Long awaited proof that G-protein-coupled receptors (GPCRs) form functional heterodimers in vivo has finally been found. Jennifer Whistler and colleagues, writing in *Proceedings of the National Academy of Sciences*, report tissue-selective expression of an opioid heterodimer that is selectively targeted by an analgesic compound. If this concept extends to other GPCR families, heterodimers could represent a large pool of unprecedented drug targets.

Although many in vitro studies have hinted at the importance of dimerization, conclusive proof of a physiological role for GPCR heterodimers has been elusive. Whistler and colleagues proposed that a ligand that selectively targeted an opioid heterodimer would provide this proof. Furthermore, as many of the side effects associated with opiate analogues are eliminated if the drug is administered directly into the spinal cord, the ability to selectively target opioid heterodimers in the spine could be beneficial.

Knowing that δ- and κ-opioid peptide receptors (DOP-R and KOP-R, respectively) coexist in spinal neurons, and that spinal-cord-selective activity of a bivalent antagonist specific for DOP/KOP-R has recently been reported, the authors proposed that DOP/KOP-R heterodimers might represent a target for the development of a spinal-selective analgesic.

Their investigation focused on an analgesic compound, 6′-guanidinonaltindole (6′-GNTI), which, although supposedly a KOP-R-selective agonist, was shown to exhibit variable agonistic activity in different tissues. This led the authors to speculate that the target for 6′-GNTI was tissue-specific and could be an opioid receptor heterodimer. To study this hypothesis they used cells stably transfected with murine opioid receptors (MOP-Rs), DOP-Rs and KOP-Rs either alone or coexpressed and measured opioid receptor signalling.

The most potent agonism was observed in cells that coexpress KOP-R and DOP-R, and could not be explained by synergistic activation. Addition of subtype-selective antagonists confirmed that the activity of 6′-GNTI requires both KOP-R and DOP-R: antagonism of either subtype abolished 6′-GNTI-mediated signalling. Because the affinities of the antagonists for the individual receptors were different to those when heterodimerized, the authors proposed that heterodimerization creates a unique signalling complex — a ‘landing pad’ for 6′-GNTI — and might also cause a change in conformation that alters ligand affinity for each receptor.

Following on from their in vitro data, the authors then went on to show that 6′-GNTI elicited analgesia when administered directly into the spinal cord, but almost no analgesia when administered directly to the brain. Moreover, this spinal-selective analgesic effect was blocked by a bivalent selective-DOP/KOP-R antagonist, confirming that the heterodimer is a functional target for analgesia in vivo.

The proof that opioid heterodimers are functionally relevant in vivo makes it reasonable to extrapolate that the same could be true for other GPCR families. The authors speculate that so-called ‘orphan’ GPCRs might actually be dimerization partners for GPCRs with known ligands, which serve to increase the complexity, and therefore subtlety, of GPCR signalling. This intriguing possibility means that the number of feasible permutations of GPCR heterodimers and their potential modes of activation provides many more avenues for refined therapeutic intervention. That many of these complexes are selectively expressed also bodes well for the future development of tissue- and subtype-selective GPCR-targeted drugs.

*Joanna Owens*
RESEARCH HIGHLIGHTS

RNA INTERFERENCE

Silence of the genes

siRNAs — short, interfering RNA duplex molecules that can mediate post-transcriptional silencing in a sequence specific manner — theoretically represent ideal drugs for the specific downregulation of unwanted gene products. However, their delivery into target cells is a key obstacle to their therapeutic application. Reporting in Nature Biotechnology, Song et al. now provide a proof-of-principle study of a systemic method to deliver siRNA into specific cell types via cell-surface receptors, with the aim of maximizing therapeutic benefit, while minimizing non-specific silencing and toxicity in bystander cells.

The authors chose HIV envelope protein (Env) as a model receptor for targeted delivery of siRNA. This was achieved with a fusion protein (F105-P) consisting of an Env-specific antibody Fab fragment (comprising the non-immunogenic antigen-recognition domains), fused to protamine, a nucleic acid-binding protein that normally nucleates DNA in sperm. Incubation with siRNAs resulted in stable fusion protein/RNA complexes, which were internalized by cells carrying the respective surface antigen and efficiently mediated the downregulation of the siRNA-targeted gene product.

INFLAMMATION

The nerve of macrophages

After abdominal surgery, paralysis of the bowel, or post-operative ileus, commonly leads to extended hospital stays, and is characterized by inflammation and delayed transit of contents of the gut, often accompanied with nausea, vomiting and pain. The economic burden of ileus is estimated to be several billion dollars per year in the US. In the August issue of Nature Immunology, De Jonge et al. demonstrate in a mouse model of gastrointestinal (GI) ileus that stimulation of the vagus nerve attenuates inflammation and ileus via a STAT3 pathway in macrophages.

The vagus nerve, the longest in the body, is a component of the parasympathetic nervous system and promotes normal body function, including gastric motility. Local inflammation causes afferent fibres of the vagus nerve to trigger an anti-inflammatory response through firing of the efferent vagus nerve and the release of acetylcholine (ACh). ACh binds to α7 nicotinic ACh receptors (nAChR) expressed by macrophages to suppress pro-inflammatory cytokine production. This pathway can be manipulated by stimulating the vagus nerve or by using cholinergic agonists, such as nicotine, to control undesirable inflammation.

The authors showed that nicotine exerts its anti-inflammatory effect on peritoneal macrophages via the tyrosine kinase JAK2 and the STAT3 transcription factor, in vitro and in vivo. After nicotine binding, JAK2 is recruited to the α7 subunit of nAChR, leading to JAK2 phosphorylation. This in turn leads to phosphorylation of the STAT3 transcription factor, which forms dimers and translocates to the cell nucleus, where it induces the expression of a number of pro- and anti-inflammatory proteins, as well as the suppressor of cytokine signalling (SOCS)-3. However, the authors found that blockade of SOCS3 expression did not prevent the anti-inflammatory action of nicotine, suggesting that the cholinergic deactivation of macrophages results from activation of STAT3 rather than SOCS3.

Manipulating the cholinergic anti-inflammatory pathway is a promising strategy for treating post-operative ileus; a number of vagus nerve stimulators are approved for the treatment of epilepsy and depression. The timing of treatment could be important, as earlier attempts to treat this condition using cholinergic agents had only limited success, perhaps because treatment was administered after the inflammatory process had progressed.

This study also has important implications for other inflammatory conditions that might be alleviated by activating the JAK2–STAT3 pathway. In particular, ulcerative colitis is associated with altered STAT3 expression and phosphorylation and, interestingly, the condition is ameliorated by cholinergic stimulation in the form of smoking or nicotine treatment. Unfortunately, the toxic effects of nicotine will undoubtedly prevent this cholinergic agonist from any long-term therapeutic use. Future studies are required to investigate the use of other α7nAChR agonists in a therapeutic setting. For example, galantamine hydrobromide (Reminyl), Johnson & Johnson, both a cholinesterase inhibitor and an allosteric enhancer of nicotinic receptors, is currently prescribed for the symptomatic treatment of schizophrenia and Alzheimer’s disease.

Melanie Brazil

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ANTIVIRAL DRUGS

Breakthrough for HCV research

Hepatitis C virus (HCV) afflicts more than 170 million people worldwide but until now HCV research has been severely hampered by the inability to produce infectious virus in cell culture. In a major breakthrough, three groups have reported the replication of full-length HCV clones in vitro, paving the way for the development of effective antiviral therapies and vaccines.

HCV primarily infects hepatocytes and causes hepatitis, cirrhosis of the liver and hepatocellular carcinoma. There is no vaccine, and drug treatments are costly and have poor efficacy. The absence of a small-animal model and a cell-culture system for HCV have been obstacles to studying this virus, and researchers have relied on studying infections in humans and chimpanzees.

In the past 5 years, the development of in vitro HCV replicon systems has enabled viral molecular biology and virus–host interactions to be probed. Such systems use genomic and subgenomic clones that are transfected into hepatocyte cell lines. The main disadvantage of these systems is that the RNAs cannot replicate in vitro without acquiring adaptive mutations, nor do these systems produce infectious virions, so their relevance to the biology of wild-type infectious HCV isolates is questionable.

Three groups set out to develop faithful in vitro replication systems for HCV. These studies build upon very recent advances: in the past 2 years, the Wakita group developed an in vitro system that replicates a subgenomic RNA that has not acquired any adaptive mutations, which formed the basis for the studies just published. All three groups used hepatocyte cell lines and, importantly, all of the full-length replicons were either the JFH-1 HCV strain that was previously isolated from a fulminant-hepatitis patient by Wakita’s group or a chimera based on that strain. None of the full-length RNA clones that were used in these studies contained adaptive mutations, which is crucial, because the Bartenschlager group had shown that these mutations interfere with virus production and infectivity in vivo. Therefore, the systems are representative of the wild-type HCV infection cycle. In all three studies, monitoring of viral RNA production by PCR, protein production by antibody labelling and classic dilution and infection studies were used to quantify RNA replication.

The different studies have common features. First, all of the in vitro systems replicate the full-length viral RNA and transfected cells produce virions — evidence of a complete virus life-cycle. Second, viruses produced in vitro can be propagated efficiently using cell passage. Third, all three groups showed that the biophysical properties of the virions that are secreted by transfected cells are comparable to virions produced in chimpanzees infected with wild-type HCV. Finally, Wakita et al. used intravenous inoculation with in vitro-produced virus suspensions to prove that the in vitro-produced virus is infectious in chimpanzees. All three groups showed that antibodies against virus proteins neutralized the infectivity of virus that was produced in vitro. Furthermore, Wakita et al. and Zhong et al. blocked a putative cellular receptor, CD81, using anti-CD81 antibody, whereas Lindenbach et al. blocked the same receptor with a soluble recombinant CD81 fragment and prevented in vitro-produced virus from infecting Huh-7.5 cells. The development of these tissue-culture systems should accelerate the pace of hepatitis research.

Susan Jones
Nature Reviews Microbiology

References and links

IN BRIEF

KINASES
Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases.

Cell-associated enveloped virions (CEVs) rely on actin to be able to move from just outside the host-cell nucleus to the cell surface, where they fuse with the cell membrane, detach and move on to infect another cell. This study shows that the CEVs require Abl and Src-family tyrosine kinases for actin motility, and specifically Abl tyrosine kinase when detaching from the cell. The authors found that the Abl-family kinase inhibitor imatinib (Gleevec; Novartis) blocks the release of CEVs and reduced viral dissemination and improved survival of infected mice.

INFECTIOUS DISEASES
Small-molecule inhibition of siderophore biosynthesis in Mycobacterium tuberculosis and Yersinia pestis.

The causative agents of tuberculosis and plague, Mycobacterium tuberculosis and Yersinia pestis, respectively, both share a common method of pathogenicity. Both use ‘siderophores’ to chelate iron from the host with extremely high affinity. This paper reports the identification of a class of non-hydrolyzable acyl-AMP analogues that inhibit a crucial step in siderophore biosynthesis called domain salicylation. One particular inhibitor, salicyl-AMS, is a promising lead compound for the development of novel antibiotics against tuberculosis and plague.

ANTICANCER DRUGS
Synthesis and identification of small molecules that potently induce apoptosis in melanoma cells through G1 cell cycle arrest.

The very features of melanocytes that protect cells against DNA damage in normal skin also protect against cell-cycle arrest caused by chemotherapy. To search for more effective melanoma therapies, the authors of this study synthesized a combinatorial library of potential pro-apoptotic compounds and identified a class of small molecules called triphenylmethylamides (TPMAs) that potently induce cell death in melanoma cell lines without causing death to normal bone-marrow cells.

PARKINSON’S DISEASE
Sumanirelo, a highly dopamine D2 selective receptor agonist: in vitro and in vivo pharmacologic characterization and efficacy in animal models of Parkinson’s disease.

The first dopamine D2-receptor-selective agonist has been reported and shows promise in animal models as a potential drug against Parkinson’s disease. Sumanirelo was shown in radioligand binding assays to have more than 200-fold greater selectivity for the D2 receptor subtype than any other dopamine receptor subtype. The authors describe how sumanirelo causes many physiological responses in animals that are attributable to D2-receptor activity, and improved disability scores and locomotor activities in rodent and primate models of Parkinson’s disease.

References and links
