Role of Nitric Oxide in the Renin and Heart Rate Responses to β-Adrenergic Stimulation

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Abstract
Studies in vitro and in vivo have implicated nitric oxide in the control of renin secretion. In the present study, the effect of inhibition of nitric oxide synthesis with N\(^\text{\textsuperscript{O}}\)-nitro-L-arginine methyl ester hydrochloride (L-NAME) on the renin secretory response to β-adrenergic stimulation was investigated in conscious, chronically prepared rabbits. Intravenous infusion of isoproterenol at 0.02 μg·kg\(^{-1}\)·min\(^{-1}\) for 30 minutes increased mean arterial pressure by 5 mm Hg (P<.05), heart rate by 51 beats per minute (P<.001), and plasma renin activity by 56% (P<.001). Intravenous infusion of L-NAME at 0.5 mg·kg\(^{-1}\)·min\(^{-1}\) increased mean arterial pressure by 6 mm Hg (P<.01) and decreased heart rate by 15 beats per minute (P<.01) and plasma renin activity by 31% (P<.05). L-NAME reduced the heart rate response to isoproterenol by 50% and inhibited the renin response. Infusion of isoproterenol at 0.05 μg·kg\(^{-1}\)·min\(^{-1}\) did not change blood pressure but increased heart rate by 62 beats per minute (P<.001) and plasma renin activity by 283% (P<.001). Treatment with L-NAME again suppressed the heart rate response to isoproterenol and inhibited the renin response. Intravenous infusion of the nitric oxide donor nitroprusside at 2 μg·kg\(^{-1}\)·min\(^{-1}\) in the presence of L-NAME decreased mean arterial pressure by 7 mm Hg (P<.05), increased heart rate by 14 beats per minute (P<.05), but did not change plasma renin activity. Nitroprusside fully restored the heart rate response to isoproterenol and partially restored the renin response. These results provide evidence that nitric oxide plays an important role in the renin and heart rate responses to β-adrenergic stimulation. (Hypertension. 1994;23[suppl I]:I-49-I-53.)

Keywords • nitric oxide • L-NAME • nitroprusside • isoproterenol • renin • heart rate • blood pressure • rabbits

Nitric oxide (NO) is synthesized in a wide variety of tissues, including the vascular endothelium, in which it regulates vascular tone, and the central and peripheral nervous systems, in which it may function as an atypical neurotransmitter or neuromodulator. NO is also involved in other physiological functions, including the regulation of endocrine secretion. For example, the secretion of insulin and growth hormone is altered by administration of L-arginine, the precursor of NO, or by arginine analogues that inhibit NO synthesis.

Recent evidence suggests that NO may also play a role in the control of renin secretion by the kidneys. Studies using renal cortical slices and isolated juxtaglomerular cells provided evidence that one or more endothelium-derived factors can influence renin secretion, and two lines of evidence suggest that one of these factors is NO. First, NO synthase and its mRNA are present in vascular and tubular elements of the kidney, including the macula densa, a component of the juxtaglomerular apparatus that plays an important role in the control of renin secretion. Second, blockade of NO synthesis with arginine analogues has been reported to alter the rate of renin secretion both in vitro and in vivo.

The aim of the present study was to investigate further the role of NO in the control of renin secretion. The experiments used chronically prepared rabbits and examined the effects of an inhibitor of NO synthesis on the renin response to β-adrenergic stimulation, a well-characterized stimulus to renin secretion. The effects of the NO donor nitroprusside were also investigated.

Methods
Experiments were performed on male New Zealand White rabbits weighing 2.5 to 3.5 kg (Nitabell Rabbitry, Hayward, Calif). The rabbits were housed in separate cages in a 12-hour light-cycled and temperature-controlled room, fed a commercial diet (Purina rabbit chow, Ralston-Purina, St Louis, Mo), and provided water ad libitum. All procedures were approved by the University of California, San Francisco, Committee on Animal Research.

Surgical Preparation
Surgery for chronic implantation of arterial and venous catheters was performed under aseptic conditions. The rabbits were anesthetized with ketamine (Parke-Davis, Morris Plains, NJ; 35 to 50 mg/kg IM) and xylazine (Lloyd Laboratories, Shenandoah, Iowa; 2 to 5 mg/kg IM). A catheter consisting of 4-in medical grade silicone elastomer (0.03-in inner diameter, 0.065-in outer diameter; Dow-Corning Corp, Midland, Mich) was inserted into a femoral artery and advanced into the aorta to a point distal to the kidneys. Two Tygon catheters (0.03-in inner diameter, 0.05-in outer diameter) were inserted into a jugular vein and positioned near the heart. The three catheters were led subcutaneously to a point between the scapulae, where they emerged through a small skin incision and were protected in a pocket of a nylon mesh jacket (Alice King Chatham, Los Angeles, Calif). The rabbits were allowed to recover for at least 3 days after surgery, during which time they were treated with trimethoprim and sulfadiazine (Di-Trim, Syntex, West Des Moines, Iowa; 0.5 mL SC SID). Catheters were flushed with sterile 0.9% saline and filled with heparin (1000 U/mL) at least every other day. During the recovery period, the rabbits were brought to the laboratory and accustomed to the experimental environment.
Experimental Protocols

On the day of an experiment, rabbits were brought to the laboratory, where they rested comfortably in a partly covered cage. Arterial blood pressure and heart rate (HR) were monitored continuously with a pressure transducer (Cobe Laboratories, Inc, Lakewood, Colo) and a custom-built cardiovascular analyzer. The cardiovascular data were simultaneously recorded on a Grass polygraph and digitized continuously at 100 Hz, stored, and analyzed with a PDP 11/23+ computer (Digital Equipment Corp, Maynard, Mass). Blood samples (2.0 mL) for analysis were collected from the femoral arterial cannula and replaced with an equal volume of sterile 0.9% NaCl. After blood pressure and HR had stabilized to their basal values for at least 15 minutes, the cardiovascular and renin secretory responses to β-adrenergic stimulation were investigated under control conditions and after inhibition of NO synthesis. In some experiments, the effects of isoproterenol were tested again after administration of the NO donor nitroprusside. The experiments were performed according to the following three protocols.

Protocol 1: Effects of Inhibition of NO Synthesis on the Responses to Isoproterenol (n=11)

Blood pressure and HR were monitored during a 15-minute control period, at the end of which a blood sample was collected. An infusion of 0.9% saline at 0.02 mL/min was then started and continued for the duration of the experiment. Fifteen minutes after the start of the saline infusion, another blood sample was collected. Isoproterenol hydrochloride (Elkins-Sinn, Inc, Cherry Hill, NJ) was then infused at 0.02 μg · kg⁻¹ · min⁻¹ IV for 30 minutes (volume, 0.02 mL/min). Blood samples were collected at 15 and 30 minutes after the start of the isoproterenol infusion and again after a 15-minute recovery period. The same procedure was performed on another day, but the saline infusion was replaced with an infusion of NO synthase inhibitor L-NAME (Sigma Chemical Co, St Louis, Mo). At 0.5 μg·kg⁻¹·min⁻¹ IV. The saline and L-NAME experiments were performed in random order.

Protocol 2: Effects of Nitroprusside (n=8)

After control measurements, isoproterenol was infused at 0.05 μg · kg⁻¹ · min⁻¹ IV for 15 minutes. This was followed by a 15-minute recovery period, and then an infusion of L-NAME was started and continued for the duration of the experiment. Fifteen minutes after the start of the L-NAME infusion, the isoproterenol infusion was repeated. An infusion of sodium nitroprusside (Elkins-Sinn) at 2 μg · kg⁻¹ · min⁻¹ was then started and continued until the end of the experiment. Fifteen minutes after the start of the nitroprusside infusion, a final infusion of isoproterenol was given.

Protocol 3: Time Controls (n=6)

In time control experiments, saline or L-NAME was infused as described in protocol 1 above, but 0.9% saline (0.02 mL/min) was infused for 30 minutes instead of isoproterenol.

Plasma Renin Activity

Plasma renin activity (PRA) was measured with a radioimmunoassay for angiotensin I and expressed as nanograms angiotensin I generated per milliliter of plasma during a 2-hour incubation at 37°C and pH 6.5.¹⁹

Statistics

Results are expressed as mean±SEM. Data were analyzed by one- and two-way analysis of variance (ANOVA) for repeated measures. When significant differences were detected by ANOVA, multiple comparisons were made by the Newman-Keuls or Dunnett's test.²⁰ Single comparisons were made by the paired t test. Changes were considered to be statistically significant at a value of P<.05.

Results

Effects of L-NAME on the Cardiovascular and Renin Responses to Isoproterenol

The effects of isoproterenol infusion at 0.02 μg · kg⁻¹ · min⁻¹ on mean arterial pressure (MAP), HR, and PRA are summarized in Fig 1. During the 30-minute isoproterenol infusion, there were increases in MAP from 76±2 to 82±4 mm Hg (P<.05) and HR from 234±4 to 286±7 beats per minute (P<.001). PRA increased from 7.1±1.9 to 11.1±2.5 ng · mL⁻¹ · 2 h⁻¹ at 15 minutes and to 10.5±2.0 ng · mL⁻¹ · 2 h⁻¹ at 30 minutes (P<.001).

Infusion of L-NAME increased MAP from 72±3 to 78±2 mm Hg (P<.01) and decreased HR from 214±9 to 199±6 beats per minute (P<.01) and PRA from 6.5±1.4 to 4.5±0.9 ng · mL⁻¹ · 2 h⁻¹ (P<.05). L-NAME did not alter the MAP response to isoproterenol but reduced the increase in HR by 50% (P<.02) and inhibited the PRA response.

Effects of Nitroprusside

The effects of L-NAME and nitroprusside on the cardiovascular and renin responses to isoproterenol are summarized in Fig 2. Infusion of isoproterenol at 0.05 μg · kg⁻¹ · min⁻¹ did not change MAP but increased HR from 224±6 to 286±8 beats per minute (P<.001) and PRA from 8.3±2.4 to 23.5±5.3 ng · mL⁻¹ · 2 h⁻¹ (P<.001).

As in the first experiment, L-NAME increased MAP and decreased HR and PRA. L-NAME again markedly reduced the HR response to isoproterenol (214±8 to 242±10 beats per minute, P<.05) and inhibited the renin response (5.9±2.5 to 7.2±2.2 ng · mL⁻¹ · 2 h⁻¹). Infusion of nitroprusside in the presence of L-NAME decreased MAP by 7 mm Hg (P<.05) and increased HR by 14 beats per minute (P<.05) but did not change PRA. Nitroprusside completely restored the HR response to isoproterenol (228±6 to 289±12 beats per minute, P<.001) and partially restored the renin response (5.8±2.1 to 12.5±3.9 ng · mL⁻¹ · 2 h⁻¹, P<.05).

Time Controls

In time control experiments, L-NAME or the saline vehicle was infused as in protocol 1, but saline was infused instead of isoproterenol. There were no changes in any of the measured variables during saline infusion. MAP increased and HR and PRA decreased during the first 15 minutes of the L-NAME infusion, with no significant changes thereafter.

Discussion

In the present study, the NO synthase inhibitor L-NAME was used to investigate the role of the L-arginine/NO pathway in the renin response to β-adrenergic stimulation. L-NAME is one of a group of closely related derivatives of L-arginine that are competitive inhibitors of the enzyme NO synthase.²⁻¹⁸ The experiments were performed in conscious rabbits to avoid the confounding effects of anesthesia, which include stimulation of renin secretion¹³ and interference with responses involving NO.²¹ As has been observed in studies in other species,¹⁶⁻¹⁸,²²⁻²⁴ L-NAME increased arterial blood pressure and decreased HR. The former response
presumably reflects removal of the vasodilator action of endogenous NO, whereas the latter has been attributed to a baroreceptor reflex response to the increase in blood pressure.\textsuperscript{23} L-NAME consistently decreased resting PRA, again confirming observations made in other species.\textsuperscript{16,17,25} There is some disagreement in the literature concerning the mechanism of this decrease in renin secretion. Sigmon et al\textsuperscript{17} proposed that it resulted from a combination of increased renal perfusion pressure and withdrawal of sympathetic neural tone to the kidney. Others, however, have provided evidence that the suppression of renin secretion by NO synthase inhibition can occur independently of the hemodynamic and neural mechanisms that control renin secretion.\textsuperscript{7,15,16}

In the present study, the effects of $\beta$-adrenergic stimulation were investigated by use of isoproterenol, which stimulates renin release in vivo and in vitro.\textsuperscript{12,13} It increases cardiac output by increasing cardiac contractility and HR, and decreases total peripheral resistance by dilating certain vascular beds.\textsuperscript{26} A major finding was that both the renin and HR responses to isoproterenol were markedly reduced by inhibition of NO synthase with L-NAME. The responses to isoproterenol could be fully (HR) or partially (PRA) restored by administration of the NO donor nitroprusside, and this provides evidence that the effects of L-NAME resulted from inhibition of NO synthesis. These findings thus provide evidence that NO participates in the renin and cardiovascular responses to $\beta$-adrenergic stimulation.

It is generally accepted that the renin response to isoproterenol is mediated by $\beta$-adrenergic receptors and involves the activation of adenylate cyclase and the formation of cyclic AMP.\textsuperscript{12,13,27,28} The mechanism by which inhibition of NO synthesis inhibited the renin response to isoproterenol was not investigated in the present study, but there are several possibilities. First, L-NAME increased blood pressure, and this may have inhibited the renin response to isoproterenol by activating the renal baroreceptor mechanism or by causing a
reflex reduction in renal sympathetic nerve activity. However, the pressor effect of L-NAME in these experiments was small (6 to 10 mm Hg), and during infusion of isoproterenol at 0.02 µg·kg⁻¹·min⁻¹, MAP was not significantly higher in the presence of L-NAME than in its absence. In addition, we have found that administration of L-NAME in conscious rabbits causes little or no reduction in renal nerve activity (K. Kumagai and I.A. Reid, unpublished observations), and others have reported that inhibition of NO synthesis in anesthetized rats increases renal nerve activity.²⁴ Another possibility is that L-NAME inhibited the renin response to isoproterenol through an action on the macula densa control of renin secretion, but further studies are required to test this possibility.

The mechanism by which L-NAME suppressed the HR response to β-adrenergic stimulation also remains to be determined. However, it is worth noting that Klimaschewski et al²⁹ recently demonstrated that NO synthase is present in endothelial cells of coronary arteries and arterioles and in nerve fibers innervating several regions of the heart, including the sinoatrial and atrioventricular nodes. They speculated that NO might be involved in the regulation of HR. In this context, it is interesting that Klabunde et al³⁰ reported that the increase in cardiac cyclic AMP concentration induced by isoproterenol was reduced by the NO synthase inhibitor N⁰-methyl-L-arginine (NMA). They also observed that NMA decreased cardiac cyclic GMP concentration and suggested that this resulted in decreased inhibition of an isoform of phosphodiesterase, which in turn increased the hydrolysis of cyclic AMP. Such a mechanism could explain the ability of L-NAME to reduce the HR response to isoproterenol in the present study. A similar mechanism could explain the suppression of the renin response.

In summary, blockade of NO synthase with L-NAME suppressed the increases in renin secretion and HR produced by β-adrenergic stimulation with two doses of isoproterenol in conscious rabbits. Administration of the
NO donor nitroprusside completely restored the HR response to isoproterenol and partially restored the renin response. These results provide evidence that there are important interactions between NO and the renin and HR responses to β-adrenergic stimulation in conscious rabbits. Additional studies are required to determine the mechanisms that underlie these interactions.

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References

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