Nitric Oxide and Physiologic Vasodilation in Human Limbs: Where Do We Go From Here?

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Abstract/Résumé

This brief review highlights human studies on the role of nitric oxide (NO) and limb vasodilation conducted at the Mayo Clinic over the last 10 years. These studies have attempted to determine whether NO is responsible for the "unexplained" limb vasodilation seen with body heating, limb ischemia, exercise, and mental stress. Our findings are placed in context with data from others, and possible future areas of study are identified.

Cet article passe en revue les faits saillants des études consacrées, à la clinique Mayo, au rôle de l'oxyde d'azote (NO) dans la vasodilatation au niveau des membres, et ce, au cours des 10 dernières années. Ces études cherchaient à établir le rôle joué par le NO dans la vasodilatation "inexpliquée" au niveau des membres au cours d'expériences sur l'augmentation de la chaleur corporelle, l'ischémie du membre, l'exercice, et le stress mental. Les observations prennent en compte les résultats d'autres études; des domaines d'étude sont également proposés.

Introduction

Over the last 10 years a group of researchers at the Mayo Clinic has attempted to explore the role that nitric oxide (NO) plays as a regulator of physiological vasodilation in human limbs. The purpose of this brief review is to highlight key findings

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from these studies, integrate these findings with observations from others, and review additional issues related to NO and the regulation of blood flow to human skeletal muscle and skin (Dietz et al., 1994a, 1994b, 1997; Dyke et al., 1995; Engelke et al., 1966; Halliwill et al., 1997; Joyner and Dietz, 1997; Reed et al., 2000; Shastry et al., 1998, 2000). These studies also represent a continuation of the long history of studies on cardiovascular physiology and vascular biology conducted in humans at the Mayo Clinic.

Emergence of Nitric Oxide as an Endogenous Vasodilating Substance

For many years the vascular endothelium was seen primarily as a barrier separating the blood vessel lumen and its contents from vascular smooth muscle cells, the interstitial space, and the tissues beyond. However, in the early 1980s it became clear that the endothelium released a number of factors which can govern blood vessel tone (Moncada et al., 1988; Shepherd, 1983; Shepherd and Katusic, 1991). The seminal observation was made by Furchgott and Zawadzki (1980) when they demonstrated that strips of rabbit aorta relaxed in response to the exogenous administration of acetylcholine (ACh) only when the vascular endothelium was intact. Subsequently, it was demonstrated that vascular endothelium can synthesize NO from L-arginine. Additionally, several isoforms of NO synthase were discovered. This enzyme was seen to be widely distributed in the vascular endothelium (eNOS), neural tissue, and skeletal muscle (nNOS); and an inducible form of NO synthase (iNOS), whose levels increased dramatically during conditions such as septic shock, was identified (Dinerman et al., 1993).

Many of the initial studies on the vascular endothelium focused on large conducting vessels that branch from the aorta such as the femoral, renal, or coronary arteries. In these studies NO was shown to be a regulator of basal vascular tone, and its release could also be stimulated by the administration of compounds such as ACh or bradykinin (Moncada et al., 1988; Shepherd, 1983; Shepherd and Katusic, 1991). In addition to NO, the vascular endothelium was also shown to release a variety of other dilating substances including vasoactive prostanoids, and perhaps a second factor known as endothelial-derived hyperpolarizing factor (EDHF; Rubanyi and Vanhoutte, 1987). So by the late 1980s, researchers had clearly made the case for participation of the endothelium in vascular regulation and the central role of NO as a vasodilating substance (Shepherd and Katusic, 1991).

Potential Mechanisms of Nitric Oxide Release

Studies during the 1980s also identified multiple ways in which NO could be released to impact vascular tone (Joyner and Dietz, 1997). First, there is basal release of NO from the endothelium. Second, mechanical stimuli, such as an increase in shear stress, can evoke NO release from the endothelium (Martin et al., 1996; Rubanyi et al., 1986). Third, metabolites and substances released from other tissues, such as contracting skeletal muscle, can cause NO release by directly vasodilating vascular smooth muscle, increasing blood flow, and evoking flow-induced NO release. The vasoactive substances might also stimulate receptors on the vascular endothelium to release NO.

A fourth way that NO can be released is from so-called nitroxidergic nerves. These nerves have emerged as key mediators of the vasodilation associated with penile erection, and they also appear to play an important role in regulating cerebral blood flow (Burnett et al., 1992; Rafjer et al., 1992; Toda and Okamura, 1991). A fifth mechanism that can evoke NO release involves the cholinergic nerves. These nerves stimulate muscarinic receptors on vascular endothelium, and there is strong evidence for sympathetic cholinergic vasodilation in the hindlimbs in a variety of animal species. As well, in the heart the ACh released from parasympathetic nerves can clearly evoke NO release (Broten et al., 1992; Matsukawa et al., 1993). Additionally, a variety of local and circulating factors including epinephrine can evoke receptor-mediated NO release from the vascular endothelium (Dawes et al., 1997). A sixth and final way that vasoactive NO might be released is from the tissues themselves. Skeletal muscle contains both nNOS and eNOS which can synthesize NO (Kobzik et al., 1994; 1995). If NO is released from the tissues and has access to the vascular smooth muscle, it could clearly cause vasodilation. Depending on the situation, all six of these NO-releasing mechanisms might contribute to one or more vasodilator phenomena typically observed in human limbs.

Does NO Explain Unexplained Vasodilation?

The information discussed above was "state of the art" when our studies in humans began. In general we sought to determine whether NO contributes to or is responsible for "unexplained vasodilation" in human limbs. We have studied four major unexplained vasodilator phenomena: (1) the cutaneous vasodilation seen during body heating; (2) reactive hyperemia; (3) exercise hyperemia; and (4) the skeletal muscle vasodilation seen during mental stress. In each case our studies were preceded by observations over many years that these stimuli could cause marked increases in skeletal muscle or skin blood flow in humans via some unknown mechanism. In the case of the cutaneous vasodilation during body heating, this response was known to be dependent on sympathetic nerves, but the neurotransmitter involved had not been identified (Roddie, 1983; Roddie et al., 1957). Along similar lines there is strong evidence from the 1950s suggesting that the forearm vasodilator response to mental stress was neurally mediated, but again, the substance responsible for the dilation was not known (Blair et al., 1959a; Roddie, 1977). With reactive hyperemia and exercise, many previous studies focused on one or more "vasodilating metabolites" (Shepherd, 1983), but little evidence had accumulated for any one obligatory vasodilator.

EXPERIMENTAL APPROACH

In our studies in healthy human subjects, we adopted an experimental paradigm that had been used since the 1950s. In this paradigm one arm is instrumented with a brachial artery catheter configured so as to permit the simultaneous measurement of arterial pressure and the selective infusion of drugs to one forearm (Blair et al., 1959a; 1959b; Roddie et al., 1957). Both forearms are then usually instrumented to measure forearm blood flow using venous occlusion plethysmography. The forearm blood flow responses to physiological stimuli are then measured. Generally a trial is performed before and after one forearm is selectively treated

with the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) given via the brachial artery. L-NMMA is an arginine analog that limits the production of NO (Vallance et al., 1989). Depending on the study, we also used the contralateral untreated forearm as an additional control. This simple approach allowed us to compare responses before and after treatment or to compare responses in treated and untreated forearms.

Skin Blood Flow. Some of our initial studies on the role of NO and vasodilation in human limbs sought to determine whether NO contributes to the marked cutaneous vasodilation seen during body heating. When humans are passively heated and core temperature rises by .5 °C or more, there is marked cutaneous vasodilation (Roddie, 1983; Roddie et al., 1957). Since the dilator response is absent in individuals after surgical sympathectomy, it is thought to be a neurally mediated "active" dilator response. The dilation starts at about the same time as sweating, which is governed by the sympathetic cholinergic nerves. By contrast, ACh released from the sympathetic nerves is not the dilating substance responsible for the sweating, nor is it a metabolic byproduct of the sweat glands (Roddie et al., 1957). However, when our studies began, there were emerging observations indicating that NO played an important role in the active cutaneous vasodilation seen in the rabbit ear during body heating (Taylor and Bishop, 1993).

In our first study, we used venous occlusion plethysmography to measure whole limb blood flow during body heating with a water-perfused suit (Dietz et al., 1994b). Since the rise in limb blood flow during the heating is confined to the skin (Roddie, 1983), changes in total forearm flow measured with plethysmography are a reasonable surrogate for changes in skin blood flow. We administered the NOS inhibitor, L-NMMA, via brachial artery catheter *before* the body heating, and gave a dose sufficient to block the dilator responses to intra-arterial ACh, demonstrating that we had inhibited endothelial NO production. While administration of the L-NMMA clearly reduced resting forearm blood flow, the rise in forearm blood flow during body heating was similar to that seen in the untreated contralateral forearm. However, there were several problems with this experiment. First, we gave the L-NMMA before body heating, so it is possible that not much of it reached the cutaneous circulation and especially the blood vessels that were dilated by the subsequent period of body heating. Second, we had concerns about whether whole-limb venous occlusion plethysmography was sensitive enough to detect the small changes in skin blood flow.

To address the concerns, we administered NOS inhibitors after marked cutaneous vasodilation had occurred to ensure that they reached the dilated blood vessels. In these studies the brachial artery administration of the NOS inhibitor, L-NMMA (Shastry et al., 1998), at the height of the cutaneous dilation caused a 20–30% reduction in the flow in some but not all subjects. Subsequently, we used microdialysis to more selectively administer NOS inhibitors (L-NMMA or L-NAME) directly to the cutaneous circulation. These studies confirmed our previous results and showed that ~30% of the cutaneous dilator response to body heating was NO-dependent (Shastry et al., 2000; also see Figure 1).

It should also be noted that our studies were in general agreement with those conducted by Dean Kellogg and colleagues (1998) at the University of Texas Health Sciences Center in San Antonio. More important, Dr. Kellogg has performed an ingenuous set of experiments using botulinum toxin to presynaptically inhibit the

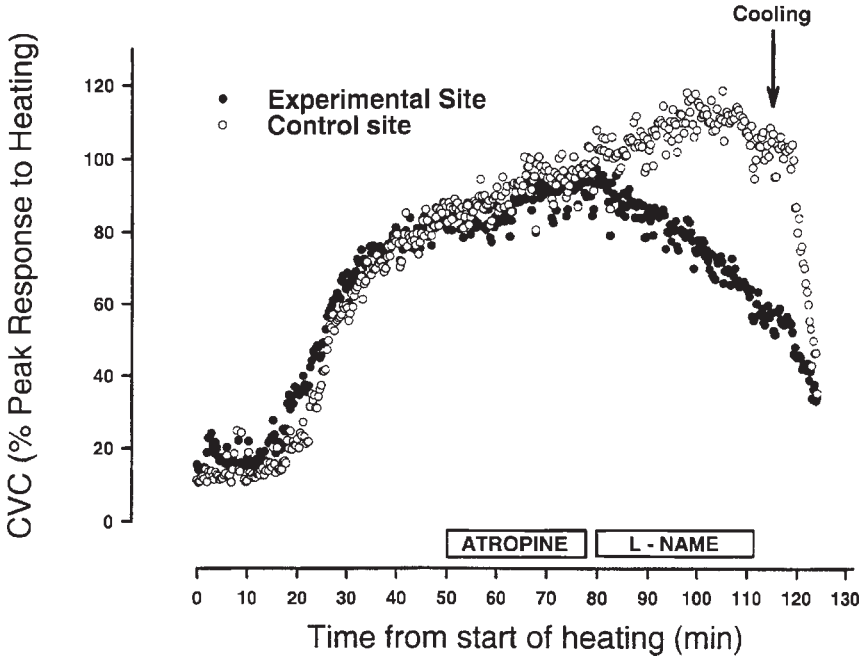


Figure 1. Effects of atropine followed by the nitric oxide synthase inhibitor, L-NAME, on the cutaneous vasodilator responses to whole-body heating in a representative subject. At time 0 the subject was heating using a water-perfused suit. After ~10–20 min of heating there was marked cutaneous vasodilation. In the experimental site, atropine was administered via microdialysis, which abolished the local sweating response but had no impact on vascular conductance. Subsequently, L-NAME was microdialyzed through the experimental site. This caused a marked reduction in the cutaneous dilator responses. When cooling was initiated there was a rapid loss of cutaneous vasodilation. CVC = cutaneous vascular conductance, our index of skin vascular tone. This observation indicates that nitric oxide contributes to the neurally-mediated vasodilation observed during whole-body heating in humans. However, it is not responsible for 100% of the dilator response. *Note*: From Shastry et al., "Effects of atropine and L-NAME on cutaneous blood flow during body heating in humans." © 2000 *J. Appl. Physiol*., 88: 467-472. Reprinted with permission.

release of ACh from the sudomotor nerves and provide strong evidence that a substance co-released with ACh from these nerves is responsible for the cutaneous dilation seen during body heating (Kellogg et al., 1995). The exact identity of this substance remains to be determined. Therefore, while NO contributes to the cutaneous vasodilator responses seen during body heating, it is not the major mediator of this response. However, local heating of the skin can cause locally-mediated vasodilation, and this dilation is largely dependent on NO (Kellogg et al., 1999; Minson et al., 2001).

Reactive Hyperemia. When an arm cuff is inflated to suprasystolic pressure for more than just a few seconds, release of this cuff is followed by a large increase in forearm blood flow, but the mechanism(s) responsible for the dilation

is unknown. Studies conducted in the 1950s indicated that the dilator response was probably not due to an oxygen debt or to the buildup of some metabolite associated with the ischemia (Blair et al., 1959b; Patterson and Whelan, 1955). However, later studies showed that vasodilating prostanoids contribute to the peak vasodilator response seen after ischemia (Carlsson et al., 1987; Kilbom and Wennmalm, 1976). In this context, we sought to determine whether NO alone or in combination with vasodilating prostanoids contributed to reactive hyperemia in the forearm.

We measured the forearm blood flow response to 5 min of ischemia. Trials were conducted before and after L-NMMA administration to inhibit NO production. In a second set of studies, trials were conducted before administration of the cyclo-oxygenase inhibitor ibuprofen, after ibuprofen, and after ibuprofen + L-NMMA (Engelke et al., 1996). We also assessed the degree of the NOS block using infusions of ACh. The main finding of our study was that L-NMMA reduced baseline blood flow and caused a modest reduction in the absolute blood flow responses following ischemia (see Figure 2). Additionally, the rate at which the blood flow returned to normal appeared to be slightly more rapid after the L-NMMA. However, when the differences in baseline blood flow were subtracted and only the "excess flow" above baseline was evaluated, we found that NOS inhibition had little effect on the reactive hyperemia response

In our studies with combined cyclo-oxygenase inhibition and L-NMMA, we showed that cyclo-oxygenase inhibition alone had little impact on baseline forearm blood flow, but that the peak dilator response following ischemia was blunted, yet combined NOS inhibition did not further reduce the peak responses. Based on these observations, we concluded that most of the reactive hyperemia response is a result of myogenic vasorelaxation associated with mechanical unloading of the resistance vessels during the period of ischemia and that NO is not obligatory to see this response (Engelke et al., 1996).

Exercise Hyperemia. A host of metabolites, ions, neurally-mediated factors, and mechanical influences have been proposed, evaluated, discussed, and analyzed as possible mediators of exercise hyperemia for more than 100 years (Shepherd, 1983). In our initial study on this topic, subjects performed mild rhythmic forearm hand-gripping until they reached a blood flow "steady-state." Blood flow to the exercising muscles was estimated on the basis of plethysmographic measurements of flow made once per minute during brief pauses in the hand-gripping. To ensure that the L-NMMA reached vasodilated forearm vessels, we started to infuse it after steady-state blood flow values had been achieved. Using this approach, we found that L-NMMA caused a modest (20–30%) reduction in forearm blood flow responses between contractions (Dyke et al., 1995).

Studies conducted by others whereby L-NMMA was given prior to exercise demonstrated a reduction in resting flow and also that, in general, the rise in flow with contractions was similar (Endo et al., 1994; Gilligan et al., 1994; Wilson and Kapoor, 1993). In most of these studies, blood flow during contractions was estimated with plethysmography during brief pauses in the contraction. Additionally, conclusions about the impact of NOS inhibition on the blood flow responses to exercise depended to some extent on whether or not the statistical analysis compared blood flow, changes in blood flow above baseline, calculated vascular resistance, or calculated vascular conductance

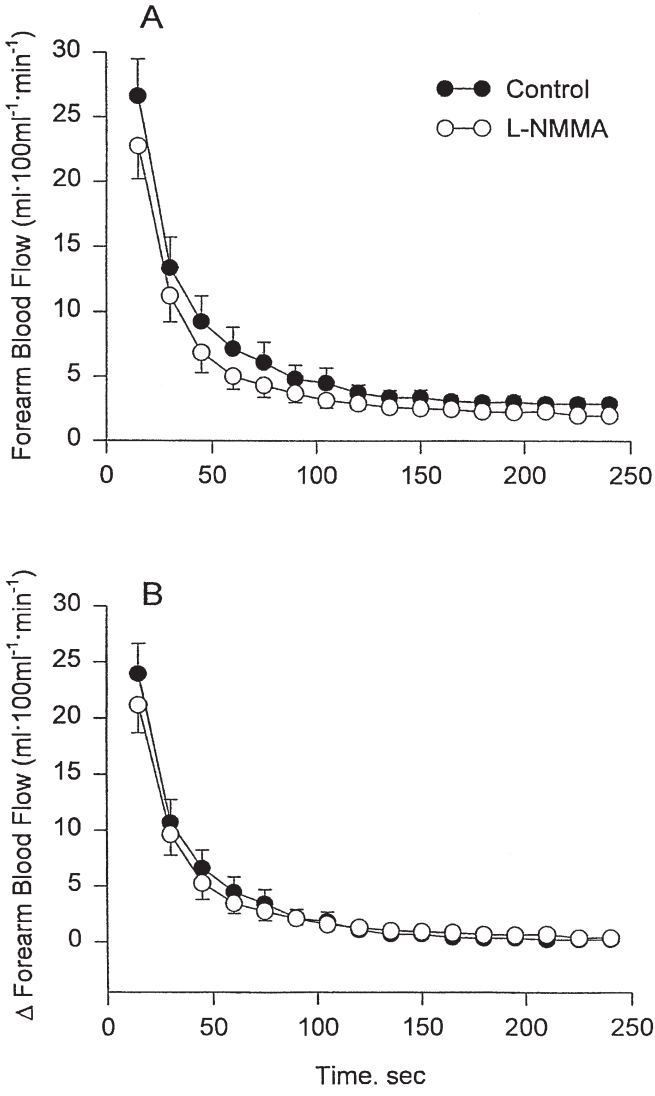


Figure 2. Forearm blood flow responses following 5 min of ischemia. Studies were conducted before (control) and after administration of NO synthase inhibitor L-NMMA via a brachial artery catheter. (A) Raw forearm blood flow responses. Demonstrates that L-NMMA had a modest impact on peak blood flow responses and that the rate of decay of the "reactive hyperemia" curve was accelerated slightly with the L-NMMA trial. Most important, resting forearm blood flow was substantially lowered after L-NMMA. (B) Identical data, except that resting flow was subtracted from hyperemic responses and the changes in forearm blood flow above baseline are compared. Using this analysis, almost no difference was seen between the control and L-NMMA treatment trials. These data indicate that nitric oxide does not play a major role in the limb vasodilator responses following ischemia. *Note*: From Engelke et al., "Contribution of nitric oxide and prostagladins to reactive hypermia in the human forearm." © 1996 *J. Appl. Physiol.*, 81: 1807-1814. Reprinted with permission.

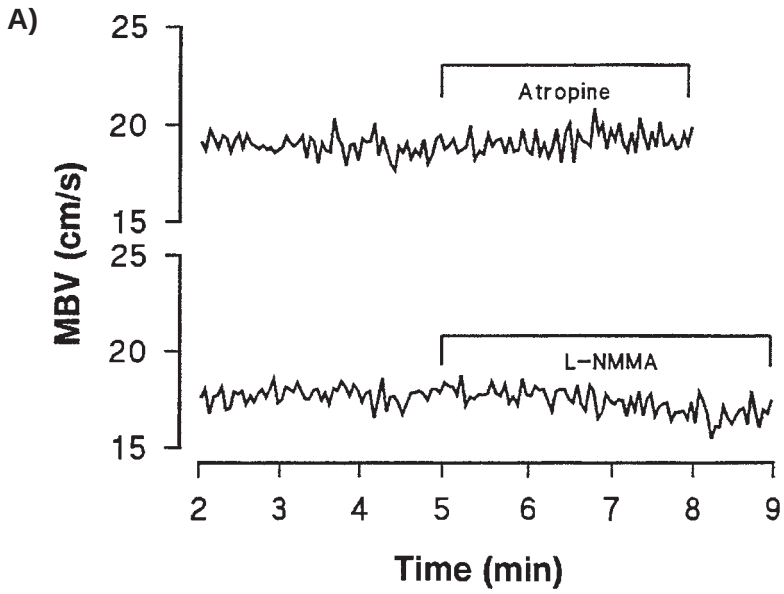
One problem with the plethysmographic approach to measuring blood flow "between contractions" is that in reality the first postexercise blood flow is being measured. To address this issue, our laboratory collaborated with the team led by Dr. Richard Hughson from the Department of Kinesiology at the University of Waterloo, Ontario. This group had mastered a variety of ultrasonic techniques to make continuous measurements of forearm blood flow *during* rhythmic contractions (Brock et al., 1998; Shoemaker et al., 1997). We combined this approach with our drug infusion protocols. The main finding was that the administration of NOS inhibitors during contraction had only a minimal impact on blood flow *during* contractions (Figure 3A). It was also noted that the characteristics of the transition in blood flow from rest to exercise remained unaltered with L-NMMA. By contrast, as soon as contractions ceased, there was a 20–30% reduction in blood flow during recovery (Figure 3B). The results in recovery were quantitatively similar to those observed in our previous study using plethysmography (Dyke et al., 1995).

In addition to the studies conducted in the isolated forearm, a number of researchers from the Copenhagen Muscle Research Centre have evaluated the impact of NO on the blood flow responses to rhythmic one-legged knee extension. In these studies, systemic doses of NOS inhibitors are given. In general the results from these studies showed that NOS inhibition has little impact on blood flow during contractions but that it reduced postexercise blood flow (Frandsen et al., 2000; Radegran and Saltin, 1999).

One continuing problem with attempting to evaluate the impact of systemic doses of NOS inhibition on blood flow to contracting muscles is the fact that the systemic NOS inhibition increases mean arterial pressure. This probably causes a baroreflex-mediated reduction in sympathetic outflow to the skeletal muscle so that any reduction in muscle blood flow associated with NOS inhibition may be masked by concurrent sympathetic withdrawal. Evidence to support this possibility comes from Sheriff et al. (2000), who studied conscious chronically-instrumented dogs running on the treadmill and used ganglionic blockade to eliminate changes in sympathetic outflow during systemic NOS inhibition. Sheriff and colleagues demonstrated that NO might be responsible for up to ~30% of the vasodilator response to exercise.

In conclusion, the best available evidence in humans suggests that NOS inhibition causes little or no reduction in the vasodilator responses to contraction. However, the confounding influences of systemic doses of NOS inhibition on blood pressure and the sympathetic nervous system need to be considered when evaluating results from some studies, and these factors should be considered again as new experiments are designed to further test the role of NO in exercise hyperemia. By contrast, it is quite clear that NO contributes to the postexercise blood flow responses in humans (Dyke et al., 1995; Frandsen et al., 2000; Radegran and Saltin, 1999; Shoemaker et al., 1997).

Mental Stress. For many years it has been known that there can be marked skeletal muscle vasodilation in the forearm during mental stress in humans. Studies in the 1950s demonstrated that this response was absent in subjects who had undergone surgical sympathectomy of the forearm (Blair et al., 1959a; Roddie, 1977). Studies in animals before and during that time also provided strong evidence for the existence of sympathetic cholinergic vasodilator fibers to skeletal



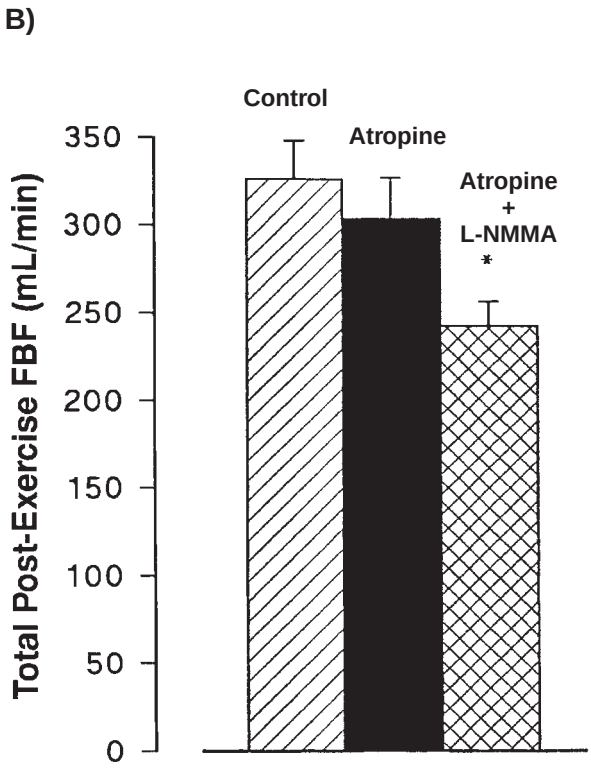


Figure 3. (A) Effects of atropine and L-NMMA on mean blood velocity (MBV) in brachial artery during moderate rhythmic forearm hand-gripping. MBV is an index of forearm blood flow. Neither atropine nor L-NMMA had a significant impact on MBV responses to rhythmic hand-gripping. (B) Total postexercise forearm blood flow after a control bout of exercise, another bout in which atropine was administered, and a third bout when both atropine and L-NMMA were administered. Additional L-NMMA caused a marked reduction in total postexercise blood flow. *Note*: Adapted from Shoemaker et al., "Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery." © 1997 *Am. J. Physiol.*, 273: H2388-H2395. Reprinted with permission.

muscle. In humans, however, it has been difficult to histologically demonstrate the existence of sympathetic cholinergic fibers in skeletal muscle (Bolme and Fuxe, 1970; Uvnäs, 1966). Additionally, while atropine and beta-blockers blunted the forearm dilator response to mental stress, they did not eliminate it. With this information as a background, we attempted to determine whether NO contributes to the forearm vasodilator responses to mental stress, and to further pharmaco-dissect this response. We also sought to measure the sympathetic traffic to the forearm during mental stress to see if we could record sympathetic dilator traffic.

Many of the original studies on mental stress employed severe emotional stress and intentional deception of the subject (for discussion, see Roddie, 1977). However, with the advent of human subjects review boards, such tactics were no longer permitted; we adopted the Stroop word color conflict test to "stress the subjects." In our version of the test, computer screens with the names of four colors were flashed at progressively faster speeds in front of the subjects. The colors of the letters used to spell the color words are different. For example, the word "red" might be spelled with blue letters, "white" with red letters, "green" with yellow letters. We asked the subjects to tell us the color of the letters and not the name of the word these letters spelled. Educated subjects tend to read, and it is difficult for them to identify the color of the letters and not read the name of the color that the letters spell. In many subjects we saw a two- to four-fold rise in forearm blood flow, a 10- to 30-beat increase in heart rate, and a 20-mmHg increase in mean arterial pressure.

In our first series of studies, led by Dr. Niki Dietz, we demonstrated that NOS inhibition in one forearm blunted the forearm vasodilator response to mental stress by ~70–80% (Dietz et al., 1994a; Figure 4). This blunting was greater than

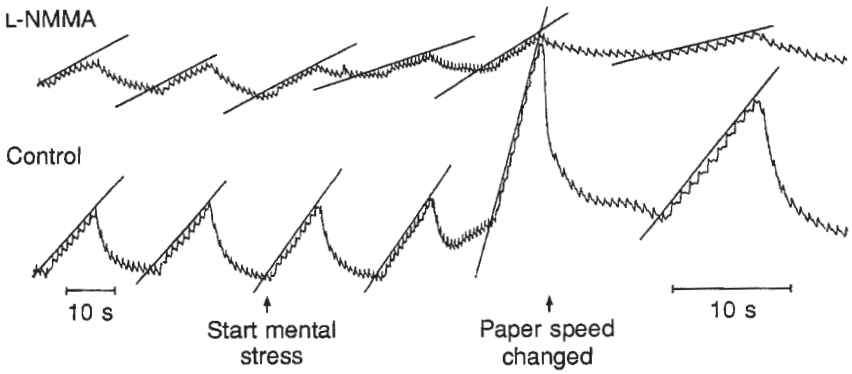


Figure 4. Effect of nitric oxide synthase inhibition on forearm vasodilator responses to mental stress. Top tracing shows an original plethysmographic record in a forearm treated with the NO synthase inhibitor, L-NMMA. Bottom tracing is from the same subject during the same trial and is the record from the untreated forearm. In response to mental stress, marked forearm vasodilation was seen in the control arm that was absent in the treated forearm. These data demonstrate that nitric oxide plays a key role in the forearm vasodilator responses to mental stress in humans. *Note*: From Dietz et al., "Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans." © 1994 *J. Physiol*., 480: 361-368. Reprinted with permission.

that seen with atropine alone (~50%). This observation was some of the first evidence that NO played a dominant role in any physiologic vasodilator response in humans. In a second study, headed by Dr. John Halliwill, we used microneurography in the radial nerve to determine whether there was actually an increase in sympathetic traffic during the vasodilation. Previous studies by others suggested there was no change in nerve traffic, but in these studies, concurrent measurements were not made of forearm blood flow and sympathetic traffic to the upper extremity (Halliwill et al., 1997).

In this context, we observed that individuals who showed the most profound skeletal muscle vasodilation during mental stress actually demonstrated a reduction in forearm sympathetic vasoconstrictor traffic and no evidence for sympathetic dilator activity. In subsequent studies we used a stellate ganglion nerve block to eliminate sympathetic traffic to the upper extremity and demonstrated that the forearm vasodilator response to mental stress was still present. Similar results were found by Lindqvist and colleagues (1996). These observations challenged the interpretation of the data from the 1950s in the subjects with sympathectomy and led us to question the existence of sympathetic vasodilator nerves to the upper extremity.

To resolve this conflict, we attempted to perform a maneuver we knew would evoke strong increases in sympathetic traffic to the upper extremity. In a second study led by Dr. Dietz, we had the subjects perform contralateral ischemic forearm hand-gripping to fatigue to cause a large increase in sympathetic traffic to the upper extremity that would be maintained during postexercise ischemia (Dietz et al., 1997). This maneuver normally causes a marked forearm vasoconstriction. However, we reasoned that if some of the sympathetic traffic to the upper extremity were vasodilator in nature, we might be able to "unmask" it by administering drugs that selectively inhibited forearm vasoconstriction. We found that administration of bretylium (which limits norepinephrine release from sympathetic nerves) and the α-adrenergic blocker phentolamine to a resting forearm converted the normal vasoconstrictor response to contralateral ischemic hand-gripping to a vasodilator response that could be blocked with L-NMMA. We initially interpreted this as potential evidence for the existence of sympathetic vasodilator fibers.

In our fourth study on this topic, we performed a similar protocol after we had used local anesthetics to block the stellate ganglion and eliminate sympathetic constrictor traffic to the upper extremity (Reed et al., 2000). We reasoned that if sympathetic dilator nerves were responsible for the vasodilation seen in our study with contralateral ischemic hand-gripping to fatigue, the dilation would be absent after stellate ganglion block. Results from this study indicated that the forearm vasodilation *was present* after the stellate ganglion block during contralateral ischemic hand-gripping to fatigue. This dilation could be blocked in part by propranolol (beta-blockade), and further blocked by L-NMMA, confirming that the vasodilation evoked by stimulation of beta-2 receptors is mediated in part by NO.

When we attempted to interpret our data and the data from others, we concluded that during sympatho-excitatory maneuvers such as mental stress, NO mediates most of the forearm vasodilator responses seen (Joyner and Halliwill, 2000). However, while there is older evidence (i.e., the sympathectomy studies) that is consistent with the idea of sympathetic vasodilator nerves to the upper extremity, our microneurographic studies and stellate ganglion block studies suggest that the dilator response may be due to circulating catecholamines acting on β_2-receptors

that can stimulate NO release and/or local factors such as increases in mean arterial pressure and heart rate that might mechanically stimulate the endothelium to release NO. Therefore, our current working hypothesis is that there are no sympathetic dilator nerves to the upper extremities in humans.

OVERVIEW

In our studies to date on unexplained vasodilation, we have demonstrated that NOS inhibition has a modest impact on the cutaneous vasodilator responses to body heating in humans. The effects on the vasodilator responses to reactive hyperemia in the human forearm are minimal, and current information suggests that NO is not an obligatory substance associated with the vasodilator responses to exercise. However, additional studies on exercise hyperemia are needed for the reasons discussed earlier. In contrast, NOS inhibition appears to play a major role in the skeletal muscle vasodilator responses to mental stress in humans. This NO release appears to be mediated by local factors and circulating catecholamines acting on beta-2 receptors. At this time it does not appear to be neurally mediated.

Where Are We Going From Here?

The extent to which sympathetic vasoconstrictor nerves can evoke constriction in contracting skeletal muscles remains controversial. Depending on the exercise model—and the experiment designed and the way the data are analyzed—it appears that contractions may limit the ability of sympathetic nerves to cause vasoconstriction in active muscles (Thomas and Victor, 1998). There are many potential explanations for this blunted constrictor response, including the possibility that substances released by the contracting muscle might cause presynaptic inhibition of norepinephrine release. There is also the possibility that these same substances interfere with the ability of the alpha-constrictors to evoke vasoconstriction. Recent evidence suggests that α_2-receptors are especially sensitive to acidosis, hypoxia, and metabolites.

A variety of studies conducted by Dr. Gail Thomas and her colleagues at the University of Texas Southwestern Medical School in Dallas suggest that NO might also be a key molecule that will inhibit α_2-mediated vasoconstriction in skeletal muscle. Therefore, studies on this topic in humans are current areas of interest for our laboratory (Tschakovsky et al., 2002). Another important area of research remains the factor or factors that cause neurally-mediated cutaneous vasodilation during body heating in humans. Research on this issue will likely take advantage of microdialysis to selectively apply various agonists and antagonists to small areas of skin (Minson et al., 2001). Of particular interest will be the contribution of numerous vasoactive peptide molecules to this response. Finally, studies in both human muscle and skin are needed to address the role of endothelial-derived hyperpolarizing factor in vascular regulation.

SUMMARY

Over the past 10 years we have attempted to determine the role of NO during physiological vasodilation in humans. We initially thought that NO might explain

a variety of forms of "unexplained" vasodilation. However, in three of the four issues we have studied, the role of NO is modest at best. In the fourth study (mental stress), NO plays a key role but the source of the NO remains unknown. There are several general conclusions that can be drawn from these observations. First, over many years a variety of new substances that cause vasodilation have been identified. Typically there has been great enthusiasm for these substances as explainers of the unexplained. After a period of initial enthusiasm, however, it has frequently been shown that many of these substances contribute or can contribute to a variety of responses, but they are not obligatory.

Second, in the case of the cutaneous vasodilator responses to body heating, and the skeletal muscle dilator responses to exercise, both of these responses are absolutely essential for survival. Therefore it is reasonable to postulate that while NO might play an important role under some circumstances in these responses, there are redundant mechanisms which govern the vasodilation associated with heating and exercise. Since NO does not appear obligatory, perhaps our findings are another demonstration of the general concept that redundant mechanisms govern physiological responses that are especially essential for survival. Third, our current focus on how NO and sympathetic vasoconstrictor nerves interact on a short- and long-term basis continues to highlight the key role that the autonomic nervous system plays in the regulation of circulation in humans.

Acknowledgments

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