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Functional characterisation of native GRPR activity in an immortalised cell line

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Introduction: Gastrin releasing peptide receptor (GRPR) is a GPCR which regulates key processes in the gastrointestinal and central nervous systems (1). Expressional and functional changes in GRPR have been associated with neurodegenerative, neurodevelopmental and psychiatric disorders (2); this highlights the potential therapeutic effect of targeting GRPR in the treatment of various brain disorders. The aim of this study was to identify and characterise a disease relevant cell line natively expressing GRPR.

Method: Gene transcription studies were used to identify CNS cell lines with relatively high transcription of the GRPR gene and these were tested functionally in calcium flux assays. The native cell assays were optimised and tool compounds, PD-176252 (PD) and RC-3095 (RC) were profiled in agonist and antagonist modes (with EC_{80} of GRP). Native GRPR activity was compared to that observed in a stable CHO-hGRPR cell line.

Results: U251-MG human neuroblastoma cells exhibited predominant GRPR gene transcription and large functional responses to GRP, neuromedin B and bombesin with the same order of potency as the stable hGRPR cell line. EC_{50} values for GRP, neuromedin B and bombesin were 1.7, 34 and 0.4 nM in U251-MG cells (n = 4); and 8.7, 13.0 and 2.7 pM in CHO-hGRPR cells (n = 2). PD and RC fully antagonised the GRP response in both cell lines; IC_{50} values (µM) for PD and RC were 0.119 and 0.003 in U251-MG cells (n = 2) and 0.6 and 0.004 in CHO-hGRPR cells (n = 2). Neither compound displayed agonism in the U251-MG assay, whereas both demonstrated a degree of agonism in the CHO-hGRPR cells. The native GRPR assay had high reproducibility with a Z' value of 0.62 (n = 4).

Conclusion: A robust, HTS amenable U251-MG calcium flux assay has been established to monitor native GRPR activity. This study has highlighted differences in compound activity and potency between native and recombinant GRPR assays. The CNS native cell system is likely to provide more disease relevant data compared to the artificial recombinant cell line and therefore may be an important tool in profiling potential drug candidates that modulate GRPR function.

References: 1. Gonzalez N *et al.* (2008). *Curr. Opin. Endocrinol. Diabetes Obes.* **15**: 58-64. 2. Roesler R and Schwartsmann G. (2012). *Front. Endocrinol.***3**: 159.