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COMMENTARY

MECHANISMS OF ENDOTHELIUM-DEPENDENT VASCULAR SMOOTH MUSCLE RELAXATION

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In recent years, it has become apparent that several agents which relax blood vessels do so by endothelium-dependent mechanisms. Among the first group of agents shown to modulate vascular tone via an interaction with the endothelium were angiotensin II and bradykinin. Subsequent studies indicated that the endothelium responded to these polypeptides by producing and releasing prostacyclin (PGI_2) which, in turn, could modulate the change in vascular tone. In 1980, Furchgott and Zawadzki [1] reported that the endothelium was requisite for acetylcholine (Ach)-induced vasodilation. The relaxing factor, however, was not PGI_2 since relaxation was not blocked by cyclooxygenase inhibitors (i.e. indomethacin or aspirin; Fig. 1). Since this initial report, numerous laboratories have confirmed the endothelium-dependence of Ach and a variety of other vasodilators (for review, see Refs. 2–4). Dr. Furchgott and his colleagues coined the term "endothelium-derived relaxing factor" (EDRF) for the substance(s) which mediates the decrease in smooth muscle contraction.

Numerous techniques have been used to remove the endothelium from the intima of arteries: (1) mechanical (i.e. rubbing, blotting with filter paper, balloon catheter, frayed nylon cord, adhesion to glass coverslip), (2) physical (blowing air on the intima, hypotonic perfusion, perfusion for up to 10 min with distilled H_2O, exposure to hypertonic medium with added K^+), (3) chemical (removal of Ca^{2+} and addition of chelators—EGTA/EDTA), and (4) enzymatic (perfusion or incubation with trypsin or collagenase).

In all cases, coincident with endothelium disruption as verified by microscopy, is the loss of a relaxation response observed in conduit and muscular arteries to the agents summarized in Table 1. In arteries, the endothelium becomes increasingly difficult to remove as the vessel diameter decreases. Some laboratories using the techniques above have been unsuccessful in removing the endothelium from isolated mesenteric arterioles (75–100 μm in diameter). In the perfused mesenteric arcade of the rat, exposure to distilled water for 5–10 min was required to destroy the endothelium. Obviously, when harsh measures are required to remove the endothelium, the medial layer of smooth muscle may also be damaged, potentially obscuring modulatory effects of endothelium on vasopressor agents.

At the present time it is not clear whether a single or numerous EDRFs exist; however, differential sensitivity of relaxing substances to blocking agents has been observed and would suggest multiple mediators. On the other hand, various compounds which act putatively via EDRF are not additive at maximal concentrations. This would be consistent with a common factor or different factors with a common mechanism of action. It is also not clear why different agents have different maximum efficacies (relaxation) but this probably reflects variation in endothelial receptor complements, in rates of production/release of EDRF, and ultimately the maximum capacity of EDRF to relax an artery. In general, the Ca^{2+} ionophore, A23187, is the most effective agent for stimulation of an EDRF-dependent vasodilation response. The magnitude of the EDRF-mediated relaxation responses (percent change) is greatest with low levels of smooth muscle tone.

Most endogenous directly-acting vasodilators (adenosine, PGI_2, epinephrine, etc.) are known to be active in some, but not all, vascular beds and to be variable among various species. In contrast, EDRFs appear to be independent of the arterial segment and mammalian species studied. The

Table 1. Classification of vasodilator substances on the basis of endothelium dependence*

(I) Endothelium-dependent agents:

A23187, Ach, ATP, ADP, bradykinin, substance P, eledoisin, thrombin, arachidonic acid (dog, rabbit, rat), histamine (rat, guinea pig), hydralazine (dog, rabbit), PAF (μM concentration in rabbit and dog).

(II) Endothelium-independent agents:

(A) Adenosine, 5′-AMP, diazoxide, minoxidil, papaverine, PAF, Ca^{2+} blockers
(B) Cyclic AMP, PGI_2, β_2-adrenergic agonists, forskolin, cholera toxin
(C) Cyclic GMP, EDRF, ANF, sodium azide, sodium nitroprusside, GTN and other nitrates

* Abbreviations: PAF = AGEPC or acetylglyceryl ether phosphorylcholine; ANF = atrial natriuretic factor or atriopeptin/auriculin; EDRF = endothelium-derived relaxing factor; and GTN = glyceryltrinitrate.

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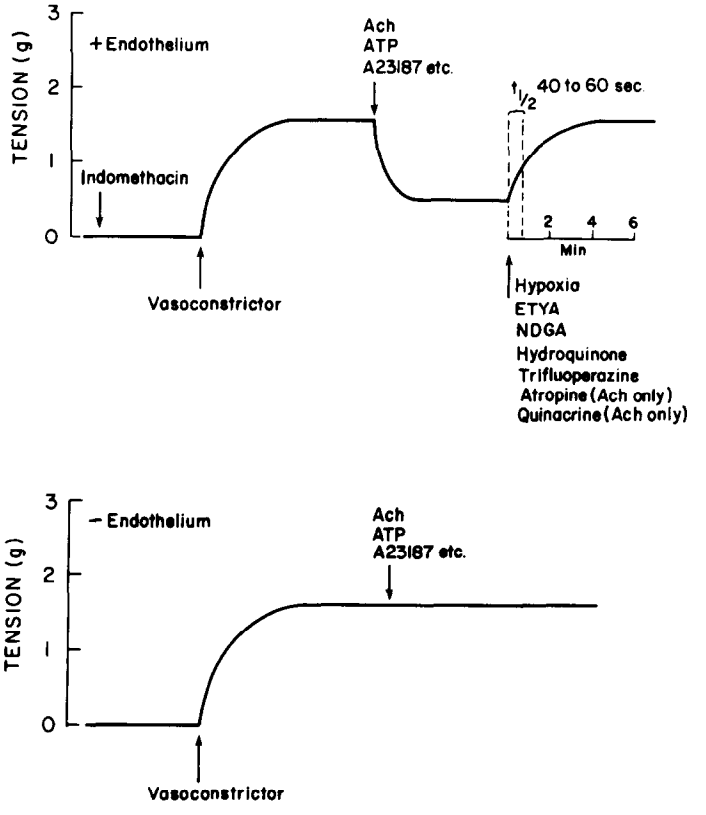

Fig. 1. Representative tension tracings for arterial rings (or strips) with the endothelium intact (top trace) or following removal of the endothelium (bottom trace). In most arteries, a vasoconstrictor must be used to induce tone before vasodilator responses can be obtained. A large number of substances (see Table 1) only produce vasodilation in arteries which contain endothelium. Note that indomethacin pretreatment does not prevent the relaxation response induced by such agents. A variety of drugs (ETYA, NDGA, hydroquinone, etc.) have been shown to block and/or reverse (see figure) endothelium-dependent vasodilation. Reversal of the relaxation is rapid with a half-time ($T_{1/2}$) of 40–60 sec with most inhibitors for recovery of tone. The $T_{1/2}$ for atropine reversal of maximal acetylcholine-induced relaxation is 43 ± 2.5 sec. The rapid $T_{1/2}$ values suggest that EDRF is a potent, labile substance.

ubiquitousness of EDRF and the multiplicity of agents that appear to act on arteries via EDRF have fostered great interest.

Given this interest which the phenomenon of endothelium-dependent vasodilation has generated, it seems surprising that the active substance(s) has not been identified. The difficulties appear to lie in the lability and/or extreme potency of the EDRF. In rabbit aorta, endothelium-dependent relaxation to Ach can be reversed rapidly ($T_{1/2}$ 40–60 sec; Fig. 1) by acute hypoxia or any of several inhibitors (i.e. atropine, quinacrine, eicosatetrayenoic acid (ETYA), etc.]. If these interventions inhibit synthesis or release of EDRF, then the rapid reversal of the response suggests a rapid turnover of the mediator substance(s). This brevity of response to EDRF could be due to a combination of potency (very little EDRF produced), lability in solution, hydrophobicity, and diffusion. In spite of these characteristics of EDRF, comparable relaxation responses are readily obtained *in vitro* with repetitive exposures to most relaxing agonists (with the exception of a receptor refractoriness which develops rapidly with tachykinins such as substance P). The potency and lability of EDRF may account for the limited number of reports which clearly demonstrate transfer of a factor from a source which contains endothelium to a recipient artery (segment, strip, or ring) that lacks endothelium. It is our personal experience that such experiments are difficult to perform. Successful demonstration of transferability of EDRF appears to require (a) close apposition between the donor endothelium and smooth muscle of the recipient (intimal vs adventitial surfaces) as first reported by Furchgott and Zawadzki [1], or (b) a large mass of donor endothelium and rapid exchange to muscle as recently shown by Griffith *et al.* [5]. Based on superfusion-cascade experiments in which the distance was varied between the endothelial source and bioassay smooth muscle denuded of endothelium, these investigators calculated a $T_{1/2}$ of 6.3 + 0.6 sec for EDRF in solution.

Transfer experiments could also be compromised by unidirectional release of EDRF from the medial surface of the endothelial cells or by the participation of endothelium-smooth muscle junctions which facilitate transfer of EDRF between the cell layers. The endothelium certainly is specialized or "sided"

with regard to several other functions. For example, platelets will not adhere to the lumenal side while the medial surfaces are thrombogenic; converting enzyme is localized on the endothelial surface against the blood stream; collagenase appears to be released toward the surface against the internal elastic lamina. Therefore, the vast majority of EDRF could be released toward the media and away from the circulation. One of the more disturbing observations regarding EDRF studies is that a significant portion of the endothelial mass can be removed without altering a relaxation response. In large arteries, 30–35% endothelial loss is tolerated while in small arteries 65–75% denudation may not affect vasodilation. Considering that the endothelium constitutes a distinct minority by total cell count, a much tighter correlation might be expected. If all endothelial cells export EDRF (potent and labile) in response to an appropriate stimulus, loss of a much smaller percentage of the endothelium should alter the sensitivity to and efficacy of the stimulus. On the other hand, if only a subpopulation of endothelial cells produce EDRF, loss of a significant portion of the endothelium could be tolerated, as might occur if the EDRF producing cells formed contacts with muscle and were, therefore, more difficult to remove. Endothelium-smooth muscle contacts may represent specialized junctions for communications between these cell types. In a large artery, the number of endothelium-smooth muscle junctions is small while in arterioles numerous cells make contact through fenestra in the basal elastic lamina. There is no doubt that smooth muscle cells are coupled to each other (as are endothelial cells); therefore, a low number of junctional complexes between the two cell types could function quite efficiently. Autoradiography for labeled ligands might also reflect a heterogeneity of endothelial cells with receptors for EDRF stimulants.

When attempting to pharmacologically characterize the endothelium-dependent relaxation response, several potential sites of action should be considered. These include: (1) signal initiation and transduction at the endothelial cell (i.e. agonist receptor binding and Ca^{2+} influx; (2) synthesis and/or release of vasodepressor factor(s) (EDRF); (3) metabolism or chemical inactivation of EDRF; and (4) physiological or pharmacological antagonism of the action of EDRF on smooth muscle. The effects of several inhibitors of EDRF responses have been interpreted assuming blockade of production of EDRF as the site of action for blockade. In some instances, such assumptions are erroneous and probably account for some of the current controversies in this area. For example, ETYA may inactivate or destroy EDRF in solution [5] and not block at the site of synthesis. In general, control experiments have been performed examining the effects of putative EDRF inhibitors on relaxation elicited by endothelium-independent vasodilators such as isoproterenol or sodium nitroprusside. The latter agent may be the best control since its mechanism of action in smooth muscle seems similar to EDRF based on the observations that both agents increase smooth muscle cyclic GMP levels (see below). However, these controls still do not pinpoint the site of inhibitor action which could be any of the three sites listed above.

Prostacyclin fits the general EDRF characteristics of potency and lability and is produced by endothelium. However, PGI_2-dependent vasodilation is blocked by inhibitors of cyclooxygenase while EDRF responses are not attenuated by steriods or by nonsteroidal antiinflammatory agents. In many arteries, Ach, bradykinin, A23187 etc. can be shown to stimulate PGI_2 production by endothelium but the smooth muscle does not respond to PGI_2. In any vessel which is susceptible to the vasodilator action of PGI_2, this eicosanoid is quite likely to be a contributor to any endothelium-dependent response.

While cyclooxygenase inhibitors do not inhibit EDRF in several blood vessels, involvement of arachidonic acid (AA) or noncyclooxygenase metabolites has received considerable attention. Phospholipase inhibitors such as quinacrine and *p*-bromophenacyl bromide have been reported to inhibit or reverse cholinergic-induced relaxation in rabbit, rat and canine arterial rings. ETYA (inhibits cyclooxygenase and lipoxygenase) and nordihydroguaiaretic acid (NDGA; an antioxidant which blocks lipoxygenase) have both been found to antagonize endothelium-dependent relaxation in several arteries and species. Arachidonate itself can stimulate endothelium-dependent relaxation. In the canine femoral artery, much of this AA-induced endothelium-dependent relaxation is blocked by indomethacin and probably reflects a PGI_2-dependent response [6] (see above). In contrast, in the rabbit aorta, pretreatment with indomethacin potentiates the relaxation response to AA most likely by blocking synthesis of contractile prostaglandins [7]. In our laboratory, pretreatment of rabbit aorta with ETYA and NDGA blocks AA-induced relaxation, suggesting some vasodilatory non-cyclooxygenase products are formed from AA by the endothelium [8]. Scavengers of free radicals (i.e. tetrahydroborate, hydroquinone, resorcinol) block EDRF and are felt to be suggestive of a role for hydroperoxy- or hydroxy fatty acids (i.e. HPETEs or HETEs) in relaxations. Thus, a body of pharmacological evidence has accumulated which is consistent with the hypothesis that, in some arterial tissues, endothelium-dependent relaxation is mediated by, or dependent on, a non-cyclooxygenase metabolite of AA.

The AA metabolite hypothesis is also consistent with the apparent Ca^{2+} dependence of the vasodilation response, since phospholipase activation and AA release have been shown to be intiated by Ca^{2+}-dependent hormones in several biological systems. The accumulation of cyclic GMP by smooth muscle in response to endothelium-dependent relaxing agonists [3] also fits with the involvement of eicosanoids. Guanylate cyclase is known to be stimulated by AA and its free radical metabolites. Regardless of the precise role of cyclic GMP in smooth muscle relaxation, the endothelium-dependent vasodilators represent a major class of naturally occurring substances whose action can be correlated with changes in smooth muscle cyclic GMP levels. At the very least, cyclic GMP accumulation represents a useful biochemical index of the interaction of EDRF with vascular smooth muscle and can be readily exploited,

unlike contraction and relaxation, in cultured smooth muscle.

If EDRF(s) is a lipoxygenase product of AA, a problem arises as to how endothelium-dependent relaxing agonists can selectively activate this pathway in a cell which is capable of producing large quantities of PGI_2 from arachidonate. The presumed rate-limiting step for either oxygenase is the availability of AA (the substrate) released from membrane phospholipids. Additional data do not support the postulated role for AA as a precursor of EDRF. Quinacrine does not antagonize the endothelium-dependent response to A23187. The effect of quinacrine on the muscarinic cholinergic response is at least partially related to inhibition of the muscarinic receptor or receptor coupling since contractile responses to Ach are also inhibited [9]. It should be pointed out that other relaxation agonists (and not just A23187) are insensitive to the inhibitory action of quinacrine and other phospholipase inhibitors (i.e. high dose glucocorticoid). Furthermore, BW755C (a lipoxygenase inhibitor) was found to have no effect on EDRF in rabbit aorta [1]. In addition to AA, other fatty acids (both saturated and unsaturated) can elicit endothelium-dependent relaxation, implying that exogenous fatty acid may act indirectly and not simply serve as a precursor of EDRF. The concentrations of ETYA (100 μM) and NGDA (25 μM) required to block EDRF responses are in vast excess of the reported K_i values of these compounds for lipoxygenase. Such high concentrations of ETYA and NDGA (equal to those for hydroquinone and resorcinol) are strongly antioxidant and may actually increase the lability of EDRF in solution [5]. Large amounts of ETYA and NDGA also are needed to block AA-induced vasodilation [8], a response which necessitates 25–100 μM concentrations of the fatty acid.

Recently, in liver and kidney, it has been shown that cytochrome P-450 monooxygenases catalyze the oxidation of arachidonate. Although the vascular activity of the oxidation products has not been reported, it seems possible that one or more of these metabolites could exert a vasodilatory action. Cytochrome P-450 activity is demonstrable in vascular endothelium, and we have obtained pharmacological evidence that P-450 inhibitors (i.e. SKF-525A and metyrapone) attenuate Ach-, A23187-, and AA-induced relaxation in rabbit aorta. Interestingly, there is also evidence that ETYA and NDGA (at high concentrations) inhibit the oxidation of AA by cytochrome P-450. The data suggest that high concentrations of AA (10 μM) are oxidized to a vasodilating substance by endothelial cytochrome P-450. However, it is unlikely that under physiological conditions the endothelial cell would ever encounter such high concentrations of archidonate. In the rat, the P-450 inhibitors are not very effective against vasodilation responses to Ach or A23187 and suggest a species variation as well.

We recently carried out experiments to determine if AA mimics ETYA as an antioxidant lipid and blocks (or destroys) EDRF. We found that the addition of 1×10^{-6} M AA reversed relaxation responses to a muscarinic agonist (Fig. 2). We also observed that pretreatment with glucocorticoid often potentiated vasodilation in response to ATP and Ach, in particular during attempts to transfer EDRF. In these experiments, ten or twelve 2-mm rings of aorta without endothelium were added to a 5-ml organ bath which contained a single assay ring (+ endothelium) mounted to record tension. The multiple rings were added to supply tissue for enhanced synthesis of lipomodulin to block phospholipase during treatment with dexamethasone. After exposure to steroid for 2–3 hr, each assay ring was contracted with phenylephrine or angiotensin II and assessed for cholinergic-induced relaxation. Relaxation responses were potentiated in assay rings which contained endothelium. Occasionally, evidence of EDRF transfer was obtained following dexamethasone treatment when the added aortic tissue contained endothelium and the assay ring did not. These data suggest that release of AA from endothelium, smooth muscle, platelets, and lymphocytes may actually attenuate EDRF-dependent vasodilation. Such an action of AA could explain the consistent but negative results from EDRF studies with cultured endothelium or with cocultures of smooth muscle and endothelial cells (see below). It is possible that free radicals generated from AA (i.e. PGG_2, PGH_2, HPETE, or HETE) inhibit the actions of EDRF or result in blockade of an enzymatic pathway requisite for EDRF formation/release. Experiments where EDRF and AA are mixed in solution prior to bioassay [5] would address this issue.

Another possible explanation for the apparent Ca^{2+} dependence of endothelium-dependent vasodilation and most of the pharmacological studies which involve drugs (e.g. ETYA and NDGA) at high concentrations which exert membrane stabilizing effects is that EDRF may be a preformed (stored) substance which is actively secreted. While there are no data directly testing this hypothesis, perhaps it should receive consideration. An examination of temperature and energy requirements (as well as other secretion blocking approaches) could shed some light on this issue. Partial depolarization with K^+ (when used as the contractile agonist) does impair relaxation induced by Ach (when compared to tone induced by noreprinephrine) and could represent K^+-induced modulation of EDRF secretion. Whether K^+ depolarization will voltage-inactivate Ca^{2+} channels in endothelium is not known. Based on the apparent lability of EDRF, fractionation of tissue and extraction of this substance (assuming storage) would seem risky. However, on a large scale with cultured endothelial cells such an approach might be fruitful.

With cultured endothelium, there is no binding of the muscarinic radioligand, [^{3}H]QNB, and no detectable vasodilator substance is released into the medium during treatment with Ach. Such negative results have been universal regardless of the species of endothelium, vessel of origin, number of passages in culture, or investigator. However, rabbit aortic endothelium, either freshly obtained by mechanical means or removed after exposure of intact aortic rings to [^{3}H]QNB demonstrates atropine sensitive (specific), saturable binding of the radioligand. It would appear, therefore, that the endothelial musca-

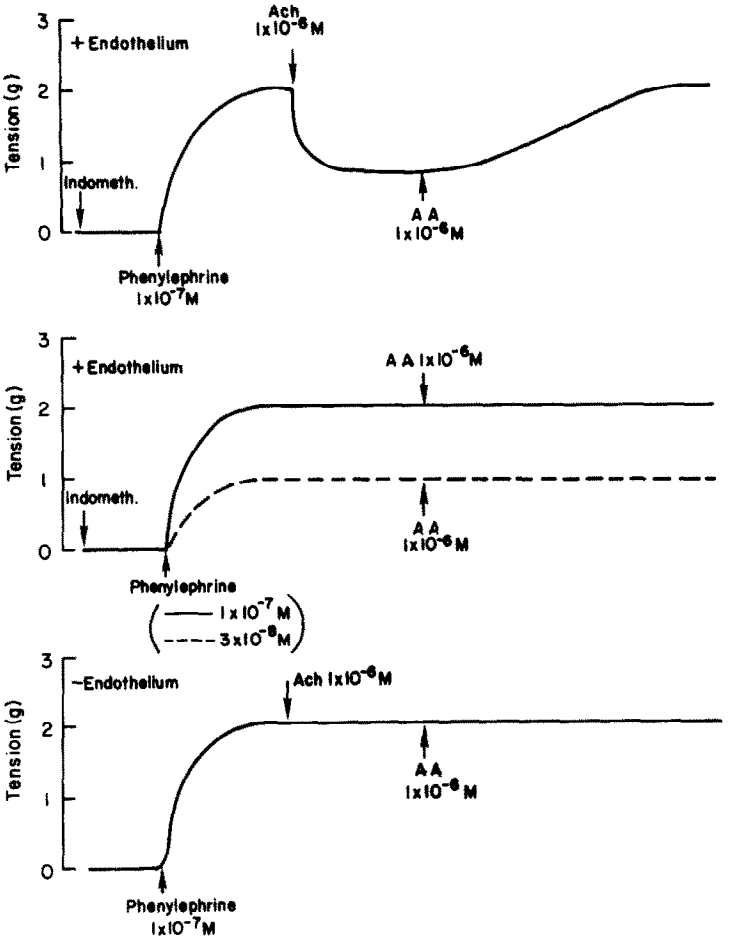

Fig. 2. Reversal of endothelium-dependent vasodilation in rings of rabbit thoracic aorta. An EC_{50} concentration of phenylephrine (1×10^{-7} M) was used to induce tone in all three preparations. The top and middle tracings are from intact aortic rings while the bottom tracing was from a ring without endothelium. Acetylcholine (Ach, 1×10^{-6} M) was used to induce endothelium-dependent relaxation (top vs bottom tracings). At low levels of smooth muscle tone, micromolar or greater concentrations of arachidonic acid (AA) induce vasoconstriction which is also endothelium-dependent but blocked by indomethacin (5 μg/ml) pretreatment (see Ref. 8). At 1×10^{-6} M, AA was not vasoactive even at low levels of induced tone (3×10^{-8} M phenylephrine curve, middle panel) regardless of the status of the endothelium. Ach-induced vasodilation was antagonized by micromolar concentrations of AA.

rinic receptor is extremely labile once the endothelial cells are isolated and that this receptor is not phenotypically expressed under conditions of culture. In fact, experiments introducing endothelium into cultures of arterial smooth muscle cells (principally by the technique of Davis and Kerr [9]) with monitoring of cyclic GMP levels in muscle or superfusing a column of endothelial cells (on carrier beads) with transfer of superfusate to a denuded arterial segment have yielded negative results with cholinergic agonists. However, we have been able to demonstrate an increase in cyclic GMP levels in response to methacholine in mixed (endothelium + smooth muscle) cultures from porcine aorta passaged up to six times (see Fig. 3). This finding suggests that phenotypic expression of the muscarinic receptor by endothelium requires the presence of vascular smooth muscle. Experiments are in progress to evaluate the presence of cholinergic receptors on endothelium grown in medium conditioned by smooth muscle cells. It should be noted that cultured porcine aortic smooth muscle cells have muscarinic receptors ([^{3}H]-QNB) and contract in response to Ach; however, muscle cyclic GMP levels do not change. The smooth muscle cultures respond to sodium nitroprusside with an increase in cyclic GMP levels. An important issue is whether or not Ach, ATP, etc. increase endothelial cyclic GMP levels. We and others have found that none of the agents which stimulate EDRF release alters cyclic GMP levels in the endothelium. This including studies on sheets of endothelium freshly removed from the aortae (either exposed to drugs before or after removal) of several species and on primary and passaged cultures of porcine aortic endothelial cells.

Unlike the muscarinic receptor, other receptor types (bradykinin, angiotensin II, ATP/ADP) are maintained by cultured endothelium (bovine, human, and porcine). The receptors are functional and coupled to a Ca^{2+} channel as determined by

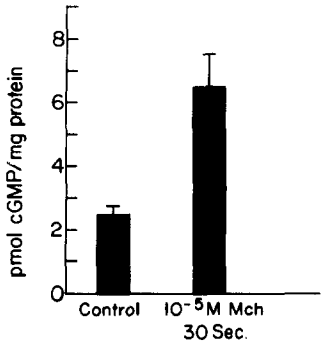

Fig. 3. Cholinergic-induced increase in cyclic GMP levels in mixed (endothelium plus smooth muscle) cultures from porcine aorta at fourth passage. Homogenous cultures of porcine aortic smooth muscle cells or endothelium (fourth passage) failed to respond to methacholine (Mch; data not shown). The cyclic GMP levels in muscle cell cultures (no endothelium) increased 3- to 4-fold (control, 2.45 ± 0.3 vs treated, 9.72 ± 1.0 pmoles/mg protein) by 30–60 sec after treatment with 1×10^{-6} M sodium nitroprusside (SNP). When the two cell types were together during culture, the cyclic GMP concentration rose rapidly (30 sec) in response to 1×10^{-5} M Mch. Mixed cultures also responded to ATP and SNP (data not shown).

$^{45}Ca^{2+}$ flux, Ca^{2+}-dependent K^+ (or Rb^+) efflux, or an induced Ca^{2+} transient detected by Quin 2 fluorescence. These cells also release PGI_2 in response to bradykinin, angiotensin, and ATP. In spite of the above, transfer experiments (adding cultured endothelial cells to cultured smooth muscle) with these peptides or nucleotides in cultures have failed to demonstrate release of EDRF on the basis of changes in cyclic GMP levels. As is the case for Ach, when porcine aortic endothelial and smooth muscle cells are in mixed-cultured (monolayer), ATP also stimulates an elevation in cyclic GMP concentration. There are at least three potential explanations for these discrepancies between results in co-cultures and mixed cultures of these cells. First, production (release) of EDRF is not phenotypically expressed by pure endothelial cultures. Since column-superfused cultured endothelial cells on carrier beads (requiring multiple passages of the cells) appear to release EDRF in response to A23187, this would seem to be unlikely. However, A23187 is extremely toxic to tissues and may result in the release of numerous substances one or more of which could mimic EDRF in some respects. Results with the Ca^{2+} ionophore and endothelial cells should be viewed with cautious enthusiasm. Second, if EDRF does have a $T_{1/2}$ in solution of 10 sec, mixing in the co-culture technique of Davis and Kerr [9], where the endothelium is in a chamber above the muscle cells, creates a major problem. We would estimate that mixing of a substance would require 90–120 sec in a stationary culture dish. On the other hand, mixing should be less of a problem utilizing column superfusion techniques. Third, these agents—angiotensin, bradykinin, and ATP—stimulate PGI_2 production in cultured endothelium and therefore must promote release of free arachidonate. This free AA may inactivate EDRF before it can diffuse from the microenvironment of the endothelial cells. In mixed cultures, adjacent smooth muscle cells could help reduce the free concentration of AA via sequestration and reacylation of membrane phospholipids with the fatty acid.

We and others have considered that vascular smooth muscle might be the initial cell involved in the ultimate formation of EDRF. Smooth muscle could supply a precursor of EDRF to the endothelium where synthesis of a vasodilator product occurs. While intriguing, such a "ping–pong" interaction between the cell types has not been supported by transfer experiments (i.e. muscle to an assay preparation which contained endothelium or muscle followed by column-superfused cultured endothelium). However, these negative results do not rule out such an interaction in the intact artery.

In blood vessels that receive cholinergic innervation, assessment of the role played by the endothelium in response to nerve stimulation has been attempted. Since the nerve terminals lie at the adventitial-medial junction, it would be impossible for neuronally released Ach to traverse the medial mass and reach the endothelium except perhaps in third and fourth order arterioles where the medial layer is only the width of one or two muscle cells. However, the density of cholinergic innervation is extremely sparse in small arteries and great in larger muscular arteries. Numerous investigators have shown in a variety of vessels that Ach depolarizes vascular smooth muscle which results in contraction. The observations suggest, therefore, that stimulation of cholinergic nerves in the arterial wall would not elicit a vasodilation. However, Dr. Bevan's laboratory [10] has reported atropine-sensitive neurogenic vasodilation in the feline isolated posterior auricular artery. This muscarinic relaxation response was not altered by removed of the endothelium from the intima. It is not clear whether or not an EDRF-like compound mediates the vasodilation response to Ach in this artery (i.e. blocked by ETYA).

It has become apparent that several endogenous vasoconstrictors [angiotensin II, III, vasopressin (ADH), epinephrine, norepinephrine (NE), 5-hydroxytryptamine (5-HT)] release a vasodilator substance from endothelial cells that in turn acts as a physiological antagonist of smooth muscle contractile activity [11]. In the absence of endothelium, these hormones also have much greater maximum efficacy as vasoconstrictors. Findings to date suggest that there may be more species and vascular variations in the presence of endothelial receptors for these pressor agents than seen with the vasodilating agents discussed previously. However, results also could be explained by variations in the arterial smooth muscle sensitivity to the dilator released by the endothelium in response to NE, 5-HT, etc. The adrenergic endothelial receptor has been identified in coronary, mesenteric, and renal arteries from pig, dog, rabbit, and rat as an α_2-receptor type. The relaxation induced by 5-HT is potentiated by ketanserin which suggests an S_1 receptor. For angiotensin II and ADH, the receptors have not been carefully characterized, but it seems likely that the endothelial response will be mediated by Ca^{2+} as the intracellular messenger. Variation in endothelial integrity or attenuation of the endothelial vasodilator response

in isolated vessels could account for the inconsistent findings regarding vascular sensitivity and reactivity to vasoconstrictors in pathophysiological models of hypertension, atherosclerosis, diabetes, etc. In addition to production of a dilator in response to vasoconstrictors, it has been apparent for many years that many pressor agents are sequestered and/or degraded by the endothelium. This means that the endothelium presents a significant barrier for circulating hormones between the vascular space and smooth muscle. The smooth muscle cells nearest the intima appear to be very sensitive to a variety of blood borne vasoconstrictor substances; thus, careful removal of the endothelium without damaging the smooth muscle would be expected to alter contractile responses.

While evaluating the effects of hypoxia on EDRF responses induced by Ach and A23187, we found that hypoxia produced an endothelium-dependent vasoconstriction as well as blockade of EDRF. Hypoxia-induced vasoconstriction also is dependent on tone in the artery and the magnitude of the change in pO_2 (Fig. 4). We obtained this response in rabbit and rat aorta whereas DeMey *et al.* [6] observed similar responses in canine arteries. In our hands, the endothelium-dependent vasoconstriction was not blocked by inhibitors of AA metabolism, adrenergic antagonists, serotonin blockers, antihistaminics or angiotensin antagonists. It is tempting to speculate that this endothelial response is the mechanism by which hypoxia produces vasoconstriction *in vivo* (e.g. pulmonary hypertension). Recently, cultured endothelium has been shown to release a vasoconstrictor into the medium. Initial characterization indicates that this material is a peptide (8500 M_r) which induces contraction of isolated arterial smooth muscle [12]. It is not clear at the present time whether one or several vasoconstrictors are released by the endothelium and exactly what conditions may modulate their production or release.

In addition to PGI_2 and EDRF(s), another class of potent vasodilators is typified by the compound 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine or platelet activating factor (PAF). The vasodilating factor that has been extracted from the kidney medulla is identical to PAF. Cultured human endothelium has been shown to produce and release PAF in response to endotoxin, A23187, angiotensin II, vasopressin, and thrombin [13]. PAF is a potent vasodilator that, unlike EDRF, causes a sustained relaxation of arterial smooth muscle. However, studies in cultured bovine aortic endothelium indicate that PAF stimulates Ca^{2+} fluxes in endothelial cells [14]. Therefore, PAF should activate the release of EDRF and/or PGI_2 which, in turn, may mediate the vasodilation induced by PAF. In the rabbit and dog, high concentrations (μM) of PAF caused endothelium-dependent vasodilation. If made and released by endothelium to act back on the endothelium, PAF would indeed represent a unique class of autacoids.

Small peptides with potent vasodilating activity have been isolated from cardiac atrial tissue and have been termed atrial natriuretic factor (ANF), atriopeptin(s), or auriculin [15]. ANF relaxes the rabbit and rat thoracic aorta and has no endothelial dependence. However, in other tissues ANF caused endothelium-dependent vasodilation and has been observed to stimulate cyclic GMP accumulation. *In vivo* ANF is relatively selective for the renal circulation causing pronounced changes in renal blood flow and glomerular filtration rate. This selectivity or specificity for the renal vasculature would indicate that, if ANF releases EDRF, it does so primarily in the kidney. On the other hand, if ANF acts directly on arterial smooth muscle and activates guanylate cyclase, such a peptide could be a candidate for EDRF(s).

In summary (see Fig. 5), it is evident that the endothelium plays a major role in the regulation of vascular smooth muscle tone by modulating the ability of muscle to contract. The identity of some

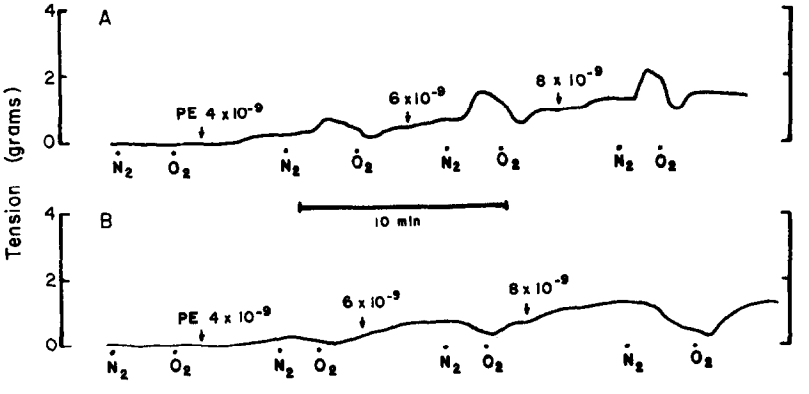

Fig. 4. Effects of hypoxia on rings of rabbit thoracic aorta. Panel A is a representative tension tracing from an intact ring; Panel B, a ring without endothelium. At resting tension (1.5 g), the gas mixture passing through the organ baths was switched from 95% O_2/5% CO_2 to 95% N_2/5% CO_2 as indicated by N_2. The gas mixture was switched back to 95% O_2/5% CO_2 (O_2), and a threshold concentration of phenylephrine (PE, 4×10^{-9} M) was used to induce tone. The anoxic insult was repeated (N_2) and initiated a contractile response *only* in preparations which contained endothelium (Panel A). As additional tone was induced by the administration of higher concentrations of PE (6×10^{-9} and 8×10^{-9} M), the magnitude of the contraction obtained in response to N_2 was enhanced.

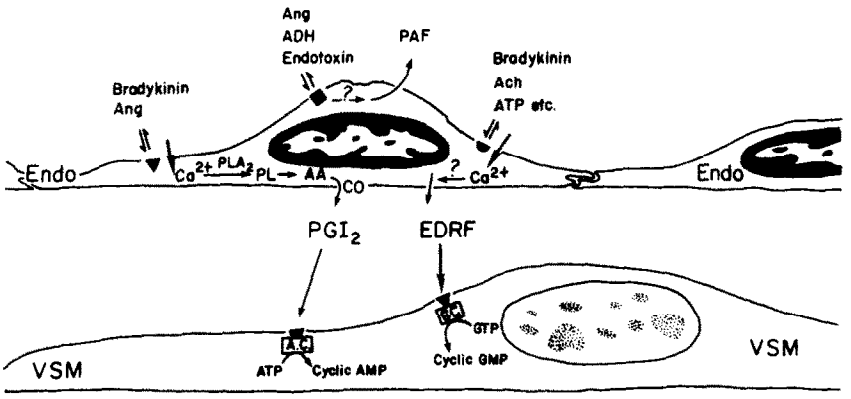

Fig. 5. Hypothetical model of known endothelial (Endo)-vascular smooth muscle (VSM) interactions based on discussion in the text.

mediators is known (i.e. eicosanoids and PAF) while others remain to be chemically identified (EDRFs). Clearly, the endothelium has receptors for a vast number of vasoactive substances, and alteration in receptor complement or capacity to make and release substances which alter smooth muscle contractile activity may play major roles in cardiovascular pathophysiology.

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