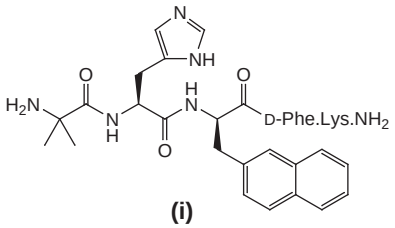
Combinatorial chemistry

Optimization of growth hormone secretagogues

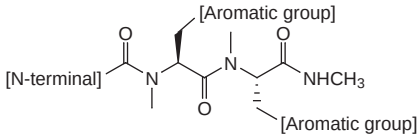
Several peptidic compounds have been discovered that have the ability to release growth hormone (GH) from the pituitary. Through this mechanism, these agents could offer the opportunity to treat several medical conditions including GH deficiency, osteoporosis and obesity. Several acylated dipeptides related to ipamorelin (**i**) have been investigated as GH releasers, and a recent



(i)

paper describes the use of combinatorial chemistry to investigate structure–activity relationships (SAR) in this series[1].

The 96 compounds in the library were individually synthesized on an *N*-methylated amine solid-phase support in a fashion that enabled variation of three positions of diversity (**ii**) and the generation of a terminal *N*-methylamide following trifluoroacetic acid-catalyzed cleavage. The *in vitro* GH-releasing



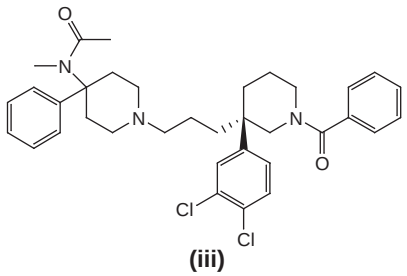
(ii)

properties of the purified products were determined in a rat pituitary cell assay, and full agonist potencies with EC_{50} values down to 1 nM were observed. It was notable that there was a distinct unpredictability in the SAR, in that some structural building blocks did not confer activity in a predictable manner. This very unpredictability emphasizes the advantages of making every compound in the combinatorial library, rather than relying on isolated compounds to develop the SAR.

1 Ankersen, M. *et al.* (1999) Demonstration of the strength of focused combinatorial libraries in SAR optimisation of growth hormone secretagogues. *Eur. J. Med. Chem.* 34, 783–790

Neurokinin-3 receptor antagonist SAR

The neurokinin-3 receptor is one of three receptors that binds the tachykinin or neurokinin family of peptides. Several non-peptide antagonists of the human receptor have been reported, and the use of combinatorial chemistry to optimize the antagonist, SR142801 (**iii**), has recently been disclosed[2].



(iii)

The 3-(3,4-dichlorophenyl)-3-propylpiperidine fragment was held constant while the amine and acylating group substituents were varied in an indexed (or orthoganol) combinatorial library. In total, 49 compounds were prepared using solution-phase methods in orthoganol pools of seven compounds. By screening the compounds while in these mixtures, it was possible to identify which of the peripheral groups made the greatest contribution to receptor affinity. These predictions were confirmed by the preparation and testing of six individual compounds, although the best compound discovered from this approach was still ≈30-times weaker than SR142801.

2 Raveglia, L.F. *et al.* (1999) Investigation of SAR requirements of SR 142801 through an indexed combinatorial library in solution. *Eur. J. Med. Chem.* 34, 825–835

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Apoptosis in immature brain: a therapeutic approach to neuronal death

Neonatal hypoxic–ischaemic (HI) encephalopathy is an easily recognized clinical syndrome in 2–4 out of a 1000 full-term newborn infants that suffer asphyxia at, or shortly before, birth. Approximately 15–20% of such asphyxiated infants die during the newborn period and, of the survivors, 25% will exhibit permanent neurological deficits[1]. Experimental models of HI in the rat pup have been used for dose–response evaluations of a series of neuroprotective agents including free radical scavengers, excitatory amino acid antagonists and voltage-sensitive calcium channel blockers[2].

The regulation of cell number is an important function for the precise control of unwanted and dangerous cells. During development or after pathological conditions, apoptosis serves as a significant regulatory mechanism whereby the organism eliminates cells by the execution of death programs[3]. Initiation of apoptotic pathways begins with death-triggering signals, followed by an execution phase where activated cellular mechanisms stimulate multiple biochemical cascades. Finally, a destruction phase is entered where morphological changes occur and cellular breakdown begins. Several lines of evidence now indicate that apoptosis is an important mechanism in cell death associated with stroke, epilepsy and neurological disorders.

Many of the neuropathological characteristics of apoptosis have been

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demonstrated by internucleosomal fragmentation of DNA, light and electron microscopy analysis of chromatin condensation, and labeling of double-strand DNA breaks *in situ* using the terminal transferase (TUNEL) assay[4]. Studies have also reported that a cell death program could be activated following ischaemia, as inhibitors of protein synthesis have been shown to significantly attenuate ischaemic damage. Furthermore, cells that exhibit DNA fragmentation and chromatin condensation appear to be localized at the inner boundary of the infarcted tissue whereas necrotic cells are found primarily in the ischemic core.

Despite the availability of these important markers, MacManus, J.P. and Linnik, M.D. have noted that ultrastructural features of apoptosis in ischaemic rat brain are only always observed in neonates[5], supporting our own observations[6]. The reasons for this are not well understood, although it has been suggested that the immature brain retains a part of the developmental cell death program while terminally differentiated neurons exhibit pyknosis (condensation of nuclear material into a homogenous body following cell death) or die by necrosis. It is now well established that constitutively expressed pro-apoptotic and anti-apoptotic proteins such as the caspases, the Bcl-2 family, and inhibitors of apoptosis protein (IAP) family members, as well as enhanced expression of Bcl-2 and IAP, ameliorate injury in experimental stroke.

The hypothesis that neuronal apoptosis occurs after HI insults to the CNS raises the possibility that interventions directed at blocking ischaemic apoptosis would constitute a potential therapeutic strategy. This might have two attractive features. Firstly, additivity with anti-excitotoxicity therapy and, secondly, a prolonged 'window of opportunity'. Recently, the potential value of combining treatment strategies (anti-excitotoxic and anti-caspase) was reported as an approach to reducing potential side effects and extending the treatment window following cerebral ischaemia in adult rodents[7]. As a cell-permeable pan-caspase inhibitor has been found to be neuroprotective after HI in neonate rats even when administered after some delay, it seems that such a combination could be more effective in immature than adult animals.

In conclusion, the developing brain responds differently to HI than the adult brain and recent data suggest that features seen in the process of apoptosis could be favored in the developing versus the adult brain in response to different brain injuries. The effects of most of these new open therapies, which appear to be efficient in adult animals on blood glucose, body temperature and/or the systemic circulation, should be measured and the protective effects confirmed in larger species prior to considering clinical applications. Furthermore, further investigations should be directed towards identifying the mechanism of global insult in immature animals and studying whether tissue treated with inhibitors of apoptosis will develop fully functional neurons.

1 Volpe, J.J. (1987) *Neurology of the Newborn* (Saunders, W.B., ed.), pp. 236–280, Philadelphia

2 Vannucci, R.C. and Perlman, J.M. (1997) Interventions for perinatal hypoxic–ischemic encephalopathy. *Pediatrics* 100, 1004–1014

3 Thompson, E.B. (1998) Special topic: Apoptosis. *Annu. Rev. Physiol.* 60, 525–532

4 Charriaut-Marlangue, C. *et al.* (1996) Apoptotic features of selective neuronal death in ischemia, epilepsy and gp120 toxicity. *Trends Neurosci.* 19, 109–114

5 MacManus, J.P. and Linnik, M.D. (1997) Gene expression induced by cerebral ischemia: An apoptotic perspective. *J. Cereb. Blood Flow Metab.* 17, 815–832

6 Renolleau, S. *et al.* (1998) A model of transient unilateral focal ischemia with reperfusion in the P7 neonatal rat – morphological changes indicative of apoptosis. *Stroke* 29, 1454–1460

7 Schulz, J.B. *et al.* (1999) Caspases as treatment targets in stroke and neurodegenerative diseases. *Ann. Neurol.* 45, 421–429

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