**Novobiocin as an Allosteric Modulator of Ste2p**

Jeffrey K Rymer, Melinda Hauser, Jeffrey M. Becker

G protein-coupled receptors (GPCRs) are the target of 30-50% of all prescribed drugs for human medicine and are therefore the subject of intense study by the scientific community. It has been recognized recently that compounds called allosteric modulators can regulate GPCR activity by binding a GPCR at sites not occupied by the normal receptor-activating molecule. Such allosteric compounds are desirable drug candidates as they may produce fewer toxic side-effects than standard drugs that target GPCRs. The purpose of this study was to determine the interaction of different allosteric modulators with Ste2p, a model GPCR expressed in the yeast *Saccharomyces cerevisiae.* An allosteric peptide, [Bio-DOPA]11-mer, was chemically cross-linked into Ste2p in the presence and absence of another allosteric modulator, the antibiotic novobiocin. The receptor was isolated, collected, and then visualized by protein immunoblot. One of the blots detected the presence of the receptor, and the second blot detected the presence of the biotinylated ligand-receptor complex containing the cross-linked [Bio-DOPA]11-mer. Analysis of the blots revealed that the receptor was present in all of the samples and that there was significantly less [Bio-DOPA]11-mer cross-linked to the receptor in the presence of novobiocin. This experiment demonstrated that both novobiocin and [Bio-DOPA]11-mer competed for a similar site of the receptor. Thus, these two compounds that are very different in their chemical structure occupy a similar allosteric site to regulate GPCR activity. Further experimental analysis may provide insights into the mechanisms utilized by these compounds to influence GPCR function. These results may prove useful in the optimization of allosteric modulators as therapeutic agents for GPCR-based pathologies.