CHARACTERIZATION OF NOVEL FLUORESCENT LIGAND FOR NEUROPEPTIDE Y RECEPTOR TYPE 1

Kadri Ligi[1], Maris-Johanna Tahk[1], Santa Veiksina [1], Sergei Kopanchuk[1], Ago Rinken[1]

[1]*Institute of Chemistry, University of Tartu, Ravila 14a, 50411 Tartu, Estonia*
e-mail of presenting author: maris-johanna.tahk@ut.ee

Neuropeptide Y (NPY) receptors are G protein-coupled receptors with several subtypes, four of which, Y1, Y2, Y4, and Y5, are expressed in humans. The human Y1 subtype is expressed mainly in the central nervous system but is also found in vascular smooth muscle cells. Hence, it regulates several different processes such as food intake and the regulation of blood pressure. [1]

The radioligand binding assay has been the method of choice for characterization of the Y1 receptor, however, in this case usually intact cells must be used, and kinetic studies are very labor-intensive. We have developed and implemented a novel fluorescence anisotropy based assay for characterization of ligand binding for several receptors [2]. In this assay, as the source of receptors, the budded baculoviruses produced using the baculovirus expression vector system can be used [3]. One of the biggest challenges that fluorescence anisotropy assays face is finding suitable fluorescence ligands.

For NPY Y1 receptor first attempts were carried out with TAMRA labeled endogenous agonist PYY (Veiksina et al unpublished), but low stability and slow kinetics of the ligand have not generated an outstanding assay system. Recently, in collaboration with Dr. M.Keller group (Univ. Regensburg, Germany), a novel nonpeptidic fluorescence ligand MC-026 was synthesized, which is derivative of the potent antagonist UR-MK299 [4]. MC-026 has a subnanomolar affinity, better signal to noise ratio and higher selectivity towards Y1 receptor compared to TAMRA-PYY. Furthermore, the association of MC-026 to the Y1 receptor is fast (k_{on}= 0.5 $min^{-1}nM^{-1}$), and the dissociation halftime is about 20 minutes which makes it to be a suitable tool for screening of new drug candidates in equilibrium assays, as well as to detect their kinetic properties.

References

1. Walther, C., Mörl, K., & Beck-Sickinger, A. G. (2011). Journal of Peptide Science, 17(4), 233-246.
2. Rinken, A., Lavogina, D., & Kopanchuk, S. (2018) Trends in Pharmacological Sciences, 39(2), 187-199.
3. Veiksina, S., Kopanchuk, S., & Rinken, A. (2014). Biochimica et Biophysica Acta (BBA)-Biomembranes, 1838(1), 372-381.
4. Keller, M., Weiss, S., Hutzler, C., Kuhn, K.K., Mollereau, C., Dukorn, S., Schindler, L., Bernhardt, G., König, B. and Buschauer, A. (2015). Journal of Medicinal Chemistry, 58(22), 8834-8849.

Euroopa Liit
Euroopa
Regionaalarengu Fond

Eesti
tuleviku heaks