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Glyxins to treat neurological disorders

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A small peptide that acts as a partial agonist on the glycine site of the brain's *N*-methyl-D-aspartate (NMDA) receptor has been shown to protect against stroke-induced neuronal damage and to alleviate neuropathic pain in animals. In addition, the compound enhances learning and memory. Clinical trials in patients with neuropathic pain are due to start later this year.

The NMDA receptor complex is a heteromeric protein with both receptor and ion channel functions. The channel is permeable to Ca^{2+} and monovalent cations and is activated by the excitatory neurotransmitter glutamate. Glutamate transmission via the NMDA receptor has a key role in neuronal processes associated with learning and memory [1]. However, excessive release of the neurotransmitter, resulting in increased calcium influx through the NMDA receptor, has been implicated in various neuronal hypoxic conditions [2]. Thus, the NMDA receptor could be involved in many neuropathologies, including stroke, Alzheimer's disease, epilepsy and schizophrenia. One in every four people worldwide develop such a mental or neurological disorder at some stage in their life [3], making the NMDA receptor complex an important drug target candidate.

Targeting the NMDA receptor

Modulating the receptor is a risky strategy. By promoting closure of the ion channel, NMDA receptor antagonists could protect from neuronal damage associated with excessive glutamate. However, attempts at achieving complete receptor blockade, for example, with drugs such as MK801 (dizocilpine; Merck, Whitehouse Station, NJ, USA), have shown that this approach can lead to unacceptable neurobehavioural side effects. Scientists have tried to circumvent this problem by targeting not the glutamate binding site on the NMDA receptor, but the glycine binding site; activation of the receptor complex requires occupation of both sites. However, although several glycine site antagonists showed promise in experimental models of stroke, they proved ineffective when evaluated in rigorous clinical studies; glavestinel (GV150526; GlaxoWellcome, Research Triangle Park, NC, USA) is such an example.

Developing agonists of the NMDA receptor is just as difficult. These compounds would enhance opening of the ion channel and could be beneficial for learning disorders or loss of memory function as a result of normal ageing or early-onset Alzheimer's disease. However, this approach carries the danger of producing the neuronal damage associated with excessive calcium flow through the NMDA receptor channel.

Scientists now hope that partial agonists will provide a solution. These agents do not produce a full pharmacological effect, even at high doses. If a glycine-like partial agonist is given in concentrations sufficient to occupy the glycine-binding sites at the brain's NMDA receptors, this could have a beneficial effect by stimulating the receptor a small amount, thereby enhancing learning. At the same time, the agent could prevent strong activation of the receptor in hypoxic conditions, such as brain ischaemia, thereby preventing neuronal damage. Michael Rogawski (National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA) believes that this approach is worth exploring. 'It remains to be seen whether this theoretical idea will work in practice. Could you really prevent toxicity? Will promoting the activity of NMDA receptors truly produce a clinically useful effect? These are open questions at this point.'

One partial agonist that targets the glycine site is D-cycloserine. The National Institute of Mental Health (NIMH; Bethesda, MD, USA) is now sponsoring a large multicentre trial to compare the effects of D-cycloserine with those of glycine or placebo in patients with schizophrenia. The trial is scheduled for completion by the end of 2003.

Glyxins

Another partial agonist is now due to enter clinical trials. NT13 (Nyxis Neurotherapies, Evanston, IL, USA) was developed using a patent-pending drug discovery method, Monoclonal Antibody Derived Custom Peptides (MADCP). Joseph Moskal (Northwestern University and Nyxis Neurotherapies), who developed this method, originally created and screened monoclonal antibodies to the hippocampus of young rats for their ability to affect learning and memory. The antibody B6B21 emerged from these experiments and was shown to act as a partial agonist on the glycine site of the NMDA receptor and to have an impact on rabbits' learning rates similar to D-cycloserine [1,4].

Moskal used B6B21 as a template to create a family of small peptides that mimic the biological activity of the antibody, which he refers to as glyxins. The tetrapeptide proline-threonine-threonine-proline (Fig. 1), or NT13, is now Moskal's prime drug candidate. The compound has been shown to cross the blood–brain barrier, and preclinical experiments demonstrated efficacy in various animal models.

Preclinical efficacy

By subjecting rats to hippocampus-dependent trace eyeblink conditioning, Moskal and colleagues demonstrated that intravenous treatment with NT13

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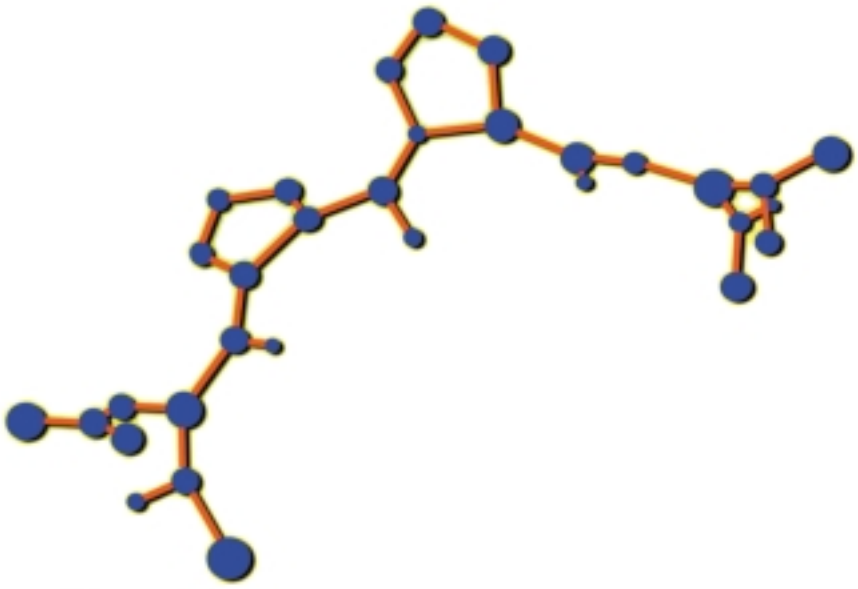

Figure 1. The peptide proline-threonine-threonine-proline (NT13) as it appears in solution. The NMDA-receptor partial agonist is due to enter clinical trials in patients with neuropathic pain. Figure kindly provided by Joseph Moskal (Northwestern University and Nyxis Neurotherapies, Evanston, IL, USA).

made animals acquire the associative learning task faster [5]. Moskal explains: 'Hippocampal lesions in humans and animals cause severe deficits in the ability to transfer information from short- to long-term stores and thus from new memories. In the trace paradigm, a blank 'trace' period intervenes between CS [conditioned stimulus; a warning tone] offset and US [unconditioned stimulus; a gentle puff of air into the eye] onset, which requires the formation of a very short-term memory of the CS in order to successfully predict US onset and perform conditioned responses [eye blink] timed properly to avoid the US.'

Using a gerbil model of ischaemia, the team demonstrated that NT13 given pre-ischaemia or 1–5 hours post-ischaemia had a neuroprotective effect equivalent to that of MK801 (Moskal, J.R. *et al.* Abstract to be presented at the *Society for Neuroscience 32nd Annual Meeting*, 2–7 November 2002, Orlando, FL, USA).

According to Moskal, NT13 can also alleviate neuropathic pain. To test this, the investigators crushed the sciatic nerves of rats to induce mechanical allodynia – a condition in which things that normally do not hurt cause pain. After 13 days, they measured the injured hindpaws' sensitivity to pressure with von Frey hairs (horsehair of increasing diameter). Moskal says that, in this model, '15 min and 60 min after injection, NT13 robustly and statistically reduced neuropathic pain'. Based on these results, the investigators plan to test proof-of-concept later on this year in patients with neuropathic pain.

References

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Peptide provides three-in-one protection

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A newly discovered peptide, derG, has been found to provide 100% protection from infection in a mouse model of malaria infection. These results, a collaboration between CEL-SCI Corporation (Vienna, VA, USA) and the Naval Medical Research Center (NMRC; Silver Spring, MD, USA), were presented at the *Experimental Biology 2002* Meeting (20–24 April 2002, New Orleans, LA, USA). Protection from disease symptoms was also reported with the herpes simplex virus (HSV) skin scarification and tumour models, after administration of this peptide [1].

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