THE ENDOTHELIUM: A KEY REGULATOR OF VASCULAR TONE

INTERNAL MEDICINE GRAND ROUNDS

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In recent years, the concept that a dynamic vasoconstrictor-vasodilator interaction controls organ perfusion and is regulated by locally produced vasoactive substances has gained acceptance, and has clear clinical correlates. For example, some of the adverse effects of non-steroidal anti-inflammatory drugs on renal function are related to the inhibition of the locally produced vasodilator prostacyclin (PGI_2) in the renal cortex. This review explores the emerging concept that other endothelium-derived substances participate in the control of systemic and regional circulations. A review of several of these endothelial products, their effects and mechanisms of action is first provided. Evidence (experimental and clinical) that they participate in the pathogenesis of known diseases is then discussed. The larger hypothesis is that the endothelium may be the key regulatory organ involved in the control of regional perfusion and may be primarily responsible for the development of several diseases.

1. ROLES OF THE VASCULAR ENDOTHELIUM AND SYSTEMS OF STUDY

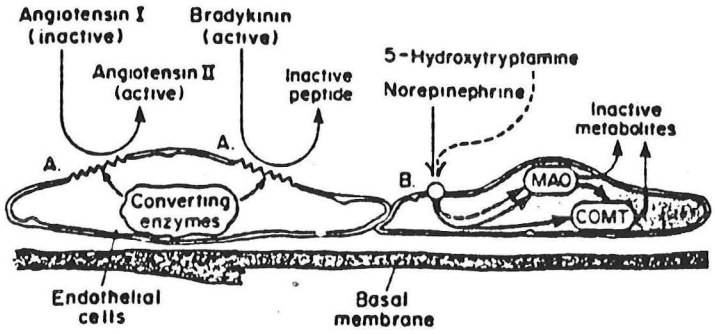
As the interface between the circulating blood and vascular smooth muscle cells, endothelial cells have several key functions (Table 1). They are the first barrier to circulating antigens and actively participate in defense reactions; they play a pivotal role in both the prevention and promotion of clotting by virtue of their complicated

TABLE 1 - FUNCTIONS OF THE ENDOTHELIUM

• Barrier to movement of fluids and solutes from blood to tissue
• Metabolic clearance of substances from the blood
• Activation/deactivation of locally active peptides
• Key role in blood coagulation, platelet adhesion, aggregation, fibrinolysis
• First barrier against circulating antigens
• Locally affects smooth muscle tone

interaction with platelets, blood proteins, and the fibrinolytic system. Endothelial cells may play a selective metabolic role by virtue of their ability to clear substances (such as norepinephrine and serotonin) from the blood. For example, endothelial cells may locally activate peptides such as angiotensin and inactivate others such as bradykinin (Figure 1). The endothelium may also be an important factor in promoting local fibrotic effects by virtue of the mitogenic activity endothelial cells possess. The recognition that the endothelium is an important regulator of local vascular tone is the main subject of this review.

Figure 1: METABOLIC FUNCTIONS OF THE ENDOTHELIUM



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It should be noted that endothelial cells are strategically located to exert effects on nearby vascular smooth muscle cells and other structures in several regional organ beds. For example, in the glomerulus of the kidney, the endothelial cells are closely situated to the mesangial cell (another cell with important contractile properties), to the juxtaglomerular cell, and to the epithelial cells which support the basement membrane (Figures 2 and 3).

Figure 2: TRANSMISSION EM OF A GLOMERULAR CAPILLARY LOOP

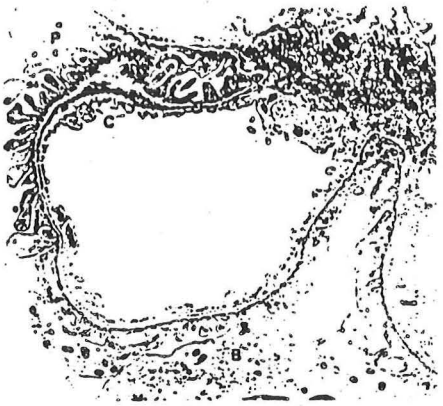

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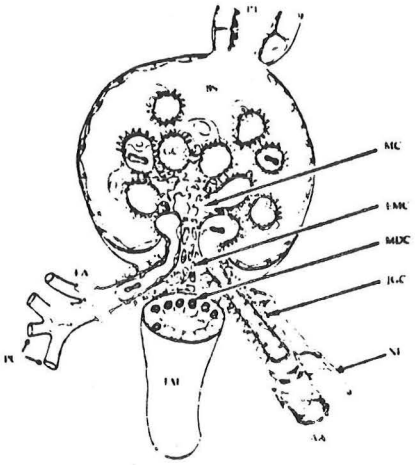
Figure 3: DIAGRAM OF GLOMERULUS AND JG APPARATUS



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The investigation of the vasoactive products of the endothelial cell has produced several ingenious experimental techniques. First, mechanical removal of the endothelium from arterial strips is easily accomplished by gentle rubbing. The most utilized model for study of vascular contractions is the vascular ring preparation (Figures 4). This

Figure 4: REMOVAL OF ENDOTHELIUM AND PREPARATION OF AN AORTIC RING EXPERIMENT

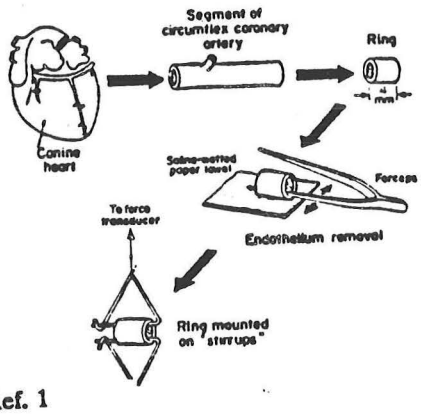

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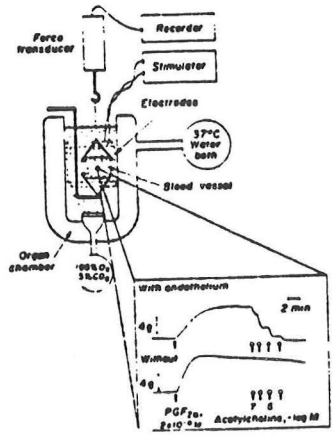
Figure 5: PERFUSION BIOASSAY SYSTEM TO DETECT ENDOTHELIUM-DERIVED SUBSTANCES FROM VASCULAR SEGMENTS



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preparation is used to study the blood vessel responsiveness to exogenously added agents. A layered preparation ("sandwich") may be used to show the release of vasoactive substances from the endothelium. A perfusion bioassay system may be used to determine the duration of effects of endothelium-derived activity (Figure 5).

Cultured endothelial cells on microcarrier bends may be also be utilized for this purpose.

2. VASOACTIVE ENDOTHELIAL FACTORS

A. VASORELAXANT FACTORS

As noted above, the endothelium is strategically located between the circulating blood and vascular smooth muscle cells; despite this position, it has been only recently that the endothelium has been recognized as a pivotal regulator of regional blood flow and vascular resistance. The growing recognition that the endothelium can be a regulatory factor in systemic events is derived from experiments in the past 10 years which have shown that the endothelial cell is a rich source of potent vasoactive substances. For example, in 1977 Moncada et al (3) demonstrated that the endothelium is the major source of prostacyclin. Perhaps the most provocative experiment that launched the investigative interest in the endothelium was performed by Furchott and Zawadzki (4), who demonstrated that the relaxation of isolated arteries induced by acetylcholine is endothelium-dependent (Figure 6). These investigators worked with isolated aortic rings and arterial strips. After constriction of the ring with norepinephrine, acetylcholine, at concentrations of 0.01 to 1.0 μM, induced vasorelaxation. Hemoglobin reversed this vasorelaxant activity. When the experiment was repeated after removing the endothelial surface by gentle rubbing, acetylcholine did not induce vasorelaxation of a contracted ring. Thus, the stimulation of muscarinic receptors on endothelial cells triggers the generation of a diffusible and transferable substance that relaxes smooth muscle--endothelium-derived relaxing factor, EDRF.

EDRF is a labile vasodilator with a half life of about 6 seconds. Generation of EDRF is not affected by cyclooxygenase inhibitors. Substances other than acetylcholine which cause EDRF release include bradykinin, angiotensin II, histamine (via H_1-type receptors), norepinephrine (via α_2 receptors), 5-hydroxytryptamine, ergotamine, calcium ionophore A23187, adenine nucleotides, thrombin, arachidonic acid, and melittin (5). In addition pulsatile pressure, visible light, and electric field stimulation also can release EDRF (Figure 7). Many of these stimulants also release PGI_2; however, only endothelial cells produce EDRF whereas PGI_2 is produced by both endothelial cells and vascular smooth muscle cells.

Figure 6: DEMONSTRATION OF EDRF

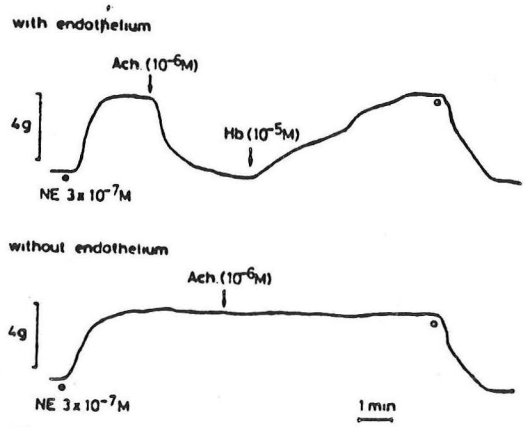

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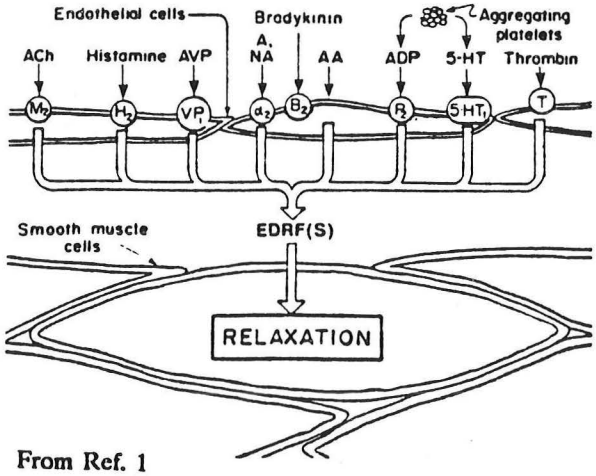
Figure 7: SUMMARY OF SEVERAL NEUROHUMORAL MEDIATORS WHICH CAUSE EDRF RELEASE



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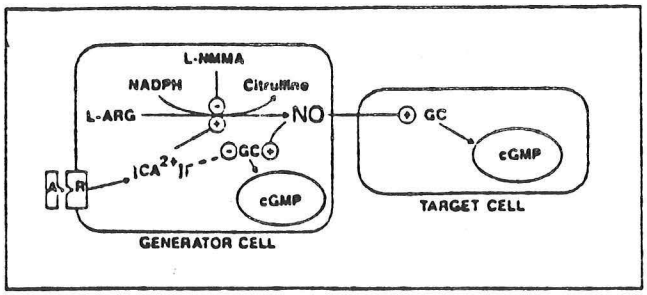
The intracellular events which lead to EDRF release may involve an increase in intracellular calcium, possibly linked to a sodium-calcium exchange (6,7) or a calcium-

activated potassium channel (8). As noted earlier, specific receptor activation of endothelial cells triggers EDRF release. The manner in which these receptors activate release may differ. For example, some agents (e.g., LTC_4, thrombin, alpha-2-adrenergic receptors) stimulate EDRF release by activation of G_i protein, whereas other stimuli (notably acetylcholine and bradykinin) do not (9,10). Thus, at least two distinct biochemical pathways lead to the release of EDRF. Likewise, some EDRF stimulators stimulate the metabolism of phosphoinositol and increase the intracellular levels of inositol triphosphate, which then leads to calcium mobilization. Post-receptor events which culminate in EDRF release include activation of guanosine 5'-triphosphate-regulatory protein and the increased breakdown of a phosphoinositide (6). Diacylglycerol activation of protein kinase C is also involved since phorbol esters inhibit EDRF release (11,12). A simplified scheme depicting these events is shown in Figure 8 (13). As shown in the Figure, an agonist (A) interacts with its receptor (R), increasing calcium concentration in the generator (endothelial) cell containing the NO synthase. The calcium stimulates the enzyme to form NO and citrulline from L-arginine. Exactly how the terminal guanido nitrogen atom(s) of L-arginine is liberated and subsequently oxidized to NO is not known (14). This NO release is inhibited by NO synthase inhibitors such as L-NMMA. Formed NO stimulates soluble guanylate cyclase (GC) to increase cyclic GMP concentration in both target (smooth muscle) and generator cells. In some cases such as the brain, the elevated cytosolic calcium has an inhibitory effect on the soluble granulate cyclase within the generator cell.

Figure 8: SCHEMATIC DIAGRAM OF THE L-ARG: NO PATHWAY



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The vascular smooth muscle cell is the target for EDRF action, but EDRF also inhibits platelet aggregation and platelet adhesion (15,16). EDRF effects are mediated by an increase in intracellular cyclic guanosine 3',5'-monophosphate (cGMP). Soluble guanylate cyclase is stimulated by other nitrovasodilators, including glycerol trinitrate (nitroglycerin). Methylene blue blocks the activation of guanylate cyclase (and thus the actions of EDRF), whereas superoxide dismutase (SOD) enhances EDRF action. This later result suggests that EDRF is destroyed (17) by superoxide and that SOD, a scavenger of superoxide, prevents EDRF from being destroyed. In fact, many of the inhibitors of EDRF action liberate superoxide, so that this mechanism of action remains attractive. Inhibitors of EDRF include BW755c, caffeic acid, quercetin, hydroquinone, phenyl hydrazine, potassium borohydride, dithiothreitol, cysteine, and metyrapone (5).

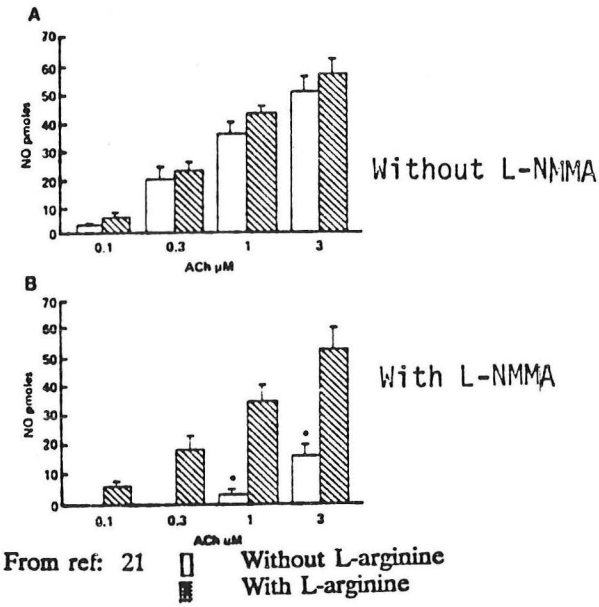
Nitrous oxide synthase has been proposed to be comprised of a family of enzymes, and consists of 2 distinct types which are present in many different cell types including endothelial cells, macrophages, adrenal cortex, brain, and neutrophils (18). Type II NO synthase (as found in endothelial cells) is a calcium and calmodulin-dependent enzyme which is rapidly activated by ligand-receptor events at the cell surface. Type I NO synthase (as occurs in macrophages) is calmodulin-independent, requires tetrahydrobiopterin as a co-factor, is slowly induced by cytokines, and by endotoxin, and remains active for many hours.

As implied by the above, similarity between the mechanisms of stimulation of guanylate cyclase by EDRF and nitrous oxide (NO) have lead investigators to conclude that EDRF is NO (19,20) (Table 2). EDRF-induced vascular relaxation and exogenous NO-induced vasorelaxation follow the same time courses, are inhibited by hemoglobin (which binds to the active ferroheme center of guanylate cyclase and inhibits activity), and are enhanced by SOD treatment. Finally, NO has been assayed chemically as the chemiluminescent product of its reaction with ozone (21, see Figure 9). NO is also released from porcine endothelium by bradykinin in amounts that account for EDRF's actions. Other forms of EDRF may exist (22).

TABLE 2 - EVIDENCE LINKING NO AND EDRF

1. Superoxide inactivates both and SOD prolongs the action of both.
2. Acidification stabilizes EDRF, generates NO from nitrite.
3. The half-lives of NO and EDRF are similar and both activate soluble guanylate cyclase.
4. Biologic activity of both blocked by hemoglobin and pyrogallol.
5. Cultured EC exposed to BK release NO.

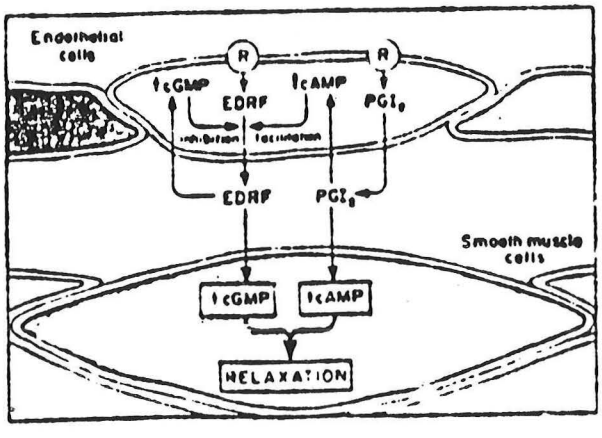
Figure 9: RELEASE OF NO FROM RABBIT AORTA



From ref: 21 ▯ Without L-arginine
▩ With L-arginine

The chemical lability of EDRF (NO) in physiologic solutions makes it highly unlikely that the substance functions as a circulating factor. Rather, EDRF diffuses from sites of local generation to immediately adjacent smooth muscle cell. NO is highly lipophilic and readily permeates plasma membranes. The cascade of events which then ensues includes NO binding to heme moieties of reduced heme-containing proteins, including soluble guanylate cyclase. This produces a NO-heme adduct which facilitates the conversion of Mg-GTP to cGMP (23); cGMP-dependent protein kinase is activated and causes dephosphorylation of myosin light chains and vasorelaxation (Figure 10).

Figure 10: EFFECTS OF CYCLIC NUCLEOTIDES ON EDRF RELEASED AND VASORELAXATION EDHF



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One additional interesting feature of endothelial cell physiology is the effect of cyclic nucleotides on release. As indicated in Figure 10, an increase in cGMP in the endothelial cell likely serves as a negative feedback stimulus to turn off EDRF release. Stimulation of cAMP (by PGI_2 or forskolin) leads to EDRF release and to vasorelaxation. Hence, cGMP and cAMP have differing effects in the endothelial cell but similar effects in vascular smooth muscle cell. EDRF is released in both luminal and abluminal directions. There is still debate over whether EDRF can traverse the blood vessel wall. Adventitial layers of smooth muscle may be less sensitive to EDRF effects than layers close to the intima. It is possible that there is polarized

release of two different EDRFs.

A number of recent studies have assessed the physiologic importance of EDRF in control of systemic and regional hemodynamics (24). Most of these studies depend on the use of L-arginine as the precursor to EDRF-NO and on the L-arginine analogue N^G-monomethyl L-arginine (L-NMMA) which competitively inhibits formation of NO and endothelium-dependent relaxation of rabbit aortic rings by acetylcholine. The influences of EDRF on basal systemic hemodynamics were assessed by Rees et al (21) in a recent study in which L-NMMA was administered intravenously to anesthetized rabbits. A concentration-dependent (3-100 mg/kg) and long-lasting (15-90 min) increase in mean blood pressure occurred (Figure 11). The D-enantiomer of L-NMMA did not produce an effect; the hypertensive actions of L-NMMA were reversed by excess L-arginine.

This finding that L-NMMA inhibits EDRF release in vivo and causes an increase in vascular tone suggests that EDRF and the endothelium plays an important role in blood pressure homeostasis.

These observations regarding regional control of the systemic circulation are also applicable to other vascular beds. Experiments in chronically instrumented awake Long-Evans rats by Gardiner et al (25) showed a dose-dependent decrease in blood flow in response to L-NMMA in renal, mesenteric, hindquarter, and internal carotid circuits (Figure 12); blood pressure also increased in response to L-NMMA. These changes in perfusion were reversed by infusion with L-arginine acetate, the EDRF precursor.

Figure 11: L-NMMA EFFECT ON BP

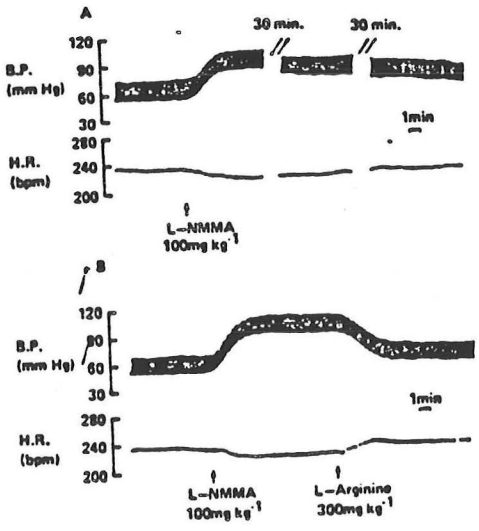

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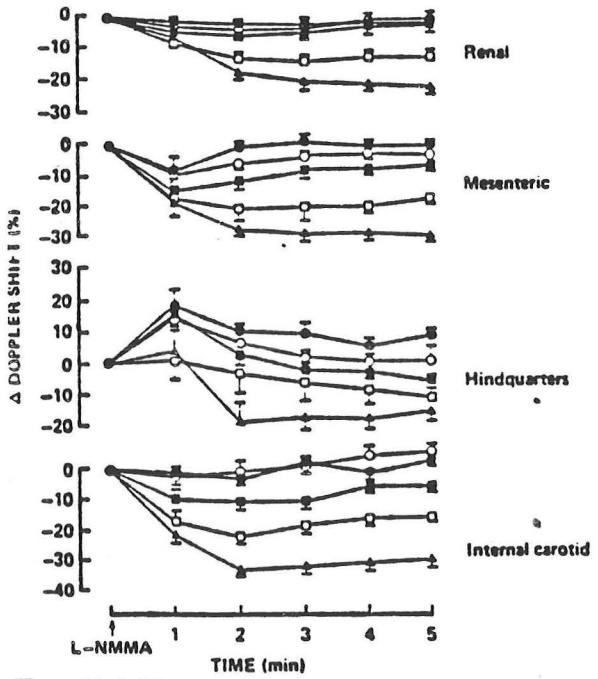
Figure 12: EFFECTS OF L-NMMA INFUSION ON REGIONAL PERFUSION



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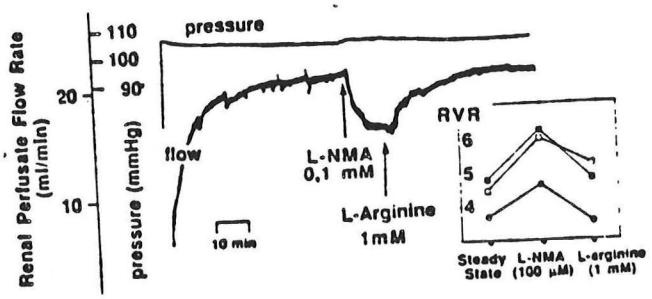
Several recent studies have probed the importance of EDRF in modulating renal function. Rademacker et al (24) have shown that resting renal vascular resistance is likely influenced by EDRF since inhibitors of EDRF action significantly increased RVR.

These studies were performed in the isolated perfused rat kidney. RVR also increased with L-NMMA infusion (Figure 13), again suggesting a tonic vasodilatory influence of renal microvasculature. Recent work by Bayliss et al (26) confirms this observation regarding the tonic influence of EDRF on renal hemodynamics.

Figure 13: EFFECTS OF L-NMMA AND L-ARGININE INFUSIONS ON RVR



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Other experiments have extended these results by showing that glomerular endothelial cells may influence the function of adjacent mesangial cells. Marsden et al (27) have performed cross incubation studies and showed that EDRF agonists caused cGMP accumulation in cultured renal cortical mesangial cells in the presence of endothelial cells. Another issue addressed by this study is the fact that there are differences between endothelial cells from different locations. For example, cultured glomerular endothelial cells do not respond to exogenous histamine (10^{-4}) by increasing intracellular calcium concentration whereas cultured bovine aortic endothelial cells do. Thus, some heterogeneity of endothelial cell populations certainly exists.

2. PROSTACYCLIN (PGI_2)

PGI_2 is a dienoic bicyclic eicosanoid which is synthesized from membrane-bound arachidonic acid. It has a stability of about 3.5 min at physiologic pH. Both phospholipase A_2 and phospholipase C accompany PGI_2 synthesis and release from both endothelial cells and vascular smooth muscle cells. Bradykinin is a notable stimulator of PGI_2, along with choline esters, substance P, arachidonate, thrombin, EGF, PDGF, IL-1, and adenine nucleotides. The activation of PGI_2 by bradykinin is particularly noteworthy because angiotensin converting enzyme (ACE) inhibitors are known to prevent the breakdown of bradykinin. This leads to increased levels of bradykinin and the stable metabolite of PGI_2, 6-keto $PGF_{1\alpha}$, in the plasma of subjects treated with ACE inhibitor drugs. Such an increase in the vasodilator PGI_2 may mediate, in part, the decrease in peripheral resistance and the hypotensive effect of these drugs.

PGI_2 increases cyclic adenosine 3', 5'-monophosphate (cAMP) levels in vascular smooth muscle cells, an action which also leads to vasorelaxation. PGI_2 also inhibits platelet adhesion and has thrombolytic properties as well. PGI_2 possess cytoprotective actions including the ability to neutralize damaging oxygen free radicals in ischemia (28).

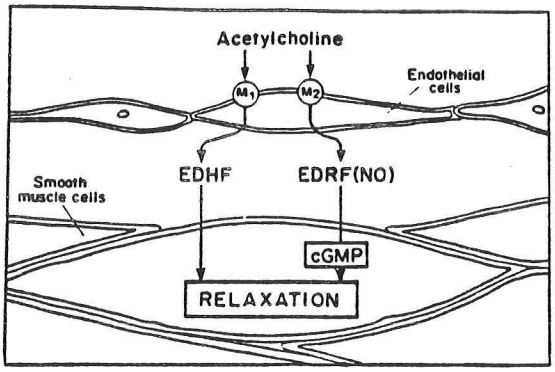
PGI_2 and EDRF potentiate each other's vascular and platelet anti-aggregatory effects even at subthreshold concentrations (29,30,31). This implies that potent vasodilators and inhibitors of platelet function (and stimulators of fibrinolysis) are released locally which ameliorate both vasoconstriction and local thrombus formation.

3. ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR (EDHF)

In canine femoral artery, the endothelium releases an EDHF in response to acetylcholine. In this experimental preparation, endothelium-dependent relaxations to acetylcholine are reduced after inhibition of sodium-potassium ATPase by ouabain (32). Exogenous NO, however, does not cause hyperpolarization of vascular smooth muscle

cells; moreover, hemoglobin does not prevent the change in membrane potential caused by acetylcholine. Hence, an endothelial-derived substance other than NO or EDRF is probably involved. Hyperpolarization of the vascular smooth muscle cells favors relaxation and decreases the responsiveness of the vascular wall to the vasoconstrictive effect of or hormones (Figure 14).

Figure 14: EDHF



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4. LIPOXYGENASE PRODUCTS

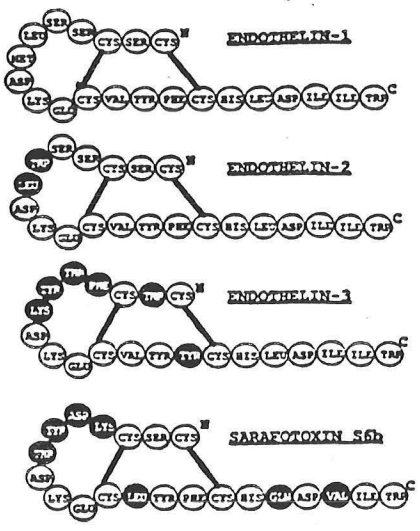
Lipid hydroperoxides may be synthesized in several ways in endothelial cells: by hydroxyl radicals that may oxidize lipids, by a co-oxidation process linked to cyclooxygenase, or by a microsomal cytochrome P-450 oxidase. The P-450 cytochrome oxidase is known to synthesize 12(R)-HETE, 19(S)-HETE, 19(R)-HETE, and 20-HETE in cornea and kidney (33,34), and endothelial cells exhibit P-450 dependent monooxygenase activity (35). Another synthetic product recently identified is 13-HODE, synthesized from linoleic acid by cytosol-associated lipoxygenase. 13-HODE posses antiadhesion actions, but is not released into the media by cultured endothelial cells. However, 13-HODE increases PGI_2 production by cultured fetal bovine aortic endothelial cells (36). Thus, 13-HODE may have a local vasodilatory action working through PGI_2.

B. VASOCONSTRICTIVE FACTORS

1. Endothelin

Endothelial cells also produce several vasoconstrictive factors. Endothelial cells in culture produce a 21 amino acid peptide called endothelin (ET) by Yanagisawa (37). Three forms of the peptide exist (Figure 15): endothelin-1 (formerly human or porcine), endothelin-2, and endothelin-3 (formerly rat endothelin). The human genome possesses three unique genes for all forms of the peptide. ET-1 is expressed in vascular tissue, while ET-3 is possibly a neural form of the peptide. Endothelin belongs to a group of highly homologous scorpion and snake venom toxins, particularly snake venom sarafotoxins. The chemical structure is characterized by a group of 21 amino acid peptides which also contain two intra-molecular disulfide bridges (38). Endothelin-1 and -2 posses similar potencies, but endothelin-3 is likely about one order of magnitude less potent. There are probably differences in the affinity for ET isoforms for ET receptors or more than one ET receptor subtype in vascular smooth muscle. ET receptors exist in vascular smooth muscle, adrenal glomerulose, renal glomeruli, medullary vascular bundles, and papilla (39). Other loci for ET receptors include cardiac atria, cardiac nerves,

Figure 15: STRUCTURE OF ENDOTHELINS



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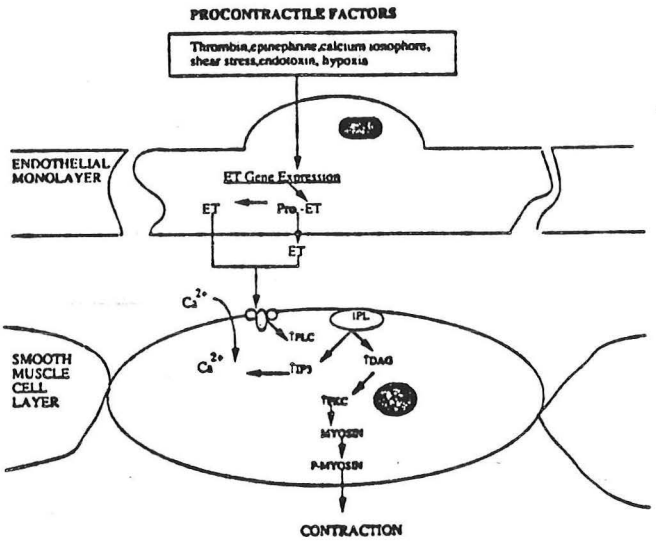
ventricles, and coronary arteries, lung (homogeneous distribution), brain (particularly the cerebellum), spinal cord, gastrointestinal tract, liver, and spleen.

Preproendothelin mRNA is expressed by cultured endothelial cells by several factors including thrombin, shear stress, epinephrine, TGF_{β}, A23187, and phorbol esters. Endothelin binds to specific membrane receptors on vascular smooth muscle cells. Phospholipase C is activated, which in turn stimulates the formation of inositol triphosphate and diacylglycerol and phospholipase A_2; an increase in intracellular calcium occurs and long-lasting contractions ensue. Figure 16 depicts endothelium/ smooth muscle pathways (40).

Figure 16: MODELS OF ENDOTHELIUM-DEPENDENT ACTIVATION OF VASCULAR SMOOTH MUSCLE



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The activation of diacylglycerol and mobilization of calcium from intracellular stores implies that ET is not dependent upon extracellular calcium for its actions. In some blood vessels, calcium channel blockers do not affect the contractions caused by endothelin. However, in other tissues such as the porcine coronary artery, the peptide can activate voltage-operated calcium channels. This explains why calcium channel blockers can attenuate, but not ablate endothelin effects in some tissue. One difference between ET and other vasoconstrictive hormones (such as angiotensin II or vasopressin via its V_1 receptor) is the fact that the vasoconstrictor action of ET is prolonged. This suggests that other late signalling mechanisms may exist. Another interesting feature of ET is that it has been shown to augment endothelium-derived NO and PGI_2 production. This finding implies that ET stimulates antagonists to its vasoconstrictor action.

In cultured rat smooth muscle cells, ET-1 is bound to cells by an apparent single class of high affinity recognition sites (dissociation constant 1.84±.3 nmol/L with a maximum binding of 62±11 fmol/10^6) (41). Down regulation of ET binding sites can occur within 30 minutes and can persist for 18 hours. The cellular binding of ET is specific and as mentioned above is not ablated by calcium channel blockers (38).

In addition to the vasoconstrictive effects of ET, an effect to cause an increase in DNA synthesis in vascular smooth muscle cells in also noteworthy (42). In these studies ^{3}H-thymidine incorporation was used to assess DNA synthesis. Also noteworthy is the fact that PDGF and ET-1 had synergistic effects on DNA growth in vascular smooth muscle cells (42). Badr et al (43) have shown that ET-1 increases mesangial cell intracellular inositol triphosphate concentration, increases intracellular calcium concentration, contracts the cell, and is a potent mesangial cell mitogen as well.

ET may be produced by endothelial cells to have an effect locally (eg. on adjacent vascular smooth muscle cells) or may act as a circulating hormone. In one recent study, ET was detected in normal human (40) plasma at a concentration of 2.36

pg/ml (Figure 17). A two-fold increase in the concentration of ET achieved by exogenous infusion results in significant increments in systemic and renal vascular resistance. These findings, in addition to the results with L-NMMA infusion, suggest that ET may contribute to the regulation of cardiovascular homeostasis. It is also possible that plasma ET values may represent the overflow of a local peptide hormonal system that participates in the regulation of local cardiovascular function.

Individual vascular beds exhibit variability with regard to ET responsiveness. Renal vasculature is the most sensitive vascular bed; a significant increase in renal vascular resistance occurs and persists long after ET is discontinued (44-53). Coronary resistance also increases after ET administration, but at doses greater than those required in the renal circulation (54). Mesenteric and hindquarter resistance also increases after ET administration, but after a period of initial hyperemia. Differences in vascular responses to ET may best be explained by differences in ET receptor populations and affinities or by opposing EDRF activity at the endothelium/vascular smooth muscle site (55,57).

ET is the most potent vasoconstrictor known. Typically, ET exerts a more profound effect on veins than on arteries (Figure 18) (56). Interestingly, the presence

Figure 17: DISTRIBUTION OF ET-1 CONCENTRATIONS IN NORMAL HUMAN PLASMA

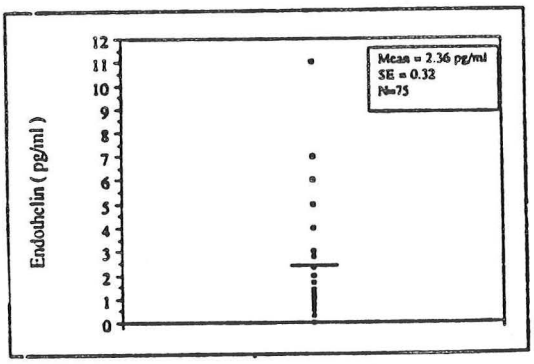

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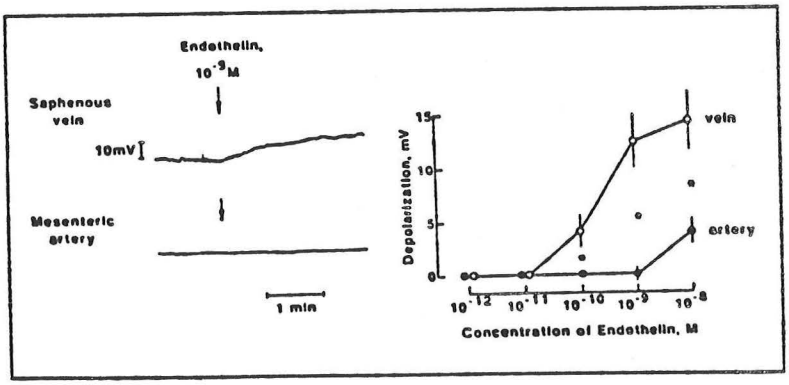
Figure 18: EFFECTS OF ET-1 ON BLOOD VESSELS



From Ref. 56

of the endothelium reduces or may even abolish the maximal vasoconstriction achieved (57). Both afferent and efferent arteriolar resistance increase in the kidney (45); in the heart, resistance vessels seem more sensitive to ET effects than conductive vessels (46).

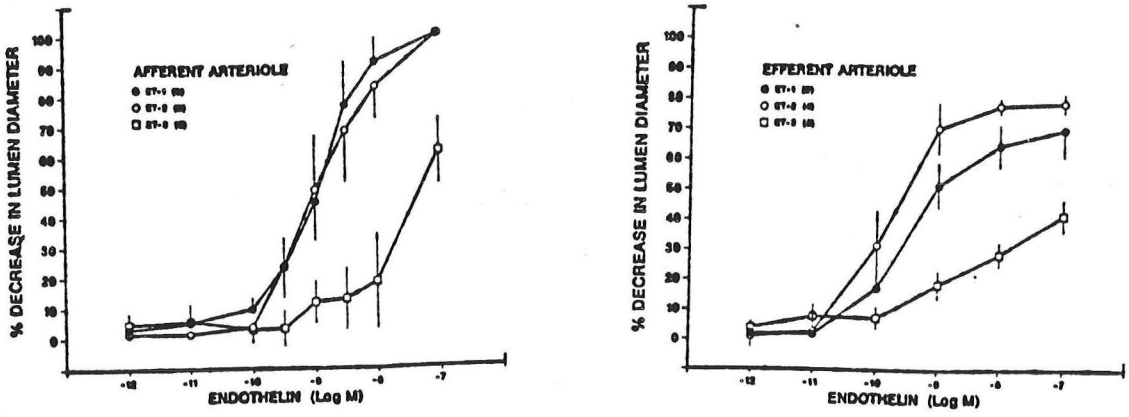
Systemic and bolus infusions and bolus infusions of ET increase systemic resistance and cause a pressor response which is maximal at 45 minutes. Bolus infusions have been associated with a biphasic response in which an initial, brief transient period of hypotension occurs. During this hypertensive period, systemic resistance declines. This reduction in systemic resistance may be due to an ET-stimulated release of EDRF, atrial natriuretic factor (ANF), or vasodilatory PGI_2. ANF has vasodilatory properties and may play a role as modulator of the pressor response to ET. ET infusion provokes ANF release and ANF can relax blood vessels preconstricted with ET. PGI_2 is also stimulated by ET, and cyclooxygenase inhibition with indomethacin enhances ET-induced vasoconstriction.

A. ET EFFECTS ON KIDNEY

Several investigators have probed the effects of ET on whole kidney function. Katoh et al (47) infused ET-1 into the left renal artery of anesthetized rats and noted decreases in urine volume, estimated renal plasma flow, and GFR. Interestingly, atriopeptin and nicardipine reduced these effects of ET when they were included in the infusion. These results suggest calcium entry may play a role in the ET effect in the kidney and that ANP can antagonize ET action. Banks (48) saw an initial vasodilatation in kidney followed by a prolonged vasoconstriction which persisted for 30 minutes post-infusion. Angiotensin II antagonists did not alter the response but captopril pre-administration did, suggesting inhibition of kinin degradation may attenuate the actions of the peptide. Similar experiments have been performed by Stacy et al (49), using intrarenal doses of ET-1 (1.15 ng/kg/min) which did not alter systemic pressure. Larger bolus doses of ET-1 have been tested, with more profound effects on systemic resistances and blood pressure (50).

The loci of ET-1 effect in renal microvasculature are likely multiple. In addition to an effect on mesangial cells (with contraction and a reduction in K_f and a subsequent fall in GFR), the effects of ET-1 on afferent and efferent arterioles have been tested (44). Both arterioles showed a 50% decrease in lumen diameter upon exposure to ET-1 at concentrations ranging from 1.4 to 0.9 nM, (51) (Figure 19a,b). Nicardipine reduced the responsiveness of afferent arterioles to ET-1. The results suggest that ET-1 is a potent renal vasoconstrictor of both the pre and post glomerular circulation; moreover, the afferent arteriole appears more dependent on calcium entry through potential-dependent channels calcium than the efferent arteriole.

Figure 19a, 19b: CONTRACTILE RESPONSES OF AFFERENT AND EFFERENT ARTERIOLE TO ET

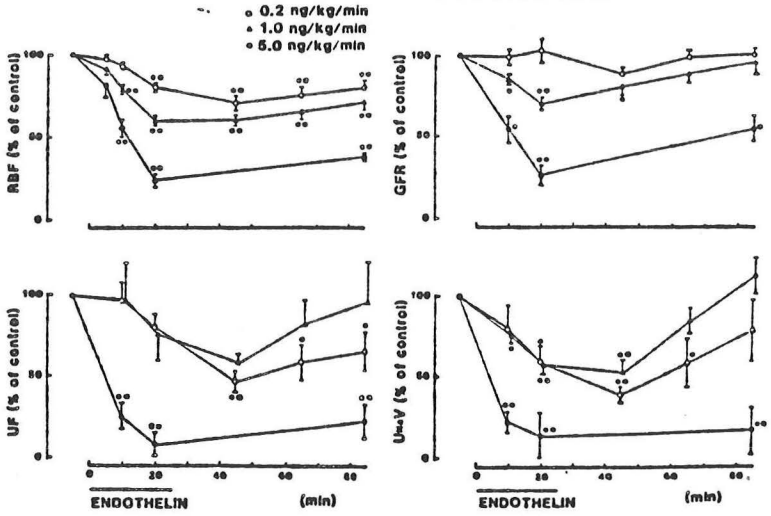


From Ref. 51

Some experimental findings are suggestive that ET-1 has a greater sustained effect on efferent vs afferent arterioles (52). Miura et al infused ET-1 into the renal artery of anesthetized dogs and noted that low dose ET-1 (.2 ng/kg/min) significantly

decreased renal blood flow but not GFR (Figure 20).

Figure 20: LOW DOSE ET-1 (0.2 ng/kg/min) LOWERS RBF BUT NOT GFR



From Ref. 52

Yasujima et al (53) have tested the effects of a chronic infusion of ET-1 into rats (60 μg/kg/day) using osmotic minipumps. Interestingly, although mean blood pressure increased, little change in urine volume and urinary sodium excretion occurred. The hypertension induced by the ET-1 chronic infusion was controlled by treatment with a calcium channel blocker.

ET effects on renal hemodynamics after a bolus infusion are listed in Table 3. ET receptors exist in renal vein, renal artery, arcuate artery, interlobular artery, glomerulus, and renal papilla (53,54). ET mRNA has been detected in cortical and medullary locations (55). The prolonged effects of ET on the renal circulation may be secondary to effects mediated via mesangial cell contraction and a lower ultrafiltration coefficient.

Table 3—Renal Hemodynamic and Excretory Effect of Administration of Endothelin*

Variable	Control	Endothelin (50 ng/kg/min) 1	2	3	Recovery (1 h after infusion)
GFR (ml/min)	38.50 ± 3.64	4.26 ± 1.31†	1.43 ± 0.39†	0.66 ± 0.22†	47.70 ± 2.10†
RBF (ml/min)	267 ± 33	136 ± 21†	87 ± 14†	63 ± 20†	173 ± 28†
RVR (mm Hg/ml/min)	0.398 ± 0.049	1.043 ± 0.172†	1.702 ± 0.212†	2.490 ± 0.656†	0.667 ± 0.115†
Urine volume (ml/min)	0.270 ± 0.082	0.030 ± 0.009†	0.012 ± 0.001†	0.008 ± 0.002†	0.300 ± 0.085
UNaV (μeq/min)	47.60 ± 14.30	4.56 ± 0.48†	1.23 ± 0.26†	‡	12.60 ± 3.61†
FENa (%)	0.95 ± 0.31	0.88 ± 0.17	0.66 ± 0.14	‡	0.18 ± 0.05†

*Values are shown as means ± SEM (N = 6). FENa = fractional excretion of sodium; GFR = glomerular filtration rate; RBF = renal blood flow; RVR = renal vascular resistance; UNaV = urinary sodium excretion.
†$P<0.05$.
‡Sodium detected; volume of urine specimen insufficient for determination.
From Miller and associates.[6] By permission of the American Society for Clinical Investigation, Inc.

Several of the endocrine effects of ET are provided in Table 4. ET stimulates aldosterone secretion directly from zona glomerulose cells. Effects on renin secretion are more complicated. In vitro studies show inhibition of ET on renin release, but in vivo studies show an increase in renin after systemic administration. The vasoconstrictive effect of ET coupled with increased sympathetic nerve activity may lead to increased renin secretion.

Table 4—Endocrine Effects of Administration of Endothelin*

Variable	Control	Endothelin (50 ng/kg/min) 1	2	3	Recovery (1 h after infusion)
ANF (pg/ml)	39.4 ± 5.90	64.9 ± 12.81†	116.8 ± 30.80†	180.5 ± 52.90†	73.7 ± 9.64†
PRA (ng/ml)	3.72 ± 1.02	7.29 ± 2.28†	7.08 ± 2.49	6.94 ± 1.74†	5.56 ± 1.56
Aldo (ng/ml)	11.6 ± 3.34	23.5 ± 6.16†	28.9 ± 8.11†	42.3 ± 9.23†	46.8 ± 10.00†
AVP (pg/ml)	8.45 ± 2.76	5.05 ± 2.60	9.43 ± 2.97	16.43 ± 3.52†	9.80 ± 2.98

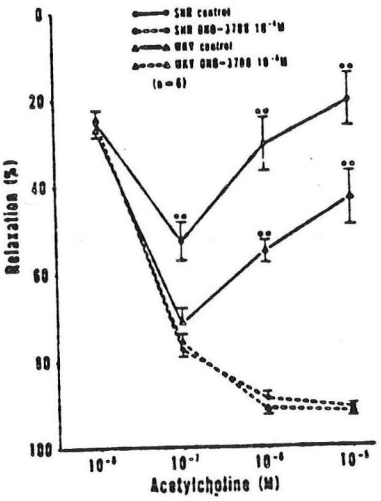
*Values are shown as means ± SEM (N = 6). Aldo = aldosterone; ANF = atrial natriuretic factor; AVP = arginine vasopressin; PRA = plasma renin activity.
†$P<0.05$.
From Miller and associates.[6] By permission of the American Society for Clinical Investigation, Inc.

2. OTHER ENDOTHELIUM-DERIVED CONTRACTING FACTORS (EDCF)

In canine coronary artery, hypoxia causes endothelium-dependent contractions (61). It is unlikely that the endothelium derived contracting factor released during hypoxia is ET because the response is much faster and can be blocked by calcium antagonists. In addition, a cyclooxygenase product (e.g., a prostanoid or superoxide anion) may also have contracting properties when exposed to vascular smooth muscle (62).

Several recent experiments have been performed to further investigate the properties of these EDCFs. In one of these studies (63), Kato et al concluded that PGH_2 is an EDCF. These studies were performed on aortic rings from the thoracic aorta of SHR and WKY rats. These workers found that ONO-3708, a specific inhibitor of PGH_2 action, caused enhanced acetylcholine-induced vasorelaxation of contracted aortic rings in both the SHR and WKY rat (Figure 21). In addition ONO-3708 (10^{-6}M) inhibited all contractile responses to various prostaglandins. The evidence indicating that the acetylcholine-stimulated, endothelium-released EDCF is PGH_2 is: exogenous PGH_2 (10^{-7}M) induced vascular contractions; 2) The contractions induced by PGH_2 were inhibited by ONO-3708; and 3) the concentration of PGH_2 in the aortic ring preparation is estimated to be about 10^{-6}M.

Figure 21: EFFECT OF PGH_2 BLOCKER ON VASORELAXATION



From Ref. 63

Another candidate to be an important local vasoconstrictor substance is epidermal growth factor (EGF). This substance is a mitogen for smooth muscle cells in vitro and also causes arterial strips to contract. Further, glomerular mesangial cells express EGF receptors, making it possible for EGF to exert a regulatory function on GFR. EGF (and its functional homolog transforming growth factor-α) are released from α-secretory granules after platelet activation and from activated macrophages (64). Some of the renal hemodynamic effects of EGF may be due in part to stimulation of both cyclooxygenase and non-cyclooxygenase arachidonate metabolites (64).

3. ROLE OF ENDOTHELIAL FACTORS IN PATHOLOGIC CONDITIONS

A. Hypertension

1) Experimental Evidence

A number of investigators have recently speculated that endothelium-derived peptides may have an abnormal physiology in disease states (65-67). For example, endothelin has been speculated to be involved in the pathogenesis of several common clinical disturbances (Table 5). Such an abnormal physiology would seem particularly likely to occur in hypertension for several reasons. First, the vasoactive factors produced by endothelium may have an altered balance between the release of vasoconstrictive and vasodilatory factors, and thereby change systemic and renal vascular resistance. Second, the endothelium may be the target of hypertension, become damaged, and this may lead to abnormal production of endothelial factors.

Finally, the endothelium may affect systemic hemodynamics by secreting substances which have effects on other key regulators - for example, endothelin causes ANP release and EDRF may change baroreceptor function. All of these pathways are potential mechanisms by which the endothelium could participate in blood pressure regulation either as the initiator or as a propagator of the primary abnormality.

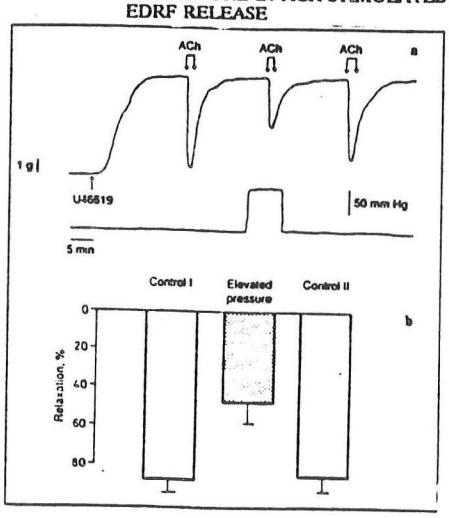
Table 5—Correlations of Endothelin With Various Clinical Conditions

Conditions associated with increased plasma endothelin levels
Cardiogenic shock
Pulmonary hypertension
Acute myocardial infarction
Major abdominal surgical procedure
Orthotopic liver transplantation
Uremia
Hypertension
Conditions in which endothelin may have a pathophysiologic role
Sepsis
Congestive heart failure
Coronary spasm
Cyclosporine nephrotoxicity
Vasculitis
Toxemia of pregnancy

Damage to the vascular endothelium is a prominent feature of hypertension. McGuire et al (68) examined aorta and large blood vessels from the SHR at several times and documented the development of a thickened subendothelial space. This acellular thickening of the subendothelium resulted from the increased synthesis and release of basement membrane macromolecules. In studies of aortic rings from animals with experimental hypertension, endothelium-dependent relaxations to acetylcholine and the calcium ionophone A23187 have been found to be attenuated (69). It was postulated in these studies that the endothelium released a cyclooxygenase-derived substance that interferes with vasodilation. Responses to NO were comparable in hypertensive and non-hypertensive rats. The reduction in these endothelium-dependent relaxations are directly related to the level of systolic blood pressure (70-72). In the Dahl rat, antihypertensive therapy normalizes blood pressure and reverses the abnormalities in the endothelium-dependent relaxations. It is not clear whether the abnormalities in the relaxations are primary or secondary to the hypertension. Evidence that a cyclooxygenase product is partly responsible for the defect in vascular relaxations in the SHR has recently been provided by Diederich et al (69). In these studies, meclofenemate restored vascular relaxations in the SHR.

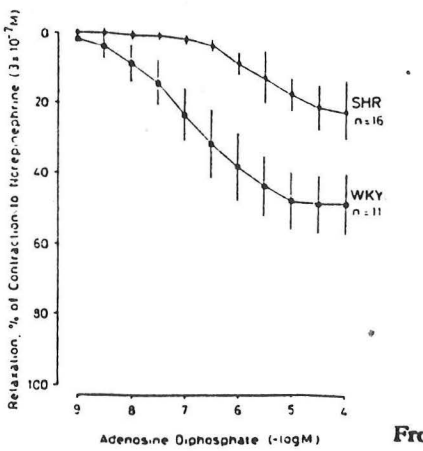
It should be pointed out that defects in vasorelaxation occur very quickly with the development of hypertension. For example, in vitro experiments have shown that acute elevations in intraluminal pressure blunts vasodilation in response to acetylcholine (73, Figure 22). These in vitro studies have been extended to the study of genetic forms of hypertension. Endothelium dependent relaxations are reduced in the SHR rat, Dahl rat, two kidney, one clip hypertension rat, and rats with aortic coarctation (75-81). A typical example of this abnormality is shown in Figure 23.

Figure 22: EFFECT OF AN ACUTE ELEVATION IN PRESSURE IN ACH-STIMULATED EDRF RELEASE



From ref. 73

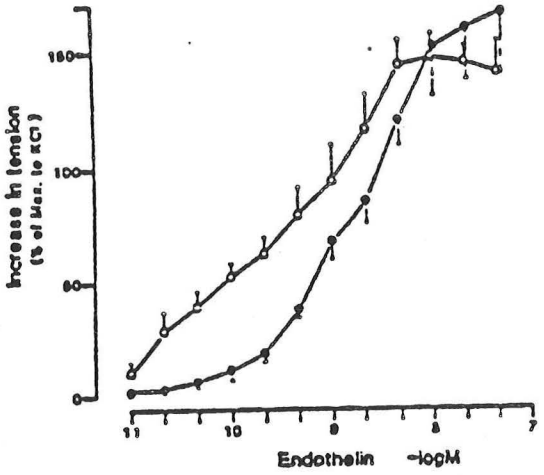
Figure 23: ENDOTHELIUM-DEPENDENT RELAXATIONS TO ADP IN THE CAROTID ARTERY OF RATS



From ref. 80

In addition to abnormalities related to smooth muscle relaxation, increased pressure induces abnormalities with regard to vessel contraction. Aortic rings from DOCA-hypertensive animals have an augmented response to stretch that can be inhibited by calcium antagonists and by removal of the endothelium (74). Acetylcholine causes endothelium-dependent contractions in the aorta of adult SHR but not in adult WKY (76,77). In older rats (12 months of age), the contractions are most pronounced in the older animals; at this age, acetylcholine induces contractions in the normotensive WKY rats as well (78). This suggests the endothelin-dependent contractions observed in the SHR aorta may be due to premature or accelerated aging of the hypertensive arterial wall. Further, the sensitivity of the renal artery to endothelium-induced contractions has been shown to be increased in the SHR compared to the WKY rat (79) (Figure 24).

Figure 24: DOSE RESPONSE OF WHY (•) AND SHR ( ) TO ET-1



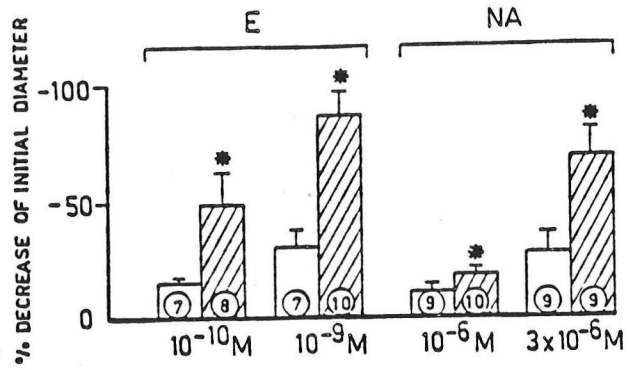
From Ref. 79

One recent study also noted the augmented sensitivity of aorta from the SHR compared to WKY, but noted that this was not due to an increased number of binding sites or a different population of receptors (82). Other studies have noted differences in arterial responses to other vasoconstrictors and dilators in the SHR and WKY rat. Luscher et al documented enhanced constriction of the carotid but not of the renal artery in response to serotonin in the SHR (83). This finding emphasizes the fact that variability in response is dependent upon the vasoconstrictor used and the vascular bed tested. The carotid artery appears to be particularly vulnerable to these endothelium-dependent effects. Levy et al recently showed that, in addition to the deranged vasoconstrictive and vasodilator properties of the vascular endothelium in hypertension, the presence of angiotensin converting enzyme activity may also be important (84). In this study, converting enzyme inhibitor in isolated preparations improved carotid compliance in WKY rats more than the SHR, suggesting an abnormality in vasorelaxation mediated via a local, intravascular renin-angiotensin system.

The issue of the primacy of the endothelium-related changes in the generation and maintenance of hypertension has been further assessed indirectly by examining vascular responses in animals rendered hypertensive by means of a renal artery clip. The responses of vascular endothelium may then be compared to genetically hypertensive animals. In one such study, Fortes and colleagues (85) examined vasoconstriction in isolated aorta of two kidney, one clip rats with hypertension. They found that enhanced endothelium-dependent vasoconstriction to norepinephrine and endothelin in these hypertensive animals (Figure 25). The results therefore suggest that hypertension per se is capable of inducing abnormalities in vascular endothelium.

Other possibilities for the role of the endothelium in the development and maintenance of hypertension exist. In one intriguing study performed by Chapleau et al (86), the relationship between the endothelium and vascular baroreceptors was examined. This relationship is important because of the key regulatory role of the baroreceptor in systemic hemo- dynamic control. Baroreceptor activity was recorded

Figure 25: CONSTRICTIVE RESPONSE OF ARTERIOLES OF HYPERTENSIVE (2K, 1C) AND NORMAL RATS TO ENDOTHELIN AND NORADRENALINE



From Ref. 85

from single fibers innervating the carotid sinus of the dog. Vascular endothelium was removed by balloon denudation, and was then replaced by bovine aortic endothelial cells which had been cultured and placed on microcarrier beads. The endothelial cells were activated with either A23187 (2μM) or bradykinin (10μM). The threshold pressure was 73 without endothelium and 96 with endothelium. A steep increase in pressure (75,125, and 175 mmHg) produced nerve activity of 14, 40, and 54 spikes/sec without endothelium and 2, 30, and 35 with endothelium. The activity was restored after replacement of the cell cultures with naked beads (no endothelium). The results suggest that the endothelial cells release an inhibitory factor that suppresses baroreceptor activity. Abnormal endothelial function secondary to hypertension could therefore lead to enhanced activity and increased efferent sympathetic output. Whether this relationship between the endothelium and baroreceptor plays a role in hypertension is not known.

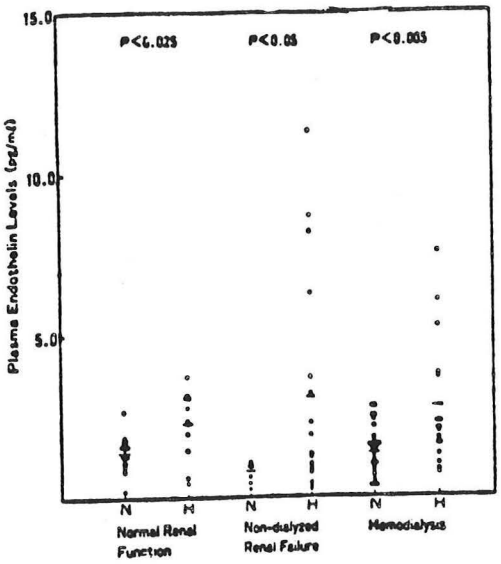
2) HUMAN STUDIES

Relatively few studies have attempted to examine the role of the vascular endothelium in hypertension. By necessity, all of these human studies are indirect because endothelium-void preparations are impossible and many inhibitory drugs are toxic. In one study which examined the potential roles of PGI_2 and thromboxane in the maintenance of hypertension, Minuz et al (87) conclude that a reduction in PGI_2 synthesis was selectively impaired in mild essential hypertension whereas thromboxane synthesis was unaltered. This conclusion was based on urinary excretion rates of 6-keto-$PGF_{1\alpha}$ in hypertensive subjects, and the correlation of the excretion rates to blood pressure.

Other studies have examined endothelin levels in subjects with hypertension. Shichiri and colleagues (88) found higher plasma ET-1 levels in patients with essential hypertension 3.08±3.45 pg/ml) vs normotensive (.73±.34 pg/ml) subjects. In addition, hypertensive subjects on dialysis had higher plasma ET-1 levels than normotensive dialysis subjects (Figure 26). A great deal of overlap in these data exist. Kohno et al (89) have also concluded plasma ET-1 levels are elevated in patients with essential hypertension. They examined ET levels in subjects with hypertension, with borderline hypertension, and normotensive. Considerable overlap was again observed, but patients who were hypertensive had higher ET-1 levels (Figure 27). In addition ET levels correlated directly with blood pressure levels in hypertensive subjects and inversely with GFR and serum creatinine in these patients (Figure 28).

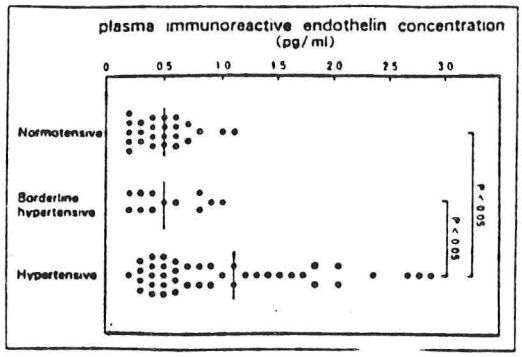
Several studies have examined the possibility of impaired vasodilatory responses in hypertensive humans. In one such study performed on human epicardial vessels studies in vitro (90), from normal atherosclerotic vessels. Coronary vessels showed

Figure 26: ET LEVELS IN NORMALS AND HYPERTENSIVES



From Ref. 88

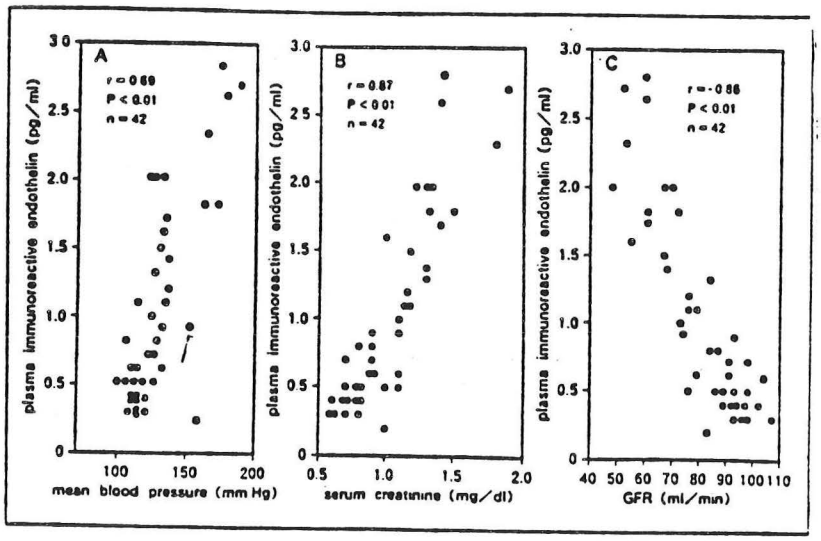
Figure 27: PLASMA ET VALUES IN HTN



From Ref. 89

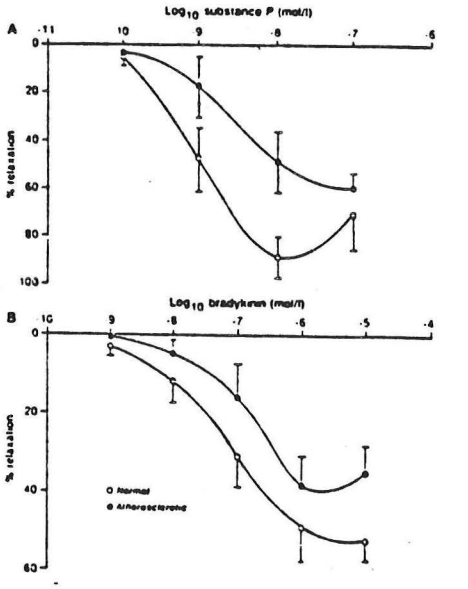
reduced vasorelaxation to substance P and bradykinin (Figure 29). Furthermore, basal secretion of NO was lower in these diseased blood vessels.

Figure 28: RELATIONSHIP OF ET LEVELS AND MBP, S_{CR}, AND GFR



From Ref. 89

Figure 29: RELAXANT EFFECT OF HUMAN CORONARY VESSELS TO SUBSTANCE P AND BRADYKININ

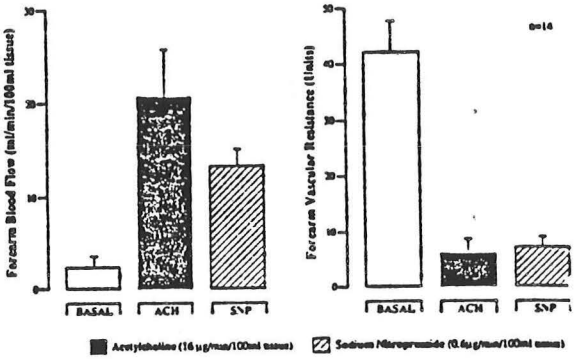


From ref. 90

Linder et al (91) have examined the role of EDRF in peripheral vascular resistance control in hypertensive and normotensive subjects. Acetylcholine infusions into the brachial artery were performed, and forearm blood blow and vascular resistance assessed (Figure 30). Acetylcholine increased blood flow and reduced resistance in

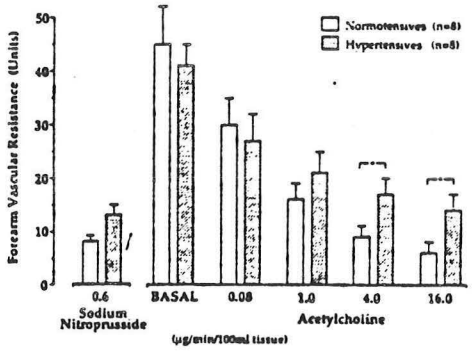
normotensive subjects, and these responses were independent of inhibition of prostacyclin synthesis (with aspirin, 500 mg I.V.) and α-adrenoreceptor blockade (with phentolamine 12 ng/min/100 mg tissue). Hypertensive subjects did not reduce forearm vascular resistance in response to acetylcholine to the levels of normotensive subjects in this study (Figure 31). Responses to nitroprusside were similar in both groups. Thus, the EDRF response elicited by acetylcholine is appeared to be blunted in subjects with hypertension.

Figure 30: INTRAARTERIAL ACH AND SNP AND EFFECTS ON FOREARM BLOOD FLOW AND RESISTANCE IN NORMAL SUBJECTS



From ref. 91

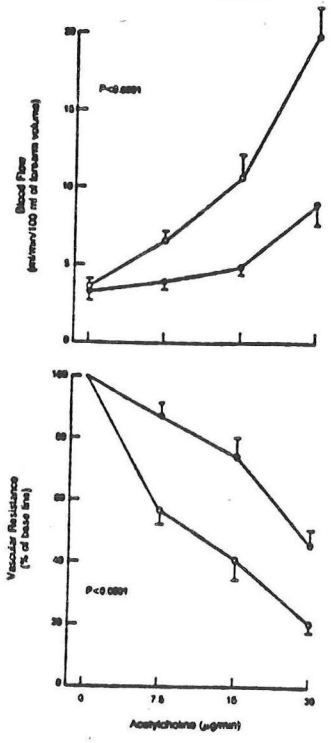
Figure 31: CHANGES IN FOREARM RESISTANCE IN NORMOTENSIVES AND HYPERTENSIVES INFUSED WITH SNP AND ACH



From Ref. 91

A similar study was performed by Panza et al and recently published (92). In age-matched hypertensive (n=18) and normotensive subjects (n=18), forearm blood flow and resistance were measured before and during infusions of nitroprusside and acetylcholine. The results (Figure 32) were clear in demonstrating a blunted vascular relaxation to acetylcholine but not nitroprusside in the hypertensive subjects. These results corroborate the earlier (92) findings and imply that defective EDRF release may underlie part of the increased peripheral resistance in subjects with hypertension.

Figure 32: FOREARM BLOOD FLOW RESPONSES TO I.A ACETYLCHOLINE



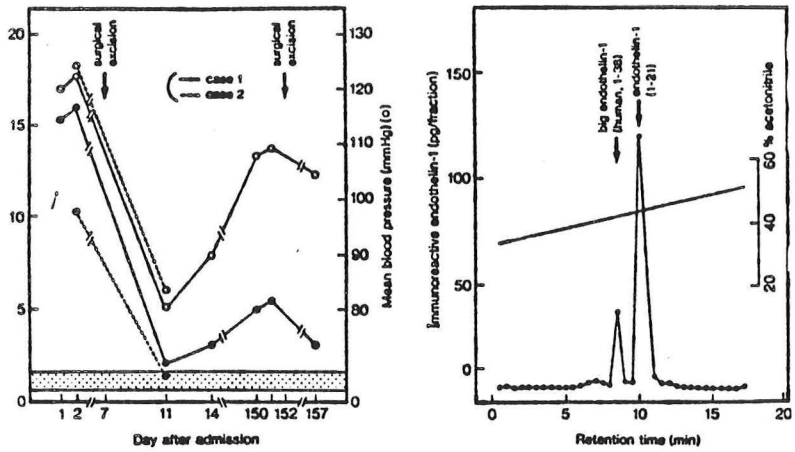
From ref. 92

Finally, a recent paper documented that in some pathologic circumstances, endothelin may be secreted and be a cause of secondary hypertension (93). In the cases reported, endothelin was secreted by a hemangioendothelioma. Excision of the tumor decreased endothelin levels and lowered blood pressure. Recurrence of the tumor in one patient reversed this result (Figure 33). Thus, secreted endothelin may cause a secondary increase in blood pressure.

B. DIABETES AND AGING

Interposed in the anatomic pathway of NO is a layer of subendothelial collagen that separates the intimal

Figure 33: ET-1 LEVELS AND BP IN 2 PATIENTS



From ref. 93

endothelial cells from the smooth muscle cells of the media. Recent studies have shown that products of advanced glycosylation accumulate in this location in aging and diabetes. These products represent the terminal abducts of the non-enzymatic glycation reaction between glucose and the amino groups of protein (94). These glycosylation products accumulate with age because matrix collagen turns over so slowly, and they form at an accelerated rate when plasma glucose is elevated. Since defective endothelium-dependent relaxations are a prominent feature in circumstances in which advanced glycosylation product accumulate, a link between the two possibilities was recently sought by Bucala et al (94). These investigators found that quenching of nitric oxide by advance glycosylation products did indeed occur (Figure 34). These authors further demonstrated an impairment in vasodilation in Lewis rats rendered diabetic with streptozotocin this impairment in endothelium-dependent vasodilation was detectable after one month of diabetes and hyperglycemia (Figure 35).

Figure 34: QUENCHING OF NITRIC OXIDE BY ADVANCED GLYCOSYLATION PRODUCTS

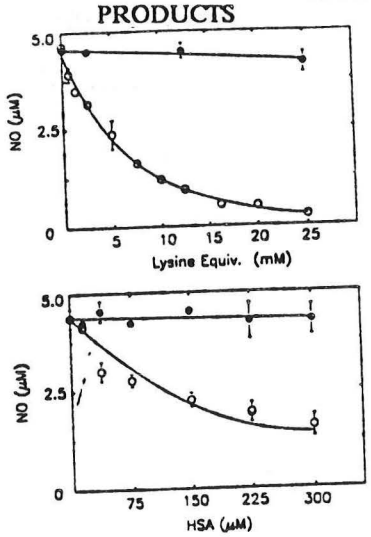


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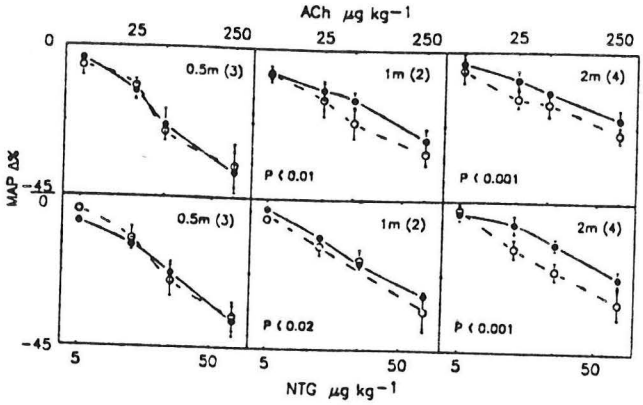
Figure 35: TIME DEPENDENT PROGRESSION OF VASODILATORY IMPAIRMENT IN DIABETIC RATS



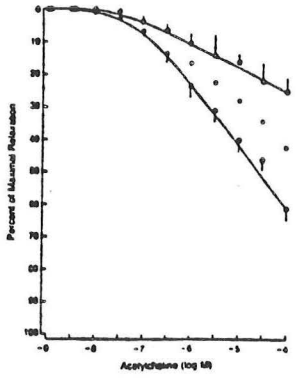
From ref. 94

This time dependent inactivation of nitric oxide by advanced glycosylation end products may represent an important mechanism in the evolution of diabetes and age-related hypertension. This work is provocative and deserving of further study.

Another important mechanism by which defective endothelial cell function may cause abnormal human physiology has to do with impotence in diabetics. In one study of smooth muscle contraction from corpus cavernosum of humans with and without diabetes (95), several key points were made. First, endothelial-dependent Figure 36 and electrical stimulation (Figure 37) induced vasorelaxation were markedly impaired in diabetics. Second, endothelium-independent relaxation of smooth muscle was similar in diabetic and non-diabetic men after administration of nitroprusside. Thus, diabetic men with impotence likely are afflicted with an impairment of endothelium-dependent

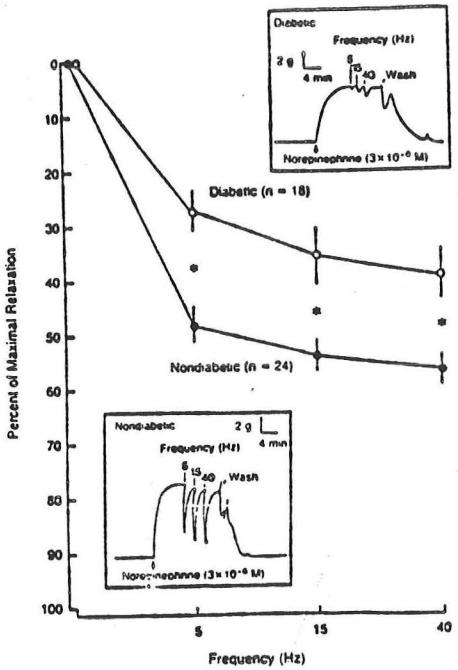
vasorelaxation as at least a partial cause for their impotence.

Figure 36: RELAXATION OF CORPUS CAVERNOSA SMOOTH MUSCLE FROM DIABETIC MEN ( ) AND NON-DIABETIC (•) MEN



From ref. 95

Figure 37: RELAXATION OF SMOOTH MUSCLE FROM CORPUS CAVERNOSA OF DIABETIC AND NON-DIABETIC MEN



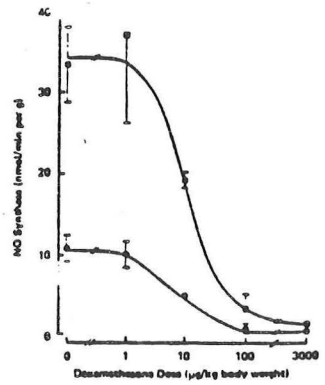
From ref. 95

C. SEPTICEMIA AND SHOCK STATES

Several recent studies have probed the potential role of endothelium-dependent events in the syndrome of septicemia. Endotoxin induces the liberation of nitric oxide from blood vessels both in vivo and in vitro (96). The resultant hypotension may be a component of the shock syndrome. Interestingly, dexamethasone has been found to block the ability of endotoxin to liberate nitric oxide from vascular tissue (96,97). This blocking effect of dexamethasone on nitric oxide synthase may be a key reason for any therapeutic effects of the drug. An example of the ability of dexamethasone to block nitric oxide synthase is provided in Figure 38.

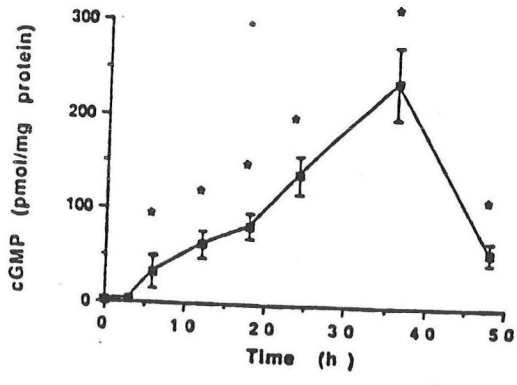
As mentioned earlier, the cytokine IL-1, a major inflammatory mediator in septic states, is capable of inducing nitric oxide synthase, type I. Thus, IL-1 may be mediator of the ability of endotoxin to produce endothelium-dependent vasodilation in vivo. IL-1 induces cGMP accumulation in cultured rat vascular smooth muscle tissues; as shown in Figure 39, this accumulation increased over a 40 hour period(18). Whether dexamethasone affects the IL-1 portion of the endothelial-dependent vasorelaxation is not known at present.

Figure 38: DOSE-DEPENDENT INHIBITION OF DEXAMETHASONE OF NO SYNTHASE IN LUNG AND LIVER



From ref. 97

Figure 39: TIME COURSE OF IL-1-INDUCED cGMP ACCUMULATION IN CULTURED VSMC



From ref. 18

D. PRE-ECLAMPSIA AND ECLAMPSIA

Pre-eclampsia is characterized by endothelial cell dysfunction, and prominent proliferation of endothelial cells in locations such as the kidney. Increased sensitivity to pressor agents and activation of the coagulation cascade are also prominent features of the disease. The primary initiator of the damage to endothelial cells in this disorder is unknown (98), but several theories exist (99). It is possible that placental tissue releases a factor that injures endothelial cells. Detailed studies of the role of the endothelial cell in the pre-eclampsia are clearly needed.

SUMMARY

The volume of information regarding the role of the endothelium as a controller of vascular tone in health and disease is expanding dramatically (100, 101). The vasoactive factors and mitogens produced by the endothelium are partly responsible for this growth, as is the recent widespread acceptance of the concept of autocoids, substances produced to exert a local effect. The vascular effects of the endothelium represent only a portion of the story, however. The ability of the endothelial factors to control other hormone events, to affect the growth of nearby cells, and to inhibit or promote coagulation are all exciting and important areas for future investigation. Hopefully, these investigations will lead to more specific and effective therapies for diseases involving the endothelium.

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