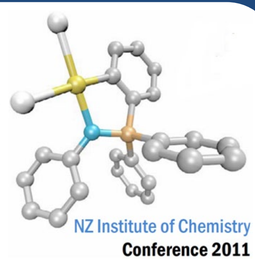
# THE QUEST FOR A GPCR SELECTIVE LIGAND - A BOTTLENECK

Johan Runesson[1], Indrek Saar[2], Kristin Karlsson[1], Rannar Sillard[1], Ülo Langel[1,2]

[1] Department of Neurochemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, Sweden

[2] Institute of Technology, University of Tartu, Estonia.




Poster board number: 29

## Introduction

The family of GPCR's has for long served as the most common pharmaceutical drug target. Even so, some receptors have failed to show any interactions when exposed to big screenings libraries in the past and consequently they also lack potent small molecules acting as ligands on these different receptors. Three of these receptors are the so called galanin receptors (GalR).

Galanin is a neuroendocrine peptide that is widely distributed in both the central and peripheral nervous systems as well as in the endocrine system. The galanin peptide family currently consists of four members, namely galanin, galanin-message-associated peptide (GMAP), galanin-like peptide (GALP) and alarin [1]. Galanin has been shown to influence several physiological processes including cognition, affective behavior, nerve injury, Alzheimer's disease, neuroregeneration, seizures, feeding, and hormone release. The co-localization with other neuromodulators and the distinct up-regulation during and after pathological disturbances has drawn attention to this peptide family. The regionally specific expression of the galanin receptors (GalR1-3) suggests different physiological roles, a feature which mostly remains unexplored due to the lack of selective ligands acting on the galanin receptor subtypes (see Fig. 1).

The first introduced receptor subtype specific ligand in the field was galanin(2-11) as a GalR2 selective ligand [2], which was an important advance in the galanin field. Unfortunately, later publications described that galanin(2-11) also binds with similar affinity to GalR3 [3], a critical limitation when delineating the interplay between galanin receptor subtypes. Even so, galanin(2-11) has been given extensive attention and been used in numerous study as a non-GalR1 ligand.

## Conclusion

| Ligand | Receptor preference | Ref. |
|---|---|---|
| Galanin | GalR1-3 | [4], Fig. 2a |
| Galanin1-16 | GalR1-3 | Fig. 2c |
| Galanin2-11 | GalR2-3 | [2-4] Fig. 2b |
| M1145 | GalR2 | [4], Fig. 2d |
| M1162 | GalR2 | Fig. 2f |
| J1 | GalR2 | Fig. 2g |
| M1154 | GalR1-2 | Fig. 2e |

We here present several promising ligands that are GalR subtype selective (see Fig. 2) and these might be future candidates to unravel the function of GalR subtypes. We are currently testing their signalling (see Fig. 3) in order to find novel agonists/antagonists for each receptor, and prepare to test them in relevant biological systems.

## Figure 1

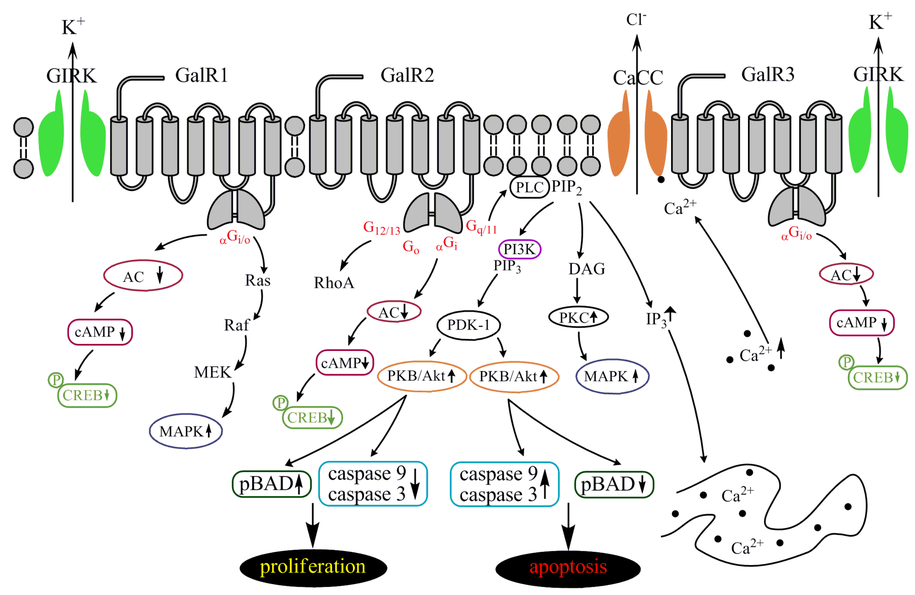



Fig 1. Intracellular signalling pathways activated by the GalR1-3.

## Figure 2

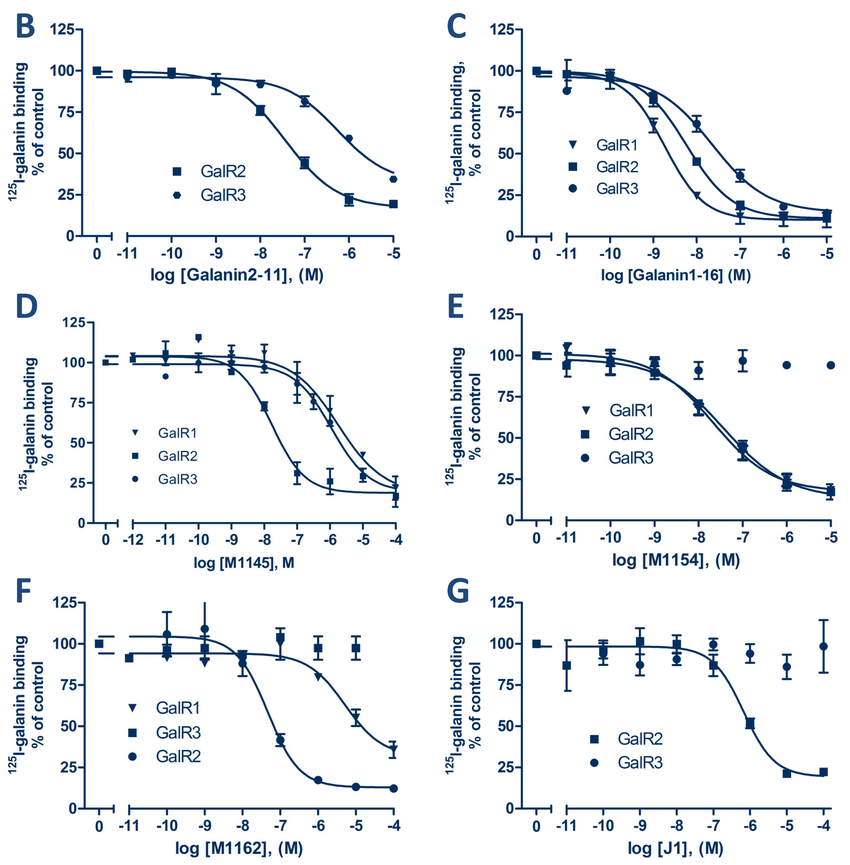



Fig 2. Galanin receptor binding studies. Displacement of porcine-[$^{125}$I]-galanin from membranes by galanin (**A**), galanin(2-11) (**B**), galanin(1-16) (**C**), M1145 (**D**), M1154 (**E**), M1162 (**F**) or J1 (**G**). Membranes were from human Bowes melanoma cells expressing GalR1, CHO cells expressing GalR2 and Flp-In T-REx 293 cells expressing GalR3. The data is from three independent experiments performed in duplicate.

## Figure 3

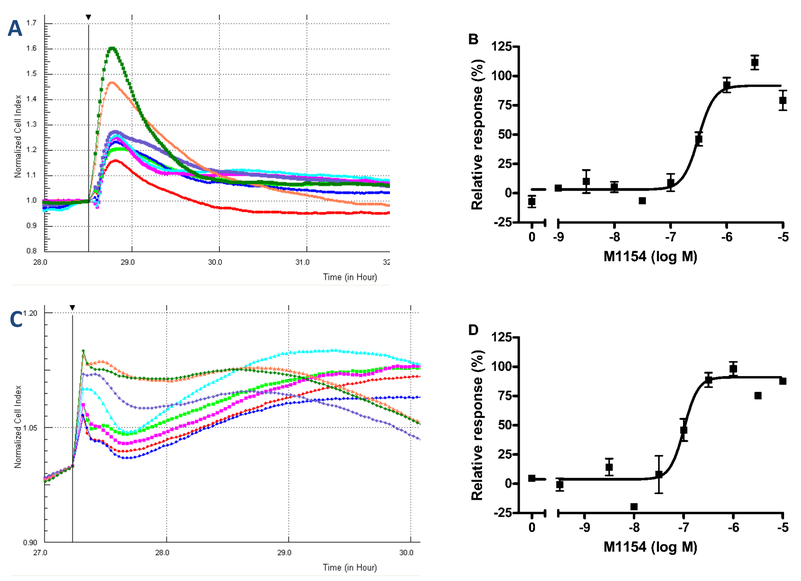



Fig 3. Example of galanin receptor signaling studies utilizing a non-label real-time instrument. Modification of impedance in Bowes melanoma cells expressing GalR1 (*A*) or CHO cells expressing GalR2 (*C*) following application of M1154, reported as normalized cell index (NCI). Dose-response curve for the induced changes in NCI by M1154 in Bowes melanoma cells expressing GalR1 (*B*) or CHO cells expressing GalR2 (*D*).

## Material & Methods

**Peptide synthesis**

The peptides were synthesized in a stepwise manner using small scale (0.1 mmol) 9-fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis strategy on an automated peptide synthesizer. Fmoc amino acids were coupled as hydroxybenzotriazole (HOBt) esters to a *p*-methylbenzylhydrylamine (MBHA) resin which will generate a C-terminally amidated peptide after the final cleavage. The peptides were finally cleaved from the resin using 95% TFA (trifluoroacetic acid), 2.5% TIS (*triisopropylsilane)* and 2.5% $H_2O$ solution for 3 h. All the peptides were purified by reverse-phase HPLC using Nucleosil 120-3 C-18 column and correct molecular weight was determined by matrix assisted laser desorption ionization time-of-flight mass spectrometry.

**Galanin receptor binding studies**

Cells for $^{125}$I-galanin-receptor displacement studies were seeded in 150 mm dishes and cultured 3–4 days until confluent. Cell dishes were washed and scraped into phosphate-buffered saline and centrifuged twice at 4 $^0$C, 3000g for 5 min. The pellet was resuspended in assay buffer (20 mM HEPES, 5 mM $MgCl_2$, pH 7.4) supplemented with 5 mM EDTA and incubated on ice for 45 min before centrifugation at 4 $^0$C, 8500x g for 15 min. After washing the pellet in assay buffer and repeated centrifugation the obtained pellet was resuspended in assay buffer supplemented with 1% protease inhibitor cocktail and stored at -80 $^0$C, until used. Displacement studies on cell membranes were performed in a final volume of 200 µl, containing 0.15 nM porcine-[$^{125}$I]-galanin, 30 µg cell membrane, and various concentrations of peptide ($10^{-4}$ to $10^{-12}$ M). Peptide solutions were made in assay buffer supplemented with 0.3% BSA using silanised tubes and pipette tips. Samples were incubated at 37 $^0$C for 30 min while shaking after which the samples were transferred and filtered through a filter plate pre-soaked in 0.3% polyethylenimine solution using vacuum. The filters were washed thrice with assay buffer and the retained radioactivity was determined in a β-counter. "IC50" values for the peptides were calculated using GraphPad Prism and converted into $K_i$ values using the equation of Cheng–Prusoff (Cheng and Prusoff, 1973).

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