Muscarinic receptors: new potential for treating chronic pain

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A new approach using small molecules that selectively activate a specific subtype of the muscarinic receptor is showing considerable promise at ACADIA Pharmaceuticals (San Diego, CA, USA) for the treatment of chronic pain.

Drugs that activate muscarinic receptors have long been implicated in the treatment of both acute and chronic pain. However the use of muscarinic agonists and cholinesterase inhibitors has been restricted by severe dose-limiting side effects that include gastrointestinal (GI) distress, excessive sweating and salivation, and bradycardia.

'In short we had drugs that could treat pain (i.e. neostigmine), but they dangerously slowed the heart rate,' says Robert E. Davis, Acadia's Executive Vice President of Drug Discovery and Development. 'These drugs must be administered directly into the spinal canal to avoid these side-effects, which severely limits the use of this class of agents for treating various pain conditions,' he explains.

Over 15 years ago, work by Mark Brann, Acadia's President and Chief Scientific Officer, revealed the existence of five different muscarinic receptor genes using classical gene hunting techniques[1]. The discovery that these receptors are expressed in distinct locations throughout the body suggested the possibility of developing receptor subtype-selective agents that are only active in the brain. Studies in receptor-knockout transgenic animals confirmed this hypothesis and showed that muscarinic-receptor agonists did not slow the heart rate in muscarinic M_2 receptor knockout animals, and did not induce excessive salivation or GI side-effects in M_3 receptor knockout animals. This suggested that M_2 and M_3 receptors should be avoided in drug discovery projects, but unfortunately also showed that muscarinic agonists act through the M_2 receptor to influence acute pain[2,3]. Using its proprietary assay technology, Receptor Selection and Amplification Technology (R-SAT™), ACADIA has now identified the first truly selective muscarinic agonists.

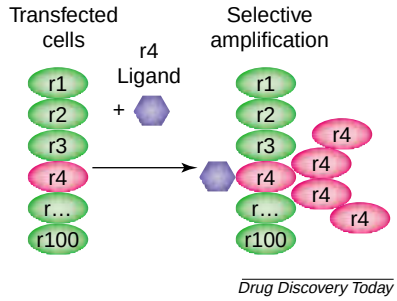

Figure 1. R-SAT™ is based on the principle of genetic selection: cells that express the receptor for a ligand will be selected and amplified relative to cells that do not express the receptor for the ligand. Figure reproduced, with permission, from ACADIA Pharmaceuticals (San Diego, CA, USA). Abbreviation: r, receptor.

Finding the drug target

Using this technology, a gene representing the desired drug target and a marker gene encoding an enzyme such as β-galactosidase, are transiently co-transfected into cultured cells. These cells are then exposed to a series of compounds. Agonists for the drug target stimulate the release of cells from contact inhibition because of the induction of oncogenic cellular transformation, resulting in the rapid proliferation of cells expressing the target, while all other cells remain quiescent (Fig. 1). Hence, the drug–target interactions can be reported in simple assays by lysing the cells and exposing them to a colorimetric substrate for the marker enzyme. 'Put simply, if the compound is an agonist for the target protein you get an increase in colour, whereas if it is an antagonist you get a decrease in colour,' says Davis.

R-SAT can be used to screen large numbers of drug targets against large numbers of compounds simultaneously. Using this method, ACADIA has found a compound that stimulated one of the muscarinic receptors, but not M_2 or M_3 receptors. The compound, which they cannot disclose for competitive reasons, has shown potent activity in two standard models of chronic pain, but not in a model of acute pain.

In vivo studies

In the Chung model of neuropathic pain – developed at the University of Texas Medical Branch (Galveston, TX, USA) – the L5 and L6 spinal nerves of a rat are tightly ligated leading to hypersensitivity to touch and heat and thus providing a model of chronic pain[4]. In the Bennett model of neuropathic pain, developed at the National Institute of Dental Research (Bethesda, MD, USA), ligatures are placed around the common sciatic nerve in rats to produce hyperalgesic responses to heat and chemogenic pain[5].

In both the Chung and the Bennett models, the time taken for the rat to remove its paw from a source of heat or touch increased more than twofold in animals given the candidate drug compared with controls. This demonstrated

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that the drug was having a beneficial effect on chronic pain. Meanwhile, no effects of the drug was seen in studies on normal rats, demonstrating that the drug had no effect on acute pain.

'The complete surprise was that we could separate the effects of acute and chronic pain. This is very exciting because people taking the drug for chronic pain will still be able to experience normal stimuli from the environment,' says Brann, suggesting that different muscarinic receptors are involved in different types of pain.

Potential for the future

ACADIA think that their drug candidates will be helpful for chronic pain associated with diabetic neuropathy, herpes lesions, metastatic cancer and pain associated with autoimmune diseases like Guillain–Barré syndrome. They could also have a role in arthritic and lower-back pain.

Research is now aimed at optimizing the candidate compounds to achieve greater potency, better *in vivo* stability and a longer duration of action. ACADIA hopes to start toxicology and safety studies in animals within a year and the company is aware that it is likely to be 18 months or more before they can start human trials

'The ability to develop small drugs that are highly selective for M_1 receptors is itself a significant achievement,' commented Alan Levey, Professor of Neurology at Emory University School of Medicine (Atlanta, GA, USA). 'This has been a goal for many pharmaceutical companies, given the potential use of an M_1 receptor-specific drug for Alzheimer's disease, schizophrenia, pain and other common problems.' He continued, 'ACADIA used a different approach to find small molecules that activate the receptor subtypes. The finding that drugs can be discovered using this strategy sets an important precedent that could lead to the more rapid discovery of many drug candidates that bind selectively to a variety of receptors, and that could be applied to many common medical conditions.'

References

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Thrombolysis without bleeding

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Plasmin, the activated form of plasminogen (a protein found in normal human plasma), has been previously overlooked as a therapeutic for conditions caused by blood clots. However, a recent preclinical study strongly suggests that plasmin is an excellent thrombolytic agent, with a striking safety profile – better than licensed agents currently in use in the clinic[1].

Lead author, Victor Marder (University of California, Los Angeles, CA, USA), together with colleagues from the Bayer Corporation (Berkeley, CA, USA and Raleigh, NC, USA), used rabbit models of local thrombolysis and fibrinolytic haemorrhage to compare plasmin with tissue plasminogen activator (TPA). TPA is the current standard 'clot-busting' treatment for the management of patients with either an acute myocardial infarction, catheter or shunt, or peripheral arterial occlusion. Although effective, TPA can cause bleeding at remote sites because it spreads systemically, even after local infusion. 'All current thrombolytics are plasminogen activators, of which TPA is the prototype. All have the same problem with bleeding, especially intracranial haemorrhage, which can be fatal,' comments Marder.

Giving plasmin a second chance

During early investigations, plasmin was recognized as a direct fibrinolytic enzyme with a potential clinical application. Desire Collen (University of Leuven, Leuven, Belgium), who is actively involved in the development of recombinant plasmin and derivatives as therapeutic agents, points out in an editorial[2] that, 'Intravenous plasmin for thrombolytic therapy was investigated in several pilot studies in humans in the 1950s and 1960s (Ref. 3).' These showed that plasmin was well tolerated, but the studies were terminated, probably because of a lack of understanding of the kinetics of plasmin inhibition by anti-plasmin, and the unavailability at that time of local catheter delivery. Subsequently, the plasminogen activators streptokinase and urokinase were discovered; both can induce plasmin generation locally within

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