NEW VIEWS ON THE ROLE OF ENDOTHELIN

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Endothelin was isolated and identified in 1988 by Yanagisawa et al. The endothelin family consists of 21 amino acid isoforms endothelin-1, endothelin-2 and endothelin-3. Endothelin receptors are present in many internal organs, e.g. heart, adrenals, kidneys, lung tissue, central nervous system. ET-1 is the main isoform which is synthetized in endothelial cells, muscular coat of arterial wall as well as in heart, kidney and central nervous system. Endothelins affect multiple organ systems and are involved in the pathogenesis of many diseases. Moreover, ET-1 raises blood pressure, induces vascular and myocardial hypertrophy. This paper is also concerned with endothelin receptor blockers which mediate relaxation of resistance arteries and, since they show a hypotensive effect, can be useful in many cardiovascular diseases.

Key words: Endothelin – cardiovascular effects – minireview

In 1985 HICKEY et al. detected a factor causing smooth muscle contractions which was produced by the endothelium. Three years later that substance was isolated by YANAGISAWA et al. (1988) from the pig arterial endothelial cell culture and called endothelin-1. It is a peptide composed of 21 amino acids, 2 disulphide bonds between amino acids 1 and 15, and 3 and 11. The loss of these bonds leads to the reduction of biological activity. Endothelin is formed by cleaving 164 amino acids from the 203 amino acid pre-pro-endothelin by means of specific endopeptidase (s), resulting in big endothelin (39 aminoacids). Big endothelin is subsequently converted to endothelin by means of an endothelin converting enzyme (PARISSIS et al. 2001; YANAGISAWA et al. 1988). In man, the endothelin gene encoding 212 amino acid pre-pro-ET is localized in chromosome 6 and consists of 5 exons and 4 introns.

In subsequent studies further endothelin isomers, referred to as endothelin-2 (ET-2) and endothelin-3 (ET-3) were identified. ET-2 is very similar to ET-1, while ET-3 differs from ET-1 at 6 out of 21 positions. These isomers are encoded by 3 independent genes. They differ in their chemical structure and potency of smooth muscle contracting effect (INOUE et al., 1989). All endothelin isomers (ET-1, ET-2, ET-3) demonstrate structural similarity to toxic venom (sapharotoxins) produced by some scorpions and snakes. ET-1 is produced mainly by endothelial cells, vascular smooth muscle cells and to a lesser extent by astrocytes and neurons in the central nervous system (CNS), Sertoli cells, mesangium and hepatocytes. ET-2 is mainly produced within the kidney and intestine, whereas the highest levels of ET-3 are found in the brain, where it is probably involved in neuronal function regulation.

The factors stimulating endothelin production by endothelial cells include: 1. mechanical stimulation of the endothelium, thrombin, calcium ions, epinephrine, angiotensin II, vasopressin, dopamine, erythropoietin, 2. cytokines: IL-1, IL-1 beta, IL-6, 3. growth factors: fibroblastic, epidermal, insulin-like, growth-transforming factor beta, endotoxins, 4. lipids: low density lipoproteins (LDL) and high density lipoproteins (HDL), stress. The substances inhibiting endothelin synthesis are nitrogen oxide (NO), cyclic

guanosine monophosphate (cGMP), atrial natriuretic peptide (ANP), prostacyclin (PGI2), bradykinin.

The blood levels of ET-1, ET-2, ET-3 and big endothelin in humans and animals range from 0.3 to 3 pg/ml (Donckier et al. 1991). Endothelin concentrations in body fluids, especially in the tissue e.g. in saliva, milk, urine, cerebrospinal fluid are several times higher than plasma concentration.

ET-1, ET-2, ET-3 demonstrate different affinity to the receptors referred to as A, B and C type receptors. Type A receptor exists mainly in vascular smooth muscle cells and responds to ET-1, but less effectively to ET-3. Type B receptor exists predominantly in endothelial cells and responds equally well to ET-1 and ET-3. ETA receptor can be further subclassified as ETA1 and ETA2 receptors, based on the susceptibility to an antagonist of ETA receptor, BQ-123, to which ETA1 receptor is sensitive and ETA2 receptor is resistant (Endoh et al 1998). Type C detected in the cutaneous glands of an Afican frog *Xenopus laevis* binds ET-3 (Tukawa 1993). Endothelin receptors can be found in many internal organs, e.g. the heart, adrenals, kidneys, lungs and the CNS. In the CNS, endothelin is present in particular in the neurons of the cerebral cortex, the hippocampus, amygdala, pituitary, hypothalamus and cerebellum (Giaid et al. 1991). In the neurons it occurs together with acetylcholine, somatostatin and neuropeptide Y, which indicates its role of a co-neurotransmitter or a neuromodulator (Giaid et al. 1991).

The effect of endothelin on blood vessels

Endothelin causes a marked and sustained vasoconstriction, exceeding in molar values the vasoconstricting properties of angiotensin II or catecholamines (Yanagisawa and Masaki 1989). The hormone causes vasoconstriction by modulating the function of dihydropyridine channel, increasing the intracellular level of calcium, or by its effect on phospholipase C. As a result, diacylglycerol (DAG) and inositol triphosphate (IP_3) are formed. DAG activates protein kinase C. IP_3 activates some calcium channels of the sarcoplasmatic reticulum, which leads to an increase of Ca^{2+} levels in the sarcoplasma and cell contraction. ET-1 also causes cellular depolarization activating the calcium current. Endothelin exerts a direct effect on the arterial smooth muscle layer, because blocking alpha-adrenergic, serotoninergic and muscarinic receptors, as well as cyclooxygenase and lipooxygenase does not abolish the vasoconstricting effect of endothelin.

Endothelin, via ETA and ETB receptors, is involved in the maintenance of baseline vascular smooth muscle tone (Haynes et al. 1995). Together with NO, it causes vasodilatation. This effect is mediated by ETB1 receptor subtype located in the vascular endothelium, whereas ETA and ETB2 receptor subtype, located in the vascular smooth muscle layer, mediate vasoconstriction (Gellai et al.1996). Under in vivo conditions, ET-1 stimulates the synthesis of prostaglandins, which exert a vasodilating effect, but it does not affect the production of the most potent vasodilator, NO. NO and ANP, as well as other vasodilators, increase the cellular levels of cGMP, which, in turn, decreases endothelin release. Vasoconstricting reactions due to ET-1 are dependent not only on its concentration, but also on the condition of the vascular endothelium. At low levels, if the endothelium is intact, stimulation of the sympathetic system increases smooth muscle contractility caused by endothelin. The vasconstricting effect of endothelin is markedly increases, when it overlaps with the effect of serotonin on damaged endothelium. The contraction of vascular smooth muscle layer is then so pronounced that the reaction cannot be further increased by stimulation of the sympathetic system. Endothelin also affects blood flow in various regions of the circulation. It reduces blood flow in mesenteric and cerebral circulation (Josko et al. 2001), whereas in pulmonary vessels it causes an increase of blood flow.

This hormone acting via the ET_A receptor contributes to basal coronary vasoconstriction tone and endothelial dysfunction (Lerman et al. 1995; Halcox et al. 2001). When endothelin was administered directly into the coronary vessels of experimental animals, arrhythmias, including even ventricular fibrillation, were observed (Yoricane et al. 1990). The mechanism of vasoconstriction caused by endothelin within the coronary circulation has not been fully elucidated. Coronary vasospasm may be associated with calcium inflow into the cells and directly correlates with the functional status of endothelial cells and walls of the coronary vessels (Kramer et al 1997; Grossman and Morgan 1997). Coronary vasospasm

may be associated with increased coronary vasoconstriction to vasoactive eicosanoids and with increased activity of protein kinase C. Cain et al (2002) indicated that physiological concentrations of ET-1 activate a Ca^{2+}-independent protein kinase C-mediated signaling pathway that involves tyrosine phosphorylation and activation of mitogen-activated protein kinase. Moreover, the enhancement of prostaglandin F (2 alpha)-induced coronary smooth muscle contraction by ET-1 involves additional activation of a Ca^{2+} sensitive protein kinase C- mediated pathway but not tyrosine phosphorylation or activation of mitogen- activated protein kinase C.

The effect of endothelin on arterial blood pressure

ET causes an increase of arterial blood pressure in a dose-dependent manner. A 2-3-fold increase of ET plasma levels causes a mean arterial blood pressure increase by 19 % . Immediately after the administration of endothelin, a drop of blood pressure is observed. This effect may be associated with increased release of NO or ANP. In the second phase, a marked increase of blood pressure due to increased vascular resistance is observed (Mortensen and Fink 1990), which persists even for several hours. The long-term pressor effect, characteristic of endothelin, is not dependent on its plasma levels, but rather on slow dissociation from the receptors.

Endothelin increases the sensitivity of vascular walls and increases the pressor reactions due to noradrenaline and serotonin (Yang et al. 1990). The expression of ET-1 gene and the pressor reaction is higher in rats with genetically determined hypertension (Yanagisawa and Masaki 1989) and ET-1 production markedly increases in transgenic hypotensive rats (Zolk et al. 2002). Also in hypertensive patients the levels of ET-1 were significantly higher than in normotensive controls. Moreover, in the hypertensives, plasma ET-1 was significantly correlated with mean arterial pressure and soluble vascular cell adhesion molecules (Parissis et al. 2001). Endogenous endothelins participate in the developent and maintenance of hypertension induced by angiotensin II (Ficai et al. 2001). Increased ET-1 levels were observed in pulmonary arterial hypertension and may contribute to the regulation of pulmonary vascular resistance, as well as to proliferative changes in the pulmonary vascular bed (Bauer et al. 2002).

The involvement of endothelin in the etiopathogenesis of hypertension aroused interest in ETA and ETB receptor blockers and their hypotensive effect. Bosentan, an ETA and ETB receptor blocker (nonspecific ET receptor antagonist), significantly reduced the mean blood pressure in the aorta, peripheral vascular blood pressure and the elevated left ventricular end-diastolic pressure (Ding et al. 2002). Bosentan reduces pulmonary arterial pressure and is beneficial in pulmonary arterial hypertension (Rubin et al. 2002).

An ETA receptor antagonist called BQ-123 prolonged the survival time of rats with cardiac failure (Love 1997). This antagonist prevents the mitogenic effect of ET on vascular smooth muscle layer. BQ-123 caused significant dilation in normal coronarty arteries and in atherosclerotic coronary arteries (Kinlay et al. 2001). Crockett et al. (2001) indicated that endothelin ETA (BQ-123) and endothelin ETB (PD1611721) receptor antagonists had an antifibrillatory effect in isolated rat heart that may be due, at least in part, to an ability to reduce the maximum following frequency.

The administration of a specific ETA receptor antagonist, ETA LU 127043, prevented swudden death due to ET-1 administration (Raschak et al. 1995). Similarly, Kojima et al. (1996) observed a reduced extent of myocardial infarctions in rats, rabbits and dogs after the administration of an ETA receptor antagonist, ETA TAK-44 before or after the onset of acute myocardial infarction. Endothelin antagonists promise to be successful as a new class of drugs for the treatment of cardiovascular diseases (Barton and Kiowski 2001).

The effect of endothelin on the heart

Ishikawa et al. (1988) were the first to describe the positive inotropic effect of ET-1 on isolated left atrium of guinea pig heart. In atrial myocytes, ET-1 causes cell membrane hyperpolarization and shortens the duration of action potential which leads to inhibition of heart excitibility. The inotropic effect of ET-1 is mediated by protein G and phospholipase C (Kramer et al. 1991). The positive inotropic effect observed in rabbit cells has been demonstrated

to be mediated in part by ET_A receptors (Endoh et al. 1996), whereas in the heart ventricles ET_A receptors are predominantly involved in the regulation of contractility induced by ET-1 (HILAL-Dandan et al. 1994). The hormone also exerts a positive chronotropic effect (Ishikawa et al. 1988, Mac Carthy et al. 2000).

It has been observed that in immature cardiac ventricle myocytes ET-1 causes a negative inotropic effect due to reduced calcium ion flow and slight intracellular acidosis (Grossmann and Morgan 1997). In mature cardiomyocytes, endothelin stimulates the hydrolysis of phosphoinositol to 1,4,5 inositoltriphosphate (IP_3) and diacylglycerol (DAG). DAG, in turn, activates protein kinase C, leading to increase of intracellular sodium and calcium levels, as well as intracellular alkalosis (Grossmann and Morgan 1997; Woo and Lee 1999), which increases myocardial contractility.

Studies on isolated atrium of guinea pig heart indicated that ET-1 demonstrates a negative chronotropic effect in the presence of isoproterenol. This result was confirmed by Zhu et al. (1997), who studied isolated rabbit heart atria and demonstrated negative inotropic and chronotropic effects of ET-1 and ET-3 in the presence of isoproterenol. The above effect is associated with a decrease of intacellular calcium level by isoproterenol. This activity is mediated by ETA receptors, activation of protein G, adenyl cyclase. The effect of ET-3 was 10 times weaker than that of ET-1. It indicates that endothelin isomers can inhibit the excitatory effect of catecholamines, especially at elevated concentrations due to certain pathologic conditions.

ET-1 is also a potent growth factor for cardiomyocytes, exert long term effects, such as myocardial hypertrophy and causes cellular injury in cardiac myocytes. In heart failure, the production of ET-1 is markedly increased. Intrapericardial administration of ET-1 can induce ventricular arrhythmias in dogs. The arrhytmogenic effect of ET-1 may be based on prolongation of action potentials duration and development of after-depolarization potentials (Geller et al. 1998). Sharif et al. (2001) indicated that the antiarrhythmic effects of endothelin-1 previously observed in vivo are not due to a direct effect on either the myocardium or the coronary blood flow.

Other effects of endothelin

Endothelin controls multiple aspects of kidney function. ET-1 increases vascular resistance, decreases renal flow and glomerular filtration even at high ANP concentrations. ET-1 effect on proximal tube H+ secretion (Langhmani et al. 2002). ET-1 reduces the production of rennin in isolated juxtaglomerular cells *in vitro* (Lin et al.1993). Endothelin reduces excretion of sodium and potassium with urine, as well as proteinuria. Bosentan prevents a marked increase in renal vascular resistance, and led to a significant increase in renal plasma flow resulting in a decrease in filtration fraction, increased urinary sodium excretion (Ding et al 2002).

Additionaly, ET-1 stimulates pulmonary fibroblasts to produce collagen, increases mucus secretion, contracts bronchial smooth muscle. It affects the conductivity of parasympathetic ganglia and nerves, regulates the function of myofibroblasts in the process of wound healing, increases the expression of protooncogens, exerts a mitogenic effect and increases human renal interstitial fibroblasts proliferation (Tian et al. 2002).

Endothelin increases the release of numerous substances such as aldosterone, catecholamines, vasopressin, atrial natriuretic peptide, prostaglandin I_2, angiotensin II, gonadotropic hormone-releasing hormone (GHRH), substance P, growth hormone.

The half-life time of endothelin in plasma is very short. After intravenous injection of ET-1 or ET-3 administered to rats, about. 60 % of endothelin is eliminated from the circulation during the first minute. Over 50 % of ET-1 administered in the infusion is taken up by the lungs during the first blood passage. It is estimated that about 90 % of circulating endothelin is eliminated by pulmonary uptake, and the remaining portion mainly through the kidneys and very small quantity through the liver.

The role of endothelin in the pathogenesis of some diseases

Elevated blood concentrations of endothelin have been observed in patients with acute and chronic renal failure (Ding et el. 2002), especially at the stage of advanced uremia and in patients on dialysis. Incresed ET values have also been observed in endotoxic and

septic shock (Wenecek et al. 2000). Increased blood levels of ET are detected in ischemic heart disease, acute myocardial infarction, especially complicated with circulatory failure and cardiogenic shock, in congenital and acquired heart defect and in patients after heart transplantation treated with cyclosporin (Letizia et al. 2001). ET-1 plays an important role in atrial (Ficai et al. 2001) and pulmonary hypertension (Bauer et al. 2002, Ivy et al. 2002), in asthma and fibrinous alveolitis. ET-1 is also involved in the CNS pathology. ET-1 is one of the causative substances in cerebral vasospam after subarachnoid hemorrhage (Mostafa et al. 2000). The involvement of ET-1 has been also demonstrated in metabolic diseases such as diabetes or atheromatosis (Migdalis et al. 2002; Ihling et al. 2001), developmental abnormalities (e.g. Hirsprung disease), during paradoxical sleep deprivation (Palma et al. 2002).

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