Nitric oxide (NO), or a molecule capable of forming NO, is a major endothelium-derived relaxing factor (EDRF) 1-4; it is synthesized from L-arginine by an enzymatic pathway that is selectively inhibited by N\textsuperscript{G}-methyl-L-arginine (NMA). 5-7 The intravenous administration of NMA to guinea pigs, 8 rabbits, 9 rats, 10 and dogs 11 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. 12 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 13 and bovine brain. 14 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. 15 These observations have raised the possibility that the EDRF/NO–cGMP system may function as an important signal transduction system in the brain. 16 Indeed, new evidence demonstrating that NO synthase is present in discrete neuronal populations further implies a neural messenger role for NO. 17

Since L-glutamate and serotonin receptors have been implicated in central autonomic reflexes, 18 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 a-chloralose (50 mg/kg i.p.) and urethane (500 mg/kg i.p.) and immobilized with gal-lamine 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-101T, 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 thiopseudouracil 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 deafferented 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 deafferented 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 deafferented 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%,
NMA or 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.

Discussion

NMA elicited a dose-dependent increase in arterial BP and a decrease in HR (20). In contrast, the increase in RNA seen with NMA was 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, 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-1 and C-2 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.

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, Nω-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, where it increases intracellular cGMP levels, thereby modulating neuronal signal transduction. 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. Since the neurotransmitter of the afferent vagal neurons is l-glutamate, which has been shown to stimulate the generation of EDRF/NO from l-arginine in the rat brain, 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.

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