NAALADase inhibitors: A novel approach to glutamate regulation

Glutamate is an excitatory amino acid neurotransmitter that is required for normal brain function. However, during strokes and in various neurological disorders, it is produced in excessive quantities, which damages or kills nerve cells. Researchers have known for some time that inhibiting the action or release of glutamate could protect neuronal cells from injury. Regulating glutamate could be the key to treating conditions as diverse as stroke, spinal cord injury, amyotrophic lateral sclerosis (ALS), chronic pain, peripheral neuropathies and possibly schizophrenia and epilepsy.

The conventional approach to regulating glutamate levels has been to block the neuronal glutamate receptors. Although several such compounds reduce injury in experimental models of cerebral ischaemia[1], none has proved effective in clinical trials with stroke patients[2]. Furthermore, rats treated with these glutamate antagonists often show behavioural abnormalities (such as causing sedation and stereotypy) and disrupted learning and memory, together with histological evidence of neurodegeneration[3].

Researchers at Guilford Pharmaceuticals (Baltimore, MD, USA), led by Barbara Slusher, have developed a novel approach by inhibiting glutamate release at the presynaptic level, which both provides effective protection against the pathological effects of glutamate, and appears to leave basal glutamate pathways unaffected[4]. Glutamate release is prevented by inhibiting the enzyme NAALADase (*N*-acetylated-α-linked-acidic-dipeptidase), which hydrolyses the neuropeptide NAAG (*N*-acetyl-aspartyl-glutamate) to *N*-acetyl aspartate and glutamate. Inhibiting NAALADase both reduces glutamate levels and increases NAAG levels[4]. Raised NAAG could itself be beneficial because it activates group II metabotropic receptors and blocks NMDA receptors[5,6]. Both of these actions are known to be neuroprotective.

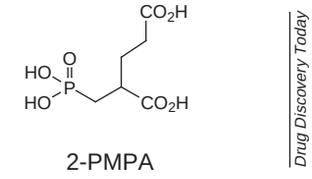

Figure 1. *Structure of the* N*-acetylated-α-linked-acidic-dipeptidase (NAALADase) inhibitor, 2-PMPA.*

Effects of NAALADase inhibitors

The role of NAALADase could not be studied in detail until the first potent inhibitor of the enzyme was characterized in 1996. This compound was 2-(phosphonomethyl)pentanedioic acid (2-PMPA or GPI5000), which appears to be selective for NAALADase (Fig. 1)[4], and is being used as a prototype compound to study the potential of NAALADase inhibitors to minimize brain damage in animal models of stroke.

In a tissue culture model of cerebral ischaemia using rat cortex, 2-PMPA showed strong, dose-dependent protection against ischaemia-induced metabolic inhibition[4]. At the highest concentration used (10 μM), there was 85% protection from cellular injury. Moreover, 2-PMPA had a significant protective effect even when treatment was delayed by 60 min after the ischaemic insult. After correlating the NAALADase K_is with the EC_{50}s of approximately 100 related compounds in the same model, the team was reasonably confident that the target of 2-PMPA was indeed NAALADase.

The effects of 2-PMPA were also assessed *in vivo*, using the MCAO (middle cerebral artery occlusion) model of stroke in rats[4]. An intraperitoneal bolus dose was given before the 2 h occlusion period, immediately followed by a 4 h intravenous infusion of 2-PMPA. The rats were killed 22 h after occlusion and the extent of brain injury was assessed using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Again, there was a dose-dependent reduction in the volume of injured brain tissue, with a reduction of 54% at the highest dose. Importantly, 2-PMPA was well tolerated, with the rats showing no signs of behavioural changes, learning deficits or neuropathology.

2-PMPA versus glutamate receptor antagonists

The Guilford team showed that the neuroprotection given by 2-PMPA is at least as good as that seen with glutamate receptor antagonists (such as CPP and MK801) in the same models, both in terms of the magnitude of the response and the time-course[6]. However, it is still unclear whether its protective effects are caused by decreased glutamate levels, higher NAAG levels or both. What is certain is that 2-PMPA does reduce gluta-

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mate concentrations *in vivo* in the MCAO model. Measurements using microdialysis probes showed an 80% reduction in extracellular glutamate concentration in the caudate nucleus of the brain and a complete prevention of ischaemia-
induced glutamate buildup in the parietal cortex[4]. By contrast, there was no significant effect on glutamate levels in normal, non-ischaemic rats. This apparent selectivity could explain the relative lack of side effects seen with 2-PMPA.

'NAALADase inhibition appears to affect glutamate only in pathological states', says Slusher. 'This is tremendously important. Basal glutamate does not seem to be affected, and we do not see the toxicities associated with other approaches to regulating glutamate. This potentially gives us a safe method to treat many disorders associated with excessive glutamate production.'

Future studies with NAALADase inhibitors

Stroke has presented various problems as a target for the first clinical application of NAALADase inhibitors, so the focus of research has now shifted to diabetic neuropathy and neuropathic pain. The high glucose levels found in patients with diabetes mellitus cause damage to the neurones and, although the mechanism is not currently understood, glutamate is known to be involved. 'We were really struck by the magnitude of protection offered in diabetic neuropathy models', Slusher said, 'both in the treatment of symptoms and in slowing disease progression.'

Guilford hopes to file an investigational new drug (IND) application for its lead NAALADase inhibitor compound for diabetic neuropathy and neuropathic pain by the end of 2000.

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Current approaches to multiple parallel synthesis

Approximately ten years ago, it was thought that organic chemistry was a mature science. Since then, however, chemistry has been rejuvenated with a technological revolution. This has been at great expense to the pharmaceutical and biotechnological industries and, so far, does not appear to have produced all the rewards it promised when combinatorial chemistry (CC) techniques were first conceptualized. An overview of current approaches to CC was given at the *Developing a successful combinatorial chemistry strategy to deliver more drug candidates* conference on 6–7 December 1999 in London.

Multiple parallel synthesis

The promise of automation

The traditional approach to synthesizing compounds and finding leads was 'linear', involving yes/no decisions at every step. If the wrong route was taken, one would return to the last stage and try a different route. Now, CC techniques enable parallel synthesis of compounds, which can generate typically 100-fold more compounds and, hence, 100-fold more data. Furthermore, it will enable the concurrent visualization of all the possible routes, enabling the correct decision to be made immediately. As highlighted by Geoff Lawton (Roche Products, Welwyn Garden City, UK), although this might actually slow down the process because the decision is more complicated, the final decision should be better.

Previously, projects were only started if there was a rationale for altering the chemistry of a particular ligand. Now, libraries can be screened and leads found rapidly. CC can increase the speed of lead generation and optimization and enable early low-cost target validation, as well as invalidation. However, to make automation beneficial, it is important to plan how to effectively use the 'saved' time, as it is very easy to waste it

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