Carbon Monoxide Promotes Endothelium-Dependent
Constriction of Isolated Gracilis Muscle Arterioles

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Running head: Carbon monoxide and endothelium-dependent constriction

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Abstract

Vascular tissues express heme oxygenase that metabolizes heme to form carbon monoxide (CO). CO promotes relaxation of vascular smooth muscle, but also inhibits nitric oxide (NO) formation. This study examines the hypothesis that CO promotes endothelium- and NO synthase-dependent vasoconstriction of isolated arterioles. Studies were conducted on pressurized first-order gracilis muscle arterioles isolated from anesthetized male Sprague-Dawley rats. Exogenous CO, as well as a heme precursor, delta-aminolevulinic acid (δ-ALA) constricted arterioles with intact endothelium pretreated with phenylephrine; these effects were abolished by endothelium removal. CO and δ-ALA-induced vasoconstrictions were converted to dilations by pretreatment with an inhibitor of NO synthase, Nω-nitro-L-arginine methyl ester (L-NAME) or by pretreatment with L-NAME and a NO donor, sodium nitroprusside. Furthermore, CO-induced vasoconstriction was prevented by pretreatment with the NO synthase substrate, L-arginine. This study shows that exogenous, as well as endogenously-formed CO can promote endothelium-dependent vasoconstriction in isolated gracilis muscle arterioles. Since CO-induced vasoconstriction is abolished by NO synthase blockade and by L-arginine, CO most likely promotes endothelium-dependent vasoconstriction by inhibiting endothelial NO formation.

Key words: heme oxygenase, carbon monoxide, nitric oxide, vascular tone, delta-aminolevulinic acid
Carbon monoxide can be generated in the body via the metabolic degradation of heme by heme oxygenase (20). There is now considerable evidence to suggest that endogenously formed carbon monoxide participates in the regulation of cardiovascular functions (2,6,24). The original hypothetical article by Marks et al. (13) speculated that endogenously-formed carbon monoxide might act like nitric oxide to stimulate soluble guanylate cyclase (3,8) and promote relaxation of vascular smooth muscle (3,22). Subsequently, endogenous carbon monoxide has been widely characterized as a vasodilatory regulator of vascular tone (2,6,24).

Despite the popular characterization of carbon monoxide as a vasodilator, there has been evidence to suggest that carbon monoxide can inhibit the nitric oxide system (6) and accordingly may also exert a vasoconstrictive influence on vascular tone (6). Biochemical studies have shown that carbon monoxide inhibits nitric oxide formation by binding to nitric oxide synthase (1,9,14,25). In addition, a recent report suggested that in isolated renal resistance vessels physiological concentrations of carbon monoxide (23) can suppress the activity of endothelial nitric oxide synthase (21). Therefore, the possibility exists that while carbon monoxide promotes a vasodilatory influence by acting directly on vascular smooth muscle, it may simultaneously exert a vasoconstrictive influence by inhibiting the formation of endothelial-derived nitric oxide.

The current study tests the hypothesis that carbon monoxide promotes endothelium- and nitric oxide synthase-dependent vasoconstriction. For this purpose, experiments were conducted using pressurized first-order gracilis muscle rat arterioles with or without intact endothelium, and arterioles with nitric oxide synthase blockade. In these preparations we examined the effects of exogenous and endogenous carbon monoxide on arteriolar diameter.

**Methods**

**Chemicals**

Carbon monoxide gas was purchased from Air Liquide (Harvey, LA). Delta-aminolevulinic acid (δ-ALA) was obtained from Frontier Scientific (Logan, UT). Nω-nitro-L-arginine methyl ester (L-NAME), L-arginine hydrochloride, sodium nitroprusside (SNP), phenylephrine and acetylcholine were purchased from Sigma Aldrich (St. Louis, MO). All other
chemicals were obtained from Fisher Scientific (Houston, TX). L-NAME, SNP, phenylephrine, L-arginine, and acetylcholine were dissolved in modified Krebs’ buffer immediately before use. Carbon monoxide saturated solution (1 mmol/l) was prepared by bubbling ice-cold modified Krebs’ buffer with carbon monoxide gas for 20 minutes. The final concentration of carbon monoxide in the microvessel chamber (0.1-100 µmol/l) was achieved by pre-dilution with ice-cold modified Krebs’ buffer in the infusion syringe (for concentrations 0.1, 1, and 10 µmol/l) and final dilution with superfusion buffer (modified Krebs’ buffer at 37 °C). Stock and final microvessel chamber concentrations of carbon monoxide were confirmed by combining samples with freshly lysed rat blood, and then calculating from the resulting carboxyhemoglobin content (OSM3, Radiometer America Inc., Westlake, OH). The composition of modified Krebs’ buffer was (mmol/l): NaCl 118.5; KCl 4.7; CaCl\textsubscript{2} 1.4; KH\textsubscript{2}PO\textsubscript{4} 1.2; MgSO\textsubscript{4} 1.1; NaHCO\textsubscript{3} 25.0; and dextrose 11.1.

**Animals**

Male Sprague-Dawley rats (250-300g; n=70; Harlan, Indianapolis, IN) were used in these studies approved by the Institutional Animal Care and Use Committee. Rats were housed in a controlled environment and had free access to commercial rat chow and tap water.

**Microvascular Preparation**

Rats were anesthetized with a single dose of thiobutabarbitral sodium (Inactin; 140 mg/kg, ip), and heparinized (1000 U/kg, iv). The gracilis anticus muscles were removed and segments of the first-order gracilis muscle arterioles were isolated as previously detailed (19). Individual arteriolar segments were cannulated at both ends with glass micropipettes in a water-jacketed vessel chamber (18 ml volume, Instrument Shop, New York Medical College, Valhalla, NY). Silicone tubing (Masterflex, Cole-Parmer, Vernon Hills, IL) connected the distal micropipette to a stopcock and the proximal micropipette to a reservoir containing modified Krebs’ buffer. The height of the reservoir was adjusted to 108.8 cm above the level of the arteriole to achieve 80 mmHg intraluminal pressure. The vessel chamber was superfused continuously via a nonrecirculating system (Masterflex L/S pump, Cole-Parmer) with oxygenated (14% O\textsubscript{2} - 5%
CO₂ - balanced with N₂) modified Krebs’ buffer (5 ml/min) at 37°C (Isotemp 2100 immersion circulator, Fisher Scientific, Houston, TX). For internal diameter measurements the vessel chamber was mounted on the stage of a microscope (Micromaster, Fisher Scientific) that was fitted with a video camera (video microscope package, Fisher Scientific) leading to a video caliper (Texas A&M, College Station, TX) and a TV-VCR (Zenith, Sears). With this setup a magnified image of the arteriolar segment was viewed on the TV screen and internal diameter was measured throughout the experiment by manually adjusting the white guides superimposed by the video caliper.

Mounted vessels were allowed to stabilize for 60 minutes before the initiation of the experiments. Only vessels that developed an active tone during the stabilization period were used for the studies. Pretreatment drugs (ie. phenylephrine, L-NAME, SNP, or L-arginine) were included in the superfusion buffer. Carbon monoxide saturated solution (1 mmol/l) or δ-ALA stock solution (80 mmol/l) was added directly to the microvessel chamber followed by a continuous infusion (SAGE pumps, model M361, Boston, MA) into the superfusion buffer to quickly achieve and maintain the desired concentration of carbon monoxide (0.1-100 µmol/l) or δ-ALA (80 µmol/l) in the chamber without interrupting chamber superfusion.

**Experimental Design**

**Arterioles with intact endothelium**

The following experiments were designed to study endothelium-dependent effects of exogenous and endogenously-formed carbon monoxide on arteriolar diameter. For these experiments, the arteriolar endothelium was left intact and microvessels were pretreated with a vasoconstrictor, phenylephrine (100 nmol/l), which was continued throughout the remainder of the experiment; this concentration of phenylephrine was used to match the internal diameter of denuded and L-NAME pretreated vessels. After 25 minutes of pretreatment, 0.1-100 µmol/l carbon monoxide or 80 µmol/l δ-ALA administration was started and internal diameter was monitored for 20 minutes. Recently, it has been reported that small amounts of carbon monoxide release nitric oxide (0.01µmol/l carbon monoxide) and promote vasodilation (0.1µmol/l carbon
monoxide), but physiological concentrations of carbon monoxide (1-10 µmol/l) inhibit nitric oxide synthesis and release in isolated renal resistance vessels (21). To explore this potentially bimodal response, we examined the effects of exogenous carbon monoxide in arterioles with intact endothelium in the concentration range of 0.1 to 100 µmol/l. Endogenous free heme levels are estimated to be around 0.5 µmol/l (6). Eight δ-ALA molecules are used for the synthesis of one heme molecule, and we used 80 µmol/l δ-ALA to enhance heme synthesis. At the end of the experiment the presence of functional endothelium was confirmed by studying the vasodilatory responses to 1 µmol/l acetylcholine.

Arterioles denuded of endothelium

The following experiments were designed to study the endothelium-independent effects of exogenous and endogenously-formed carbon monoxide on arteriolar diameter. After the stabilization period, the arteriolar endothelium was removed by perfusing 2 ml of air through the lumen, as previously described (19). After an additional 40 minute stabilization period, 50 µmol/l carbon monoxide or 80 µmol/l δ-ALA administration was started and internal diameter was monitored for 20 minutes. At the end of the experiments the absence of functional endothelium was confirmed by the lack of vasodilatory response to 1 µmol/l acetylcholine.

Arterioles with nitric oxide synthase blockade

Experiments were designed to study the nitric oxide system-independent effects of exogenous- and endogenously-formed carbon monoxide on arteriolar diameter. For these experiments, the microvessel endothelium was left intact. After the stabilization period, microvessels were exposed to an inhibitor of nitric oxide synthase, L-NAME (1 mmol/l). Exposure to the inhibitor was continued throughout the remainder of the experiment. Previous experiments have found that this concentration of L-NAME was required to cause maximal constriction of isolated gracilis muscle arterioles (Johnson FK, and Johnson RA unpublished data, [2000]) suggesting maximal achievable blockade of the nitric oxide system. After 55 minutes of pretreatment, 50 µmol/l carbon monoxide or 80 µmol/l δ-ALA administration was started and internal diameter was monitored for 20 minutes.
Arterioles with “nitric oxide clamp”

The following experiments were designed to identify the nitric oxide synthase-independent effects of exogenous carbon monoxide on arteriolar diameter. For these experiments, the microvessel endothelium was left intact. After the stabilization period, arterioles were pretreated with L-NAME (1 mmol/l) to block nitric oxide synthase activity and with a nitric oxide donor, SNP (10-30 nmol/l), to maintain internal diameter at before-pretreatment levels (“nitric oxide clamp”). Exposure to the clamp was continued throughout the remainder of the experiment. With this design we achieved maximal blockade of the nitric oxide synthase enzyme while maintaining the activity of the vascular nitric oxide signaling system by replacing endogenous nitric oxide with a nitric oxide donor. After a 55 minute pretreatment period, 50 µmol/l carbon monoxide administration was started and internal diameter was monitored for 20 minutes.

Arterioles pretreated with L-arginine

The following experiments were designed to study the L-arginine-dependent effects of exogenous carbon monoxide on arteriolar diameter. For these experiments, the microvessel endothelium was left intact. After the stabilization period, arterioles were pretreated with a vasoconstrictor, 100 nmol/l phenylephrine, and the substrate for nitric oxide synthesis, 1 mmol/l L-arginine. This pretreatment regime was continued throughout the remainder of the experiment. With this design we provided excess substrate for nitric oxide synthesis. After 60 minutes of pretreatment, 50 µmol/l carbon monoxide administration was started and internal diameter was monitored for 20 minutes.

Statistics

Data are expressed as mean ± S.E.M. Data showing time-related vascular responses were analyzed by analysis of variance (ANOVA) and then further post-hoc comparisons were made by performing orthogonal contrasts (18) using a statistical package (SYSTAT), when appropriate. Data showing concentration-related vascular responses to exogenous carbon monoxide were analyzed by t-tests. P<0.05 was considered statistically significant.
Results

During the stabilization period, internal diameter of isolated gracilis muscle arterioles decreased spontaneously from 195±2 µm to 131±3 µm (n=70; P<0.05). Phenylephrine (100 nmol/l) pretreatment promoted vasoconstriction (132±3 to 98±3 µm; n=35; P<0.05). Endothelium removal resulted in a decrease in internal diameter (144±10 to 110±6 µm; n=12; P<0.05). Pretreatment with an inhibitor of nitric oxide synthase, 1 mmol/l L-NAME, resulted in a comparable vasoconstriction (123±7 to 85±9 µm; n=10; P<0.05). However, the nitric oxide clamp - pretreatment with a nitric oxide synthase inhibitor, 1 mmol/l L-NAME, and a nitric oxide donor, 10-30 nmol/l SNP - caused a moderate increase in arteriolar diameter (101±12 to 115±10 µm; n=6; P<0.05). Arterioles pretreated with 100 nmol/l phenylephrine and 1 mmol/l L-arginine had larger internal diameters than vessels pretreated with phenylephrine only (99±6 µm; n=7 vs. 91±5 µm; n=14; P<0.05).

In arterioles with intact endothelium (Figure 1, panel A), exogenous carbon monoxide (50 µmol/l) promoted a sustained vasoconstriction (85±5 to 66±4 µm; n=8; P<0.05). Endothelium removal (Figure 1, panel B) abolished the vasoconstrictor response to carbon monoxide (111±9 to 117±9 µm; n=8; P>0.05). Figure 2 shows concentration-dependent responses to exogenous carbon monoxide in arterioles with intact endothelium measured as the average of the last 4 measurements (1 measurement/minute) at the end of the twenty-minute exposure. The figure shows that carbon monoxide promoted concentration-dependent vasoconstriction that was evident at 1 µmol/l (Δ_max=-6±1 µm; n=5; P<0.05 vs. vehicle) and maximum at 50 µmol/l (Δ_max=-17±3 µm; n=8; P<0.05 vs. vehicle) concentrations. In arterioles with intact endothelium (Figure 3, panel A) pretreatment with a nitric oxide synthase inhibitor, 1 mmol/l L-NAME, converted carbon monoxide-induced vasoconstriction into vasodilation (91±14 to 128±11 µm; n=6; P<0.05). Similarly, in arterioles with nitric oxide clamp (Figure 3, panel B), pretreated with a nitric oxide synthase inhibitor (1 mmol/l L-NAME) and a nitric oxide donor (10-30 nmol/l SNP), carbon monoxide caused vasodilation (115±10 to 123±9 µm; n=6; P<0.05). The heme synthesis precursor, δ-ALA (80 µmol/l), promoted vasoconstriction in
arterioles with intact endothelium ($\Delta_{\text{max}}-9\pm1$ µm; $n=6$; $P<0.05$; Figure 4). δ-ALA -induced vasoconstriction was converted to vasodilation by endothelium removal ($\Delta_{\text{max}}7\pm1$ µm; $n=4$; $P<0.05$) and by pretreatment with an inhibitor of nitric oxide synthase, 1 mmol/l L-NAME ($\Delta_{\text{max}}7\pm1$ µm; $n=4$; $P<0.05$). In arterioles with intact endothelium (Figure 5, panel B) pretreatment with the nitric oxide synthase substrate, 1 mmol/l L-arginine, prevented carbon monoxide-induced vasoconstriction ($100\pm6$ to $95\pm6$ µm; $n=7$; $P>0.05$).

Discussion

The current study shows that carbon monoxide and a heme precursor, which drives endogenous carbon monoxide formation, can promote vasoconstriction in a manner that is endothelium-dependent, and L-NAME sensitive.

Carbon monoxide is commonly characterized as a vasodilatory regulator of vascular tone, as carbon monoxide has been shown to relax vascular smooth muscle (2,3,6,22,24). In addition, numerous studies have reported that both exogenously applied and endogenously-formed carbon monoxide promoted dilation of different vascular beds (2,3,6,22,24). With regard to these findings, we have similarly observed that heme-derived carbon monoxide dilated isolated gracilis muscle arterioles pretreated with an inhibitor of nitric oxide synthase (10). Still, a commonly overlooked effect of carbon monoxide is to inhibit nitric oxide synthesis by binding to the nitric oxide synthase (1,9,14,25). Seminal findings by Marletta et al. (25), and Mayer et al. (9) led to the recognition of nitric oxide synthase as a cytochrome P-450 type hemoprotein. More recently, it has been reported that small amounts of carbon monoxide release nitric oxide (21), but physiological concentrations of carbon monoxide (23) inhibit nitric oxide synthesis in isolated renal resistance vessels (21).

Our current study shows that exogenous carbon monoxide promotes concentration-dependent vasoconstriction in isolated rat arterioles with intact endothelium. Since the vasoconstrictive effect of carbon monoxide was abolished by endothelium removal, we reasoned that carbon monoxide might a) stimulate the release of an endothelium-derived constricting factor or b) inhibit the release of an endothelium-derived relaxing factor. Since previous studies
have suggested that carbon monoxide interferes with nitric oxide synthesis (1,9,14,25), we examined the effects of exogenous carbon monoxide in arterioles with intact endothelium pretreated with an inhibitor of nitric oxide synthase. Consistent with our previous reports (10), we have found that pretreatment with L-NAME converted carbon monoxide induced vasoconstriction into vasodilation. These data suggest that carbon monoxide-induced endothelium-dependent vasoconstriction is most likely due to interference with the vasodilatory effects of the endothelial nitric oxide system.

While previous studies have shown that carbon monoxide directly inhibits the nitric oxide synthase enzyme (1,9,14,25), a recent report suggested that endogenous carbon monoxide may also promote hypertension by serving as a partial agonist/antagonist for soluble guanylate cyclase (4). In that case, in arterioles pretreated simultaneously with a nitric oxide synthase inhibitor and with a nitric oxide donor (“nitric oxide clamp”), carbon monoxide should promote vasoconstriction. However, we found that carbon monoxide promotes vasodilation in vessels subjected to the nitric oxide clamp. Even so, the response was marginally attenuated when compared to arterioles pretreated only with the nitric oxide synthase inhibitor. While this attenuation may in part be due to carbon monoxide interfering with nitric oxide-induced vasodilatory mechanisms, we did not find vasoconstriction in these arterioles with nitric oxide clamp; this feature strongly suggests that carbon monoxide-induced vasoconstriction does not arise primarily from inhibition of soluble guanylate cyclase activity.

The major source of endogenous carbon monoxide production is from the enzymatic degradation of heme by heme oxygenase (20). Delta-aminolevulinic acid (δ-ALA) is a precursor for heme synthesis: eight δ-ALA molecules are required for the synthesis of one molecule of heme (15,17). In the heme synthetic cascade, δ-ALA formation is the primary point of regulation (15,17). Provision of exogenous δ-ALA drives heme synthesis (11) and consequently increases substrate availability for heme oxygenase. Radiolabeled δ-ALA has been shown to generate radiolabeled carbon monoxide in a reaction which is mediated by heme oxygenase (11). Because heme oxygenase activity appears to be regulated by substrate availability (6,16),
provision of δ-ALA increases heme oxygenase activity and consequently endogenous carbon monoxide formation (12,26) in a concentration-dependent manner (12) and the iron released during heme degradation is available to be recycled into the formation of new monomeric heme (17). Our previous studies (6,10) have used heme preparations to provide substrate for heme oxygenase (6,16). However, heme contains iron and its use may potentially contribute to detrimental iron loading of the tissues. To minimize the chances of these unwanted effects, we decided to use the heme synthesis precursor, δ-ALA to drive heme synthesis and consequently drive the endogenous formation of carbon monoxide. In this current study, we observed that the δ-ALA promoted effects that were similar to those of exogenous carbon monoxide. In arterioles with intact endothelium, δ-ALA promoted vasoconstriction, and this vasoconstrictive effect was converted to vasodilation by endothelium removal or by pretreatment with an inhibitor of nitric oxide synthase. The highly unique actions of this well established heme precursor, in conjunction with the highly unique actions of exogenously-formed carbon monoxide, strongly suggests that δ-ALA drives carbon monoxide formation in these isolated microvessels, and that its vasoconstrictive actions arise from the subsequent generation of carbon monoxide by inhibiting the vasodilatory effects of the nitric oxide system.

A previous study using isolated nitric oxide synthase has suggested that excess substrate (L-arginine) levels decrease the affinity of carbon monoxide binding to nitric oxide synthase (1,14) by binding close to the heme and/or altering the confirmation of the distal heme pocket and hence constraining carbon monoxide binding to the heme moiety (1,14). To investigate if L-arginine protects against carbon monoxide-induced vasoconstriction, we conducted experiments on arterioles with intact endothelium pretreated with L-arginine. We found that in isolated gracilis muscle arterioles pretreatment with 1 mmol/L L-arginine abolished carbon monoxide-induced vasoconstriction. These data show that L-arginine can protect against carbon monoxide-induced vasoconstriction, which further supports the notion that carbon monoxide might promote vasoconstriction by inhibiting endothelial nitric oxide synthase.

The vasodilatory effects of exogenous carbon monoxide and δ-ALA in arterioles
pretreated with L-NAME appear to reflect their actions on vascular smooth muscle. Our findings show that the vasodilatory effects of carbon monoxide may dominate in the absence of nitric oxide synthase activity when the endothelium is present. However, our findings also show that exogenous carbon monoxide, at the examined concentration, has little vasodilatory action in the absence of endothelium. Since both L-NAME-pretreated and endothelium-denuded vessels should lack nitric oxide synthase activity, the difference in carbon monoxide-induced responses suggest that perhaps other endothelium-dependent systems may be involved. Nitric oxide has been suggested to interact with other vasoactive mediators produced in the vascular endothelium, such as endothelin, and prostaglandins. Many of these systems may be altered when nitric oxide synthesis is blocked. Carbon monoxide has also been suggested to interact with some of these other endothelium-dependent vasoactive mediators (6). In the face of these observations, the possibility exists that, in L-NAME pretreated vessels, increased release of an endothelium-dependent vasodilator or a decreased release of an endothelium-dependent vasoconstrictor factor may contribute to the vasodilatory effects of carbon monoxide. However, exploration of such potential interactions is beyond the scope of the current study.

A recent study (21) reported that small amounts of carbon monoxide release nitric oxide (0.01µmol/l carbon monoxide) and promote vasodilation (0.1µmol/l carbon monoxide), but physiological concentrations of carbon monoxide (1-10 µmol/l) inhibit nitric oxide synthesis and release in isolated renal resistance vessels. We found that in our preparation 0.1µmol/l carbon monoxide did not produce statistically significant changes in arteriolar diameter compared to the matched unbubbled ice-cold vehicle controls. At larger concentrations exogenous carbon monoxide promoted concentration-dependent vasoconstriction of arterioles with intact endothelium that was evident at 1 µmol/l and maximal at 50 µmol/l concentration. Since tissue contents of carbon monoxide were reported to be between 1 and 50 pmol/mg fresh weight (23), which roughly translates to 1 to 50 µmol/l concentration, it appears that carbon monoxide can affect nitric oxide synthase activity at “physiological” concentrations.

It also appears that the inhibitory effect of carbon monoxide on endothelial nitric oxide
synthase may become more pronounced during certain pathophysiological conditions when heme oxygenase expression and/or endogenous carbon monoxide formation are increased. Specifically, induction of heme oxygenase-1 has been shown to attenuate muscarinic agonist-induced nitric oxide release (21) and vasodilation (7) in isolated renal resistance vessels. We recently reported that vascular heme oxygenase-1 levels were increased in Dahl/Rapp salt-sensitive rats with salt-induced hypertension (5). Skeletal muscle arterioles isolated from these hypertensive rats showed abolished endothelium-dependent vasodilatory responses, which were completely restored by acute in vitro treatment with an inhibitor of endogenous carbon monoxide formation (5). Therefore, it appears that endogenous levels of carbon monoxide are able to inhibit endothelial nitric oxide synthase activity under physiological conditions, but this inhibitory effect becomes more severe during certain pathophysiological conditions when endogenous carbon monoxide formation is increased.

In summary, our data show that in isolated gracilis muscle arterioles with intact endothelium carbon monoxide and a heme synthesis precursor, δ-ALA, promote endothelium-dependent vasoconstriction. In arterioles pretreated with an inhibitor of nitric oxide synthase, as well as in arterioles pretreated with an inhibitor of nitric oxide synthase and a nitric oxide donor, carbon monoxide promotes vasodilation. Furthermore, pretreatment with the nitric oxide synthase substrate abolishes carbon monoxide-induced vasoconstriction. These data suggest that in isolated arterioles both exogenous and endogenously-formed carbon monoxide can promote endothelium-dependent vasoconstriction most likely by inhibiting endothelial nitric oxide synthesis.
Acknowledgments

This work was supported by National Heart, Lung and Blood Institute grant 1R01HL64577 (P.I.-Robert A. Johnson, PhD) and by the American Heart Association, Southeast Affiliate postdoctoral fellowship 0020335B (P.I. - Fruzsina K. Johnson, MD).
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**Figure Legends**

Figure 1. Effects of exogenous carbon monoxide (CO; 50 µmol/l) on internal diameter of isolated pressurized first-order gracilis muscle arterioles in the presence of endothelium pretreated with 100 nmol/l phenylephrine (PE) (panel A), and in the absence of functional endothelium (panel B). Data are expressed as mean±S.E.M. * indicates P<0.05 relative to control diameter before carbon monoxide treatment.

Figure 2. Effects of exogenous carbon monoxide (CO; 0.1-100 µmol/l) or matched ice-cold buffer vehicle (Veh) on internal diameter of isolated pressurized first-order gracilis muscle arterioles in the presence of endothelium pretreated with 100 nmol/l phenylephrine. Maximal response calculated as the average of the last 4 measurements (1 measurement/minute) at the end of the twenty-minute exposure period. Multiple observations each: n=5 for vehicle, n=4 for 0.1, n=5 for 1, n=4 for 10, n=8 for 50 and n=3 for 100 µmol/l carbon monoxide. Data are expressed as mean±S.E.M. * indicates P<0.05 relative to vehicle control.

Figure 3. Effects of exogenous carbon monoxide (CO; 50 µmol/l) on internal diameter of isolated pressurized first-order gracilis muscle arterioles in the presence of functional endothelium pretreated with a nitric oxide synthase inhibitor, 1 mmol/l Nω-nitro-L-arginine methyl ester (L-NAME) (panel A), and pretreated with 1 mmol/l L-NAME and a nitric oxide donor, sodium nitroprusside (SNP; 10-30 nmol/l) (panel B). Data are expressed as mean±S.E.M. * indicates P<0.05 relative to diameter before carbon monoxide treatment.

Figure 4. Effects of a heme synthesis precursor, delta-aminolevulinic acid (δ-ALA; 80 µmol/l) on internal diameter of isolated pressurized first-order gracilis muscle arterioles in the absence of functional endothelium (open circles), in the presence of endothelium pretreated with 100 nmol/l phenylephrine (PE) (closed circles), and in the presence of endothelium pretreated with an inhibitor of nitric oxide synthase, 1 mmol/l Nω-nitro-L-arginine methyl ester (L-NAME) (closed circles).
squares). Data are expressed as mean±S.E.M. * indicates P<0.05 relative to control diameter before δ-ALA treatment in endothelium denuded and PE-pretreated vessels. † indicates P<0.05 relative to control diameter before δ-ALA treatment in L-NAME-pretreated vessels.

Figure 5. Effects of exogenous carbon monoxide (CO; 50 µmol/l) on internal diameter of isolated pressurized first-order gracilis muscle arterioles in the presence of endothelium pretreated with 100 nmol/l phenylephrine (PE) (panel A), and pretreated with 100 nmol/l phenylephrine and the nitric oxide synthase substrate 1 mmol/l L-arginine (panel B). Data are expressed as mean±S.E.M. * indicates P<0.05 relative to control diameter before carbon monoxide treatment.
Figure 1.

A) Intact Endothelium

Internal Diameter (µm)

B) Denuded of Endothelium

Internal Diameter (µm)
Figure 2.
Figure 3.
Figure 4.
Figure 5.