

Report on the ISN Symposium “Ligand Directed Signalling at Opioid Receptors” held as part of the 44th International Narcotics Research Conference in Cairns, Australia, July 14-19 2013.

The 44th INRC was a highly successful meeting drawing 125 participants from all over the world. Approximately 40 were from Australia/New Zealand, 35 from the US and Canada, 20 plus from Japan and a dozen or so from each of the rest of Asia and Europe. Attendees included 2 scientists who attended the very first INRC in Aberdeen, Scotland, in 1971 – Graeme Henderson and Brian Cox, both of who remain at the cutting edge of research into the biology of opioids and opioid receptors.

The ISN Symposium featured 3 leading researchers specializing in G protein-coupled receptor biology – Arthur Christopoulos from the Monash Institute of Pharmaceutical Sciences in Melbourne, Australia, Eamonn Kelley from the University of Bristol, U.K, and Graciela Pineyro from the Université de Montréal, Canada. Short talks selected from submitted papers rounded out the Symposium, and these also contained some exciting new findings.

Together with the realization that most if not all GPCR are likely to have targetable allosteric modulatory sites, the concept of ligand directed signaling is currently dominant in Pharmacology. In short, the idea is that ligand action at GPCR is not differentiated simply by efficacy, but that different ligands can promote (or inhibit) signaling to different degrees through distinct intracellular pathways; in some cases drugs can essentially be an agonist at one signaling pathway while inhibiting signaling through another. Importantly, these patterns of preference are ligand dependent, and do not simply reflect differences in the ease with which a given receptor will couple to an intracellular signaling pathway. Given that for the most part the analgesic and rewarding as well as constipating and respiratory depressant properties of opioids are mediated through the μ -

opioid receptor, of which there is one generally recognized type, the idea that subsets of the multiple signaling cascades activated by this one receptor could be selectively targeted by just the right ligand has proven highly attractive to the field. For better or worse, the idea of a non-addictive/over-dose proof opioid analgesic is back on the table....

The Symposium began with a stroll through the basic ideas of bias and allostery by Arthur Christopoulos, illustrated by his groups work on the muscarinic receptor (for the most part). Receptor mutagenesis, highly powered molecular dynamic simulations and chemistry came together to illustrate how it is ultimately possible to identify the amino acids forming an allosteric site on a GPCR and then design a new ligand to fit snugly into this site. The idea that functionally linked amino acids would be needed to transmit the allosteric modulation to the orthosteric signal was explored, together with the new ways of grappling with and presenting data arising from testing complex interactions. An important idea was being able to differentiate intrinsic mechanisms of bias from preferences introduced by the system in which the experiments were being done, such preferences may arise from differing relative levels of of effector or regulatory molecules influencing the strength of coupling.

Eamonn Kelley then explored the important area of quantifying ligand bias. Christopoulos and colleagues have developed innovative schemes for visually displaying relative bias for multiple ligands and multiple effectors, but several mathematical schemes exist for putting a number on the bias. Eamonn used a comprehensive μ -opioid receptor data set generated in cider country to illustrate the pros and cons of each method. A rigorous determination of efficacy at each of the pathways being investigated for bias is necessary, as it is the ratio of efficacy for individual ligands at different pathways that forms the basis of bias calculations. The necessity to chose (and stick

with) a reference ligand for was clearly illustrated – bias is of course always relative to other drugs, and not an absolute quantity.

Graciela Pineyro then shifted attention to signaling through the δ -opioid receptor, where ligand selectivity has been a feature of research for a number of years. Using a comprehensive series of assays of receptor signaling, cell surface expression and direct receptor/effector interaction determined through BRET, ligand-dependent differences in coupling and receptor regulation were starkly illustrated. Importantly, Graciela also showed the distinct differences between cell types, attributable to differential action of G protein receptor kinase 2 and protein kinase C (both important in neurons rather than HEK 293 cells). While the efficacy for inhibition of adenylyl cyclase, activation of protein kinase C and recruitment of β -arrestin seemed to vary consistently with δ -ligands across different cell types, the recruitment of GRK2 did not, suggesting a key role for this kinase in differentiating delta ligands.

These 3 speakers were followed by 5 short presentations drawn from abstracts submitted to the meeting. Meritxell Canals from Monash Institute of Pharmaceutical Sciences presented data illustrating the utility of quantifying μ -opioid receptor activity across a range of effectors in a number of cell types to derive bias factors. Andrew Alt from Bristol Myers Squibb presented data on the first series of positive allosteric modulators (PAMs) of μ -opioid receptors to be identified. Some compounds were purely allosteric enhancers (BMS-986121) and significantly increased the potency of a range of opioid agonists, some also had intrinsic efficacy at higher concentrations than the PAM activity (BMS-986-122) while others acted as antagonists of PAM activity without displaying any efficacy themselves. Andrew introduced a new pharmacological term to the INRC – the “shifty fifty” (SH_{50})– the concentration at which a PAM exerted 50 % of its enhancing effect. INRC President John Traynor followed with more data on the BMS compounds, showing that they

increased agonist affinity as well as efficacy, and demonstrating that the effects could still be seen on purified receptor homomers reconstituted with G proteins. In the honoured tradition of students and supervisor everywhere, AA showed a picture of JT apparently channeling Annie Lennox, while JT hit back with AA looking like an escapee from Twisted Sister...look them up if the '80s were too long ago....The final presentations from Erin Bobeck (Washington State University/OHSU) and James Zadina (Tulane University) examined the potential in vivo consequences of ligand bias. James Zadina presented a series of endomorphin analogs with reduced reward, dependence and glial activation liability paired with prolonged analgesic efficacy – when compared to morphine. Erin Bobeck reported that manipulations designed to variously attenuate G protein coupling or μ -receptor internalization differentially affected the anti-nociceptive effects of morphine, DAMGO or fentanyl injected into the PAG of rats, potentially pointing to different receptor mechanisms underlying the effects of these ligands.

The ISN-sponsored Symposium was great success, and the presentations and discussions indicated that the paradigms of ligand bias and allosteric modulation of signaling continue to produce new insights into GPCR function and drug action. The INRC thanks the ISN for its generous support.

Budget:

The money from ISN went towards economy airfares for Eamonn Kelly - AUD 2509.46 (from Bristol); Graciela Pineyro - AUD 3248.19 (from Montreal). The balance of speaker expenses totalling (\$700 registration for each speaker, \$350 airfare for Arthur Christopoulos, ~\$1000 accommodation for AC) were paid out of funds raised from Registrations and other general sponsorship.