



Molecular Pharmacology Lab

Group Head

Prof Roger Summers

BPharm(Hons, Lond) PhD(Lond)

Head of Department of Pharmacology

Tage Erlander Visiting Professor of Swedish Research Council 2003

Michael Rand Medallist 2003

Tel: +61 3 9905 1440

Fax: +61 3 9905 819

Specific Interests

Analysis of drug-receptor interactions by receptor binding, autoradiography, cell biology, molecular biology, second messenger and organ bath approaches. Discovery of novel receptors related to Family A G-protein coupled receptors by database screening and pharmacological analysis with particular emphasis on adrenoceptors. Signal transduction pathways utilized by these receptors and their alteration by drugs. The laboratory is one of the founder members of the Consortium for G-protein coupled receptor Research (CGR) formed in 2001 to facilitate collaboration in this important area of drug discovery and is a member of the Drug Discovery Research Strength of the Faculty of Medicine, Nursing and Health Sciences at Monash University.

Goal

Our goal is to identify and validate drug targets based on knowledge of the structure and function of G protein-coupled receptors (GPCRs). X-ray crystal structures of GPCR's and associated proteins are determined in collaboration with the Structural Biology Facility. This will utilize the synchrotron due to be commissioned in 2006 as well as key technologies already in place including X-ray crystallography, nuclear magnetic resonance and cryo-electron microscopy. The ligand binding properties of particular GPCRs are examined by high throughput radioligand binding to determine affinities at recombinant receptors expressed in cell culture systems. The ability of selected compounds to stimulate or block receptors or interact with allosteric sites is studied using high through put cell signalling assays including cAMP, IP3 (α -screen, Perkin Elmer), cytosensor microphysiometer and calcium mobilisation assays.



Current projects

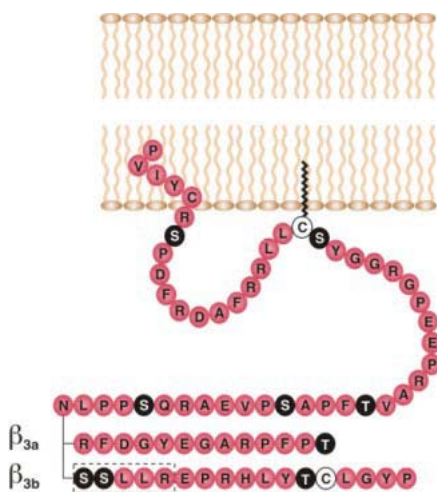
Receptor polymorphisms

In humans, mutations in GPCRs (single nucleotide polymorphisms – SNPs) can modify disease susceptibility and severity as well as responses to drugs. SNPs are common (e.g. 18 for the β 1-AR; 17 for the β 2-AR) and some of these examples are associated with diseases such as asthma, cardiac failure and obesity. SNPs may also be associated with modified responses to drugs and receptor regulation. If the GPCR polymorphism is relatively common this may lead to the rejection of

potentially useful drugs due to side effects or lack of efficacy. A better understanding of GPCR polymorphisms and their relationship to function will therefore lead to the design of drugs which are effective at mutant and wild type receptors or to the application of simple screening techniques to identify patients that may display abnormal responses. The laboratory is studying the factors that define the relationship between structural features of GPCRs and their response to drugs.

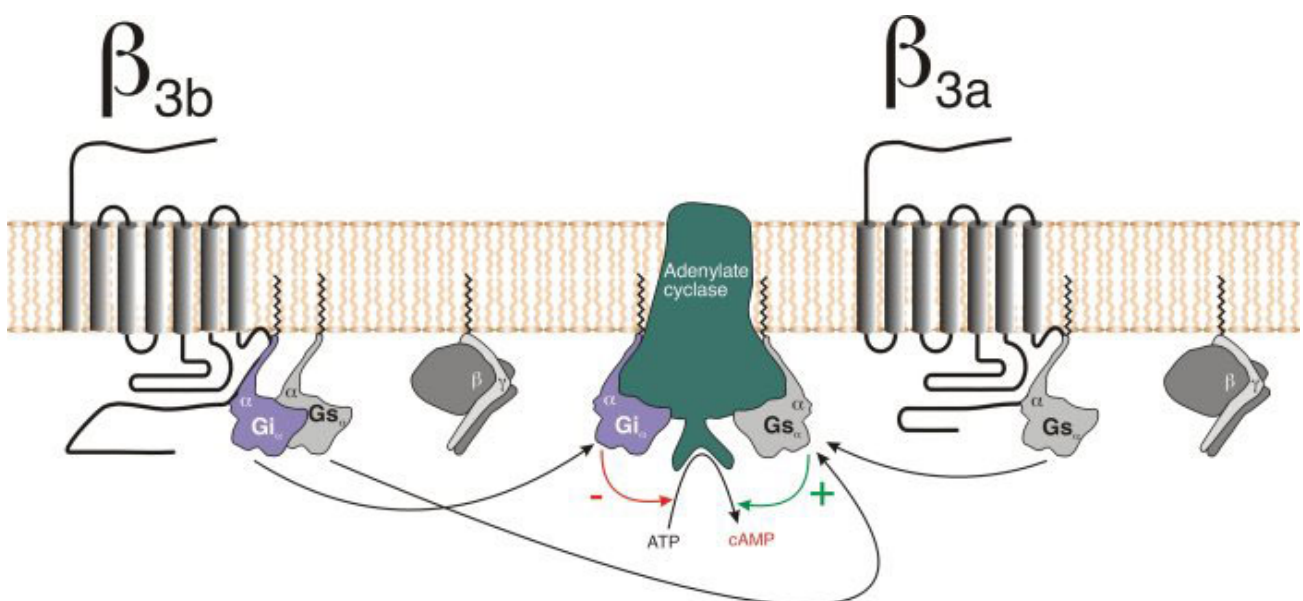
Drug interaction with GPCRs at allosteric sites: In addition to the classic agonist (and antagonist) binding site, some (and potentially most) GPCRs contain secondary, "allosteric", sites that modulate the activity of the classic binding site. We are conducting studies in collaboration with Dr Arthur Christopoulos at the University of Melbourne to study allosteric modulators that have been identified for α -adrenoceptors, and may provide an explanation for the novel pharmacology displayed by the α 1L-AR and β 1-AR. The separation of functional properties of orthosteric and allosteric sites may provide significant potential for therapeutic exploitation. Theoretically, allosteric drugs have a number of advantages over classical, orthosteric drugs, including greater specificity (allosteric sites tend to show greater sequence divergence across receptor subtypes), modulation of ongoing physiological events (thus maintaining spatial and temporal patterns of neurotransmitter or hormone responses) and minimal or differential effects on receptor desensitisation/regulation.

Splice variants



One of the ways in which a number of receptors can be derived from a single gene is by alternative splicing of the gene. The splice variants of GPCRs can vary in ligand recognition, signal transduction properties and trafficking. In the case of the α 1A-AR, there are at least 12 splice variants that share similar pharmacology, consistent with retention of identical transmembrane regions forming the ligand-binding pocket. Alternative splicing of the mouse β 3-AR mRNA produces 2 isoforms with different C-terminal tails. These isoforms have similar affinity and potency for β -AR ligands, but the β 3b-AR and a truncated β 3-AR couple to G_s and G_i , whereas the β 3a-AR couples only to G_s . We are generating mutant receptors by site-directed mutagenesis and these are being used to examine receptor specificity, G protein-coupling, signalling, and trafficking with a view to identifying functional domains of GPCRs.

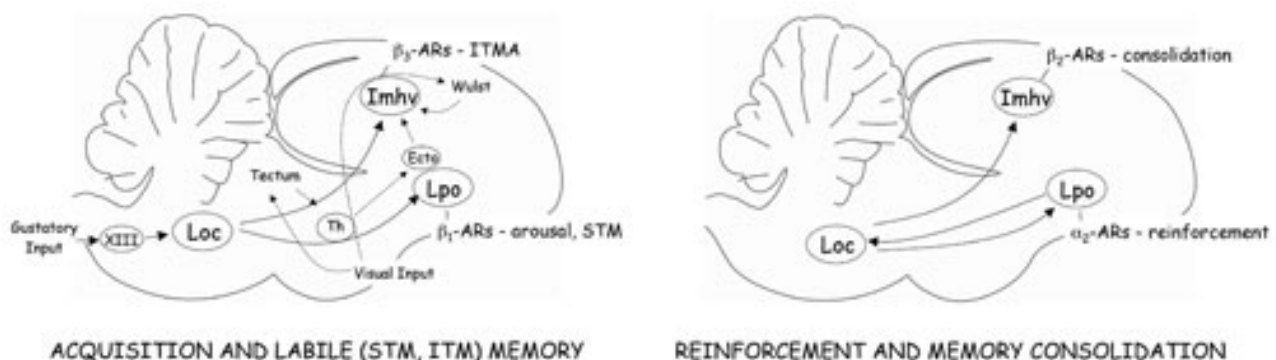
Dimer Formation



G-protein coupled receptors (GPCRs) are the major group of cell surface receptors activated by hormones and neurotransmitters. Until recently it was thought that GPCRs operated as single entities and that each receptor contained domains responsible for interaction with ligands,

coupling to G-proteins and receptor regulation. However, recent studies indicate that GPCRs interact with like-receptors, other GPCRs, or other receptors such as EGF and insulin receptors, or with accessory proteins such as RAMPs (receptor activity modifying proteins). There is evidence that these interactions influence pharmacological properties, coupling to G-proteins, and trafficking to and from the cell membrane. Our studies presently focus on adrenoceptors but the principle and methods developed will be applicable to any of the 616 genes coding for GPCRs. At present we are determining which adrenoceptor (AR) subtypes form homo- or heterodimers, whether they interact with RAMPs (in collaboration with Dr Patrick Sexton of the Howard Florey Institute) and whether this is driven by particular agonists or antagonists; whether protein/protein interactions affect pharmacological, signal transduction and internalisation properties and whether known novel receptor pharmacology such as β_4 - and α_{1L} -ARs can be explained by protein/protein interactions of known ARs. We are using Bioluminescence Resonance Energy Transfer (BRET) and functional studies employing TM3 and TM6/7 mutant receptors to provide approaches for testing drugs and determining whether compounds favouring or inhibiting dimerisation or association with RAMPs produce differential signalling via pathways such as PKA or MAPK activation. Drugs that have these properties would facilitate studies on the importance of AR protein/protein interactions in animal tissues with endogenous ARs, for example cardiomyocytes, skeletal muscle, adipocytes, and urinary tract smooth muscle. κ -opioid receptor and dopamine D2R-somatostatin SSTR5 heterodimers have been shown to have enhanced sensitivity to combinations of agonists. Thus drugs or combinations of drugs targeting receptor homo- or heterodimers may have significant therapeutic benefits.

Adrenoceptors in memory formation and consolidation



In collaboration with Dr Marie Gibbs studies are being carried out of the role of adrenoceptors in modulating memory formation. There are two important periods in memory processing where noradrenaline plays an essential role. The first occurs at the time of acquisition, where attentional or arousal factors are important and related to noradrenaline release; the second occurs at the time of consolidation, when labile memory is stored into a permanent form. The role of noradrenaline in the modulation of memory formation depends on the populations of ARs in different brain locations and the times at which they are activated in the sequence of memory processing. We are currently investigating the mechanisms of action of adrenoceptors using both in vivo (behaviour) and in vitro (cell culture and molecular pharmacology) techniques. The mechanism by which activation of β_3 -ARs promotes memory consolidation is related to the uptake of glucose at specific times after training. Similar studies are also being carried out in mice to enable the effects of removal of specific genes (knock-out mice) on memory formation and consolidation to be studied in a mammalian model. We are also studying the involvement of noradrenaline in the effect of prenatal compromise (hypoxia) on subsequent learning disabilities.

Relaxin receptors

In collaboration with Professor Geoffrey Tregear, Dr John Wade, Dr Ross Bathgate and Dr Chrisan Samuel of the Howard Florey Institute we are studying the pharmacology of the hormone relaxin. We have developed a receptor autoradiographic method with phosphorimaging detection and used this technique to identify and characterize relaxin receptors in the heart, uterus and brain. Current studies are examining the signalling pathways and functional domains of the relaxin receptors (LGR-7, LGR-8, GPCR135 and GPCR142). The studies are aimed at understanding the role of relaxin and related molecules and development of small molecules that demonstrate relaxin-like activity.

A wide range of student projects are available that utilize the techniques of molecular biology, bioinformatics, cell and tissue culture, high throughput receptor binding and second messenger screens as well as organ bath pharmacology.

Members of Laboratory

Dr Bronwyn Evans – Senior Research Fellow

Dr Marie Gibbs – Senior Research Fellow

Dr Michelle Porritt – NH&MRC Peter Doherty Fellow

Dr Dana Hutchinson – NH&MRC C.J. Martin Research Fellow (currently Wenner-Gren Institut, Stockholm)

Mrs Maria Papaioannou – Research Assistant

Ms Julia Nevzorova – Ph.D. Student

Ms Natalie Phiri - Ph.D. Student

Mr Ben Popp - Ph.D. Student

Ms Candice Rodricks - Ph.D. Student

Mr Damian Anderson - Ph.D. Student

Mr Masaaki Sato - Ph.D. Student

Ms Mitch Halls - Ph.D. Student

Ms Emma Zumpe - Ph.D. Student

Ms Kylie Longstaff – B.Sc. Honours Student

Funding

National Health & Medical Research Council

Australian Research Council

Wellcome Trust

Monash Research Fund

ANZ Trust

Collaborations

Professor Geoff Tregear, Dr John Wade, Dr Ross Bathgate, Dr Chrisan Samuel, Dr Patrick Sexton (Howard Florey Institute of Experimental Physiology and Medicine) Dr Arthur Christopoulos (University of Melbourne), Professor Barbara Cannon, Professor Jan Nedergaard, Dr Tore Bengtsson (Wenner Gren Institute, University of Stockholm), Professor Mike Cawthorne (University of Buckingham, UK), A/Prof Frank Ng (Monash University)

Recent selected publications

1. Evans, B.A., Papaioannou, M., Hamilton, S. & Summers, R.J. Alternative splicing generates two isoforms of the β_3 -adrenoceptor which are differentially expressed in mouse tissues. *Brit. J. Pharmacol.* 127, 1525-1531 (1999)

2. Kompa, A., & Summers, R.J. Desensitisation and resensitisation of β_1 - and β_4 -adrenoceptor mediated responses occur in parallel in a rat model of cardiac failure. *Brit.J.Pharmacol.* 128, 1399-1406 (1999).

3. Tan, Y.Y., Wade, J.D., Tregear, G.W. & Summers, R.J. Comparison of relaxin receptors in rat isolated atria and uterus by use of synthetic and native relaxin analogues. *Brit. J. Pharmacol.* 123, 762-770, (1998)

4. Tan, Y.Y., Dawson N.F., Kompa, A.R., Bond, C., Claasz A.A., Wade, J.D. Tregear, G.W. & Summers, R.J. Structural Requirements for the Interaction of Sheep Insulin-like Factor 3 (INSL3) with Relaxin Receptors in Rat Atria. *Eur. J. Pharmacol* 457, 153-160, (2002)

5. Gibbs, M.E. & Summers, R.J. Separate roles for β_2 - and β_3 -adrenoceptors in memory consolidation *Neuroscience*, 95, 913-922 (2000)

6. Hutchinson, D.S., Evans, B.A. & Summers, R.J. β_1 -adrenoceptors compensate for β_3 -adrenoceptors in ileum from β_3 -adrenoceptor knockout mice. *Brit.J. Pharmacol.* 132, 433-442 (2001)

7. Evans, B.A., Brown, K.J. & Summers, R.J. β -Adrenoceptors and their variants. *Analytical Pharmacology*. 2, 62-78 (2001).

8. Gibbs, M.E. & Summers, R.J. Role of adrenoceptors in memory consolidation in the chick. *Progress in Neurobiology*, 67, 345-391 (2002).

9. Hutchinson, D.S., Bengtsson, T., Evans, B.A. & Summers, R.J. Mouse β_{3a} - and β_{3b} -adrenoceptors expressed in Chinese hamster ovary cells display identical pharmacology but utilise distinct signaling pathways. *Brit. J.Pharmacol.* 135, 1903-1914 (2002)

10. Nevzorova, J., Bengtsson, T., Evans, B.A. & Summers, R.J. b₂-Adrenoceptors mediate increases in glucose transport in rat skeletal muscle cell line L6. *Brit. J. Pharmacol.* 137, 9-18, (2002)
11. Bathgate R A.D. , Samuel C.S., Burazin T.C.D., Sharon Layfield, S., Claasz A.A., Reytomas I.G.T., Dawson N.F., Zhao, C., Bond, C., Summers, R.J., Parry,L.J.,Wade, J.D., & Tregear, G.W.Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene: Novel members of the relaxin peptide family. *J. Biol. Chem.* 277, 1148-1157 (2002)
12. Samuel, C.S., Parry, L.J. & Summers, R.J.Physiological or pathological – a role for relaxin in the cardiovascular system? *Curr Opin Pharmacol.* 3, 152-158, (2003)
13. Heffernan, M., Summers, R.J., Thorburn, A., Ogru, E., Gianello, R., Jiang, W.J., Ng,F.M. The effects of hGH and its lipolytic fragment (AOD9604) on lipid metabolism following chronic treatment on ob/ob mice and b₃-AR knockout mice. *Endocrinology*,142, 5182-5189 (2001)
14. Gibbs,M.E. & Summers, R.J. Effects of glucose and 2-deoxyglucose on memory formation in the chick: interaction with b₃-adrenoceptor agonists *Neuroscience*, 113, 69-79 (2002)

Patents

1. Summers ,R.J. & Evans, B.A. Novel beta₃-adrenoceptor Australian Patent PP0620.1997
2. Summers, R.J. & Gibbs, M.E. Enhancement of memory with beta₃-adrenoceptor agonists Australian Patent PP8409, 1999.
3. Belyea, C, Heffernan, M & Summers, R.J. Australian Patent FP14944, 2001
4. Belyea, C, Heffernan, M & Summers, R.J. US Patent PCT/AU01/01086, 2003