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and proteins can be reliably reduced without loss of biological activity or changes to the stability of their native structures.'

Future applications

Zyentia has no plans to take human calcitonin variants into clinical development. Instead, the company has successfully applied its technology to other biopharmaceuticals that are hardly used because of aggregation problems, for example, glucagon. 'This peptide is sometimes used by diabetics to treat hypoglycemic shock but it is extremely insoluble and difficult to handle', notes Zurdo. Zyentia also seeks to license its technology to other pharmaceutical and biotechnology companies.

Finally, Zurdo and Schymkowitz are using their algorithms to search for inhibitors of the pathogenic aggregation that underlies Alzheimer's, Parkinson's and other neurodegenerative diseases [3]. 'By understanding which regions of amyloid-forming proteins drive their aggregation, we have been able to generate compounds that halt the pathogenic process', says Zurdo, adding that these are now in pre-clinical development at Zyentia.

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Endocannabinoids mediate age-old pain suppression

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Stress-induced analgesia (SIA) is a phenomenon where the affected individual does not feel the effects of a physically harmful experience. Although long-documented, it was not until the 1970s that researchers discovered that about half the effects of SIA are mediated by endogenous opioids [1]. 'The question is', says Daniele Piomelli (http://www.ucihs.uci.edu/pharmaco/people/faculty/piomelli.html) from the University of California, Irvine, USA, 'what about the rest?' Now he and Andrea Hohmann (http://www.uga.edu/psychology/faculty/ahohmann.html) from the University of Georgia, USA, have shown that endogenous cannabinoids also contribute to SIA [2].

'endocannabinoids are produced in brain areas involved in emotions'

Piomelli describes the most famous episode of SIA, experienced by the British explorer David Livingstone as a lion attacked him on an expedition in Africa. 'The lion grabbed him and as he was being bounced up and down, he felt nothing, he was very calm. The body shuts off pain signals, because it makes more sense to focus your attention and resources toward escaping the danger.'

Targets for drug development

The brain produces two endogenous cannabinoids: anandamide and 2-arachidonylglycerol (2-AG); both affect pain. Hohmann targeted a known relay station for SIA, the periaqueductal grey matter (PAG). When she injected antagonists to the cannabinoid receptor CB1 into the PAG of rats, SIA was blocked. Then the team looked directly at endocannabinoid production in the PAG, finding anandamide and 2-AG present.

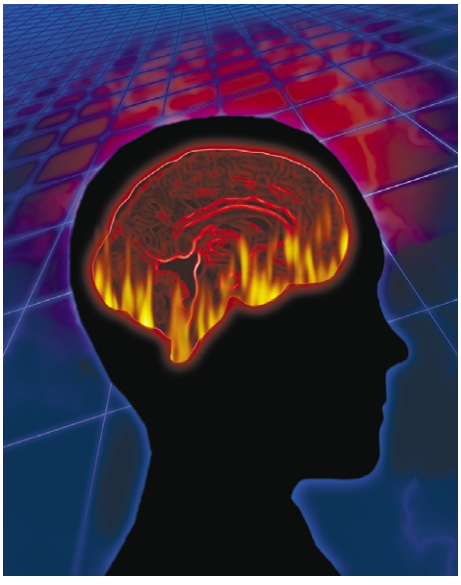
They wanted to show that endocannabinoids were directly mediating SIA, 'could we boost the system up?' asked Piomelli. Each of the endogenous compounds is deactivated by a hydrolyzing enzyme: anandamide by fatty-acid amide hydrolase (FAAH) and 2-AG by monoacylglycerol lipase (MGL). If these deactivators can be shut down, the analgesic effects of the endocannabinoids might increase.

Piomelli previously developed an inhibitor to FAAH that has turned out to have anti-anxiety properties [3]. They used this molecule as a scaffold to construct a specific inhibitor of MGL. Because the inhibitor is not potent enough to work systemically, they injected the compound directly into the brain. Rats whose tails were placed on a hotplate, lingered longer when injected with the inhibitors – evidence of increased analgesia.

Raphael Mechoulam (http://paincenter.huji.ac.il/mechoulam.htm) from the Hebrew University of Jerusalem (not involved in the current work) is hopeful about future uses of MGL inhibitors. 'I can certainly make use of them in neuroprotection', he said. 'They may possibly be used in various neurological states where 2-AG is important'. Hohmann too predicts potential broad-spectrum use of the new inhibitors. 'MGL inhibitors may be useful therapeutically not just for pain but for post-traumatic stress and anxiety-related disorders. We now have a tool to address these possible clinical applications'.

Two endocannabinoids: individual roles

The finding that these endocannabinoids were released in the PAG seemed at first redundant. 'Nature doesn't do things this way, with such a blatant overlap', said Piomelli. He wondered whether they were serving different functions. Intriguingly, the two endocannabinoids were temporally segregated. Whereas, the concentration of 2-AG increased immediately with stress and decreased within a few minutes, 'it was a completely different situation with anandamide, it took seven minutes to go up'. The compounds differed spatially as well. 'When we look for the compounds released in the brain, we see a lot of 2-AG closely associated



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to the areas of release, whereas anandamide moves farther away'. These differences provide 'a one–two punch', comments Piomelli.

The results left Piomelli with questions about the individual roles of the two endocannabinoids. He speculates, '2-AG may act as a fast modulator, followed by longer-lasting and more far-reaching actions of anandamide', perhaps even corresponding to the nociceptive and emotional aspects of pain, although he calls this 'pure speculation'. Mechoulam agrees that these endogenous molecules might be tethered to emotion. 'As endocannabinoids are produced in brain areas involved in emotions and are involved in physiological states like pain, appetite and stress, which heavily involve emotion', he said, 'I believe that ultimately they will be shown to be central players in this important area. How exactly', he added, 'is still beyond us. Maybe the endocannabinoids will be good tools.'

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Battle against malaria could involve anti-HIV drugs

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Preliminary studies suggest that antiretroviral protease inhibitors (ARPIs) could be useful in the prophylaxis and treatment of malaria, particularly in individuals co-infected with HIV-1. Kathy Andrews of the Queensland Institute of Medical Research (Herston, QLD, Australia) presented some of her group's results during the Medical Research Week organized by the Australian Society for Medical Research in June this year.

A common target

HIV protease inhibitors target an aspartic protease in HIV that has significant homology with plasmepsins that reside in the food vacuole of *Plasmodium falciparum*. 'Plasmodium aspartic proteases are now recognized as a novel drug target for future research', says Andrews. 'For scientists interested in rational drug-design of antimalarials, this finding opens another window with enormous potential', comments Vijay Sharma (Washington University Medical School, St. Louis, MO, USA). 'This finding has also generated considerable excitement and debate on mechanism(s) of action involving ARPIs in antimalarial research', he adds.

Dual purpose

Malaria is one of the deadliest parasitic diseases world-wide, killing over 2 million people annually. The global emergence of drug resistance is making efforts for treatment less effective. 'The literature contains several reports of antimalarial drugs having some efficacy against HIV but this is the first report of antiretrovirals being used to manage malaria', comments Frank Romanelli (University of Kentucky, Lexington, KY, USA). Romanelli notes that 'studies involving ARPIs are often limited by their high pill burdens, cost, side-effect profiles and propensity to cause drug–drug interactions'. Sharma agrees that 'although cost may be a critical determinant for making them a routine first-line choice, wide deployment of these drugs for HIV and malaria patients could be immensely beneficial in underdeveloped regions of the world'.

Future studies

Brian Greenwood (London School of Hygiene and Tropical Medicine, UK) predicts that identification of the exact target of ARPIs and their mode of action might allow some modified molecules to be developed as specific antimalarials. 'These could be even more powerful than the drugs currently being used to treat HIV', he says. It is possible that antiprotease ARPIs could provide some background protection against malaria for people on long-term ARPI treatment. 'However, more work is needed to determine whether blood taken from people on ARPIs at different periods after receiving treatment can inhibit parasite growth', he explains. If ARPI treatment maintains protective levels in the blood throughout a course of treatment, then it could provide a useful form for malaria chemoprophylaxis for at-risk groups. However, he warns that if blood concentrations fluctuate above and below inhibitory concentrations, then this would be a very effective way of selecting for parasite resistance and could quickly make this group of drugs unsuitable for use as a primary malaria treatment.

> '…this is the first report of antiretrovirals being used to manage malaria…'

A natural experiment?

'A natural experiment is already taking place in that patients living in highly malarial regions are now receiving ARPIs long-term and it would be helpful to measure the effect of this on the incidence of malaria both in individual subjects and at the population level', adds Greenwood. However, Andrews points out that in areas where *P. falciparum* is prevalent, protease inhibitors are not currently the first choice for HIV treatment. 'The NNRT [non-nucleoside reverse transcriptase] inhibitor nevirapine is commonly in use, particularly to treat pregnant women. We have found no antimalarial effect at therapeutic concentration for the HIV drug nevarapine', she reports. Greenwood also stresses that any large-scale studies would be difficult because of ethical concerns about having a non-ARPI-treated control group.

1359-6446/05/$ – see front matter PII: S1359-6446(05)03601-9