Update on endothelins

The latest developments in the endothelin field were the topic of a one-day symposium held in London last December. The symposium, entitled *Endothelins; New Therapeutic Opportunities*, was organized by the Society for Medicines Research.

Eight presentations, all from scientists well-respected in their discipline, covered the entire field from endothelin receptor structure and multiplicity, through the chemical design of antagonists to their evaluation in the clinic.

Endothelium-derived contracting factors

The day commenced with a review of the role of endothelium-derived contracting factors (EDCF) by Professor P.M. Vanhoutte (Servier, Paris, France). He explained that endothelial cells can control underlying smooth muscle tissue by releasing either relaxing factors, such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF), or EDCFs, such as superoxide anions, thromboxane A_2 and endothelin-1 (ET-1).

ET-1 is a 21-amino-acid peptide that is synthesized from preproendothelin in the endothelial cells. The preproendothelin is cleaved to big-endothelin-1, which is converted by endothelin-converting enzyme (ECE) to ET-1. The production of ET-1 can be stimulated by various neurotransmitters and hormones, such as thrombin and angiotensin II, and is strongly inhibited by nitric oxide (NO), which can also inhibit the action of endothelin-1 on smooth muscle cells. The role of ET-1 in vascular pathology is not completely clear yet. Professor Vanhoutte thinks that ET-1 does play a role in vascular disease, but believes that the increased levels of ET-1 that are observed in diseased tissue are a result of a disturbed NO system.

ET receptor subtypes

This introduction to EDCFs and the production of endothelin was followed by a review of endothelin receptors by Dr A.P. Davenport (Clinical Pharmacology, University of Cambridge, UK). Dr Davenport's group set out to characterize the ET_A and ET_B receptor subtypes and determine their localization in the human cardiovascular system. Using ligand binding studies, they found that in the smooth muscle cells of the blood vessels of the heart, lungs, brain and kidney the ET_A subtype predominates; approximately 90% of the ET receptors were found to be of the ET_A and 10% of the ET_B subtype[1]. The receptors were localized in the plasma membrane. ET_B receptors were mainly detected in endothelial cells, at sites of neovascularization and in nerves.

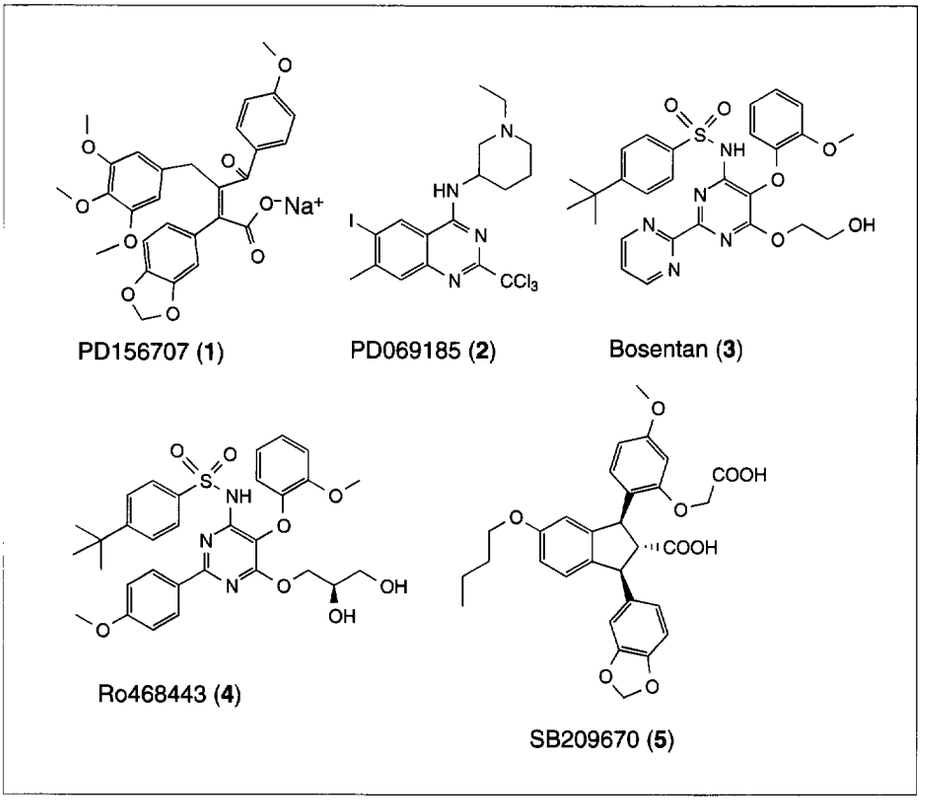
The group also investigated disease-associated changes in the expression of ET-receptor subtypes and found that ET_A receptors predominate in atherosclerotic vessels[2]. In agreement with the ligand binding studies, functional studies on isolated vessels showed that ET-1 induced vasoconstriction is mainly mediated by the ET_A receptors, which suggests that selective ET_A receptor antagonists could be valuable new therapies for cardiovascular disease.

Big endothelin-1

Dr R. Corder (William Harvey Research Institute, London, UK) discussed the structure of big endothelin-1 (big ET-1) and mechanism of action of ECE, an endopeptidase that selectively cleaves the Trp21-Val22 bond. Dr Corder's group produced modified forms of big ET-1 and monitored their conversion to ET by ECE. The results were used to develop a molecular model of big ET-1 (Ref. 3). The localization and regulation of ECE was also discussed. ECE is found both integrated in membranes and in soluble form in secretory vesicles. Immunoelectron microscopy has shown that the latter form produces ET-1. The relationship of the integral membrane ECE to ET-1 is not known.

ET receptor antagonists

In 1990, both the ET_A and ET_B receptor subtypes were cloned. This made it



possible to screen vast numbers of compounds for ET-receptor affinity and obtain leads for the development of ET receptor antagonists.

Dr Annette Doherty (Parke-Davis, Ann Arbor, MI, USA) reviewed the discovery and development of several potent, nonpeptide ET antagonists that have evolved from the screening programmes. Scientists at Parke-Davis have synthesized an ET_A-selective antagonist from a library lead, PD156707 (**1**) (see figure), which will be ready for IND filing in 1997. This compound has a very high affinity for the human ET_A receptor (IC_{50} = 0.3 nM) and moderate affinity for the ET_B subtype (420 nM). It was shown to reduce damage after focal cerebral ischaemia by 45% by reducing cerebral blood flow. A number of analogues of PD156707 were found to be potent ET antagonists with varied ET_A/ET_B receptor selectivity, some displaying improved solubility and/or longer duration of action. Scientists at Parke-Davis are also developing an ECE inhibitor. PD069185 (**2**; IC_{50} = 0.9 μM) was identified as an ECE inhibitor by library screening. In CHO/preproendothelin-1 cells it inhibits the conversion of big ET-1 with an EC_{50} of 3.8 μM. Other quinazoline derivatives have been prepared and a number were found to be selective ECE-1 inhibitors over other closely related metalloproteinases.

A presentation by Dr V. Breu (Hoffmann-La Roche, Basel, Switzerland) on the endothelin antagonists developed by Roche followed. In the endothelin field, Roche is concentrating on two compounds: bosentan (**3**), their clinical candidate, and Ro468443 (**4**), an ET_B-selective antagonist. Bosentan is a mixed ET antagonist, with an K_i of 5 nM for the ET_A receptor and 100 nM for the ET_B receptor[4]. It showed efficacy in animal models for renal ischaemia and cerebral vasospasm after subarachnoidal haemorrhage (acute treatment) and also in models of chronic heart failure and systemic and pulmonary hypertension (chronic situations). Dr Breu and coworkers found that in certain animal models the simultaneous blockade of ET_A and ET_B receptors is more effective than selective blockade of the ET_A receptor. This could be the result of the upregulation of ET_B receptors, which is observed in certain pathological conditions. In coronary heart disease, for example, equal amounts of ET_A and ET_B receptors were found, while in healthy arteries only 17–20% of ET receptors are of the ET_B subtype. Using selective ET_B receptor antagonist Ro468443 [$K_i(ET_A)$ = 5 μM, $K_i(ET_B)$ = 5 nM], it was demonstrated in rat models of hypertension that both ET_B-dependent vasoconstriction and vasodilation may become upregulated[5].

(Patho)physiological role of endothelin

Many groups have investigated the role of endothelin in pathophysiological processes, and the results of this research implicate that endothelin is involved in diseases such as systemic and pulmonary hypertension, congestive heart failure, hypercholesterolaemia and atherosclerosis. However, little is known yet about endothelin's role in normal physiological processes. Dr T. Warner (The William Harvery Research Institute, London, UK) reviewed work to establish this role using gene-knockout mice. ET-$1^{+/-}$ knockout mice were found to have a higher blood pressure, but lower levels of ET-1 than normal mice, whereas ET-$1^{-/-}$ mice showed craniofacial abnormalities, could not breathe properly and died within 30 minutes[6]. ET-3 knockout mice appeared normal, but died within 3–4 weeks. This suggests that both ET-1 and ET-3 are important for the development of neural crest cells. The role of ET in mature species is not clear yet; ET receptor antagonists often have little or no effect on the blood pressure in animals, but localized administration of ET has been shown to affect vascular tone in healthy humans.

Winner of the Prix Galien 1997, Dr Eliot Ohlstein (SmithKline Beecham Pharmaceuticals, King of Prussia, PA, USA) discussed more research into the pathophysiological role of endothelin. Scientists at SmithKline Beecham effected overexpression of preproendothelin in rats using an adenovirus

construct and found this leads to elevated levels of ET and hypertension. They also created lesions in rat carotid artery using balloon angioplasty, and observed that injection with ET worsened these lesions (i.e. more neointima developed). Two weeks after the angioplasty, the expression of both ET_A and ET_B receptors was found to have increased by 40%. Treatment with ET antagonist SB209670 [**5**; $K_i(ET_A)$ = 0.2 nM, $K_i(ET_B)$ = 18 nM] reduced the neointimal growth[7]. Both magnetic resonance imaging (MRI) of the rat carotid artery and histological studies indicated 45% inhibition of neointima formation.

The meeting closed with a discussion of endothelin in the clinical area by Dr David J. Webb (University of Edinburgh, UK). Dr Webb's group studied the effects of the administration of ET_A receptor antagonist BQ123 and combined $ET_{A/B}$ receptor antagonist TAK044 directly into the forearm in healthy volunteers. Both drugs were found to cause forearm vasodilation. TAK044 was also administered systemically, which resulted in a reduction in vascular resistance and blood pressure lowering[8]. However, an increase in heart rate and stroke volume partially offset the effect on systemic vascular resistance. These studies show that ET is important for the maintenance of basal vascular tone in man. Using the forearm technique, Dr Webb and coworkers also showed that endothelin antagonists cause additional vasodilation in heart failure patients who are receiving treatment with an ACE inhibitor and diuretic. This result should encourage studies into the benefit of ET antagonist/ACE inhibitor combination therapy.

The Society for Medicines Research is organizing a meeting in April 1997 on *New Frontiers in the Treatment of Epilepsy*. A one-day meeting entitled *Pain and Analgesia* is scheduled for July. For more information contact Barbara Cavilla, tel: +44 171 581 8333, fax: +44 171 823 9409.

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Race to displace Taxol

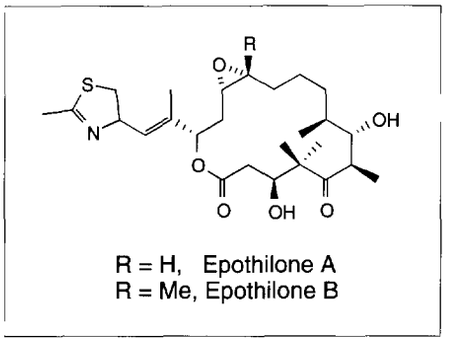
The race is over between three independent groups of chemists who spent much of last year trying to find ways to make the novel anticancer antifungal agents, the epothilones. These compounds promise to take the anticancer crown from Taxol in the next few years.

The epothilones were discovered in a bacterial brew in the 1980s by natural product chemist Gerhard Höfle (National Biotechnology Research Institute, Braunschweig, Germany). He found that they had powerful effects on runaway cell division *in vitro*. Further research showed that the epothilones' mode of action is very similar to that of Taxol (paclitaxel) and Taxotere (docetaxel), the major new anticancer agents derived from extracts of yew tree bark and needles. These compounds affect the microtubules that are assembled and disassembled during normal cell division.

In vitro tests on standard US National Cancer Institute cancer cell lines for breast and colon tumours showed the epothilones to be even more potent than paclitaxel in spite of their simpler structures. The epothilones have two properties that may make them more attractive as drugs than paclitaxel. First, the epothilones are slightly soluble in water (the solubility of paclitaxel is very poor), which should facilitate formulation. Second, and more important, they have shown high *in vitro* efficacy against certain drug-resistant cancer cells, a problem that is plaguing the clinical development of paclitaxel.

In July 1996, Höfle and his team established the exact structure of the epothilones using X-ray crystallography and effectively fired the starting gun in the race to find a synthetic route to the compounds.

First past the publication post was a team led by organic chemist Professor



R = H, Epothilone A
R = Me, Epothilone B

Samuel Danishefsky (Sloan-Kettering Institute for Cancer Research, New York, USA) and his colleagues at Columbia University [*Angew. Chem. Int. Ed. Engl.* (1996) 35, 2801–2803] with Professor K.C. Nicolaou of the Scripps Research Institute (La Jolla, CA, USA) a very close second [*Angew. Chem. Int. Ed. Engl.* (1997) 36 (1/2), 166–168]. Nicolaou and his team were winners in the race to synthesize paclitaxel itself in 1994. Dr Dieter Schinzer (Technical University of Braunschweig, Germany) will probably be third with his team's version of the synthetic epothilones.