Regulation of Renovascular Adenosine 3′,5′-Cyclic Monophosphate in Spontaneously Hypertensive Rats

Edwin K. Jackson, Zaichuan Mi

Abstract—This study tested the hypothesis that regulation of 3′,5′-cAMP levels in the kidney vasculature is abnormal in spontaneously hypertensive rats. In isolated, perfused kidneys from adult rats (16 weeks of age), isoproterenol similarly increased renal venous 3′,5′-cAMP secretion from kidneys of hypertensive versus normotensive Wistar-Kyoto rats. However, a broad-spectrum phosphodiesterase inhibitor (isobutyl-1-methylxanthine) augmented isoproterenol (3 μmol/L)-induced increases in renal venous 3′,5′-cAMP secretion more so in kidneys from adult hypertensive versus age-matched normotensive rats (31-fold and 5-fold, respectively; P<0.0001). In contrast to isoproterenol, broad-spectrum phosphodiesterase inhibition augmented forskolin-induced increases in renal venous 3′,5′-cAMP secretion similarly in kidneys from adult hypertensive versus age-matched normotensive rats. In kidneys from adults of both strains, the effects of isobutyl-1-methylxanthine on isoproterenol-induced 3′,5′-cAMP responses were mimicked by the inhibition of phosphodiesterase 4 (RO 20-1724) but not by the inhibition of phosphodiesterase 1 (3,8-methoxymethyl-3-isobutyl-1-methylxanthine) or phosphodiesterase 3 (milrinone). In kidneys from young (5 weeks of age), adult, and old (39 weeks of age) rats, RO 20-1724 augmented isoproterenol-induced renal 3′,5′-cAMP secretion more so in kidneys from hypertensive rats. In adult hypertensive rats, arterial blood pressure and renal vascular resistance were elevated compared with age-matched normotensive rats, and intravenous infusions of RO 20-1724 reduced blood pressure and renal vascular resistance in hypertensive rats but had little effect on these variables in normotensive rats. We conclude that, in the renal vasculature of spontaneously hypertensive rats (young, adult, and old), there is increased activity of a compartment of phosphodiesterase 4. Selective inhibition of renal vascular phosphodiesterase 4 may represent a new strategy for improving renal hemodynamics in genetic hypertension. (Hypertension. 2009;54:00-00.)

Key Words: 3′,5′-cAMP ▪ adenyl cyclase ▪ phosphodiesterase ▪ isoproterenol ▪ forskolin ▪ spontaneously hypertensive rats ▪ Wistar-Kyoto rats

3′,5′-cAMP importantly regulates renal vascular tone, and dysregulation of 3′,5′-cAMP levels in the renal microcirculation may contribute to the pathophysiology of genetic hypertension. However, whether receptor-induced 3′,5′-cAMP in the renal vasculature is normal, reduced, or elevated in animal models of genetic hypertension is unclear. In renal microvessels freshly isolated using intrarenal artery delivery of magnetized iron oxide particles, prostaglandin E2, prostaglandin I2, and isoproterenol stimulated 3′,5′-cAMP levels less in arterioles from spontaneously hypertensive rats (SHRs) versus normotensive Wistar-Kyoto rats (WKYs). It is conceivable, however, that injury of the arterioles by luminal iron oxide particles altered the responses to agonists and more so in arterioles from SHRs. In contrast to the results in freshly isolated arterioles, isoproterenol-induced 3′,5′-cAMP was significantly greater in cultured preglomerular vascular smooth muscle cells obtained from SHRs compared with similar cells obtained from WKYs. However, it is possible that the regulation of 3′,5′-cAMP is much different in cultured preglomerular vascular smooth muscle cells compared with similar cells in vivo. At odds with the results obtained in either freshly isolated preglomerular arterioles or cultured preglomerular vascular smooth muscle cells, a study in isolated, perfused kidneys reported that isoproterenol-induced renal venous 3′,5′-cAMP secretion (which likely represents mostly vascularly derived 3′,5′-cAMP) was similar in kidneys obtained from SHRs versus WKYs. However, this study was performed in the presence of a low concentration of isobutyl-1-methylxanthine, a broad-spectrum phosphodiesterase (PDE) inhibitor, whereas the aforementioned studies in freshly isolated microvessels and cultured cells were performed with 100-fold and 10-fold higher concentrations of isobutyl-1-methylxanthine, respectively. Therefore, there may have been incomplete inhibition of PDEs in the latter study.

Because previous studies have reported decreased, increased, or no change in the response of the renal vasculature to agonist-induced stimulation of 3′,5′-cAMP in genetic hypertension, we decided to investigate this question in more...
ureter was cannulated with polyethylene (PE) 10 tubing, and the left ureter were dissected free from surrounding tissue. The left kidney, left renal artery, abdominal aorta, and D(-)-glucose, 5.6) containing heparin (100 U/mL). The left kidney was isolated without interrupting perfusion and placed in a water-jacketed organ chamber maintained at 37°C with a thermostatically controlled water circulator (Thermocirculator, Harvard Apparatus). Kidneys were perfused with oxygenated Tyrode’s solution at 5 mL/min in a nonrecirculating manner with a Harvard model 1210 peristaltic pump. Before entering the kidney, the perfusate was pumped through a warming coil (37°C) that was fitted with a bubble trap. Perfusion pressure was measured with a Statham pressure transducer (model P23ID, Statham Division, Gould Inc) connected to an access port located above the kidney on the perfusion cannula and a Grass model 79D polygraph (Grass Instruments).

After the initiation of perfusion, kidneys were allowed to stabilize for 1.5 hours and then were treated with isoproterenol: 3 and then 10 μmol/L of isoproterenol were added directly to the Tyrode’s solution for 5 minutes at each concentration. One minute before and during the last 1 minute of the treatment with each concentration of isoproterenol, perfusate exiting the renal vein was collected on ice for later analysis of 3',5'-cAMP. Next, the treatment with isoproterenol was stopped, and kidneys were treated with one of the following: (1) 1,3-isobutyl-1-methylxanthine (IBMX), a "broad-

### Table. Basal Renal Venous cAMP Secretion in the Absence of Isoproterenol or Forskolin

<table>
<thead>
<tr>
<th>Group</th>
<th>0, μmol/L</th>
<th>10, μmol/L</th>
<th>30, μmol/L</th>
<th>100, μmol/L</th>
<th>300, μmol/L</th>
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<tbody>
<tr>
<td><strong>IBMX (μmol/L)</strong></td>
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<tr>
<td>Basal levels for isoproterenol experiment</td>
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<tr>
<td>16 WOA WKY</td>
<td>&lt;DL</td>
<td>0.0051 ± 0.0004</td>
<td>0.0060 ± 0.0010</td>
<td>0.0074 ± 0.0007</td>
<td>0.0103 ± 0.0012</td>
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<tr>
<td>16 WOA SHR</td>
<td>0.0066 ± 0.0011</td>
<td>0.0081 ± 0.0006</td>
<td>0.0095 ± 0.0010</td>
<td>0.0105 ± 0.0009</td>
<td>0.0132 ± 0.0014</td>
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<tr>
<td><strong>mmIBMX (μmol/L)</strong>, basal levels for isoproterenol experiment</td>
<td></td>
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<tr>
<td>16 WOA WKY</td>
<td>0.0040 ± 0.0010</td>
<td>0.0030 ± 0.0010</td>
<td>0.0030 ± 0.0010</td>
<td>0.0040 ± 0.0010</td>
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<tr>
<td>16 WOA SHR</td>
<td>0.0050 ± 0.0020</td>
<td>0.0080 ± 0.0010</td>
<td>0.0070 ± 0.0010</td>
<td>0.0080 ± 0.0010</td>
<td>0.0100 ± 0.0020</td>
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<tr>
<td><strong>Minoxidine (μmol/L)</strong>, basal levels for isoproterenol experiment</td>
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<tr>
<td>16 WOA WKY</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>0.0050 ± 0.0010</td>
<td>0.0050 ± 0.0010</td>
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<tr>
<td>16 WOA SHR</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>0.0080 ± 0.0010</td>
<td>0.0080 ± 0.0010</td>
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<td><strong>R0 20–1724 (μmol/L)</strong></td>
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<td>Basal levels for isoproterenol experiment</td>
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<tr>
<td>16 WOA WKY</td>
<td>&lt;DL</td>
<td>0.0086 ± 0.0008</td>
<td>0.0085 ± 0.0010</td>
<td>0.0085 ± 0.0007</td>
<td>0.0092 ± 0.0005</td>
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<tr>
<td>16 WOA SHR</td>
<td>&lt;DL</td>
<td>0.0134 ± 0.0009</td>
<td>0.0134 ± 0.0009</td>
<td>0.0128 ± 0.0013</td>
<td>0.0127 ± 0.0015</td>
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<tr>
<td>Basal levels for isoproterenol experiment</td>
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<tr>
<td>5 WOA WKY</td>
<td>&lt;DL</td>
<td>0.0130 ± 0.0001</td>
<td>0.0160 ± 0.0020</td>
<td>0.0160 ± 0.0020</td>
<td>0.0150 ± 0.0030</td>
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<tr>
<td>5 WOA SHR</td>
<td>&lt;DL</td>
<td>0.0240 ± 0.0020</td>
<td>0.0240 ± 0.0020</td>
<td>0.0250 ± 0.0010</td>
<td>0.0210 ± 0.0030</td>
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<tr>
<td>Basal levels for isoproterenol experiment</td>
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<tr>
<td>39 WOA WKY</td>
<td>0.0090 ± 0.0030</td>
<td>0.0160 ± 0.0060</td>
<td>0.0150 ± 0.0030</td>
<td>0.0160 ± 0.0030</td>
<td>0.0170 ± 0.0030</td>
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<tr>
<td>39 WOA SHR</td>
<td>0.0110 ± 0.0020</td>
<td>0.0160 ± 0.0030</td>
<td>0.0150 ± 0.0020</td>
<td>0.0140 ± 0.0020</td>
<td>0.0130 ± 0.0020</td>
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WOA indicates weeks of age; <DL, less than the assay detection limit. Values are mean ± SEM (n = 5 to 8).

Methods

**Animals**

Studies used male SHRs and WKys of different ages (5, 16, and 39 weeks of age) that were obtained from Taconic Farms (Germantown, NY). The Institutional Animal Care and Use Committee approved all of the procedures. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

**Experiments in Isolated, Perfused Kidneys**

Rats were anesthetized with sodium pentobarbital (45 mg/kg, IP injection), and the left kidney, left renal artery, abdominal aorta, and left ureter were dissected free from surrounding tissue. The left ureter was cannulated with polyethylene (PE) 10 tubing, and the abdominal aorta below the left kidney was cannulated with PE-50 tubing. After the suprarenal aorta was ligated, the left kidney was rapidly flushed, via the PE-50 cannula, with oxygenated (95% O₂/5% CO₂) Tyrode’s solution (composition in mM: NaCl, 137.0; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 1.1; NaHCO₃, 12.0; NaH₂PO₄, 0.42; and D(-)-glucose, 5.6) containing heparin (100 U/mL). The left kidney was isolated without interrupting perfusion and placed in a water-jacketed organ chamber maintained at 37°C with a thermostatically controlled water circulator (Thermocirculator, Harvard Apparatus). Kidneys were perfused with oxygenated Tyrode’s solution at 5 mL/min in a nonrecirculating manner with a Harvard model 1210 peristaltic pump. Before entering the kidney, the perfusate was pumped through a warming coil (37°C) that was fitted with a bubble trap. Perfusion pressure was measured with a Statham pressure transducer (model P23ID, Statham Division, Gould Inc) connected to an access port located above the kidney on the perfusion cannula and was displayed on a Grass model 79D polygraph (Grass Instruments).

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spectrum” PDE inhibitor; (2) Ro 20-1724, a selective PDE4 inhibitor; (3) 8-methoxymethyl-3-isobutyl-1-methylxanthine (mIBMX), a selective PDE1 inhibitor; or (4) milrinone, a selective PDE3 inhibitor. A low concentration of each inhibitor was added to the Tyrode’s solution, and 30 minutes later the kidney was again treated with 3 and then 10 µmol/L of isoproterenol added directly to the Tyrode’s solution for 5 minutes at each concentration. One minute before and during the last 1 minute of the treatment with each concentration of isoproterenol, perfusate exiting the renal vein was collected on ice for later analysis of 3',5'-cAMP. The isoproterenol treatment was stopped, the concentration of inhibitor was increased 3-fold, and, 30 minutes later, isoproterenol treatments and collections of venous effluent were repeated. This procedure was repeated twice more as the concentration of each inhibitor in the Tyrode’s solution was increased 3-fold each time. Thus, perfusate exiting the renal vein was collected from isoproterenol-stimulated kidneys in the absence and presence of 4 different concentrations of a single PDE inhibitor, with each concentration of inhibitor being administered for 30 minutes before stimulating with isoproterenol and collecting the perfusate. In 1 set of experiments, forskolin (1 and 3 µmol/L), rather than isoproterenol, was used as the agonist to stimulate 3',5'-cAMP production. The concentration range of each inhibitor was selected such that the lowest concentration was approximately the IC_{50}, and the highest concentration was ∼30 times the IC_{50}. At the end of each experiment, the kidneys were blotted dry and weighed.

The concentration of 3',5'-cAMP in the renal venous effluent was determined with a high-pressure liquid chromatographic-fluorometric assay, as described previously. Renal venous 3',5'-cAMP secretion was calculated by multiplying the concentration of 3',5'-cAMP in the renal venous perfusate by the volume of collected perfusate and by dividing this by the collection time and kidney weight (to give nanomoles of 3',5'-cAMP per gram of kidney per minute). The agonist-induced (either isoproterenol or forskolin) venous secretion of 3',5'-cAMP was calculated by subtracting the basal 3',5'-cAMP venous secretion (ie, the 3',5'-cAMP secretion observed just before the agonist treatment) from the 3',5'-cAMP secretion observed during the agonist treatment. Previously, we demonstrated that agonist-induced venous secretion of 3',5'-cAMP from isolated, perfused SHR and WKY kidneys was reliable over ∼4 hours of perfusion, and although responses to isoproterenol tended to decrease over time, the time-related reductions were similar in SHR versus WKY kidneys.

Experiments In Vivo

Rats were anesthetized with Inactin (100 mg/kg, IP), placed on an isothermal pad, and body temperature was monitored with a rectal probe thermometer and kept at 37°C with a heat lamp. The trachea was cannulated with PE-240 to facilitate respiration, and a PE-50 cannula was inserted into the left carotid artery cannula and connected to a digital blood pressure analyzer (Micro-Med, Inc) for continuous measurement of mean arterial blood pressure (MABP) and heart rate (HR). A PE-50 cannula was placed in the left jugular vein, and an infusion of 0.9% saline was begun at 100 µL/min. A transit-time flow probe (model 1 RB, Transonic Systems, Inc) was positioned around the left renal artery and connected to a transit-time flow probe (model 1 RB, Transonic Systems, Inc) was to monitor renal blood flow (RBF). All of the animals were allowed a stabilization period of 1 hour before the experimental protocol was performed. MABP, HR, and RBF were measured during the last 30 minutes of three 60-minute experimental periods in which RO 20-1724 was infused IV at increasing doses (0, 3, and 10 µg/kg per min; 100 µL/min in 0.9% saline). Renal vascular resistance (RVR) was calculated by dividing MABP by RBF normalized to body weight.

Statistical Analysis

Variables were compared with either an independent-sampling 1-factor ANOVA followed by a Fisher’s least significant difference test, a repeated-measures 2-factor ANOVA, or a repeated-measures 3-factor ANOVA. Statistical analysis was performed by using the Number Cruncher Statistical System, and all of the values in the text and figure refer to mean ± SEM.

Figure 1. Line graphs illustrate concentration-dependent effects of IBMX, a broad-spectrum PDE inhibitor, on isoproterenol-induced renal venous secretion of 3',5'-cAMP in kidneys from 16-week-old SHRs vs WKYs. A and B summarize results for the 3- and 10-µmol/L concentrations of isoproterenol, respectively.

Results

Experiments in Isolated, Perfused Kidneys

Basal levels of renal venous 3',5'-cAMP secretion were very low (less than the assay detection limit in some samples), similar in kidneys from WKYs and SHRs and increased slightly or not at all by PDE inhibitors (Table). Basal perfusion pressures were low (∼50 mm Hg) in all of the groups, because the isolated, perfused kidneys were maximally vasodilated in the absence of endogenous and exogenous vasoconstrictors. Perfusion pressures were not affected by any of the treatments (data not shown) because of the low basal tone.

In kidneys from 16-week–old SHRs and WKYS, in the absence of PDE inhibitors, isoproterenol-induced renal venous 3',5'-cAMP secretion was similar in kidneys from SHRs versus WKYS (Figure 1A and 1B for 3 and 10 µmol/L of isoproterenol, respectively). IBMX, in a concentration-dependent manner, augmented isoproterenol-induced renal venous 3',5'-cAMP at both the 3 and 10 µmol/L concentrations of isoproterenol (Figure 1A and 1B, respectively; P<0.0001). However, the augmentation by IBMX of both concentrations of isoproterenol was significantly (P<0.0001) greater in kidneys from 16-week–old SHRs compared with kidneys from aged-matched WKYS.

In kidneys from 16-week–old SHRs and WKYS, in the absence of PDE inhibitors, forskolin-induced renal venous 3',5'-cAMP secretion was similar in kidneys from SHRs
versus WKYs (Figure 2A and 2B for 1 and 3 µmol/L of forskolin, respectively). In contrast to the strain-dependent effects of IBMX on isoproterenol-induced renal venous 3′,5′-cAMP secretion, IBMX significantly (*P<0.0001) increased forskolin-induced renal venous 3′,5′-cAMP secretion, but in a strain-independent manner (Figure 2A and 2B for 1 and 3 µmol/L of forskolin, respectively).

In kidneys from both 16-week-old SHRs (Figure 3) and WKYs (Figure 4), IBMX caused concentration-dependent increases in isoproterenol-induced renal venous 3′,5′-cAMP secretion (Figure 3A and 3B for SHR kidneys with 3 and 10 µmol/L of isoproterenol, respectively, and Figure 4A and 4B for WKY kidneys with 3 and 10 µmol/L of isoproterenol, respectively). RO 20-1724 also augmented isoproterenol-induced renal venous 3′,5′-cAMP secretion but was ≈30-fold more potent than IBMX. In kidneys from SHRs (Figure 3) and WKYs (Figure 4), at the highest concentrations of IBMX and RO 20-1724 that were investigated, the isoproterenol-induced renal venous 3′,5′-cAMP secretions were nearly identical in IBMX-treated versus RO 20-1724–treated kidneys. Stated differently, high concentrations of RO 20-1724 mimicked the effects of high concentrations of IBMX in kidneys from both 16-week-old SHRs and WKYs. In contrast to IBMX and RO 20-1724, neither mmIBMX nor milrinone affected isoproterenol-induced renal venous 3′,5′-cAMP secretion (Figures 3 and 4).

In kidneys from 5-, 16-, and 39-week-old SHRs and WKYs, RO 20-1724 significantly (*P<0.0001) augmented isoproterenol-induced renal venous 3′,5′-cAMP at both the 3- and 10-µmol/L concentrations of isoproterenol (Figure 5A, 5B, and 5C, respectively, for the 3-µmol/L concentration of isoproterenol and Figure 6A, 6B, and 6C, respectively, for the 10-µmol/L concentration of isoproterenol). As with IBMX, the augmentation by RO 20-1724 of both concentrations of isoproterenol was significantly (*P<0.0001) greater in kidneys from all ages of SHRs compared with kidneys from aged-matched WKYs (Figures 5 and 6 for the 3- and 10-µmol/L concentrations of isoproterenol, respectively). The interaction between RO 20-1724 and rat strain (*P<0.0001) was independent of age of the animal from which the kidneys were obtained (*P=0.6293 and *P=0.6345 for isoproterenol at 3 and 10 µmol/L, respectively).
Experiments In Vivo

As illustrated in Figure 7A, RO 20-1724 administered IV did not alter MABP in WKYS; however, the same doses of RO 20-1724 decreased the elevated levels of MABP in SHRS. In this regard, the interaction between rat strain and IBMX was significant \((P=0.0203)\). RO 20-1724 increased HR similarly in SHRS (399 ± 5, 422 ± 6, and 414 ± 9 bpm at 0, 3, and 10 g/kg per min, respectively) versus WKYS (398 ± 12, 426 ± 7, and 431 ± 8 bpm at 0, 3, and 10 g/kg per minute, respectively).

As shown in Figure 7B, RO 20-1724 increased RBF despite the fall in MABP induced by RO 20-1724 in SHRS. This effect tended to be larger in SHRS compared with WKYS. Although the interaction between strain and RO 20-1724 was not statistically significant with respect to RBF, this interaction was statistically significant with respect to RVR \((P=0.0377)\). In this regard, RVR was clearly higher in SHRS compared with WKYS and was nearly normalized to that of WKYS by RO 20-1724, whereas RO 20-1724 had little, if any, effect on RVR in WKYS (Figure 7C).

**Figure 4.** Bar graphs illustrate concentration-dependent effects of IBMX (a broad-spectrum PDE inhibitor), mmIBMX (a selective PDE1 inhibitor), milrinone (a selective PDE3 inhibitor), and RO 20-1724 (a selective PDE4 inhibitor) on isoproterenol-induced renal venous secretion of 3',5'-cAMP in kidneys from 16-week-old WKYS. A and B summarize results for the 3- and 10-μmol/L concentrations of isoproterenol, respectively. “a” and “b” indicate \(P<0.05\) compared with all other groups or compared with mmIBMX and milrinone, respectively, at the indicated concentration level of PDE inhibitor.

**Figure 5.** Line graphs illustrate concentration-dependent effects of RO 20-1724, a selective PDE4 inhibitor, on isoproterenol (3 μmol/L)-induced renal venous secretion of 3',5'-cAMP in kidneys from 5- (A), 16- (B), and 39- (C) week-old SHRS vs WKYS.
regulation of 3',5'-cAMP, we selected the isolated, perfused rat kidney for study to avoid potential artifacts and pitfalls related to isolating renal microvessels with iron oxide particles or culturing renovascular smooth muscle cells in vitro. We decided to study SHRs and WKYs as the model of genetic hypertension and normotension, respectively, because of the contrasting reports regarding the regulation of 3',5'-cAMP levels in the renal vasculature of these rat strains.5,7,8 We selected renal venous secretion of 3',5'-cAMP as the index of renal vascular 3',5'-cAMP production because 3',5'-cAMP in vascular smooth muscle cells5,16 and vascular endothelial cells17 is robustly transported to the extracellular space and, therefore, would have immediate access to the vascular lumen. In contrast, 3',5'-cAMP produced by tubules and renal interstitial cells would have to negotiate diffusion and metabolic barriers between the interstitial space and the vascular lumen. Indeed, in the isolated, perfused rat kidney, stimulation of tubular adenylyl cyclase with parathyroid hormone increases renal tissue 3',5'-cAMP and urinary 3',5'-cAMP by 10-fold and >100-fold, respectively but has negligible effects on perfusate 3',5'-cAMP.18 Limitations, however, of the model system are that it cannot resolve whether the source of renal venous 3',5'-cAMP is the vascular smooth muscle or vascular endothelium or both or whether the source of renal venous 3',5'-cAMP is preglomerular versus postglomerular or both. Also, renal venous 3',5'-cAMP could derive in part from nonvascular cell types.

Because both the production and metabolism of 3',5'-cAMP would influence levels of 3',5'-cAMP in the kidney vasculature, it is likely that the relative levels of 3',5'-cAMP in the renal vasculature in SHR versus WKY kidneys would depend critically on whether renovascular PDE activity is inhibited. Indeed, in the first experimental series, renal venous 3',5'-cAMP secretion in response to isoproterenol (a β-adrenoceptor agonist) was similar in SHR versus WKY kidneys from adult (16-week-old) animals in the absence of IBMX, a broad-spectrum PDE inhibitor, yet was much higher in SHR kidneys in the presence of IBMX. The concentrations of isoproterenol used in this study achieved maximum activation of β-adrenoceptors, as evidenced by the fact that the 10-μmol/L concentration did not give 3',5'-cAMP responses greater than those observed with the 3-μmol/L concentration. Thus, any differences between SHRs and WKYSs with respect to the ability of β-adrenoceptors to augment 3',5'-cAMP could not have been attributed strain-dependent shifts in the concentration-response relationship to isoproterenol. We interpret these findings to imply that, in the adult SHR renal vasculature, there is an increased coupling of vascular β-adrenoceptors to adenylyl cyclase, but the effect of this increased coupling on 3',5'-cAMP levels is masked by concomitant increases in the activity of renal vascular PDE activity. Thus, inhibition of renal PDE activity with IBMX increases 3',5'-cAMP to a much higher level in kidneys from adult SHRs versus kidneys from age-matched WKYSs.

The results with isoproterenol (a receptor-mediated stimulus to adenylyl cyclase) and forskolin (a direct activator of adenylyl cyclase) were qualitatively different. In this regard, IBMX augmented isoproterenol-induced 3',5'-cAMP more so in

Discussion

The main objective of this research was to characterize the regulation of 3',5'-cAMP levels in the renal vasculature of genetically hypertensive and normotensive rats. The model system was the isolated, perfused rat kidney using kidneys from the SHR and WKY strains of genetic hypertension and normotension, respectively, and the response variable was the renal venous secretion of 3',5'-cAMP. We selected the isolated, perfused rat kidney for study to avoid potential artifacts and pitfalls related to isolating renal microvessels with iron oxide particles or culturing renovascular smooth muscle cells in vitro. We decided to study SHRs and WKYs as the model of genetic hypertension and normotension, respectively, because of the contrasting reports regarding the regulation of 3',5'-cAMP levels in the renal vasculature of these rat strains.5,7,8 We selected renal venous secretion of 3',5'-cAMP as the index of renal vascular 3',5'-cAMP production because 3',5'-cAMP in vascular smooth muscle cells5,16 and vascular endothelial cells17 is robustly transported to the extracellular space and, therefore, would have immediate access to the vascular lumen. In contrast, 3',5'-cAMP produced by tubules and renal interstitial cells would have to negotiate diffusion and metabolic barriers between the interstitial space and the vascular lumen. Indeed, in the isolated, perfused rat kidney, stimulation of tubular adenylyl cyclase with parathyroid hormone increases renal tissue 3',5'-cAMP and urinary 3',5'-cAMP by 10-fold and >100-fold, respectively but has negligible effects on perfusate 3',5'-cAMP.18 Limitations, however, of the model system are that it cannot resolve whether the source of renal venous 3',5'-cAMP is the vascular smooth muscle or vascular endothelium or both or whether the source of renal venous 3',5'-cAMP is preglomerular versus postglomerular or both. Also, renal venous 3',5'-cAMP could derive in part from nonvascular cell types.

Because both the production and metabolism of 3',5'-cAMP would influence levels of 3',5'-cAMP in the kidney vasculature, it is likely that the relative levels of 3',5'-cAMP in the renal vasculature in SHR versus WKY kidneys would depend critically on whether renovascular PDE activity is inhibited. Indeed, in the first experimental series, renal venous 3',5'-cAMP secretion in response to isoproterenol (a β-adrenoceptor agonist) was similar in SHR versus WKY kidneys from adult (16-week-old) animals in the absence of IBMX, a broad-spectrum PDE inhibitor, yet was much higher in SHR kidneys in the presence of IBMX. The concentrations of isoproterenol used in this study achieved maximum activation of β-adrenoceptors, as evidenced by the fact that the 10-μmol/L concentration did not give 3',5'-cAMP responses greater than those observed with the 3-μmol/L concentration. Thus, any differences between SHRs and WKYSs with respect to the ability of β-adrenoceptors to augment 3',5'-cAMP could not have been attributed strain-dependent shifts in the concentration-response relationship to isoproterenol. We interpret these findings to imply that, in the adult SHR renal vasculature, there is an increased coupling of vascular β-adrenoceptors to adenylyl cyclase, but the effect of this increased coupling on 3',5'-cAMP levels is masked by concomitant increases in the activity of renal vascular PDE activity. Thus, inhibition of renal PDE activity with IBMX increases 3',5'-cAMP to a much higher level in kidneys from adult SHRs versus kidneys from age-matched WKYSs.

The results with isoproterenol (a receptor-mediated stimulus to adenylyl cyclase) and forskolin (a direct activator of adenylyl cyclase) were qualitatively different. In this regard, IBMX augmented isoproterenol-induced 3',5'-cAMP more so in

Figure 6. Line graphs illustrate concentration-dependent effects of RO 20-1724, a selective PDE4 inhibitor, on isoproterenol (10 μmol/L)-induced renal venous secretion of 3',5'-cAMP in kidneys from 5- (A), 16- (B), and 39- (C) week-old SHRs vs WKYSs. 

3-Factor ANOVA

Age: p=0.3025
Strain: p=0.0002
RO 20-1724: p<0.0001
Age x Strain: p=0.4926
Age x RO 20-1724: p=0.5274
Age x RO 20-1724 x Strain: p=0.0001
Age x RO 20-1724 x Strain: p=0.6345

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SHR versus WKY kidneys, yet it augmented forskolin-induced 3′,5′-cAMP similarly in SHR versus WKY kidneys. These data suggest that the increased PDE activity in SHR kidneys is restricted in cellular location so as to act in a targeted fashion on the isoproterenol-induced pool of 3′,5′-cAMP.

The results with IBMX left open the question as to what type of PDE is masking the effects of isoproterenol in the SHR renal vasculature. There are ≥11 different PDE families. One isozyme family, the cGMP-stimulated PDE (PDE2), has a low affinity for 3′,5′-cAMP and, therefore, would not likely participate importantly in the regulation of 3′,5′-cAMP levels in the renal vasculature. Another isozyme family, the cGMP-specific PDE (PDE5), does not hydrolyze 3′,5′-cAMP and, therefore, would not participate directly in the regulation of intracellular levels of 3′,5′-cAMP. PDE6 appears to be involved exclusively in visual transduction. Although the function of PDE7 is unknown, expression of this enzyme has been observed only in skeletal muscle and in some lymphocytes. Little is known regarding PDEs 8 to 11, in part because of the lack of specific pharmacological inhibitors.

In an attempt to elucidate the isozyme of PDE responsible for the apparent increased metabolism of 3′,5′-cAMP in the SHR renal vasculature, we compared the ability of IBMX (broad-spectrum PDE inhibitor), mmIBMX (selective PDE1 inhibitor), milrinone (selective PDE3 inhibitor), and RO 20-1724 (selective PDE4 inhibitor) to augment isoproterenol-induced 3′,5′-cAMP in SHR and WKY kidneys. Importantly, neither mmIBMX nor milrinone affected the ability of isoproterenol to increase the renal venous secretion rate of 3′,5′-cAMP, thus ruling out any roles for PDE1 and PDE3, respectively. It is noteworthy that RO 20-1724 was ≈30-fold more potent than IBMX with respect to increasing isoproterenol-induced 3′,5′-cAMP secretion and that high concentrations of RO 20-1724 mimicked the effects of high concentrations of IBMX in kidneys from both 16-week–old SHRs and WKYs. Therefore, we conclude that, in both strains, it is PDE4 that is linked to the regulation of receptor-induced levels of 3′,5′-cAMP in the renal vasculature, and, therefore, it is likely PDE4 activity that is upregulated in the renal vasculature and is responsible for masking the elevated isoproterenol response in SHR kidneys.

Figure 7. Bar graphs illustrate dose-dependent effects of RO 20-1724, a selective PDE4 inhibitor, on MABP (A), RBF (B), and RVR (C) in 16-week–old SHRs vs WKYs.
To directly test the conclusion that PDE4 activity is upregulated in the renal vasculature and is responsible for masking the elevated isoproterenol response in SHR kidneys, we examined the effects of PDE4 inhibition with RO 20-1724 on isoproterenol-induced renal venous 3',5'-cAMP secretion. These experiments were conducted in isolated, perfused kidneys from SHRs and WKYs that were harvested from young, adult, and old animals (5, 16, and 39 weeks of age, respectively) to also determine whether any overactivity of PDE4 was restricted to a particular age range or was observable throughout the life cycle of the animals. As with the IBMX experiments, we observed that RO 20-1724 unmasked a greater isoproterenol response in kidneys from SHRs regardless of the age of the SHR from which the kidneys were obtained. These data suggest that, indeed, PDE4 is the isoform of PDE4 that limits the ability of receptors to stimulate 3',5'-cAMP levels in the renal vasculature and that this may be a genetically determined trait, because the effect can be observed even in young SHRs in which hypertension is very mild. However, another interpretation is that these effects are observable in 5-week-old SHRs because even very mild elevations in MABP may markedly alter the role of PDE4 in the renal vasculature. Because Tawar et al reported that the primary renal isoform of PDE4 is PDE4B4, it would be interesting to determine whether this is the isoform of PDE4 responsible for masking the ability of isoproterenol to induce 3',5'-cAMP in the renal vasculature of SHRs.

Although RFB is similar in adult SHRs versus WKYs, RVR is higher. The next question that we chose to address was whether the increased PDE4 activity in the SHR renal vasculature had any role to play in the elevated RVR in SHR kidneys. Consistent with other studies, we observed that RVR was elevated in SHR kidneys. Importantly, intravenous infusions of RO 20-1724 lowered RVR in SHRs but had little effect on RVR in WKYs. In fact, RO 20-1724 nearly normalized RVR in SHR kidneys compared with WKY kidneys. Notably, intravenous infusions of RO 20-1724 also caused a marked reduction in MABP in SHRs but not in WKYs.

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**References**
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