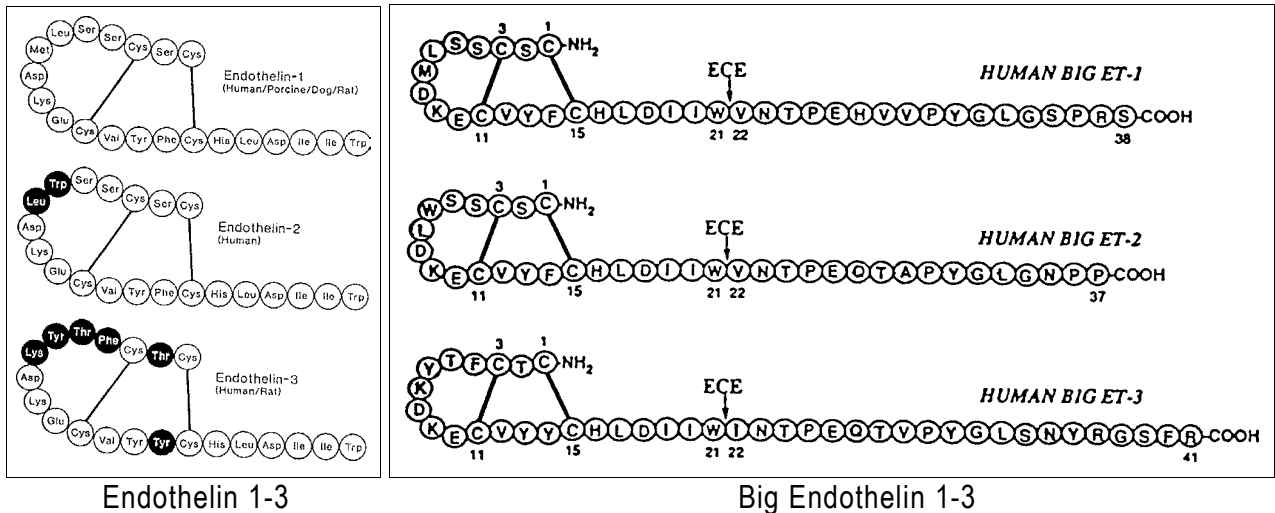


ENDOTHELIN AND BIG ENDOTHELIN

1. Characteristics of ENDOTHELIN

Endothelin (ET), a 21-amino acid peptide, is the most potent vasoconstrictor known today. Since the discovery of Endothelin in 1985 and its first detailed characterisation in 1988, three ET isoforms have been described: ET-1, ET-2 and ET-3.

The precursors of ET in their biosynthesis are called Big Endothelin (Big ET).



Vasoconstrictor potency is highest with ET-1.

All ET isoforms are composed of 21-amino acids with two intra-chain disulfid bridges, linking paired cysteine amino acid residues.

ET-2 exhibits the closest structural similarity to ET-1, differing by only two amino acid residues, while ET-3 differs by six amino acids.

In normal plasma samples, ET-2 circulates at less than 20%, while ET-3 circulates at 50% of the ET-1 level. The concentration of Big ET is 2-3 times higher than the ET-1 level.

From now on, (Big) ET is used as a synonym for (Big) ET-1,2,3.

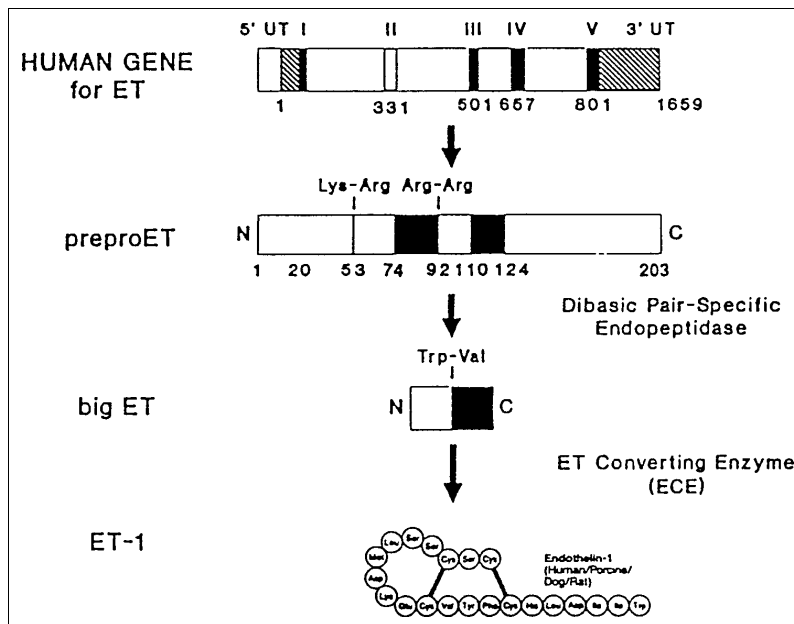
ET regulates systemic and local blood flow and it plays a role in the maintenance of cardiovascular and renovascular homeostasis.

2. Biosynthesis of ENDOTHELIN

The biosynthesis of ET is predominantly performed by vascular endothelial cells. But also heart, brain, spinal chord, pulmonary epithelial cells, glomerular mesangial cells, renal epithelial cells, monocytes and macrophages synthesize ET.

The three isoforms are encoded on three different genes and show highly conserved sequences in several species. They have been described in the human, porcine and rat genome.

The biosynthesis, starting from DNA transcription to the mature ET-1 is shown in the next graph.

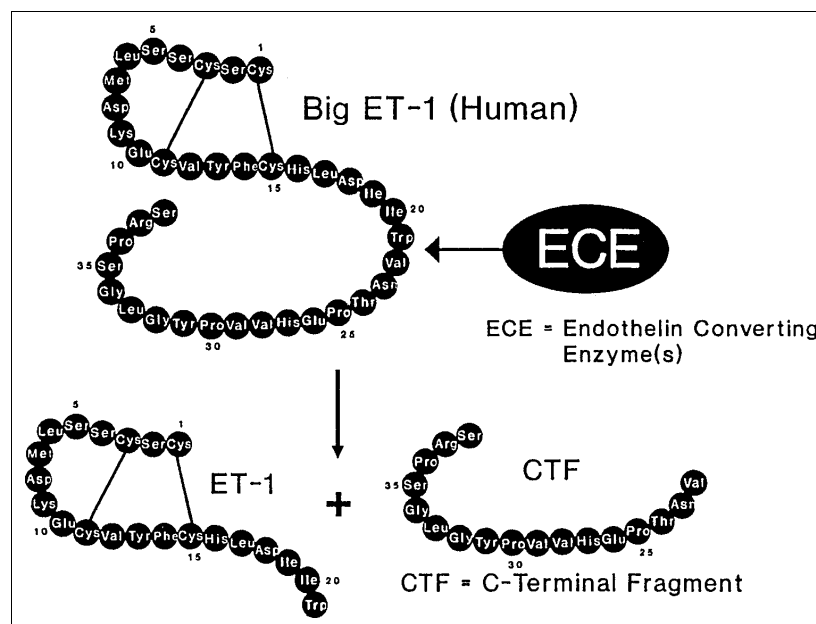


Transcription and maturation of ET-1

All of the ET peptides are synthesized through proteolytic processing of a 203-residue peptide termed prepro ETs. The polypeptides are termed Big ETs and consist of amino acid residues with different lengths.

The Big ETs are cleaved, after synthesis in the cytoplasm, by the proteolytic action of membrane-bound metalloproteinases, the ET converting enzymes. The cleavages take place in the intracellular component and on the cell surfaces and lead to the active ET hormones and to the C-terminal fragments.

The cleavage process of Big ET-1 to ET-1 is shown schematically in the following graph.



Enzymatic cleavage of Big ET-1 to ET-1

The physiological importance of cleavage of Big ET is indicated by the reported 140-fold increase in vasoconstrictor potency upon cleavage to ET.

The half-life of ET in vivo is less than one minute, whereas that of Big ET is much longer, approx. 20-25 min.

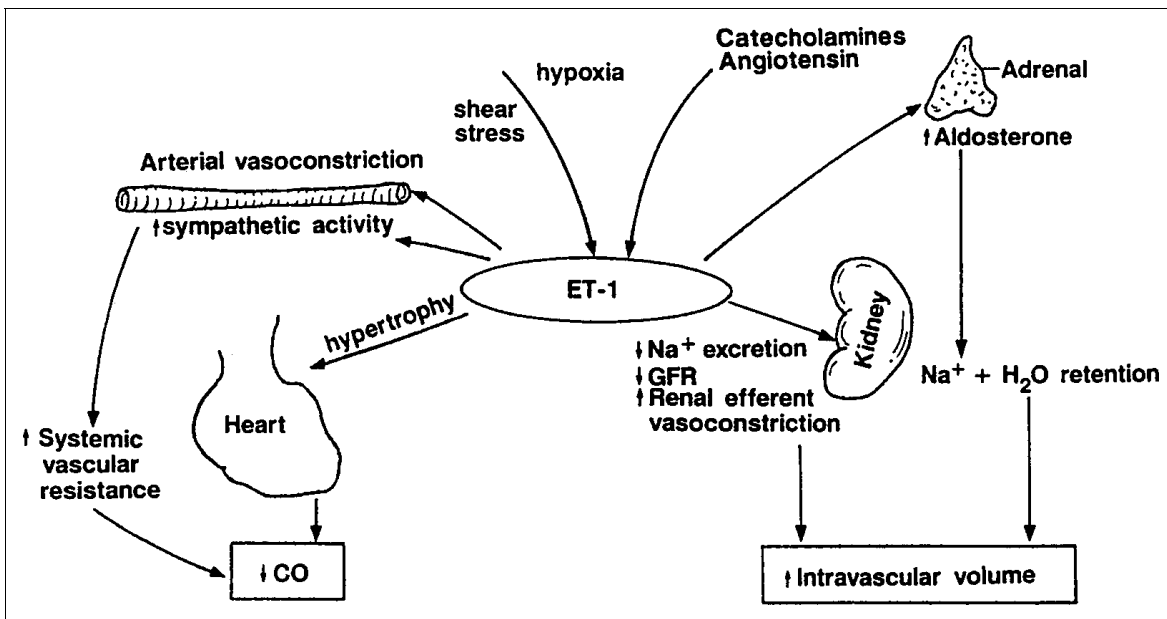
ET is cleared from the circulation by the kidney, the lung and by degradation in endothelial cells.

3. Function of Endothelin

After biosynthesis, ET is released into the circulation or it diffuses towards underlying vascular smooth muscle cells.

ET acts by binding to specific receptors, found on smooth muscle cells, myocytes, and fibroblasts. The binding to the receptor leads to various cellular actions e.g. efflux of cellular calcium, release of intracellular stored calcium, activation of Phospholipase C, and inhibition of Na/Ka ATPase.

A summary of the physiological actions of ET-1 is shown below.



Physiological actions of ET-1

Besides its vasoconstrictor function, ET acts also as a neurotransmitter and as a growth factor. The peptide interacts in an autocrine / paracrine manner.

Main biologic actions of ET

- Systemic and local homeostasis:
 - regulation of blood volume
 - regulation of blood pressure
 - regulation of blood flow
 - regulation of blood viscosity
- Renal volume homeostasis:
 - regulation of renal blood flow
 - regulation of ion and water excretion
 - regulation of urine composition
- Cardiovascular homeostasis:
 - regulation of cardiac output
 - regulation of coronary blood flow
- Wound healing in tissue injury and inflammation
- Intestinal tract movement
 - vasoactive intestinal contractor peptide (VIC)
- Neurotransmitter action in brain
- Regulation of pulmonary vascular constriction
- Closure of umbilical vessels
- Control of menstruation
- Induces release of:
 - endothelium derived relaxing factor (EDRF)
 - prostacycline
 - catecolamines
 - atrial natriuretic peptide (ANP)
 - vasopressin
 - alderone
 - inhibition of Renin

4. Clinical Applications and Pathophysiology

The ET levels are elevated in many disorders:

- ⇒ cardiovascular disorders:
 - acute myocardial infarction
 - congestive heart failure
 - angina pectoris
 - cardiogenic shock
 - coronary vasospasm

- ⇒ vascular disorders:
 - ischemia
 - atherosclerosis
 - pregnancy induced hypertension
 - hypercholesteremia
 - scleroderma
 - Buerger's disease, Takayasu arteritis, Raynaud's phenomenon, Wegener-Klinger granulomatosis
 - complications in diabetes

- ⇒ renal diseases:
 - acute / chronic renal failure
 - uremia
 - hemodialysis

- ⇒ neuronal diseases:
 - cerebral vasospasm
 - subarachnoid vasospasm

- ⇒ endocrine diseases:
 - preeclampsia

- ⇒ bronchoconstriction:
 - pulmonary hypertension
 - asthma

- ⇒ other diseases:
 - liver cirrhosis
 - rejection of transplants, surgical stress
 - septic shock

ET and Big ET are investigated as potential candidates for the assessment and treatment of heart diseases.

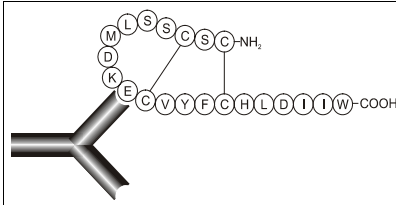
Veterinary research results lead to the hypothesis, that ET prevents the heart from overstimulation after a myocardial infarct.

ET and Big ET could be a valuable parameter in the supervision and monitoring of clinical studies.

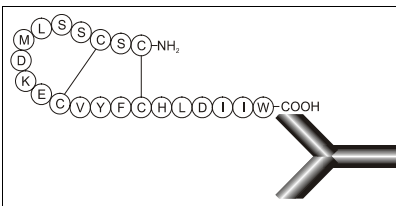
5. Endothelin antibodies used in Biomedical ELISAs

5.1. ET (1-21) antibodies

The monoclonal anti-ET antibody recognizes the „loop“ part of the ET-1 and ET-2 peptides. This antibody also recognizes Big ET (1-38):

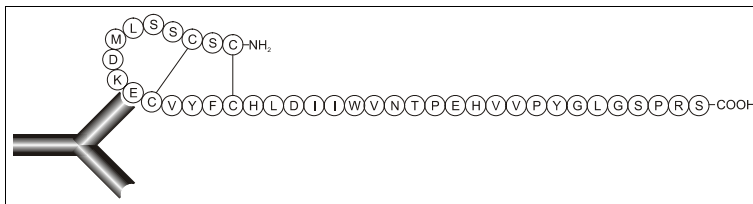


The polyclonal antibody binds to the C-terminal region of the Endothelin peptide and recognizes the last few aminoacids and the COOH-group. This antibody is specific for ET-1 and ET-2:

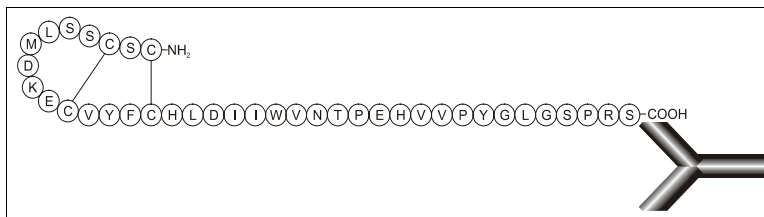


5.2. Big ET (1-38) antibodies

The monoclonal anti-Big ET antibody recognizes the „loop“ part of the Big ET (1-38) peptide. This antibody also recognizes ET-1 and ET-2:



The polyclonal antibody binds to the C-terminal fragment (22-38) of the Big ET region. This antibody is specific for ET (1-38):



The paracrine endothelin system: pathophysiology and implications in clinical medicine

(1) Hocher et al., *Eur. J. Clin. Chem. Clin. Biochem.* (1997) 35: 175-189

In this review, the impact of the endothelin system for various cardiovascular pathophysiological states, especially atherosclerotic vascular disease, restenosis, myocardial infarction, congestive heart failure, and arterial hypertension.

New targets for heart-failure therapy: endothelin, inflammatory cytokines, and oxidative stress

(2) Givertz et al., *The Lancet* (1998) 352: 34-38

Clinical trials have begun to address the potential therapeutic role of endothelin-receptor antagonists in heart failure. Studies have established that endothelin exerts measurable haemodynamic effects in heart failure.

Effect of endothelin-1 on exercise-induced vasodilation in normal subjects and in patients with heart failure

(3) Krum et al., *American Journal of Cardiology* (1998) 81: 355-358

This study was undertaken to determine the effects of administration of endothelin-1 in the brachial artery on forearm blood flow at rest and during rhythmic handgrip exercise in normal subjects and patients with chronic heart failure.

Cardiac release and kinetics of endothelin after uncomplicated percutaneous transluminal coronary angioplasty (PTCA)

(4) Krüger et al., *American Journal of Cardiology* (1998) 81: 1421-1426

This study was designed to assess the release kinetics of endothelin after PTCA and to prove the coronary endothelium as the source of the endothelin release.

Dynamic changes of endothelin-1, nitric oxide, and cyclic GMP in patients with congenital heart disease

(5) Bando et al., *Circulation* (1997) 96 suppl. II: 346-351

Endogenous NO may decrease vascular tone and maintain low pulmonary pressure in HF-LP (high flow - low pressure) patients. High levels of ET-1, inadequate NO production, and/or impaired responses to NO may increase pulmonary pressure in HF-HP (high flow - high pressure) patients.

Role of endogenous endothelin in chronic heart failure

(6) *Mulder et al., Circulation (1997) 96: 1976-1982*

This study describes the effect of long-term treatment with an endothelin antagonist on survival, hemodynamics, and cardiac remodeling.

Clinical implications of a sandwich enzyme immunoassay for big endothelin-1

(7) *Mathew et al., Clinical Chemistry (1997) 43: 9-10*

Assays for the ET pathway are a crucial component of ongoing research investigating the physiological effects of this peptide as well as measuring the therapeutic effects of potential ET-receptor blockers and endothelin converting enzyme inhibitors.

Prognostic impact of big endothelin-1 plasma concentrations compared with invasive hemodynamic evaluation in severe heart failure

(8) *Pacher et al., J. Am. Coll. Cardiol. (1996) 27: 633-641*

In advanced heart failure, plasma Big ET-1 is strongly related to survival and appears to predict 1-year mortality better than hemodynamic variables and levels of atrial natriuretic peptide, and established neurohumoral prognostic marker in chronic heart failure.

Plasma big endothelin-1 concentrations in congestive heart failure patients with or without systemic hypertension

(9) *Pacher et al., Am. J. Cardiol. (1993) 71: 1293-1299*

Patients with severe chronic heart failure had significantly greater Big ET-1 values than did those of moderate chronic heart failure.

Prognostic value of plasma endothelin-1 in patients with chronic heart failure

(10) *Pousset et al., European Heart Journal (1997) 18: 254-258*

Elevated ET-1 plasma levels are associated only with a poor prognosis, but routine plasma ET-1 determination provides important prognostic information in mild to moderate heart failure.

Direct enzyme immunometric measurement of plasma big endothelin-1 concentrations and correlation with indicators of left ventricular function

(11) *Haug et al., Clinical Chemistry (1998) 44:2 239-243*

Plasma Big ET concentrations seem to be inversely correlated to the left ventricular ejection fraction.

Plasma endothelin and big endothelin levels in patients undergoing percutaneous transluminal coronary angioplasty

(12) *Heins et al., (1995) 39th Ann Meeting of the GTH. Posterpresentation Abstract 366*

Increase of ET and Big ET induced by PTCA and mediated by endothelial injury may contribute to transient increase of vasomotor tone. ET and Big ET could therefore be a target for pharmacological interventions to reduce the incidence of early reocclusion.

Endothelin in human congestive heart failure

(13) *Wei et al., Circulation (1994) 89: 1580-1586*

Elevation of plasma ET in severe human CHF represents principally elevation of big ET.

Plasma endothelin determination as a prognostic indicator of 1-year mortality after acute myocardial infarction

(14) *Omland et al., Circulation (1994) 89: 1573-1579*

Plasma endothelin concentrations are strongly related to outcome after myocardial infarction and provide prognostic information independent of clinical and biochemical variables previously associated with a poor prognosis.

Endothelin in acute myocardial infarction and early restenosis in patients undergoing percutaneous transluminal coronarangioplasty (PTCA)

(15) *Heins et al., (1995) XV Congress of the Int Soc. on Thrombosis and Haemostasis, Jerusalem / Israel*

- a. ET and Big ET are elevated in coronary artery disease and show a positive correlation to the severity of CAD.
- b. As a result of ischemia and endothelial lesion after PTCA a short term increase of ET and Big ET and in early re-occlusion a further elevation is observed. So a renew increase can indicate an insufficient result after PTCA.
- c. ET and Big ET are early and sensitive markers for myocardial ischemia after PTCA and / or acute myocardial infarction.

Racial differences in plasma endothelin-1 concentrations in individuals with essential hypertension

(16) *Ergul et al., Hypertension (1996) 28: 652-655*

BIOMEDICA Endothelin ELISA (BI-20052) Reference.

Comparison and clinical evaluation of immunoreactive endothelin, big endothelin and C-terminal big endothelin

(17) *Heins et al., unpublished*

The aim of this study was to investigate the clinical value of endothelin measured by three methods. Our data confirmed previous findings of the variation in endothelin and big endothelin concentrations depending on the specificity of the assay used.

Reduced pulmonary clearance of endothelin-1 in pulmonary hypertension

(18) *Dupuis et al., American Heart Journal (1998) 135: 614-620*

Pulmonary hypertension is associated with a reduced pulmonary clearance of ET-1 that contributes to the increase in circulating levels.

Down-regulation of ET_B receptor, but not ET_A receptor, in congestive lung secondary to heart failure

(19) *Kobayashi et al., Life Sciences (1998) 62: 185-193*

Since lung ET_B receptors play a role in the clearance of circulating ET-1, it is proposed that down-regulation of lung ET_B receptors partly contributes to marked increases in circulating ET-1 and that increased ET-1 in the circulating plasma as well as in the lung is involved in the progression of pulmonary hypertension in CHF.

Endothelin levels are elevated in atherosclerosis, congestive heart failure and renal insufficiency (and some of these patients may be hypertensive). The mechanism and significance of these raised levels is not known. However, ET as an endothelium-derived peptide, is a sensitive marker of endothelial injury and thus these elevated levels may simply reflect diffuse endothelial injury.

The role of endothelin in coronary atherosclerosis

(20) *Mathew et al., Mayo Clin. Proc. (1996) 71: 769-777*

Endothelin has been implicated in the pathogenesis of atherosclerosis and is involved in the cellular and humoral mechanisms responsible for its progression.

Endothelin in coronary endothelial dysfunction and early atherosclerosis in humans

(21) *Lerman et al., Circulation (1995) 92: 2426-2431*

This study strongly supports a role for ET as an early participant in and marker for coronary atherosclerosis and coronary endothelial dysfunction in humans.

Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis

(22) *Lerman et al., New Engl. J. Med. (1991) 325: 997-1001*

ET plasma levels are normal in most patients with hypertension, except in the presence of renal failure and atherosclerosis.

The endothelium is a crucial factor in cardiovascular disease. Three important risk factors, ageing, hypertension and hypercholesterolemia are associated with the decreased basal and stimulated release of ET-derived nitric oxide. In contrast, the release of ET-1 appears to increase with age, while the sensitivity to this peptide decreases with age. In patients with hyperlipaemia and atherosclerosis, circulating levels of ET is increased. In endothelial cells in culture, oxidized low density lipoprotein

induces the expression of prepro ET mRNA and the release of this peptide. Currently, circulating levels of ET may be a useful marker of end-organ damage in atherosclerosis for example.

ET is elevated in every disease that involves injury to vascular walls. The peptide causes vasoconstriction, which causes vascular damage, which in turn causes more ET to be released - a classic vicious cycle.

Increased plasma endothelin-1 in acute ischemic stroke

(23) *Ziv et al., Stroke (1992) 23: 1014-1016*

Ischemic stroke is associated with acute and marked increases in plasma levels of ET-1. This may reflect enhanced production by damaged endothelial cells within the infarcted tissue.

ET is a peptide that has an important role in the control of renal function and in renal disease. It has raised interest in its potential role in the development and progression of diabetic nephropathy and ultimately glomerulosclerosis.

A role for endothelin in the pathogenesis of hypertension: fact or fiction?

(24) *Pinto-Sietsma et al., Kidney International (1998) 54: 115-121*

Investigation of conditions associated with hypertensive end-organ damage, such as chronic renal failure, has led to a re-evaluation of the role of the ET system in hypertension.

Physiological role of the endothelin system in human cardiovascular and renal haemodynamics

(25) *Webb, Current Opinion in Nephrology and Hypertension (1997) 6: 69-73*

ET-1 is now emerging as a central player in the maintenance of systemic vascular resistance and blood pressure in healthy people, probably acting in major part through the ET_A receptor.

Endothelins in diabetic kidneys

(26) *Koide et al., Kidney International (1995) 48: 45-49*

ET induces mesangial cell proliferation and contraction and increases the expression of extracellular matrix component mRNA.

Role of endothelin-1 in diabetes mellitus

(27) *Sármán et al., Diabetes Metab. Rev. (1998) 14: 171-175*

The available data of the role of endothelin-1 in Type 1 and Type 2 diabetes mellitus and the development of diabetic complication are summarized.

Elevated plasma endothelin in patients with diabetes mellitus

(28) *Takahashi et al., Diabetologia (1990) 33: 306-310*

Progression of vascular complications in diabetes is accompanied by activation of the ET system, becoming manifest with an increase in plasma ET levels.

Clinical significance of plasma endothelin-1 in patients with chronic liver disease

(29) *Matsumoto, Digestive Diseases and Sciences (1994) 39: 2665-2670*

Plasma ET-1 increased in proportion to the severity of liver damage and may be causally related with the derangement of systemic / renal hemodynamics and fluid and electrolyte homeostasis seen in advanced liver disease.

Plasma endothelin immunoreactivity in liver disease and the hepatorenal syndrome

(30) *Moore et al., New Engl. J. Med. (1994) 327: 1774-1778*

Endothelin in organ transplantation

(31) *Watschinger et al. (1996) 27: 151-161*

After successful renal transplantation ET plasma levels return to baseline with the restoration of renal function. There is ample evidence that local ET production as well as circulating plasma levels are increased during transplant rejection and after administration of immunosuppressive drugs used in clinical transplantation.

Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate

(32) *Nelson et al., Nature Medicine (1995) 1: 944-949*

Endothelin may be a mediator of the osteoblastic response of bone to metastatic prostate cancer.