# Nitric Oxide and Cardiovascular Regulation
## Beyond the Endothelium

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The importance of nitric oxide (NO) for normal cardiovascular regulation and health has been well established. However, the large majority of the focus and knowledge about NO has revolved around the endothelium and endothelial derived NO. Aside from its importance for blood flow and blood pressure via endothelium-dependent vasodilation,[1,2] endothelial NO synthase (eNOS) has been shown to have numerous other vascular protective effects including, but not limited to, inhibition of platelet aggregation and adhesion, promotion of angiogenesis, anti-inflammation, and inhibition of the atherosclerotic process.[3,4] In addition, eNOS has important cardiac effects. Thus, it is clear that impairments in eNOS-derived NO can have deleterious consequences and play a role in the disease process. Although the aforementioned functions of eNOS cannot be overstated, the synthesis of NO via neuronal NOS (nNOS) may also be critical for cardiovascular regulation and health. Indeed, nNOS along with eNOS is constitutively expressed in mammalian cells, and an emerging body of research, mainly performed in animals, has indicated that nNOS may also be important for the regulation of vasomotor tone and blood pressure.[5] However, much less is known about nNOS in humans.

In the current issue of *Hypertension*, Shabeeh et al[6] report results from the first human study to systemically infuse the selective nNOS inhibitor, S-methyl-L-thiocitrulline (SMTC). In healthy young men, SMTC was intravenously infused in escalating dosages with each dose administered >10 minutes. In comparison to placebo (saline infusion), SMTC at low dosages (0.1 and 0.3 μmol/kg) had no effect on blood pressure, whereas higher dosages (1.0 and 3.0 μmol/kg) elicited dose-dependent increases in mean arterial pressure and diastolic blood pressure that were accompanied by reductions in heart rate. The highest dose of SMTC was also shown to evoke a reduction in cardiac output and an increase in systemic vascular resistance. Thus, these studies indicate that nNOS is playing a role in resting blood pressure and basal vascular tone.

These studies are predicated on the selectivity of SMTC for nNOS inhibition. To address this, during SMTC and placebo infusions, the authors performed measures of flow-mediated dilation, which is an index of eNOS-dependent vasodilation. Flow-mediated dilation measures were unaffected by the systemic infusion of SMTC, indicating that eNOS was not inhibited. These results are consistent with previous work by this same group demonstrating that local brachial artery infusion of SMTC had no effect on radial artery flow-mediated dilation in healthy subjects.[7] Combined with other studies identifying the selectivity of SMTC for nNOS over eNOS, the hemodynamic and pressor responses elicited in the current study can be ascribed to inhibition of nNOS.

The key question now with these results is how SMTC is eliciting changes in systemic vascular resistance and blood pressure (Figure). This is a challenge with a systemic infusion and the presence of nNOS in the central nervous system, autonomic and perivascular nerves, smooth muscle, skeletal muscle, and cardiac muscle.[8] As alluded to by the authors, local effects of SMTC may include inhibiting the release of NO from perivascular nitrergic nerves or inhibiting the release of hydrogen peroxide, a vasodilator in the microvasculature. Both of these mechanisms would be suggestive of a local inhibition of nNOS in the microcirculation, which is consistent with previous studies demonstrating a reduction in forearm blood flow during local brachial artery infusion of SMTC.[7] However, additional studies are needed to test such effects of SMTC in humans. Likewise, given the systemic infusion of SMTC used by Shabeeh et al,[6] an activation of the sympathetic nervous system via inhibition of NO in the brain warrants consideration. Indeed, a growing body of research indicates that NO is a key signaling molecule in the tonic restraint of central sympathetic outflow.[9,10] Plasma norepinephrine or direct recordings of sympathetic nerve activity during systemic infusion of SMTC would be needed to determine whether sympathetic activation is involved. It will also be important to consider the impact of arterial baroreflex activation from the SMTC-induced rise in blood pressure on systemic vascular resistance. A control experiment with a different pharmacological agent in which blood pressure is elevated to a similar extent as during nNOS inhibition would be helpful to begin to elucidate potential baroreflex and non–baroreflex-mediated effects of SMTC. This experiment would also be important in understanding the significant reduction in heart rate (and cardiac output) observed during SMTC infusion. As with all good studies, a fundamental foundation is built for which additional questions and experiments arise. Shabeeh et al[6] have provided this



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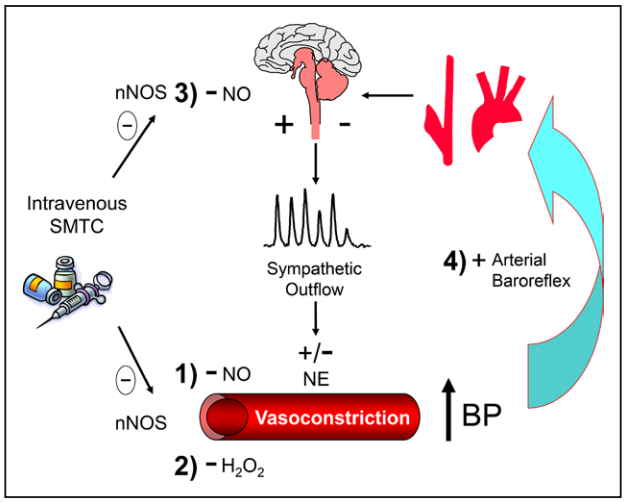


**Figure.** Schematic representation of some of the mechanisms to consider for peripheral vasoconstriction with systemic infusion of S-methyl-L-thiocitrulline (SMTC). (1) The most direct effect would be inhibition of neuronal NO synthase (nNOS) and NO release from perivascular nitrergic nerves; (2) inhibition of the release of hydrogen peroxide ($H_2O_2$), a vasodilator in the microvasculature; and (3) inhibition of NO in the brain and activation of the sympathetic nervous system and release of norepinephrine (NE). Importantly, these effects of SMTC to induce vasoconstriction may be offset by arterial baroreflex activation because of the SMTC-induced rise in blood pressure (BP; 4).

foundation. Notably, the participants for the current studies were all men so caution should be used in extrapolating these findings to women until additional studies are performed and potential sex differences are elucidated.

Regardless of the mechanisms involved, these exciting, novel data force us to think beyond eNOS in terms of NO in human health and disease. Likewise, it is important to now reconsider previous work infusing nonselective NOS inhibitors systemically and the potential contribution of nNOS to the reported findings. Nonetheless, although it is clear that endothelium-derived NO is critical for vascular health and has prognostic value in terms of cardiovascular disease risk and mortality,[11,12] nNOS-derived NO may also be contributing importantly. Indeed, an emerging body of literature is advancing a role for nNOS in the regulation of blood flow not only in skeletal muscle but also in the cerebral, renal, and coronary circulations.[5] Thus, aside from its known importance for synaptic plasticity, learning, and memory, nNOS may also be important for a healthy vasculature to prevent hypertension and atherosclerosis.[8,13] In summary, the NO story continues, and by performing the first systemic infusion of SMTC in humans, Shabeeh et al[6] provide novel data that further call for thinking beyond eNOS-derived NO production in human health and disease.

## Disclosures

None.

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