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PHARMACOLOGY OF THE ENDOTHELIUM IN ISCHEMIA-REPERFUSION AND CIRCULATORY SHOCK

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BASIC PHYSIOLOGY OF HUMORAL AGENTS OF ENDOTHELIAL ORIGIN

During the past 16 years, great strides have been made in elucidating the regulatory role of the endothelium in vascular dynamics. The endothelium was previously thought of only as a permeability barrier to ions and organic molecules, and as a regional site of metabolic effects, particularly in the pulmonary microvasculature (i.e. converting angiotensin I to angiotensin II and removing prostaglandins from the circulation) (1). Although these effects are of considerable significance, they did not foretell the far-reaching role of the entire vascular endothelium as an important organ in regulating vascular tone.

Cytoprotective Agents Produced by the Endothelium

In 1976, Vane, Moncada, and colleagues (2) discovered PGI_2 (prostacyclin), a unique eicosanoid produced by the endothelium. PGI_2 exerts a remarkable constellation of actions, including vasodilation of most vasculatures, inhi-

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bition of platelet aggregation, prevention of polymorphonuclear (PMN) leukocyte adherence, and stabilization of membranes (3–5). Prostacyclin has a short half-life (i.e. about 1–2 min), and exerts its effects via activation of adenylate cyclase and the resulting increased production of cyclic AMP (6). There are no specific inhibitors of either PGI_2 synthesis, or PGI_2 action, since cyclooxygenase inhibitors block a variety of prostaglandins, endoperoxides, and thromboxanes (7), and putative PGI_2 receptor antagonists also block PGE_2. Nevertheless, valuable data have been accumulated showing a key role of PGI_2 in ischemia/reperfusion (8, 9) and circulatory shock states (10, 11).

In 1980, Furchgott & Zawadski (12) discovered a substance generated from the endothelium that mediated the vasorelaxing effect of acetylcholine. This substance was termed endothelium-derived relaxing factor (EDRF). EDRF, subsequently found to be nitric oxide (13, 14), is produced in endothelial cells by a constitutively present enzyme, NO synthase (15), which requires calcium and calmodulin for full expression of its activity. NO exerts a biological profile virtually identical to that of PGI_2, including vasodilation, inhibition of platelet aggregation, and attenuation of neutrophil adherence (16). These effects are mediated via activation of guanylate cyclase and the resulting formation of cyclic GMP (17). In addition, NO appears to directly quench superoxide radicals. NO has a biological half-life of only 10–20 seconds (13, 14, 18), and thus is even more labile than PGI_2. NO and PGI_2 can exert synergistic effects when given together in low doses (19). Recently, NO has been found to be useful in ischemia-reperfusion states (20, 21).

A third important protective agent formed by the endothelium is the purine nucleoside, adenosine. Adenosine is an important regulator of cell metabolism (22). Adenosine is produced normally from extracellular adenylate and from S-adenosyl homocysteine (23), and is rapidly metabolized by the enzymes adenosine deaminase and adenosine kinase (23). Adenosine, like PGI_2 and NO, is a vasodilator in most vascular beds. Adenosine also attenuates PMN adherence to endothelial cells (24) and inhibits platelet aggregation. Adenosine exerts its effects via activation of P_1 purinergic receptors coupled to adenylate cyclase by a GTP-dependent mechanism. There are two well-recognized adenosine receptors based on agonist affinity studies: the A_1 and A_2 receptors (23, 24). The A_2 receptor is thought to mediate the vasodilator effect of adenosine, whereas the A_1 receptor mediates the bradycardia produced by adenosine. There are specific blocking agents for both the A_1 and A_2 adenosine receptors. Adenosine also inhibits superoxide radical production, particularly in PMNs (25), and appears to increase endothelial cell production of PGI_2. Similarly to the other endothelially released vasodilators (e.g. PGI_2, NO), adenosine exerts beneficial effects in ischemia/reperfusion and shock states (23).

Substances Produced by the Endothelium that Mediate Cell Injury

The endothelium also produces substances that either increase vascular tone or release vasoconstrictors and are pro-thrombogenic. These pro-inflammatory substances tend to promote ischemia and its consequences (i.e. provoke cell injury).

One of the potentially important vasoconstrictor substances produced by the endothelium is endothelin (26). Endothelin is actually a family of potent biologically active peptides containing three members: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3) (27), of which the most important is ET-1, the form released by endothelial cells. ET-1 is synthesized as a precursor polypeptide comprising 203 amino acids. This precursor is subsequently cleaved to a 38 or 39 amino acid intermediate called "big endothelin". This intermediate is then processed to its active form, a 21 amino acid peptide, ET-1, by an "endothelin converting enzyme", somewhat analogous to the converting enzyme of the renin-angiotensin system (27).

ET-1 is a powerful, albeit slowly acting, coronary vasoconstrictor, as well as a positive cardiac inotrope, constrictor of pulmonary and intestinal smooth muscle, mitogen, releaser of aldosterone from the adrenal cortex, and stimulator of sympathetic tone in the central nervous system. ET-1 is thought to constrict vascular tissue either by directly or indirectly moderating dihydropyridine-sensitive calcium channels (28). Our knowledge of the role of ET-1 in pathophysiological states is only rudimentary, since few studies have demonstrated any significant increases in circulating ET-1 concentrations in blood in any major circulatory disease state. Moreover, ET-1 may actually release EDRF and thus moderate the endothelin-induced constriction. Nevertheless, one cannot eliminate ET-1 from consideration as a mediator since we do not know if it accumulates locally in the extracellular fluid, or is taken up by tissues where it acts.

Platelet activating factor (PAF), also known as acetyl-glycerol-ether-phosphorylcholine (AGEPC) or 1-0-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine, is a virtually ubiquitous phospholipid (29). PAF is produced by endothelial cells, and upon release can induce vasodilation. The mechanism of this vasodilation is not well understood, and may involve release of vasodilator eicosanoids (e.g. PGI_2) or other vasodilators (30). In rat hearts, PAF releases LTC_4 and LTD_4 (31, 32) and in rabbit hearts, PAF releases TxA_2 (33), all of which are vasoconstrictors. Thus, the net effect of PAF is species dependent and is perhaps also dependent on an intact blood perfused circulation as well. However, PAF is a potent inducer of platelet aggregation (34) and also activates PMNs (35). Moreover, PAF also increases vascular permeability (36), thus enhancing other pro-inflammatory actions. A variety

of PAF receptor antagonists are now available with which to study the biological effects of PAF (37, 38).

Superoxide radicals ($^{-}O\bullet_2$) are also produced by the vascular endothelium under a variety of circumstances, particularly following ischemia-reperfusion (39, 40) and hypoxia-reoxygenation (40). Superoxide radicals exert a profound effect on NO, causing a rapid inactivation of this EDRF. In this manner, $^{-}O\bullet_2$ appears to exert a vasoconstrictor effect. However, it is in reality acting to overcome vasodilator tone produced by NO (41). In the absence of an intact endothelium, or in the presence of an NO synthase inhibitor (e.g. L-NMMA or L-NAME) $^{-}O\bullet_2$ fails to alter vascular tone (41). Since a variety of substances induce $^{-}O\bullet_2$ formation (e.g. LY-85853, methylene blue), one must be careful in interpreting some pharmacologic data. Superoxide radicals can be scavenged effectively by superoxide dismutase (SOD) as well as by other antioxidants (e.g. ascorbic acid) (42).

Endothelial Ligands for Adhesive Molecules

In addition to vasoactive mediators produced by endothelial cells that have a direct effect on vascular smooth muscle tone, endothelial cells interact with a complex system of adhesive molecules. The adhesive molecules are either proteins (e.g. integrins) (43) or carbohydrate-containing macromolecules (i.e. lectin adhesion molecules, or LECCAMS), now called selectins (44). In each case, leukocytes contain a component of the adhesive molecule system (e.g. CD11/CD18, LAM-1 or L-selectin). The adhesive molecules on leukocytes (e.g. PMNs, lymphocytes, monocytes) bind to corresponding ligands on endothelial cells (e.g. ICAM-1, ELAM-1 or E-selectin, GMP-140 or P-selectin) (45–47). This process of adherence is a crucial step in the activation of leukocytes. Once activated, leukocytes release a variety of humoral mediators that can damage any cell with which they are in contact (e.g. endothelial cell, cardiac myocyte, intestinal parenchymal cell, etc). Among the various mediators released from leukocytes, particularly PMNs, are oxygen-derived free radicals (e.g. superoxide radicals, H_2O_2), cytokines (e.g. TNFα, IL-1β), and proteases (e.g. elastase) (48).

In summary, endothelial cells are capable of releasing cytoprotective agents or endothelial modulators (e.g. PGI_2, NO, adenosine), as well as pro-inflammatory mediators that contribute to cellular injury, including that of endothelial cells (e.g. endothelin, PAF, oxygen-derived free radicals). Endothelial cells can also express a series of ligands for adhesive molecules (e.g. ICAM-1, ELAM-1, GMP-140) on their cell surface. How these molecules interact is of vital importance to understanding ischemia-reperfusion injury. Figure 1 represents a schematic diagram interrelating these modulators and mediators.

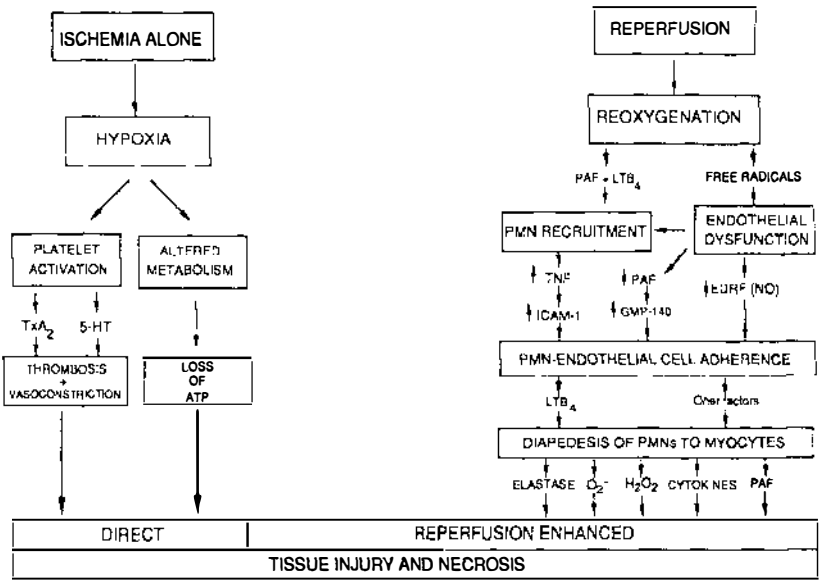

Figure 1 Schema of pathophysiologic mechanisms of ischemia/reperfusion injury including ischemia alone and reperfusion pathways. TxA_2 = thromboxane A_2; 5-HT = 5-hydroxytryptamine; ATP = adenosine triphosphate; PAF = platelet activating factor; LTB_4 = leukotriene B_4; TNF = tumor necrosis factor (X; ICAM-1 = intercellular adhesion molecule-1; GMP-140 = granular membrane protein 140; NO = nitric oxide; $^{-}O_2$ = superoxide radicals.

Role of the Endothelium in Ischemia-Reperfusion

Ischemia leads to hypoxia, which initiates a series of events primarily related to activation of platelets and release of their vasoconstrictor mediators (e.g. thromboxane A_2, TxA_2, and 5-hydroxytryptamine, 5-HT) that further restrict blood flow to the ischemic area (49). If the ischemia is severe enough, the rate of metabolism is diminished and the generation of high energy compounds subsequently declines (e.g. ATP). The reduced energy metabolism eventually leads to a slow but significant degree of tissue injury and necrosis. This degree of tissue injury is further enhanced and accelerated by reperfusion (See Figure 1, right portion).

Reperfusion leads to reoxygenation and the formation and activation of a variety of humoral mediators of injury and inflammation, including oxygen-derived free radicals (e.g. superoxide radicals, hydroxyl radicals, hydrogen peroxide), lipid mediators (e.g. platelet activating factor, PAF, and leukotriene B_4, LTB_4), as well as polypeptide mediators (e.g. C5a). Super-

oxide radicals and PAF originate to a large extent from endothelial cells. Endothelial cell dysfunction is thought to be the "trigger" of reperfusion injury (50). This leads to a marked reduction in nitric oxide release (51). Decreased NO along with chemotactic factors (e.g. PAF, LTB_4, C5a) promote PMN recruitment to the reperfusion site and adherence to the dysfunctional endothelium (52). This adherence is also facilitated by cytokine upregulation of ICAM-1 and ELAM-1 receptors and PAF-induced upregulation of GMP-140 receptors on the endothelial cell surface (53). These adhesive molecules accentuate PMN adherence to the endothelium and promote PMN diapedesis through the endothelium. Since cardiac myocytes express ICAM-1 on their surface (54), PMNs that have diapedesed can adhere to cardiac cells where they release a host of pro-inflammatory mediators, which promote cell injury (e.g. elastase, superoxide cytokines, and PAF). These factors enhance reperfusion injury due to the short diffusion distance to the myocyte from the adhered neutrophil. Thus, there is "neutrophil amplification" of the "endothelial trigger" (50).

The critical event in the early phase of reperfusion injury appears to be endothelial dysfunction (e.g. reduced NO and PGI_2). This dysfunction is compounded by endothelial activation of adhesive molecule ligands (e.g. GMP-140, ICAM-1) occurring sequentially over a time period of several minutes to several hours. Therefore, the remainder of this review is devoted to the pharmacology of the endothelium, with particular reference to agents that protect the ability of endothelial cells to synthesize and release EDRF during ischemia and shock. EDRF has been selected as the "marker molecule" of the endothelium to analyze for the following reasons: it is normally released due to shear stress on the blood vessel wall; it exerts such an important profile of biological effects; and the tools are currently available with which to study this substance.

PHARMACOLOGY OF THE ENDOTHELIUM: AGENTS THAT PRESERVE ENDOTHELIAL FUNCTION DURING ISCHEMIA-REPERFUSION

A variety of pharmacologic agents have been studied in an attempt to preserve endothelial cells from the severe dysfunction that occurs after ischemia and reperfusion. These agents are of diverse chemical structure and have varied mechanisms of action. We can arbitrarily categorize these beneficial agents as substances that either *(a)* replace cytoprotective agents of endothelial origin, or *(b)* inhibit pro-inflammatory agents of endothelial origin, or *(c)* as agents that inhibit neutrophils or their mediators.

Substances that Replace Cytoprotective Agents of Endothelial Origin

The major endothelial generated agents that are cytoprotective and thus preserve endothelial function are prostacyclin, nitric oxide, and adenosine. All three cytoprotective agents are rapidly metabolized and have short half-lives in vivo (i.e. 10 sec to 3 min) (3, 13, 23). Thus, one must either continuously infuse these substances or employ stable analogs of these substances.

Taprostene, a stable analog of prostacyclin, was one of the first substances employed in ischemia-reperfusion in an attempt to preserve endothelial function. Taprostene was selected because it is significantly less potent than PGI_2 as a vasodilator, yet it is more potent than PGI_2 in its cytoprotective and antiplatelet effects (55). One rationale for use of PGI_2 or its analogs is that PGI_2 production by the endothelium is reduced following anoxia and reoxygenation (56). Taprostene was employed at 100 ng/kg/min, an infusion rate just at the threshold of its vasodilator activity (57), and was administered starting 60 min after the onset of myocardial ischemia (i.e. 30 min pre-reperfusion). Not only did taprostene preserve myocardial tissue against necrosis (i.e. reduce infarct size from 20% of area-at-risk (AAR) to 5% of AAR (57), but it also dramatically attenuated the endothelial dysfunction observed following reperfusion. Figure 2 illustrates a representative example of the responses of coronary artery rings isolated from cats subjected to ischemia-reperfusion and given either taprostene or its vehicle (i.e. 0.9% NaCl). Taprostene given just prior to reperfusion clearly preserved the vasorelaxant response to acetylcholine in coronary rings isolated from cats subjected to LAD occlusion for 90 min and reperfusion for 270 min. Thus, replacement of PGI_2 usually produced by the normal endothelium can preserve endothelial function following ischemia-reperfusion. Not only does a stable prostacyclin analog preserve endothelial function in ischemia-reperfusion, but a novel PGI_2-enhancing agent is also effective in this regard. This PGI_2-enhancing agent, defibrotide, a natural polydeoxyribonucleotide isolated from mammalian lung (58), also maintains endothelium-dependent responses in the coronary (59) and mesenteric vasculature (60) following ischemia-reperfusion in the rat.

Exogenous administration of nitric oxide or organic NO donors that release NO provides another means of preserving the endothelium by replacing a naturally occurring endothelially produced humoral agent. In this regard, several of a series of NO-generating compounds called sydnonimines (61) have been studied in ischemia-reperfusion. These compounds (i.e. SIN-1 and C87-3754) markedly preserve the ability of the coronary endothelium to relax to acetylcholine (62). Similar responses occurred in superior mesenteric artery

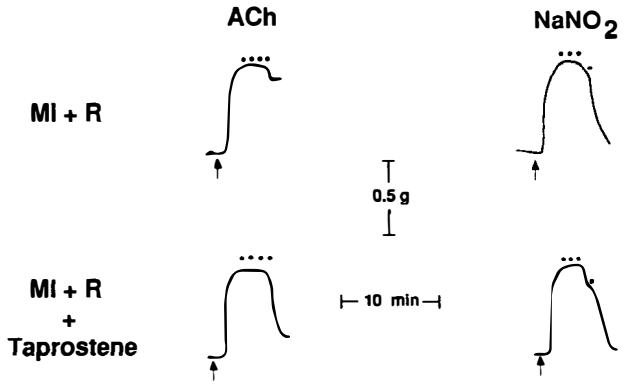

Figure 2 Representative recordings of vasorelaxant responses of isolated cat coronary artery rings from ischemic-reperfused cats to acetylcholine (ACh) and acidified sodium nitrite ($NaNO_2$).

rings after SMA occlusion and reperfusion (63). Figure 3 illustrates representative recordings of these responses in isolated cat superior mesenteric artery rings. C87-3754, infused intravenously just prior to reperfusion, markedly preserved the endothelium-dependent vasorelaxation to ACh without altering the normal response to the endothelium-independent dilator, acidified $NaNO_2$. Not only was the endothelium protected, but these sydnonimines significantly retarded circulatory shock in these cats. These studies have recently been confirmed employing a new class of cysteine-containing NO donors (e.g. SPM-5185) (64). SPM-5185 also maintained the coronary endothelium and protected the myocardium from necrosis

Thus, replacement of either PGI_2 or NO during the reperfusion phase of ischemia-reperfusion preserves the endothelium of the ischemic vasculature, both in large conduit vessels (57, 60, 62), and in the microvasculature (59), as well as protects the underlying parenchymal tissue from reperfusion injury.

Inhibition of Endothelial Generated Pro-Inflammatory Mediators

A second mode of protecting the ischemic-reperfused endothelium is to inhibit pro-inflammatory mediators of endothelial origin. Two such endothelial mediators are platelet activating factor (PAF) and superoxide radicals. Although these mediators can be generated by a variety of cell types, reoxygenated endothelial cells produce significant quantities of superoxide radicals and PAF immediately following reperfusion of an ischemic vascular bed (40, 66). Their effects are later augmented by superoxide radical formation generated by activated neutrophils adhering to the endothelium (41, 67).

Over the past five years, a variety of PAF receptor antagonists have become

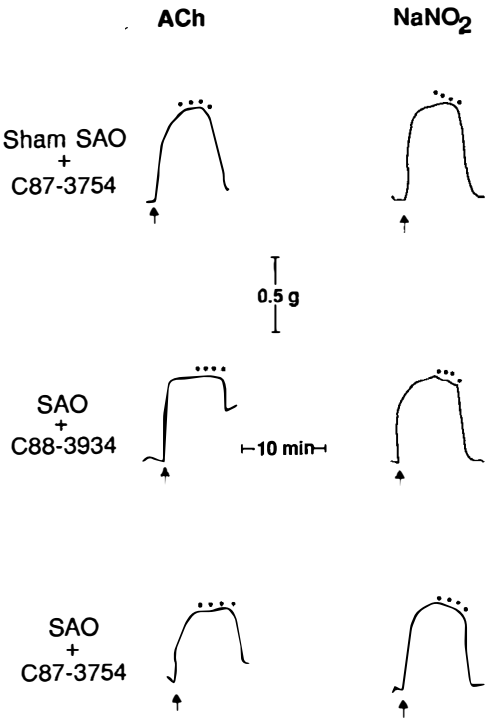

Figure 3 Representative recordings of vasorelaxant responses of isolated cat superior mesenteric artery rings to acetylcholine (ACh) and acidified sodium nitrite ($NaNO_2$)

available (30, 37, 38) that have enabled precise studies of the role of PAF in pathophysiologic processes. There are now available second and third generation PAF receptor antagonists that are very potent and highly specific agents. PAF exerts a variety of pathophysiologic actions including: aggregation of platelets, induction of hypotension, cardiac depression, enhancement of microvascular permeability (36), and, more recently, promotion of leukocyte adherence to the vascular endothelium (52).

PAF antagonists have been shown to attenuate PAF-induced microvascular leakage in the coronary circulation (31, 68), renal circulation (69), and the systemic circulation (70). PAF receptor antagonists (e.g. CV-6209 and WEB-2086) have been found to protect against fluid leakage. Recently, WEB-2170 was reported to preserve EDRF release in the ischemic-reperfused coronary vasculature (71). WEB-2170, infused at 2 mg/kg/h starting 10 minutes before reperfusion, preserved vasorelaxation responses in isolated LAD coronary arteries isolated 270 min post-reperfusion. The degree of

protection was comparable to that observed with taprostene. These findings point to the important role of PAF as a mediator of endothelial dysfunction and reperfusion injury. In this regard, Lorant et al (52) have shown that PAF and GMP-140 are co-expressed on endothelial cells when stimulated with histamine or thrombin. GMP-140 is a very rapidly activated adhesive protein released from Weibel-Palade bodies and expressed on endothelial cell surface in about 5 min (72). Thus, PAF may play a significant role as a signaling molecule in the regulation of neutrophil trafficking and adherence to the endothelium.

In addition, endothelial cells generate superoxide radicals (40, 66) that appear to feed back upon the endothelium to impair endothelium-mediated vasorelaxation either by quenching NO (73–75), or by impairing the ability of the endothelium to generate NO. In either case, administration of recombinant human superoxide dismutase (hSOD) just prior to reperfusion (76), or low doses of hSOD in combination with taprostene (77), or low doses of hSOD along with acidified $NaNO_2$, as a source of NO (78), dramatically protected against coronary vascular endothelial dysfunction. Thus, hSOD alone at moderate doses, or hSOD at low doses along with low doses of other endothelial protective agents, markedly preserves endothelial function.

Agents Inhibiting Neutrophils or Neutrophil-Derived Mediators

Neutrophils express substances on their surface or produce a variety of inflammatory mediators in addition to oxygen-derived free radicals, which have already been discussed as an endothelial-generated mediator (79). Thus, neutrophils produce cytokines (e.g. TNFα, IL-1β), proteases (e.g. elastase), and leukotrienes (e.g. LTB_4), all of which participate in the ischemia-reperfusion process and contribute to reperfusion injury. In addition, neutrophils also express adhesive glycoproteins on their surface (e.g. CD11/CD18 complex), which interact with ligands on the endothelial cell surface (e.g. ICAM-1, GMP-140) during ischemia-reperfusion. Although these substances of neutrophil origin are now well-established entities (80), therapeutic agents to inhibit or antagonize these mediators and adhesive proteins are only recently becoming available for study. Several of these therapeutic agents are monoclonal antibodies (MAbs) directed against specific adhesion molecules, while others are chemical antagonists against lipid or protein mediators.

One of the important chemotactic factors produced by neutrophils is leukotriene B_4 (LTB_4). LTB_4 is a potent chemotactic agent that activates neutrophils and promotes adherence of PMNs to the endothelium (81, 82). Recently, specific LTB_4 receptor antagonists have become available (83, 84). There is some controversy whether LTB_4 antagonists protect the ischemic-reperfused heart (85), but they clearly preserve the ischemic-reperfused splanchnic viscera against neutrophil-induced endothelial dysfunction (86, 87).

In this regard, LY-255283, a LTB_4 receptor antagonist, protected against post-reperfusion circulatory collapse, and preserved mesenteric artery endothelial function (e.g. vasorelaxation to endothelium-dependent vasodilators) in isolated rat mesenteric artery rings (87). These effects were absent in a stereoisomer of LY-252283, lacking LTB_4 receptor antagonistic properties (87). Another interesting aspect that inhibits PMN infiltration following ischemia and reperfusion is perfluorochemical (Fluosol^R), which has been found to be very effective in reducing infarct damage following myocardial ischemia and reperfusion (88). TNFα is one of the most important pro-inflammatory cytokines produced by leukocytes. It is well known that TNFα is an important mediator of endotoxic shock and itself can produce cachexia and shock (89). It is not therefore surprising that TNFα also induces endothelial dysfunction (90, 91) and inhibits EDRF release. This endothelial dysfunction occurs by a mechanism involving protein synthesis, perhaps via receptor activation (90). TGF-β has recently been shown to be an important naturally occurring protein that exerts effects opposite to that of TNFα in a variety of biological systems (92). TGF-β has been shown to protect the ischemic-reperfused heart and to preserve the ability of the coronary endothelium to release EDRF in response to endothelium-dependent vasodilators (93). Figure 4 illustrates representative recordings of the protective effects of TGF-β in the isolated perfused rat heart, showing preservation of the vasorelaxant response to acetylcholine (ACh). This protective effect was dose-dependent and occurred both pre- and post-reperfusion (93). Recently, TGF-β was also shown to preserve endothelial PGI_2 release from the ischemic reperfused cerebral circulation (94).

Another approach to preserving endothelial integrity in the face of ischemia-reperfusion injury is the use of specific monoclonal antibodies to adhesive proteins. MAbR15.7, a monoclonal antibody directed against the β-chain of the adhesive glycoprotein complex present on neutrophils (95, 96), and MAbRR1/1, a monoclonal antibody directed against the major endothelial ligand to CD11/CD18, ICAM-1 (97) have been successfully used. Both antibodies protected against myocardial necrosis, reduced PMN migration into the ischemic area, and also preserved endothelial function as evidenced by agonist-induced EDRF release (e.g. to ACh and A23187). The protection against PMN adherence and activation appeared to preserve endothelial function as well.

Thus, a variety of mechanisms are available to protect against the endothelial dysfunction occurring following ischemia-reperfusion or in shock states, a severe form of ischemia-reperfusion. These endothelial protective agents range from simple molecules such as nitric oxide (NO) up to and including large immunoglobulins (e.g. antibodies to adhesive proteins). Virtually all these agents are effective when given after the onset of ischemia and just before reperfusion, and thus it is clear that the endothelial dysfunction is a

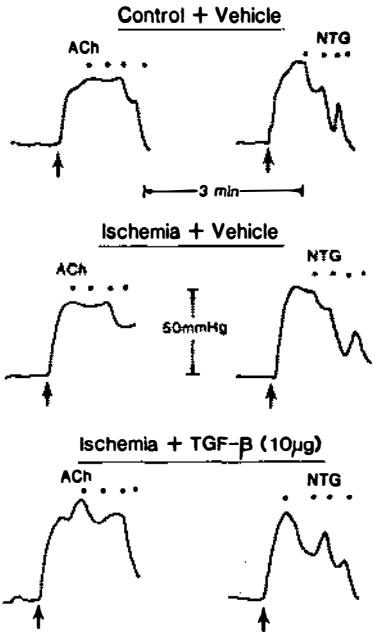

Figure 4 Representative recordings of vasorelaxant responses of isolated perfused rat hearts to acetylcholine (ACh) and nitroglycerin (NTG).

reperfusion-induced phenomenon (51). Moreover, early occurring endothelial dysfunction leads to tissue injury at a later stage. Thus, endothelial dysfunction acts as a trigger for subsequent tissue injury, which is amplified by a process involving neutrophils (50, 51).

HYPOPERFUSION STATES LEADING TO ENDOTHELIAL DYSFUNCTION

Experimental ischemia-reperfusion can be produced in animal models by different means: for example, in vitro in isolated perfused hearts (40) and the isolated perfused mesentery (98) of the rat. Secondly, ischemia-reperfusion can be studied in vivo in situations in which a regional ischemia and reperfusion occur. Situations in which regional ischemia-reperfusion have resulted in endothelial dysfunction include myocardial ischemia-reperfusion (76, 99–101), splanchnic ischemia-reperfusion (60, 87, 102), renal ischemia-reperfusion (103), and pulmonary ischemia-reperfusion (104). Finally, endothelial dysfunction can be studied in whole body ischemia-reperfusion (i.e. circulatory shock states). These whole body ischemia-reperfusion states in

which endothelial dysfunction has been observed include endotoxic shock (105, 106), traumatic shock (107), and hemorrhagic shock (108). The overall consensus is that in all these situations, ischemia-reperfusion, in the absence or presence of circulatory shock, results in a significant degree of endothelial dysfunction. Most investigators have studied the responses of large arteries since they are technically easier to study than microvessels or thin-walled veins. However, endothelial dysfunction following ischemia-reperfusion also occurs in the microcirculation (50, 109), as well as in large veins (110). Endothelial dysfunction was correlated to enhanced PMN adherence to venous endothelium (110, 111). In one case, blockade of NO synthesis with an analog of L-arginine enhanced adherence of neutrophils to the endothelium of venules (111), and in the other case, an NO donor inhibited adherence of neutrophils to cardiac veins (110).

The effects of three circulatory shock states on endothelial dysfunction in rat superior mesenteric artery (SMA) rings are summarized in Figure 5 and compared to control (i.e. sham shock SMA rings). These studies were all carried out in pentobarbital-anesthetized rats subjected to either endotoxin lipopolysaccharide (LPS) given intravenously at 30 mg/kg, splanchnic artery occlusion (SAO) and reperfusion, or Noble-Collip drum trauma (trauma). Rat SMA rings were studied 90–120 min following reperfusion (i.e. SAO + R) or induction of trauma or administration of LPS. All three conditions resulted in a comparable degree of impairment of the vasorelaxant responses to ACh, (and not shown, also to A23187), but all exhibited normal responses to the endothelium-independent dilator, acidified $NaNO_2$. SMA rings were selected for study, since all these forms of shock result in severe splanchnic hypoperfusion (112), followed by reperfusion. Thus, not only regional ischemia-reperfusion, but whole body ischemia-reperfusion results in a marked degree of endothelial dysfunction. Clearly, circulatory shock triggers a very severe form of endothelial dysfunction. Preliminary results suggest that superoxide radicals play a major role in this phenomenon since hSOD administration can significantly attenuate the endothelial dysfunction (106, 107). Although endothelial dysfunction occurs within the first two hours following a shock-inducing injection of lipopolysaccharide, others have reported that lipopolysaccharide injection can result in a delayed overproduction of NO by macrophages and vascular smooth muscle cells due to activation of an inducible NO synthase in these cells (113, 114). However, this inducible NO synthase is not significantly activated until 8–12 hr following LPS administration (115), and thus is unlikely to account for the early hypotension following LPS administration, nor would it be likely to oppose the early endothelial dysfunction occurring in endotoxin shock (105, 106). This has been elegantly shown by Myers et al (116) who measured reductions in NO formation in endothelial cells subjected to endotoxin treatment.

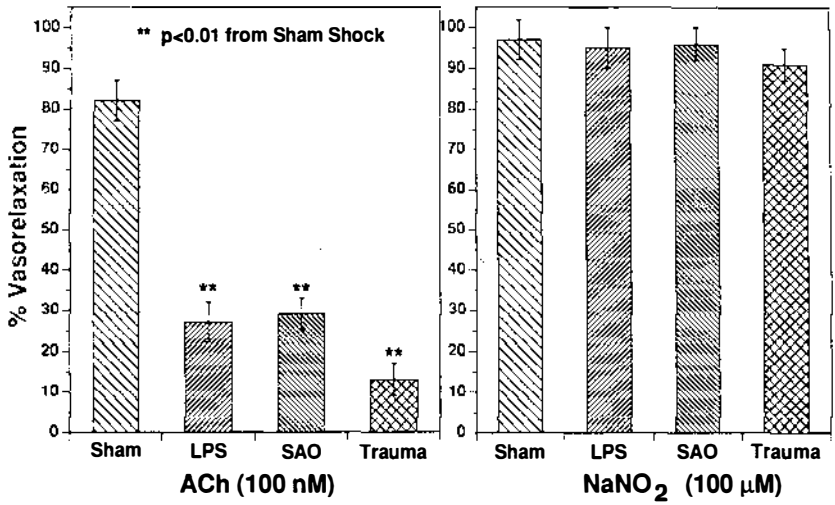

Figure 5 Bar graph of vasorelaxant responses of isolated rat superior mesenteric artery (SMA) rings to acetylcholine (ACh) and acidified sodium nitrite ($NaNO_2$). SMA rings were isolated from sham shock rats, and from rats subjected to endotoxic shock (LPS), splanchnic artery occlusion shock (SAO), and traumatic shock (trauma). All values are means ± SEM for 8 to 10 rings in each group.

SUMMARY AND CONCLUSIONS

Endothelial dysfunction is an important early-recurring phenomenon in virtually all forms of ischemia-reperfusion, including a variety of circulatory shock states. The dysfunction appears to be triggered within 2.5 min of the endothelial generation of a large burst of superoxide radicals (40, 76, 117). However, the initial dysfunction may be amplified by neutrophil-generated factors including oxygen-derived free radicals, cytokines, proteases, and lipid mediators. Moreover, adhesive molecules on the surface of the PMN, along with their ligands on the endothelial cell membrane, appear to promote endothelial dysfunction in ways that may go beyond the adherence of neutrophils on the endothelial surface. These interactions remain to be elucidated but may involve intricate cell signaling pathways.

A variety of pharmacologic agents exert endothelial protective effects in ischemia-reperfusion and circulatory shock states. Table 1 summarizes these agents and indicates the major mechanism of preservation of the endothelium. These substances can be classified into three broad categories: (*a*) substances replacing endogenous cytoprotective agents of endothelial origin including prostacyclin (PGI_2), endothelium-derived relaxing factor (EDRF), and adenosine: the endothelium protecting agents include these substances as well as

Table 1 Pharmacologic agents that preserve endothelial function following ischemia/reperfusion

Substance	Mechanisms of endothelial preservation
PGI_2 or stable analogs	Potentiates action of SOD, increases cAMP
Defibrotide	Increases PGI_2 production
Adenosine	Inhibits PMNs, increases cAMP
Nitric oxide (NO) or NO donors	Overcomes superoxide radicals, increases cGMP, inhibits PMN activation
PAF receptor antagonists	Prevents increased EC permeability and GMP-140 activation
Superoxide dismutase	Prevents degradation of EDRF (NO)
LTB_4 receptor antagonists	Blocks LTB_4-induced PMN activation
5-lipoxygenase inhibitors	Inhibits leukotriene production
Thromboxane synthetase inhibitors	Increases PGI_2 production
Transforming growth factor-β	Inhibits TNF and superoxide production
Anti-CD18 antibodies	Prevents PMN adherence by blocking PMN adhesive glycoproteins
Anti-ICAM-1 antibodies	Prevents PMN adherence by blocking EC ligands for adhesive glycoproteins
Perflurochemical	Inhibits PMN infiltration

stable analogs of PGI_2, and nitric oxide donors; (*b*) substances that inhibit pro-inflammatory mediators of endothelial origin: the pro-inflammatory agents are primarily platelet activating factor (PAF) and oxygen-derived free radicals (e.g. superoxide radicals) although other mediators may be involved. The therapeutic agents useful in this area are PAF receptor antagonists and free radical scavengers (e.g. superoxide dismutase); (*c*) substances that inhibit neutrophils or neutrophil-derived mediators: the major neutrophil-derived mediators are oxygen-derived free radicals, cytokines (e.g. TNFα and IL-1β), proteases (e.g. elastase), and lipid mediators (e.g. LTB_4). In addition, adhesive molecules on the neutrophil surface and their endothelial ligands promote endothelial dysfunction and the action of adherent neutrophils. Agents that inhibit some of these mediators are transforming growth factor-β (TGF-β), elastase inhibitors, leukotriene B_4 (LTB_4) receptor antagonists and monoclonal antibodies to adhesive proteins (e.g. anti-CD18, anti-ICAM-1). Further work is needed to clarify these findings and to determine the physiologic and pathophysiologic interactions among these diverse agents.

This topic of endothelial dysfunction represents a fertile area for further investigation to elucidate the complex mechanisms of neutrophil-endothelial interactions. These interactions lead to neutrophil adherence to the endothelium, neutrophil migration into the underlying tissues, and subsequent tissue injury (e.g. myocardial reperfusion injury). Understanding these mechanisms will provide important insight into the pathophysiologic processes of reperfusion injury as well as offer important targets for therapeutic intervention in these important disease states.

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