

## **NOP RECEPTOR AND FUNCTIONAL SELECTIVITY**

1)Ruzza C. 2)Ferrari F. 3)Malfacini D. 4)Asth L. 5)Rizzi A. 6)Zaveri NT. 7)Guerrini R. 8)Calò G.

*University of Ferrara Section of Pharmacology, Department of Medical Sciences*

G protein coupled receptors (GPCRs) are cell-surface proteins that control a large number of biological functions via G protein signaling. Recent evidence demonstrated that these receptors are also able to signal via  $\beta$ -arrestins. The ability of a ligand to selectively stimulate G protein or  $\beta$ -arrestin pathways is named functional selectivity. A biased agonist (BA) is a ligand able to selectively promote the interaction of the receptor with one effector e.g. G protein but not  $\beta$ -arrestin or vice versa. It is now evident that Bas may deliver more effective and/or better tolerated innovative drugs. The neuropeptide nociceptin/orphanin FQ (N/OFQ) is the endogenous ligand of an opioid like receptor now named N/OFQ peptide (NOP) receptor. NOP receptor activation promotes activation of Gi proteins as well as recruitment of  $\beta$ -arrestin 2. Via NOP receptor activation N/OFQ controls a large variety of biological functions in the central nervous system (pain transmission, stress and anxiety, mood, locomotor activity, drug abuse, learning and memory) and in the periphery (cardiovascular and renal functions, gastrointestinal motility, and some reflexes such as micturition and cough). Considering its pleiotropic nature, the N/OFQ – NOP system represents a useful model for the investigation of functional selectivity and to challenge the hypothesis that BAs can be developed as innovative drugs. For the in vitro identification and pharmacological characterization of NOP BAs a bioluminescence resonance energy transfer (BRET) assay, that measures NOP/G-protein and NOP/ $\beta$ -arrestin 2 interactions, has been recently set up and validated in our laboratories by testing a large panel of standard NOP ligands together with some new compounds. This analysis led to the identification of some pharmacological tools useful for in vivo studies. In particular, AT-403 and AT-90 behave as unbiased NOP full and partial agonists, respectively. Moreover the full agonists Ro 65-6570 and MCOPPB showed a moderate bias toward G protein while UFP-113 and [F/G]N/OFQ(1-13)NH<sub>2</sub> behaved as partial agonists at NOP/G protein interaction and as pure antagonists in the  $\beta$ -arrestin 2 assay. To transduce cellular responses into behavioral output, a first in vivo study in mice has been performed, testing AT-90, Ro 65-6570, UFP-113 and [F/G]N/OFQ(1-13)NH<sub>2</sub> in the elevated plus maze (EPM), as assay predictive for anxiety, and in the forced swim test (FST), as assay predictive for depression. Ro 65-6570 and AT-090 induced anxiolytic-like effects in the EPM but were inactive in the FST. Opposite results were obtained with UFP-113 and [F/G]N/OFQ(1-13)NH<sub>2</sub>, that produced antidepressant-like effects in the FST, being inactive in the EPM. Thus NOP ligands producing similar effects on NOP/G protein interaction (agonism) but showing different effects on  $\beta$ -arrestin 2 recruitment (agonism vs antagonism) elicited different actions on anxiety and mood. These results suggest that the action of a NOP ligand on emotional states is better predicted based on its  $\beta$ -arrestin 2 rather than G-protein efficacy. We are confident that, in the near future, the use of genetic (i.e. mouse lacking the  $\beta$ -arrestin 2 protein) and pharmacological (i.e. NOP ligands highly biased toward G protein or  $\beta$ -arrestin 2) tools will allow to deeply understand the biological relevance and the therapeutic implications of NOP receptor functional selectivity.

