Interaction of the Xanthine Nucleotide Binding Goα Mutant with G Protein-coupled Receptors*

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We constructed a double mutant version of the α subunit of Go that was regulated by xanthine nucleotides instead of guanine nucleotides (GoX). We investigated the interaction between GoX and G protein-coupled receptors in vitro. First, we found that the activated m2 muscarinic cholinergic receptor (MACHr) could facilitate the exchange of XTPγS for XDP in the GoXβγ heterotrimer. Second, the GoXβγ complex was able to induce the high affinity ligand-binding state in the N-formyl peptide receptor (NFPR). These experiments demonstrated that GoX was able to interact effectively with G protein-coupled receptors. Third, we found that the empty form of GoX, lacking a bound nucleotide and βγ, formed a stable complex with the m2 muscarinic cholinergic receptor associated with the plasma membrane. Finally, we investigated the interaction of GoX with receptor in COS-7 cells. The empty form of GoX bound tightly to the receptor and was not activated because XTP was not available intracellularly. We tested the ability of GoX to inhibit the activities of several different G protein-coupled receptors in transfected COS-7 cells and found that GoX specifically inhibited Go-coupled receptors. Thus the modified G proteins may act as dominant-negative mutants to trap and inactivate specific subsets of receptors.

Hundreds of seven-transmembrane receptors activate heterotrimeric G proteins and transduce signals across cell membranes in eukaryotic cells. The stimulated receptors catalyze the exchange of GTP for GDP bound to G protein α subunits. Activated GTP-bound α subunits and free βγ subunits regulate a variety of cellular effectors including enzymes and ion-channels (1–3). Signaling is normally initiated by the binding of agonist to receptor, which stabilizes the receptor in an active conformation. Receptors function to stimulate the dissociation of GDP bound to the G protein α subunits. The subsequent binding of GTP to the empty α subunit promotes the conformational change of Gα and dissociation of the βγ subunits. The G protein α subunit in the nucleotide-free state appears to be an important intermediate in the activation. From studies of rhodopsin and transducin, it has been postulated that the empty G protein (nucleotide-free) forms a stable complex with the receptor (4). Both empty forms of Gi and Go α subunits have been purified under harsh conditions (1 M (NH₄)₂SO₄ and 20% glycerol), and they were unstable (5).

We recently reported that a mutant version of Goa, GoX (GoaD273N/Q205L), was regulated by xanthine nucleotides, not by guanine nucleotides (6). GoX bound XDP₁ and XTP instead of GDP or GTP. GoX bound G protein βγ subunits only in the presence of XDP, and XTP stimulated dissociation of the GoXβγ heterotrimer. XTP-bound GoX underwent a conformational change similar to the activated wild-type Goα. In the present study, we investigated the interaction between GoX and G protein-coupled receptors. We found that GoX mutant proteins retained the receptor binding specificity of the wild-type Goα and were able to interact with Go-coupled receptors, such as the m2 muscarinic cholinergic receptor (MACHr), N-formyl peptide receptor (NFPR), and thrombin receptor, but not with m1 MACHr or thyrotropin-releasing hormone (TRH) receptor which do not couple to wild-type Go. Because the concentrations of XDP and XTP are relatively low in vivo (7), GoX mutant proteins are essentially nucleotide-free unless exogenous xanthine nucleotides are provided. Thus, GoX provides an excellent model to study the receptor interaction of empty G protein α subunits. Consistent with the previously reported studies on the empty form of transducin (4), our data are most readily interpreted as showing that “empty” GoX can form a stable complex with appropriate receptors on the membrane.

EXPERIMENTAL PROCEDURES

Materials—Purified bovine retinal transducin βγ were generous gifts from Dr. O. Nakanishi (Division of Biology, Caltech). Xanthine and guanine nucleotides were from Sigma. All the radioactive ligands including [35S]ATPγS, [35S]GTPγS, [3H]QNB, and [3H]HIP were from NEN Life Science Products.

Expression and Purification of His₆-tagged Goα—Both wild-type Goα and mutant GoX were subcloned into the Escherichia coli expression vector pET-15b (Novagen) with His₆ tag at the N terminus. These clones were used to transform the E. coli strain BL21(DE3), and proteins were expressed. Expression and purification of these proteins was previously described (6, 8). After harvesting the culture, the cell extracts were resuspended in the binding buffer (5 mM imidazole, 0.5 mM NaCl, 160 mM Tris-HCl, pH 7.9, and 1 mM DTT). The His₆-tagged proteins were purified from Ni²⁺–NTA column according to the protocol provided by Novagen. Purified proteins were stored in TED buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 1 mM diithiothreitol) with 0.1 mM MgCl₂ and 0.1 mM nucleotide diphosphate (GDP or XDP as appropriate).

Membrane Preparation from Baculovirus-infected Sf9 Cells—Sf9 cells were grown and infected with recombinant baculoviruses encoding either m2 MACHr or NFPR (9–11). Infected cells were centrifuged and resuspended at <10⁵ cells/ml in HME/PI buffer (20 mM NaHepes, pH 8.0, 2 mM MgCl₂, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, and 10 μg/ml leupeptin). The cell suspension was homogenized by 10 strokes in a glass homogenizer followed by passing through a 27 gauge hypodermic needle several times. The homogenate was briefly centrifuged at 3,000 × g for 10 min, and then the supernatant was collected and centrifuged at 30,000 × g for 30 min. The pellet was washed once with HME/PI, and the final pellet was resuspended in HME/PI at a protein concentration of 5 mg/ml.

Synthesis of XTPγS—XTPγS was synthesized from XDP and ATPγS

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1 The abbreviations used are: XDP, xanthine diphosphate; XTP, xanthine triphosphate; MACHr, muscarinic cholinergic receptor; NFPR, N-formyl peptide receptor; TRH, thyrotropin-releasing hormone; NDK, nucleotide diphosphate kinase; IP₃, inositol 1,4,5-triphosphate; PLC, phospholipase C; GTPγS, guanosine 5′-O-(3-thiotriphosphate); XTPγS, xanthine 5′-O-(3-thiotriphosphate); [3H]QNB, quinuclidinylbenzilate; [3H]HIP, formyl-methionyl-leucyl-phenylalanine.

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with nucleotide diphosphokinase (NDK) as described previously (12). To produce $^{35}$S-labeled XTPγS, the reaction contained 10 μM XDP, 1 μM $[^{35}]$SATP, and 10 units of NDK (Sigma) in 100 μl of NDK buffer (1 mM MgCl₂, 5 mM dithiothreitol, and 20 mM Tris-HCl, pH 8.0). The mixture was incubated at room temperature for 2 h. The resulting concentration of $[^{35}]$S-ATPγS was about 1 μM (1 μCi/pmol). The radiochemical purity of XTPγS was monitored by TLC on Avicel/PEAE plates (Analtech) in 0.07 N HCl.

**Nucleotide Binding of Purified Goα**—Binding of $[^{35}]$S-ATPγS and $[^{33}]$P-γTPγS to the recombinant Goα or the mutant proteins was performed as described (6). The binding reaction contained 0.5 μg of purified protein in TED buffer, with 0.1 mM MgCl₂, 1 μM ATP, and 0.1 μM GTPγS or XTPγS (20,000 cpm/pmol). For the time course experiments, 20-μl aliquots were withdrawn from a 200-μl reaction, diluted 10-fold with ice-cold TED buffer containing 0.1 mM MgCl₂, filtered through 45-μm nitrocellulose, washed, and dried. The amount of bound radioactivity was determined by scintillation counting.

**Radioligand Binding of Receptors**—The ligand binding assays of membrane-bound receptors were performed as described (9–11). The total concentration of m2 MACHR and the affinities of NFPR were determined by incubating membranes with 2 nM $[^{3}H]$QNB or various concentrations of fMLP$[^{3}H]$ for 1 h in 20 mM Tris-HCl, pH 7.4, 12.5 mM MgCl₂, and 1 mM EDTA at 30°C in a final volume of 0.5 ml. Nonspecific binding was defined as binding that was not displaced by 10 μM atropine for m2 MACHR or 10 μM fMLP for NFPR. Unbound ligands were removed by filtration through Whatman GF/F filters and washing four times using ice-cold binding buffer. The amount of bound radioactivity was determined by scintillation counting.

**Binding of Goα on Sf9 Cell Membranes**—0.2 μg of purified Goα or Goα mutant proteins were incubated with 100 μg of Sf9 cell membranes in TED buffer of a final volume of 100 μl at room temperature for 1 h. The membranes were centrifuged and subjected to Western blot using antibodies against Goα.

**Cell Culture and Transfection**—COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. 1 μg of DNA was mixed with 5 μl of LipofectAMINE (Life Technologies, Inc.) in 0.5 ml of Opti-MEM (Life Technologies, Inc.), and 5 h later, 0.5 ml of 20% fetal calf serum in Dulbecco’s modified Eagle’s medium was added to the medium. After 48 h, cells were assayed for inositol phosphate levels as described previously (13, 14).

**RESULTS**

**Stimulation of XTPγS Binding of GoαX by Activated M2 Muscarinic Receptor**—To test if GoαX could interact with G protein-coupled receptors, we investigated the receptor-stimulated nucleotide binding of GoαX. Activated G protein-coupled receptors are known to facilitate the binding of GTPγS to G protein α subunits. It has been reported that recombinant m2 MACHR from Sf9 cells stimulated the binding of GTPγS to wild-type Goα 2–3-fold in response to muscarinic agonists (9, 10). We infected Sf9 cells with recombinant baculoviruses encoding m2 MACHR and prepared membranes. The concentration of receptor was about 20 pmol/mg of membrane protein, determined from $[^{3}H]$QNB binding. We have previously shown that GoαX mutant proteins bind only xanthine nucleotides, but not guanine nucleotides. In this experiment, we reconstituted purified GoαX with Sf9 cell membranes containing m2 MACHR in the presence of XDP and G protein βγ subunits purified from bovine retina, and followed agonist-dependent stimulation of XTPγS binding to GoαX. We found that carbachol accelerated the XTPγS binding of GoαX, in a fashion similar to the acceleration of GTPγS binding observed with wild-type Goα (Fig. 1a).

In a set of experiments using wild-type Sf9 cell membranes, both atropine and carbachol had no effect on the XTPγS binding of GoαX (Fig. 1a). βγ subunits were required for the carbachol-dependent stimulation of nucleotide binding (Fig. 1b), suggesting that only the trimeric complex of GoαX with βγ can be activated to exchange XDP for XTPγS by the ligand-bound receptors.

**High Affinity Ligand Binding of N-formyl Peptide Receptor**

**Fig. 1.** M2 MACHR stimulated XTPγS binding of GoαX. a, 0.5 μg of purified GoαX was incubated with 1 μg of βγ, 100 μg of m2 MACHR membranes, or control Sf9 cell membranes with and 10 μM XDP in TEDM buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, and 1 mM MgCl₂) for 30 min at room temperature, and then the mixture was diluted 10-fold with TEDM buffer containing 0.1 μM $[^{35}]$S-ATPγS (20,000 cpm/pmol) and 100 μM carbachol or 2 μM atropine at time 0. 20-μl aliquots were withdrawn and assayed for the bound nucleotides at the indicated times. b, 0.5 μg of purified wild-type Goα or GoαX were subjected to the similar nucleotide binding assay as in panel a with GTPγS or XTPγS under indicated conditions. Only data at 20 min were shown as the percentage of maximum binding.

**Induced by GoαX**—Another well documented indication of receptor–G protein interaction is that GTP or GTPγS inhibits the high affinity binding of G protein-coupled receptors to their agonists. NPFR receptors expressed in Sf9 cells are known to exist in the low ligand affinity state (20–60 mM MBL) (11), presumably because of the lack of mammalian Gi-like G proteins in Sf9 cells, whereas NPFR in neutrophils and NPFR expressed in mouse L cells exhibited high affinity ligand binding (0.5–3 mM). Therefore, we reconstituted GoαX with NPFR from Sf9 cells and investigated whether GoαX could induce the high affinity ligand binding state in NPFR receptors. Sf9 cell membranes containing NPFR receptors were prepared as de-
wild-type Go showed low ligand affinity binding of fMLP (varieties of nucleotides and determined their affinities for the membrane when it was coincubated with βγ. Interestingly, both XDP and XTP stayed on the membrane when both XDP and XTP were incubated with Sf9 cell membranes. Furthermore, similar experiments using Sf9 cell membranes containing m2 MACHR and released GoX from the membrane association. In the experiments using membranes containing m2 MACHR, we found somewhat surprisingly that GoX bound to receptor-containing membranes even in the absence of carbachol and without βγ (Fig. 3, lane 12), whereas wild-type GoX did not (Fig. 3, lane 1), suggesting GoX alone was able to bind to receptor. Interestingly, both XDP and XTP abolished the interaction between GoX and m2 MACHR-containing membranes and released GoX from the membrane fraction (Fig. 3, lanes 7 and 8), whereas GDP or GTP had no effect (data not shown), suggesting that the nucleotide-free form of GoX can recognize and bind to Go-mediated receptor. As expected, GoX stayed on the membrane when both XDP and βγ were present (Fig. 3, lane 10), and XTP promoted dissociation of the GoXβγ complex (Fig. 3, lane 9). In the case of wild-type Go, the binding pattern was the same between membranes with or without the receptors, and XDP or XTP had no effect on binding (Fig. 3, lanes 1-5). In a titration experiment, quantitation of GoX revealed that the amount of GoX bound to the membrane increased linearly until it reached saturation, and the level of saturation was proportional to the amount of receptor incubated in the reaction (Fig. 4, a-c). Furthermore, similar experiments using Sf9 cell membranes containing NFPR were also performed, and the results were similar (data not shown). These experiments indicated that the empty form of GoX, without a bound nucleotide and βγ, could form a stable complex with receptor. In summary, these data described under “Experimental Procedures.” The expression level of NFPR was about 20 pmol/mg of membrane protein, determined by fMLP[3H] binding. We incubated the NFPR with wild-type GoX or mutant GoX in the presence of βγ and varieties of nucleotides and determined their affinities for the agonist fMLP. As expected, NFPR expressed in Sf9 cells showed low ligand affinity binding of fMLP (100 nM), and GoX alone did not affect ligand binding (Fig. 2a). More interestingly, NFPR exhibited high affinity ligand binding (10 nM) when GoX, βγ, and XDP were present (Fig. 2a). Both βγ and XDP were required to induce the high ligand affinity state of NFPR, and XTP inhibited the fMLP binding of the receptors (Fig. 2b). In the control experiments, wild-type Go αβγ heterotrimer was also found to convert the NFPR to the high affinity ligand binding state, which was inhibited by GTPγS (Fig. 2b). These experiments demonstrated that the heterotrimeric complex of GoXβγ can interact efficiently with NFPR.

**Binding of GoX with M2 Muscarinic Receptor on Sf9 Cell Membranes**

These experiments showed that GoXβγ heterotrimer could interact with the G protein-coupled receptors efficiently and that the interaction was similar to the interaction between wild-type Go and receptors. To investigate receptor interaction of GoX more directly, we studied binding of GoX to receptor containing Sf9 cell membranes. Purified wild-type Go or GoX were incubated with Sf9 cell membranes containing m2 MACHR in the presence of different reagents. The membranes were then pelletted and subjected to Western blotting using antibodies against GoX to see if GoX remained bound to the membrane. In the control experiments using wild-type Sf9 cell membranes without m2 MACHR, both wild-type Go and GoX did not remain associated with the membrane. However, wild-type GoX was bound to membrane when it was coincubated with βγ. Similarly, GoX stayed on the membrane when in complex with βγ in the presence of XDP (data not shown). These experiments using wild-type Sf9 cell membranes showed that GoX bound to the membranes only in the αβγ complex form, presumably because βγ facilitates membrane association. In the experiments using membranes containing m2 MACHR, we found somewhat surprisingly that GoX bound to receptor-containing membranes even in the absence of carbachol and without βγ (Fig. 3, lane 12), whereas wild-type GoX did not (Fig. 3, lane 1), suggesting GoX alone was able to bind to receptor. Interestingly, both XDP and XTP abolished the interaction between GoX and m2 MACHR-containing membranes and released GoX from the membrane fraction (Fig. 3, lanes 7 and 8), whereas GDP or GTP had no effect (data not shown), suggesting that the nucleotide-free form of GoX can recognize and bind to Go-mediated receptor. As expected, GoX stayed on the membrane when both XDP and βγ were present (Fig. 3, lane 10), and XTP promoted dissociation of the GoXβγ complex (Fig. 3, lane 9). In the case of wild-type Go, the binding pattern was the same between membranes with or without the receptors, and XDP or XTP had no effect on binding (Fig. 3, lanes 1-5). In a titration experiment, quantitation of GoX revealed that the amount of GoX bound to the membrane increased linearly until it reached saturation, and the level of saturation was proportional to the amount of receptor incubated in the reaction (Fig. 4, a-c).

**FIG. 2. High affinity ligand binding of NFPR induced by GoX.** a, 10 μg of NFPR membranes or wild-type Sf9 cell membranes were incubated with various concentrations of fMLP[3H] for 1 h in 20 mM Tris-HCl, pH 7.4, 12.5 mM MgCl₂, and 1 mM EDTA at 30 °C in a final volume of 0.5 ml, in the presence of 0.1 μg of GoX, 0.2 μg of βγ, and 100 μM XDP, or GoX alone. The amount of bound radioligand was then determined. Nonspecific binding was defined as binding in the presence of 10 μM cold fMLP, which was less than 10% of total ligand binding, and was subtracted before analyzing. b, NFPR was incubated with 50 nM fMLP[3H] and various reagents under the same conditions as panel a.

**FIG. 3. Binding of GoX to m2 MACHR on Sf9 cell membranes.** 0.2 μg of wild-type GoX (with 100 μM GDP) or GoX were incubated with 100 μg of m2 MACHR membranes in TED buffer of a final volume of 100 μl at room temperature for 1 h with indicated reagents. The membrane then was centrifuged and subjected to Western blot using antibodies against GoX. All nucleotide concentrations were 100 μM, and the amount of βγ was 0.5 μg. Lane 13 shows the total amount of GoX used in each assay.

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suggest that GoX with XDP bound and βγ bind to membranes, whereas the XTP form is found to be cytoplasmic. The nucleotide-free form is able to bind to Go-mediated receptors.

Dominant-negative Effect of GoX on Receptor Activation in COS-7 Cells—Because our experiments suggested that empty GoX was able to bind to the receptor in vitro, we went on to test for this interaction in intact cells. Indeed, we found that GoX was able to interact with receptors and inhibit their activities in COS-7 cells consistent with the observation that GoX did not dissociate from the receptors without xanthine nucleotides. Thrombin receptors are known to couple with G proteins from both the Gi and Gq families (17). In COS-7 cells transfected with the thrombin receptor, endogenous Gq is activated by the addition of thrombin and stimulates PLCβ isoforms to elevate cellular IP3 concentration. Inhibition of receptor activation in transfected cells by wild-type G proteins was observed before (18). Thus if cells are cotransfected with both the thrombin receptor and wild-type Goα, we looked into the interaction between GoX and NFPR receptors expressed in Sf9 cells. The NFPR expressed in Sf9 cell membranes was used to maintain a constant amount of membranes of 200 μg in each binding reaction.

All these experiments suggested that GoX was able to interact with G protein-coupled receptors in cells and retained the receptor specificity of wild-type Goα; it coupled with thrombin receptor and m2 MACHR, but not with m1 MACHR or TRH receptor. Furthermore, GoX exhibited dominant-negative inhibitory effects against these receptors in cells.

**DISCUSSION**

GoX (GoαD273N/Q205L) was the first reported mutant of heterotrimeric G protein α subunits that bound xanthine nucleotides, not guanine nucleotides (6). It bound βγ only in the presence of XDP and could be activated by XTP. We continued to study the interaction of GoX with G protein-coupled receptors in this report. The interaction of G proteins and their receptors is best demonstrated in two experiments: agonist-stimulated GTPγS binding of G protein α subunits and inhibition of high affinity ligand binding of the receptors by GTPγS. To test if GoX can interact with G protein-coupled receptors and be activated by their agonists, we reconstituted purified GoX with Sf9 cell membranes containing m2 MACHR or NFPR. First, we found that binding of XTPγS to GoX was inhibited by the muscarinic agonist carbachol. In both cases, βγ was required for the carbachol-dependent nucleotide binding, suggesting that only GoXβγ heterotrimer could interact with the receptors effectively. Second, we tested GoX to determine whether it could induce the high affinity state in NFPR receptors expressed in Sf9 cells. The NFPR expressed in these cells is known to be in the low affinity state probably because of lack of mammalian Gi-like proteins in Sf9 cells (11). In our experiments, we found that GoX could convert NFPR into the high affinity state in the presence of βγ and XDP, and this effect was inhibited by XTP. These two experiments demonstrated that GoX, when in complex with βγ and XDP, could interact with G protein-coupled receptors effectively and be activated by the agonists.

Because cells lack xanthine nucleotides, GoX provides an excellent model to study empty G protein α subunits. The empty form of Goα is an important intermediate in receptor activation and has long been proposed to form a stable complex with activated receptors. However stable interaction between empty G proteins and their receptors was only reported in the transducin-rhodopsin system. Empty transducin apparently formed a stable complex with light-activated rhodopsin and the thrombin receptor and wild-type Goα observed before (18). Thus if cells are cotransfected with both m2 MACHR and GoX, we concluded that GoX inhibited thrombin receptor stimulation by competitive binding to the receptor.

To test if GoX could bind to other Go-coupled receptors in cells, we looked into the interaction between GoX and m2 MACHR. Because m2 MACHR couples only to the Gi family of Go proteins and not to the Gq family (9, 21), we could not assay their interaction in the same way as the thrombin receptor by monitoring PLC activities in COS-7 cells transfected with the receptor and GoX. Therefore, we constructed an artificial pathway by cotransfecting both m2 MACHR and G15α into COS-7 cells. G15α is known as a promiscuous G protein that can be activated by all kinds of G protein-coupled receptors, and G15α also activates PLCβ isoforms (21). In cells cotransfected with both m2 MACHR and G15α, we were able to activate endogenous PLCβ isoforms by the addition of the muscarinic agonist carbachol. We found that this m2 MACHR stimulation pathway could also be inhibited by GoX (Fig. 5d).
stayed on the rod outer segment membrane. Interestingly, deactivation of the rhodopsin did not lead to the dissociation of transducin from the complex (4). In this report, we showed that empty GoaX was able to bind to the receptor on the membrane in the absence of bg subunits and without agonists, and the interaction could be abolished by either XDP or XTP. The amount of GoaX associated with the membranes with m2 MACHR was proportional to the amount of receptor at saturation. Interestingly, binding of GoaX alone did not convert the receptor to the high ligand affinity conformation, which required the abg complex. Therefore, the binding of GoaX alone to the receptor is not functional in contrast with the binding in the presence of bg and XDP.

Because GoaX appears to form a stable complex with the receptor, we tested whether GoaX could inhibit receptor activation in cells. In transfected COS-7 cells, we showed that GoaX was able to inhibit thrombin receptor or m2 MACHR stimulated PLCβ activities via the Gq or G15 pathway, but had no effect on m1 MACHR or TRH receptor stimulation. Because both thrombin receptor and m2 MACHR are known to couple with wild-type Go, and m1 MACHR and TRH receptor only couple with Gq, we interpret the data to mean that GoaX retained the receptor specificity of wild-type Go and was able to interact with Go-coupled receptors in cells. The inhibitory binding of GoaX enables us to specifically block Go-coupled receptors in certain systems. This could be a useful means to analyze
different receptor-stimulated signal transduction pathways, and could perhaps be useful in drug screening associated with G protein-coupled receptors.

In the previous report (6), we showed that the single Goa mutant, GoaD273N, lost the ability to bind either guanine nucleotides or xanthine nucleotides and could not bind \( \beta \gamma \) under any conditions. Surprisingly, GoaD273N can still bind to receptors. In transfected COS-7 cells, we found that GoaD273N inhibited thrombin receptor and m2 MAChR activation, in a fashion similar to GoaX (Fig. 5, a and d). GoaD273N also retained the same receptor specificity as wild-type Goa; i.e. it had no effect on m1 MAChR or TRH receptor stimulated pathways (Fig. 5, b and c). In the Sf9 cell membrane binding assay, it only bound to the m2 MAChR membranes, not to the control wild-type Sf9 cell membranes. However in contrast to GoaX, GoaD273N was not released from the m2 MAChR membranes by XDP or XTP, consistent with its inability to bind nucleotides (data not shown). The reason that GoaD273N mutant proteins do not bind xanthine nucleotides is not clear. Apparently it must have a structure similar to that of the empty Goa which enables it to bind receptors, but the structure is probably not stable locally around the nucleotide binding pocket. Nevertheless, GoaD273N may also be useful as a dominant-negative inhibitor of receptor functions.

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