Construction and Analysis of α2A-Adrenoceptor Gγ6/Go12-Subunit Fusion Proteins.

Richard Ward and Graeme Milligan
Molecular Pharmacology Group, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, Davidson Building, University of Glasgow, Glasgow, G12 8QQ.

Constructs expressing fusion proteins between the porcine α2A adrenoceptor and the rat Gγ1, Gγ2 Gγ3 and Gγ1 α-subunits were produced in the vector pcDNA3. Pertussis toxin resistant (for example Gγ1αC351G) and selective uncoupling (for example α2A D79N) mutations were introduced into some of the constructs. The constructs were transiently transfected into COS-7 cells and membrane preparations were analysed by tritiated ligand binding, high affinity GTPase assays and Western blotting.

The fusion proteins were found to function as agonist dependent GTPases. Using adrenaline at a maximally effective concentration agonist induced turnover numbers were calculated. These were shown to have a rank order of activation by the receptor of Gγ1>Gi3>Giγ2>Giγ1.

The authors would like to acknowledge the contribution to this work made by Dr Vicky Jackson.

Regulation of A3 Adenosine Receptor Internalisation by Receptor Phosphorylation

Gail Ferguson and Timothy M Palmer
Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ

Upon binding adenosine or inosine released from cells during a hypoxic insult, activated A3 adenosine receptors initiate signalling cascades which protect the heart and brain from ischemic injury. However, these pathways are simultaneously turned-off or "desensitised" by a series of intracellular mechanisms which have just recently been defined. Within seconds of agonist exposure, the receptor is phosphorylated within its C-terminal domain by members of the G-protein-coupled receptor kinase (GRK) family. This is followed by a dramatic translocation of cell-surface receptors into intracellular compartments. Using a series of molecular biological and biochemical approaches, we are determining the identity of the sites within the receptor C-terminal domain phosphorylated by GRKs, the identity of the GRK family members responsible, and the molecular events linking receptor phosphorylation to the membrane trafficking events involved in receptor internalisation.

Characterisation of Three Subtypes of Rat Thyrotropin Releasing Hormone Receptor

Tomas Drmota and Graeme Milligan
Molecular Pharmacology Group, Dep. Biochemistry and Molecular Biology, Institute of Biomedical Sciences, University of Glasgow, Glasgow G12 8QQ

To study differences between three subtypes of rat thyrotropin releasing hormone receptor (rTRHR) the rTRHR1-Long was taken as a starting model. A spectrum of C-terminal truncated rTRHR1-Long mutants transiently expressed in HEK293 cells was analysed and further compared with both rTRHR1-Short and rTRHR2 subtypes. The characterisation was based on estimation of the rate of agonist-induced receptor internalisation and the production of inositol phosphates. The C-terminal truncated mutants of rTRHR1-Long showed lowering of internalisation rate in a length dependent manner. The internalisation rate of rTRHR1-Short and rTRHR2 was also modified depending on their C-terminal length but additional information may play role in this process because the C-terminus of rTRHR2 shares only 42% similarity with rTRHR1-Long and rTRHR1-Short. TRH stimulation of inositol phosphates production was again dependent on C-terminal length suggesting an important role in receptor desensitisation.

COTRANSFECTION OF PSD-95 WITH CLONED NMDA RECEPTOR SUBTYPES RESULTS IN A SELECTIVE INCREASE IN NR2 SUBUNIT IMMUNOREACTIVITIES

A.R. Rutter, P.L. Chazot, and F.A. Stephenson
School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX

The N-methyl-D-aspartate subclass of glutamate receptor is a heteromeric ligand-gated cation channel composed of NR1 and NR2 subunits. The C-termini of NR2 subunits are known to interact with membrane-associated guanylate kinases, notably postsynaptic density-95 (PSD-95), thought to cluster NMDA receptors at postsynaptic sites [1]. Here, the effects of coexpression of PSD-95γ95 with NR1a, NR2A, NR1a/NR2A and NR1a/NR2B receptors in human embryonic kidney (HEK) 293 cells were investigated. HEK 293 cells were transfected with NR1a, NR2A, NR1a/NR2A, or NR1a/NR2B in the presence and absence of PSD-95γ95. The coexpression of PSD-95γ95 with NR1a had no effect on the level of NR1a as determined by quantitative immunoblotting, whether NR1a was expressed alone or in NR1a/NR2B combinations. However when NR2A was expressed alone, PSD-95γ95 induced a 3.5±0.5-fold (n=2) increase in NR2A immunoreactivity compared to controls. When coexpressed with either NR1a/NR2A or NR1a/NR2B a 3.1±1.2 and a 3.1±1.0-fold (n=6) increase in NR2 immunoreactivity respectively was induced. [3H]MK801 radioligand binding revealed a 51±17% (n=6) increase in specific binding to HEK 293 cells transfected with NR1a/NR2A in the presence of PSD-95γ95 compared to controls together with an approximate 3-fold decrease in affinity. Inhibition constants were KI = 67.6 ± 10.3 nM and 20.1 ± 0.4 nM in the presence and absence of PSD-95γ95, respectively. No change in [3H]MDL105,519 binding was observed. Thus, coexpression of PSD-95γ95 with NR2B subunits results in both an increased level of NR2 subunit and a decreased affinity of the assembled receptor for [3H]MK801.