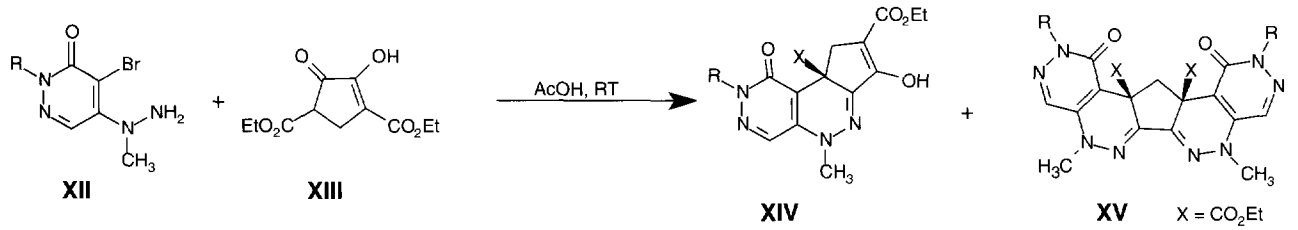
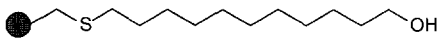
Scheme 5



High fluidity supports for solid phase synthesis



XVI

The need for improved solid-phase supports has been driven by the development of combinatorial and parallel syntheses. Traditional supports often have low capacity and in many cases the chemistry that can be readily performed using these supports is limited by the low accessibility of reaction sites on the solid support. Brown, J.M. and Ramsden, J.A. [*Chem. Commun.* (1996) 2117–2118] have described the synthesis of a novel support **XVI** with both a high functionality and fluidity by modification of chloromethylpolystyrene particles.

Angiotensin in psychiatry – a possible role

Angiotensin II is a peptide known predominantly for its effects on the cardiovascular system. However, it is also a neurotransmitter and neuromodulator, having effects not only on fluid intake but also on the hypothalamico-pituitary sex hormone axis and on animal behaviour. The availability of selective, nonpeptide antagonists for the angiotensin (AT) receptor has resulted in the identification of two pharmacologically distinct high-affinity binding sites for angiotensin II, designated AT_1 and AT_2 receptors. The majority of pharmacological actions of angiotensin II appear to be mediated by the AT_1 receptor, however both AT_1 and AT_2 receptors have been identified in the human brain, with the AT_1 subtype predominating [Barnes, J.M. *et al. Eur. J. Pharmacol.* (1993) 230, 251–258]. In rat brain, AT_1 receptors account for approximately 90% of the angiotensin binding, although AT_2 receptors are the major subtype in the midbrain [Höhle, S. *et al. Pharmacol. Toxicol.* (1995) 77, 306–315].

Effects of angiotensin on animal behaviour

Angiotensin has been implicated in the control or modification of several aspects of behaviour with both receptor subtypes playing roles. One of the earliest behavioural effects to be reported was that of drinking in rats. Subcutaneous administration of angiotensin II to rats results in a rapid (30 minute) increase in drinking that lasts for about 20 minutes. Fluid intake increases about fourfold above controls. Interestingly, we have been unable to elicit this response in mice, an observation corroborated by another laboratory. This response in rats is mediated by AT_1 receptors located in the circumventricular organs. These brain areas have little protection by the blood–brain barrier (BBB), thus explaining how a peripherally administered peptide can produce a central response. Other behaviours that are affected by angiotensin are memory, learning and locomotor activity [Braszko, J.J. *et al. Behav. Brain Res.* (1987) 25, 195–203]. Again the responses primarily involve AT_1 receptors, though there is now data to suggest involvement of the AT_2 receptors. Several of the central responses mediated by AT_1 receptors appear to be under inhibitory control by AT_2 receptors [Höhle, S. *et al. Pharmacol. Toxicol.* (1995) 77, 306–315] and mice that lack the gene encoding for AT_2 receptors exhibit decreased locomotor activity, suggesting that these receptors may normally be involved in the control of locomotor or exploratory behaviour [Ichiki, T. *et al. Nature* (1995) 377, 748–750].

Control of psychiatric disease

As many of these studies of minute aspects of rodent behaviour appear esoteric and of little relevance to human medicine, what evidence is there to suggest that the ability to modify the central actions of angiotensin may pay dividends in the ability to control human psychiatric diseases?

There are two disorders worthy of investigation: anxiety and depression. In the case of anxiety it has been shown that the AT_1 receptor antagonist losartan produces positive results in the mouse light–dark box [Barnes, N.M. *et al. NeuroReport* (1990) 1, 15–16], a test used in the identification of potential anxiolytic agents, although we have found that these effects of losartan in the light–dark box only occurred in one of the strains of mice tested, the other four strains of mice failed to respond. We have also been able to demonstrate a positive effect of losartan in rats and mice in another test used for the study of anxiolytic agents, the elevated plus maze [unpublished data]. To my knowledge there have been no studies of the effects of AT_1 antagonists on anxiety in humans, although the angiotensin-converting-enzyme inhibitor ceranapril (SQ29852), which reduces the conversion of the nonapeptide angiotensin I to the octapeptide angiotensin II, was found to be comparable to diazepam in the treatment of generalized anxiety disorder [Smith, W.T. *et al. Proc. 17th Congr. Coll. Int. Neuropsychopharmacol.* (1990) 12–1–12].

Evidence for the involvement of angiotensin in the treatment of depression is somewhat stronger than in the case of anxiety. In rats the angiotensin-induced

drinking response is significantly reduced by the clinically proven, chemically dissimilar antidepressants desipramine, fluoxetine and tranylcypromine. Similarly, when studied in isolated tissues, these three drugs were all reversible, but insurmountable, pharmacological antagonists of angiotensin II [Gard, P.R. *et al. Eur. J. Pharmacol.* (1994) 264, 295–300], although neither desipramine nor fluoxetine bind directly to the AT_1 receptor [Mandy, A. *et al. J. Pharm. Pharmacol.* (1994) 46, 1056], suggesting that the effect is due to interference with a post-receptor event, for example generation of a second messenger.

The first clinical indication of an involvement of angiotensin II in the treatment of depression came with the report that the anti-hypertensive ACE inhibitor captopril had a mood-elevating effect in two out of three depressed patients treated [Zubenko, G.S. and Nixon, R.A. *Am. J. Psychiatry* (1984) 141, 110–111]. Since then there have been several other clinical case reports of depressed patients, satisfying DSM-III or scoring highly on the Beck Depression Inventory and the Hamilton Rating Scale for Depression, being treated successfully with captopril [e.g. Deicken, R.F. *Biol. Psychiatry* (1986) 21, 1425], and another ACE inhibitor, enalapril, has been seen to produce mood elevation in normal volunteers [Cohen, L. *et al. Am. J. Psychiatry* (1984) 141, 1012–1013]. As yet there have been no large-scale controlled clinical trials of the antidepressant effects of reducing angiotensin II activity but in the forced swim test in mice both captopril and losartan produce effects characteristic of clinically proven antidepressants [Giardina, W.J. and Ebert, D.M. *Biol. Psychiatry* (1989) 25, 697–702; Gard, P.R. *et al. J. Pharm. Pharmacol.* (1994) 46, 1056] and in the learned helplessness paradigm in rats, captopril was equipotent with imipramine [Martin, P. *et al. Biol. Psychiatry* (1990) 27, 968–974]. Thus, not only do known antidepressants reduce angiotensin activity, but drugs that reduce angiotensin (probably AT_1) function also have antidepressant actions.

The future

These findings, in both animals and humans, suggest that drugs that modify the function of the neurochemical angiotensin II, or of the AT_1 or AT_2 receptors, may have potential for use in the treatment of certain psychiatric illnesses. The challenge will be to obtain selective effects in the brain without modulating the peripheral renin–angiotensin system. Obvious approaches are the development of prodrugs that freely cross the BBB, or coadministration of drugs that inactivate the novel compound peripherally but do not cross the BBB, in a way analogous to the coadministration of L-dopa and a peripheral decarboxylase inhibitor in the treatment of Parkinson's disease, but these are problems for the medicinal chemists to solve!

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Combinatorial chemistry

Molecular framework analysis

An analysis of drug molecule structures has recently been described by chemists from Vertex Pharmaceuticals (Cambridge, MA, USA) [Bemis, G.W. and Murcko, M.A. *J. Med. Chem.* (1996) 39, 2887–2893]. Although not strictly a combinatorial chemistry paper, the findings of this analysis may have an impact on the future design of scaffolds for lead discovery libraries.

Over 5,000 different drug molecules were analysed using both graph and atomic properties. Using graph theory analysis, 1,179 different frameworks were found, of which 783 (66%) were represented by only one drug each. In contrast, 32 frameworks accounted for 50% of all drug molecule structures. In a second analysis that considered atom type, hybridization and bond order, 2,506 different frameworks were identified with just 42 frameworks accounting for a quarter of all drug structures. This analysis raises the intriguing question of whether drug molecules are constrained by receptors and enzymes to take up these particular frameworks, or whether the frequency of some frameworks reflects particular biases on the part of medicinal chemists.

Novel encoding strategy

A number of ways now exist to resolve the structures of combinatorial library compounds via the analysis of an encoding molecule synthesized in parallel. However, the encoding molecule can interfere with biological screening of the library unless it is present in very small quantities. An alternative solution to this problem has been developed by Barany and colleagues [Barany, G. *et al. Proc. Natl. Acad. Sci. U. S. A.* (1996) 93, 8194–8199]. This paper explains that it is possible to use chymotrypsin to proteolyse peptide substrates bound to the surface of a polyethyleneglycol-grafted polystyrene bead, without affecting the molecules held within the bead. Thus the surface residues have been distinguished from the internal residues and using the orthogonal Fmoc- and Boc-protecting strategies for peptide synthesis, library compounds can be assembled on the surface, and a complementary encoding sequence assembled in the interior.

This technique has been used to synthesize an encoded peptide library of 100,000 members and these were screened against three different receptors: an anti-β-endorphin antibody, streptavidin and thrombin. In each case the library was assayed on the solid-phase such that the biological protein target only had access to the surface residues and the encoding peptides could not interfere with the assay. Beads bearing active peptide sequences could be distinguished by a colour change and the encoding peptide sequenced using Edman degradation. The expected recognition motifs were found for anti-β-endorphin antibody and streptavidin, and a new thrombin ligand (Arg-Gly-Arg-Pro-DPhe, K_i = 5.7 μM) was identified.

This enzyme-mediated spatial segregation strategy allows a ready differentiation between the surface and interior residues on resin beads. Although the technique was validated by the synthesis of peptide-encoded peptide library, the approach holds promise for the synthesis of non-peptide combinatorial libraries.

ACE-MS for on line screening

Affinity capillary electrophoresis-mass spectrometry (ACE-MS) is described as a new methodology for the on-line screening and identification of solution combinatorial libraries [Karger, B.L. *et al. J. Am. Chem. Soc.* (1996) 118, 7827–7835]. ACE-MS was demonstrated using the binding of vancomycin to libraries of

