M1154 – A novel galanin ligand to delineate the galaninergic system

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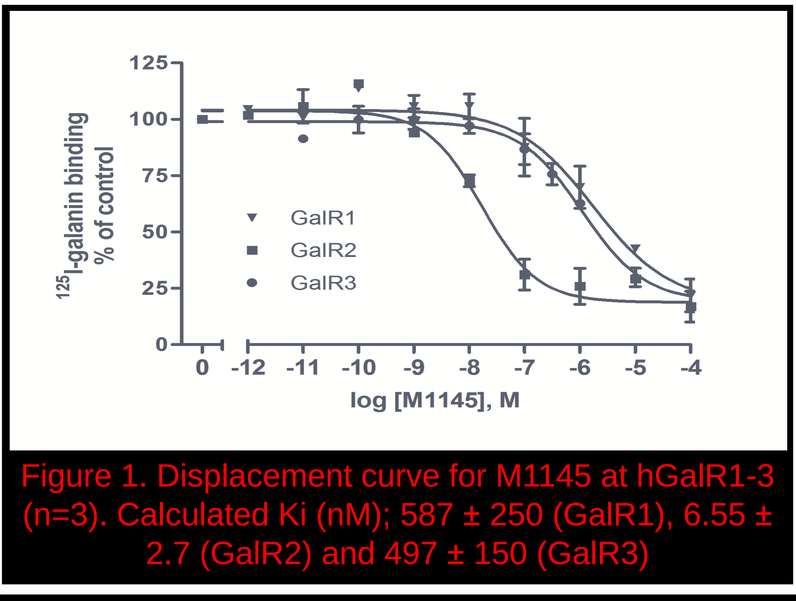

Figure 1. Displacement curve for M1145 at hGalR1-3 (n=3). Calculated Ki (nM); 587 ± 250 (GalR1), 6.55 ± 2.7 (GalR2) and 497 ± 150 (GalR3)

SUMMARY

M1154, a GalR1/2 selective agonist, could be used to delineate the galanin receptor subtypes involved in pathological processes, preferentially together with the already published M1145. Furthermore, there's therapeutic benefits could be evaluated.

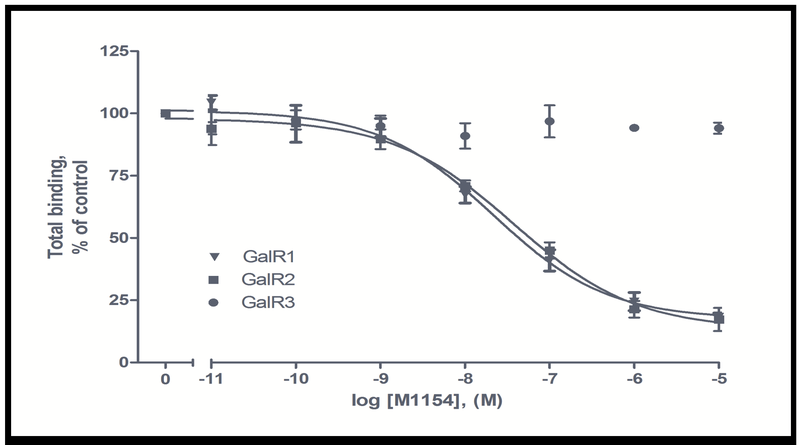

Figure 2. Displacement curve for M1154 at hGalR1-3 (n=3). Calculated Ki (nM); 11.7 ± 7.2 (GalR1), 14.4 ± 4.2 (GalR2) and no detectable binding (GalR3)

INTRODUCTION

The 30 amino acid long neuropeptide galanin has been shown to be involved in a variety of physiological and pathological processes [1].

The galanin-peptide family consist of galanin, galanin message-associated peptide (GMAP), galanin-like peptide (GALP) and alarin.

To distinguish between the three receptor subtypes, GalR1-3 (Fig 3), in biological experiments, selective ligands are of great importance, particularly since subtype specific antibodies are not available [2]. The peptide M1145 (Figure 1) was published 2009 [3].

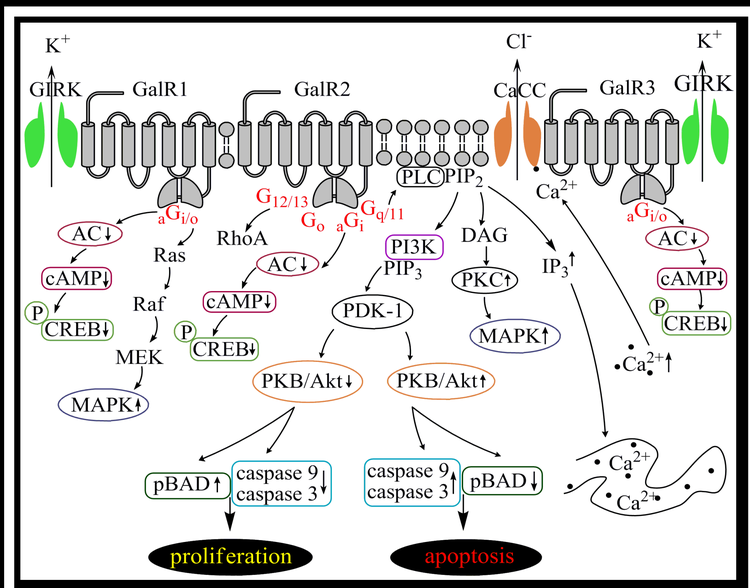

Figure 3. Signaling induced by activation of GalR1-3

MATERIAL & METHODS

Binding

^{125}I-galanin-receptor Binding displacement experiments with M1145 and M1154 were performed on cell membranes from human Bowes melanoma cells endogenously expressing hGalR1, CHO cells stably transfected with hGalR2 and Flp-In TREx 293 cells with inducible expression of hGalR3. Studies were performed in a final volume of 200 µl, containing 0.1 nM porcine-[^{125}I]-galanin (2200 Ci/mmol), 30 µg cell membrane, and various conc. of peptide (10^{-4}-10^{-11} M). Samples were incubated at 37 °C for 30 min while shaking after which the samples were transferred and filtered through a MultiScreen-FB filter plate and the retained radioactivity was determined in a β-counter. IC_{50} values for the peptides were converted into K_i values using the equation of Cheng–Prusoff.

Signalling

Signal transduction through hGalR1 and hGalR2 was examined by uses of the xCELLigence system. Cells were seeded 24 h before addition of various concentration of M1154 (10^{-5}-10^{-9} M) in a final volume of 200 µl. Data were collected during one hour and the data were analysed based on the area under curve value.

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[2]. Lu, X., Bartfai, T. (2009). Analyzing the validity of GalR1 and GalR2 antibodies using knockout mice. *Naunyn Schmiedbergs Arch. Pharmacol.* 379(4), 417-420.
[3]. Runesson, J., Saar, I., Lunström, L., Järv, J., Langel, Ü. (2009). A novel GalR2-specific peptide agonist. *Neuropeptides*. 43, 187-192.

RESULTS

The peptide M1154 retains high affinity binding to GalR2 (14.4 nM), comparable to the published M1145 (Ki 6.55 nM).

M1154 binds with similar affinity to GalR1 (Ki 11.7 nM), disparate with the loss of affinity towards GalR1 seen for M1145 (Ki 587 nM).

M1154 has no detectable binding to GalR3 at all tested concentrations.

M1154 were shown to induce a concentration dependent response through both GalR1 (Figure 4; EC50 220 nM) and GalR2 (Figure 5; 370 nM).

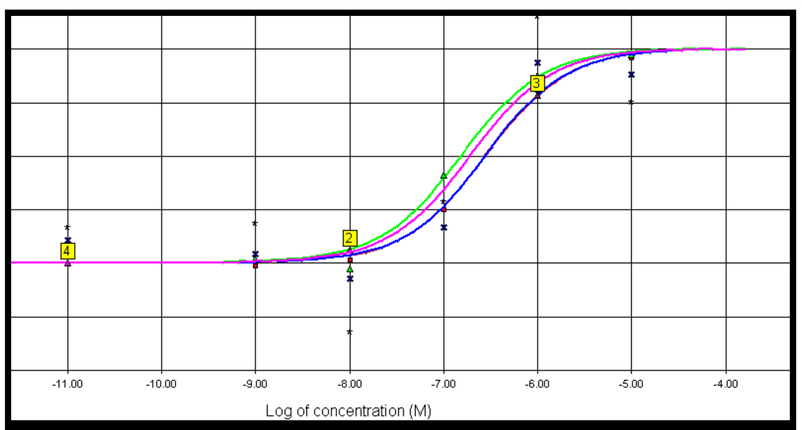

Figure 4. Induced cAMP response after hGalR1 activation by peptide M1154. EC50 220 ± 60 nM (n=3).

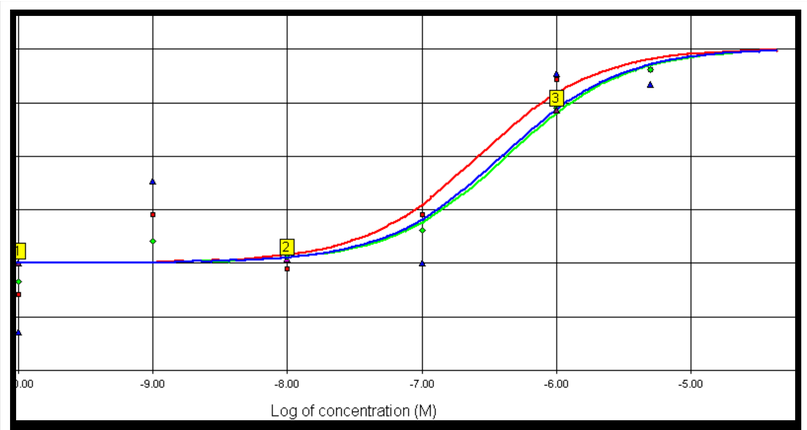

Figure 5. Induced IP response after hGalR2 activation by the peptide M1154. EC50 370 ± 87 nM (n=3).