Brief Communications

$N^G$-Methyl-L-Arginine, an Inhibitor of L-Arginine-Derived Nitric Oxide Synthesis, Stimulates Renal Sympathetic Nerve Activity In Vivo

A Role for Nitric Oxide in the Central Regulation of Sympathetic Tone?

Ichiro Sakuma, Hiroko Togashi, Mitsuhiro Yoshioka, Hideya Saito, Miwa Yanagida, Mamoru Tamura, Takeshi Kobayashi, Hisakazu Yasuda, Steven S. Gross, and Roberto Levi

Continuous production of endothelium-derived nitric oxide (NO) in peripheral vessels has been shown to modulate vascular resistance and blood pressure. NO is also formed in the brain upon activation of glutamate receptors, which are thought to mediate central autonomic reflexes. In the present study we assessed whether NO plays a role in central autonomic regulation. For this, we have investigated the effects of $N^G$-methyl-L-arginine (NMA), a selective inhibitor of NO synthesis from L-arginine, on sympathetic renal nerve activity (RNA), blood pressure, and heart rate in the anesthetized rat. NMA elicited a dose-dependent sustained increase in blood pressure (approximately 20 and 30 mm Hg, 5 minutes after 10 and 50 μmol/kg i.v., respectively). Heart rate and RNA decreased transiently (15 beats per minute and 40%, respectively); RNA subsequently increased (100%) while blood pressure remained elevated. Baroreceptor deafferentation markedly altered these responses to NMA; the transient decreases in heart rate and RNA were abolished, whereas the increases in RNA and blood pressure were significantly potentiated. After spinal C-1-C-2 transection, there was no increase in RNA, and blood pressure increased to a smaller extent. L-Arginine blocked the NMA-induced increases in blood pressure and RNA. Thus, in addition to modulating vascular resistance by a peripheral action, NO may also play a role in the central regulation of sympathetic tone. (Circulation Research 1992;70:607–611)

KEY WORDS • nitric oxide • $N^G$-methyl-L-arginine • central sympathetic regulation • blood pressure regulation • vasoconstrictors • renal nerve activity

Nitric oxide (NO), or a molecule capable of forming NO, is a major endothelium-derived relaxing factor (EDRF). It is synthesized from L-arginine by an enzymatic pathway that is selectively inhibited by $N^G$-methyl-L-arginine (NMA). The intravenous administration of NMA to guinea pigs, rabbits, rats, and dogs elicits a sustained increase in arterial blood pressure (BP); L-arginine reverses this response. These findings indicate that basal EDRF/NO release from peripheral vessels elicits a continuous vasorelaxant action in vivo.

EDRF is also produced in the brain; Garthwaite et al. found that, on activation of L-glutamate receptors, rat cerebellar cells release EDRF/NO, which in turn elevates intracellular cGMP. Additional investigations have provided evidence for the enzymatic formation of NO from L-arginine in cytosol from rat forebrain synaptosomes and bovine brain. More recently, evidence was presented in favor of the view that serotonin induces EDRF/NO production in NG108-15 mouse neuroblastoma-rat glioma hybrid cells. These observations have raised the possibility that the EDRF/NO-cGMP system may function as an important signal transduction system in the brain. Indeed, new evidence demonstrating that NO synthase is present in discrete neuronal populations further implies a neural messenger role for NO.

Since L-glutamate and serotonin receptors have been implicated in central autonomic reflexes, it is conceivable that sympathetic nerve activity would be modulated by EDRF/NO. If so, blocking EDRF/NO production in the brain with NMA might be expected to alter
sympathetic tone by a central mechanism and consequently elicit changes in BP and heart rate (HR). The present study investigates the role of EDRF/NO in the regulation of sympathetic outflow and the impact of centrally formed NO on cardiovascular regulation.

**Materials and Methods**

*Measurements of Hemodynamic Parameters*

Male Wistar rats weighing between 350 and 400 g were anesthetized with α-chloralose (50 mg/kg i.p.) and urethane (500 mg/kg i.p.) and immobilized with gallamine triethiodide (10 mg/kg i.p.). Ventilation was maintained artificially (model 683, Harvard Apparatus, South Natick, Mass.) using room air via a tracheal cannula. Temperature was monitored intrarectally and maintained close to 38°C with a heating pad. The left femoral vein was cannulated for the injection of drugs; the left femoral artery was cannulated and connected to a pressure transducer (model TP-10T1, Nihon Koden, Tokyo) for the continuous measurement of phasic and mean BP, as well as HR. In some rats, total deafferentation was performed by bilateral surgical section of vagal and sinoaortic nerves. For this, the carotid sinus, aortic depressor, and vagal nerves were cut bilaterally at their junctions with the glossopharyngeal and superior laryngeal nerves.10

*Nerve Recordings*

Postganglionic sympathetic renal nerves and, in some rats, preganglionic sympathetic adrenal nerves were approached retroperitoneally and dissected under a microscope. The exposed nerves were cut and covered with liquid paraffin. Efferent activity was recorded from the central cut end of the nerve with a bipolar platinum-iridium electrode. Impulses were amplified by a preamplifier (model AVB-10, Nihon Koden) with high and low cut-off frequency of 1 kHz and 150 Hz, respectively. The amplified signals were separated from noise levels with a window discriminator and counted every 10 seconds using a real-time data analyzer (model ATAC-450, Nihon Koden). Nerve activity is expressed as the percent of control value measured before drug administration.

**Chemicals**

NMA was prepared from l-ornithine and a thiosulfon-dourea by adaptation of the procedure of Corbin and Reporter21; NMA was chromatographically pure as determined by amino acid analysis by high-performance liquid chromatography. The flavianate salt of NMA was converted to the hydrochloride salt by stirring with Dowex-1-chloride (Sigma Chemical Co., St. Louis, Mo.). All other compounds were of the highest grade available from Sigma.

**Statistics**

Results, expressed as mean±SEM, were analyzed by analysis of variance. Differences of means were further assessed with Dunnett’s test (when comparing two groups, Figures 2 and 4) or Duncan’s multiple comparison test (when comparing three groups, Figure 3). A value of p<0.05 was considered statistically significant.

**Results**

Intravenous bolus administration of NMA (10–50 µmol/kg) to anesthetized rats elicited a dose-dependent increase in BP that lasted >10 minutes (Figures 1 and 2). The pressor response to NMA was associated with a decrease in HR and a biphasic change in renal nerve activity (RNA) (Figures 1 and 2). RNA decreased initially until the concomitant increase in BP reached a plateau and subsequently increased beyond the pre-NMA level while BP remained elevated (Figures 1 and 2).

After total deafferentation (i.e., vagotomy plus sinoaortic denervation), the pressor response to NMA was potentiated, and no decrease in HR occurred (Figure 1; also compare columns a and b in Figure 3). Moreover, in the deafferentated rat, there was no initial decrease in RNA but only a monophasic increase (Figure 1; also compare columns a and b in Figure 3). Spinal transection in the deafferentated rat abolished the NMA-induced increase in RNA (compare columns b and c in Figure 3). Changes in adrenal nerve activity in response to NMA in the deafferentated rat were similar to those in RNA: the percent increase at 1, 5, and 10 minutes after the administration of an intravenous bolus of NMA (50 µmol/kg) was 0±10%,
FIGURE 2. Bar graphs depicting time courses of changes in renal nerve activity (RNA), mean arterial blood pressure (mBP), and heart rate (HR) after a bolus intravenous administration of either saline (column a) or Nω-methyl-L-arginine (NMA) (10 μmol/kg in column b and 50 μmol/kg in column c). Bars represent mean ± SEM of changes from basal values before NMA injection measured at 1, 5, and 10 minutes after NMA. Basal mBP values in rats injected with saline (n=6), 10 μmol/kg NMA (n=9), and 50 μmol/kg NMA (n=6) were 91±8, 86±10, and 85±8 mm Hg, respectively; basal HR values were 426±9, 396±15, and 389±16 beats per minute, respectively. *p<0.05, **p<0.01, and ***p<0.001 vs. saline-injected control at corresponding time.

FIGURE 3. Bar graphs depicting modification by bilateral vagotomy (Vag), sinoaortic denervation (SAD), and spinal transection between C-1 and C-2 (spinal cut) of the effects of an intravenous bolus of Nω-methyl-L-arginine (NMA, 50 μmol/kg) on renal (RNA) or adrenal (ANA) nerve activity, mean arterial blood pressure (mBP), and heart rate (HR) in the anesthetized rat. Bars represent mean ± SEM of changes (measured at 1, 5, and 10 minutes after NMA) from basal values before NMA injection. Basal mBP values in rats with nerve intact (n=6, column a), Vag+SAD (n=6, column b), and Vag+SAD+spinal cut (n=6, column c) were 85±8, 86±6, and 54±3 mm Hg, respectively. Basal HR values were 389±16, 436±20, and 349±8 beats per minute, respectively. Basal mBP and HR values in rats with Vag+SAD (n=10, column d) were 91±8 mm Hg and 425±12 beats per minute, respectively. **p<0.01 vs. nerve intact; #p<0.05 vs. Vag+SAD at corresponding time.

Discussion

NMA elicited a dose-dependent increase in arterial BP and a decrease in HR in the intact α-chloralose/urethane-anesthetized rat. Similar changes were previously reported to occur in barbiturate-anesthetized guinea pigs, rabbits, rats, and dogs. In contrast, an NMA-induced increase in RNA had not been described previously. We observed an initial transient decrease in RNA, followed by a substantial increase despite a continuous elevation in BP. Inasmuch as an elevation in BP by NMA should elicit a reflex decrease in sympathetic outflow, which would complicate our analysis of NMA action on RNA, we sought to eliminate this reflex by total deafferentation. Abolition of the baroreceptor reflex uncovered a slow-onset monophasic increase in RNA and potentiated the increase in BP seen with NMA; both effects were abolished by spinal transection.
These findings indicate that NMA crosses the blood-brain barrier and directly stimulates sympathetic nerve activity.

Since NO/EDRF may be capable of modulating efferent neurotransmission at neuronal junctions,16 the site of the NMA-induced increase in RNA could be either at the spinal level or the celiac ganglion. However, spinal transection between C-2 and C-5 abolished the increase in postganglionic RNA caused by NMA. Furthermore, in the deafferentated rat, NMA elicited similar increases in postganglionic (renal) and preganglionic (adrenal) nerve activity. Accordingly, the site of the neural action of NMA appears to be central rather than peripheral; indeed, we have obtained preliminary evidence of a pressor effect of NMA injected into the cisterna magna at doses lower than those shown to increase BP upon intravenous administration.22

To investigate whether the effects of NMA on RNA were due to specific inhibition of EDRF/NO synthesis, the deafferentated rat was pretreated with L-arginine. L-Arginine elicited a transient dose-dependent decrease in RNA associated with a small reduction in BP and HR at the higher dose tested (500 μmol/kg). It is conceivable that these effects of L-arginine arise from an enhanced NO synthesis in the brain. We found that the administration of NMA to L-arginine-pretreated rats (after the direct effects of L-arginine had waned) failed to elicit an increase in RNA (see Figure 4). We interpret this to indicate that the NMA-induced increase in RNA is due to NO synthase inhibition via substrate competition. Furthermore, we have also found (data not shown) that another specific inhibitor of EDRF/NO synthesis, NGL-nitro-L-arginine, increases both BP and RNA in the deafferentated rat. Collectively, our data suggest that NMA increases sympathetic nerve activity by inhibiting neuronal EDRF/NO synthesis.

Most likely, EDRF/NO is generated in central neurons,17 where it increases intracellular cGMP levels, thereby modulating neuronal signal transduction.16 A possible site for the central action of NMA on RNA is the nucleus tractus solitarii, which receives a large population of primary afferent fibers from systemic baroreceptors and can regulate sympathetic outflow.17 Since the neurotransmitter of the afferent vagal neurons is L-glutamate,17 which has been shown to stimulate the generation of EDRF/NO from L-arginine in the rat brain,12 it is conceivable that NO is required for the baroreceptor reflex. Nevertheless, we have found that, in response to the pressor effect of NMA, the baroreceptor-mediated decreases in RNA and HR were still operative. Thus, the contribution, if any, of the nucleus tractus solitarii as a site of action of NMA seems to be small. Further investigations to specify the site of action of NMA are now being undertaken.

It is conceivable that NMA-induced cerebral ischemia caused by local vasoconstriction may have stimulated sympathetic outflow. However, we found that intravenous administration of NMA (50 μmol/kg) to rats does not alter cerebral blood flow (measured by laser-Doppler flowmetry) or cerebral oxygenation.
(monitored by near-infrared spectrophotometry) (Reference 24 and authors' unpublished observations). These findings suggest that autoregulation of cerebral blood flow is preserved even in the presence of NMA and that NMA does not cause detectable cerebral ischemia.

Multiple factors are responsible for the regulation of BP. Recently, it has become apparent that EDRF/NO may be a very important factor. It had been previously thought that the role of EDRF/NO was solely peripheral; its synthesis by vascular endothelium provided a continuous vasodilating tone to the underlying smooth muscle. Inhibition of EDRF/NO synthesis by NMA therefore caused vasoconstriction and an increase in BP. We now report that EDRF/NO formed in the brain may also play a role in the central regulation of BP by influencing sympathetic nerve activity. Because of our finding that NMA increases central sympathetic outflow by an action that can be prevented by L-arginine, we propose that centrally formed EDRF/NO acts to reduce sympathetic outflow and may lower BP. It is tempting to speculate that a defect in central NO synthesis may contribute to essential hypertension in humans.

Acknowledgments
We thank Dr. Owen W. Griffith for synthesizing N⁰-Methyl-L-arginine hydrochloride. The expert secretarial assistance of Ms. Rebecca Zilke is gratefully acknowledged.

References
18. Reis DJ, Granata AR, Joh TH, Ross CA, Ruggiero DA, Park DH: Brain stem catecholamine mechanisms in tonic and reflex control of blood pressure. Hypertension 1984;6(suppl II):II-7–II-15
NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone?

I Sakuma, H Togashi, M Yoshioka, H Saito, M Yanagida, M Tamura, T Kobayashi, H Yasuda, S S Gross and R Levi

Circ Res. 1992;70:607-611
doi: 10.1161/01.RES.70.3.607

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/70/3/607

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/