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

all D-tri- and tetrapeptides. Peptide sequences that bound strongly to vancomycin could be recognized by the longer capillary electrophoresis retention times of these compounds, and on-line mass spectral analysis gave immediate identification of the compound sequences. This technique was studied for libraries of 500–1,000 components, but with more sensitive mass spectrometry detection larger libraries could be analysed directly.

Isotopic encoding of bead libraries

A novel approach to combinatorial library compound structure elucidation has been proposed by a group from Glaxo Wellcome [Geysen, M. *et al. Chemistry & Biology* (1996) 3, 679–688]. As mass spectrometry is a highly sensitive technique for the characterization and quantification of organic compounds, this approach has been used to encode for the individual compounds in a library. As organic compounds appear in the spectrum as a singly charged ion, any isotopic variation in the compound's composition will show up as a predictable shift in molecular weight. This principle has been used to generate several methods for the isotopic or mass encoding of bead libraries.

One approach uses a coding sequence of two glycine monomers each containing either zero, one or two ^{13}C-atoms. Using these monomers either alone or in equimolar quantities gives rise to 25 different highly predictable mass spectrum 'bar code' patterns generated over a very small region of the mass spectrum.

This and other isotope encoding strategies are most effective for 'one bead–one compound' libraries, and particular care needs to be taken in matching the encoding strategy with the bead size and the chemical loading being employed.

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Emerging molecular targets

Tissue-specific estrogen therapy

Estrogen replacement therapy prevents resorption of bone and provides a strong protective effect against cardiovascular disease in postmenopausal women. However, estrogen therapy is not without danger. Some cervical and breast tumor cells are stimulated to grow by estrogen, requiring women to make a difficult choice. If they take estrogen, they will benefit from its well-known protective effects while at the same time they increase their risks of developing cervical or breast cancer.

If they decide to forgo estrogen therapy, they will almost certainly have some bone loss, which puts them at risk of developing osteoporosis, and they will increase their risk of developing cardiovascular disease. Recent research on the molecular mechanism of action of estrogen suggests some new strategies for developing drugs that will provide only the beneficial actions associated with estrogen therapy.

Bone resorption occurs in postmenopausal women because the lack of estrogen permits the overgrowth of osteoclasts, large multinucleated cells that perforate the bone and resorb the minerals that make up the bone structure. Estrogen prevents the growth of osteoclasts in bone, but until the recent studies of David E. Hughes and coworkers at the University of Texas Health Science Center (San Antonio, TX, USA) and the University of Sheffield (Sheffield, UK) the mechanism of its action was unknown. They found that 17β-estradiol triggers apoptosis, or programmed cell death, of osteoclasts grown in cell culture, and *in vivo* studies in ovariectomized mice suggest that this is how estrogen controls osteoclast growth in bone [*Nat. Med.* (1996) 2, 1132–1136].

Apoptosis of osteoclasts is a newly discovered pathway for the action of estrogen, and most of the details remain to be established. The investigators believe that the induction of apoptosis by estrogen is mediated by transforming growth factor-β (TGF-β). They found that estrogen induces the synthesis of TGF-β and antibodies against TGF-β blocked the action of estrogen on the osteoclasts. The source of the TGF-β is unclear; the studies were conducted with cultured cells consisting of a mixture of osteoclasts and stromal cells.

Another recent discovery regarding the mechanism of action of an estrogen analogue, raloxifene, also suggests that tissue-specific estrogen analogs are possible. Raloxifene acts as an estrogen antagonist in the uterus and the breast, but it acts as an estrogen agonist in the preservation of bone and in reducing cholesterol levels. Na Yang and coworkers at Lilly Research Laboratories (Indianapolis, IN, USA) found that activation of the TGF-β3 gene by raloxifene in osteosarcoma cells requires the estrogen receptor, but the raloxifene-estrogen receptor complex does not act by binding to the estrogen response element of DNA as might be expected. Instead, the raloxifene–estrogen receptor complex utilizes a cellular adapter protein to bind to a newly discovered polypurine DNA sequence on the TGF-β3 gene [*Science* (1996) 273, 1222–1225]. This polypurine sequence has now been termed the raloxifene response element or RRE. The nature of the cellular adapter protein remains to be determined.

Both of these discoveries – that estrogen protects women from developing osteoporosis by limiting the lifespan of osteoclasts and the presence of multiple DNA response elements for the estrogen receptor – help explain the wide range of effects of estrogen on the female body. Most important, however, they provide a rational approach for the development of tissue-selective estrogen therapies that will allow postmenopausal women to enjoy the positive aspects of estrogen therapy without the danger of developing cervical or breast cancer.

Robert W. Wallace

About Bob Wallace...

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