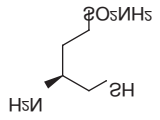
has described the synthesis of a library of pseudotripeptides that have micromolar affinity for TeNt [Martin, L. *et al.* (1999) *J. Med. Chem.* 42, 515–525].



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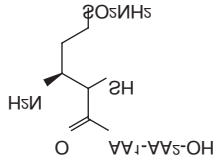

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The thiol compound (**3**, K_i = 100 μM), recently reported as an inhibitor of TeNt, was the starting point for this study. Making the assumption that the thiol is the zinc-chelating group led to the design of extended analogues that might interact with the S′ subsites of the enzyme's active site. The combinatorial library of 19 mixtures (**4**) were prepared by solid-phase mix and split peptide synthesis on 2-chlorotrityl chloride resin. From the deconvolution of the library it was determined that a preferred TeNt inhibitor had AA_1 = Tyr and AA_2 = His. Separating the diastereoisomers revealed one compound with K_i = 5 μM. *Ex vivo* and *in vivo* studies of these and other compounds are now in progress.

D-peptide antigens

Synthetic antigen mimetics, which are recognized by antibodies, are of great interest as they may provide clues to the understanding of antigen recognition, and ultimately lead to the design of more effective immunodiagnostics and synthetic vaccines. Epitope mapping, more recently using combinatorial chemistry, has been invaluable in revealing the peptide sequence of antigens, but a recent study has demonstrated the discovery of novel all D-amino acid peptides that bind an antibody with high affinity [Pinilla, C. *et al.* (1998) *J. Mol. Biol.* 283, 1013–1025].

Positionally scanning libraries of both all-L and all-D hexapeptides were generated and screened for inhibition of monoclonal antibody HGAC 39 G3 that binds to an antigen displaying *N*-acetyl-D-glucosamine residues. It was found that the all-D sequences were the most potent inhibitors with the sequence Ac-yryygl-NH_2 recognized with a relative affinity of 300 nM. The study supports the concept that some monoclonal antibodies are functionally polyspecific as they can recognize multiple antigens with distinct chemical characteristics.

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Contribution of oxidative stress to excitotoxicity-induced deleterious iNOS in the CNS

Nitric oxide (NO) is a gaseous messenger which is involved in several physiological processes in the brain [Dawson, T.D. and Dawson, V.L. (1994) *Neuroscientist* 11, 9–20]. It is synthesized by the NO-synthases (NOS), of which three isoforms have been cloned [Kerwin, J.F. and Heller, M. (1994) *Med. Res. Rev.* 14, 23–74]. The neuronal (NOS-1) and endothelial (NOS-3) isoforms are Ca^{2+}-dependent and constitutively expressed whereas NOS-2, also called iNOS, is Ca^{2+}-independent and inducible. However, evidence has accumulated during the last decade that demonstrates a deleterious role for NO under pathophysiological conditions in the brain, such as cerebral ischaemia. Nowicki, J.P. and coworkers [*Eur. J. Pharmacol.* (1991) 204, 339–340] first demonstrated the neuroprotective effect of NOS-1 inhibition on mice exposed to cerebral ischaemia. A few years later, a delayed NO production originating from iNOS was described in the formation of ischaemic lesions [Iadecola, C. *et al.* (1995) *Am. J. Physiol.* 268, R286-R292]. Although iNOS has been shown to be induced by multiple mechanisms *in vivo* and *in vitro*, the pathways that lead to iNOS expression during cerebral ischaemia remain unclear.

We have therefore focussed our work in determining the mechanisms responsible for iNOS expression in such a neuropathology. In particular, we were interested in excitotoxicity, a deleterious event that occurs in the very early stages of cerebral ischaemia. Excitotoxicity results from an accumulation in the synaptic cleft of the excitatory amino acid glutamate. Supra-physiological concentrations of glutamate activate post-synaptic glutamate receptors, in particular the NMDA receptor subtype. This NMDA receptor activation has been shown to result in an immediate and detrimental increase in NO production by the activation of NOS-1. Of particular interest was whether this NMDA-induced excitotoxicity is able to trigger delayed NO-synthesis due to its ability to activate iNOS under these conditions.

In a model of excitotoxicity in the rat where NMDA was perfused in the striatum through a microdialysis probe, Ca^{2+}-independent NOS activity appeared 48 hours after NMDA exposure [Lecanu, L. *et al.* (1998) *Br. J. Pharmacol.* 125, 584–590]. In addition, this activity was accompanied by an increase in the production of the NO metabolite nitrite, as measured in microdialysate samples. We showed that this NOS activity as well as nitrite production was reduced by dexamethasone, a glucocorticoid known to block iNOS gene expression, and by aminoguanidine, a direct inhibitor of the iNOS enzyme. These results provided the first evidence of an iNOS induction

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secondary to an excitotoxic insult. Moreover, both these pharmacological tools reduced the striatal lesion, indicating a deleterious role for iNOS during excitotoxicity. These last results are of interest because they indicate that the excitotoxicity-triggered NO production is biphasic: an early production occurs because of NOS-1 followed by a delayed production due to iNOS, and both are detrimental to neuronal survival.

iNOS is expressed in several cell subtypes and is ubiquitously distributed in the body. In peripheral tissues, iNOS was shown *in vivo* and *in vitro* to be expressed after oxidative stress was induced. Because NMDA receptor overactivation was shown to generate excessive free radical production [Hammer, B. *et al.* (1993) *NeuroReport* 5, 72–74], we assessed whether oxidative stress contributes to iNOS induction during excitotoxicity. We initially studied the ability of oxidative stress to induce iNOS in the CNS of the rat. Oxidative stress was induced by intra-striatal injection of malonate, a mitochondrial toxin that inhibits the respiratory chain leading to free radical production. Forty-eight hours after malonate injection, an aminoguanidine-sensitive Ca^{2+}-independent NOS was expressed, suggesting that iNOS had been activated [Lecanu, L. *et al.* (1998) *NeuroReport* 9, 559–563]. These results were supported by the use of a specific immunostaining technique to show iNOS localization in neurons, microglia and vascular endothelial cells. This iNOS activity was reduced by α-phenyl-tert-butyl-nitrone (PBN), a free radical scavenger, and by N-acetyl-cysteine (NAC), a glutathione precursor, providing evidence, for the first time, for redox control of iNOS expression in the CNS.

In a second series of experiments, we studied the involvement of this oxidative stress in excitotoxic-induced iNOS expression. A number of different anti-oxidants were employed, including PBN, NAC and the pineal hormone melatonin (MEL). The excitotoxic model was created by direct injection of NMDA into the striatum of the rat. NMDA exposure resulted in a decrease in the concentration of striatal glutathione within 48 hours, indicating exposure to oxidative stress. Concomitantly, the appearance of aminoguanidine-sensitive Ca^{2+}-independent NOS activity, associated with specific iNOS immunostaining in the neurons, microglia and vascular endothelial cells, provided evidence for iNOS expression. All the anti-oxidants reduced the decrease in glutathione levels, suggesting that both oxidative stress, and iNOS activity was reduced. Taken together, these results suggest that NMDA-triggered iNOS induction in the CNS involves excessive free radical formation. In addition, because aminoguanidine, PBN, NAC and MEL reduced the excitotoxic neuronal damage, it would appear that the induction of oxidative stress and of the iNOS pathway has a detrimental effect.

These results raise the question of the occurrence of such a pathophysiological pathway during cerebral ischaemia. In this context, the determination of oxidative stress/iNOS contribution to ischaemic lesion formation will be of interest for the development of new therapeutic approaches. Moreover, because excitotoxicity, oxidative stress and iNOS have been shown to be extensive in the brain during neurodegenerative processes such as Alzheimer's and Parkinson's disease, our results may improve the understanding of the pathophysiological mechanisms that contribute to neuronal death in such neurological pathologies.

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