2+} and CaM. Each experiment was repeated twice with similar results.

It is possible that functionally distinct regions of $G\beta\gamma$ subunits mediate the stimulatory and inhibitory effects of $G\beta\gamma$ on different ACs. We therefore tested the effect of the QEHA peptide on regulation of brain ACs by calmodulin (*8*). The QEHA peptide completely suppressed inhibition of the calmodulin-stimulated activity of rat brain membrane ACs by $G\beta\gamma$, whereas the TEMS and SKEE peptides had no such effect (Fig. 2). Thus, the QEHA peptide specifically blocks $G\beta\gamma$ regulation of ACs, irrespective of whether the effect is stimulatory or inhibitory.

We tested whether the QEHA peptide regulated $G\beta\gamma$ interactions with $G\alpha$ subunits (Fig. 3). Biotinylated $G\beta\gamma$ subunits were bound to avidin beads and washed, and $G\alpha_{oA}$ was added to allow reassociation. $G\alpha_{oA}$ binding was detected by analysis of the protein adsorbed to the avidin beads by electrophoresis on SDS-polyacrylamide gels (*9*). Only beads with adsorbed biotinylated $G\beta\gamma$ bound $G\alpha_{oA}$, and this binding was inhibited by excess free unbiotinylated $G\beta\gamma$. Addition of 100 μM QEHA peptide did not affect $G\alpha_{oA}$ binding to the biotinylated $G\beta\gamma$; addition of the peptides SKEE or TEMS also had no effect. The QEHA peptide by itself did not have any effect on the interaction of biotinylated $G\beta\gamma$ with the avidin beads. Hence, the QEHA peptide does not appear to affect the interaction between $G\beta\gamma$ and $G\alpha_{oA}$. This result implies that the sites on $G\beta\gamma$ responsible for $G\alpha$ binding and AC interaction are distinct.

To determine whether the QEHA peptide affected $G\beta\gamma$ regulation of other effectors, we tested its effects on $G\beta\gamma$ stimulation of β-ARK, atrial K^+ channels, and PLC-110, which is a truncated version of PLC-β3 that is stimulated only by $G\beta\gamma$. The QEHA peptide, but not the SKEE peptide, blocked $G\beta\gamma$ stimulation of β-ARK (*10*) (Fig. 4A). The QEHA peptide also specifically blocked $G\beta\gamma$ stimulation of PLC-110 (*11*) (Fig. 4B) and chick atrial K^+ channels (*12*) (Fig. 4, C to F). In the cell-attached patch clamp mode

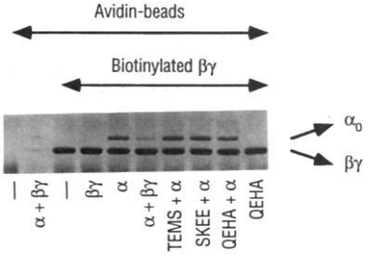

Fig. 3. Effects of QEHA, TEMS, and SKEE peptides on $G\alpha_{oA}$ interaction with $G\beta\gamma$ subunits. Proteins were visualized by Coomassie blue staining. The 32- to 42-kD region of the gel is shown. The experiment was repeated three times with similar results.

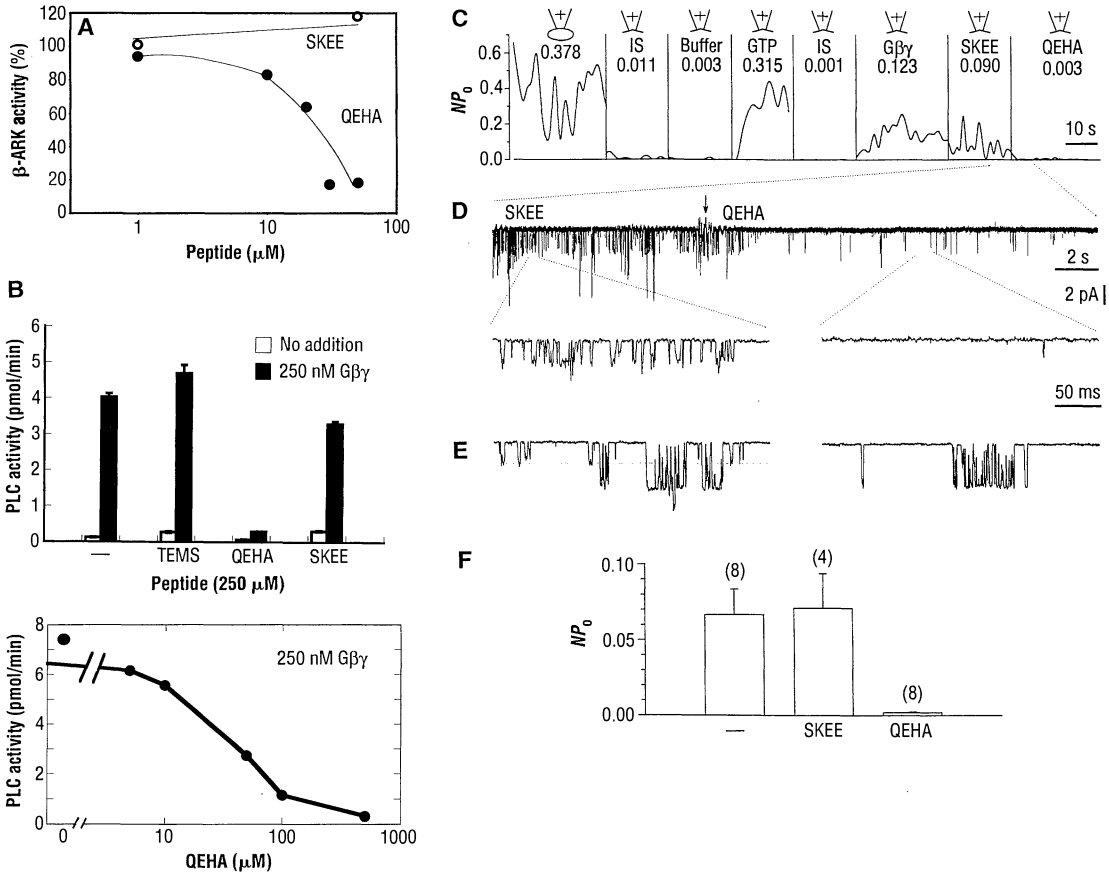

Fig. 4. Effects of QEHA, TEMS, and SKEE peptides on Gβγ stimulation of β-ARK (**A**), PLC-110 (**B**), and muscarinic K^+ channels (**C** to **F**). Experiments in (A) and (B) were repeated at least twice. In (**C**), the product of the number (*N*) of channels in the patch and the single-channel open probability (P_o) is shown as a function of time. The overall average NP_o is shown at the top of the plot for each condition. (D) Continuous primary single-channel activity before and after application of the QEHA peptide. Inward current is downward. Membranes were held at −80 mV. Representative segments for conditions before and after application of QEHA are also shown on an expanded time scale (lower traces) to resolve single-channel openings. (E) Same conditions as in (D) but with a different patch containing both muscarinic and ATP-sensitive K^+ channels. The single-channel amplitude level for the muscarinic K^+ channel is indicated by the dotted line. (F) Summary of the effects of the peptides QEHA and SKEE on Gβγ-stimulated muscarinic K^+ channel activity. Values are means ± SEM for the number of experiments indicated in parentheses.

with acetylcholine in the pipette (extracellular side), the average K^+ channel activity in the patch was 0.378 (Fig. 4C). Excision of the patch into the inside-out mode and bath perfusion (intracellular surface) with guanosine triphosphate (GTP)–free solution resulted in a decrease in channel activity to ~3% of that apparent in unexcised patches. Bath perfusion with GTP increased the activity of the K^+ channels in the patch to a level comparable to that observed in cell-attached patches. Removal of GTP again decreased channel activity. Perfusion with a solution containing Gβγ (10 nM) in the previously tested buffer solution resulted in channel activation. During perfusion of the patch with SKEE peptide (50 μM), channel activity decreased by 27%; perfusion with 50 μM QEHA peptide reduced channel activity by 98% relative to the activity apparent in the presence of Gβγ. This effect of QEHA is evident in single-channel recordings from around the time of peptide application (Fig. 4D). The QEHA peptide had no effect on the activity of adenosine triphosphate (ATP)–sensitive K^+ channels (Fig. 4E) (*13*). Data from several experiments are summarized in Fig. 4F. These data suggest that the QEHA peptide, by interacting with Gβγ subunits, can selectively block

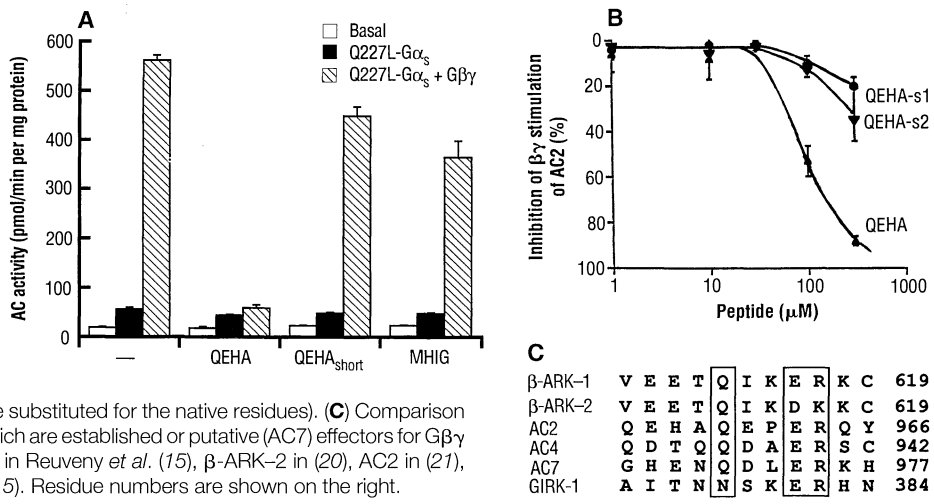

Fig. 5. Localization of determinants within the QEHA peptide that are important for modulation of Gβγ effects on AC2. (**A**) Effects of QEHA, $QEHA_{short}$, and MHIG peptides on stimulation of AC2 activity by Q227L-$G\alpha_s$ (1 nM) and Gβγ (100 nM). All peptides were used at a final concentration of 300 μM. $QEHA_{short}$, QEHAQEPERQYMHI; MHIG peptide, MHIGTMVEFAYALVGK. (**B**) Effects of various concentrations of QEHA, QEHA-s1, and QEHA-s2 peptides on Gβγ stimulation of AC2 activity in Sf9 cell membranes. Percentage inhibition by peptide was calculated as 1 minus the ratio of the activity in the presence of peptide to that in the absence of peptide. Values are means ± SEM from three seperate experiments. QEHA-s1, QEHA**AA**PERQYMHIGTMVEFAYALVGK; QEHA-s2, QEHAQEP**AA**QYMHIGTMVEFAYALVGK (bold Ala residues are those that were substituted for the native residues). (**C**) Comparison of amino acid sequences from the indicated proteins, which are established or putative (AC7) effectors for Gβγ subunits. The sequences for β-ARK–1 and GIRK-1 are in Reuveny *et al.* (*15*), β-ARK–2 in (*20*), AC2 in (*21*), AC4 in Gao and Gilman (*2*), and AC7 in Watson *et al.* (*15*). Residue numbers are shown on the right.

Gβγ interaction with effectors.

To determine the structural specificity of the QEHA peptide–Gβγ interaction, we substituted potentially important residues within the QEHA peptide with alanine and also synthesized two peptides corresponding to different segments of QEHA (*14*). Neither the 14– ($QEHA_{short}$) nor the 16– (MHIG) amino acid unsubstituted peptides inhibited stimulation of AC2 by Gβγ (Fig. 5A). Two 27–amino acid substituted peptides—QEHA-s1 and QEHA-s2—also showed greatly reduced capacities to inhibit stimulation of AC2 by Gβγ (Fig. 5B). Neither the QEHA-s1 nor QEHA-s2 peptide inhibited basal or $G\alpha_s$-stimulated AC2 activity (*17*). Thus, although the $QEHA_{short}$ peptide containing the amino acids targeted for substitution was not active, the presence of these amino acids in the longer QEHA peptide was crucial for inhibition of Gβγ regulation of AC2. The QXXER motif is conserved in AC2, AC4 (*2*), and AC7 (*15*). AC7, which is closely related to AC2, has recently been shown to be regulated by protein kinase C (*15*), as is AC2 (*18*), and the occurrence of the QXXER motif in AC7 suggests that this enzyme also might be susceptible to stimulation by Gβγ subunits. Furthermore, this or a similar motif also appears in regions of β-ARK–1, β-ARK–2, and the G protein–gated potassium channel GIRK-1 that are thought to be required for regulation by Gβγ (Fig. 5C) (*15*), suggesting that the motif may participate in signal transduction from Gβγ subunits. The Q and the ER residues are important in AC2, but it remains to be determined if they are also important in the other proteins.

Signal transmission in G protein–regulated pathways appears to be achieved by interactions between discrete regions of the signaling components. Such regions on receptors and G proteins have been mapped in several pathways (*16*). Regions of effectors that interact with G protein subunits are less well characterized. Our data delineate a region of AC2 that may participate in the interaction with Gβγ subunits.

Given the diverse effectors regulated by Gβγ subunits, it might be expected that distinct sites on βγ subunits interact with the different effectors. However, we have shown that a relatively short, 27–amino acid peptide corresponding to a region of AC2 blocks Gβγ regulation of five effectors: AC2, AC1, phospholipase C–β3, K^+ channels, and β-ARK. Such blockade probably occurs because the peptide binds to Gβγ and prevents Gβγ interaction with effectors, thus indicating that the regions of Gβγ that interact with the different effectors are very close to each other if not overlapping. Because the peptide corresponding to the AC2 sequence did not affect Gβγ interaction with Gα subunits, the region of Gβγ that participates in the interaction with Gα is functionally, if not physically, distinct from the Gβγ effector region. The similar potencies with which the AC2 peptide blocked Gβγ regulation of several unrelated effectors and the similar concentrations at which Gβγ regulates the different effectors (*17*) suggest that differential effector affinities for Gβγ are unlikely to direct signals through Gβγ subunits to different effector pathways. Rather, the relative abundance of the effectors and their relative proximity to the released Gβγ subunits are likely to be more crucial factors in determining whether receptors successfully use Gβγ subunits to transmit signals to different effector pathways.

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6. AC2 was expressed in Sf9 cells with a baculovirus vector. Two to 3 days after infection, cells were lysed and membranes were prepared (*18*). AC2 activity in Sf9 cell membranes (1 to 2 μg of protein per assay) was measured in the presence of 0.1 mM [α-^{32}P]ATP (adenosine triphosphate), 5 mM $MgCl_2$, and other reagents for 20 min at 30°C as described (*18*) [R. T. Premont, O. Jacobowitz, R. Iyengar, *Endocrinology* **131**, 2774 (1993)]. Q227L-$G\alpha_s$ [J. Chen and R. Iyengar, *Science* **263**, 1278 (1994)] protein was synthesized in vitro with the use of a T7 polymerase–coupled TNT reticulocyte lysate kit (Promega). Gβγ was purified from bovine brain (*19*). Peptides were synthesized with an Applied Biosystems model 430A peptide synthesizer. The identity of the QEHA peptide was verified by mass spectrometry. All peptides were dissolved in water. For the fixed-concentration assays, peptides (300 μM) were preincubated on ice for 20 min with Gβγ or buffer in the absence of membranes. AC activity is expressed as picomoles of adenosine 3′,5′-monophosphate formed per milligram of membrane protein per minute.
7. J. Chen and R. Iyengar, unpublished observations.
8. Membranes were prepared from freshly dissected rat brains. Tissue was homogenized in 20 mM Hepes-NaOH (pH 8.0), 150 mM NaCl, 5 mM EDTA, and 1 mM EGTA. After centrifugation (1000*g* for 10 min) to remove cell debris, the supernatant was centrifuged at 100,000*g* for 30 min. The resulting pellet was resuspended in 50 mM Hepes-NaOH (pH 8.0), 1 mM EDTA, and 8% (w/v) sucrose, and stored at −70°C. The rat brain membranes were assayed for AC activity as described (*6*), with the exception that the reaction mixture also contained either 1 μM $CaCl_2$ and calmodulin (33 μg/ml), or 1 mM EGTA. Bovine brain Gβγ (100 nM) or buffer was preincubated on ice for 20 min with peptide (300 μM or as indicated) in the absence of membranes.
9. $G\alpha_{oA}$ was purified as described (*19*). Biotinylated Gβγ subunits were prepared and bound to beads as previously described [J. Dingus, M. D. Wilcox, R. Kohnken, J. D. Hildebrandt, *Methods Enzymol.* **237**, 457 (1994)], with the exception that UltraLink Immobilized NeutrAvidin Plus beads (Pierce) were used. Beads containing biotinylated Gβγ were preincubated for 15 min at 22°C in 20 mM Hepes (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol, 0.1% Lubrol, 10 μM guanosine diphosphate, and 100 mM NaCl, in the absence or presence of 100 μM peptide. After addition of $G\alpha_{oA}$ (1 μg) in the absence or presence of free Gβγ (4 μg), the samples were incubated for 1 hour. The incubation was terminated by centrifugation at 14,000 rpm for 1 min and aspiration of the supernatant. The beads were resuspended in sample buffer, heated to 95°C, and resolved on an 11% SDS-polyacrylamide gel. Protein bands were visualized by staining with Coomassie blue.
10. Phosphorylation of rhodopsin by β-ARK was measured as described [W. J. Koch, J. Inglese, W. C. Stone, R. J. Lefkowitz, *J. Biol. Chem.* **268**, 8256 (1993); J. Inglese, W. J. Koch, M. G. Caron, R. J. Lefkowitz, *Nature* **359**, 147 (1992)]. In the absence of added βγ subunits, 25,532 cpm were incorporated into rhodopsin; in the presence of 100 nM βγ subunits, 538,464 cpm were incorporated. The latter activity was set as 100%, and percentage activities in the presence of peptides were determined.
11. PLC-110 was purified as described [J. L. Blank and J. H. Exton, *Methods Enzymol.* **238**, 237 (1994)] and its activity measured with [^{3}H]phosphatidylinositol 4,5-bisphosphate as substrate as described [M. D. DeVivo, *ibid.*, p. 131]. When present, the concentration of βγ subunits was 250 nM and that of the peptides was 250 μM or as indicated. Activity is expressed as picomoles of inositol 1,4,5,-trisphosphate formed per minute.
12. All experiments were performed at 20° to 22°C on atrial cells from a single 14-day-old chick embryo. Cells were enzymatically dispersed, plated, and used within 1 to 2 days of culture. The symmetrical ionic composition of the pipette and bath (denoted IS for intracellular) solutions was 140 mM K^+, 140 mM Cl^-, 5 mM EGTA, 2 mM Mg^{2+}, and 10 mM Hepes (pH 7.2). Acetylcholine (10 μM) and 1 mM Ca^{2+} were present in the pipette. Guanosine triphosphate (200 μM) was added as indicated. Data were sampled at a rate of 5 kHz with the Axon Instruments DIGIDATA 1200 interface and an AXOPATCH 200A amplifier, and analyzed with a low-pass filter set at 2.0 kHz with pCLAMP 6.0 software. Purified Gβγ subunits in 0.3% CHAPS detergent were dialyzed with the intracellular solution containing 0.3% CHAPS, 1 mM dithiothreitol, and 300 mM NaCl. The final concentrations of these additional reagents in intracellular solution were 0.015% (230 mM) CHAPS, 50 μM dithiothreitol, and 15 mM NaCl (denoted as Buffer). The peptides were diluted in intracellular solution.
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14. With the exception of QEHA, peptides used in the experiments shown in Fig. 5 were synthesized commercially by Bio-synthesis (Lewisville, TX) and purified by high-performance liquid chromatography in the laboratory of R.I.
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