Commentary

The endothelium – the body’s largest endocrine gland?

E. E. Ånggård

Think of the endothelium not only as an inert blood container but as a vast endocrine gland. It stretches over the entire vascular tree with a surface area of about 400 square metres, of which most are in the capillaries. Its weight in an adult is about 1·5 kg and it contains an estimated 1·2 trillion endothelial cells (for review see Gimbrone, 1986; Ryan, 1988). In addition to the obvious barrier and transport functions, the endothelium influences its environment by the secretion of a wide range of biologically active mediators regulating immune responses, vascular tone and coagulation. The endothelium of each organ could therefore be considered to be an endocrine gland in its own right responding to external stimuli by the production of paracrine hormones and growth factors which act on neighbouring smooth muscle cells, monocytes, macrophages, fibroblasts and organ specific cells.

The endothelial cells are remarkably versatile. They can rapidly organize and maintain a monolayer, phagocytose foreign particles, make way for or stop other cells from penetrating into the subendothelial space and co-ordinate the building of capillaries in vascularization.

Endothelial pathology is contributory to conditions such as atherosclerosis, hypertension, vasospastic disorders, diabetes, acute respiratory distress syndrome, inflammation and autoimmune diseases, thrombosis and tumour growth rate. In this commentary I shall focus on nitric oxide and endothelin, two recently discovered endothelial paracrine hormones and their influence on cardiovascular function and possible role in pathophysiology.

Endothelium derived relaxing factor–nitric oxide

The role of the endothelium in vasodilatation has only emerged in the last 10 years (for review see Vane, Ånggård & Botting, 1990). In 1980 Furchgott & Zawadski showed that the endothelium was essential for the vasodilator action of acetylcholine in isolated arterial strips or rings. Removal of the endothelium by gentle rubbing prevented the relaxant effect of acetylcholine and even led to a contraction. Thus stimulation of muscarinic receptors on the endothelial cells triggered the release of a substance, which Furchgott named endothelium derived relaxing factor (EDRF). EDRF was found to be highly unstable with a half-life of seconds in buffer solutions. Its action was destroyed by haemoglobin and potentiated by superoxide dismutase, indicating that superoxide anions could inactivate it.

Many endogenous substances were found to cause release of EDRF and to elicit endothelium-dependent vasodilatation (Gryglewski, Botting & Vane, 1988). Among them are bradykinin, histamine, adenine nucleotides, thrombin and 5-hydroxy tryptamine. Also factors such as mechanical stretching, hypoxia and shear stress augment release of EDRF from endothelial cells.

The chemical nature of EDRF was not known until 1987. Khan & Furchgott (1987) and Ignarro, Byrns, Buga & Wood (1987) first pointed out that EDRF and nitric oxide (NO) were closely similar in the perfusion cascade bioassay system. Soon after, Palmer, Ferrige & Moncada (1987), using simultaneous chemical assays and bioassay, showed that NO accounted for most if not all of the biological activity of EDRF. It seemed at first surprising that such a simple chemical could have a transmitter function. As was pointed out by Vane, Gryglewski & Botting (1987) NO is after all simply ‘a one-to-one combination of the two main elements of the atmosphere’.

One of the leads pointing to NO was the similarity between the actions of EDRF and the nitrovasodilators. It was known that these drugs were being metabolized in the target cells to a nitrosothiol and NO (Katsuki, Arnold, Mittal & Murad, 1977; for review see Abshagen, 1985). Both EDRF and the nitrovasodilators act through the stimulation of the soluble guanylate cyclase and subsequent formation of cyclic GMP (cGMP) (for reviews see Waldman & Murad, 1987; Ignarro, 1988). cGMP activates cGMP-
dependent protein kinases and leads ultimately to dephosphorylation of myosin light chains and muscle relaxation.

How is NO generated in the body? The clue came from work on the source of the formation of endogenous nitrates. It was already known since 1916 that the body excreted more nitrates than were ingested, but it was thought that the balance originated from the gut micro-organisms. However Tannenbaum and his colleagues were able to show endogenous formation of nitrates (Green, Ruiz de Luzuriaga, Wagner et al. 1981; for review see Marletta, 1988). In one of their experiments a human subject was found to have a greatly enhanced nitrate excretion during an episode of intercurrent infection. Subsequent experiments showed that urinary nitrate levels could be elevated about tenfold when fever was induced by injection of E. coli lipopolysaccharide (Wagner, Young & Tannenbaum, 1983). It was found by serendipity that macrophages activated by endotoxin produced high amounts of nitrite and nitrate (Stuehr & Marletta, 1985). The generation of nitric oxides was dependent on the presence of L-arginine and could be inhibited by the analogue N⁶-mono-methyl-L-arginine. Interestingly the nitrate production was linked to the killer activity of macrophages (Hibbs, Taintor & Vavrin, 1987). Moncada and co-workers (Palmer, Ashton & Moncada, 1988) were the first to show that endothelial synthesis of NO also originates from L-arginine and could be blocked by the methyl analogue.

By that time it was becoming obvious that several fields were converging to reveal an important regulatory mechanism with EDRF–NO as the central factor. Non-adrenergic/non-cholinergic nerve stimulation was associated with formation of nitrite and could be blocked by NO-synthesis inhibitors (Gillespie, Liu & Martin, 1989). It was recognized that the earlier reports of stimulation of guanylate cyclase in brain synaptosomes by L-arginine and L-arginine-containing peptides (Deguchi, 1977) could be due to conversion to NO. Independently Garthwaite, Charles & Chess-Williams (1988) showed that N-methyl-D-aspartate receptor stimulation in the cerebellum caused release of an NO-like factor and elevation of cyclic GMP. NO has now been found in neutrophils, mast cells, renal epithelial cells and liver Kupffer cells (for review see Moncada, Palmer & Higgs, 1989). The formation of NO from L-arginine therefore seems to be part of a widely distributed cell-to-cell communication system. The receptor for NO is the soluble guanylate cyclase, which therefore is the target for the endogenous nitrate derived from L-arginine as well as the exogenous nitrate derived from nitrovasodilators.

Endocrinologists will recognize that L-arginine has multiple and potent secretagogue actions, e.g. the release of growth hormone, prolactin, insulin, glucagon and vasopressin (for review see Barbul, 1986). It will be interesting to see whether any of the secretagogue actions of intravenous L-arginine are mediated through the arginine-NO-guanilate cyclase system.

The availability of L-arginine analogues as inhibitors of NO formation has been instrumental in uncovering its physiological role. The most potent NO-synthesis inhibitor is N-nitro-L-arginine. Intravenous injections of these inhibitors of the NO formation causes an immediate and substantial rise in blood pressure, which can be reversed by L-arginine (Rees, Palmer & Moncada, 1989). This indicates that there is a continuous basal release of NO from endothelial cells to keep the vasculature dilated. There is also strong evidence that the vessels of healthy humans are continuously dilated by NO produced from endothelial cells. Infusion of Ng-methyl-L-arginine in the brachial artery caused a large increase in vascular resistance and blunted the dilator responses to acetylcholine (Vallance, Collier & Moncada, 1989).

The EDRF–NO is clearly a typical paracrine hormone acting only in its immediate environment. Released into the bloodstream it would be immediately inactivated by haemoglobin. There seems to be close functional relationship with prostacyclin, another labile vasodilator hormone. They act synergistically with respect to antiaggregation. Whereas NO acts on the guanylate cyclase, prostacyclin acts on the adenylate cyclase. It is therefore likely that these two endothelial-derived local hormones act in concert to defend the integrity of the vasculature (Fig. 1).

There is ever increasing evidence that a hypofunctioning NO system could contribute to a number of diseases such as hypertension, atherosclerosis, diabetes and vasospastic disorders. Decreased responses of endothelium-dependent vasodilators have been found in these conditions, both in animal disease models and in humans (for review see Vane et al. 1990). Prostacyclin and NO-generating drugs inhibit proliferation in cultured fibroblasts and smooth muscle cells. An antiproliferative action of these mediators could prevent the smooth muscle hypertrophy which takes place during the development of hypertension and atherosclerosis. There is therefore good reason to explore how various preventative and therapeutic measures could be developed to preserve the protective functions of the vascular endothelium.

Endothelins

Another recently discovered group of endothelial mediators are the endothelins. The endothelins (ETs) are a family of peptides with potent vasoconstrictor properties discovered by Yanagisawa, Kurihara, Kimura et al. (1988). They have 21 amino acids and
are structurally related to the sarafotoxins found in the venom of the Israeli burrowing asp. They are also related to the gut peptide vasoactive intestinal contractor (VIC) (Saide, Mitsui & Ishida, 1989).

The ETs originate from a large prepropeptide, from which a 38 amino acid, big ET, is generated by a proteolytic cleavage. Big ET is biologically less active but is transformed to the active amino acid peptide ET by a putative endothelin-converting enzyme (ECE). ET-1 has two disulphide bridges and a C-terminal Trp. Three isopeptides have so far been found, called ET-2, ET-3 (Inoue, Yanagisawa & Kimura, 1989) and VIC (Saide et al. 1989). ET-1 and ET-2 resemble each other in their pharmacological actions and in their binding characteristics to receptors. ET-3, however, has weaker vasoconstrictor action but is a more potent inhibitor of platelet aggregation than ET-1 (Lidbury, Thiemermann, Thomas & Vane, 1989). It also appears to bind to a different receptor. VIC is a more active intestinal contractor than ET-1.

The distribution of the ET system is currently being studied using mRNA probes (Northern analysis), radioimmunoassay for big ET and ET isopeptides, and binding sites using both quantitative histochemistry and conventional binding techniques (Power, Wharton, Zhao et al. 1989). The occurrence of ETs has been demonstrated in animals and in humans (Nunez, Brown, Davenport et al. 1990). In the rat, most tissues (brain, lung, heart, aorta, spleen, pancreas, kidney, stomach, intestine and bladder) contain more ET-1 than ET-3: ET-2 levels are universally lower. However, some tissues contain more ET-3 than ET-1, e.g. the pituitary. Also, some tissues like brain and intestine contain more ET-3 (50- to 100-fold) than others, e.g. heart. The highest levels of ET-1 are in the lung, about 3400 pg/g. This could either be a consequence of the generation of ET-1 in the lung or the binding and internalization of circulating big ET or ET-1.

Big ET, ET-1 and ET-3 are present in circulating blood. The levels (about 1 pg/ml) are too low to exercise systemic effect and probably represent overflow from locally released peptides (for review see Vane et al. 1990). Elevated levels of ETs have been found in cardiogenic and septic shock, acute myocardial infarction, diabetes mellitus and renal failure. ET-like immunoreactive material is also present in human urine in levels higher than those observed in blood (Berbinschi & Ketelsleger, 1989). Although plasma ET-1 appears to be substantially cleared through the kidney it is possible that the urinary ET-like material originates from the kidney. Urinary ET-1-like radioactivity could in this case be an indicator of renal ET formation.

The mechanism of action of the ETs involves binding to specific receptors coupled to G-proteins, activation of phospholipases with elevation of inositol phosphates, diacylglycerol, eicosanoids and calcium (Nayler, 1990). ET-1 binding and action is, however, mostly not inhibited by the dihydropyridine-type calcium channel antagonists.

The widespread availability of synthetic peptides have led to an explosive increase in publications on
the pharmacology of the endothelins (Whittle & Moncada, 1990). The outstanding property of ET-1 is its potent and long-lasting hypertensive actions. It is the most active hypertensive factor so far discovered, being about ten times more potent that angiotensin II. The kidney appears to be particularly sensitive to the vasoconstrictor actions of endothelin and it is interesting to note that in-situ hybridization studies have shown localized endothelin mRNA in close proximity to ET-1 binding sites in the rat kidney and other tissues (Power et al. 1989; Nunez et al. 1990). This vascular localization provides evidence to support a physiological or pathophysiological role for endogenous endothelins.

Some of the actions of pharmacological doses of endothelin are likely to be indirect. In conscious dogs, the peptide increases the blood pressure as well as the plasma levels of vasopressin, renin, aldosterone, norepinephrine, epinephrine and atrial natriuretic peptide (Goetz, Wang, Madwed et al. 1988). In rabbits, indomethacin potentiates the hypertensive actions of ET-1 (De Nucci, Thomas, D’Orleans-Juste et al. 1988) and blunts the antiaggregatory effects indicating the release of vasodilator eicosanoids—most likely prostacyclin (Thiemermann, Lidbury, Thomas & Vane, 1989). These actions may well be relevant to the physiological actions of endothelin as quite high doses were used.

One of the most interesting effects of endothelin reported to date may be its stimulatory effect on proliferation of vascular smooth muscle cells (Komuro, Kurihara, Sugiyama et al. 1988). This trophic effect could account for the development of the fibrous lesions seen in atheromas or the smooth muscle wall hypertrophy in hypertension.

The endothelins are most likely local mediators. Released endothelins are effectively and rapidly cleared from the blood by the lung, kidney and liver (Anggärd, Galton, Rae et al. 1989). The circulating levels of endothelins are too low to have a systemic effect and probably represent an overflow. Factors influencing the release of endothelins are thrombin, catecholamines and anoxia. Interestingly, there seems to be a link between a release of endothelin and the activity of the EDRF—NO system. Activation of EDRF release led to an inhibition of the release of endothelin from porcine aorta (Boulanger & Luscher, 1990). It would be fascinating if it could be verified in further experiments that the regulation of endothelin expression and release was under the control of the EDRF—NO system. This could explain the prolonged vasospasm seen in subarachnoid haemorrhage. The EDRF—NO would be inhibited by the haemoglobin from the haemorrhage, stimulating local release of the powerful vasoconstrictor endothelin. Further studies will hopefully clarify the relationship between these two local endothelial mediators.

The roles of the endothelins are as yet unknown. The most likely hypothesis is that the release represents a response to inadequate perfusion of vital tissues. Thus, increased levels of immunoreactive endothelin have been demonstrated in conditions such as myocardial infarction, cardiogenic shock and endotoxin shock. Strategies aimed at a pharmacological control of these powerful vasoconstrictor peptides will be either to inhibit endothelin formation, possibly by blocking the putative endothelin-converting enzyme, by using receptor antagonists or monoclonal antibodies to ETs. Efforts are underway in several academic and industrial laboratories to develop such tools to evaluate the endothelin system.

ACKNOWLEDGEMENTS

This work was supported by a grant from Glaxo Group Research Ltd.

REFERENCES


Journal of Endocrinology (1990) 127, 371–375

The William Harvey Research Institute, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.