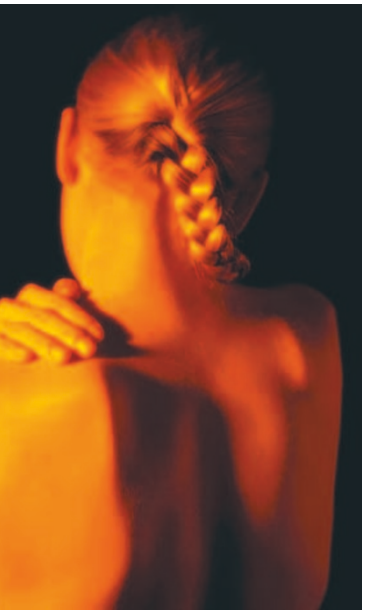
TARGET IDENTIFICATION

Unravelling the problem of neuropathic pain



The detection of thermal, mechanical and other stimuli, and their interpretation as pain (that is, nociception), serves the useful adaptive purpose of guiding us through the world while accumulating a minimum of physical damage. But when the nerves that carry pain signals become damaged — for example, through diabetes, surgery or infection — maladaptive pain signals, termed neuropathic pain, can be generated from within the nervous system. This can, in the case of mechanical allodynia, turn normal activities involving touch into agonizing ordeals. Two papers recently published by Tsuda *et al.* in *Nature* and by Ibrahim *et al.* in the *Proceedings of the National Academy of Sciences* throw further light on the receptors involved in propagating neuropathic pain, and provide potential targets for therapeutic intervention.

Tsuda and colleagues used a rat model of allodynia to show that the $P2X_4$ receptor on spinal microglial cells, which has not previously been studied as a pain receptor, has a role in a form of neuropathic pain. The rat model involved cutting a peripheral sensory nerve, and then looking at the rats' response to tactile stimuli (that is, measuring mechanical allodynia); after a week, the rats in this study withdrew their paws from a light touch as if responding to a painful event.

P2X receptor (P2XR) antagonists were then infused into the spinal cords of the treated rats to see whether allodynia would be reduced. There are seven subtypes of P2XR ion channels: subtypes $P2X_3$ and $P2X_2$ are found in high numbers on specific nociceptive neurons, thereby providing the rationale for studying P2XR antagonists. Surprisingly, Tsuda *et al.* found that PPADS, an antagonist of P2XRs on nociceptive neurons but not of $P2X_4$ and some other subtypes, did not reduce allodynia, whereas TNP-ATP, which blocks all P2XR subtypes, did. So Tsuda and colleagues determined which receptor was being blocked, and its location. Fluorescently labelled antibodies to $P2X_4$ localized to microglia on the side of the spine where the nerve was cut; this, combined with the inhibition data and knowledge that microglia only express the receptors $P2X_7$ (which is inhibited by PPADS) and $P2X_4$, led to the identification of $P2X_4$ as the key receptor. Furthermore, an antisense oligonucleotide against $P2X_4$ infused into rats' spinal cords after nerve damage reduced the severity of allodynia. These results identify a new receptor in the pain process, and provide strong evidence of the functional role of microglia.

Ibrahim *et al.* also explored neuropathic pain, and set out to address the hypothesis that activation of the CB_2 cannabinoid receptor would reduce pain hypersensitivity by using AM1241, a CB_2-receptor-selective agonist. AM1241 was found to dose-dependently reverse tactile and

HIV

No entry!

The recent approval of the 36-amino-acid peptide enfuvirtide (T-20; Trimeris/Roche) heralds a new class of HIV-1 therapeutics: viral entry inhibitors. Before the launch of enfuvirtide in March 2003, all marketed HIV drugs targeted one of two viral enzymes — protease and reverse transcriptase. However, despite the significant success achieved with standard combination therapy, the lack of patient compliance due to adverse side effects and the emergence of resistant viral strains has resulted in many therapeutic failures and a significant unmet clinical need. Now, in the *Proceedings of the National Academy of Sciences*, a group of researchers at Bristol-Myers Squibb reveal details of a small molecule, BMS-378806, which targets the HIV-1 envelope protein and inhibits CD4 receptor binding, thereby preventing HIV-1 entry into the host T cell.

The viral entry process provides new anti-HIV-1 targets, and the potential for developing novel classes of anti-HIV drugs. The HIV-1 envelope consists of the exterior glycoprotein gp120 and the transmembrane domain gp41, both of which are processed from a gp160 precursor. After attachment of HIV-1 to a target T cell carrying the CD4 receptor, the interaction of gp120 with the CD4 receptor initiates a series of conformational changes in both gp120 and gp41 that allow the insertion of a region of gp41 into the host cell. Further changes in the conformation of gp41 bring the viral and cellular membranes close enough for membrane fusion, although the mechanism of fusion is still unresolved. Enfuvirtide binds to a region of gp41 that mediates part of the conformational change, whereas BMS-378806 binds to gp120 to inhibit CD4 binding.

A whole-cell infection screen against a strain of HIV-1 identified an indole analogue as an initial hit, which after optimization to improve potency, specificity and pharmacokinetic parameters resulted in the lead candidate BMS-378806, a 4-methoxy-7-azaindole derivative. The compound showed potent inhibitory activity against a panel of R5, X4, R5/X4 (viruses using the CCR5, CXCR4 or both chemokine receptors, respectively) HIV-1 laboratory and clinical isolates. BMS-378806 was also selective for HIV-1, and inactive against other viruses, including HIV-2 and simian immunodeficiency virus. Importantly, BMS-378806 is effective against HIV strains resistant to existing HIV-1 protease and reverse transcriptase inhibitors.

The authors carried out studies on the mechanism of action, and confirmed that BMS-378806 binds to gp120 and prevents further interaction of the viral protein with cellular CD4 receptors. Further confirmation was obtained by analysing the amino-acid substitution of resistant virus strains, isolated by passaging virus in the presence of the drug and selecting for variants resistant to BMS-378806. These experiments revealed that almost all the substitutions spanned the gp120 region, with a couple of changes residing in the gp41 region, and none outside the envelope. Significantly, the two key substitutions are situated in the CD4-binding pocket of gp120.

BMS-378806 showed good oral bioavailability and a clean safety profile in initial animal studies. Whereas other HIV entry inhibitors in early clinical trials block co-receptors and membrane fusion, BMS-378806 is the first small molecule to block the gp120–CD4 binding event.

Melanie Brazil

References and links

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thermal hypersensitivity produced by cutting the L5 and L6 spinal nerves in rats. In addition, these effects were ablated by an antagonist selective for CB_2 receptors, but not by a CB_1-receptor-specific antagonist. Furthermore, AM1241 reversed allodynia in mice lacking CB_1 receptors, establishing that CB_2 is the crucial receptor.

Some of the current treatment options for neuropathic pain have unwanted side effects on the central nervous system (CNS), and so drugs directed against targets not found in the CNS would make attractive agents. CB_2 receptors, which are not expressed in the brain or CNS, fit the bill.

Daniel Jones

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LEAD DISCOVERY

RAMPing up NMR screening

Nuclear magnetic resonance (NMR)-based screening approaches that directly detect the binding of compounds to a target are becoming increasingly popular in lead discovery, as they have several valuable advantages over established high-throughput functional assays. First, they can be used for highly novel targets for which functional assays are not readily available; second, they can detect weak-binding 'fragments' that might be more easily developed into potent lead compounds; and last, they can yield detailed structural data that indicate where on the protein the compound is binding, thereby aiding compound optimization. However, the NMR spectra resulting from such screens are often complex and hard to analyse, leading to relatively low throughput. As described in the *Journal of the American Chemical Society*, Zartler *et al.* have now developed a new NMR-based screening approach that yields greatly simplified spectra that not only allow increased throughput owing to easier spectral analysis, but also allow — for the first time in any format — multiple proteins to be screened simultaneously in one tube.

Like several other NMR-based screening methods, such as 'SAR by NMR', the authors approach — dubbed 'RAMPED-UP NMR' — is based on labelling the protein target with ^{15}N, an NMR-active nitrogen isotope (^{14}N, which has a natural abundance of 99.6%, is not NMR-active, whereas the most common isotope of hydrogen, ^{1}H, is NMR-active). Ligand binding is then assessed by observing the movements induced in the chemical shifts of ^{1}H–^{15}N resonances in the protein NMR spectra; resonances that shift will usually originate from residues in or close to the binding site.

However, typically only a relatively small percentage of the ^{1}H–^{15}N resonances in a uniformly ^{15}N-labelled protein will be significantly affected by ligand binding; the many resonances that do not shift merely add complexity to the spectra and provide no useful information. Zartler *et al.* decided to address this issue by only labelling specific amino acids with ^{15}N, which can be achieved by expressing the protein in bacteria grown in media containing the chosen labelled amino acid, or by using *in vitro* expression systems and supplying the chosen labelled amino acid.

The authors selected three unrelated targets to illustrate their methodology: protein tyrosine phosphatase 1B (PTP1B), kRAS and green fluorescent protein (GFP). A key issue with selective labelling is which amino acids should be labelled so as to maximize the likelihood of detecting a binding event. In the case of PTP1B, the location of the binding site is known, and it was decided to label Trp residues with ^{15}N, as a Trp residue is close to this site. For the other two targets — as would probably be done for targets for which the position of the binding site is uncertain — the authors chose to label Ile and Ala, respectively, as these provide a good sampling of the primary protein sequence, meaning that there is a good probability that a labelled residue is close to any binding sites.

The authors then showed that the NMR spectra of a solution containing these three targets could be easily used to quickly and cleanly discriminate the binding of known weak ligands for PTP1B and kRAS, highlighting the power of the approach. In this case, the targets are not related, but the strategy would clearly also be valuable when aiming to discover ligands that are selective for particular members of a family with highly homologous active sites — for example, kinases — a scenario in which the ability to analyse potential binding events for multiple family members simultaneously in the same tube would be especially desirable.

Peter Kirkpatrick

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