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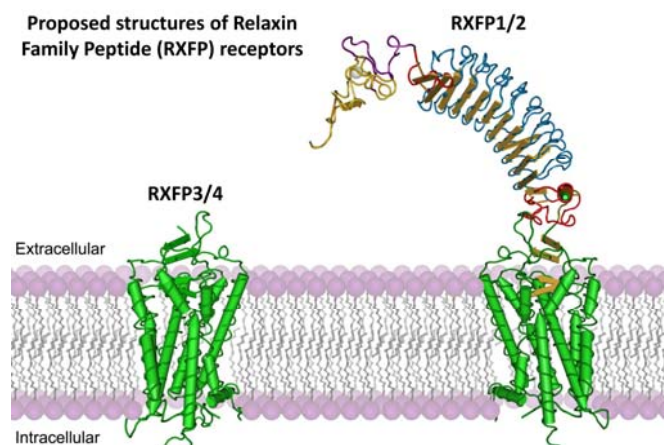
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Studies on novel G-protein coupled receptors; Relaxin family peptide receptors, evolution, structure, function and drug development

My research focuses on the relaxin peptide family and their G-protein coupled receptors RXFP1-4. The peptides relaxin, relaxin-3, insulin-like peptide 3 (INSL3) and INSL5 have numerous essential biological roles. Relaxin induces its effects by regulating collagen turnover, stimulating tissue growth and angiogenesis and inducing blood vessel dilatation. It is currently being used in a Phase III clinical trial for acute heart failure being performed by Novartis. INSL3 is essential for germ cell maturation and drugs targeting its receptor RXFP2 have considerable potential as fertility regulators in both males and females. INSL5 is a gut hormone that has potential roles in fat and glucose metabolism and we are working with Takeda Cambridge to develop compounds targeting its receptor RXFP4 which may be useful for treating obesity and/or diabetes. Relaxin-3 is a specific neuropeptide which our laboratory recently discovered (7) and has potential roles in regulating behaviours which are perturbed in mental illnesses including anxiety, depression, sleep disorders, and memory deficits. Hence drugs targeting the relaxin-3 receptor RXFP3 may be potential therapeutics to treat these mental illnesses. We are working with pharmaceutical industry partners (Johnson and Johnson, Takeda and Novartis) to determine the biological roles of the peptides and to develop drugs targeting their receptors.

Receptor projects:

The receptors for these peptides are all G-protein coupled receptors (GPCRs) which are the largest class of cell surface signaling molecules and major drug targets. The receptors for relaxin and INSL3, RXFP1 and RXFP2 are leucine rich-repeat containing GPCRs with large extracellular domains (see figure). Relaxin-3 and INSL5 interact with unrelated receptors RXFP3 and RXFP4 which are more like classic peptide GPCRs and lack a large ectodomain. We are using various molecular and pharmacological techniques to determine the ligand binding specificities of the receptors, the mechanisms of receptor activation as well their cell signaling characteristics. Furthermore we are working with A/Prof Paul Gooley using various biochemical and NMR techniques to study aspects of the receptors structure and the functions of the individual receptor protein domains. A complete understanding of the mechanism of ligand binding and activation is required to design drugs targeting these receptors.



Adeno-associated virus mediated modulation of neuropeptide function in brain

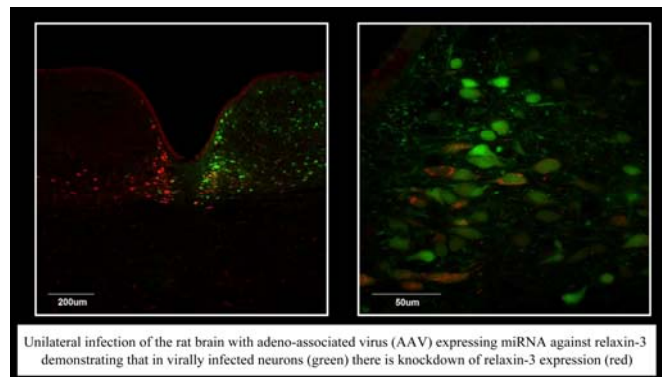
(Collaboration with A/Prof Andrew Gundlach, Florey Neuroscience Institutes)

Mental illness is a large and increasing health and economic burden in Australia and worldwide and more research is urgently required to identify new and innovative therapies. In this regard, GPCR neuropeptide receptors may be better therapeutic targets than receptors for the 'primary' transmitters (amino acids and monoamines), as they offer reduced side-effects, due to their modulatory rather than primary excitatory or inhibitory actions. Studies by our group have shown that relaxin-3 has potential roles in regulating behaviours which are perturbed in mental illnesses including anxiety, depression, sleep disorders and memory deficits. The study of neuropeptide systems is complicated by their modulatory actions such that gene knockout animals are potentially susceptible to developing functional compensation. We utilize viral gene transfer to transduce specific neuronal populations allowing the chronic modulation of neuropeptide or neuropeptide receptor function by either gene silencing or by overexpression of peptide agonists or antagonists in adult animals thus avoiding compensation.

We have successfully employed gene silencing to modulate the expression of the neuropeptide relaxin-3. We have produced adeno-associated viral (AAV) particles expressing microRNA targeting relaxin-3 which when infused at the site of relaxin-3 production, the nucleus incertus resulted in ablation of relaxin-3 expression. Co-expression of EmGFP simultaneously confirmed injection sites and labelled transduced neurons (see figure above).

We are using this technology to elucidate the role of relaxin-3 in behaviours disrupted in mental

illnesses. Additionally, we have utilized AAV and lentiviral particles overexpressing a relaxin-3 agonist to modulate feeding and arousal in rat models. This research will identify influences of relaxin-3 signaling on parallel animal behaviours to those disrupted in mental illnesses such as anxiety, depression, sleep disorders, and memory deficits; and support future studies to identify effects of altered relaxin-3 activity in experimental models of human disease to uncover new therapies for these disorders. We have intellectual property (IP) and commercial links in the area that will facilitate therapeutic opportunities.



Recent Publications:

1. Bathgate RAD, et al., (2006) International union of pharmacology (IUPHAR); Recommendations for the nomenclature of receptors for relaxin family peptides. *Pharmacological Reviews* 58: 7-31.
2. Callander GE, Thomas WG and Bathgate RAD (2009) Prolonged RXFP1 and RXFP2 signaling can be explained by poor internalization and a lack of β arrestin recruitment. *American Journal of Physiology, Cell Physiology* 296: C1058-66
3. Hossain MA, et al., and Bathgate RAD (2008) The A-chain of human relaxin family peptides has distinct roles in the binding and activation of the different relaxin family peptide receptors. *Journal of Biological Chemistry* 283: 17287 - 17297.
4. Yan Y, et al., and Bathgate RAD (2008) Identification of the N-linked Glycosylation Sites of the Human Relaxin Receptor and the Effect of Glycosylation on Receptor Function. *Biochemistry* 47: 6953-6968
5. Scott D, Wilkinson TN, Zhang S, Wade JD, Tregear GW and Bathgate RAD (2007) Identification of the INSL3 binding site in its receptor LGR8. *Molecular Endocrinology*, 21: 1699-1712.
6. Scott D, Layfield S, Hsueh A, Tregear GW and Bathgate RAD (2006) Characterization of novel splice variants of LGR7 and LGR8 reveals that receptor signaling is mediated by their unique LDLa modules. *Journal of Biological Chemistry* 281: 34942-34954.
7. Bathgate RAD, et al., (2002) Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene: Novel members of the relaxin peptide family. *Journal of Biological Chemistry* 277: 1148-1157.
8. Callander GE, Thomas WG, Bathgate RAD (2009) Development and Optimization of miRNA against relaxin-3. *Annals of the New York Academy of Sciences* 1160: 261-264.