Endothelins come home to roost

The reports elsewhere in this issue of the structure of two receptors for the potently vasoconstrictive endothelins are a reminder of the power molecular biology is bringing to pharmacology (and much else besides).

CELL membrane receptors transmit specific messages to alter the function of a cell. On pages 730 and 732 of this issue[1,2], two independent Japanese groups describe the structure of two different receptors for the family of peptides known as endothelins, the most powerful of all substances in raising the blood pressure and closing down the circulation.

The endothelin story is remarkable. Highsmith and his colleagues[3] found that endothelial cells in culture elaborate into their medium a peptide vasoconstrictor substance. In searching for a topic for his PhD thesis, Masashi Yanagisawa, with Hiroki Kurihara, found Highsmith's papers and suggested to his supervisor at Tsukuba University, Tomoh Masaki, that he should take up the problem. Masaki agreed and, as the project developed, brought a sizeable team into the work, including not only Yanagisawa as the molecular biologist but also Katsutoshi Goto as the pharmacologist and Sadao Kimura as the biochemist. The result was that endothelin exploded into our consciousness in a paper published in *Nature*[4] on 30 March 1988. It was so complete and thorough that one of my colleagues, who read it at the weekend, was convinced that it must have been intended by the editors as an elaborate scientific April fool's joke.

Endothelin (ET) is a 21-amino-acid peptide made by the endothelial cell from pre-pro endothelin (200 amino acids) and pro endothelin (38 amino acids; called 'big ET') by an unusual cleavage performed by an endothelin converting enzyme (ECE). The structure of ET-1 is twisted into a conical spiral by two disulphide bridges and it is the most potent vasopressor compound yet discovered, easily beating the previous record holder, angiotensin II, by some tenfold. What is more, the pressor effects of ET-1 are long-lasting, so that a single small injection into the circulation of a rat increases the blood pressure for an hour or more. Clearly, the endothelial cells lining the blood vessels would secrete a substance so potently active on vascular smooth muscle only for some crucial physiological (or pathological?) purpose. Just as clearly, we do not yet understand how endothelin earns its living.

All the classical hormones were discovered by bioassay of active principles in extracts of different tissues. Only later did the chemists and biochemists come along to tell us the actual chemical structures and the biosynthetic route by which the compound was made. Not so with the endothelins. A few months after the first publication, a second isopeptide was identified, initially called rat endothelin. With the advent of the third[5], Masaki's team disclosed the existence of all three peptides (now called ET-1, ET-2 and ET-3) in man and other species. They differ from each other by a few amino acids, but quite enough to give them different properties. Interestingly, they also have high homology with the sarafotoxins found in the venom of the Israeli burrowing asp, suggesting that they have a long genealogical history.

Endothelin-1 is the only one of the three made by the endothelial cell. Its action as a vasoconstrictor and other effects suggest that it is designed to function as a local hormone, released by the endothelial cell to contract the underlying vascular smooth muscle. Fitting with this hypothesis, ET-1 is rapidly removed from the blood. Circulating ET-1 also releases the potent vasodilators prostacyclin and endothelin-derived relaxing factor (EDRF) from endothelial cells, thereby limiting its own vasoconstrictor effects[6]. The peptide also has proliferative effects, for instance on mesangial cells of the kidney[6]. Remarkably, and because of the techniques by which the endothelins were discovered, we do not yet know which cells make endothelins 2 and 3, although their distribution is quite widespread.

Last week, the Second International Conference on Endothelins was held in their home in Tsukuba City, Japan. Despite burgeoning interest (there were almost 400 participants) the functions of the endothelins are still far from clear, although many expect excess ET-1 to be linked with hypertension, heart attacks and similar conditions. As with other hormones, their precise effects will become clearer only when receptor antagonists or ECE inhibitors are available. Pharmaceutical companies, racing each other to find such potentially therapeutic agents, are keeping their compounds close to their chests and none was reported at the Tsukuba meeting. However, a neutral protease inhibitor, phospharamidon, not only prevents the conversion of big endothelin to ET-1 but also prevents the rise in blood pressure induced by injections of big endothelin in rats. Excitingly, this crude inhibitor of ECE also gradually lowers the blood pressure of spontaneously hypertensive rats[7].

The two distinct receptors described[1,2] probably serve different functions. Each belongs to the superfamily of rhodopsin-like receptors, with seven transmembrane domains. Each is coupled to a G protein. One[1] shows high specificity for ET-1 and the messenger RNA is widely distributed in the central nervous system, the heart and the lungs. Might this be the vascular smooth muscle receptor? The other[2] equally accepts all three endothelins, as well as sarafotoxins, and is coupled through a G protein to phospholipase C, leading to transient increases in intracellular free Ca^{2+}. The messenger RNA is not found in vascular smooth muscle. Such characteristics suggest it may (amongst others) be the endothelin receptor on endothelial cells responsible for the release of prostacyclin and EDRF. A receptor nomenclature subcommittee of IUPHAR met in Tsukuba City and will recommend that the specific receptor[1] is called ET_A and the non-selective one[2] ET_B.

These two groups were lucky not only to submit their manuscripts for publication within two days of each other, but also to end up cloning two different endothelin receptors. Let us hope that the next groups will be just as lucky in identifying the other receptors involved.

At a ceremony associated with the Tsukuba City meeting, Masaki and his colleagues were awarded the second Tsukuba Prize consisting of a huge silver medal and a research grant of ¥5 million (£20,000). This recognition of a truly outstanding discovery will surely not be the last to do with the endolethins. Oh yes, I should mention that Yanagisawa was awarded his doctorate.

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