Winston Sanders

**A Novel Method to Study Differential G-protein Activation States Utilizing X-mutant G-proteins**

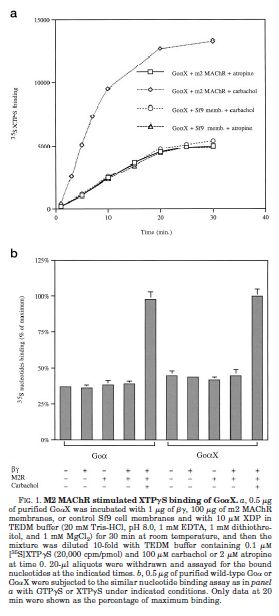
1. **Introduction**

Considering that for decades the U.S. has been spending over $325 billion dollars in prescription drugs1, opportunities for more efficient methods of ailment treatment should be ripe for investment. So, what if the medicinal effects of a drug could be amplified and its unwanted side effects diminished? For example, the mu opioid receptor (MOR) mediates the effects of pain relieving opioid drugs, such as morphine, but also mediates unwanted side effects such as respiratory depression, which can be fatal2. In order to accomplish selective activation of receptors, the pathway of cellular regulation that the drugs activate need to be closely examined. The ability of certain drugs, or agonists, to specifically activate one pathway over another is called stimulus trafficking. These agonists can be naturally occurring or synthetic compounds which bind to and activate receptor molecules on the surface of the cell. Once bound, the agonist activates the receptor, which in this case, is a MOR, a 7-transmembrane protein defined as a GPCR (G-protein coupled receptor)3.

GPCRs are the link between an extracellular drug stimulating effect, and the body’s internal response. They have an extracellular N-terminus and 3 extracellular loops that interact with external stimuli, such as small molecule agonists, and an intercellular C-terminus, and 3 intracellular loops which mediate communication with intracellular proteins. Once activated, a GPCR is then able to activate a G-protein, a heterotrimeric protein that is attached to the surface of the cell membrane, and binds to the intracellular C-terminus and/or the 3rd intracellular loop of a GPCR4. Upon binding, agonist ligands stabilize a particular conformation of the receptor which favors the activation of specific G-protein types, such that intracellular signaling can vary based on the possible pathways using different agonists.

In the past, methods of examining the receptor coupling preferences to different G-protein (Ga subtypes) were investigated using bacterial toxins. In particular, treatment of cells with the *Bordetela pertussis* toxin (PTX), which ADP-ribosylates Gi/o proteins and renders then unresponsive to GPCR-mediated activation, was utilized to measure how different agonists may generate specific activation states of the receptor. After creating and transfecting PTX-insensitive mutants of Gi/o proteins, comparisons of potency (measured by the drug concentration required to produce 50% maximal effect, or EC50) along with relative efficacy (measured as the maximal stimulation of G-protein activation, or Emax) across MOR agonists were made between cells expressing different PTX-insensitive G subtypes. Though the findings from these experiments confirmed that while the PTX-insensitive mutants couple with the agonist-activated MOR after PTX treatment, whereas endogenous subtypes (PTX-sensitive) were not, there were no differences in the order of relative agonist efficacy or potency between different agonists among the different G subtypes tested. However, PTX ADP-ribosylates the G proteins at a Cysteine residue in the C-terminal region, which can interfere with the communication between GPCR and G-protein. Thus, PTX mechanisms result in unnatural covalent modification of the G-protein5. This proposal addresses an alternative methodological approach to investigate the ability of GPCRs, activated by distinct ligands, to activate specific G-protein subtypes.

1. **Experiment**

The different pathways of GPCR activation used would need to be observed; but how would the different pathways be able to be identified once activated? When active, G-proteins “turn on” when the subunit replaces bound GDP with GTP from the cytoplasm and dissociating from their bound -subunit dimer, and turn off by hydrolyzing the GTP back to GDP and re-associating with The article *Interaction of the Xanthine Nucleotide Binding Goa Mutant with G Protein-coupled Receptors* gives results of a constructed subunit double-mutant ((mutant Go (Goa) mutated into GoaX)) in the Go family of G-proteins, GoaX, which binds xanthine nucleotides rather than guanine nucleotides. This mutant subunit would only bind to its -subunit binding partner while in the presence of XDP, and only dissociate from Gwhen XDP was exchanged for XTP. These experiments further demonstrated that the mutant -subunit’s ability to interact effectively with a GPCR remained conserved , in that agonist-activated muscarinic acetylcholine receptors (MAChRs) in Sf9 cells could stimulate XDP-XTP exchange similarly to the way the receptor normally stimulates GDP-GTP exchange in wild-type G-proteins. Part a in the results from Fig. 1 depicts data for the binding of of [35S]XTPS, a radiolabelled hydrolysis-resistant XTP analog, by purified GoaX when incubated by G, M2-MAChR or control (untransfected) Sf9 cell membranes in the presence of the agonist ligand, carabachol, or the antagonist ligand, atropine. The results show a time-dependent activation of the Go X-mutant, as measured by stimulation of [35S]XTPS binding, only when the M2-MAChR is present and activated by carbachol. Section b of the results of Fig 1. again illustrates the binding of [35S]XTPS however, both purified wild-type subunit from the Go family (Go) and the double mutant (GoX) were compared under similar experimental conditions which indicated that they both are activated in the same way by agonist-bound receptor, except that the wild-type G-protein binds and exchanges guanine nucleotides whereas the X-mutant G-protein binds and exchanges xanthine nucleotides. These results suggest that mutant GoX is able to maintain the same natural responses as a wild-type Gsubunit to receptor activation.6

1. **Discussion**

With this knowledge, one may expectedly be able to utilize the xanthine nucleotide-binding mutant G proteins to act as efficient indicators for the specific activation states of the receptor towards different agonists without the experiment being complicated by the presence of multiple guanine nucleotide-binding G-protein types that are normally present in cell membranes. This would be accomplished by individually co-transfecting specific X-mutant G protein subtypes with our receptor of interest, in this case the MOR, and measuring the potency and efficacy of different agonists to activate the different mutant G subtypes. Furthermore, these experiments show that this approach is unlikely to interfere with receptor coupling to the mutant G, unlike the PTX-insensitive point mutation. Certainly this would be a model method of study that deserves immediate attention.

**References**

# Ressler T, (2013) Prescription Drug Spending Drops As Struggling Americans Are Forced To Cut Back On Health Care. (<http://thinkprogress.org/health/2013/05/10/1993841/prescription-drug-spending-drops/>)

# Saidak Z, Palmer K, Hay D, Northup J, Glass M (2006) Differential activation of G-proteins by μ-opioid receptor agonists. (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1751342/>)

# Milligan G, Kostenis E (2006) Heterotrimeric G-proteins: a short history. (<http://www.ncbi.nlm.nih.gov/pubmed/16402120>)

# Hamm H, (2001) How activated receptors couple to G proteins. (<http://www.pnas.org/content/98/9/4819.full.pdf>)

# Clark MJ, Furman CA, Gilson TD, Traynor JR (2006) Comparison of the relative efficacy and potency of mu-opioid agonists to activate Galpha(i/o) proteins containing a pertussis toxin-insensitive mutation.( <http://www.ncbi.nlm.nih.gov/pubmed/16436499>)

# Yu B, Simon M (1998) Interaction of the Xanthine Nucleotide Binding Goa Mutant with G Protein-coupled Receptors\* (<http://www.jbc.org/content/273/46/30183.abstract>)

# 