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In-vivo evidence of a role for nitric oxide in regulating the activity of the norepinephrine transporter

Joseph Simaan, Ramzi Sabra *

Department of Pharmacology and Toxicology, Faculty of Medicine, American University of Beirut, P.O. Box 11-0236/37, Beirut, Lebanon

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ABSTRACT

We examined the role of nitric oxide (NO) in the regulation of neuronal uptake of norepinephrine (uptake-1) in rats under anesthesia. The effect on systolic blood pressure of two pressor drugs that work by different mechanisms, norepinephrine and angiotensin II, was explored in anesthetized rats under control conditions and after prevention of NO synthesis with Nw-nitro-L-arginine (L-NNA). The results showed that whereas the pressor effects of increasing doses of norepinephrine were potentiated by L-NNA, those of angiotensin II were not affected, which implied that NO was selectively involved in modulating the pressor effect of norepinephrine. To explore the mechanisms involved in this potentiation, we examined the effect of L-NNA on the pressor effect of tyramine, a purely-indirectly-acting sympathomimetic amine which enters nerve terminals thorough uptake 1 and liberates norepinephrine from storage vesicles. Increasing doses of tyramine produced pressor effects which, in contrast to those of norepinephrine, were significantly attenuated by pretreatment with L-NNA. Similarly, pretreatment with cocaine, the classical inhibitor of uptake 1, significantly decreased the pressor effect of tyramine; however, the response to tyramine was then restored when L-NNA was administered, thus reversing the effect of cocaine. We conclude that NO plays a major role in the adrenergic system by enhancing the activity of uptake 1 in sympathetic nerve terminals. Blockade of uptake 1 by cocaine is also partly dependent on NO. The stimulus for the mobilization of the NO synthase pathway in adrenergic neurons and the subsequent steps involved in modulating uptake 1 deserve further exploration.

1. Introduction

Since the first report on the endothelium-derived relaxing factor by Furchgott and Zawadski (1980), later discovered to be nitric oxide (NO), many reports have appeared which provide evidence that NO is a fundamental mediator of numerous physiological and pathophysiological events in the biological system. Among these mediator functions are its effects on the cardiovascular system, including its autonomic control (Balligand, 1999; Chowdhar and Townend, 1999; Greenberg et al., 1990; Han et al., 1994; Scrogin et al., 1992; Simaan, 2002). The most reproducible effect of NO on the circulation is revealed by the prompt and drastic elevation in blood pressure following inhibition of its synthesis, implying that under resting conditions, NO is involved in the control of blood pressure (Rees et al., 1989). Several studies have addressed the crosstalk between NO and the sympathetic nervous system. Nitric oxide was found to be involved in the control of vascular tone mediated by the adrenergic system by interfering with the release of norepinephrine (Greenberg et al., 1990) and by reducing its uptake by the uptake-1 catecholamine transporter (Kaye et al., 1997). It was also found to inhibit the release of norepinephrine from sympathetic cardiac nerves (Schwartz et al., 1995). Opposite effects were also reported, whereby NO synthase inhibition enhanced the vasoconstrictor response to norepinephrine and sympathetic nerve stimulation (Tesfamariam et al., 1987). Involvement of NO in the control of baroreflex activity continues to be controversial: some studies showed that systemic blockade of NO synthase increased the gain of the baroreceptor reflex sympathetic nerve activity in conscious rabbits (Liu et al., 1996) and rats (Kumagai et al., 1993); similar findings were reported after administration of NO synthase inhibitors in the cerebral ventricles in conscious rabbits (Katsumura et al., 1998). Other studies reported no effect of NO on baroreflex control of sympathetic nerve activity (Jimbo et al., 1994; Murakami et al., 1998). Finally, sympathetic stimulation was found to contribute to a limited degree to hypertension produced by chronic exposure to moderate NO synthase inhibition (Scrogin et al., 1992). These observations led us to examine whether or not NO is involved in the compensatory mechanisms which are aroused by a sudden rise in blood pressure in-vivo, using two different pressor agents, and in the baroreflex compensatory mechanisms that ensue. Since preliminary findings showed that the pressor effect of norepinephrine, but not that of angiotensin II, was potentiated after blockade of NO synthesis, we focused on exploring the effect of NO on the activity of uptake 1 in sympathetic nerve terminals using classical in-vivo pharmacological approaches. For this purpose, we used

* Corresponding author at: Department of Pharmacology and Toxicology, American University of Beirut, P.O. Box 11-0236/37, Beirut, Lebanon. Fax: +961 1 744464.
E-mail addresses: jsimaan@aub.edu.lb (J. Simaan), rsabra@aub.edu.lb (R. Sabra).

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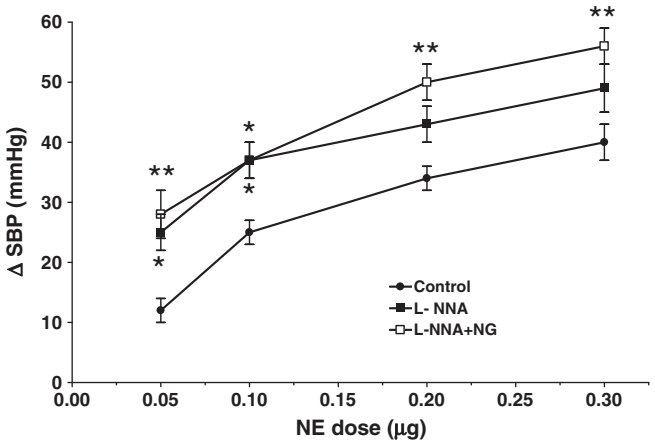

Fig. 1. Change in systolic pressure in response to increasing doses of norepinephrine in anesthetized rats (n = 12) in the control period and after sequential administration of L-NNA and nitroglycerin (NG). Two-way ANOVA: $P<0.0001$ for the effect of both Group factor and Dose factor on the response. *: $P<0.05$ compared with the control value; **: $P<0.001$ compared with the control value. No significant differences were observed in the responses to NE between L-NNA and L-NNA + NG periods at any dose.

tyramine, a purely indirectly-acting sympathomimetic amine which enters across uptake 1 to release norepinephrine from its vesicular storage sites and produce a pressor effect (Burn, 1932; Fawaz and Simaan, 1965), and examined its pressor effects under control conditions and after blockade of uptake 1 by cocaine (Carmichael and Israel, 1973; Hartling et al., 1961; Iversen, 1965), in a NO-intact state and NO-deficient state. The hypothesis was advanced that if NO is involved in the activity of uptake 1, then blockade of its synthesis will modify uptake of tyramine and thus its pressor effect, and it may further modify binding of cocaine to uptake 1 that will be reflected by a change in the pressor effect of tyramine.

2. Material and methods

This study was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, at the American University of Beirut. Sprague Dawley rats of both sexes (N = 38), weighing 383 ± 7 g, were anesthetized with pentobarbital Na intraperitoneally (35 mg/kg). The carotid artery was cannulated and connected to a Gould P23XL pressure transducer to measure arterial pressure. One femoral vein was cannulated for single injections and the other for continuous infusions. The trachea was cannulated, but the animals were allowed to breathe spontaneously. Recordings of arterial pressure were made on a Gould TA11 recorder. The preparations were divided into 3 series. The first series (N = 12) received single injections of norepinephrine (0.05, 0.1, 0.2, 0.3 μg) followed by single injections of angiotensin II (0.0125, 0.025, 0.05, 0.1 μg) and the blood pressure measured. For each dose of drug, the blood pressure was allowed to return to the baseline level before the following dose was administered. Nw-nitro-L-arginine (L-NNA) was then administered (15 mg/kg, i.v.). A period of ten minutes was allowed for stabilization before the test doses of norepinephrine and angiotensin II were repeated and the blood pressure measured. Nitroglycerin (200 μg/ml) was then infused at a rate necessary to restore the rise in blood pressure induced by L-NNA back to the baseline level (average dose = 19 ± 3 μg/kg/min); the test doses of norepinephrine and angiotensin II were then repeated and the blood pressure was measured. The purpose of restoring the starting pressure with an infusion of nitroglycerin, a NO donor, was to determine if a higher baseline blood pressure per se influenced the absolute rise in pressure in response to the pressor drugs, while preserving the blockade of NO synthase by L-NNA. The second series of rats (N = 14) received single injections of tyramine (0.025, 0.05, 0.1 mg) and the change in blood pressure was measured. The same doses were repeated and the blood pressure measured after treatment with L-NNA then after an infusion of nitroglycerin to restore arterial pressure to baseline levels. The third series of rats (N = 12) was treated with the same doses of tyramine and the blood pressure measured under control conditions, after treatment with cocaine (3 mg/kg i.v. over 5 min), after cocaine and L-NNA and after cocaine, L-NNA and an infusion of nitroglycerin.

The drugs used in this study were purchased from Sigma, Switzerland (norepinephrine, tyramine, angiotensin II, L-NNA); American Regent, USA (nitroglycerin); American University of Beirut Medical Center Pharmacy (cocaine, pentobarbital Na). All the drugs were dissolved in saline. The systolic blood pressure under control conditions and after various treatments was expressed as the mean ± the standard error of the mean.

Statistical comparisons among groups were conducted using GraphPad Prizm software by two way analysis of variance (ANOVA) with repeated measures, with one factor being Group and the other factor being Dose, followed by the Bonferroni test for individual comparisons at specific doses. A P-value less than 0.05 was considered significant.

3. Results

3.1. The pressor effects of norepinephrine and angiotensin II before and after blockade of NO synthesis

Increasing doses of norepinephrine produced progressive increases in systolic arterial pressure (Fig. 1). Treatment with L-NNA increased the blood pressure significantly in this and in all subsequent series (change in systolic pressure = 36 ± 3 mm Hg, n = 34, $P<0.0001$). After L-NNA administration, the pressor effect of norepinephrine was potentiated over a range of 23% to 128% at the doses used ($P<0.001$). Further treatment with an infusion of nitroglycerin to restore the rise in blood pressure induced by L-NNA did not modify this potentiation. Fig. 2 shows that treatment with angiotensin II produced increases in blood pressure similar to those produced by norepinephrine. However, in contrast to the norepinephrine responses, L-NNA did not potentiate the pressor effect of angiotensin II. Further treatment with nitroglycerin to restore arterial pressure to baseline levels was also without effect on the pressor action of angiotensin II.

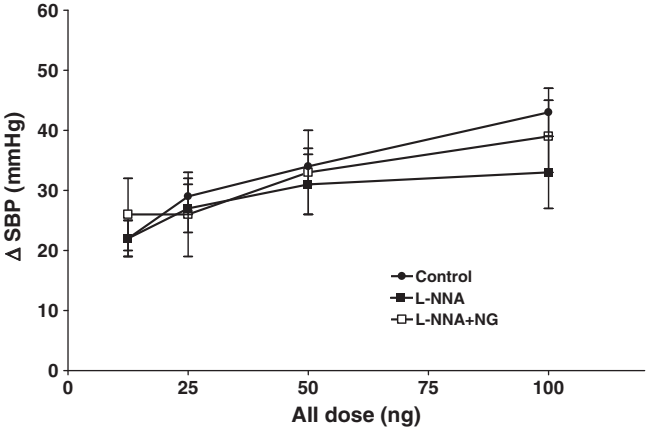

Fig. 2. Change in systolic pressure in response to increasing doses of angiotensin II in anesthetized rats (n = 12) in the control period and after sequential administration of L-NNA and nitroglycerin (NG). Two-way ANOVA: P = 0.002 for effect of dose on the response. No significant differences were found between groups at any dose.