G-PROTEIN-COUPLED RECEPTORS

It takes two…

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 analgesics 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′-guanidinonaltrindole (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

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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.

In *in vitro* experiments, F105-P complexed with siRNA targeting HIV *gag* (encoding an essential viral capsid protein) significantly reduced viral replication and release of viral particles in HIV-infected primary CD-4 T cells compared with cells treated with F105-P alone. Using the same fusion protein construct, these studies were extended to *in vivo* mouse tumour models with melanoma cells that were transfected with vectors for HIV Env before their implantation in the host. Here, intratumoural or intravenous injection of F105-P complexed with a cocktail of siRNA directed against several oncogenes significantly slowed down tumour growth in Env-expressing tumours, without exerting any effect on non-transfected tumours. Furthermore, the complex seemed to be completely non-immunogenic, and was not trapped by any cells of the reticuloendothelial system that could interfere with systemic delivery. Demonstrating the flexibility of the targeting strategy, further siRNA/fusion proteins were generated that consisted of a single-chain antibody directed against ErbB2 (an antigen on many breast cancer cell lines), which also mediated targeted delivery of siRNAs.

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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FURTHER READING Ulloa, L. The vagus nerve and the nicotinic anti-inflammatory pathway. *Nature Rev. Drug Discov.* **4**, 673–684 (2005)

This systemic, cell-type specific antibody-mediated siRNA delivery method might not only hold potential for the treatment of infection or cancer, but could also be used to modulate the function of normal cells in disease settings. As it does not require covalent linkage of siRNA or specialized chemistry, the same reagent can be flexibly used to deliver changing mixtures of different siRNAs, and the delivery strategy can be modified to target any of a variety of cells.

While there is ample room for optimization, and the pharmacokinetics and trafficking pathways of the fusion protein/siRNA complexes remain to be understood, the targeted delivery should raise the therapeutic index of siRNA, reduce the amount of drug required and minimize concerns about off-target effects.

Alexandra Flemming

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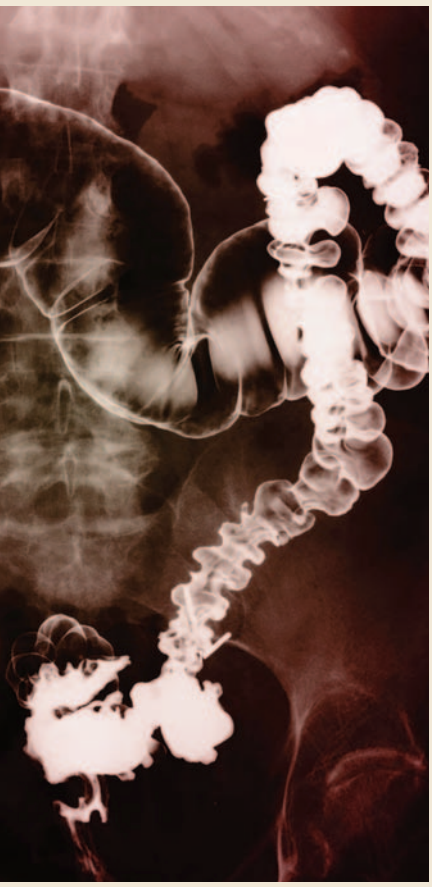

ANTIVIRAL DRUGS

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 chimaera 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

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IN BRIEF

KINASES

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

Reeves, M. P. *et al. Nature Med.* **11**, 731–739 (2005)

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*.

Ferreras, J. A. *et al. Nature Chem. Biol.* **1**, 29–32 (2005)

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.

Dothager, R. S. *et al. J. Am. Chem. Soc.* **127**, 8686–8696 (2005)

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

Sumanirole, a highly dopamine D2 selective receptor agonist: *in vitro* and *in vivo* pharmacologic characterization and efficacy in animal models of Parkinson's disease.

McCall, R. B. *et al. J. Pharm. Exp. Ther.* 24 Jun 2005 (doi:10.1124/jpet.105.084202)

The first dopamine D_2-receptor-selective agonist has been reported and shows promise in animal models as a potential drug against Parkinson's disease. Sumanirole was shown in radioligand binding assays to have more than 200-fold greater selectivity for the D_2 receptor subtype than any other dopamine receptor subtype. The authors describe how sumanirole causes many physiological responses in animals that are attributable to D_2-receptor activity, and improved disability scores and locomotor activities in rodent and primate models of Parkinson's disease.

SCREENING

Won't get fooled again

The prevalence of nonspecific or 'promiscuous' inhibitors that seem to be hits in multiple high-throughput screening (HTS) campaigns, but which turn out to be dead ends when attempts are made to optimize their activity, is a key problem in the field of HTS. Shoichet and colleagues, writing in *Nature Chemical Biology*, now describe two high-throughput assays that aim to address this issue by aiding the detection of promiscuous inhibitors.

Various explanations have been put forward to account for promiscuous compounds, including chemical reactivity and interference with assay readouts. Recent work from the Shoichet lab has also identified another possible mechanism to explain promiscuous inhibition: formation of colloid-like aggregates of the compounds, which sequester and thereby inhibit enzymes nonspecifically. Hits from HTS, leads and even some drugs seem to inhibit various enzymes through this mechanism at the micromolar concentrations typically used in HTS.

To facilitate investigation of the extent of this problem, the authors developed two rapid assays based on a standard 96-well format for detecting aggregate-based inhibition. The first assay exploits the detergent-sensitive nature of aggregate formation; compounds that only inhibit β-lactamase in the absence of detergent are considered likely to be promiscuous. The second assay uses dynamic light scattering to detect aggregate formation.

Shoichet *et al.* then selected 1,030 drug-like compounds and screened these molecules at micromolar concentrations in both assays, and a number of significant results were obtained. First, 19% of a subset of the compounds selected at random were detergent-sensitive inhibitors at screening-relevant concentrations (30 μm) — a percentage sufficiently high that it could dominate a screen that did not control for this effect. Second, both assays (whose reliability was confirmed using more sensitive, low-throughput versions of each assay) were able to robustly detect promiscuous aggregates, although of the two the detergent-sensitive assay seems best-suited for larger-scale applications. Finally, computational models for predicting aggregation-based promiscuity exploiting the results from the assays also showed some potential, and the data provided freely by the authors should aid the development of further such models.

Peter Kirkpatrick

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