Pharmacological Evaluation of CNO as a Tool to Determine *in vivo* GPCR Function

We have developed a chemical genetic approach to study the function of the M_1 muscarinic receptor *in vivo*. This method involves mutating the receptor to remove receptor responsiveness to its natural ligand, acetylcholine (ACh), while simultaneously engender activity to an otherwise inert compound, Clozapine-N-oxide (CNO). These mutations, $Y^{3.33}C$ and $A^{5.46}G$, were initially developed within the human M_3 muscarinic receptor (1) and are conserved residues located within the binding pocket of members of the muscarinic receptor family. Prior to the generation of a knock-in mouse model expressing the mutant receptor (termed M_1 RASSL) we have characterised the pharmacological properties of CNO *in vitro*.

In competition radioligand binding studies at the human M_1 receptor using ^{3}H-NMS, CNO competitively bound to the orthosteric site, producing a K_d of -5.35 ± 0.04, compared to ACh binding with a K_d of -4.85 ± 0.07 Using an inositol phosphate accumulation assay, figure 1, we show that CNO was able to cause inositol phosphate accumulation with EC_{50} of -5.61 ± 0.23 and -5.29 ± 0.27 at the human and mouse wild-type M_1 muscarinic receptors, respectively. The compound produced maximal response ~30% of the response produced by ACh and hence behaved as a partial agonist. Functional antagonism of the mouse and human M_1 receptors using inositol phosphate accumulation show CNO functionally antagonise the ACh response at the mouse M_1, with a pA_2 of -5.35 ± 0.09, when the two ligands were co-added.

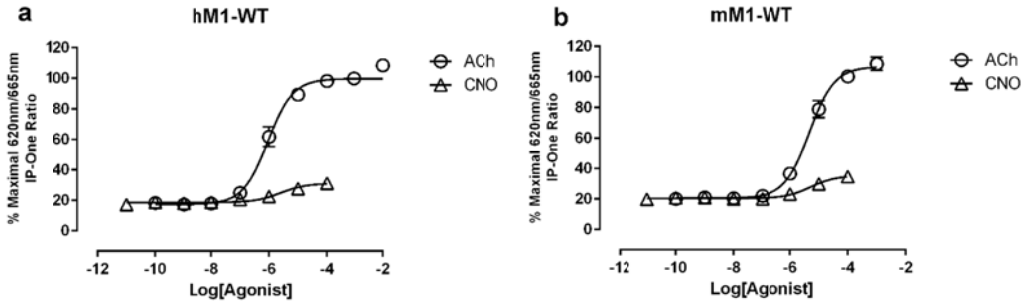

Figure 1. Inositol phosphate accumulation concentration response curves for human M_1-WT (**a**) and mouse M_1-WT (**b**) receptors with ACh and CNO. Data are shown as mean ± SEM n=3.

These data suggest that CNO, unlike previously thought, possesses pharmacological activities at the M_1 muscarinic receptors. It is therefore important to select the right dosage for *in vivo* studies in order to avoid toxic or unwanted off target effects. To further evaluate CNO we will characterise its pharmacological properties at other members of the muscarinic receptor family, as any interactions between CNO and these receptors will also have an effect on the *in vivo* dosing.

Armbruster BN *et al.* (2007) PNAS **104:** 5163–5168.