cGMP stimulates renin secretion in vivo by inhibiting phosphodiesterase-3

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Beierwaltes, William H. cGMP stimulates renin secretion in vivo by inhibiting phosphodiesterase-3. Am J Physiol Renal Physiol 290: F1376–F1381, 2006.—The interaction between renin, nitric oxide (NO), and its second messenger cGMP is controversial. cAMP is the stimulatory second messenger for renin but is degraded by phosphodiesterases (PDEs). We previously reported that increasing endogenous cGMP in rats by inhibiting its breakdown by PDE-5 stimulated renin secretion rate (RSR). This could be reversed by selective inhibition of neuronal nitric oxide synthase (nNOS). PDE-3 metabolizes cAMP, but this can be inhibited by cGMP, suggesting that renal cGMP could stimulate RSR by diminishing PDE-3 degradation of cAMP. Rats were anesthetized with Inactin before determination of blood pressure (BP), renal blood flow (RBF), and sampling of renal venous and arterial blood to determine RSR. In 13 rats, basal BP was 104 ± 2 mmHg, RBF was 6.1 ml·min⁻¹·g kidney wt⁻¹ and RSR was 2.9 ± 1.4 ng ANG I·h⁻¹·min⁻¹. Inhibiting PDE-5 with 20 mg/kg body wt ip Zaprinast did not change hemodynamic parameters but increased RSR fivefold to 12.2 ± 2.3 ng ANG I·h⁻¹·min⁻¹ (P < 0.05). Renal venous cAMP was increased by Zaprinast from 93.8 ± 27.9 to 149.2 ± 36.0 pM·min⁻¹·g kidney wt⁻¹ (P < 0.05). When another 10 rats were treated with the PDE-3 inhibitor Milrinone (0.4 μg/min over 30 min, which did not affect hemodynamics), RSR was elevated to 10.4 ± 4.4 ng ANG I·h⁻¹·min⁻¹. Milrinone also increased renal venous cAMP from 212 ± 29 to 304 ± 29 pM·min⁻¹·g kidney wt⁻¹ (P < 0.025). Administration of Zaprinast to rats pretreated with Milrinone (n = 10) did not further increase in RSR (7.5 ± 3.3 ng ANG I·h⁻¹·min⁻¹). These results are consistent with endogenous renin cGMP inhibiting PDE-3, which diminishes renal metabolism of cAMP. The resulting increase in cAMP serves as an endogenous stimulus for renin secretion. This suggests a pathway by which NO can indirectly stimulate RSR through its second messenger cGMP. cAMP; nitric oxide; Zaprinast; Milrinone; furosemide

Renin, the rate-limiting enzymatic step in formation of the potent vasoconstrictor angiotensin, is synthesized and stored in the juxtaglomerular (JG) cells of the renal afferent arteriole. Formation of the cyclic nucleotide cAMP is the second messenger, which integrates a variety of stimulatory signals with renin secretion (8). There has been considerable debate over the role of another nucleotide, cGMP in renin secretion. cGMP is the second messenger of nitric oxide (NO). Since Vidal et al. (30) first showed that NO inhibited renin release in vitro in 1988, numerous studies in a variety of systems have confirmed the inhibitory effect of NO or cGMP on renin (4, 30) or, alternatively, suggested that NO stimulates renin (16, 18, 24). The answer may lie in the location of specific isoforms of nitric oxide synthase (NOS). Stimulation of the endothelial isoform (eNOS) by increased renal perfusion and shear stress results in increased endothelium-derived NO and cGMP activation of a specific GII kinase in the JG cells that inhibits renin release (31). Alternatively, NO derived from the neuronal isoform of NOS (nNOS) has been implicated in mediating renin stimulation through the macula densa pathway. Sodium restriction upregulates nNOS in the macula densa (29). Studies in vitro by He et al. (14) and in vivo by Beierwaltes (2, 3) and by Reid and Chou (26) suggested that the neuronal isoform (nNOS), selectively found in the macula densa, is a component of this renin-stimulation pathway. However, there remained the question of how cGMP itself could result in dual actions of either inhibition or stimulation of renin release from the same JG cells. It was Ian Reid who, in a review article in 1994 (25), proposed that cGMP derived from NO might not act directly on renin release, but rather act on the cGMP-inhibitable phosphodiesterase (PDE)-3 to alter its hydrolysis of cAMP. This possibility was indirectly supported by their observation that the PDE-3 inhibitor Milrinone both increased basal plasma renin activity in rabbits and potentiated the renin response to cAMP-mediated stimulation by isoproterenol (7). Kurzt et al. (21), using an isolated perfused kidney preparation, also reported that PDE-3 inhibition with either Milrinone or Trequinsin stimulated renin secretion, consistent with a role for PDE-3 in degrading the endogenous cAMP-mediated signal for renin. Additional evidence was provided by Friis et al. (10) using isolated rat JG cells. They reported that JG cells expressed both isoforms of PDE-3, and further that Trequinsin increased cellular CAMP production and stimulated renin release from these cells. Thus PDE-3 is in the right place to mediate the interaction of cGMP and cAMP, and it appears that all the necessary components of the hypothetical pathway are in place to support Reid’s hypothesis (25).

The present study was designed to test the hypothesis that NO-mediated stimulation of renin takes place via an indirect pathway in which cGMP inhibits PDE-3 in the JG cells, potentiating cAMP stimulation of renin secretion. Because PDE-3 is cAMP selective but cGMP inhibitable (1), we repeated our previous observation (27) that increasing endogenous cGMP by selectively inhibiting its degradation by PDE-5 stimulates renin through its subsequent action on PDE-3. We carried out these studies in vivo to ensure that all necessary components are in the proper juxtaposition. Because changes in renal hemodynamics can influence renin secretion (17), we designed these studies to minimize any changes in renal hemodynamics or renal perfusion pressure. We provide evidence that endogenous cGMP, derived from macula densa stimulation of nNOS, inhibits PDE-3 in the JG cells, potentiating cAMP-mediated stimulation of renin secretion.
MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) weighing 300–400 g fasted overnight but were allowed free access to water. They were 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. A tracheotomy was performed with PE-240 tubing to allow spontaneous breathing of room air. The femoral vein was catheterized with PE-50 tubing for maintenance infusion of 40 μl/min 0.9% NaCl. The femoral artery was catheterized with PE-50 tubing attached to a Statham pressure transducer (Viggo-Spectramed, Oxnard, CA, calibrated using a mercury manometer) and a Gould recorder (Gould Instruments, Valley View, OH) for continuous monitoring of blood pressure (BP) and sampling of arterial blood.

The abdominal cavity was opened via a midventral incision. The intestines were wrapped in warm moist gauze and tucked under the right ventral wall, and the left renal artery and vein were dissected from the surrounding tissues. Renal venous blood was sampled with a 25-gauge needle bent to 90°, fitted to PE-50 tubing attached to a 1-ml syringe filled with heparinized saline. The needle was carefully placed in the left renal vein at its bifurcation with the vena cava, advanced to 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 to measure renal blood flow (RBF). 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, St. Louis, MO) 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 RBF.

All procedures using animals were reviewed by our Institutional Animal Care and Use Committee (IACUC) and adhere to the guiding principles in the care and use of experimental animals. Henry Ford Hospital’s animal facility is AAALAC approved.

Selective pharmacological inhibition of PDE-5 (22) was carried out with a single bolus of 20 mg/kg body wt ip Zaprinast (Biomol, Plymouth Meeting, PA) suspended in peanut oil by sonication and delivered via PE tubing placed within the gauze-wrapped intestine. We previously reported that this was the highest dose we could give without affecting either BP or RBF and produced a significant increase in both urinary cGMP excretion and renin secretion (27). PDE-3 was inhibited with (7, 13) Milrinone (Biomol) at a dose of 0.4 μg/min iv given over 30 min. During this period, we again determined BP, HR, and RBF and sampled blood for renin. To confirm the effect of Milrinone on cAMP degradation, in this protocol we measured urinary cAMP excretion and the renal venous cAMP effluent during the initial control period and again after Milrinone treatment.

Renin response to inhibition of PDE-3 with Milrinone. The next protocol was run to determine whether the PDE-3 inhibitor Milrinone could stimulate RSR and increase renal cAMP production. During the initial 30-min control period, BP, HR, and RBF were recorded and urine was collected to determine renal cAMP excretion. At the end of this period, femoral arterial and renal venous blood were collected for determination of basal RSR. Then the PDE-3 inhibitor Milrinone (or, in controls its saline vehicle) was delivered intravenously at a dose of 0.4 μg/min given over 30 min. During this period, we again determined BP, HR, and RBF and sampled blood for renin. We previously reported that this was the highest dose we could give so that layering additional treatments could not further stimulate renin.

To address this directly, the eight rats previously treated with furosemide (after the furosemide samples were completed) were given an additional 20-min period in which the β-adrenergic agonist isoproterenol was run for determination of BP, HR, and RBF, after which arterial and renal venous blood were sampled for RSR.
enol (Aldrich) was infused at a rate of 100 ng·kg body wt⁻¹·min⁻¹. At the end of the 20-min infusion, collections for RSR were repeated, and these values compared with RSR from the previous samples with furosemide alone.

**Analysis.** RVR was calculated as renal perfusion pressure (mmHg) divided by RBF (ml·min⁻¹·g kidney wt⁻¹) and given in units of milligrams Hg per milliliter per minute per gram kidney wt, hereafter referred to as resistance units or RU. RSR was calculated from the difference in plasma renin activity (PRA) between the renal vein and femoral arterial samples multiplied by renal plasma flow (RPF). The RPF was calculated from RBF by correcting for kidney weight and multiplied by 1 minus the hematocrit. RSR units are nanograms ANG I per hour per minute per gram kidney wt. PRA was determined by radioimmunoassay for generation of ANG I using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN). cAMP was determined in plasma and urine samples using an R&D Systems colorometric immunoassay kit (R&D Systems, Minneapolis, MN). Blood samples were collected in EDTA as an anticoagulant for later analysis according to the kit protocol. Urine was collected in the presence of IBMX (1 mM) to diminish cAMP degradation, diluted 1:200 and stored at −70°C until the assay. Responses to pharmacological interventions or vehicle were analyzed using paired t-tests. Comparisons between the experimental response and vehicle treatment were analyzed using an unpaired t-test, taking $P < 0.05$ as significant. All values are presented as means ± SE.

**RESULTS**

**Renin response to inhibition of PDE-5 with Zaprinast.** In 13 rats, basal BP was 104 ± 2 mmHg, HR was 300 ± 17 beats/min, RBF 6.14 ± 0.14 ml·min⁻¹·g kidney wt⁻¹, and RVR 17.9 ± 1.3 RU. Basal RSR was 2.90 ± 1.38 ng ANG I·h⁻¹·min⁻¹. Administration of Zaprinast had no effect on any hemodynamic parameter (BP, 99 ± 2 mmHg; HR, 278 ± 16 beats/min; RBF, 6.74 ± 0.14 ml·min⁻¹·g kidney wt⁻¹; RVR, 16.6 ± 1.7 RU), but RSR was increased fourfold to 12.22 ± 4.97 ng ANG I·h⁻¹·min⁻¹ ($P < 0.05$). Hemodynamic values in untreated time controls ($n = 10$) were unchanged (BP, 106 ± 4 mmHg; HR, 310 ± 26 beats/min; RBF, 6.13 ± 0.43 ml·min⁻¹·g kidney wt⁻¹; RVR, 17.4 ± 0.8 RU), and RSR did not increase, as it was 3.89 ± 2.82 ng ANG I·h⁻¹·min⁻¹ in the first period and 0.72 ± 1.10 ng ANG I·h⁻¹·min⁻¹ in the second period.

In the second set of eight rats run with Zaprinast for determination of cAMP and RSR, as before, there were no changes BP (101 ± 4 mmHg), HR (295 ± 13 beats/min), RBF (7.00 ± 0.51 ml·min⁻¹·g kidney wt⁻¹), or RVR (15.2 ± 1.5 RU). Urinary cAMP excretion was unchanged from basal to Zaprinast periods (58.6 ± 26.5 to 68.3 ± 24.1 pM/min, respectively). However, renal venous cAMP was increased significantly by Zaprinast treatment (Fig. 1) from a basal value of 93.8 ± 27.9 to 149 ± 36 pM·min⁻¹·g kidney wt⁻¹ ($P < 0.05$). RSR was increased twofold by Zaprinast (Fig. 1) from 4.45 ± 2.42 to 9.99 ± 4.15 ng ANG I·h/min/g kidney wt ($P < 0.05$).

**Renin response to inhibition of PDE-3 with Milrinone.** In 10 rats, basal BP was 107 ± 3 mmHg, HR was 309 ± 9 beats/min, RBF 7.77 ± 0.64 ml·min⁻¹·g kidney wt⁻¹, and RVR 14.6 ± 1.3 RU. Basal RSR was 4.23 ± 2.01 ng ANG I·h⁻¹·min⁻¹. Milrinone had no effect on any hemodynamic parameter (BP, 105 ± 3 mmHg; HR, 294 ± 11 beats/min; RBF, 7.61 ± 0.46 ml·min⁻¹·g kidney wt⁻¹; RVR, 14.2 ± 0.8 RU, but RSR was increased 2.5-fold (Fig. 2) to 10.11 ± 3.62 ng ANG I·h⁻¹·min⁻¹ ($P < 0.05$). Values in 10 untreated time controls were unchanged. RSR was 2.62 ± 1.22 ng ANG I·h⁻¹·min⁻¹ in the first period and 2.99 ± 1.53 ng ANG I·h⁻¹·min⁻¹ in the second period.

Urine cAMP excretion was unchanged by Milrinone treatment; it was 248 ± 24 pM/min in the control period and 225 ± 38 pM/min with Milrinone. However, renal venous cAMP increased by over 40% with Milrinone treatment, from 212 ± 8.7 to 309 ± 10.8 ng ANG I·h⁻¹·min⁻¹. Administration of Milrinone had no further RSR response to Milrinone (Fig. 1).
Parameter (BP, 114 mmHg, HR was 330 ± 14 beats/min, RBF 7.35 ± 0.45 ml·min⁻¹·g kidney wt⁻¹, and RVR 17.5 ± 1.1 RU. The RSR in the Milrinone-treated rats was 10.36 ± 4.41 ng ANG I·h⁻¹·min⁻¹, not different from the result of Milrinone treatment in the group represented in the left. Giving Zaprinast to Milrinone-treated rats had no effect on any hemodynamic parameter (BP, 114 ± 4 mmHg; HR, 337 ± 10 beats/min; RBF, 7.51 ± 0.47 ml·min⁻¹·g kidney wt⁻¹; RVR, 16.1 ± 1.4 RU). RSR remained elevated, as in the first period (7.53 ± 3.26 ng ANG I·h⁻¹·min⁻¹) but did not increase further any with Zaprinast (Fig. 2). This contrasts with a significant fourfold increase in RSR seen when Zaprinast was given to vehicle-treated rats.

Furosemide stimulation of renin and the effect of PDE-3 inhibition. In 15 rats, basal BP was 116 ± 2 mmHg, HR was 361 ± 9 beats/min, RBF 6.50 ± 0.47 ml·min⁻¹·g kidney wt⁻¹, and RVR 19.1 ± 1.5 RU. Basal RSR was 2.92 ± 1.06 ng ANG I·h⁻¹·min⁻¹ (Fig. 3). In the second control period, no hemodynamic parameters changed, and RSR also remained unchanged at 3.03 ± 1.25 ng ANG I·h⁻¹·min⁻¹. In the third period, administration of furosemide did not provide any significant hemodynamic changes (BP, 105 ± 3 mmHg; HR, 371 ± 13 beats/min; RBF, 5.64 ± 0.50 ml·min⁻¹·g kidney wt⁻¹; RVR, 21.2 ± 2.1 RU), but RSR was increased fourfold (Fig. 3) to 12.11 ± 5.78 ng ANG I·h⁻¹·min⁻¹ (P < 0.05).

In a second set of six rats, basal BP was 119 ± 6 mmHg, HR was 338 ± 12 beats/min, RBF 8.52 ± 0.49 ml·min⁻¹·g kidney wt⁻¹, and RVR 14.2 ± 1.1 RU. Basal RSR was 1.17 ± 0.96 ng ANG I·h⁻¹·min⁻¹ (Fig. 3). In the second period, Milrinone had no effect on hemodynamics (BP, 118 ± 6 mmHg; HR, 356 ± 13 beats/min; RBF, 10.20 ± 0.53 ml·min⁻¹·g kidney wt⁻¹; RVR, 11.8 ± 0.7 RU), but RSR increased significantly to 14.52 ± 2.04 ng ANG I·h⁻¹·min⁻¹ (P < 0.05). In the third period, furosemide treatment in the Milrinone group slightly decreased RBF (8.68 ± 0.53 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) and increased RVR (13.5 ± 0.8 RU; P < 0.05) while BP and HR remained unchanged. Furosemide had no additional stimulatory effect on RSR in Milrinone-treated rats (12.80 ± 10.54 ng ANG I·h⁻¹·min⁻¹; Fig. 3), and it was not different from the rats treated only with furosemide during the same period.

In an additional set of eight rats, furosemide stimulation of renin was accompanied by measurements of renal venous cAMP. A group of eight time controls was run without furosemide treatment (Fig. 1). Basal RSR was 3.14 ± 1.33 ng ANG I·h⁻¹·min⁻¹·g kidney wt⁻¹, and this was increased threefold with furosemide treatment to 9.13 ± 3.45 ng ANG I·h⁻¹·min⁻¹·g kidney wt⁻¹ (P < 0.05). RSR in unstimulated time controls was unchanged from 3.18 ± 1.92 to 1.76 ± 1.11 ng ANG I·h⁻¹·min⁻¹·g kidney wt⁻¹ in sequential unstimulated periods. Renal venous cAMP was also increased by furosemide (Fig. 1), from a basal value of 64.2 ± 10.8 to 99.6 ± 15.5 pmol·min⁻¹·g kidney wt⁻¹ (P < 0.05), while it remained unchanged in untreated time controls, from 59.2 ± 11.0 to 46.4 ± 7.8 pmol·min⁻¹·g kidney wt⁻¹.

In the eight rats previously treated with furosemide, additional isoproterenol treatment resulted in a further fourfold increase in RSR to 36.89 ± 11.28 ng ANG I·h⁻¹·min⁻¹·g kidney wt⁻¹ (P < 0.02). Thus this magnitude of renin stimulation by ~10 times the basal levels suggests the possible responses reported above are not truncated by some limiting maximal secretion.

**DISCUSSION**

We have found that inhibition of renal PDE-3 with Milrinone stimulated a significant three- to fivefold increase in renin secretion in vivo, as well as an increase in renal venous cAMP (the stimulatory second messenger for renin). This suggests that endogenous renal degradation of cAMP is an important component in overall regulation of renin secretion. Similar to our previous report (27), inhibition of PDE-5 degradation of renal cGMP with Zaprinast also stimulated renin secretion in vivo, as well as renal venous cAMP, but this response was eliminated if PDE-3 was already blocked. This supports Reid’s (25) hypothesis that cGMP acts indirectly to increase renin secretion by inhibiting PDE-3 degradation of cAMP. We believe the present study provides the first comprehensive in vivo support of Reid’s hypothesis in that it reflects every expected step; cGMP formation, increased cAMP mediated by inhibiting PDE-3, and consequent stimulation of RSR. It is also consistent with previous in vitro studies in isolated, perfused kidneys (21) and isolated JG cells (10).

To carry out these studies in vivo, it was essential to establish doses of both of the PDE inhibitors that would act on PDEs within the kidney without significantly changing renal hemodynamics, thereby affecting renin, while still eliciting a change in renal cyclic nucleotide production. We previously reported (27) that the current dose of the PDE-5 inhibitor Zaprinast would elicit a significant increase in renal cGMP excretion without changing blood pressure or renal hemodynamics. Additionally, before carrying out the present studies we established a dose of Milrinone that also significantly

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**Fig. 3.** RSR in rats increases significantly after Milrinone treatment (●, period 2, P < 0.05 vs. paired control values in period 1). In period 3, furosemide significantly increased RSR in vehicle-treated rats (○; P < 0.05 vs. paired control values in period 2) but did not stimulate RSR further in rats pretreated with Milrinone (●). Full circles in the key indicate treatment during that period; halved circles indicate that both groups received similar treatment.
increased renal venous cAMP concentration (although not affecting renal cAMP excretion) without significantly altering blood pressure or renal hemodynamics. Blocking degradation of either cyclic nucleotide resulted in a significant increase in renin secretion. However, if we first inhibited PDE-3 with Milrinone, then added Zaprinast, we found that it prompted no further increase in renin secretion, suggesting that cGMP stimulation of renin is mediated indirectly through its selective effect to block PDE-3 breakdown of cAMP (9) independent of any hemodynamic changes.

We previously found that inhibition of the cGMP-degrading PDE-5 with Zaprinast stimulated renin secretion (27). We also found that nonselective inhibition of NOS with l-NAME completely reversed the stimulation of renin secretion induced 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 in the macula densa, may play a significant role in this renin-stimulating pathway. The role of cGMP in influencing cAMP is based on Dousa’s observations that PDE 3 is inhibitable by cGMP (9). Inhibition of either PDE 3 or PDE 4, both found in the JG cells, resulted in increased PRA in rabbits (7) and humans (6). Additionally, frequensin inhibition of PDE-3 in isolated JG cells increased cellular cAMP content and renin release (10), as did inhibition of PDE-4. This in vitro release could be blocked by a protein kinase A inhibitor, suggesting that cAMP acts through stimulation of cAMP-sensitive protein kinase. Our observation that PDE-3 inhibition preempts subsequent cGMP stimulation of renin as well as macula densa-mediated furosemide stimulation of renin suggests that a major component in the macula densa pathway for regulating renin is mediated by PDE-3 and its inhibition by cGMP derived from nNOS. This is consistent with reports in vivo in which NOS inhibition using l-NAME (26) or selective nNOS inhibition by 7-NI (2) blocked furosemide-stimulated renin secretion in vivo.

Because we report that the renin responses to pharmacological interventions were similar, the question arises as to whether the lack of any additive effect (as with Zaprinast plus Milrinone) might not be due to the redundancy in the system, but rather to having reached a maximal stimulation of renin such that any further response was not possible. To address this potential limitation, we took rats that had just been treated with furosemide and added to this the adrenergic agonist isoproterenol. Isoproterenol evoked a profound additional renin secretory response, fourfold greater than the effect found with furosemide (and 10-times the basal level). Thus it is clear that the results we report are not due to a truncated response of renin in our model.

There is considerable controversy as to whether NO or its second messenger cGMP inhibits renin secretion (4, 15, 19, 30, 31), stimulates it (16, 18, 20, 21, 24), or both (14, 28). Exogenous cGMP suppresses renin release from isolated JG cells (19), cortical kidney slices (15), and the isolated perfused kidney (31). Endothelium-derived NO, which stimulates cGMP production, can also inhibit renin (4, 30). Inhibition of renin by cGMP has been characterized by Wagner et al. (31) as acting through G kinase-II found in the JG cells. The most likely source of this cGMP is eNOS-derived NO in the arteriolar endothelium, stimulated by the elevated shear stress that accompanies increased renal perfusion. Notably, these conditions of renin inhibition occur in the absence of coincident stimulation of endogenous renal cAMP or any renin-stimulating signals. In contrast, NO stimulation of renin seems to involve the macula densa pathway. Chronic sodium restriction results in coincident increases in COX-2 and nNOS (13) in the macula densa. By generating PGE2, COX-2 stimulates adenylate cyclase and causes cAMP-mediated renin secretion from the JG cells. Inhibition of COX-2 has been shown to block the increase of renal renin content, renin mRNA, and renin release in vitro (12) and retard chronic stimulation of renin in vivo (11) in response to dietary sodium restriction. In nNOS knockout mice (12), or following selective nNOS inhibition, the renin response to sodium restriction persists, but basal levels of renin are reduced. This suggests that NO influences but does not regulate the magnitude of the renin response to cAMP. Castrop et al. (5) reported that PRC in nNOS knockout mice was significantly lower than in wild-type controls, but the renin response to chronic or acute loop diuretics was not markedly altered. They concluded that nNOS from the macula densa does not play a specific role in macula densa-dependent renin secretion, but NO does play a permissive role allowing the macula densa to function normally. These reports are consistent with the idea that cGMP may exaggerate cAMP-mediated renin stimulation through its effect on PDE-3, but in the absence of stimulated cAMP, cGMP acts as an inhibitory regulator (4, 31). Thus the stimulation of renin by cAMP is a function of both its synthesis and its breakdown within the JG cell, and the effect of cGMP on PDE 3 contributes to this balance but does not exclusively regulate it.

In summary, we found that PDE-5 inhibition with Zaprinast increases urinary cGMP excretion and increases renin secretion and renal venous cAMP. Additionally, PDE-3 inhibition with Milrinone increases both renal venous cAMP and renin secretion. However, initial inhibition of PDE-3 blocks any further increase in renin secretion by subsequent PDE-5 inhibition. We believe increased renal cGMP inhibits PDE-3 metabolism of cAMP, thereby serving as a potent endogenous stimulus of renin secretion. Our data are consistent with the hypothesis that cGMP may stimulate renin indirectly by inhibiting PDE-3 cAMP degradation, facilitating an increase in this potent second messenger for renin secretion.

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GRANTS

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REFERENCES


