Nitric oxide and vasodilation in human limbs

MICHAEL J. JOYNER, AND NIKI M. DIETZ
Department of Anesthesiology, Mayo Clinic and Foundation, Rochester, Minnesota 55905

Joyner, Michael J., and Niki M. Dietz. Nitric oxide and vasodilation in human limbs. J. Appl. Physiol. 83(6): 1785–1796, 1997.—Both the skeletal muscle and skin of humans possess remarkable abilities to vasodilate. Marked vasodilation can be seen in these vascular beds in response to a variety of common physiological stimuli. These stimuli include reactive hyperemia (skin and muscle), exercise hyperemia (muscle), mental stress (muscle), and whole body heating (skin). The physiological mechanisms that cause vasodilation in response to these stimuli are poorly understood, and the substance(s) responsible for it remain unclear. In this context, recent attention has been focused on the possible contribution of nitric oxide (NO) to the regulation of hyperemic responses in human skin and skeletal muscle. The emerging picture is that NO is not an essential component of the dilator response seen during reactive hyperemia. However, it does appear that NO may play a modest role in exercise hyperemia. NO appears to play a major role in the skeletal muscle vasodilation seen in response to mental stress in humans. Preliminary evidence also indicates that NO is not essential for the normal dilator responses observed in the cutaneous circulation during body heating in humans, but this issue needs further study. There are a number of possible mechanisms that might mediate NO release in humans, and the role of these mechanisms in the various hyperemic responses is also poorly understood. The role of altered NO-mediated vasodilation in some disease states is also discussed. Whereas NO is a potent vasodilating substance, the actions of NO alone do not explain a variety of poorly understood vasodilator mechanisms in conscious humans. Much work remains for those interested in the role of NO in the regulation of blood flow to the skin and skeletal muscle of humans.

Magnitude of Limb Hyperemia

At rest in a thermoneutral environment, blood flow to the forearm or calf is usually 2–5 ml·100 ml⁻¹·min⁻¹ (22, 95). About one-half of the total flow goes to skin, and one-half to muscle, which together comprise 50–75% of limb volume (22, 44, 84, 95). Blood flow to fat or bone is usually considered negligible (95). After ischemic exercise, blood flow can increase 10- to 20-fold (100, 104). During rhythmic quadriceps exercise, muscle blood flow values of ~300 ml·100 ml⁻¹·min⁻¹ have been reported (2, 119). With whole body hyperthermia, forearm blood flow values of up to 20 ml·100 ml⁻¹·min⁻¹ can occur, with the increases in flow confined exclusively to the skin (22, 86, 112). Additionally, total skin flow can reach values of 5–10 l/min distributed in ~2–4 kg of skin (87). This means that skin and muscle blood flow can increase 50- to 100-fold above baseline. Because these increases in flow occur with little change in perfusion pressure, most of the rise in flow is due to vasodilation.

Overview of NO

In 1980, Furchgott and colleagues demonstrated that acetylcholine (ACh)-mediated vasorelaxation in the...
isolated rabbit aorta required the presence of intact vascular endothelial cells (37, 96). Previously, the effects of ACh on vascular smooth muscle tone in isolated preparations were inconsistent, but infusions of ACh in the brachial artery of humans consistently evoked marked dilation that could not be explained by known mechanisms (30, 95). These conflicting observations could be explained if the vascular endothelium released a vasodilating factor in response to ACh administration (95, 116). This substance was later identified as NO or a closely related compound that evokes vasodilation by stimulation of guanosine 3',5'-cyclic monophosphate pathways in vascular smooth muscle (71, 90). The pharmacological vasodilators nitroglycerin and sodium nitroprusside both cause vasodilation by donation of exogenous NO or NO-like compounds (52).

Vascular endothelial cells also possess the enzyme NO synthase (NOS) that catalyzes the synthesis of NO from L-arginine (27). At least three isoforms of NOS have been identified: endothelial, neuronal, and an inducible form that can be expressed in vascular smooth muscle after exposure to endotoxin. Arginine analogs have proved useful as pharmacological tools to inhibit the actions of NOS and reduce the synthesis of NO (10, 83, 115).

**Mechanisms of NO Release (Fig. 1)**

There is basal NO release from the vascular endothelium, and NO release can be evoked by stimulation of cholinergic (muscarinic) and other receptors on the endothelium (9, 96, 115, 116). Mechanical interactions between blood flowing in vessels and the vascular endothelium can cause release of NO from the endothelial cells (65, 88). NO may also be released directly by nitrooxidergic (autonomic) nerves, and ACh released from autonomic nerves can evoke NO release from endothelial cells (7, 8, 27, 67, 82, 114). Tissues such as skeletal muscle also contain substantial NOS and might release NO in a manner that can increase blood flow (58). Finally, NO can be bound to hemoglobin in an oxygen-sensitive manner so that when oxygen saturation falls, NO is liberated from hemoglobin (53). This mechanism might promote local vasodilatation and augment flow to tissues with a high demand for oxygen such as exercising skeletal muscle. Little is understood about the contribution of NO release from skeletal muscle or from hemoglobin in regulating vascular tone and blood flow in humans or other species.

**Mechanically Induced NO Release (Fig. 2)**

Mechanical interactions (i.e., increased shear stress) between blood flow and the vascular endothelium can evoke the release of NO in isolated blood vessels and in cultured endothelial cells (9, 60, 65, 88). Several studies have provided evidence that shear stress activates potassium channels in the vascular endothelial cells that evoke NO release (18, 75). Mechanical factors might also cause local release of ACh from a subpopulation of endothelial cells, which then stimulates release of NO from their neighbors via the activation of muscarinic receptors (65, 69, 70, 79).

**Neurally Mediated Release of NO**

Neurally mediated NO release can occur in two ways. First, NO may be released directly from nitrooxidergic (or nitrodergic) nerves. This occurs in the cat cerebral circulation and may also govern penile erection in many species (8, 82, 114). It is not known whether human skeletal muscle or skin is innervated by nitroxi-
dergic nerves. Stimulation of several types of vasodilating cholinergic nerves including vagal fibers to the canine coronary and feline pulmonary vessels can cause NO-mediated vasodilation (7, 67). Similarly, sympathetic-cholinergic vasodilation of the cat hindlimb is also due to NO release (67). This means that ACh from various autonomic nerves can reach the vascular endothelium and stimulate NO release.

Basal NO Release in Humans

In humans, N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) is the only arginine analog that has been used extensively to inhibit NOS. When this compound is infused via the brachial artery at rates of 1–4 mg/min for periods of up to 10 min (total dose 10–50 mg), forearm blood flow in the arm receiving the L-NMMA is reduced by 25–50%, and it appears that flow to both forearm muscle and skin is reduced similarly. There is also no change in blood flow in the contralateral untreated forearm, and systemic arterial pressure does not change (25, 26, 32, 40, 109, 115, 120). This fall in blood flow has been attributed to reduced basal release of NO.

Similarly, when systemic doses of L-NMMA (3.0–6.7 mg/kg over 15 min) are given to humans, there are both systemic and pulmonary vasconstriction and a rise in mean and pulmonary arterial pressures (6, 48, 106). There are also baroreflex-mediated reductions in heart rate and sympathetic vasoconstrictor outflow similar to those observed when mean arterial pressure is increased by infusions of phenylephrine (48, 106). It has generally been assumed that the source of the NO that is “blocked” in these studies is from the vascular endothelium, but Toda (113) has postulated that NO tonically released from vasodilating autonomic nerves is a major source of the NO that regulates baseline vascular tone. The possible contribution of tonic NO released from autonomic nerves to baseline blood pressure regulation has yet to be addressed in humans.

Release of NO by ACh in Humans (Fig. 3)

When ACh or other cholinergic agonists are infused into the brachial artery, there is marked endothelium-mediated forearm vasodilation, and dilation occurs in both muscle and skin (25, 29, 86, 115). Treatment of the forearm with L-NMMA frequently, but not always, blunts the vasodilator responses to ACh in a highly variable manner among subjects (25, 26, 33, 115). This variable effect of L-NMMA on ACh-mediated vasodilation appears to be independent of the changes in baseline flow. Additionally, in some subjects, L-NMMA can inhibit vasodilator responses to some physiological stimuli, even though the vasodilator response to ACh is only minimally affected (24, 33). Administration of arginine analogs that inhibit NOS to the rabbit hindlimb causes a marked vasoconstriction but has little impact on the rise in flow with intra-arterial ACh (72). These observations suggest that ACh evokes the release of multiple vasodilating substances, including one that may not be inhibited by L-NMMA (72, 89), and mean that ACh-mediated vasodilation cannot be assumed to be identical to NO-mediated vasodilation. The identity of this second vasodilating substance, if it exists, is unknown (89).

Comparisons between the endothelium-dependent dilation caused by ACh and the endothelium-independent dilation caused by nitroprusside administration
have been used to make inferences about how various interventions or conditions might alter endothelial synthesis and release of NO vs. vascular responsiveness to NO. ACh- but not nitroprusside-mediated vasodilation of the human forearm is augmented by acute brachial artery administration of estrogen, suggesting that estrogen causes greater endothelial synthesis or release of vasodilating compounds. These findings might explain some of the “cardioprotective” effects of estrogen (39, 41). ACh- but not nitroprusside-mediated vasodilation has also been reported to be blunted in hypertensive humans as well as those with a strong family history of hypertension, diabetes, heart failure, various forms of hyperlipidemia, and other vascular diseases (11, 13, 15, 16, 19, 28, 54, 62–64, 76–78, 107, 108, 117). In some pathological conditions, the blunted vasodilator response to ACh can be restored by infusions of L-arginine, a treatment that normally does not augment ACh-mediated dilation in normal subjects (14, 18, 74, 105). These observations suggest that altered NO synthesis or release may be important in many forms of human vascular disease and related conditions.

Contribution of NO to Hyperemic States in Humans

Reactive hyperemia probably does not occur solely to repay an oxygen-related or metabolic “debt” in the previously ischemic tissues, since the flow after release of ischemia exceeds by a wide margin any oxygen or metabolic debt incurred (5). Additionally, no single mediator or class of mediators (i.e., metabolites) has been identified that can satisfactorily explain reactive hyperemia, and it is not dependent on intact innervation of the previously ischemic tissues (30, 33, 95). Vasodilating prostanooids may play a modest role as mediators of the reactive hyperemia in the human forearm. Inhibition of cyclooxygenase with nonsteroidal anti-inflammatory drugs has no effect on resting blood flow but can reduce the peak blood flow response during reactive hyperemia by 15–30% (12, 33, 56). The effect of these compounds on the blood flow responses after release of ischemia is unclear. Some reports indicate that they reduce flow for several minutes, while others show they have little effect or cause a modest increase (33, 73).

When reactive hyperemia trials are conducted before and after treatment of one forearm with L-NMMA (Fig. 3), resting forearm blood flow is selectively reduced in the treated arm (33, 109). However, peak forearm blood flow responses following periods of ischemia ranging from 3–10 min are only minimally affected by L-NMMA, and the peak rise in flow above baseline is not affected by L-NMMA (33, 109). By contrast, there is some modest blunting of the blood flow response during the second and third minutes after ischemia, and total excess flow is reduced 15–30% after L-NMMA when the differences in baseline flow are not considered (33, 109). However, when the effects of L-NMMA on baseline forearm blood flow are considered, NOS inhibition appears to have little impact on total excess flow (33).

Brachial artery diameter can increase by 5–15% during reactive hyperemia, and this dilatation is thought...
to be a result of increased brachial artery flow evoking release of NO (13, 62, 99). L-NMMA blunts the changes in brachial artery diameter that are seen after release of ischemia (62).

In patients with coronary artery and other vascular diseases, the vasodilator and blood flow responses after forearm ischemia (i.e., reactive hyperemia) are blunted (62). In these patients, the dilator responses to ACh and the increase in brachial diameter after ischemia are also blunted (13–15, 19, 28, 54, 62, 77–79, 107, 108, 117). However, caution must be exercised when ascribing altered NO-mediated mechanisms to these deficits. ACh-mediated dilation may not be due solely to NO release from the endothelium, and NO does not appear to be essential to see a nearly normal reactive hyperemia response in otherwise healthy subjects (33, 72, 89, 109). Caution must also be exercised when comparing results from the large conduit arteries with responses that reflect changes primarily in the resistance vessels. For example, estrogen therapy can augment the increase in brachial artery diameter after forearm ischemia without augmenting the peak vasodilator response of the resistance vessels (63).

Exercise Hyperemia (Fig. 4)

The factor or factors that cause skeletal muscle vasodilation with exercise remain unknown after more than 100 yr of investigation (95). Several basic mechanisms have been proposed. These include dilating actions of 1) metabolites or other factors released by the skeletal muscle in proportion to the contractile activity; 2) substances released from nerves; 3) some substances carried by the blood (i.e., carbon dioxide or oxygen); and 4) mechanical interactions between the muscle contractions and the blood vessels (41, 61, 95, 97). To date, multiple efforts to block or eliminate one or more of these proposed mechanisms have been ineffective in eliminating the rise in blood flow with exercise (41, 95). In some cases, it is possible to slow the rise in flow at the onset of contractions or cause a mild reduction in the steady-state blood flow values as contraction continues. However, when reductions are seen, they frequently are transient, suggesting that if one vasodilating mechanism is blocked, other mechanisms are available to restore the flow to the normal value (31, 41, 95).

When L-NMMA is infused into the brachial artery before rhythmic handgrip exercise, baseline forearm blood flow is reduced, but the rise in flow has been reported to be both blunted and unaffected (40, 120). When L-NMMA is infused during mild or moderate levels of rhythmic handgrip exercise (Fig. 4) in the human forearm, blood flow is reduced by 25–30% (31). NOS inhibition also blunts modestly the blood flow responses seen after static handgrip exercise (32). However, static exercise can compress skeletal muscle vessels, and the blood flow response following static exercise can represent a response to a combined exercise and reactive hyperemia stimulus (105). These observations suggest that NO is not essential to observe the normal rise in blood flow at the onset of exercise but that it may contribute to the flow during or after more prolonged exercise. One possible mechanism is that the increased flow associated with exercise causes release of NO from the vascular endothelium that contributes to the continued dilation (31, 88).

In animal studies, NO can reduce the vasodilation associated with exercise in conscious dogs, but it is unclear if this is simply due to a reduction in baseline flow (74). When arginine analogs that inhibit NOS are infused into the phrenic artery in spontaneously breathing dogs, diaphragmatic blood flow is reduced (51). When exercising rats are treated with arginine analogs, there is a fiber type-specific inhibition of vasodilation that is confined to the vessels perfusing slow-twitch fibers (49). The role of NO as a key vasodilator during heavy exercise is difficult to evaluate because, if any NO-mediated dilation is “blocked,” there might be a mismatch between blood flow and metabolism that could lead to the buildup of other vasodilating factors and restore the flow to the normal value (31). Taken together with studies in humans, the animal studies indicate that NO may have a modest role in exercise hyperemia but that its presence is not essential to see a nearly normal response.

NOS is present in skeletal muscle, and it is tempting to speculate that NO is released by the skeletal muscle in proportion to its metabolic oxygen consumption (58). Such a mechanism, if present, could contribute to the matching of blood flow and muscle metabolism during exercise. NO also binds to oxygenated hemoglobin, and NO can be released from hemoglobin as oxygen is extracted from the blood. NO is then reloaded to hemoglobin as the systemic venous blood passes through
the lungs (53). If this mechanism were active in skeletal muscle, it might serve to augment flow to metabolically active tissues with high levels of oxygen extraction. It is not known whether NOS inhibitors administered by brief local infusions effectively inhibit intramuscular NOS. Additionally, it seems unlikely that local infusions of arginine analogs that block NOS would limit systemic shuttling of NO via hemoglobin. The possible contribution of these mechanisms to vascular regulation needs further evaluation in both humans and animals.

Finally, while sympathectomized or denervated skeletal muscle can clearly vasodilate in response to contractions, it is still possible that nerves play a role in exercise hyperemia. Segal and Kurjiaka (92) have recently proposed that “acetylcholine spillover” from motor nerves might play a role in exercise vasodilation. This ACh might evoke release of NO from the vascular endothelium.

NO and exercise training. Chronic increases in blood flow can enhance the synthesis and release of NO from the vascular endothelium of the vessels subject to the high flow (68). In animal models, endurance exercise training can evoke an increase in NOS gene expression in the endothelial cells of conducting arteries (i.e., the aorta) that are subject to increased flow during exercise (21, 91). These vessels also show increased endothelial dependent vasodilation. However, it has been difficult to determine whether exercise training enhances endothelium-dependent vasodilation in humans (42, 43). Studies that have compared the forearm dilator responses to brachial artery infusions of ACh before and after training or in the dominant and nondominant arms of tennis players have not shown dramatic differences in the two forearms, suggesting that training may not augment the synthesis and release of NO in forearm resistance vessels (42, 43). This lack of response is seen, even though forearm vasodilation and blood flow after combined exercise and ischemia are augmented after training (42, 43, 98, 100, 101).

The differences in human and animal studies could be due to the fact that the dilator responses to ACh given via the brachial artery in humans reflect events at the resistance vessels and not the conducting arteries that have been studied in animal models. It might also represent an inadequate training stimulus or the previously discussed limitations of intra-arterial ACh as a “test” drug for endothelial function in humans. Additionally, endurance-trained subjects may have augmented ACh-mediated vasodilation in their “untrained” limbs (i.e., the arms of runners) (57). The proposed mechanism for this observation is related to training-induced reduction in blood lipid levels, so if training has an effect in humans, it may be due to systemic and not local adaptations (57).

Shen et al. (93, 94) have proposed that augmented synthesis of NO after exercise training might increase mitochondrial efficiency and reduce the muscle oxygen demand associated with a given absolute exercise level. However, this would seem unlikely because oxygen consumption at the same absolute work rate is not reduced after training, which indicates that the oxygen demand required to generate ATP at the mitochondria is not altered by training (50).

NO in Skin Blood Flow (Figs. 5 and 6)

During body heating in humans, there is a marked thermoregulatory vasodilation in the skin of body areas not directly subject to the heating (84, 86). In acral skin such as the fingertips, this dilation is mediated by withdrawal of sympathetic vasoconstrictor tone (84). In nonacral (hairy) skin, which is that covering the fore- and most of the body surface area, intact autonomic innervation is required to see the rise in skin blood flow with body heating (55, 84, 86). This dilation occurs at about the same time as the onset of sweating, which is mediated by ACh released from sympathetic-cholinergic nerves (55, 86). Local treatment of one forearm with atropine during body heating can delay the onset of the dilation but has little impact on its total magnitude, despite the fact that sweating is almost completely eliminated (55, 86). This means that it is unlikely that either direct cholinergic stimulation of blood vessels or some substance released by active sweat glands (such as bradykinin) is essential to observe the cutaneous vasodilation with body heating in humans (36). However, when botulinum toxin is selectively injected into a small area of skin to presynaptically block ACh release from sympathetic-cholinergic nerves, the cutaneous dilation is absent in the treated portion of the skin (55). This suggests that some factor(s) cotransmitted by sympathetic-cholinergic nerves plays a key role in the cutaneous dilation seen in hairy skin during whole body heating.

Only one study has attempted to evaluate the contribution of NO to active cutaneous vasodilation during body heating in humans. Dietz and colleagues (26) infused l-NMMA into one forearm of human volunteers and then subjected them to whole body heating sufficient to evoke a marked vasodilator response (i.e., ~1.0°C increase in core temperature). In these subjects, the l-NMMA reduced baseline blood flow to the forearm, but there was little difference in the rise in forearm blood flow seen in the l-NMMA-treated arm and the untreated arm. On the basis of this study, it appears that NO is not essential to observe the cutaneous vasodilation with body heating in humans.

There is also neurally mediated cutaneous vasodilation in the rabbit ear (111). This dilation does appear to be blocked by arginine analogs that inhibit NO synthesis (110). It appears that NO per se is not the substance solely responsible for the cutaneous dilation in the rabbit ear but that NO plays a “permissive” role and is required (along with some other transmitter) to observe the full dilator response (34). In these studies, rabbits are heated until a “full” vasodilator response is seen, and then the arginine analogs are given selectively in the auricular artery. This contrasts with the approach used in the previously described human study in which l-NMMA was given before heating.

However, the rabbit ear is probably somewhat more analogous to “acral” skin, since there is little or no
thermoregulatory sweating, as is the case in human hairy skin. This means that it is unclear whether the differences between humans and rabbits reflect species differences, differences in the type of skin under study, or are a result of different experimental protocols and the timing of NOS inhibition. The mechanisms responsible for the neurally mediated cutaneous vasodilation during body heating in humans remain unknown, and additional attempts to study the possible contribution of NO to this phenomenon are clearly warranted (26, 55).

Mental or Emotional Stress (Fig. 7)

During mental or emotional stress in humans, there can be skeletal muscle vasodilation in the forearm (4, 85). The presence, magnitude, and duration of forearm muscle vasodilation during mental stress are variable and dependent on a wide variety of subject-specific factors (85). However, this dilation is absent after sympathectomy and it is also blunted by intra-arterial infusions of atropine (4, 85). These observations suggest that there might be sympathetic-cholinergic innervation to skeletal muscle in humans and that these nerves can be active during mental or emotional stress. Whereas there are pharmacological data to support sympathetic-cholinergic dilation in humans, there is no histological evidence for sympathetic-cholinergic nerves in human skeletal muscle.

In control conditions, there is also a marked rise in skin blood flow [cutaneous vascular conductance (CVC)] during body heating that appears to occur at about the same time as increase in sweating. Iontophoresis of atropine delays onset of sweating and blunts the overall magnitude of dilator response but does not eliminate it. These observations, in conjunction with earlier studies on sympathectomized subjects, demonstrate that sympathetic dilator fibers evoke cutaneous vasodilation seen during general body heating but that this dilation is not a cholinergic event. By contrast, selective intradermal injection of botulinum toxin to a small area of skin permits the normal increase in sympathetic dilator traffic to forearm but limits release of ACh and any cotransmitters. It also eliminates rise in skin blood flow during body heating. These data suggest that some factor(s) released in conjunction with ACh from sympathetic-cholinergic nerves is responsible for cutaneous vasodilator responses observed during body heating in humans. These data highlight the neurally mediated basis of this response, its noncholinergic nature, and the new observation that a vasodilating cotransmitter released from sympathetic-cholinergic nerves may contribute to cutaneous vasodilation seen during body heating. Whether this cotransmitted substance is NO remains to be determined. (Data from Ref. 55.)
dilation as a result of circulating epinephrine has also been proposed to contribute to the dilation (1, 45, 85).

When NOS inhibitors are administered to one forearm in conscious humans, the rise in forearm blood flow during mental stress and the associated vasodilation are almost completely eliminated in the treated forearm but remain normal in the contralateral untreated side (25). The blunting of the dilatation by L-NMMA is also greater than that seen by atropine alone (25). These observations suggest that there might be activation of sympathetic-cholinergic nerves that stimulate the vascular endothelium to release NO. Such a mechanism has been demonstrated in the cat hindlimb (35, 66). There might also be activation of nitroxidergic nerves or corelease of NO by other nerves (20, 114). One problem with cholinergic nerves stimulating NO release from the endothelium is that ACh would have to travel from the adventitial side of the blood vessel to the endothelium. Such a large distance and the presence of various forms of cholinesterase make this mechanism seem less likely. However, Broten and colleagues (7) have provided evidence that cholinergic dilation in the coronary circulation is NO dependent and may involve adventitial ACh release and subsequent stimulation of muscarinic receptors on the vascular endothelium.

Mental stress is also associated with arterial hypertension and tachycardia, and it is possible that these hemodynamic changes mechanically stimulate NO release from the endothelium. Flow-induced NO release may be active in the human hand, where NOS blockade can reduce finger blood flow in a normothermic human, but not in a cool human with vasoconstricted fingers (17) and limited blood flow to stimulate NO release. This last possibility is attractive in light of recent observations showing that there can be selective sympathetic withdrawal to the forearm in some subjects who show forearm vasodilation during mental stress (47). Under these circumstances, the dilatation associated with sympathetic withdrawal might be amplified by mechanically induced NO release (65, 88). The observation that forearm vasodilation is absent after chronic surgical sympathectomy might then be explained by a lack of sympathetic withdrawal in conjunction with chronic changes in endothelial and vascular function that can be associated with autonomic denervation.

Marked skeletal muscle vasodilation is seen during fainting in humans. Like mental stress, this dilatation is absent after surgical sympathectomy of the forearm or local anesthetic nerve block of the forearm (3, 47). These observations suggest that skeletal muscle vasodilation during fainting is an active, neurally mediated process. In contrast, later studies demonstrated the abrupt onset of sympathetic silence to skeletal muscle during fainting and gave rise to the concept that sympathetic withdrawal was responsible for the dilatation (46, 103, 118). These findings are qualitatively similar to those associated with vasodilation during mental stress. However, unlike mental stress, vasodilation during fainting does not appear to be attenuated by cholinergic blockade with atropine (46).

When fainting is provoked in human volunteers by lower body negative pressure (venous pooling), the magnitude of the forearm dilatation is unaffected by selective infusions of L-NMMA to one forearm, suggesting that NO is not responsible for the skeletal muscle vasodilation seen with fainting (24). Studies in animals confirm this concept and indicate that NO is not essential to the systemic vasodilation during vasovagal responses caused by acute hemorrhage (59).

Summary

The role of NO released from the vascular endothelium in regulating basal vascular tone in human skin...
and muscle is clear (23, 25, 26, 33, 38, 115, 121). However, NO is not essential to observe the peak blood flow response during reactive hyperemia in the human forearm, and its role in the overall response is probably modest (33, 109). The contribution of NO to the regulation of skeletal muscle vasodilation during exercise is more difficult to study as a result of multiple mechanisms that might contribute to exercise hyperemia and due to the many potential sources of NO that might contribute to vasodilation in active skeletal muscles during exercise (31, 41, 53, 58, 61, 95). It does appear reasonable to suggest that locally released NO is not essential for the normal rise in flow at the onset of exercise but that it may contribute modestly as exercise continues, particularly during mild or moderate levels of exercise and in slow-twitch fibers (31, 49, 121). NO is probably not essential to observe cutaneous vasodilation in hairy skin during body heating in humans (26). However, this observation needs to be confirmed as a result of conflicting findings from animal studies (110, 111).

The studies on the role of NO during skeletal muscle vasodilation during mental stress indicate that NO contributes to human skeletal muscle vasodilation during mental stress (25). However, the anatomic and neurophysiological basis for this release remains obscure (45). It is reasonable to postulate that NO is released as a result of increased activity by a poorly understood sympathetic vasodilator system. It is also reasonable to suggest that sympathetic withdrawal, in conjunction with mechanically evoked local release of NO in the forearm, might explain much of this response (65). In each physiological vasodilator response that might be caused by NO, it is not clear whether increased NO synthesis is the final mediator of the vasodilation or whether NO plays a permissive role and is required in a modest amount to see the full expression of dilator responses caused by other factors (34).

The contribution of “endothelial dysfunction” to altered vasodilator responses in pathophysiological conditions needs to be viewed with caution, and the differences between events in conducting arteries and vasodilation in resistance vessels needs to be appreciated. In many cases, there is little evidence that endothelial factors such as NO play an essential role in various physiological vasodilator responses such as reactive hyperemia (33, 109). This makes it less likely that endothelial dysfunction alone can explain blunted vasodilator responses seen with disease. Interactions between vasoactive substances (including NO) released by autonomic nerves and the vascular endothelium are also emerging as important mechanisms that might regulate vascular tone in vivo, and it is almost inconceivable that the autonomic nervous system and NO will not have major modulating influences on each other (113, 114). These emerging mechanisms will need to be addressed in humans.

Finally, NO is an important vasodilating substance when studied in large conducting arteries in vitro (37, 96, 116). In various other isolated preparations and animal models, NO has been shown to have potentially profound effects on local and systemic circulatory control mechanisms (27, 96, 116). By contrast, the human studies performed to date suggest a more modest role for NO in the overall circulatory control scheme and in the vasodilator responses to various forms of physical and mental stress (33, 46). These modest responses reinforce the general concept that multiple redundant regulatory systems operate (23–26, 31) at a variety of levels to govern the circulatory system in humans. In this context, NO is a new piece of the puzzle for those attempting to gain an integrative understanding of human cardiovascular regulation.

The authors thank Janet Bedman for her skillful preparation of the manuscript, the many subjects who have participated in the studies on this topic, and the ongoing technical support provided by Darrell Loeffler and Tamara Eichhoff. The authors also acknowledge the encouragement of Prof. J ohn T. Shepherd over many years.

This work was supported by National Institutes of Health Grants HL-46493, NS-32352, and RR-00585–25; by the Glen L. and Lyra M. Eb ling Cardiology Research Endowment, and by the Mayo Foundation. M. D. Dietz was also supported by Division of Research Resources Grant RR-00585–2452.

Address for reprint requests: M. J. Joyner, Dept. of Anesthesiology, Mayo Clinic, 200 First St. SW, Rochester, MN 55905 (E-mail: joyner.michael@mayo.edu).

REFERENCES


