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Specificity of Metabotropic Glutamate Receptor 2 Coupling to G Proteins

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ABSTRACT

Metabotropic glutamate receptor 2 (mGluR2) is a class 3 G protein-coupled receptor and an important mediator of synaptic activity in the central nervous system. Previous work demonstrated that mGluR2 couples to pertussis toxin (PTX)-sensitive G proteins. However, the specificity of mGluR2 coupling to individual members of the $G_{i/o}$ family is not known. Using heterologously expressed mGluR2 in rat sympathetic neurons from the superior cervical ganglion (SCG), the mGluR2/G protein coupling profile was characterized by reconstituting coupling in PTX-treated cells expressing PTX-insensitive mutant $G\alpha$ proteins and $G\beta\gamma$. By employing this method, it was demonstrated that mGluR2 coupled strongly with $G\alpha_{ob}$, $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$, although coupling to $G\alpha_{oa}$ was less efficient. In addition, mGluR2 did not seem to couple to the most divergent member of the $G_{i/o}$ family, $G\alpha_z$, although $G\alpha_z$ coupled strongly to the endogenous $\alpha 2$ adrenergic receptor. To determine which $G\alpha$ proteins may be natively expressed in SCG neurons, the presence of mRNA for various $G\alpha$ proteins was tested using reverse transcription-polymerase chain reaction. Strong bands were detected for all members of the $G_{i/o}$ family ($G\alpha_o$, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_z$) as well as for $G\alpha_{11}$ and $G\alpha_s$. A weak signal was detected for $G\alpha_q$ and no $G\alpha_{15}$ mRNA was detected.

Metabotropic glutamate receptors (mGluRs) are members of the class 3 G protein-coupled receptor family, which includes the calcium sensing receptor and the $GABA_B$ receptor, among others (Conn and Pin, 1997). There are eight known mammalian mGluR genes (mGluR1–8), which play diverse roles in the nervous system, including the modulation of synaptic transmission from both pre- and postsynaptic locations and regulation of synaptic plasticity. In addition, mGluRs also play a role in mediating sensory transduction (Bortolotto et al., 1994; Conn and Pin, 1997; Wilsch et al., 1998). mGluRs have been divided into three groups based on sequence homology, sensitivity to pharmacological agents, and G protein-coupling specificity (De Blasi et al., 2001). Group II (mGluRs 2 and 3) and group III mGluRs (mGluRs 4, 6–8) are known to couple exclusively to the pertussis toxin (PTX)-sensitive $G_{i/o}$ family of G proteins (Tanabe et al., 1992, 1993; Saugstad et al., 1994), whereas group I mGluRs couple to multiple classes of G proteins (Abe et al., 1992; Aramori and Nakanishi, 1992; Pin et al., 1992; Joly et al., 1995).

The mechanism of mGluR/G protein coupling has been examined in several studies (Pin et al., 1995; Gomeza et al., 1996; Blahos et al., 1998; Mary et al., 1998). Clearly, activation of G proteins by mGluRs in response to agonist binding involves regions of the receptor that are distinct from those of the class 1 G protein-coupled receptors. Coupling of mGluRs to G proteins seems to involve the proximal end of the intracellular C-terminal tail (Mary et al., 1998) and part of the second intracellular loop (Pin et al., 1995; Gomeza et al., 1996). Chimeric group II/group I mGluRs in which these regions from a group I mGluR were inserted into mGluR3 were able to couple to phospholipase C (similar to wild-type group I mGluRs; Gomeza et al., 1996). In addition, residues on the C-terminal tail of group I mGluRs seem to be involved in coupling to $G\alpha_{q/11}$, because this region has been shown to participate in phospholipase C activation (Mary et al., 1998). Thus, although many studies have examined the molecular basis of mGluR coupling to distinct G protein families (Gomeza et al., 1996; Blahos et al., 1998), detailed studies of the G protein coupling specificity of an mGluR *within* a single G protein family have not been performed. Such studies may begin to shed light on the molecular basis for specificity in systems such as synaptic terminals in the central nervous system, where several types of G protein-coupled receptor are present.

PTX is a valuable tool for the study of heterotrimeric G proteins. By ADP-ribosylating the last cysteine residue in the extreme C terminus of $G\alpha_{i/o}$ proteins (present only on $G\alpha$ proteins in this family), PTX treatment selectively uncouples

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ABBREVIATIONS: mGluR, metabotropic glutamate receptor; PTX, pertussis toxin; RT-PCR, reverse transcription-polymerase chain reaction; SCG, superior cervical ganglion.

these G proteins (Milligan, 1988). Consequently, mutation of this cysteine to another residue renders the resulting Gα PTX-insensitive. Therefore, after treating cells expressing a given $G_{i/o}$-coupled receptor with PTX to inactivate endogenous $G\alpha_{i/o}$ proteins, the G protein-coupling specificity of the receptor to G proteins within this family can be examined by heterologously expressing individual Gα CG or CI mutants and examining coupling. This method has been used to examine the coupling of other G protein-coupled receptors (Taussig et al., 1992; Senogles, 1994; Wise et al., 1997; Jeong and Ikeda, 2000).

In this study, the G protein specificity of mGluR2 for Gα proteins in the $G_{i/o}$ family was examined by reconstituting mGluR coupling in PTX-treated cells through expression of PTX-insensitive Gα (C-terminal Cys to Gly) mutants in sympathetic neurons from the rat superior cervical ganglion (SCG). In addition, to determine which subtypes of Gα proteins may be endogenously expressed in SCG neurons, the presence of mRNA for nine different Gα subunits was determined using RT-PCR.

Materials and Methods

Cell Isolation, DNA Injection, and Plasmids. A detailed description of the cell isolation and cDNA injection protocol is published elsewhere (Ikeda, 1997). The animal protocols used were approved by the Institutional Animal Care and Use Committee. Briefly, both SCGs were removed from adult Wistar rats (175–225 g) after decapitation, and incubated in Earle's balanced salt solution (Invitrogen, Carlsbad, CA) containing 0.45 mg/ml trypsin (Worthington Biochemicals, Freehold, NJ), 0.6 mg/ml collagenase D (Roche Applied Science, Indianapolis, IN), and 0.05 mg/ml DNase I (Sigma Chemical, St. Louis, MO) for 1 h at 35°C. Cells were then centrifuged (50 g), transferred to minimum essential medium (Fisher Scientific, Pittsburgh, PA), plated on poly(L-lysine)-coated 35-mm polystyrene tissue culture dishes and incubated (95% air/5% CO_2; 100% humidity) at 37°C before DNA injection. After injection, cells were incubated overnight at 37°C and patch-clamp experiments were performed the following day. Where indicated, neurons were incubated overnight with PTX (0.5 μg/ml; List Biological, Campbell, CA) in the culture media.

Injection of cDNA was performed with an Eppendorf 5246 microinjector and 5171 micromanipulator (Madison, WI) 4 to 6 h after cell isolation. Plasmids were stored at −20°C as a 1 μg/μl stock solution in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8). Rat mGluR2 was injected at 50 ng/μl (pCI; Promega, Madison, WI). Construction of the PTX-insensitive mutants of $G\alpha_{i1-3}$ and $G\alpha_{oa,b}$ has been described previously (Jeong and Ikeda, 2000). For reconstitution experiments, all Gα cDNAs (pCI; Promega) were injected at 5 to 6 ng/μl with bovine Gβ1 and Gγ2 (from M. I. Simon, Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA) injected at 10 ng/μl each (pCI; Promega). Neurons were coinjected with "enhanced" green fluorescent protein cDNA (0.005 μg/μl; pEGFP-N1; BD Clontech Laboratories) to facilitate later identification of successfully injected cells.

All inserts were sequenced using an automated DNA sequencer (ABI 310; Applied Biosystems, Foster City, CA). PCR products were purified with QIAGEN (Valencia, CA) silica membrane spin columns before restriction digestion and ligation. Plasmids were propagated in XL1-blue bacteria (Stratagene, La Jolla, CA) and midipreps prepared using QIAGEN anion exchange columns.

Electrophysiology and Data Analysis. Patch pipettes were made from 7052 glass (Garner Glass, Claremont, CA) and had resistances of 1 to 4 MΩ. Series resistances were 2 to 6 MΩ before electronic compensation, which was typically ≥80%. Ruptured patch whole-cell recordings were made with an Axopatch 200A patch-clamp amplifier (Axon Instruments, Union City, CA). Voltage protocol generation and data acquisition were performed using custom software on a Macintosh Quadra series computer (Apple Computer, Cupertino, CA) with a MacADIOS II data acquisition board (G.W. Instruments, Somerville, MA). Currents were low-pass–filtered at 5 kHz using the four-pole Bessel filter in the patch-clamp amplifier, digitized at 2 to 5 kHz and stored on the computer for later analysis. Experiments were performed at 21 to 24°C (room temperature). Data analysis was performed using Igor software (Wavemetrics, Lake Oswego, OR).

The external (bath) solution contained 155 mM Tris, 20 mM HEPES, 10 mM glucose, 10 mM $CaCl_2$, and 0.0003 mM tetrodotoxin, adjusted to pH 7.4 with methanesulfonic acid; osmolality, 320 mOsm/kg. The internal (pipette) solution contained: 120 mM *N*-methyl-D-glucamine, 20 mM tetraethylammonium methanesulfonic acid, 11 mM EGTA, 10 mM HEPES, 10 mM sucrose, 1 mM $CaCl_2$, 4 mM MgATP, 0.3 mM Na_2GTP, and 14 mM Tris-creatine phosphate, pH 7.2; osmolality, 300 mOsm/kg. PTX was obtained from List Biological (Campbell, CA) and applied to cells overnight at 500 ng/ml as indicated. L-Glutamate (100 μM) was used as the agonist for mGluR2. All drugs and control solutions were applied to cells using a custom gravity-driven perfusion system, positioned ~100 μm from the cell, that allowed rapid solution exchange (≤ 250 ms). The degree of mGluR-mediated calcium current inhibition (and norepinephrine-mediated inhibition, where indicated) was calculated as the maximal inhibition of the current in the presence of drug compared with the last current measurement before application of the drug.

RT-PCR. To test for the presence of mRNA coding for each of the nine Gα subunits (i1–3, o, z, q, 11, s, and 15), unique 18- to 25-base primer pairs from coding or 3′ noncoding sequences were identified using MacVector software (Accelrys, Inc., Princeton, NJ). Potential primers were constrained by length, GC content, melting temperature, and product size (see Table 1 for primer list and expected product sizes). Where possible, longer PCR products that were more likely to span introns were selected to reduce the contribution of genomic DNA. In addition, samples were treated with DNase as part of the RNA isolation procedure. Each potential primer was then BLAST-searched to rule out the presence of homologous or identical

TABLE 1
RT-PCR primers and expected product sizes

Primers	Expected Product Size
	nt
Gαo	300
5′-CGTGGAGTATGGTGACAAGGAGAG-3′	
5′-AAGGTGAAGTGGGTTTCTACGATG-3′	
Gαi1	482
5′-TGACTATGACCTGGTTCTTGCTGAG-3′	
5′-ACACTACATTCTCTGTTGCTGGGAG-3′	
Gαi2	210
5′-CAAGATGTTTGATGTGGGTGGTC-3′	
5′-AGGATGATGGAGGTGTCTGTGAAC-3′	
Gαi3	453
5′-TGAGTAAAGAGCCCAGGATTGC-3′	
5′-CAAAGCAGTTCTGACCACCAACC-3′	
Gαz	229
5′-CAGGGAATGACTACGGCAAATC-3′	
5′-TGAAATGTGGGCTGGTATGACG-3′	
Gαq	250
5′-TGTGGCTGACCCTTCCTATCTG-3′	
5′-CTCCATTCGGTTCTCATTGTCTG-3′	
Gα11	432
5′-GGTTGATGTGGAGAAGGTCACGAC-3′	
5′-ATGATTGTGCGGAACAGGGC-3′	
Gαs	531
5′-GAATCTTTGAGACCAAGTTCCAGG-3′	
5′-TGATGTCACGGCAGTCGTTG-3′	
Gα15	147
5′-TCTCTGAGCGAGTATGACCAGTGTC-3′	
5′-CAGGATGTCTGTCTTGTTGAGGAAG-3′	

sequences present in other known rat mRNAs. The primer sets for each Gα, as well as the size (number of bases) of the expected product are shown in Table 1. RT-PCR was performed using the QIAGEN One-Step RT-PCR kit on 40 ng of isolated total RNA (annealing temperature of 60–63°C, for 30–35 cycles) from dissociated rat SCG cultures using the Qiagen RNeasy total RNA isolation kit. As a positive control, primers were constructed to detect the ubiquitously expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) message (5′- CCAAAAGGGTCATCATCTCCG-3′, and 5′- AGACAACCTGGTCCTCAGTGTAGC-3′, producing an expected product of 501 bases). Negative controls were performed with the GAPDH primers in the absence of RNA. Primers were obtained from Operon Technologies (Alameda, CA). RT-PCR products were run on 3% agarose precast gels from Bio-Rad (Hercules, CA).

Western Blotting. Homogenates (10%, w/v) were made from combined SCGs dissected from an adult rat, and protein concentrations were determined using the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Polyacrylamide gels (10%) were used for protein fractionation and parallel gels were stained with Coomassie blue to verify loading of proteins, separation, and sample integrity. Proteins were then transferred to polyvinyl difluoride membranes for immunodetection. The membranes were blocked for ≥1 h with 5% powdered milk in 25 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20, then probed with anti-Gα antibodies at dilutions in the same medium. Antibodies used were anti-$G\alpha_z$ [1:200, 4°C overnight; Santa Cruz Biotechnology (Santa Cruz, CA) and Calbiochem (San Diego, CA)] and anti-$G\alpha_s$ (1:5,000, 1 h, 22°C; PerkinElmer Life Sciences, Boston, MA), anti-$G\alpha_{q/11}$ (1:10,000, 1 h, room temperature; Calbiochem), polyclonal anti-$G\alpha_{i3}$ (Calbiochem) and monoclonal anti-$G\alpha_{i2}$ (both at 1:1000, 4°C overnight; LabVision, Fremont, CA). Horseradish peroxidase-conjugated donkey anti-rabbit (Cell Signaling Technology, Beverly, MA) was used (1:1000) for detection. Peroxidase activity was detected with SuperSignal West Pico (Pierce Biotechnology) and visualized using a Kodak Image Station 1000 or with X-ray film (XAR-2; Eastman Kodak, Rochester, NY).

Results

Calcium Current Inhibition by Heterologously-Expressed mGluR2. Neurons isolated from the rat SCG do not functionally express mGluRs (Ikeda et al., 1995). Heterologous expression of distinct subtypes of mGluRs in SCG neurons is therefore a useful model system for studying the properties of individual mGluR subtypes. Cells expressing mGluR2 after intranuclear cDNA injection respond to application of 100 μM L-glutamate with a fast and potent inhibition of the predominantly N-type (Zhu and Ikeda, 1994) whole-cell calcium current (Ikeda et al., 1995; Kammermeier et al., 2000). The time course of this inhibition is illustrated in Fig. 1A (see inset for samples of control and Glu-inhibited current traces). The 'triple-pulse' voltage protocol (Elmslie et al., 1990) was used to illustrate the voltage-dependent nature of the modulation and to measure basal facilitation as an indicator of free G$\beta\gamma$ levels (see below). The average magnitude of mGluR2-mediated calcium current inhibition was 59 ± 2% (n = 31; Fig. 1C). Cells expressing mGluR2 and treated overnight with 500 ng/ml PTX did not exhibit any detectable calcium current inhibition in response to Glu application (Fig. 1B). Calcium current inhibition in PTX-treated cells was 2 ± 0.3% (n = 24; Fig. 1C). This result confirms previous observations that mGluR2 couples to PTX-sensitive, $G_{i/o}$ G proteins (Chavis et al., 1994; Ikeda et al., 1995).

Reconstitution of G Protein Coupling after PTX Treatment. The strategy for examining the specificity of mGluR2 coupling to $G\alpha_{i/o}$ proteins is illustrated in Fig. 2A. First, cells were intranuclearly injected with cDNA for mGluR2 plus $G\beta_1$, $G\gamma_2$ (this G$\beta\gamma$ combination was chosen for its ability to robustly modulate N-type calcium currents when expressed in SCG neurons), and a PTX-insensitive mutant (or naturally PTX-insensitive wild-type) Gα. Next, cells were treated overnight with PTX to inactivate endogenous $G\alpha_{i/o}$ proteins. Finally, calcium current facilitation was examined to determine the Gα/G$\beta\gamma$ stoichiometry.

The calcium current modulatory pathway used by mGluR2 here is G$\beta\gamma$-mediated and voltage-dependent (Herlitze et al., 1996; Ikeda, 1996). This is evident from the slowed activation kinetics of the inhibited currents and from the 'facilitation' observed after a strong depolarizing prepulse (Bean, 1989; Elmslie et al., 1990) (Fig. 1A, inset). These features are hallmarks of the G$\beta\gamma$-mediated calcium current inhibitory pathway. Commonly, facilitation is defined as the current in the postpulse divided by the current at the same time in the prepulse (the first test pulse to +10 mV). Thus, facilitation can be used as a quantitative measure of relative free G$\beta\gamma$ levels in the cell. Overexpression of G$\beta\gamma$ alone mimics this modulation and produces basal currents with slow activation and strong basal facilitation (≫ 1) (Ikeda, 1996; Garcia et al.,

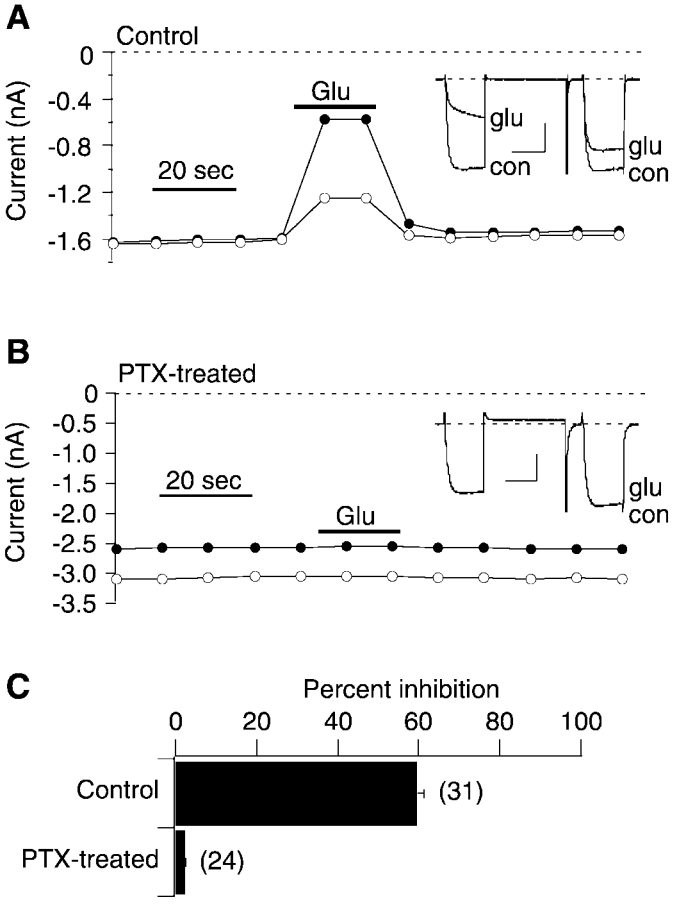


Fig. 1. Calcium current modulation mediated by mGluR2 activation in SCG neurons. A, time course and sample currents (inset) of glutamate-mediated calcium current inhibition in an mGluR2-expressing SCG neuron. Glu indicates application of 100 μM L-glutamate. Scale bars in inset denote 0.5 nA and 20 ms. B, same as A, but for a PTX-treated cell. Scale bars in inset denote 1 nA and 20 ms. C, bar graph illustrating average (+S.E.M.) inhibition in control (mGluR2-expressing) and PTX-treated cells. Number of cells is indicated in parentheses.

1998; Ruiz-Velasco and Ikeda, 2000). Overexpression of Gα subunits alone produces basal currents with facilitation <1, because of strong buffering of endogenously expressed Gβγ. Under these conditions, agonist-induced Gβγ-mediated calcium current modulation is occluded.

As illustrated in Fig. 2B, heterologous expression of Gα and Gβγ resulted in cells with currents that were placed into three functional categories. In the first category were placed all cells that exhibited strong basal facilitation (> 1.3, chosen arbitrarily because basal facilitation this high was rare in control cells in this study: 1 of 55 cells). This level of facilitation was an indication of excess free Gβγ. The second category included those cells with basal facilitation < 1, indicating excess Gα, resulting from Gβγ buffering by expressed Gα. The third category included cells with basal facilitation between 1 and 1.3, indicating a good functional stoichiometric balance of Gα and Gβγ. Therefore, Gα/Gβγ-expressing cells with basal facilitation in this range were chosen for analysis (Jeong and Ikeda, 2000). In addition to determining stoichiometric balance, these criteria provide a control for expression levels of different Gα subunits, assuming that levels of $G\beta_1\gamma_2$ remain relatively constant.

mGluR2 Coupling to $G_{i/o}$ G Proteins. After treatment with PTX, cells expressing mGluR2 exhibited no detectable calcium current inhibition in response to 100 μM L-glutamate (Glu; as described in Fig. 1B). Over this background, PTX-insensitive $G_{i/o}$ proteins [with a Cys-to-Gly mutation in the extreme C terminus, denoted GαC351G (or GαC352G)] were expressed with Gβγ to reconstitute coupling and examine the specificity of mGluR2/G protein interactions. Calcium current inhibition in PTX-treated cells expressing $G\alpha_{ob}$C351G (and $G\beta_1\gamma_2$) was strong (Fig. 3A, a), indicating that GluR2 couples efficiently to $G\alpha_{ob}$. The magnitude of calcium current inhibition in these cells was indistinguishable from paired control cells (recorded the same days; Fig. 3B, ▨). Calcium currents in

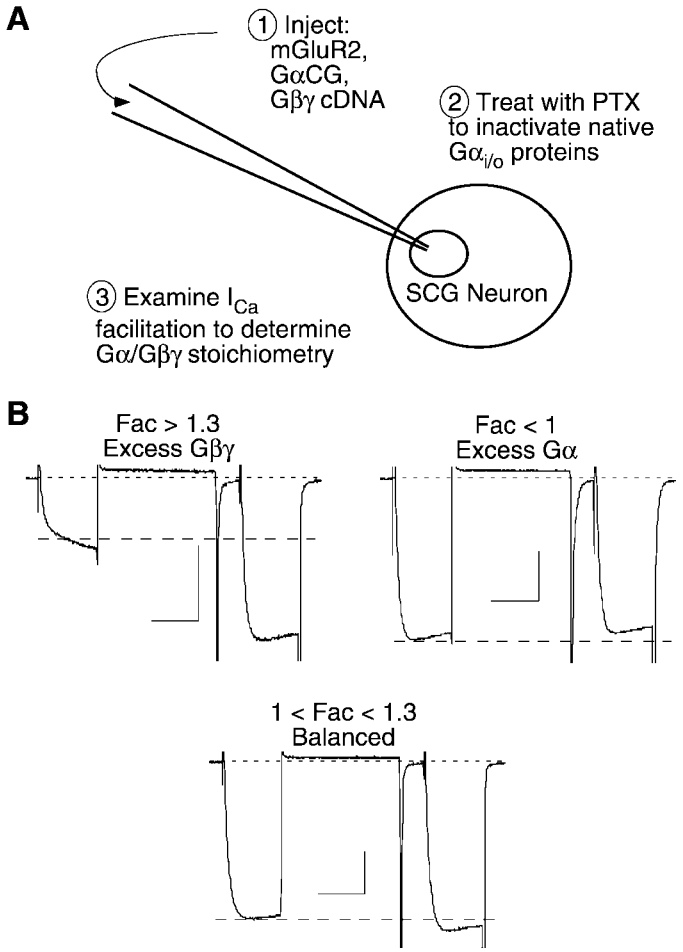


Fig. 2. Strategy for PTX-insensitive Gα reconstitution experiments. A, graphic depiction of the reconstitution procedure. First, cells were injected intranuclearly with cDNA for mGluR2, a PTX-insensitive mutant Gα (or a naturally PTX-insensitive Gα), and $G\beta_1\gamma_2$. Second, cells were treated overnight with PTX to inactivate endogenous Gα proteins that normally couple to mGluR2. Third, the Gα/Gβγ stoichiometry was examined by measuring basal facilitation (postpulse current/prepulse current). B, illustration and implication of currents from cells in each of the three categories: 1 (upper left), high basal facilitation indicated excess Gβγ. 2 (upper right), basal facilitation less than 1 indicated excess Gα (because of buffering of Gβγ). 3 (lower), basal facilitation between 1 and 1.3 indicated good stoichiometric balance of Gα and Gβγ.

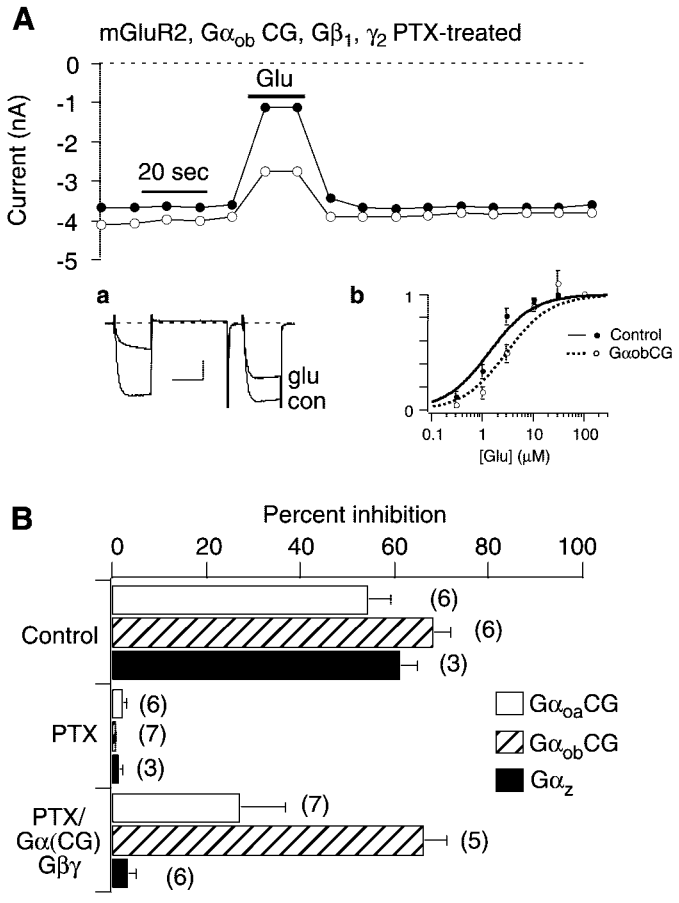


Fig. 3. Reconstitution of mGluR2 coupling with $G\alpha_{oa}$C351G, $G\alpha_{ob}$C351G, and $G\alpha_z$. A, time course and sample currents (a) of calcium current inhibition in a PTX-treated SCG neuron expressing mGluR2, $G\alpha_{ob}$CG, $G\beta_1$, and $G\gamma_2$. Scale bars in a denote 1 nA and 20 ms. b, Dose dependence of the calcium current inhibitory response for glutamate of wild-type mGluR2-expressing cells (●) and cells expressing mGluR2 reconstituted with PTX-insensitive $G\alpha_{ob}$C351G (○). Each data set was fit to the equation B_{max}[Glu]/(EC_{50} + [Glu]), with B_{max} fixed at 1. Data were normalized to inhibition at 1 μM within each cell. Fits revealed EC_{50} values for the control and reconstituted conditions of 1.3 and 3.8 μM, respectively. B, bar graph illustrating average (+S.E.M.) calcium current inhibition in PTX-untreated controls (Control), PTX-treated controls (PTX; both mGluR2-expressing), and Gα reconstituted (PTX/Gα(CG) Gβγ; PTX-treated, expressing mGluR2, GαCG - or wild type $G\alpha_z$, $G\beta_1$ and $G\gamma_2$) for G_{oa} (□), G_{ob} (▨), and G_z (■). Number of cells is indicated in parentheses.

PTX-treated cells reconstituted with $G\alpha_{ob}$C351G were inhibited 66 ± 5% (n = 5) upon Glu application. Calcium currents in paired control cells (PTX-untreated) were inhibited 68 ± 4% (n = 6), whereas currents in paired PTX-treated control cells were inhibited 0.5 ± 0.4% (n = 6). Dose-response curves for calcium current inhibition in control and $G\alpha_{ob}$C351G-reconstituted cells are illustrated in Fig. 3A, b. EC_{50} values for the two groups, determined by fitting to a single-site binding isotherm equation (see Fig. 3 legend), were comparable. Control cells had an EC_{50} of 1.3 μM, compared with 3.8 μM for $G\alpha_{ob}$C351G-reconstituted cells. These data provide evidence that the CG mutation in the C terminus of the $G\alpha$ subunit did not detectably alter the receptor-G protein interaction.

Reconstitution with $G\alpha_{oa}$C351G was less efficient, exhibiting calcium current inhibition of only 27 ± 10% (n = 7) compared with 54 ± 5% (n = 6) in control cells from the same experimental days (Fig. 3B). PTX-treated control cells from the same preparations were inhibited 2 ± 1% (n = 6). These data indicate that mGluR2 couples more efficiently to $G\alpha_{ob}$C351G than to $G\alpha_{oa}$C351G, a surprising result because the extreme C terminus, a region demonstrated to be critical in receptor/$G\alpha$ interaction (Hamm et al., 1988; Conklin et al., 1993), is nearly identical in these two splice variants. Poor expression of the $G\alpha_{oa}$C351G construct cannot sufficiently explain these results because $G\alpha$ expression levels were balanced with $G\beta\gamma$ expression. Finally, mGluR2 seemed to be unable to couple to $G\alpha_z$. Calcium current inhibition in PTX-treated cells reconstituted with $G\alpha_z$ (a naturally PTX-insensitive member of the $G_{i/o}$ family) was virtually undetectable at only 3 ± 2% (n = 6), compared with 61 ± 4% (n = 3) in PTX-untreated cells, and 1 ± 0.6% (n = 3) in PTX-treated control cells (Fig. 3B).

As negative controls, similar reconstitution experiments were performed using wild-type $G\alpha_q$ or wild-type $G\alpha_{ob}$ (Fig. 4). As expected, no detectable calcium current inhibition was evident in PTX-treated cells expressing either $G\alpha_q$, which is not coupled to mGluR2, or $G\alpha_{ob}$, the PTX-sensitive wild-type $G\alpha$. Stoichiometrically balanced PTX-treated cells coexpressing mGluR2, $G\beta_1\gamma_2$, and $G\alpha_q$ were not inhibited by Glu (0 ± 0.3%, n = 5), compared with inhibitions of 61 ± 4% (n = 3) in PTX-untreated paired control cells and 1 ± 0.6% (n = 3) in PTX-treated paired control cells. Similarly treated cells coexpressing mGluR2, $G\beta_1\gamma_2$, and wild-type $G\alpha_{ob}$ were inhibited −1 ± 2% (n = 2) by Glu, compared with 47 ± 12% (n = 3) and 3 ± 0.2% (n = 3) in PTX-untreated and -treated control cells, respectively.

As a positive control for expression, $G\alpha_z$ was coexpressed with $G\beta_1\gamma_2$ to reconstitute coupling to the natively expressed α_2 adrenergic receptor in PTX-treated cells. Coupling of the α_2 adrenergic receptor to $G\alpha_z$ in SCG neurons has been demonstrated previously (Jeong and Ikeda, 1998). In PTX-treated cells coexpressing $G\beta\gamma$ and $G\alpha_z$ (in functional stoichiometric balance), 10 μM norepinephrine (NE) inhibited calcium currents 69 ± 3% (n = 3). Paired PTX-untreated and -treated cells were inhibited 74 ± 1% (n = 3) and 13 ± 5% (n = 3), respectively. These data demonstrate that $G\alpha_z$ is expressed and is capable of coupling a G protein-coupled receptor to calcium channels in SCG neurons.

Finally, the remaining members of the $G_{i/o}$ family were examined. Figure 5A, inset, illustrates the time course and sample currents from a PTX-treated, mGluR2-expressing cell reconstituted with $G\beta\gamma$ and $G\alpha_{i2}$C352G. Glu-mediated calcium current inhibition in this cell was potent, indicating that mGluR2 couples efficiently to $G\alpha_{i2}$ in this system. On average, PTX-treated cells whose mGluR2 coupling was reconstituted with $G\alpha_{i2}$C352G were inhibited 48 ± 10% (n = 8)

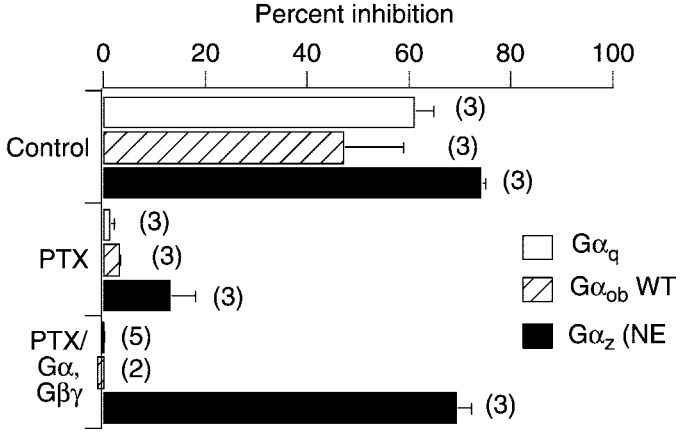


Fig. 4. Negative control for mGluR2 reconstitution and $G\alpha_Z$ reconstitution with NE. Bar graph illustrating average (+S.E.M.) calcium current inhibition in PTX-untreated controls (Control), PTX-treated controls (PTX; both mGluR2-expressing), and $G\alpha$ reconstituted (PTX/$G\alpha$(CG) $G\beta\gamma$; PTX-treated, expressing mGluR2, $G\alpha$, $G\beta_1$, and $G\gamma_2$) for G_q (□), wild-type G_{ob} (▨) and G_z reconstitution with endogenous α2 adrenergic receptor (■). Number of cells is indicated in parentheses.

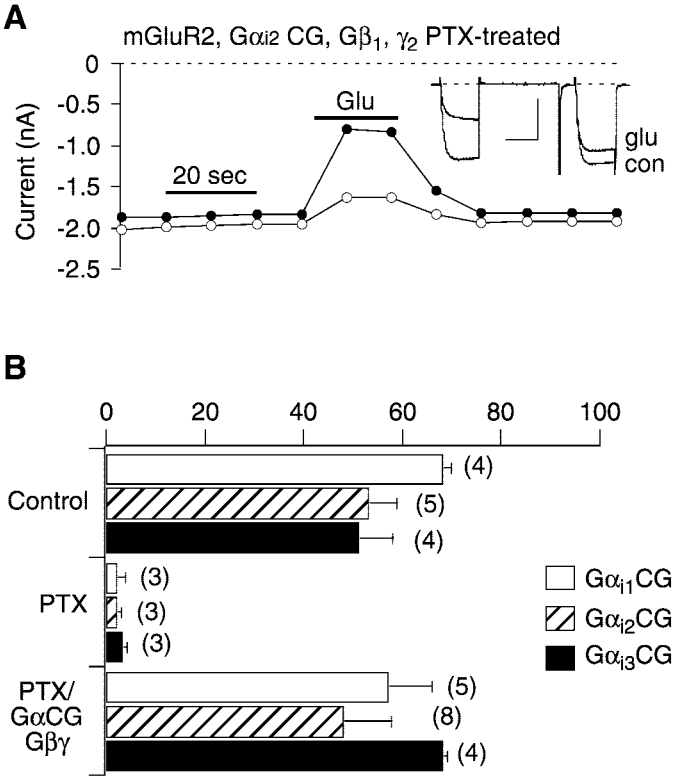


Fig. 5. Reconstitution of mGluR2 coupling with $G\alpha_{i1}$C352G, $G\alpha_{i2}$C352G, and $G\alpha_{i3}$C352G. A, time course and sample currents (inset) of calcium current inhibition in a PTX-treated SCG neuron expressing mGluR2, $G\alpha_{i2}$CG, $G\beta_1$, and $G\gamma_2$. Scale bars in inset denote 1 nA and 20 ms. B, bar graph illustrating average (+S.E.M.) calcium current inhibition in PTX-untreated controls (Control), PTX-treated controls (PTX; both mGluR2-expressing), and $G\alpha$ reconstituted (PTX/$G\alpha$(CG) $G\beta\gamma$; PTX-treated, expressing mGluR2, $G\alpha$CG, $G\beta_1$ and $G\gamma_2$) for G_{i1} (□), G_{i2} (▨) and G_{i3} (■). Number of cells is indicated in parentheses.

by Glu, compared with 53 ± 6% ($n = 5$) and 2 ± 1% ($n = 3$) in paired PTX-untreated and -treated control cells, respectively (Fig. 5B). In addition, mGluR2 seemed to be similarly capable of coupling to $G\alpha_{i1}$ and $G\alpha_{i3}$ (Fig. 5B). Reconstitution using the CG mutants of these Gα proteins also exhibited efficient coupling to calcium currents. PTX-treated cells coexpressing mGluR2, $G\alpha_{i1}C352G$, and $G\beta_1\gamma_2$ were inhibited 57 ± 9% ($n = 5$) by Glu. Inhibition in PTX-untreated, paired control cells was 68 ± 2% ($n = 4$) and in PTX-treated, paired control cells inhibition was 2 ± 2% ($n = 3$). PTX-treated cells coexpressing mGluR2, $G\alpha_{i3}C352G$, and $G\beta_1\gamma_2$ were inhibited 68 ± 1% ($n = 4$) by Glu. Inhibition in PTX-untreated, paired control cells was 51 ± 7% ($n = 4$), and in PTX-treated, paired control cells, inhibition was 3 ± 1% ($n = 3$).

Endogenous Expression of Gα Proteins in SCG Neurons. To determine which Gα proteins may be expressed natively in SCG neurons, and to shed some light on possible receptor/G protein interactions of natively-expressed receptor/G protein pairs (or in the case of mGluR2, heterologously expressed receptor/native G protein pairs), RT-PCR was used to detect mRNA for several Gα proteins. Unique primer sets were designed for several Gα proteins, including o, i1–3, z, q, 11, s, and 15. In addition, primers for the housekeeping gene GAPDH were used as a positive control. Table 1 lists the primer sequences and predicted product size for each Gα primer set. Figure 6A shows the results of RT-PCR reactions targeting each of the $G_{i/o}$ Gα proteins. In each case, a clear band at the predicted product size was detected. In addition, the GAPDH positive and negative (no RNA) controls are shown. These data suggest that SCG neurons may potentially express each member of the $G_{i/o}$ Gα protein family.

In addition to the $G_{i/o}$ Gα proteins, the presence of message for several other Gα proteins was tested using RT-PCR. As Fig. 6B indicates, bands were detected at the predicted product sizes for $G\alpha_q$, $G\alpha_{11}$, and $G\alpha_s$. However, the $G\alpha_q$ band seemed much weaker than that of the other Gα proteins. This suggests that the $G\alpha_q$ mRNA is unstable or perhaps present at lower levels than the other Gα proteins tested, but poor hybridization by the selected primers is the more likely cause of the weak signal. Therefore, one can only infer that $G\alpha_q$ message is present in rat SCGs. In addition, previous studies have confirmed the presence of $G\alpha_q$ in SCG neurons, as well as $G\alpha_o$ and $G\alpha_{11}$ (Haley et al., 1998). Finally, $G\alpha_{15}$ message was undetectable in RNA from SCG neurons. This result was expected because $G\alpha_{15}$ expression is confined to hematopoietic cells (Wilkie et al., 1991).

Western blotting was used to confirm the results of RT-PCR experiments where specific antibodies were available, as judged by detection of recombinant proteins (Fig. 6C). Of the antibodies tested, only three (anti- $G\alpha_{i2}$, anti-$G\alpha_{q/11}$, and anti-$G\alpha_s$) seemed specific as judged by detection of recombinant proteins (see Fig. 6C). Others, namely $G\alpha_{i1}$, $G\alpha_{i3}$, and $G\alpha_o$, detected protein from SCG near the appropriate molecular weight, but also detected at least one inappropriate recombinant control (data not shown), so the presence of specific proteins could not be determined with confidence. The positive result for $G\alpha_z$ RNA was surprising. Previously, $G\alpha_z$ protein has been shown to be present in brain and few other tissues at low levels (Fong et al., 1988; Matsuoka et al., 1988; Casey et al., 1990) and, to our knowledge, has not been demonstrated in sympathetic neurons. However, when Western blots were performed, $G\alpha_z$ could not be detected with protein from either SCG or hippocampus using any of three commercially available antibodies (see *Materials and Methods*). Thus, the RT-PCR experiment suggesting the presence of $G\alpha_z$ in SCG neurons could be neither confirmed nor refuted with Western blotting experiments.

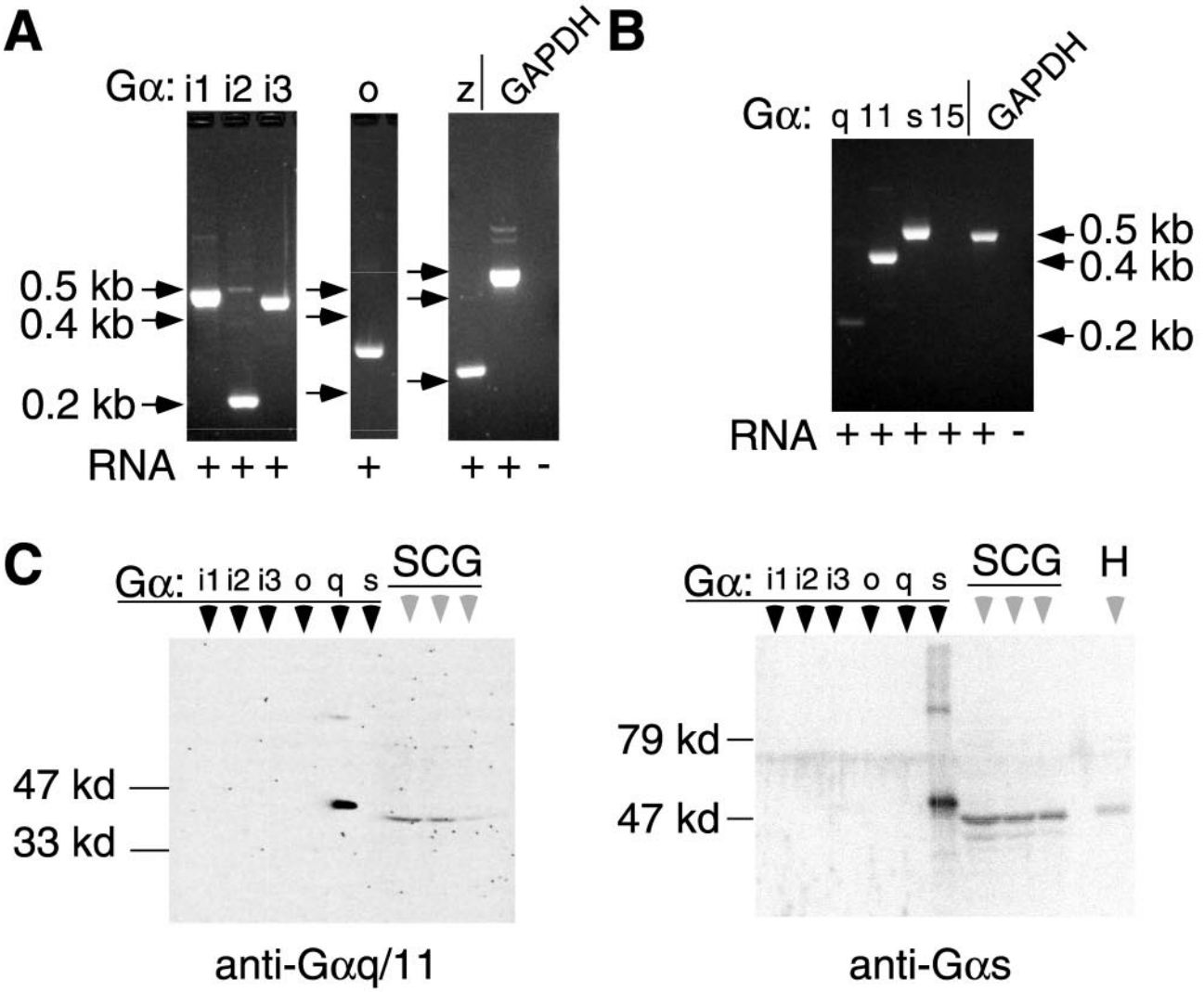
Discussion

The aim of this study was to characterize the G protein coupling profile of mGluR2, a $G_{i/o}$-coupled receptor. This was achieved by treating mGluR2-expressing cells with PTX to inactivate endogenous $G_{i/o}$ proteins and coexpressing various PTX-insensitive mutants of Gα proteins with Gβγ to reconstitute coupling, measured as the degree of calcium current modulation upon Glu application. In addition, RT-PCR was used to detect messenger RNA coding for various Gα proteins endogenously expressed in SCG neurons. The RT-PCR results were confirmed with Western blots where possible.

Although mGluR2 is known to couple exclusively to the $G_{i/o}$ family of G proteins, a comprehensive characterization of coupling with members within this family has not been reported. Such data may shed light on a potential source of specificity, particularly in systems in which several G protein-coupled receptors are known to coexist. For example, the existence of a subset of Gα proteins in a nerve terminal coupled with the knowledge of G protein coupling capabilities of the expressed receptors could lead to a more complete understanding of the specific roles of individual receptors. To date, tools are unavailable to distinguish between the individual members of every G protein family, but there is some evidence that Gα proteins may be selectively localized in nerve terminals. In the large calyx preparation of the chick ciliary ganglion, several Gα proteins have been shown to express in the synaptic terminals and associate with the active site, whereas other Gα proteins (namely $G\alpha_s$ and $G\alpha_z$), known to express elsewhere in the cells, are excluded from close association with the membrane at release sites (Mirotznik et al., 2000). If similar selective localization of Gα proteins within a family also occurs, then this in combination with unique receptor/G protein coupling profiles may underlie the specific physiological roles of the receptors.

The data presented here demonstrate that mGluR2 can couple efficiently to $G\alpha_{ob}$, $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ and less efficiently to $G\alpha_{oa}$. Additionally, mGluR2 does not seem to couple to the more divergent member of the $G_{i/o}$ family, $G\alpha_z$ (Fong et al., 1988; Matsuoka et al., 1988). Particularly intriguing is the finding of selectivity between $G\alpha_{ob}$ and $G\alpha_{oa}$. This is surprising because there are few amino acid changes between these two splice variants and only one in the extreme C terminus (N/K at −10 from the C-terminal end of the

Fig. 6. Detection of Gα mRNA with RT-PCR. A, products of RT-PCR reactions for the indicated members of the $G_{i/o}$ families using 40 ng of isolated SCG total RNA. Also shown are positive (GAPDH primers, +RNA) and negative (GAPDH primers, −RNA) controls. B, products of RT-PCR reactions for other (indicated) Gα proteins using 40 ng of isolated SCG total RNA. Also shown are positive (GAPDH primers, +RNA) and negative (GAPDH primers, −RNA) controls. Compare products with predicted size in Table 1. C, Western blot experiments illustrating results with anti-$G\alpha_{q/11}$ and anti-$G\alpha_s$. The first six lanes in each blot show 20 ng of recombinant, His-tagged Gα i1, i2, i3, o, q, and s, respectively, as indicated. The next three lanes (SCG) show 10, 5 and 2.5 μg of SCG protein. H indicates 5 ng of protein from rat hippocampus as a positive control. D, aligned amino acid sequences of $G\alpha_{oa}$ and $G\alpha_{ob}$. The N-terminal portion of the sequences that are not illustrated here are identical.


A
Gα: i1 i2 i3 o z GAPDH
0.5 kb
0.4 kb
0.2 kb
RNA + + + + + + -
B
Gα: q 11 s15 GAPDH
0.5 kb
0.4 kb
0.2 kb
RNA + + + + + -
C
Gα: i1 i2 i3 o q s SCG
47 kd
33 kd
anti-Gαq/11
Gα: i1 i2 i3 o q s SCG H
79 kd
47 kd
anti-Gαs
D
250 260 270
. . . E S L M L F D S I C N N K F F I D T S I I L F L N
. . . E S L K L F D S I C N N K W F T D T S I I L F L N
E S L L F D S I C N N K F D T S I I L F L N
280 290 300
K K D L F G E K I K K S P L T I C F P E Y P G S N T Y E D A
K K D I F E E K I K K S P L T I C F P E Y T G P S A F T E A
K K D . F E K I K K S P L T I C F P E Y G . . A
310 320 330
A A Y I Q T Q F E S K N R S P N K E I Y C H M T C A T D T N
V A H I Q G Q Y E S K N K S A H K E V Y S H V T C A T D T N
A I Q Q E S K N . S K E . Y . H . T C A T D T N
340 350
N I Q V V F D A V T D I I I A N N L R G C G L Y N
N I Q F V F D A V T D V I I A K N L R G C G L Y N
N I Q . V F D A V T D . I I A N L R G C G L Y N

mouse sequence used for expression in this study; Fig. 6C). The extreme C terminus is believed to play an important role in receptor/G protein interaction and is generally thought to be critical for selectivity in receptor interactions (Hamm et al., 1988; Conklin et al., 1993). In fact, several studies have shown that receptor selectivity can be conveyed to Gα chimeras by swapping only the most distal five amino acids (Conklin et al., 1993; Gomeza et al., 1996; Blahos et al., 1998). Therefore, the finding that mGluR2 coupling is selective for Gα_{ob} over Gα_{oa} suggests that other regions of Gα may also be important in coupling to receptors, at least to class 3 G protein-coupled receptors such as mGluRs. The small number of amino acid changes across these variants (of which just 15 are nonconservative changes) could provide a useful starting point for investigation into the molecular basis for mGluR/G protein interaction.

Although the measured signal (calcium current modulation) is G$\beta\gamma$-mediated, the identity of $\beta\gamma$ released from the various Gα subunits can be ruled out as the source of observed differences in signal strength because the same G$\beta\gamma$ subunits (Gβ_1,γ_2) were used in each experiment in this study. Although these subunits have been shown to produce robust voltage dependent calcium current modulation, specificity of signaling does not seem to come from specific G$\beta\gamma$ subunit combinations (Ruiz-Velasco and Ikeda, 2000). In addition, the two Gα subunits that displayed inefficient coupling with mGluR2 in this study (Gα_z and Gα_{oa}) have been demonstrated to couple strongly to endogenous receptors in this system by a previous study from this laboratory (Jeong and Ikeda, 2000), using the same Gα_{oa}C351G and Gα_z constructs that were used in the present study. Finally, the differences in coupling specificity between the heterologously expressed mGluR2 in this study and the endogenous α2 adrenergic receptor may lead to speculation that differences in coupling result from differential access to molecular scaffolds. However, because distinct coupling profiles have been reported for various endogenously expressed receptors (Jeong and Ikeda, 2000), this explanation is unlikely to account for all of the observed differences in G protein coupling across receptor types. Changes in mGluR2 expression levels might have also influenced coupling. However, this did not seem to be the case here. Results from each group were consistent despite the variability in expression levels that normally results from cDNA injection as judged by GFP expression.

A tacit assumption of these studies is that the C-to-G mutation in Gα does not greatly influence receptor-G protein coupling fidelity. However, the mutated residue lies within a region of Gα identified as a critical determinant of receptor/G protein coupling (Hamm et al., 1988; Conklin et al., 1993). Thus, the mutation may influence G protein coupling to mGluRs. Although we cannot completely rule out this possibility, studies of PTX-resistant Gα subunits indicate that although the efficacy of partial agonists is altered, general characteristics of coupling are maintained (Bahia et al., 1998). Moreover, in the current study, neither the EC_{50} nor the maximal effect of reconstituted Gα_{ob} was significantly altered from control (Fig. 3). Thus, the strategy is clearly useful for determining G protein coupling profiles within the context of the required mutation. However, extrapolation of these data to native proteins requires some caution and alternative approaches will be required to definitively establish a coupling profile.

Results from this study confirm the conclusions from some recent studies. Gomeza et al. (1996) and Blahos et al. (1998) demonstrated that Gα chimeras containing the N terminus of G_q and the extreme C terminus of either G_o or G_i could couple to phospholipase C via mGluR2, but similar G_q/G_z chimeras could not. These data indicate that mGluR2 is capable of coupling to variants of G_o and G_i, but not to G_z, as was demonstrated here. It should be noted, however, that the experiments in the Gomeza et al. (1996) and Blahos et al. (1998) papers were unable to distinguish coupling to G_{oa} from that of G_{ob}, or among G_{i1}, G_{i2}, and G_{i3}. Also, the assay for coupling in those studies was dependent on PLC activation by chimeric receptors. Therefore, only differences in Gα coupling resulting from sequence variations in the C-terminal 5 amino acids could be detected. It should also be noted that under the conditions described here, mGluR2 seemed unable to couple to similar G_q/G_o chimeras as described in the above studies (not shown).

One recent study examined coupling of several endogenously expressed receptors in cultured hippocampal neurons using a strategy similar to that described here (Straiker et al., 2002). Although mGluR2 was not examined, a natively expressed group III mGluR was tested and seemed unable to couple to the PTX-insensitive Gα proteins tested (G_{oa}, G_{i1-3}). However, the authors note that the initial signal (synaptic inhibition by a group III mGluR agonist) was small, which may have contributed to a difficulty in reconstitution.

The RT-PCR results described above demonstrate the presence of mRNA from rat SCG for Gα_o, Gα_{i1}, Gα_{i2}, Gα_{i3}, and Gα_z. In addition, mRNA coding for Gα_q, Gα_{11}, and Gα_s was detected, although the Gα_q signal seemed weaker than the other Gα subunits tested. These data are interesting considering the findings regarding G protein coupling specificity of heterologously expressed mGluR2. For example, although Gα_o seems to be expressed strongly in SCG neurons, it is likely that any coupling between mGluR2 and Gα_o is primarily via Gα_{ob}, because mGluR2/Gα_{oa} coupling seems inefficient. In addition, although Gα_z may be present in SCG neurons, it does not seem to contribute to calcium current modulation via heterologously expressed mGluR2 in this system. Regarding the natively expressed α_2 adrenergic receptor, previous work has demonstrated strong coupling to Gα_{oa}, Gα_{ob}, Gα_{i2}, and Gα_z, but weak coupling to Gα_{i1} and Gα_{i3} (Jeong and Ikeda, 2000). This is particularly interesting in light of the finding that all members of the $G_{i/o}$ family seem to be expressed in SCGs. The implication of these findings is that in native neuronal systems, signal specificity may arise, at least in part, from selective coupling to individual members within G protein families. It should be noted, however that although care was taken to minimize the number of glial cells in the SCG preparation from which the RNA was isolated, it is likely that some were present and may have contributed to results. Therefore, future studies should be performed using RNA isolated from single SCG neurons to confirm the results presented here.

The presence of Gα_z mRNA from SCG neurons was unexpected. Previously, Gα_z protein has been shown to be present in brain and some other tissues at low levels (Fong et al., 1988; Matsuoka et al., 1988; Casey et al., 1990), but not in sympathetic neurons. Here, we show that Gα_z message is present in sympathetic neurons from the rat SCG, but we were unable to confirm (or refute) this result by demonstrat-

ing the presence of the $G\alpha_z$ protein with Western blotting. Western blots were also performed to confirm the presence of other $G\alpha$ proteins. However, because of the lack of specificity of most anti-$G\alpha$ antibodies tested (as judged by recognition of various recombinant $G\alpha$ proteins), many of these experiments produced less than meaningful results. Exceptions were $G\alpha_{i2}$ (which was detected in SCG and did not recognize even the closely related $G\alpha_{i1}$ or $G\alpha_{i3}$) $G\alpha_q$ (although this antibody did not distinguish recombinant $G\alpha_{11}$), and $G\alpha_s$ (Fig. 6).

In summary, the G protein-coupling profile of mGluR2 was characterized using heterologous expression in SCG neurons treated with PTX and reconstituting coupling to calcium currents by coexpressing PTX-insensitive $G\alpha_{i/o}$ proteins with $G\beta\gamma$. mGluR2 was found to couple strongly to $G\alpha_{ob}$, $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$, and less strongly to $G\alpha_{oa}$. No coupling with $G\alpha_z$ was observed. Finally, several $G\alpha$ mRNAs were detected in rat SCG with RT-PCR, including $G\alpha_o$, $G\alpha_{i1-3}$, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_s$. Finally, message for $G\alpha_{15}$ was absent, as expected, because of its unique expression in hematopoietic tissue (Wilkie et al., 1991).

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References

Abe T, Sugihara H, Nawa H, Shigemoto R, Mizuno N, and Nakanishi S (1992) Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca^{2+} signal transduction. *J Biol Chem* **267:**13361–13368.

Aramori I and Nakanishi S (1992) Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. *Neuron* **8:**757–765.

Bahia DS, Wise A, Fanelli F, Lee M, Rees S, and Milligan G (1998) Hydrophobicity of residue351 of the G protein Gi1 alpha determines the extent of activation by the alpha 2A-adrenoceptor. *Biochemistry* **37:**11555–11562.

Bean BP (1989) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature (Lond)* **340:**153–156.

Blahos J, 2nd, S Mary, J Perroy, C de Colle, I Brabet, J Bockaert and JP Pin (1998) Extreme C terminus of G protein alpha-subunits contains a site that discriminates between Gi-coupled metabotropic glutamate receptors. *J Biol Chem* **273:**25765–25769.

Bortolotto ZA, Bashir ZI, Davies CH, and Collingridge GL (1994) A molecular switch activated by metabotropic glutamate receptors regulates induction of long-term potentiation. *Nature (Lond)* **368:**740–743.

Casey PJ, Fong HK, Simon MI, and Gilman AG (1990) Gz, a guanine nucleotide-binding protein with unique biochemical properties. *J Biol Chem* **265:**2383–2390.

Chavis P, Shinozaki H, Bockaert J, and Fagni L (1994) The metabotropic glutamate receptor types 2/3 inhibit L-type calcium channels via a pertussis toxin-sensitive G-protein in cultured cerebellar granule cells. *J Neurosci* **14:**7067–7076.

Conklin BR, Farfel Z, Lustig KD, Julius D, and Bourne HR (1993) Substitution of three amino acids switches receptor specificity of Gq alpha to that of Gi alpha. *Nature (Lond)* **363:**274–276.

Conn PJ and Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* **37:**205–237.

De Blasi A, Conn PJ, Pin JP, and Nicoletti F (2001) Molecular determinants of metabotropic glutamate receptor signaling. *Trends Pharmacol Sci* **22:**114–120.

Elmslie KS, Zhou W, and Jones SW (1990) LHRH and GTP-γ-S modify calcium current activation in bullfrog sympathetic neurons. *Neuron* **5:**75–80.

Fong HK, Yoshimoto KK, Eversole-Cire P, and Simon MI (1988) Identification of a GTP-binding protein alpha subunit that lacks an apparent ADP-ribosylation site for pertussis toxin. *Proc Natl Acad Sci USA* **85:**3066–3070.

Garcia DE, Li B, Garcia-Ferreiro RE, Hernandez-Ochoa EO, Yan K, Gautam N, Catterall WA, Mackie K, and Hille B (1998) G-protein beta-subunit specificity in the fast membrane-delimited inhibition of Ca^{2+} channels. *J Neurosci* **18:**9163–9170.

Gomeza J, Joly C, Kuhn R, Knopfel T, Bockaert J, and Pin JP (1996) The second intracellular loop of metabotropic glutamate receptor 1 cooperates with the other intracellular domains to control coupling to G-proteins. *J Biol Chem* **271:**2199–2205.

Haley JE, Abogadie FC, Delmas P, Dayrell M, Vallis Y, Milligan G, Caulfield MP, Brown DA, and Buckley NJ (1998) The α subunit of G_q contributes to muscarinic inhibition of the M-type potassium current in sympathetic neurons. *J Neurosci* **18:**4521–4531.

Hamm HE, Deretic D, Arendt A, Hargrave PA, Koenig B, and Hofmann KP (1988) Site of G protein binding to rhodopsin mapped with synthetic peptides from the alpha subunit. *Science (Wash DC)* **241:**832–835.

Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, and Catterall W (1996) Modulation of Ca^{2+} channels by G-protein $\beta\gamma$ subunits. *Nature (Lond)* **380:**258–262.

Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by G-protein $\beta\gamma$ subunits. *Nature (Lond)* **380:**255–258.

Ikeda SR (1997) Heterologous expression of receptors and signaling proteins in adult mammalian sympathetic neurons by microinjection, in *Methods in Molecular Biology* (Challis RAJ ed.) pp 191–202, Humana Press, Inc., Totowa, NJ.

Ikeda SR, Lovinger DM, McCool BA, and Lewis DL (1995) Heterologous expression of metabotropic glutamate receptors in adult rat sympathetic neurons: subtype-specific coupling to ion channels. *Neuron* **14:**1029–1038.

Jeong S-W and Ikeda SR (1998) G protein α subunit $G\alpha_z$ couples neurotransmitter receptors to ion channels in sympathetic neurons. *Neuron* **21:**1201–1212.

Jeong SW and Ikeda SR (2000) Effect of G protein heterotrimer composition on coupling of neurotransmitter receptors to N-type Ca^{2+} channel modulation in sympathetic neurons. *Proc Natl Acad Sci USA* **97:**907–912.

Joly C, Gomeza J, Brabet I, Curry K, Bockaert J, and Pin JP (1995) Molecular, functional and pharmacological characterization of the metabotropic glutamate receptor type 5 splice variants: comparison with mGluR1. *J Neurosci* **15:**3970–3981.

Kammermeier PJ, Xiao B, Tu JC, Worley PF, and Ikeda SR (2000) Homer proteins regulate coupling of group I metabotropic glutamate receptors to N-type calcium and M-type potassium channels. *J Neurosci* **20:**7238–7245.

Mary S, Gomeza J, Prezeau L, Bockaert J, and Pin JP (1998) A cluster of basic residues in the carboxyl-terminal tail of the short metabotropic glutamate receptor 1 variants impairs their coupling to phospholipase C. *J Biol Chem* **273:**425–432.

Matsuoka M, Itoh H, Kozasa T, and Kaziro Y (1988) Sequence analysis of cDNA and genomic DNA for a putative pertussis toxin-insensitive guanine nucleotide-binding regulatory protein alpha subunit. *Proc Natl Acad Sci USA* **85:**5384–5388.

Milligan G (1988) Techniques used in the identification and analysis of function of pertussis toxin-sensitive guanine nucleotide binding proteins. *Biochem J* **255:**1–13.

Mirotznik RR, Zheng X, and Stanley EF (2000) G-Protein types involved in calcium channel inhibition at a presynaptic nerve terminal. *J Neurosci* **20:**7614–7621.

Pin JP, Gomeza J, Joly C, and Bockaert J (1995) The metabotropic glutamate receptors: their second intracellular loop plays a critical role in the G-protein coupling specificity. *Biochem Soc Trans* **23:**91–96.

Pin J-P, Waeber C, Prezeau L, Bockaert J, and Heinemann SF (1992) Alternative splicing generates metabotropic glutamate receptors inducing different patterns of calcium release in *Xenopus* oocytes. *Proc Natl Acad Sci USA* **89:**10331–10335.

Ruiz-Velasco V and Ikeda SR (2000) Multiple G-protein betagamma combinations produce voltage-dependent inhibition of N-type calcium channels in rat superior cervical ganglion neurons. *J Neurosci* **20:**2183–2191.

Saugstad JA, Kinzie JM, Mulvihill ER, Segerson TP, and Westbrook GL (1994) Cloning and expression of a new member of the L-2-amino-4- phosphonobutyric acid-sensitive class of metabotropic glutamate receptors. *Mol Pharmacol* **45:**367–372.

Senogles SE (1994) The D2 dopamine receptor isoforms signal through distinct Gi alpha proteins to inhibit adenylyl cyclase. A study with site-directed mutant Gi alpha proteins. *J Biol Chem* **269:**23120–23127.

Straiker AJ, Borden CR, and Sullivan JM (2002) G-protein alpha subunit isoforms couple differentially to receptors that mediate presynaptic inhibition at rat hippocampal synapses. *J Neurosci* **22:**2460–2468.

Tanabe Y, Masu M, Ishii T, Shigemoto R, and Nakanishi S (1992) A family of metabotropic glutamate receptors. *Neuron* **8:**169–179.

Tanabe Y, Nomura A, Masu M, Shigemoto R, Mizuno N, and Nakanishi S (1993) Signal transduction, pharmacological properties and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. *J Neurosci* **13:**1372–1378.

Taussig R, Sanchez S, Rifo M, Gilman AG, and Belardetti F (1992) Inhibition of the omega-conotoxin-sensitive calcium current by distinct G proteins. *Neuron* **8:**799–809.

Wilkie TM, Scherle PA, Strathmann MP, Slepak VZ, and Simon MI (1991) Characterization of G-protein alpha subunits in the Gq class: expression in murine tissues and in stromal and hematopoietic cell lines. *Proc Natl Acad Sci USA* **88:**10049–10053.

Wilsch VW, Behnisch T, Jager T, Reymann KG, and Balschun D (1998) When are class I metabotropic glutamate receptors necessary for long- term potentiation? *J Neurosci* **18:**6071–6080.

Wise A, Watson-Koken MA, Rees S, Lee M, and Milligan G (1997) Interactions of the alpha2A-adrenoceptor with multiple Gi-family G- proteins: studies with pertussis toxin-resistant G-protein mutants. *Biochem J* **321:**721–728.

Zhu Y and Ikeda SR (1994) Modulation of Ca^{2+}-channel currents by protein kinase C in adult rat sympathetic neurons. *J Neurophysiol* **72:**1549–1560.

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