Differential Coupling of G Protein α Subunits to Seven-helix Receptors Expressed in *Xenopus* Oocytes*

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Xenopus oocytes were used to examine the coupling of the serotonin 1c (5HT1c) and thyrotropin-releasing hormone (TRH) receptors to both endogenous and heterologously expressed G protein α subunits. Expression of either G protein-coupled receptor resulted in agonist-induced, Ca^{2+}-activated Cl^- currents that were measured using a two-electrode voltage clamp. 5HT-induced Cl^- currents were reduced 80% by incubating the injected oocytes with pertussis toxin (PTX) and inhibited 50–65% by injection of antisense oligonucleotides to the PTX-sensitive Go α subunit. TRH-induced Cl^- currents were reduced only 20% by PTX treatment but were inhibited 60% by injection of antisense oligonucleotides to the PTX-insensitive Gq α subunit. Injection of antisense oligonucleotides to a novel *Xenopus* phospholipase C-β inhibited the 5HT1c (and Go)-induced Cl^- current with little effect on the TRH (and Gq)-induced current. These results suggest that receptor-activated Go and Gq interact with different effectors, most likely different isoforms of phospholipase C-β. Co-expression of each receptor with seven different mammalian G protein α subunit cRNAs (Goa, Gob, Gq, G11, Gs, Golf, and Gt) was also examined. Co-expression of either receptor with the first four of these Gα subunits resulted in a maximum 4–6-fold increase in Cl^- currents; the increase depended on the amount of Gα subunit cRNA injected. This increase was blocked by PTX for Gαoa and Gαob co-expression but not for Gαq or Gα11 co-expression. Co-expression of either receptor with Gs, Golf, or Gt had no effect on Ca^{2+}-activated Cl^- currents; furthermore, co-expression with Gs or Golf also failed to reveal 5HT- or TRH-induced changes in adenylyl cyclase as assessed by activation of the cystic fibrosis transmembrane conductance regulator Cl^- channel. These results indicate that in oocytes, the 5HT1c and TRH receptors do the following: 1) preferentially couple to PTX-sensitive (Go) and PTX-insensitive (Gq) G proteins and that these G proteins act on different effectors, 2) couple within the same cell type to several different heterologously expressed G protein α subunits to activate the oocyte's endogenous Cl^- current, and 3) fail to couple to G protein α subunits that activate cAMP or phosphodiesterase.

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are responsible for transducing signals between cell surface seven-helix receptors and intracellular second messengers (for reviews, see Ross (1989), Simon *et al.* (1991), and Hille (1992)) or ion channels (Pfaffinger *et al.*, 1985; Logothetis *et al.*, 1987; Yatani and Brown, 1989) (for reviews, see Brown and Birnbaumer (1990), Kurachi *et al.* (1992)). Cloning and characterization of the α subunits of these G proteins resulted in subtype classifications based upon amino acid identity (Simon *et al.*, 1991), second messenger activation, and pertussis toxin (PTX)[1] sensitivity. Gs and Golf constitute a subfamily of G proteins that activate adenylyl cyclase and are not modified by PTX (Graziano *et al.*, 1987; Jones and Reed, 1989). The Gq subfamily of G proteins activate phospholipase C-β (PLCβ) and are also unmodified by PTX (Strathmann and Simon, 1990; Smrcka *et al.*, 1991; Conklin *et al.*, 1992). Another subfamily shares significant amino acid homology, but its members are PTX-sensitive. These include G proteins that inhibit adenylyl cyclase (Gi) (Taussig *et al.*, 1993), activate cGMP phosphodiesterase (Gt, transducin) (Vuong *et al.*, 1984; Chabre *et al.*, 1988), and activate PLC in *Xenopus* oocytes (Go) (Moriarty *et al.*, 1990).

The large number of different seven-helix receptors, G proteins, and effectors that are often present in the same cell suggests that in principle, a large number of distinct signal transduction pathway combinations can be formed. The extent to which pathway specificity is achieved for given receptors, heterotrimeric G proteins, and effectors in different cell types is not known. Recent investigations have focused upon this question using several different approaches. These include the following: 1) examining GTP hydrolysis following reconstitution of purified receptor and G proteins in vesicles (Cerione *et al.*, 1986; Ueda *et al.*, 1989; Senogles *et al.*, 1990), 2) using antibodies raised against the C-terminal receptor-interacting portion of G protein α subunits to inhibit coupling (Cerione *et al.*, 1988; McFadzean *et al.*, 1989; Shenker *et al.*, 1991; Aragay *et al.*, 1992), and 3) using different combinations of PTX treatment, antisense "knock-out," and heterologous expression (Moriarty *et al.*, 1989; Kleuss *et al.*, 1991; Coupry *et al.*, 1992; Duzic *et al.*, 1992; Blitzer *et al.*, 1993) and then examining their actions on downstream effectors.

These approaches have been used to identify which receptors couple to which G proteins, to assess the relative affinities of receptors for various G proteins, and to determine mechanistic details of this interaction. Studies of the actions of the same seven-helix receptor in different endogenous tissues established that coupling to second messenger systems occurs in a tissue-specific manner (Bonner *et al.*, 1988; Cantiello and Lanier, 1989). Cloning and heterologous expression of specific seven-helix receptors in different cell types established that the cell-specific coupling was not necessarily due to different receptor isoforms but could also be due either to cell-specific inter-

[1] The abbreviations used are: PTX, pertussis toxin; CFTR, cystic fibrosis transmembrane regulator; 5HT, serotonin; PLC, phospholipase C; TRH, thyrotropin-releasing hormone.

actions of the same G protein subtype with different second messenger pathways or with different G protein subtypes present in the cells. For example, the cloned neuropeptide Y receptor expressed in human embryonic kidney cells inhibits accumulation of cAMP; in Chinese hamster ovary cells, this receptor acts to elevate intracellular Ca^{2+} levels (Herzog *et al.*, 1992). Moriarty *et al.* (1989) expressed the PLC-activating liver AVP receptor (which couples to a PTX-*insensitive* G protein in its native environment) in *Xenopus* oocytes and found that it activated PLC via a PTX-*sensitive* G protein. This finding suggests that the same receptor can couple to different G proteins in different cell types. However, such a conversion to PTX sensitivity is not a necessary result of expression in oocytes. The α1-adrenergic receptor that in liver is PTX-insensitive (Lynch *et al.*, 1986) retains its PTX insensitivity when expressed in oocytes (Blitzer *et al.*, 1993).

Xenopus oocytes provide a convenient and quantitative system for investigating the coupling of seven-helix receptors and G proteins to pathways involving PLC and adenylyl cyclase. To examine receptor/G protein activation of PLC and its downstream effectors, the oocyte's endogenous Ca^{2+}-activated Cl^- conductance can be measured using the standard two-electrode voltage-clamp configuration. The Cl^- current results from PLC-activated inositol 1,4,5-trisphosphate release of Ca^{2+} from internal stores (McIntosh and Catt, 1987; Gillo *et al.*, 1987; Oron *et al.*, 1988). To examine receptor/G protein activation of adenylyl cyclase and its downstream effectors, the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel can be expressed in oocytes to provide an assay system for adenylyl cyclase that can also be assessed electrophysiologically. This channel is activated by protein kinase A (Welsh *et al.*, 1992) and is sensitive to small changes in cAMP concentration (Uezono *et al.*, 1993). In this article, we have examined the receptor/G protein specificity for the serotonin 1c (5HT1c) and thyrotropin releasing hormone (TRH) receptors, two seven-helix receptors that in their endogenous tissues, couple to G proteins that activate PLC. We find the following: 1) the 5HT1c and TRH receptors preferentially couple to endogenous, PLC-activating G proteins in the oocyte that are PTX-sensitive (Go) and PTX-insensitive (Gq), respectively; 2) these two G proteins act through distinct downstream effectors (most likely different PLC isoforms) that effect Ca^{2+}-activated Cl^- currents; 3) the two receptors can couple to other, heterologously expressed mammalian G protein α subunits to activate inositol 1,4,5-trisphosphate-mediated Cl^- currents; and 4) these two receptors fail to couple to heterologously expressed G protein α subunits that activate cAMP or phosphodiesterase.

EXPERIMENTAL PROCEDURES

Preparation of Sense and Antisense Oligonucleotides—The oligonucleotides were synthesized by the Caltech Oligonucleotide Synthesis facility. The sequences encoding the sense and the corresponding antisense complementary sequence were as follows: *Xenopus* Gαo, nucleotides 891–918 (Olate *et al.*, 1989); *Xenopus* Gαq, nucleotides 901–931; and *Xenopus* PLCβ, nucleotides 928–958 (Ma *et al.*, 1993). The oligonucleotides were phenol-chloroform extracted, recovered by ethanol precipitation, dissolved in H_2O at 25 μM, and aliquoted and stored at −20 °C.

In Vitro Synthesis of RNA—*In vitro* transcription of sense RNA was carried out as described previously (Guastella *et al.*, 1990) with few modifications. Recombinant plasmids containing cDNA inserts were linearized by digestion with appropriate restriction enzymes. The transcription of linearized templates was performed in 7.6 mM Tris-HCl, pH 7.6, 6 mM $MgCl_2$, 0.6 mM NaCl, and 10 mM dithiothreitol containing 0.5 mM each of adenosine triphosphate, cytidine triphosphate, and uridine triphosphate, 0.1 mM of guanosine triphosphate, 0.5 mM of 5′-(7-methyl)-guanosine-guanosinetriphosphate, and 180 units of the respective polymerase as follows: p3Gq (Strathmann and Simon, 1990), T3; pBSG11 (Strathmann and Simon, 1990), T3; pML52Goa (Strathmann *et al.*, 1990; Slepak *et al.*, 1993), pML52Gob (Strathmann *et al.*, 1990; Slepak

et al., 1993), pBSmTRHR (Straub *et al.*, 1990), and pBS5HT1c, T7; in a total volume of 250 μl. The reaction mixture was incubated for 150 min at 37 °C. The DNA template was subsequently removed by treatment with 5 units of RNase-free DNase I (Promega) for 15 min at 37 °C. Free nucleotides were removed using a Sephadex G50 column. The mRNA was phenol-chloroform-extracted and recovered by ethanol precipitation. The RNA was dissolved in RNase-free water, aliquoted, and stored at −70 °C until used.

Oocyte Expression and Electrophysiology—These procedures are described in detail elsewhere (Quick and Lester, 1994). Briefly, oocytes were defolliculated and maintained at 18 °C in incubation medium containing ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES pH 7.4), 1.8 mM $CaCl_2$, 50 μg/ml gentamycin, and 5% horse serum (Quick *et al.*, 1992). Twenty-four hours prior to electrophysiological assay, the oocytes were transferred to incubation medium without horse serum. Unless otherwise noted, measurements were performed 48–72 h post-injection. Whole cell currents were measured at room temperature using a Dagan 8500 amplifier in a standard two-microelectrode voltage-clamp configuration. Current was measured on-line by oscilloscope and chart recorder. Electrodes were filled with 3 M KCl and had a resistance of 1–2 MΩ. During the experiment, the oocytes were clamped at −80 mV and superfused continuously in ND96 medium; all drugs were applied in this solution.

Materials—TRH was from Peninsula Laboratories (Belmont, CA), 5HT was from Research Biochemicals Incorporated (Natick, MA), and PTX was from List Biological Laboratories (Campbell, CA). All other drugs and reagents were from Sigma.

RESULTS

Xenopus oocytes expressing either 5HT1c or TRH receptors voltage clamped at negative holding potentials (−80 mV) produced an inward current when superfused with the appropriate agonist. Examination of the waveforms (Fig. 1*A*) of these responses showed that they are characteristic of the PLC- and inositol 1,4,5-trisphosphate-mediated Ca^{2+}-activated Cl^- conductance (Miledi and Parker, 1984; Dascal *et al.*, 1986; Moriarty *et al.*, 1989), namely, a rapid transient inward current that returns to pre-agonist background levels in the presence of agonist. The waveforms generated by the two receptors did not show any noticeable differences. Application of either 5HT or TRH to uninjected oocytes failed to elicit these currents (data not shown).

Injection of increasing amounts of either receptor resulted in larger agonist-induced peak inward currents (Fig. 1*B*) that otherwise exhibited the same waveform. As little as 5 pg of 5HT1c receptor cRNA and 50 pg of TRH receptor cRNA produced inward currents of several hundred nanoamps (827 ± 128 nA and 273 ± 90 nA, respectively). With cRNA injection amount plotted on a log scale, there was an approximately linear increase in the peak inward currents for both receptors as a function of the log of the amount of injected cRNA. For oocytes injected with 500 pg of 5HT1c receptor cRNA, application of 1 nM 5HT resulted in peak currents of approximately 3 μA (3050 ± 270 nA); for oocytes injected with 1 ng of TRH receptor cRNA, application of 10 nM TRH resulted in comparable peak currents (2260 ± 218 nA). In the experiments described below, oocytes were injected with amounts of receptor cRNA that would produce responses in this log-linear range in order to allow for sensitive detection of changes in the agonist-induced inward currents as a result of various experimental treatments.

The effect of PTX treatment on the agonist-induced inward currents of oocytes injected with both receptors is shown in Fig. 2. PTX treatment resulted in a strong inhibition of the 5HT-induced current (18% of non-PTX treated control oocytes) with little effect on the TRH-induced current (83% of non-PTX treated controls). Agonist applications were performed at 5-min intervals, and the order of agonist presentations was alternated on different oocytes; this was done to eliminate the possibility that the measured effects were due to desensitization of the oocyte as a result of multiple agonist applications. It should also be noted that neither application of 5HT to TRH receptor-
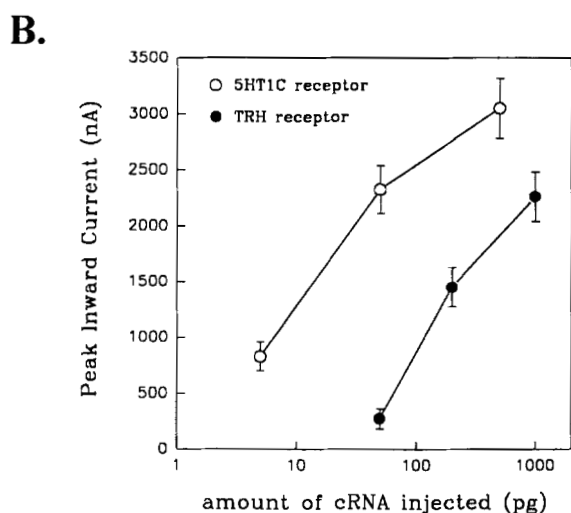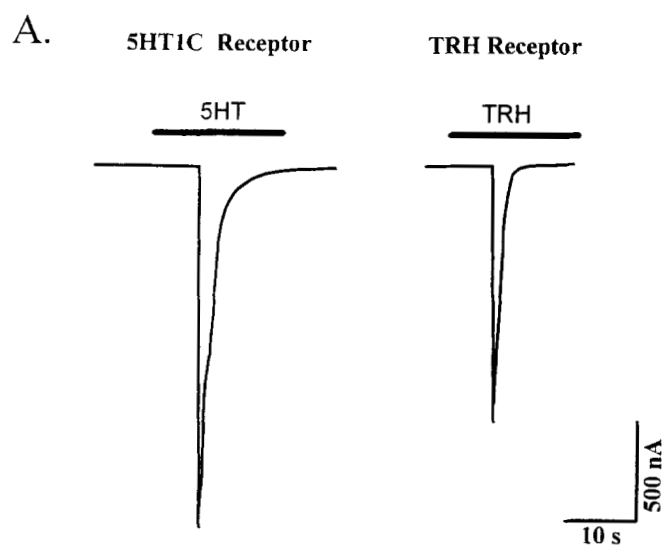
A.

B.

FIG. 1. **Agonist-induced inward currents in oocytes injected with cRNA encoding either the 5HT1c or TRH receptor.** *A,* recordings from individual oocytes injected with either 50 pg of 5HT1c or 200 pg of TRH receptor cRNA. The *solid bars above* each *trace* represent the start of a 20-s application of either 1 nM 5HT or 10 nM TRH. *B,* dose-response relations for the magnitude of inward current elicited for various amounts of injected receptor cRNA. 5HT and TRH concentrations were 1 and 10 nM, respectively. Data are the mean ± S.E. for 7–12 oocytes/data point.

FIG. 2. **The effect of PTX incubation on the inward currents in oocytes injected with 5HT1c and TRH receptor cRNA.** Oocytes were injected with *both* 5 pg of 5HT1c and 50 pg of TRH receptor cRNA. Approximately 1 day after injection, oocytes were placed in medium containing 5 µg/ml PTX for 24 h. Currents induced by 5HT (1 nM) or by TRH (10 nM) were then measured and compared with those for oocytes not incubated in PTX. Multiple agonist applications were made at 5-min intervals. The data are mean ± S.E. for an individual oocyte batch, five to eight oocytes/condition. Similar results were obtained in three other batches.

injected oocytes nor application of TRH to 5HT1c receptor-injected oocytes elicited any currents (data not shown), suggesting that each receptor is specific for its appropriate ligand. The differential reduction due to PTX in the 5HT1c-induced current *versus* the TRH-induced current in oocytes injected with both receptors eliminates systematic errors due to possible nonspecific toxic effects produced by PTX. These findings suggest that in co-injected oocytes, the 5HT1c receptor preferentially couples to an endogenous PTX-*sensitive* G protein that activates PLC, and the TRH receptor preferentially couples to an endogenous PTX-*insensitive* G protein that activates PLC.

Go is a PTX-sensitive G protein subtype that is linked to phosphoinositol turnover in *Xenopus* oocytes (Moriarty *et al.*, 1990) and also activates a novel PLC-β isoform in oocytes (Ma *et al.*, 1993). These findings suggested that the 5HT1c receptor-mediated response might occur via the oocyte's endogenous Go. This hypothesis was tested using antisense oligonucleotides to the Go α subunit, and the results are shown in Fig. 3A. When
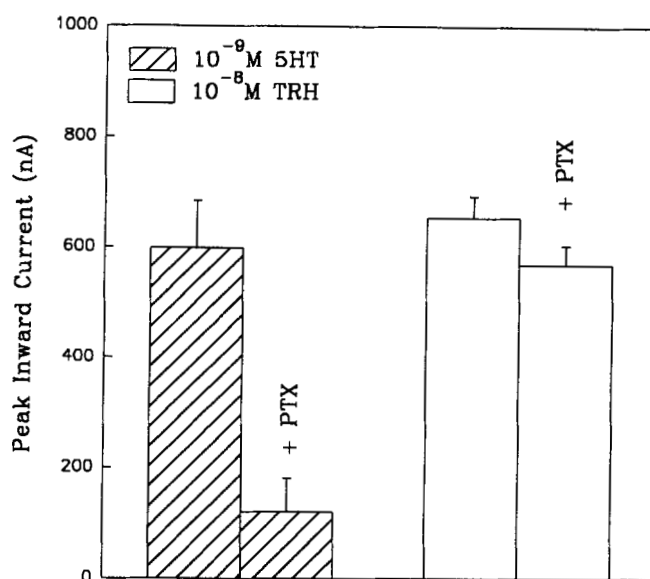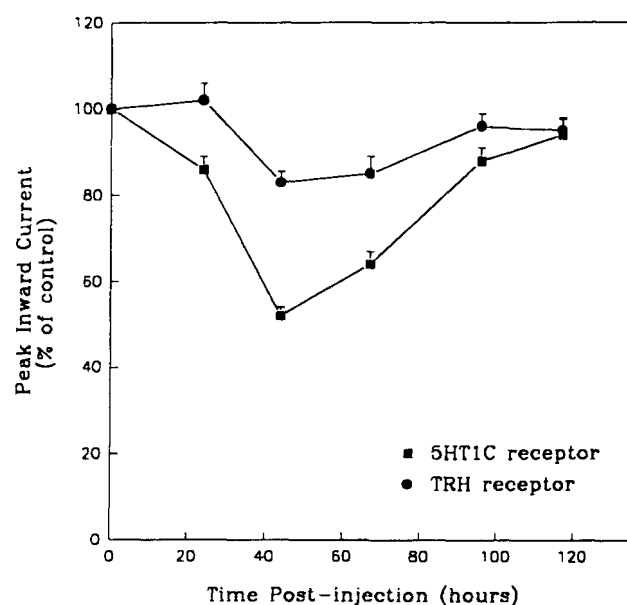
compared with receptor-injected oocytes that were co-injected with 25 ng of the sense oligonucleotide corresponding to the Go α subunit, oocytes co-injected with the same amount of antisense oligonucleotide showed reduced peak inward Cl⁻ currents (Fig. 3A). The maximum effect of this reduction occurred approximately 1–2 days following injection of the oligonucleotide, which agrees with previous studies examining antisense knock-out of G protein α subunits (Kleuss *et al.*, 1991; Blitzer *et al.*, 1993). The ligand-induced current found in antisense-treated oocytes recovered to the levels found in control oocytes (sense oligonucleotide-injected) 3–4 days following injection. These effects are due to the low turnover time and synthesis rate of the G protein. As was seen with the effect of PTX treatment, oocytes expressing the 5HT1c receptor were much more sensitive to the effects of Go α subunit antisense oligonucleotides than oocytes expressing the TRH receptor (Fig. 3A).

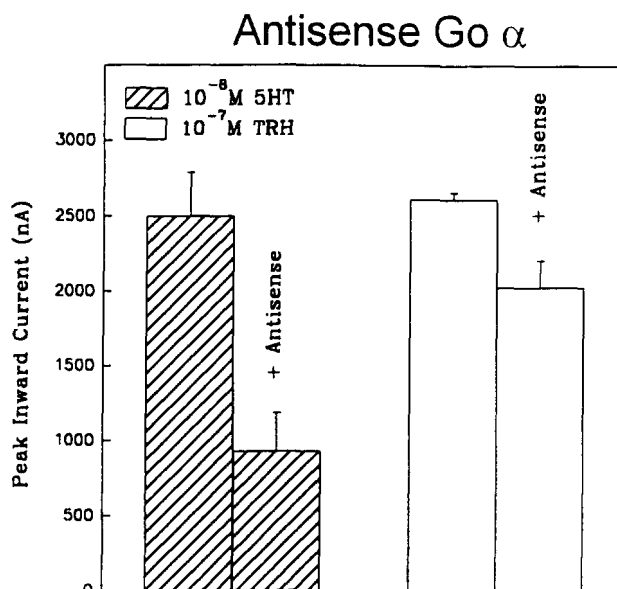The difference in coupling preference between the 5HT1c and TRH receptors for endogenous G proteins in oocytes as revealed by antisense knock-out experiments is more clearly observed in oocytes co-injected with both receptors. In measurements made 48 h following oligonucleotide injection, oocytes injected with 25 ng of Go α subunit antisense (Fig. 3B) showed 5HT-induced peak inward currents that were 34% of control, sense-injected oocytes. On the other hand, these same oocytes showed peak inward currents that were 77% of sense-injected oocytes when superfused with TRH. These results suggest that a Go subtype(s) endogenous to the oocyte preferentially couples to the 5HT1c receptor.

Gq is a PTX-insensitive G protein that is found in oocytes; the limited effect of PTX treatment on the TRH-induced currents in oocytes co-expressing 5HT1c and TRH receptors suggested that Gq may be mediating the TRH response. We tested this hypothesis directly using antisense oligonucleotides to the oocyte Gq α subunit, and the results are presented in Fig. 3C. TRH-induced currents in oocytes injected with Gq α antisense oligonucleotides 48 h prior to measurement were reduced by 62% compared with sense-injected controls. By comparison,
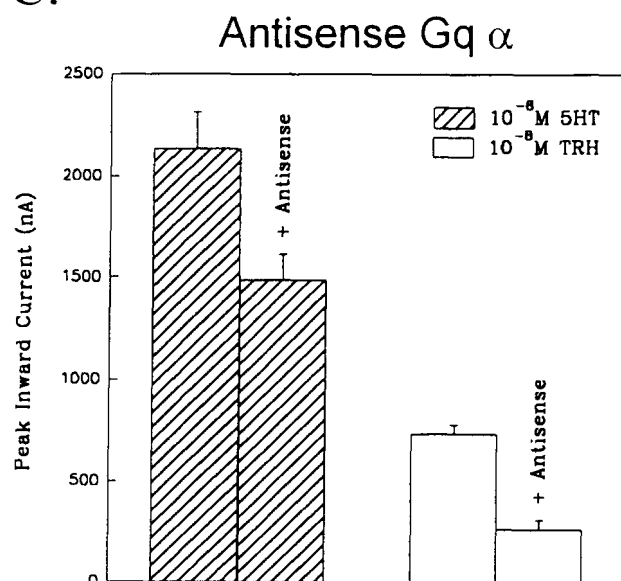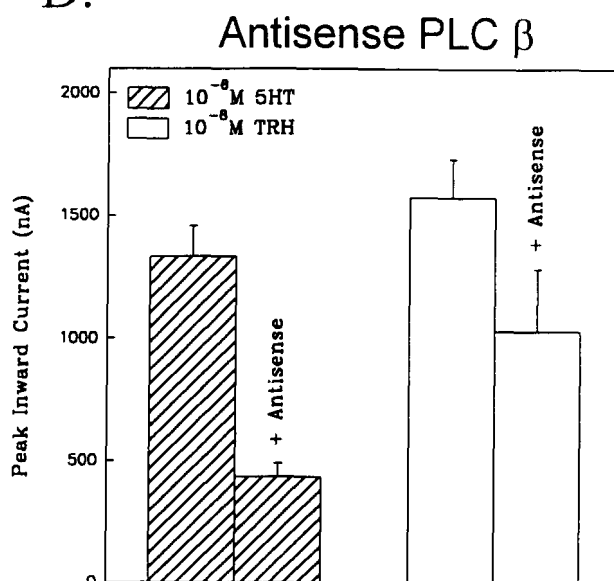
A.

B.

C.

D.

FIG. 3. **The effect of Go α, Gq α, or PLCβ antisense oligonucleotide injection on the inward currents in oocytes injected with 5HT1c and/or TRH receptor cRNA.** A, oocytes were first injected with either 10 pg of 5HT1c or 50 pg of TRH receptor. Twenty-four hours later, the oocytes were injected with 25 ng of Go α antisense oligonucleotide. Agonist-induced inward currents (10 nM 5HT or 100 nM TRH) were measured, and the results are plotted as a function of time following antisense injection (mean ± S.E. for three batches of oocytes, four to seven oocytes/data point). B–D, oocytes were injected with both 5 pg of 5HT1c and 50 pg of TRH receptor cRNA and injected 24 h later with 25 ng of antisense oligonucleotides to Go α (B), Gq α (C), or PLC-Xβ (D). Multiple agonist applications were made in 5-min intervals, 48 h after antisense oligonucleotide injection. The data (with and without antisense) are mean ± S.E. for an individual oocyte batch, five to eight oocytes/condition. Similar results were obtained for three other batches. In all cases, the induced currents were compared with receptor-injected oocytes co-injected with 25 ng of the comparable sense oligonucleotide.

5HT1c-induced currents were reduced by only 28% compared with control oocytes treated with the corresponding sense oligonucleotide. Therefore, in dual receptor-injected oocytes, the majority of the TRH response is mediated through Gq.

Because both 5HT1c (Go) and TRH (Gq) receptor stimulation results in activation of the same Ca^{2+}-activated Cl^- response in oocytes, the most obvious question was whether the Go and Gq activation converged directly on the same effector. To examine
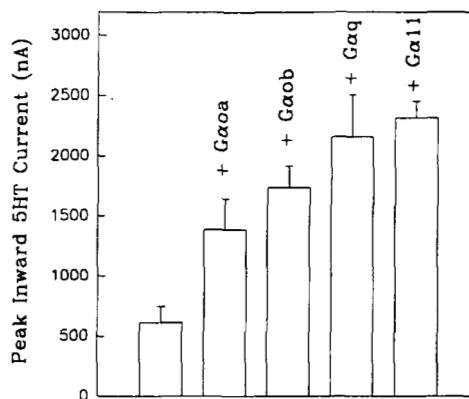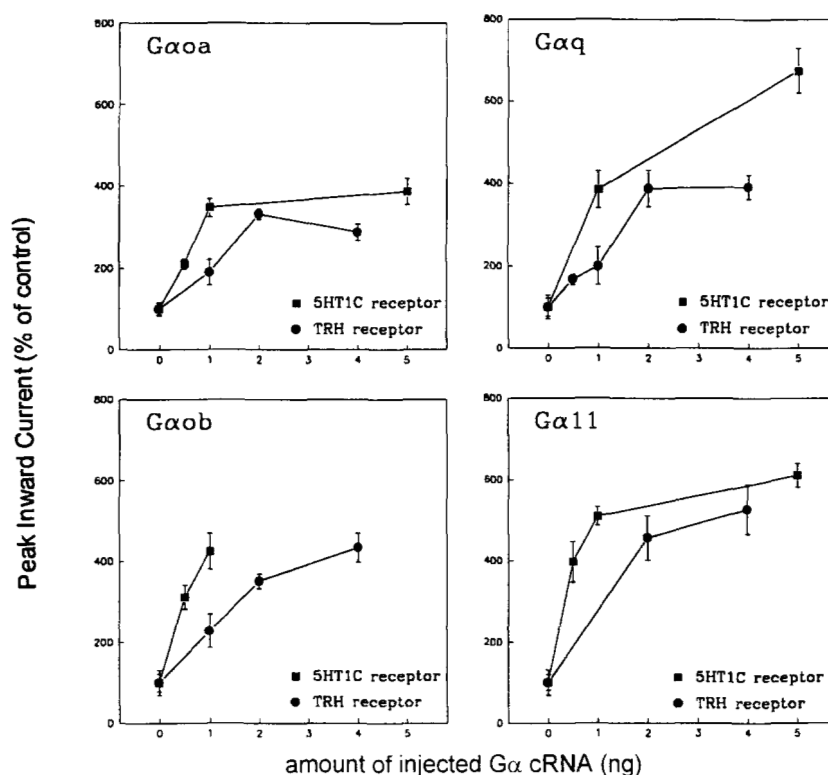
A.

B.

amount of injected Gα cRNA (ng)

FIG. 4. **Co-injection of cRNA for re-ceptors and cRNA for the mammalian α subunits of Goa, Gob, Gq, and G11 (effect on agonist-induced inward currents).** *A*, oocytes were injected with 10 pg of 5HT1c receptor alone or in combination with 1 ng of various mammalian G protein α subunits. The 5HT concentration was 10 nM. Data are mean ± S.D. for an individual oocyte batch, five oocytes/condition. Comparable results were obtained in experiments using the TRH receptor. *B*, oocytes were injected with 10 pg of 5HT1c or 100 pg of TRH receptor cRNA alone or in combination with various amounts of G protein α subunit cRNA. Data are mean ± S.E. for two to three oocyte batches/G protein subtype injected, four to nine oocytes/condition.

this, we injected dual receptor-injected oocytes with antisense specific for a novel *Xenopus* PLCβ (PLC-Xβ) that in oocytes mediates the PTX-sensitive response. This antisense oligonucleotide corresponds to a region that is distinct for PLC-Xβ in comparison with mammalian PLC-β1, -β2, and -β3, and injection of this oligonucleotide in oocytes has been shown to reduce the amount of PLC-Xβ mRNA (Ma *et al.*, 1993). Measured 48 h after antisense oligonucleotide injection, 5HT-induced currents were reduced by 66%; TRH-induced currents were reduced by 32% (Fig. 3*D*).

The preferential coupling of the 5HT1c and TRH receptors to native, presumably PLC-activating G proteins in the oocyte that are PTX-sensitive (Go) and PTX-insensitive (Gq), respectively, prompted us to ask whether these receptors could also couple to other G proteins that activate PLC (*i.e.* could this preference be overridden by heterologous expression of other G protein α subunits?). This was accomplished by co-injecting oocytes with receptor cRNA and cRNA encoding different mammalian G protein α subunits (each cloned from mouse). The results for oocytes injected with 10 pg of 5HT1c receptor alone

or in combination with 1 ng of individual G protein α subunits are shown in Fig. 4*A*. Both of the PTX-sensitive Go α subunits (Goa and Gob, two splice variants that differ in the receptor-interacting C-terminal portion of the deduced protein sequence) (Strathmann *et al.*, 1990), the PTX-insensitive Gq α subunits, and the PTX-insensitive G11 α subunits increased the 5HT-induced peak inward currents severalfold over oocytes injected with 5HT1c receptor alone. Comparable results were found for oocytes injected with the TRH receptor and these same G protein α subunits (data not shown). The effect of different amounts of injected G protein α subunit cRNA on the agonist-induced currents mediated by both the 5HT1c and TRH receptors is shown in Fig. 4*B*. Increases in peak inward currents of 4–6-fold (above control oocytes injected with receptor alone) were observed with each G protein α subunit tested, suggesting that these receptors can couple to several different mammalian G proteins that activate PLC.

Further evidence for coupling of these seven-helix receptors to different mammalian G protein α subunits in oocytes comes from experiments in which heterologous G protein α subunit
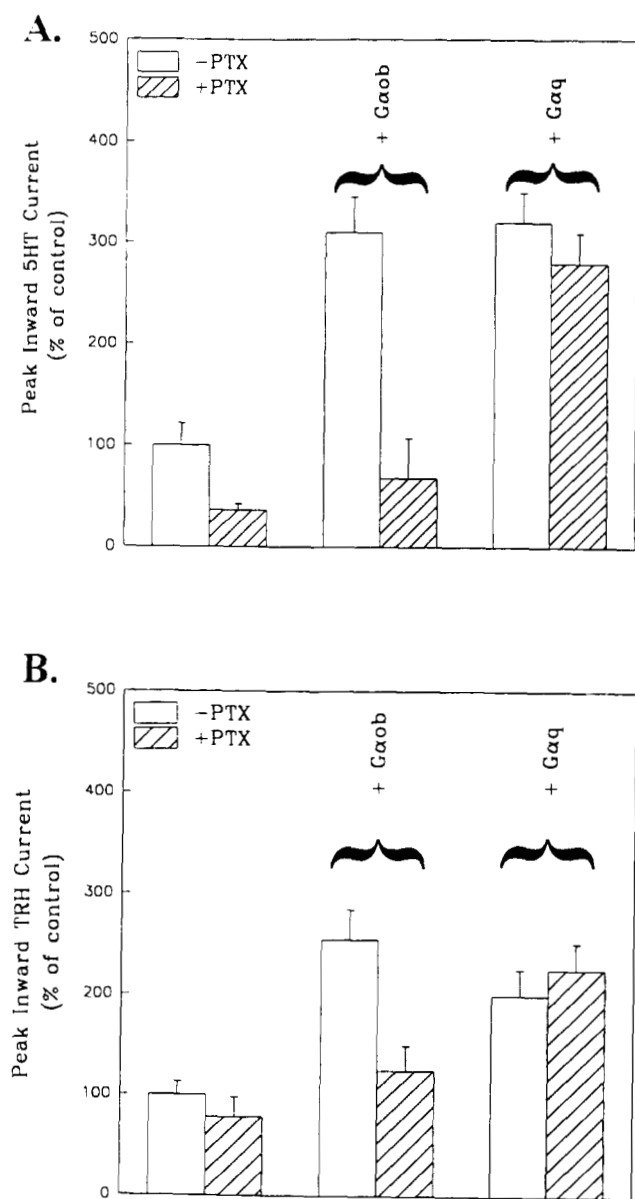
FIG. 5. **Effect of PTX treatment on the agonist-induced inward currents in oocytes injected with receptor and mammalian G protein α subunit cRNA.** *A*, oocytes were injected with 10 pg of 5HT1c receptor cRNA alone or in combination with 2 ng of Gob or Gq α subunit cRNA. A subset of oocytes was then incubated with 5 μg/ml PTX for 24 h prior to measurement. The 5HT concentration was 10 nM. The data are mean ± S.E. for two oocyte batches, five to eight oocytes/condition. *B*, same as *A*, except the oocytes were injected with 100 pg of TRH receptor. The agonist concentration was 100 nM.

expression was combined with PTX treatment. These results for 5HT1c receptor-injected oocytes are illustrated in Fig. 5*A*. As shown above, 5HT-induced inward currents were reduced 74% following 24 h of PTX treatment. When 2 ng of cRNA for the PTX-sensitive Gob α subunit was co-expressed with the 5HT1c receptor, the 5HT-induced currents were increased 3-fold. These currents were blocked by 78% with PTX treatment. However, the 3-fold increase in 5HT-induced inward currents due to co-expression of 2 ng of the PTX-insensitive Gq α subunit were blocked by only 13% following PTX treatment. Comparable results were found in similar experiments involving the TRH receptor (Fig. 5*B*). Treatment of oocytes injected with receptor alone resulted in TRH-induced currents that were mostly PTX-insensitive (22% reduction). PTX sensitivity of the TRH-induced currents could be achieved with co-injec-

tion of the Gob α subunit; the greater than 2-fold increase in inward currents was eliminated by PTX treatment. On the other hand, the 2-fold increase in TRH-induced currents following co-expression of the Gq α subunit was unaffected by PTX treatment.

These results demonstrate that several different exogenous G protein α subunits can couple to exogenously expressed seven-helix receptors. However, under these conditions, the receptor cannot distinguish between PTX-insensitive and PTX-sensitive G protein α subunits. The experiments using antisense oligonucleotides to PLC-Xβ demonstrated a significant reduction in Cl⁻ currents via the 5HT1c/Go-mediated pathway and less of an effect upon TRH/Gq-mediated Cl⁻ currents, suggesting that the oocyte's endogenous Go and Gq may act through different effectors. These two results prompted us to ask whether a similar pathway segregation occurs with heterologous expression of mammalian Go and Gq α subunits. Oocytes were injected with 10 pg of 5HT1c alone or in combination with 2 ng of Gob or Gq. Twenty-four hours later, a subset of these oocytes was injected with 25 ng of antisense oligonucleotide to PLC-Xβ (Ma *et al.*, 1993). Measured 48 h later, co-expression of both Go and Gq resulted in 2–2.5-fold increases in 5HT-activated Cl⁻ currents compared with oocytes injected with 5HT1c receptor alone. Injection of the antisense oligonucleotide to PLC-Xβ resulted in a 50–65% reduction in the Cl⁻ currents of mouse Gob-injected oocytes; oocytes injected with mouse Gq showed a reduction in Cl⁻ currents of 0–40% (data not shown). These results, although variable, suggest that the heterologously expressed G protein α subunits are acting on separate downstream effectors, as was seen with the endogenous G protein α subunits.

The ability of both the 5HT1c and TRH receptors to couple to several different exogenously expressed G protein α subunits raised the possibility that heterologous overexpression could result in a complete loss of specificity between receptor and G protein. Thus, G protein α subunits that typically exert their effects on other second messenger pathways might also couple to these receptors when overexpressed in the oocyte system. Therefore, oocytes were injected with either receptor alone or in combination with various amounts of the α subunits for Gs, Golf, or Gt. Results for experiments involving the 5HT1c receptor are shown in Fig. 6*A* (similar results were found for experiments using the TRH receptor). The currents elicited in the presence of 5HT for oocytes co-injected individually with these three G proteins were indistinguishable from the currents for oocytes injected with receptor alone. These results demonstrate either that these G proteins do not couple to the receptors or, if they couple, they fail to activate the endogenous Ca²⁺-activated Cl⁻ conductance.

For oocytes injected with Gs or Golf, it was more likely that coupling to the 5HT1c or TRH receptors would reveal itself through changes in the normal effector of these G proteins, adenylyl cyclase. To examine this possibility, oocytes were injected with CFTR, a Cl⁻ channel that can be activated via the adenylyl cyclase-cAMP-protein kinase A pathway, resulting in a slow inward current that can be assessed electrophysiologically and distinguished from the faster, endogenous Ca²⁺-activated Cl⁻ response (Uezono *et al.*, 1993). When 1 ng of CFTR cRNA was co-injected with 10 pg of cRNA encoding the β2-adrenergic receptor, a seven-helix receptor that couples to Gs to activate adenylyl cyclase, application of 100 μM isoproterenol resulted in inward currents of approximately 500 nA. Co-injection of 5 ng of Gs α subunit cRNA increased this response 2–3-fold (data not shown). These results verified that we could use CFTR as a sensor for Gs activity and established that our Gs cRNA was being functionally expressed in oocytes.
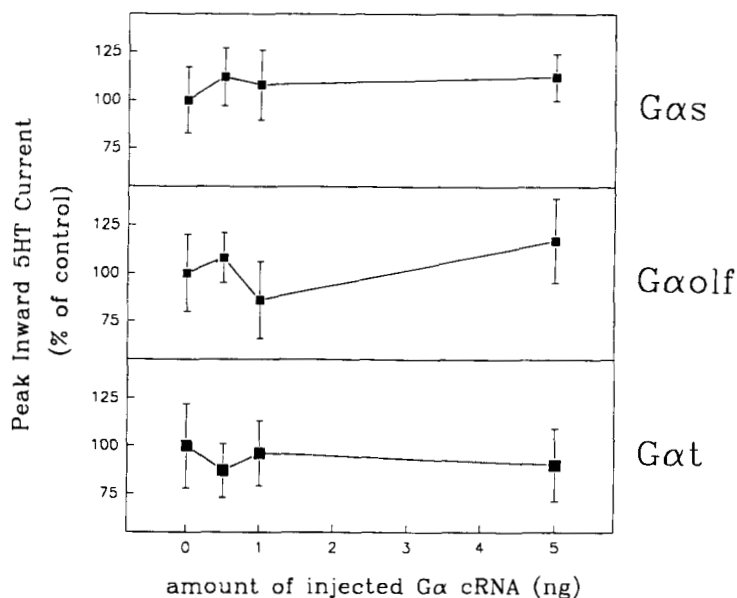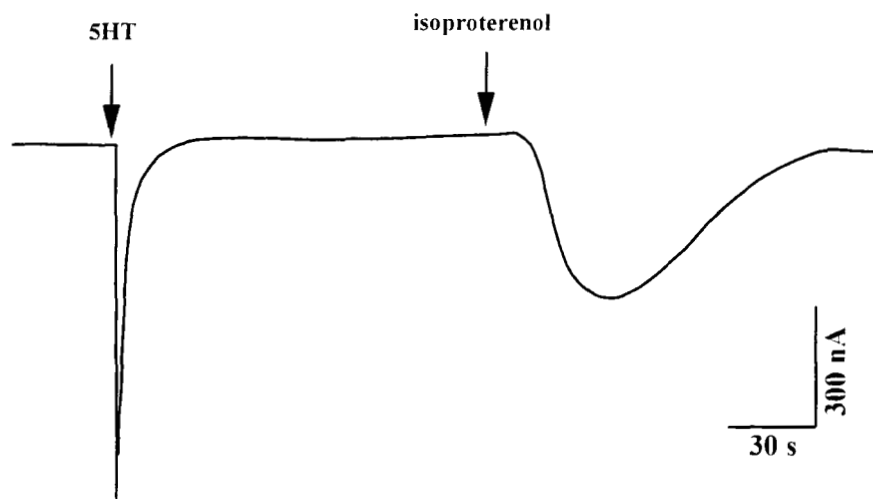
A.

B.

Fig. 6. **Effect of co-injection of 5HT1c receptor cRNA and cRNA for the α subunits of Gs, Golf, and Gt on 5HT-induced inward currents.** *A*, oocytes were injected with 10 pg of 5HT1c receptor cRNA alone or in combination with various amounts of mammalian G protein α subunit cRNA. The 5HT concentration was 10 nM. The data are mean ± S.E. for three oocyte batches/each G protein subtype injected, five oocytes/condition. *B*, recording for a representative oocyte injected with 10 pg of 5HT1c receptor, 10 pg of β₂-adrenergic receptor, 5 ng of Gs α subunit, and 1 ng of CFTR cRNA. The *arrows* above each trace represent the start of a 15-s application of either 100 nM 5HT or 100 μM isoproterenol.

To test for coupling between the 5HT1c receptor and Gs, oocytes were injected with cRNA encoding the 5HT1c receptor, the α subunit of Gs, and CFTR. Application of 100 nM 5HT resulted in inward currents that were associated with the endogenous Cl⁻ channel; however, there was no CFTR response (data not shown) (see also, Uezono *et al.* (1993)). To verify that all heterologous components were functionally expressed, oocytes were injected with cRNAs encoding the 5HT1c and β2-adrenergic receptors, the α subunit of Gs, and CFTR. The results are illustrated in Fig. 6B. Application of 5HT resulted in the expected Ca²⁺-activated Cl⁻ current; once again, there was no Gs activation of CFTR. However, application of isoproterenol (and activation of the β2-adrenergic receptor) produced a slowly activating inward current that is associated with CFTR stimulation (Uezono *et al.*, 1993). Similar results were obtained in experiments using the TRH receptor and the α subunit of Golf (data not shown). These findings suggest that the 5HT1c (and TRH) receptor fails to couple to G proteins that activate

non-PLC pathways in oocytes, even when these non-PLC pathways can be activated *via* other seven-helix receptors.

DISCUSSION

The *Xenopus* oocyte has been a model system for understanding how specificity is achieved among seven-helix receptors, heterotrimeric G proteins, and downstream effectors. Exogenous receptors can be coupled to either endogenous or exogenous G proteins to evoke native or experimentally-created second messenger cascades that can be assayed electrophysiologically with extreme sensitivity. The best characterized of these pathways is Go/PLCβ-mediated and results in Ca²⁺-activated Cl⁻ currents; most mammalian receptors that lead to phospholipase C activation elicit a PTX-sensitive response (via Go) when expressed in the *Xenopus* oocyte system, although many of these receptors are PTX-insensitive in their native environment (Blitzer *et al.*, 1993). In this article, we present evidence that two different receptors (5HT1c and TRH), when
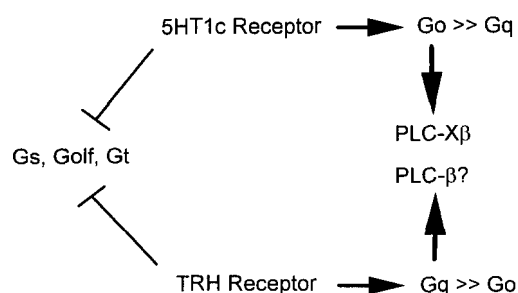
5HT1c Receptor ⟶ Go >> Gq

Gs, Golf, Gt

PLC-Xβ

PLC-β?

TRH Receptor ⟶ Gq >> Go

Fɪɢ. 7. **Summary diagram of our data examining the interaction among receptors, G proteins, and effectors in *Xenopus* oocytes.** The 5HT1c preferentially couples to Go, which activates a recently cloned *Xenopus* PLCβ (PLC-Xβ) (Ma *et al.*, 1993). The TRH receptor preferentially couples to Gq. Gq does not act through PLC-Xβ but most likely through some other PLCβ isoform. Although each receptor shows a preference for a particular G protein, they also appear to couple to other, but not all, G protein subtypes.

co-injected in oocytes, activate Cl⁻ channels via separate pathways. The 5HT1c receptor preferentially couples to the Go/PLCβ pathway; on the other hand, the TRH receptor preferentially couples to Gq and induces Cl⁻ currents that are largely independent of the Go-activated PLCβ. These results are summarized in Fig. 7. Interestingly, the preference of these receptors for the oocyte's endogenous PTX-sensitive and PTX-insensitive G proteins, respectively, can be eliminated by co-expression of some, but not all, individual exogenous G protein α subunits.

When the 5HT1c receptor is expressed in oocytes, agonist application results in the activation of a PTX-sensitive pathway leading to Cl⁻ currents. This is consistent with findings that many exogenously expressed seven-helix receptors preferentially couple to an endogenous Go subtype in the oocyte (Blitzer *et al.*, 1993). Using antisense oligonucleotides to the α subunit of the oocyte's Go, we have similarly found that the majority of the 5HT-induced Cl⁻ current is due to Go; these results are consistent with the PTX results. Neither the PTX experiments nor the antisense experiments resulted in a complete abolition of the 5HT-induced current. Although the residual current (approximately 20%) is very likely caused by incomplete elimination of the Go-mediated response, it is also possible that the residual current is due to activation of a PTX-insensitive G protein by the 5HT1c receptor.

The TRH receptor in its native environment (mammalian pituitary cells) is insensitive to PTX; interestingly, we find that this preference is retained when the cloned receptor is co-expressed in oocytes along with the 5HT1c receptor. The CCK receptor also stimulates PLC through a PTX-insensitive pathway (Moriarty *et al.*, 1989), and this characteristic is similarly retained when the oocytes are injected with total brain RNA. However, in the CCK experiments there was no assessment of the role of exogenous G protein subunits encoded by the total brain RNA in mediating the PTX-insensitive response. Using antisense oligonucleotides to the oocyte's endogenous Gq α subunit, we find that the majority of the TRH-induced current is mediated via the PTX-insensitive Gq subtype. Our results show a clear difference in the behavior of the 5HT1c and TRH receptors; this difference cannot be due to variability in the *Xenopus* oocyte response, because individual oocytes co-injected with cRNA for both receptors show significant differences in their specific ligand-induced responses. We conclude that we have detected a preference for the coupling of the TRH receptor and the 5HT1c receptor to Gq and Go, respectively.

Despite the preference in coupling of the TRH receptor to Gq and the 5HT1c receptor to Go, a portion of the current elicited by either receptor could not be inhibited by the appropriate G

protein α subunit antisense oligonucleotide. These results may be due to nonspecific effects of the treatment conditions or could be due to coupling of these receptors to several different G protein subtypes in the oocyte. This promiscuity may be related to cell-specific interactions between individual receptors and G proteins. Changes in the relative concentrations of receptors and G proteins or co-localization of individual receptors and G proteins in different cell types may influence the signaling pathway for a given receptor. This hypothesis is consistent with our results obtained from the co-injection of receptors with cRNAs encoding various mammalian G protein α subunits. In these experiments, each receptor preferentially coupled to the over-expressed G protein α subunit as though a change in the concentration of G protein α subunits in the oocyte alters the nature of complexes formed between a given receptor and its preferred G protein. However, even though there is cross-talk between some G proteins and receptors, the amount of G protein is not the single determinant in receptor/G protein coupling. For example, neither the TRH nor the 5HT1c receptor couples to exogenously expressed Gs or Golf in oocytes, suggesting that there are also specific recognition sites between receptor and G protein.

We detected no differences in TRH-induced currents between oocytes co-injected with the TRH receptor and G11 or Gq, in contrast to previous reports (Lipinsky *et al.*, 1992). This discrepancy may be caused by different levels of expressed TRH receptor or by different ratios of injected receptor:G protein α subunit. In our experiments, the injected TRH receptor cRNA concentration was 5–200 times less than that used by Lapinsky *et al.* (1992). Furthermore, the ratio of injected receptor:G protein cRNA in our experiments was much less than 1, whereas this ratio was greater than or equal to 1 in the experiments described by Lapinsky *et al.* (1992). Thus, large quantities of receptor may saturate downstream effectors that would otherwise be increased with G protein co-expression.

Our experiments tested whether both the Go-mediated 5HT response and the Gq-mediated TRH response were elicited via a recently cloned *Xenopus* PLCβ that is known to be activated by Go (Blitzer *et al.*, 1993; Ma *et al.*, 1993). Using antisense oligonucleotides specific to this PLCβ isoform, we found that there is specificity between various G protein subtypes and effectors, *i.e.* PLC-Xβ antisense injections strongly inhibited the Go-mediated 5HT-induced current and only weakly inhibited the Gq-mediated TRH-induced current. The approximately 20% inhibition of the TRH-induced response by PLC-Xβ antisense is consistent with the idea that a fraction of this receptor couples to the Go subtype. At present, we do not know what effector is being activated by the Gq subtype; however, the most straightforward hypothesis is that it is acting upon another PLC, perhaps the oocyte counterpart to the mammalian Gq-activated PLCβ1 (Berstein *et al.*, 1992). We also do not know whether the observed G protein-mediated responses are occurring via α subunits or βγ subunits. The βγ subunit has been recently implicated in the activation of some isoforms of mammalian PLCβ (Blank *et al.*, 1992; Camps *et al.*, 1992a, 1992b; Katz *et al.*, 1992), and it is possible that similar activation occurs in *Xenopus* oocytes. We do know that when the cloned *Xenopus* PLCβ is transfected in COS cells along with various mammalian βγ subunits, no activation of PLCβ is observed.[2]

In summary, our results demonstrate that specificity exists both at the level of receptor/G protein interactions and at the level of G protein/effector interactions. It will be of interest to determine the molecular mechanisms underlying this specificity; such findings may also elucidate the mechanisms responsible for the loss of specificity that can be achieved with G

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[2] A. Aragay, unpublished results.

protein α subunit overexpression.

REFERENCES

Aragay, A. M., Katz, A., and Simon, M. I. (1992) *J. Biol. Chem.* **267**, 24983–24988

Berstein, G., Blank, J. L., Jhon, D.-Y., Exton, J. H., Rhee, S. G., and Ross, E. M. (1992) *Cell* **70**, 411–418

Blank, J. L., Brattain, K. A., and Exton, J. H. (1992) *J. Biol. Chem.* **267**, 23069–23075

Blitzer, R. D., Omri, G., De Vivo, M., Carty, D. J., Premont, R. T., Codina, J., Birnbaumer, L., Cotecchia, S., Caron, M. G., Lefkowitz, R. J., Landau, E. M., and Iyengar, R. (1993) *J. Biol. Chem.* **268**, 7532–7537

Bonner, T. I., Young, A. C., Brann, M. R., and Buckley, N. J. (1988) *Neuron* **1**, 403–410

Brown, A. M., and Birnbaumer, L. (1990) *Annu. Rev. Physiol.* **52**, 197–213

Camps, M., Carozzi, A., Schnabel, P., Scheer, A., Parker, P. J., and Gierschik, P. (1992a) *Nature* **360**, 686–689

Camps, M., Hou, C., Sidiropoulus, D., Stock, J. B., Jakobs, R. H., and Gierschik, P. (1992b) *Eur. J. Biochem.* **206**, 821–832

Cantiello, H. F., and Lanier, S. M. (1989) *J. Biol. Chem.* **264**, 16000–16007

Cerione, R. A., Kroll, S., Rajaram, R., Unson, C., Goldsmith, P., and Spiegel, A. M. (1988) *J. Biol. Chem.* **263**, 9345–9352

Cerione, R. A., Regan, J. W., Nakata, H., Codina, J., Benovic, J. L., Gierschik, P., Somers, R. L., Spiegel, A. M., Birnbaumer, L., Lefkowitz, R. J., and Caron, M. G. (1986) *J. Biol. Chem.* **261**, 3901–3909

Chabre, M., Bigay, J., Bruckert, F., Bornancin, F., and Deterre, P. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53(P1)**, 313–324

Conklin, B. R., Chabre, O., Wong, Y. H., Federman, A. D., and Bourne, H. R. (1992) *J. Biol. Chem.* **267**, 31–34

Coupry, I., Duzic, E., and Lanier, S. M. (1992) *J. Biol. Chem.* **267**, 9852–9857

Dascal, N., Ifune, C., Hopkins, R., Snutch, T. P., Lubbert, H., Davidson, N., Simon, M. I., and Lester, H. A. (1986) *Mol. Brain Res.* **1**, 201–209

Duzic, E., Coupry, I., Downing, S., and Lanier, S. M. (1992) *J. Biol. Chem.* **267**, 9844–9851

Gillo, B., Lass, Y., Nadler, E., and Oron, Y. (1987) *J. Physiol.* **392**, 349–361

Graziano, M. P., Casey, P. J., and Gilman, A. G. (1987) *J. Biol. Chem.* **262**, 11375–11381

Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M., Davidson, N., Lester, H. A., and Kanner, B. I. (1990) *Science* **249**, 1303–1305

Herzog, H., Hort, Y. J., Ball, H. J., Hayes, G., Shine, J., and Selbie, L. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 5794–5798

Hille, B. (1992) *Neuron* **9**, 187–195

Jones, D. T., and Reed, R. R. (1989) *Science* **244**, 790–795

Katz, A., Wu, D., and Simon, M. I. (1992) *Nature* **360**, 686–689

Kleuss, C., Hescheler, J., Ewel, C., Rosenthal, W., Schultz, G., and Wittig, B. (1991) *Nature* **353**, 43–48

Kurachi, Y., Tung, R. T., Ito, H., and Nakajima, T. (1992) *Prog. Neurobiol.* **39**, 229–246

Lipinsky, D., Gershengorn, M. C., and Oron, Y. (1992) *FEBS Lett.* **307**, 237–240

Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., and Clapham, D. E. (1987) *Nature* **325**, 321–326

Lynch, C. J., Prpic, V., Blackmore, P. F., and Exton, J. H. (1986) *Mol. Pharmacol.* **29**, 196–203

Ma, H., Blitzer, R. D., Healy, E. C., Premont, R. T., Landau, E. M., and Iyengar, R. (1993) *J. Biol. Chem.* **268**, 19915–19918

McFadzean, I., Mullaney, I., Brown, D. A., and Milligan, G. (1989) *Neuron* **3**, 177–182

McIntosh, R. P., and Catt, K. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 9045–9048

Miledi, R., and Parker, I. (1984) *Proc. R. Soc. London Ser. B Biol. Sci.* **215**, 491–497

Moriarty, T. M., Padrell, E., Carty, D. J., Omri, G., Landau, E. M., and Iyengar, R. (1990) *Nature* **343**, 79–82

Moriarty, T. M., Sealfon, S. C., Carty, D. J., Roberts, J. L., Iyengar, R., and Landau, E. M. (1989) *J. Biol. Chem.* **264**, 13524–13530

Olate, J., Jorquera, H., Purcell, P., Codina, J., Birnbaumer, L., and Allende, J. E. (1989) *FEBS Lett.* **244**, 188–192

Oron, Y., Gillo, B., Straub, R. E., and Gershengorn, M. (1988) *Mol. Endocrinol.* **1**, 918–925

Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M., and Hille B. (1985) *Nature* **317**, 536–538

Quick, M. W., Corey-Naeve, J., Davidson, N., and Lester, H. A. (1992) *Biotechniques* **13**, 358–362

Quick, M. W., and Lester, H. A. (1994) in *Methods in Neuroscience: Ion Channels of Excitable Cells* (Conn, P. M., ed) pp. 261–279, Academic Press, San Diego

Ross, E. M. (1989) *Neuron* **3**, 141–152

Senogles, S. E., Spiegel, A. M., Padrell, E., Iyengar, R., and Caron, M. G. (1990) *J. Biol. Chem.* **265**, 4507–4514

Shenker, A., Goldsmith, P., Unson, C. G., and Spiegel, A. M. (1991) *J. Biol. Chem.* **266**, 9309–9313

Simon M. I., Strathmann, M. P., and Gautam N. (1991) *Science* **252**, 802–808

Slepak, V. Z., Wilkie, T. M., and Simon, M. I. (1993) *J. Biol. Chem.* **268**, 1414–1423

Smrcka, A. V., Hepler, J. R., Brown, K. O., and Sternweis, P. C. (1991) *Science* **251**, 804–806

Strathmann, M., Wilkie, T. M., and Simon, M. I. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 6477–6481

Strathmann, M., and Simon, M. I. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 9113–9117

Straub, R. E., Frech, G. C., Joho, R. H., and Gershengorn, M. C. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 9514–9518

Taussig, R., Iniguezlluhi, J. A., and Gilman, A. G. (1993) *Science* **261**, 218–221

Ueda, H., Yoshihara, Y., Misawa, H., Fukushima, N., Katada, T., Ui, M., Takagi, H., and Satoh, M. (1989) *J. Biol. Chem.* **264**, 3732–3741

Uezono, Y., Bradley, J., Min, C., McCarty, N., Quick, M., Riordan, J. R., Chavkin, C., Zinn, K., Lester, H. A., and Davidson, N. (1993) *Recept. Channels* **1**, 233–241

Vuong, T. M., Chabre, M., and Stryer, L. (1984) *Nature* **311**, 659–661

Welsh, M. J., Anderson, M. P., Rich, D. P., Berger, H. A., Denning, G. M., Ostedgaard, L. S., Sheppard, D. N., Cheng, S. H., Gregory, R. J., and Smith, A. E. (1992) *Neuron* **8**, 821–829

Yatani, A., and Brown, A. M. (1989) *Science* **245**, 71–74