Nitric oxide synthase and cGMP-mediated stimulation of renin secretion

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Sayago, Cecilia M., and William H. Beierwaltes. Nitric oxide synthase and cGMP-mediated stimulation of renin secretion. Am J Physiol Regulatory Integrative Comp Physiol 281: R1146–R1151, 2001.—The interaction between nitric oxide (NO) and renin is controversial. cAMP is a stimulating messenger for renin, which is degraded by phosphodiesterase (PDE)-3. PDE-3 is inhibited by cGMP, whereas PDE-5 degrades cGMP. We hypothesized that if endogenous cGMP was increased by inhibiting PDE-5, it could inhibit PDE-3, increasing endogenous cAMP, and thereby stimulate renin. We used the selective PDE-5 inhibitor zaprinast at 20 mg/kg body wt, which we determined would not change blood pressure (BP) or renal blood flow (RBF). In thiobutabarbital (Inactin)-anesthetized rats, renin secretion rate (RSR) was determined before and 75 min after administration of zaprinast or vehicle. Zaprinast increased cGMP excretion from 12.75 ± 1.57 to 18.67 ± 1.87 pmol/min (P < 0.003), whereas vehicle had no effect. Zaprinast increased RSR sixfold (from 2.95 ± 1.74 to 17.62 ± 5.46 ng ANG I·h⁻¹·min⁻¹, P < 0.024), while vehicle had no effect (from 4.08 ± 2.02 to 3.87 ± 1.53 ng ANG I·h⁻¹·min⁻¹). There were no changes in BP or RBF. We then tested whether the increase in cGMP could be partially due to the activity of the neuronal isoform of NO synthase (nNOS). Pretreatment with the nNOS inhibitor 7-nitroindazole (7-NI; 50 mg/kg body wt) did not change BP or RBF but attenuated the renin-stimulating effect of zaprinast by 40% compared with vehicle. In 7-NI-treated animals, zaprinast-stimulated cGMP excretion was attenuated by 48%, from 9.17 ± 1.85 to 13.60 ± 2.15 pmol/min, compared with an increase from 10.94 ± 1.90 to 26.38 ± 3.61 pmol/min with zaprinast without 7-NI (P < 0.04). This suggests that changes in endogenous cGMP production at levels not associated with renal hemodynamic changes are involved in a renin-stimulatory pathway. One source of this cGMP may be nNOS generation of NO in the kidney.

phosphodiesterase; zaprinast; cyclic nucleotides

RENIN IS THE ENZYMATIC rate-limiting step in the formation of the potent vasoconstrictor angiotensin. Renin is synthesized and stored in the juxtaglomerular (JG) cells of the renal afferent arteriole, and its release or secretion is regulated by several well-established pathways, including the renal baroreceptor, β-adrenergic stimulation (8, 10), and the macula densa feedback pathway (16, 21). The nucleotide cAMP is a stimulatory second messenger that integrates different signals with renin secretion (7).

Although renin stimulation is regulated by cAMP, renin release has been shown to be inhibited by another nucleotide, cGMP (13, 18). The JG cells are surrounded by various cells containing different isoforms of the enzyme nitric oxide synthase (NOS) with a high capacity for nitric oxide (NO) formation, and the second messenger of NO is cGMP. It has been shown that endothelium-derived NO, which stimulates cGMP production, can also inhibit renin (4, 30). However, contrary reports have suggested that NO may stimulate renin (18, 17, 25). The possible regulatory effects of NO on renin are controversial, but the diversity of the data suggests that there may be inhibitory and stimulatory pathways characterizing the interactions between these dilator and constrictor systems. Various studies have now implicated NO derived from the neuronal isoform of NOS (nNOS) in mediating renin stimulation by the macula densa pathway in response to chronic sodium depletion (3, 11) or acute furosemide administration (2, 26). Sodium restriction upregulates nNOS in the macula densa (29), but it is not clear whether NO produced at this site somehow acts directly on the JG cells or whether it participates as an intermediary signal in some undefined stimulatory cascade.

Beavo (1) proposed that cAMP in the JG cells may be regulated or degraded by two isoforms of phosphodiesterase (PDE), PDE-3 and PDE-4. Chiu and Reid (6) noted that the stimulatory second messenger for renin, cAMP, was regulated in the kidney by its production and its metabolism. Furthermore, they reported that inhibition of the PDE-selective PDE-3, which should diminish cAMP degradation, resulted in increased basal and isoproterenol-stimulated renin. Kurzt et al. (20) also reported that stimulation of renin secretion by NO is mediated by PDE-3. Likewise, these authors have reported that PDE-3 activity may partially account for the vasodilator effect of NO in the renal vasculature (27). These studies led us to look at the control of endogenous NO-stimulated cGMP as a renin-regulatory factor. cGMP is degraded by PDE-5 in the kidney (9). One in vitro report (24) suggested a biphasic action of exogenous cGMP on renin, stimulating it at
low concentrations but inhibiting it at higher levels. Because PDE-3 is cAMP selective but cGMP inhibitable (1), we tested whether increasing endogenous cGMP by decreasing its degradation might stimulate renin, perhaps by its effect on the activity of PDE-3. In the present study, we hypothesize that increasing endogenous cGMP by selectively inhibiting its degradation by PDE-5 may inhibit PDE-3, increasing cAMP and stimulating renin secretion in vivo. Because renal hemodynamic changes are well established as potent regulatory signals for renin secretion (16), we designed these studies to test the renin response to PDE-5 inhibition in the absence of any changes in renal hemodynamics or renal perfusion pressure. Additionally, we tested whether NOS activity, and particularly nNOS, could be the source of this endogenous cGMP activity.

**MATERIALS AND METHODS**

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA; 250–400 g body wt) were fasted overnight but allowed free access to water and then anesthetized using thiobutabarbital (125 mg/kg body wt ip, Inactin; Sigma, St. Louis, MO) and placed on a heating pad to maintain constant body temperature. To facilitate spontaneous breathing of room air, rats were tracheotomized using PE-240 tubing. The femoral vein was catheterized with PE-50 tubing for maintenance infusion of 0.9% NaCl at 40 μl/min. The femoral artery was catheterized with PE-50 tubing attached to a Statham pressure transducer (Viggo-Spectramed, Oxnard, CA) and a recorder (Gould Instruments, Valley View, OH) for continuous monitoring of blood pressure (BP) and sampling of arterial blood. The pressure transducer was calibrated using a mercury manometer.

A midventral incision was made in the abdominal cavity, the intestines were wrapped in moist gauze and folded under the right ventral wall, and the left renal vein and artery were dissected from the surrounding tissues. To sample renal venous blood, a 25-gauge needle was bent to 90°, removed from its hub, and fitted to PE-50 tubing attached to a 1-ml syringe. It was filled with heparinized saline. The needle was carefully placed in the left renal vein at its bifurcation with the vena cava, advanced into the vein toward the kidney, and fixed in place with a drop of Vetbond tissue adhesive (3-M, St. Paul, MN) over a 2-mm2 gauze patch. Femoral arterial and renal venous blood were slowly sampled in 250-μl volumes and then replaced with an equal amount of 6% heat-inactivated BSA. A noncannulating electromagnetic flow probe with an internal circumference of 1.5 mm (Carolina Medical Electronics, King, NC) was placed on the renal artery. The flowmeter was calibrated by direct cannulation of the renal artery and by gravimetric determination of blood flow over timed intervals. Urine was collected directly from the bladder. After surgery, the rats received a supplemental bolus of 1.0 ml of 6% heat-inactivated BSA (Sigma) in normal saline and stabilized for 60 min. When the experiments were completed, the rats were killed by pneumothorax and aortic transection, and the kidneys were decapsulated, excised, and weighed for normalization of renal blood flow (RBF).

Selective pharmacological inhibition of PDE-5 (23) was carried out with a single bolus of zaprinast (20 mg/kg body wt ip; Biomol, Plymouth Meeting, PA) suspended in peanut oil by sonication and delivered via polyethylene tubing placed within the gauze-wrapped intestine. In preliminary studies, we determined that this was the highest dose we could give without affecting BP or RBF.

**Inhibition of PDE-5 with zaprinast.** The first protocol (n = 20) was designed to test whether PDE-5 inhibition would increase cGMP excretion and renin secretion rate (RSR). During an initial 30-min control period, BP, heart rate (HR), and RBF were recorded. At the end of this period, femoral arterial and renal venous blood were collected for determination of plasma renin activity (PRA) and hematocrit. Urine was collected throughout the 30-min period for measurements of cGMP excretion. Then a single bolus of the PDE-5 inhibitor zaprinast or its peanut oil vehicle was delivered intraperitoneally, and the animal was allowed to stabilize for 45 min. Then, in a final 30-min experimental period, BP, HR, and RBF were determined, arterial and renal venous blood were sampled, and urine was collected.

**Inhibition of nNOS.** The second protocol (n = 23) was designed to see whether blocking NO formation would alter cGMP excretion and RSR. After surgery, 11 rats were given a single intravenous bolus of 7-nitroindazole (7-NI, 50 mg/kg, n = 16; Biomol) or its peanut oil vehicle (n = 18) and were allowed to stabilize for 45 min before zaprinast treatment as described above.

Neuronal NOS was selectively inhibited by a dose of 7-NI (50 mg/kg body wt). 7-NI was suspended in peanut oil and injected intraperitoneally. We and others have demonstrated that this dose of 7-NI completely blocks nociceptive responses known to be mediated by nonadrenergic, noncholinergic neurons where NO is the neurotransmitter. Additionally, 7-NI completely blocks cerebellar conversion of L-arginine to citrulline, without altering BP or RBF. Furthermore, this dose does not alter BP or RBF and does not attenuate the renal hemodynamic response to nonselective NOS inhibition with L-NAME, suggesting that it has no effect on endothelial NOS (eNOS) (2, 3).

**Analysis.** Renal vascular resistance (RVR) was calculated as renal perfusion pressure (mmHg) divided by RBF (ml/min·100 g kidney wt−1) and given in units of mmHg·g−1·s−1 (g kidney wt−1) and given in units of mmHg·g−1·s−1·103 (ml·min−1·g kidney wt−1). RVR was calculated from the difference in PRA between the renal vein and femoral arterial samples multiplied by renal plasma flow, which was calculated from RBF, corrected for kidney weight, and multiplied by 1 minus the hematocrit. Units are nanogramsANG I per hour per minute (g kidney wt−1). PRA was determined by radioimmunoassay for generation of ANG I using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN). Urine was collected in the presence of 3-isobutyl-1-methylxanthine (1 mM) to avoid cGMP degradation by urinary PDEs and stored at −70°C until assayed. cGMP was determined by a competitive enzyme immunoassay kit (R & D Systems, Minneapolis, MN). Control responses to zaprinast or vehicle were analyzed using paired t-tests. Comparisons between the response to zaprinast and vehicle treatment were analyzed using an unpaired t-test. P < 0.05 was considered significant. Values are means ± SE.

**RESULTS**

**Inhibition of PDE-5 by zaprinast.** During the control period, before treatment, BP was the same in zaprinast and vehicle groups (103 ± 2 vs. 103 ± 2 mmHg). Likewise, there were no significant differences in HR

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(305 ± 19 vs. 290 ± 9 beats/min), RBF (5.56 ± 0.41 vs. 6.21 ± 0.47 ml·min⁻¹·g kidney wt⁻¹), or RVR (19.73 ± 1.60 vs. 18.54 ± 2.25 RU) between zaprinast and vehicle groups, respectively. Zaprinast did not alter BP or renal hemodynamics, nor were there any significant differences between zaprinast and vehicle-treated animals after treatment (Fig. 1).

Urinary cGMP excretion was 12.75 ± 1.57 pmol/min during the control period; after zaprinast treatment, it increased significantly to 18.67 ± 1.87 pmol/min (P < 0.03). In controls, cGMP excretion was 12.02 ± 1.43 pmol/min and remained unchanged during vehicle treatment (Fig. 2).

RSR was 2.95 ± 1.74 ng ANG I·h⁻¹·min⁻¹ before treatment and increased sixfold after zaprinast to 17.62 ± 5.46 ng ANG I·h⁻¹·min⁻¹ (P < 0.024; Fig. 2). In controls, basal RSR was 4.08 ± 2.02 ng ANG I·h⁻¹·min⁻¹, and this remained unchanged during vehicle treatment.

Inhibition of NOS. As expected, treatment with the NOS inhibitor L-NAME increased BP from 110 ± 4 to 128 ± 6 mmHg (P < 0.01). Likewise, NOS inhibition decreased HR by 17% (from 286 ± 10 to 239 ± 10 beats/min) and RBF by 27% (from 5.42 ± 0.38 to 3.93 ± 0.24 ml·min⁻¹·g kidney wt⁻¹) and increased RVR by 60% (from 21.39 ± 1.89 to 34.29 ± 3.03 RU). In L-NAME-treated rats, zaprinast had no effect on BP (130 ± 8 mmHg); however, HR reverted to pre-L-NAME values (to 286 ± 18 beats/min), while RBF decreased to 3.20 ± 0.28 ml·min⁻¹·g kidney wt⁻¹ and RVR increased by 30% to 44.37 ± 4.94 RU (P < 0.014). However, treatment with zaprinast after NOS inhibition had no effect on RSR, inasmuch as it was 1.90 ± 0.52 and 2.48 ± 1.14 ng ANG I·h⁻¹·min⁻¹ during the control period and after zaprinast treatment, respectively (Fig. 3). We do not have urinary cGMP excretion data for this protocol.

Inhibition of nNOS. Selective nNOS inhibition with 7-NI did not alter BP (96 ± 2 vs. 97 ± 3 mmHg with vehicle). Similarly, there were no significant differences in HR (275 ± 7 vs. 297 ± 11 beats/min), RBF (5.87 ± 0.34 vs. 6.44 ± 0.35 ml·min⁻¹·g kidney wt⁻¹),
or RVR (17.20 ± 1.10 vs. 15.80 ± 1.26 RU) between 7-NI- and vehicle-treated rats, respectively. Treatment with zaprinast after 7-NI did not affect BP or renal hemodynamics (Fig. 4).

Urinary cGMP excretion increased from 10.94 ± 1.90 to 26.38 ± 3.61 pmol/min after zaprinast treatment (P < 0.004). Treatment with 7-NI had no effect on basal urinary cGMP excretion; it was 9.17 ± 1.85 pmol/min and remained virtually unchanged after zaprinast treatment (13.60 ± 2.15 pmol/min; Fig. 5).

RSR during the control period was 4.46 ± 0.94 and 4.41 ± 0.93 ng ANG I·h⁻¹·min⁻¹ in 7-NI- and vehicle-treated rats, respectively. Zaprinast increased RSR significantly to 9.79 ± 2.31 ng ANG I·h⁻¹·min⁻¹ in the vehicle-treated group (P < 0.04). However, in rats pretreated with 7-NI, the response of RSR to zaprinast was attenuated to 5.58 ± 1.73 ng ANG I·h⁻¹·min⁻¹, diminishing zaprinast stimulation of RSR by 40% compared with vehicle (Fig. 5).

DISCUSSION

We have found that inhibition of the cGMP-degrading PDE-5 with zaprinast increased endogenous cGMP (as indicated by increased cGMP excretion) and stimulated renin secretion sixfold in the absence of any systemic or renal hemodynamic changes. We also found that nonselective inhibition of NOS with L-NAME completely reversed the stimulation of RSR by zaprinast, suggesting that NO stimulation of guanylate cyclase is a major source of cGMP in this pathway. Moreover, selective inhibition of nNOS attenuated zaprinast-stimulated renin secretion, indicating that cGMP produced by NO originating from nNOS may be a factor in this renin-stimulating pathway.

Although the effect of NO on renin remains controversial, the in vitro literature supports direct inhibition by its second messenger, cGMP. Direct addition of exogenous cGMP to isolated JG cells (18), cortical kidney slices (13), and the isolated perfused kidney (31) suppresses renin release. Although some reports have indicated that endothelium-derived NO, which stimulates cGMP production, can also inhibit renin (4, 30), other reports have suggested that NO may actually stimulate renin (15, 17, 25). A possible biphasic effect of cGMP, both stimulating...
and inhibiting renin in a dose-dependent manner, has been suggested. High levels of cGMP inhibited renin release, whereas low levels stimulated it (28). Moreover, adding L-arginine to the bath or the macula densa of the isolated JG apparatus produced opposite renin responses (11). When agents that affect NO availability were present in the bath, renin release was inhibited, whereas applying them to the macula densa stimulated renin. These last results are consistent with the possibility that renin secretion may be influenced by NO from at least two different sources, the action of which may be directionally opposite. Thus NO could inhibit or stimulate renin, depending on its source; however, our studies cannot distinguish between such sources but can only suggest it.

In the present study, inhibition of cGMP degradation by zaprinast increased cGMP excretion and stimulated renin release without altering BP or renal hemodynamics. Changes in renal perfusion (particularly vasodilatation) and systemic BP are powerful stimuli for renin secretion (14, 16), and a role for PDEs in the regulation of hemodynamics has also been proposed (27). We believed that it was important to eliminate hemodynamic changes in vivo to clarify the effect of endogenous cGMP on renin secretion. We were able to control pressure and RBF and still show that endogenous cGMP increased in concert with RSR.

Studies in vivo using L-NAME to block NOS have shown that renin is suppressed (25), indirectly suggesting that NO mediates renin secretion. There has been some controversy over whether the L-NAME-mediated decrease in renin is due to diminished NO, the potent renal baroreceptor-mediated inhibition of renin, or both. We observed an 18-mmHg increase in BP with L-NAME and a 60% decrease in PRA in response to L-NAME. Importantly, zaprinast did not change BP further, nor did it stimulate RSR in L-NAME-treated rats. These data suggest that a primary source of increased cGMP in response to zaprinast is NO stimulation of guanylate cyclase.

The possibility that NO in the macula densa is involved in stimulating renin release was first suggested by He et al. (12). In an isolated perfused JG preparation, they found that a low-NaCl perfusate increased renin fourfold compared with high NaCl (12). In response to nonselective NOS inhibition delivered to the tubular lumen (and ultimately to the macula densa), the renin stimulation evoked by reduced macula densa NaCl delivery was severely depressed. In related studies, selective inhibition of nNOS with 7-NI blocked acute renin stimulation by the diuretic furosemide (2) and chronic stimulation by dietary sodium restriction (3). These studies suggest that nNOS-derived NO plays an important role in the macula densa pathway for renin stimulation. Likewise, our data showed that selective inhibition of nNOS-derived NO production attenuated the stimulation of renin secretion produced by increases in endogenous cGMP. Accordingly, elevated cGMP excretion was attenuated by nNOS inhibition. Taken together, these results support an important role for cGMP produced by nNOS-derived NO in this stimulation of renin.

It should be noted that the attenuation of excreted cGMP we observed with 7-NI is too great to be accounted for by nNOS in the macula densa. It could reflect drug-mediated changes in eNOS, except we saw no renal hemodynamic changes, or it could reflect altered medullary nNOS production of cGMP (22).

Another possible explanation of our result is that zaprinast, which is a highly selective PDE-5 inhibitor, may have some undisclosed effect on a cAMP-degrading PDE, stimulating renin independently of changes in cGMP. However, when we blocked NOS with L-NAME or 7-NI, we were able to suppress zaprinast-stimulated renin without observing a drug effect independent of cGMP generation, suggesting that the drug was acting as we have proposed.

The stimulatory second messenger for renin is cAMP (7). Kurtz et al. (19) suggested that stimulation of renin using NO donors was related to increasing cAMP, but they were unable to show the same effect using exogenous cGMP. Chiu and Reid (6) reported that inhibition of the cAMP-selective PDE-3, which should decrease cAMP degradation, increased basal and isoproterenol-stimulated renin. Kurtz et al. (20) also presented evidence that cAMP-stimulated renin is mediated by PDE-3. Because PDE-3 is a cAMP-selective but a cGMP-inhibitable enzyme, increases in endogenous cGMP might inhibit PDE-3 degradation of cAMP as we have found. This would increase endogenous cAMP and, therefore, the stimulus for renin release, similar to what has been shown with pharmacological inhibition of PDE-3 (6, 20). Our data suggest that inhibition of endogenous cGMP degradation provokes a mild, yet significant, increase in cGMP together with a remarkable increase in RSR. Although we have not measured cAMP, we know that increased cAMP is an essential step in the stimulation of renin (7). Thus, although we did not directly address the role of PDE-3, our data are consistent with the hypothesis that renin may increase through cGMP-mediated inhibition of PDE-3 degradation of cAMP.

In summary, we found that increases in endogenous cGMP, resulting from selective PDE-5 inhibition, significantly stimulated renin secretion in the absence of changes in BP or renal hemodynamics. We also found that inhibition of NO synthesis reversed the renin stimulation caused by PDE-5 inhibition, suggesting that NO is a primary source for this endogenous cGMP production. Additionally, selective inhibition of nNOS greatly attenuated the stimulation of RSR seen when PDE-5 is blocked. Thus our data suggest that NO derived from NOS, including a component dependent on the neuronal isoform, is a primary source of cGMP-mediated renin stimulation. We propose that this stimulatory pathway is consistent with a model by which endogenous cGMP inhibits PDE-3 degradation of cAMP, enhancing the stimulatory signal for renin secretion in vivo.
Perspectives

For 13 yr, there has been an ongoing and spirited debate over whether NO and its second messenger cGMP can stimulate or inhibit renin release or secretion. Well-established laboratories in the field have produced compelling data on both sides of the issue, and many have produced evidence that NO may, under the right circumstances, do both. Intuitively, increased renal perfusion, which is typically characterized by renin suppression, is associated with increased renal arteriolar shear stress. This is the predominant stimulus of eNOS. Alternatively, sodium restriction that stimulates renin via the macula densa pathway is associated with local upregulation of nNOS in the macula densa. If such pathways are mediated by NO, it seems logical that stimulatory and inhibitory pathways can exist and could differentiate due to the isoform activated or the origin of synthesized NO. However, the mechanisms by which such pathways might work and the conditions through which they might be differentiated have not been convincingly established. The present work, inspired by previously published and cited observations of other laboratories, provides some insight as to one possible mechanism by which renal NO synthesis, through the interaction of different cyclic nucleotide second messengers, might stimulate renin.

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