Development of Novel Corticotropin Releasing Factor Binding Protein Allosteric Modulators

Carolina L Haass-Koffler, Chase T Francis, Douglas J Sheffer, Mohammad Naemmuddin, Carsen K Nilsen, Selena E Bartlett, Nicholas DP Cosford, Antonello Bonci and Lorenzo Leggio

Many factors contribute to the development and maintenance of alcohol use disorder (AUD) and growing attention has been paid to stress pathways as potential pharmacological targets. There are not, however, FDA-approved medications for AUD that specifically target the stress system. The brain stress response is several pathways, among which a key role is played by the corticotrophin releasing factor (CRF). CRF exerts its effects on both the hypothalamic-pituitary-adrenal (HPA) axis and extrahypothalamic regions through binding to two receptors (CRFR1 and CRFR2) and a secreted 37 kD CRF binding protein (CRFBP). Our group was the first to show that CRF modulates synaptic input by potentiating *N*-methyl-D-aspartate-mediated excitatory postsynaptic currents through CRFBP/CRFR2 interactions in the ventral tegmental area (VTA). We have also demonstrated that loss of the CRFBP gene *CRHBP* leads to increased ethanol consumption in mice, and that a selective downregulation of *CRHBP* in the center nucleus of the amygdala (CeA) decreases brain hemodynamic activity. Recently, by utilizing a novel cell-based assay, we showed that CRFBP(10 kD) fragment is able to potentiate CRF-intracellular Ca^{2+} release, demonstrating that CRFBP may possess excitatory roles in addition to the inhibitory role established by CRFBP(27kD). To provide preliminary translation of our findings, we further conducted a case-control study with a clinically-relevant sample of patients affected by alcohol dependence and demonstrated that genetic variants related to CRFBP(10kD) were associated with greater risk for alcohol dependence and anxiety, while other genetic variants were associated with reduced risk for anxiety. There are currently no small molecule ligands available that selectively interact with either CRFBP or CRFR2.

Methods: We miniaturized the developed cell-based assay, where we have expressed CRF-BP(10kD) on the plasma membrane fused as a chimera with CRFR2 in order to develop a high throughput screening (HTS) assay. We screened >350,000 compounds, run a secondary assay for specificity, and selected two negative allosteric modulators (NAMs). These two compounds were then tested in an electrophysiology tertiary assay.

Results: The two identified NAMs are able to blunt CRF-induced potentiation of NMDAR-mediated synaptic transmission in dopamine neuron in the VTA.

Discussion: These results provide novel evidence for a specific role of CRFR2 and CRFBP in the modulation of neuronal activity. These results further suggest that NMDARs in the VTA may be a target for both addiction and stress.

References: Haass-Koffler, C.L., Henry, A. T., Melkus, G., Simms, J. A., Naemmuddin, M., Nielsen, C. K., Lasek, A. W., Magill, M., Schwandt, M. L., Momenan, R., Hodgkinson, C. A., Bartlett, S. E., Swift, R. M., Bonci, A. & Leggio, L. Defining the role of corticotropin releasing factor binding protein in alcohol consumption. *Transl Psychiatry,* 6**,** e953 (2016)

Funding was provided by NIH grants K01AA023867 (CLH-K), R21DA209966 & R33DA029966 (NDPC); NIDA Intramural Research Program (AB, CTF, LL); NIAAA Division of Intramural Clinical and Basic Research (LL); State of California and NIH Fast Track Award (SEB).