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EWDOTHELIUM-DERIVED BIOLOGICALLY ACTIVE SUBSTANCES: VON WILLEBRAND FACTOR AND ENDOTHELIN

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ABSTRACT

• *Endothelial cells from the inner lining of the vascular wall and create a continuous nonthrombogenic and semipermeable layer. They are capable of regulating vas-cular tone, haemosthasis, inflammation etc. by synthesis and secretion of various biologically active substances like substance P, acetylcholine, ABO-antigens, EDRF, EDCF, NO, Endothelins, Tlirombospondin, von Willebrand factor and others. In the following review we describe briefly the biology of von Willebrand factor (vWF), a potent adhesiveglycoprotein, and the constrictorpeptide Endothelin, recently isolated from endothelial cell cultures. VWF is the major constituent ofFVIII/vWF complex. It is synthetized by endothelial cells and megakaryocytes. Endothelial cell Weibel-Palade bodies act as storage compartments of the large multimeric forms of vWF. Its unique secretion is maintained by constitutive and regulated pathway. VWF is secreted into the lumen and periendothelial matrix and mediates addhesion of platelets to the subendothelium after vascular injury. Structuraly defective or immature subunits circulate in the plasma of patients, suffering from von Willebrand's disease. Endothelins are a group of 21-aminoacidpeptides secreted mainly by endothelial cells. Synthesis of peptides of the Endothelin family is located also in nonedothelial cells like pulmonary and tracheal epithelium, dorsal root ganglia etc. They act as potent vasoactive substances causing susstained elevation of blood pressure as well as powerful bronchoconstrictors, via two distinct receptors. Judging from their physiological effects, they may take part in various diseases like atherosclerosis, hypertension and asthma. The clinical relevance of this is also disscussed.*

INTRODUCTION

• Endothelial cells (EC) create a continuous, nonthrombogenic layer on the inner surface of blood vessels. Forming a huge surface (over 1000 m^2, they occupy a unique place in regulation of imporant physiological processes by the synthesis and secretion of various biochemical substances. They take part in the mechanisms of coagulation and thrombolysis, vascular tone, immunity, inflammation, angiogenesis etc. (Table I). They synthetize also the structural components of the periendothelial matrix. In this way the endothelium could be accepted as the biggest endocrine organ in the human body. Blood vessels are influenced by various humoral, neural or local signals, which are mediated mostly by the EC. After the isolation of prostacyclin by Monkada and Vane (1), a new insight was given to the importance of the vasoactive substances, synthetized by the endothelium itself. The works of Furchgott and Zawadzki (2) concerning the obligatory role of EC in the mechanisms of vasodilation, as well as the investigations made by Burnstock et al (1985) (3), created a big interest in the role of EC in maintaining the vascular responce and made the endotheliium one of the most attractive objects of today's cardiovascular research.

In our minireview we shall discuss two endothelium derived products - the von Willebrand factor (vWf) and endothelin (ET).

EHDOTHELIAL CELL VOH WILLEBRAHP FACTOR (VWF)

• The history of the problem begins with the studies on two diseases - haemophilia A and von Willebrand disease (vWd). Early data on that subject is found in the Thalmud (4,5). In 1828 a bleeding disorder, occuring only

Table I

Biologicaly active substances - synthetized by the EC

Vasoactive substances	EDRF EDCF ACh ACE ATP NO SP ET
Substances with procoagulant activity	vWf factor V collagen IV laminin fibronectin
Substances with anticoagulant activity	thrombomodulin protein S PA prostacyclin
Substances participating in immunity and inflammation	IL-1 GMCF PAF PDGR-like ABO EDGF-like

EDRF-endothelium derived relaxing factor, EDCF-endothelium derived contracting factor, NO-nitric oxide, SP-substance P, ACh-acetyl-choline, ATP-adenosine, triposphate, PA-plasminogen activator, GMCF-granuIocyte monocyte colony-stimulating factor, PAF-platelet-activating factor, PDGF-platelet derived growth factor, ABO-ABO-antigens, EDGF-endothelium derived growth factor, IL-l-interleukin-1

in males, was called haemophilia (6). A different disfunction of the coagulation, occuring, in both sexes, was described by E. von Willebrand in 1927 (7), It was much later that vWf was recognized as a separate entity in the process of haemostasis. VWf is a part of the factor VHI/von Willebrand factor complex (FVIII/vWf) which consists of two circulating plasma proteins, linked to one another by non-covalent bonds. The complex is controlled by two different chromosomes. F VIII participates, together with factor IX, in the middle part of the intrinsic coagulation cascade. The liver seems to be the major source of F VIII. Wion et al (1985) demonstrated by hybridization probes the presence of F VIII mRNA in hepatocytes, kidney, spleen and lymph nodes (8). The majority of patients with haemophilia A have low values of F VIII (VIII:C). Haemophilia A is an X-linked hereditary bleeding.

Von Willebrand factor is a plasma glycoprotein, synthetized in endothelial cells (EC) (9) and megakaryocytes (10). EC contribute more than 75% of the circulating vWf (11). It is found also in the basement membranes of EC. Storage organelles are situated in platelet a-granules and in the endothelium specific Weibel-Palade bodies (WPb) (12). They contribute about 5% of the circulating pool of vWf (13). The remainder is secreted by constitutive pathway. VWf is one of the largest and most complex proteins in the human body (14). Its synthesis is controlled by a gene, situated in the short arm of XII chromosome (15). The initial transl ation product is a precursor polypeptide (741 aminoacids, 300 - 350 000 Daltons) (16). Cotranslationally 12 high mannose N-linked oligosaccharide chains are added. After the initial glycosylation, the propolypeptide forms dimers through disulfide bridge formation at the carboxy-termina end. This process takes place in the endoplasmic reticulum. The pro-vWf dimers are then transported to the Golgi complex, where high mannose glycans are processed to the complex form. Also an inorganic sulphate is incorporated. Dimers multimerize and the prosequence is cleaved. Pulse-chase experiments have shown that this is done at the same time that vWf dimer multimerize (17). By formation of disulfide bonds at the amino-terminal ends of dimers, a series of differently sized multimers are formed, ranging from 225 000 to 12 x 10^6 daltons. It has to be noted, that although the majority of vWf processing is intracellular, the cleavage of the propolypeptide may continue extracellulary (17). The plasma vWf circulates as an array of differently sized multimers. This fact is important in differentiating Wd. In EC, vWf is stored in WPb (12). These endothelium-specific organelles are membrane-bound bodies 0,1 um thick and up to 4 um long. Inside the organelle, several longitudinally arranged tubules (15 nm thick), embedded in dense matrix, are present. The specificy of these organelles for the EC was first recognized by Weibel et al (1964) (18). They originate from the trans-Golgi complex (19) and their content is released by exocytosis. They contain also the vEf-propolypeptide (vWAg II). It appears that vWf molecules are packed into WPb in their precursor form (17). The storage of vWf is independent of its covalent multimeric structure, while the vWf propolypeptide is necessary for the formation of vWf storage granules (20). It is also found in plasma. Like platele *a* - granule membrane, all WPb are found to contain GMP140 (21). VWf is secreted from EC by two different pathways: constitutive and regulated. The first pathway can be inhibited by

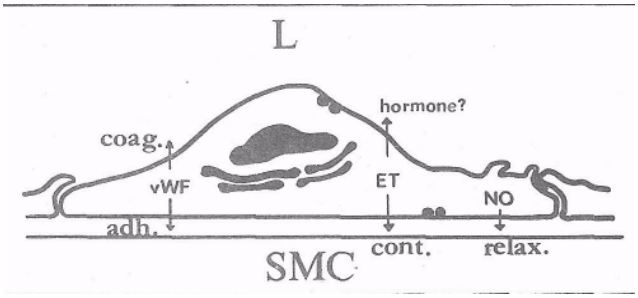

FIGURE 1. Schematic representation of some endothelial functions. L - lumen, SMS - smooth muscle cell, ET - endothelin, vWF - von Willebrand factor, NO - nitric oxide, cont. - contraction, adh. - adhesion, coag. - coagulation, relax - relaxation.

protein synthesis inhibitors, while the regulated pathway is induced by diffferent secretagogues like calcium ionophore A 23187, phorbol myristate acetate (PMA), thrombin and histamine. The latter process requires intact microtubules and is highly polarized (17). The molecular forms of vWf released from EC by the two secretory pathways are different. VWf secreted constitutively is composed off dimers and small multimers, while vWf released from the storage compartments (WPb) is of high molecularweight. There is variation in the number of WPb per cell in the endothelium of different vessels. It is, however, proportional to the diameter of the vessels and inversly related to the distance of the vessels to the heart. Increased number of WPb has been detected in proliferating EC, after reendothelization, in glaucoma, Behchet's disease, rheumatic diseases, atherosclerosis. Increased number is encountered also after in vivo treatment with vasoactive agents like 5-HT, histamine, thrombin (22). LTE4 (Dikranian, unpublished observations) in terminal vessels of the gut. It has been proposed also that the increased plasma levels of vWf could be a marker of endorhelial injury (23). VWf is important in hemostasis and repair of vascular injury. It participates by gluing platelets to the exposed subendothelium which is followed by spreading of platelets. These processes involve surface proteins of platelets and subendothelial matrix vWf, which bind to platelet glycoproteins Ib, Ilb/IIIa complex, collagens, acidic proteoglycans, heparin and thrombin. VWf assists the entangling of platelets into polymerizing fibrin. The basement membrane maintains EC adhesion to the vessel wall (17). Binding to vWf induces the organization of microfilaments and attachment plaques to EC (24). During that process vWf is needed at two levels. At the first it is used as a constituent of the basement membrane and the exposed tissue after vascular damage where it mediates platelet adhesion. At the second level vWf is needed during the formation of the platelet aggregates and their interaction with the fibrin clot (D. Wagner, personal communication). The first level is maintained by the release of vWf, while the second most probably is mediated by the circulating plasma vWf and oc-granule pool.

Analysis of vWf is based on different methods and functional tests. They include the measurement of vWf (vWf:Ag) and are based mostly on immunologic techniques like radioimmunoassay (RIA), electroimmunoassay (EIA), immunoradiometric assay (IRMA), ELISA, immunoelectrophoresis (CIE). Functional tests include the glycopeptide antibiotic ristocetin-initiated binding of vWf to platelet glycoprotein Ib. This assay is important in the diagnosis of vWD. Methods for qualitative analysis of vWf are important in the classification of vWD. The first evidence for the heterogeneity of vWf was obtained by crossed immunoelectrophoresis (CIE). They led to the discovery of two variants of vWD - called subtype IIA and IIB, both accompanied by lack of high molecular weight molecules (HMW). VWd in fact is a complex of disorders, whose common clinical symptom is the prolonged bleeding time. In all cases the amount and/or function of vWf is abnormal or decreased. Classification of vWd is based on vWf multimeric characterization. It is divided into three major groups denoted I, II and III. Type I has the full range of multimers seen in normal plasma, type II lacks the HMW, while type III has either no multimers or only traces of them. According to the classification of Hoyer et al (1983) (25), type I is subdivided to: IA - the common dominantly inheritted vWD, characterized by decreased intensity of multimers of plasma vWf and normal relative proportion of multimers; IIB - characterized by the relative defficiency of HMW, and 1C - where all multimers are found in plasma, but each oligomer is structurally abnormal, lacking in satelite bonds. Other classifications also exist. The group of heterozygots of the autosomal recessive patient is not included in these classifications. It represents the asymptomatic carriers. It is important to note that in some cases the genetic defect seems to be expressed differently in plasma and in platelets. Apart from the recessive variant, all type I variants are dominantly inherited. Type II is characterized by the lack of HMW multimers. It is subdivided to various types - IIA, IIB, IID, IIP, IG and OH. Exept IIC and IIH all subtypes have dominant inheritance. Type III cases are homozygotes of the recessively inherrited vWd. In these cases vWF:Ag is undetectable or very low. No multimers are seen and VIII:C is low too. In 1982 a platelet-type vWd was described by Miller and Castella (26). It is an autosomal dominant bleeding disorder, in which the pathogenetic mechanism relates to an itrinsic platelet abnormality, resulting in increased binding of normal vWf to both Gp-IB and Ilb/IIIa.

COMCLUDIHG REMARKS AMD FUTURE ASPECTS

• Although remarkable progress in recent years in understanding the nature and biology of vWF and vWf/FVIII has been achieved, many questions are still unanswered. There are problems in the way of treatment of vWd like prevention of proteolitical degradation of vWf and depletion of HMW from the transfusion concentrates. From a cellular point of view it is important to be seen how vWF polymers are targeted to WPb and which vessel EC are mostly involved. It is interesting to know via what receptors are vWf activities mediated and what is the exact role of various secretagogues and inflammatory mediators on its synthesis and secretion. From a functional and clinical point how does the binding of vWf to the matrix make it complement to interact with glycoproteins on platelets. In what way the complex FVIII/vWf is formed and how FVIII from that complex supports the coagula-

tion cascade. The application of various monoclonal antibodies in the identification of various kinds of vWD is now in progress.

EHDOTHELIHS - HEW GENERATION OF VASOACTIVE AGENTS

• The history of the isolation of endothelins (ET) is very interesting. It begins with the discovery that EC in culture elaborate a vasoconstrictor peptide into the medium (27). The problem was extended by M. Yanagisawa, H. Kurihara and their supervisor T.Masaki from the University of Tsukuba. Their paper in Nature (1988) was

relative isopeptides (29). lonomycin, thrombin. interleukin-1 and TGF-3 lead to substantial rise in the level of pre-pro-ET-1. In 1989, another peptide was added to the family of ET - the vasoactive intestinal contractor (VIC), which differs from ET-1 only in 3 aminoacid residues (33). In that way the group of ET increased and probably VIC is not the last peptide in the list. VIC was isolated only in gut tissue but not in the vascular system. However its exact location is not clear yet. Immunoelectron microscopical investigation revealed that ET-1-immunoreactivity was localized in gut vascular EC and colonic epithelium of rats under chronic hypoxia (Dikranian et al, unpublished

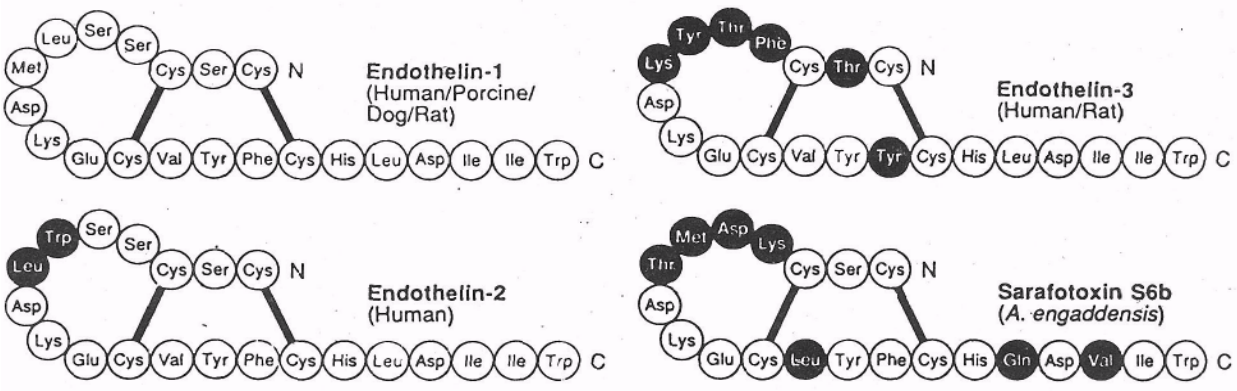

***FIGURE** 2.* Araino acid sequences of peptides of the endothelin family.

the first in a series of serious publications regarding the cell biology and activity of one the most powerfull vasoconstrictors in the human body (28). ET is a 21-aminoacid peptide originally identified in a culture supernatant of porcine aortic endothelial cells (fig. 2).

It is synthetized as a precursor peptide of 203 aminoacids (preproendothelin). Then it is proteolyticaly cleaved to form a 38- or 39-aminoacid intermediate product called "big ET" (29). A protease, called "endothelin converting enzyme" cleaves Trip21-Val22 of big ET to form the mature peptide. After the discovery of ET-1, other peptides of similar structure were isolated (29). They were related to the family of endothelins, named ET-2 and ET-3 (fig. 2). All ET isopeptides share a common design - two disulfide bonds Cysl-Cysl5 and Cys3-Cysll, three polar charged side chains on aminoacid residues 8-10, and a hydrophobic C-terminal, containing the aromatic indole side chain at Trip21 (30). ET share close sequence homology and similar actions with Serafotoxins isolated from the venom of the burrowing asp Atractaspis engaddensis (31) (fig. 2). The C-terminal is very important for the biological activity of these peptides. Like many other disulfide containing peptides, destruction of the interrchain disulfide results in a marked decrease of vasoconstrictor activity (32). Three genes encode the

observations). Recent publications show that tissue content of ET-1 is highest in the lungs and large intestine (34). Tracheal epithelial cells in culture also synthetize and secrete ET, Thus it is prompting to speculate that various epithelial cells are also capable of ET synthesis and secretion. Pre-pro-ET-1 mRNA is found in lungs, kidney, uterus and placenta. Pre-pro-ET-2 mRNA is localized un intestine, suprarenal gland, brain and pancreas. There are serious reasons to assume that ET-3 is the potent neurogenic representative of the ET family.

Despite the vast number of investigations and data on ET, it is extremely hard to generalize the biological role of these vasoactive substances. That is why we shall limit ourselves only to some aspects of the biological role of ET in different conditions.

IN VITRO STUDIES.
HEART AMD ISOLATED BLOOD VESSELS

• ET-1 exerts a possible chronotropic and inotrpic effect. At nanomolar concentrations it leads to contraction of the atria. This effect involves L-type voltage sensitive calcium channels. ET have potent contractile activity on a variety of mammalian blood vessels (35). It is more powerful than epinephrin, angiotensin II and vasopressin.

The constrictor effect is not influenced by phenotolamine, diphenhydramine, methysergide and indomethacine. That means that its action is not mediated through ce-adrenergic, histaminergic and serotoninergic receptors as well as by arachidonic acid stimulation. Constriction is blocked by isoproterenol and nitroglycerine. These data suggest that ET (ET-1) does not influence relaxation of conducting vessels through the action of cAMP and cGMP. Vasoconstriction by ET-1 is blocked by the calcium entry blocker nicardipin. The contraction is dependent on the extracellular calcium. However, the data is conflicting. Diltiazem, niphedipine and methoxyverapamil, representatives of three distinct classes of calcium channel blockers have little effect on the contractile responce to ET-1 in rat aorta (35). It has been proposed that ET promote the formation and release of endothelium derived relaxing factor (EDRF). It also releases TXA_2, PGI_2 from isolated perfused lungs.

Two distinct receptors, described in the action of ET, probably serve different functions. Each belongs to a family of rhodopsin-like receptors with seven transmembrane domains (27,36). Each is coupled to a G-protein. The first receptor shows high specificity to ET-1, while the other reacts with all three ET and is coupled through a G-protein to phospholipase C, which leads to a transient increase in intercellular Ca^{2+}.

OTHER TISSUES AMD ORGAHS

• The contractile action of ET is not limited to the vascular tissue alone. Similar contractile effect is exerted to the smooth muscle coat of the stomach, duodenum, trachea and bronchi. ET-1 contractile effect is independent of T- and N-type voltage-sensitive calcium channels or the activation of sodium channels. Binding sites for ET are localized in alveoli, tracheal and bronchial smooth muscle cells, as well as in the glomeruli (37). All these facts lead to the suggestion of the paracrine ection of ET on neighbouring structures (27). These facts could have pathogenetic role in the mechanisms of coronary and bronchial spasms, hypertension and myocardial ischemia. In other organs (uterus, bladder) ET induce their contractile effects via different way.

BIOLOGICAL ACTION OF ET IN VIVO. CARDIOVASCULAR SYSTEM

• In vivo effects of ET are complex and dependent upon various circumstances. The hemodynamic responce is greatly influenced by the structure of the vascular segment, mode of administration, dose, as well as the initial vascular tone. The intial study of Yanagisawa et al (1988) points that ET-1 produces a systemic pressor responce in anestetized rat (28). However most of the in vivo studies reveal an initial, short lasting decrease in systemic arterial pressure, followed by pronounced and prolonged systemic hypertension (28,35). The ability of ET to decrease blood pressure is most probably due to induction of EDRF release. The vasoconstrictor action of ET is most powerful on the mesenteric artery. In the pulmonary circulation (i.e. in the lobar arterial flow) this effect is much more attenuated. In vitro studies show that atrial myocytes release angiotensin II after treatment with ET-1. The same phenomenon appears in vivo, but the exact mechanism of action is still unknown. On microcirculatory level ET is also a potent constrictor.

OTHER ORGAHS AMD SYSTEMS

• In guinea-pigs ET increase the inspiratory pressure like histamine and platelet activating factor. The bronchoconstrictor effect of an aerosol of ET-1 is not associated with changes in arterial pressure, while intravenous administration of ET-1 produces systemic rise in blood pressure (35) without causing bronchoconstriction. Therefore it must be accepted that ET-1 causes contraction to both types of smooth muscle cells (bronchial and vascular) via two different mechanisms within one and the same organ.There are proofs that ET-1 takes part in various inflammatory processes. Intradermal administration causes a long-lasting pruritus and rise of local temperature. ET-3 may be released from neural tissue.

ENDOTHELINS AND THE PATHOGENESIS OF CARDIOVASCULAR AMD RESPIRATORY DISEASES

• During the last two years many studies have accumulated evidence for the possible involvement of ET in the pathogenesis of such diseases. The recent reviews on ET clearly point out the possible role of ET on these systems (35,36,38).

The first pathological state that may involve ET is hypertension (27,36). The rapid rise in systemic arterial pressure after intravenous administration of ET-1 shows, that it penetrates the endothelial barrier quite rapidly to act on the vascular smooth muscle. Another fact in that suggestion is that renal vessels of SHR are very sensitive to ET-1(39). Despite these and other facts, the role of ET in hypertension is far from clear. Like other paracrine substances or hormones, their effects will become clearer after the application of possible receptor antagonists or endothelin-converting enzyme inhibitors, which are still a pharmaceutical secret. However a neural protease inhibitor phospharamidon was found to exibit such properties (27). It also lowers blood pressure of spontaneously hypertensive rats. It may be that this has put a start of a new class of antihypertensive drugs. A role of ET in cell proliferation and atherosclerosis is suggested too. The fact

that ET increase the reactivity of the vascular wall both in vitro and in vivo, induce a multifactor influence on vascular cells, induce the secretion of atrial natriuretic factor and are found to be increased in plasma during acute myocardial infarction show, that they can act as primary regulators of cardiovascular effects.

Autoradiographic studies have shown that after i.v. administration, ET-1. is detected in lungs, kidney, spleen, liver. Practically ET-1 is a powerful bronchoconstrictor. Lungs are the major target organs for accumulation and ET clearance (35).

Quantitative autoradiographic studies have shown that the pulmonary smooth muscle cells possess specific binding sites. ET is released after an attack of asthma and during hypoxia - states, which are often encountered in obstructive pulmonary diseases.

The airway epithelial cells are also capable of ET synthesis. They are much more powerful spasmogens compared to carbachol and histamine! They also stimulate the synthesis of TXA_2 - a powerful bronchoconstrictor agent. All that makes ET a class of peptides that are capable of maintaining a high muscular tone in airways under appropriate conditions.

Although the role of ET in physiological and pathophysiological conditions is far from clarified, it is probably soon that we will witness the discovery of a new regulatory system not only concerning cardiovascular and pulmonary system, but other systems of the body too.

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