Reciprocal Effects of Dexamethasone on Vasodilatory Responses to Arachidonic Acid and Prostanoids in the Isolated Perfused Rabbit Kidney

William C. Sessa, Lang Lin, and Alberto Nasjletti

We reported that dexamethasone treatment of rabbits causes a reduction in renal vasoconstrictor responses to prostaglandin \(\text{F}_2\), and U46619, an agonist at the thromboxane-endoperoxide receptor, but not to phenylephrine. The purpose of this study was to examine if dexamethasone treatment can affect the renal vasodilatory responses to prostacyclin (PGI\(\text{i}\)) and prostaglandin \(\text{E}_2\) (PGE\(\text{i}\)) in isolated Krebs-perfused kidneys constricted with phenylephrine. In kidneys from dexamethasone-treated rabbits, the vasodilatory response to PGI\(\text{i}\) was reduced by 57\%, whereas that to PGE\(\text{i}\) was converted to a vasoconstrictor response. This effect of dexamethasone appears to be specific in that the renal vasodilatory responses to forskolin and to sodium nitroprusside were not affected by the steroid. Contrasting with the inhibitory effect of dexamethasone on prostanoid-induced renal vasodilation, treatment with dexamethasone augmented the renal vasodilatory response to arachidonic acid; for example, arachidonic acid, at 10 \(\mu\)g decreased perfusion pressure by 24.8±5.4 and 49.0±5.6 mm Hg in kidneys from vehicle- and dexamethasone-treated rabbits, respectively. The enhanced vasodilatory effect of arachidonic acid could not be attributed to increased renal formation of PGE\(\text{i}\) and PGI\(\text{i}\). In conclusion, dexamethasone interferes with prostanoid-mediated renal vasodilation, which is not associated with an impairment in renal responsiveness to direct activators of adenylate cyclase and guanylate cyclase. The reciprocal effect of dexamethasone on the renal vascular responses to arachidonic acid and vasodilatory prostanoids are indicative of a previously unrecognized influence of glucocorticoids on the renal arachidonate-prostaglandin system. (Hypertension 1990;15(suppl I):I-93–I-96)

Consistent with a role for glucocorticoids in the regulation of vascular functions, glucocorticoids have been shown to bind to specific receptors in blood vessels\(^1\) and to affect vascular reactivity\(^2\)\(^3\) as well as systemic and renal hemodynamics.\(^4\) For example, glucocorticoids have been shown either to increase or to decrease blood pressure,\(^4\)\(^5\) to increase renal blood flow,\(^6\) and to reduce vascular smooth muscle contractile responses to prostaglandin (PG) \(\text{F}_2\) and U46619, a synthetic agonist for the thromboxane \(A_2\) and PG endoperoxide receptor.\(^7\)

Recently, we reported that treatment of rabbits with dexamethasone diminishes the vasoconstrictor effect of PGE\(\text{i}\) and U46619 in the isolated kidney perfused with Krebs-bicarbonate buffer.\(^7\) Because the glucocorticoid did not interfere with the vasoconstrictor response to phenylephrine, its inhibitory influence on vascular responsiveness to prostanoids appears to be specific. Yet the possibility that dexamethasone also affects the renal vascular actions of vasodilatory eicosanoids cannot be excluded. Therefore, the present study was designed to investigate the effects of dexamethasone treatment on the expression of renal vasodilatory responses to PGE\(\text{i}\), PGI\(\text{i}\), and arachidonic acid in the isolated perfused rabbit kidney.

Methods

Animals

Male New Zealand white rabbits (2.5–3.0 kg) were treated for 6 consecutive days with daily subcutaneous injections of dexamethasone 21-acetate (2.5 mg/kg) suspended in sesame oil. Control rabbits received injections (0.5 ml) of sesame oil.
only. On the sixth day of treatment, a control and a dexamethasone-treated rabbit were anesthetized by intramuscular injection of 50 mg/kg ketamine HCl (Ketaset, Aveco Co., Fort Dodge, Iowa) and 8 mg/kg xylazine (Rompun, Haver Mobay Corp., Shawnee, Kansas), and the left kidneys were isolated and perfused, in parallel.

**Isolated Perfused Kidney**

After anesthesia, the left kidney was exposed, left renal artery cannulated, renal vein and ureter cut, and the kidney freely suspended in a water-jacketed chamber maintained at 37°C. Kidneys from vehicle- and dexamethasone-treated rabbits were perfused in parallel at a constant flow of 12 ml/min with oxygenated (95% O₂ and 5% CO₂) Krebs-bicarbonate buffer of the following composition (mmol/l): NaCl 118.5, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 7H₂O 1.1, NaHCO₃ 25.0, and dextrose 5.6. Perfusion pressure was monitored by means of a Statham P23 pressure transducer coupled to a Grass polygraph (model RPS 7C8A, Grass Instr. Co., Quincy, Massachusetts).

After a 60-minute equilibration period, the perfusion pressure was 54.2±3.7 mm Hg in the kidneys of control rabbits (n=25) and 50.5±4.2 mm Hg in the kidneys of dexamethasone-treated rabbits (n=25). To establish a vascular tone appropriate for the examination of agonist-induced vasodilatory responses, phenylephrine was infused close-arterially at 3.4±0.5 μg/min in the kidneys from control rabbits, and at 3.6±0.5 μg/min in the kidneys from dexamethasone-treated rabbits, raising renal perfusion pressure to 111.4±5.3 and 107.5±4.3 mm Hg, respectively, which pressures were maintained throughout the experiments except during administration of vasodilatory agonists. Renal perfusion pressure responses to PGE₂ (0.25–10.0 μg), PGI₂ (0.10–1.0 μg), forskolin (0.5–5.0 μg), sodium nitroprusside (0.5–5.0 μg), and arachidonic acid (10.0 μg) were then studied, with each dose of agonist injected as a bolus, in a volume of 5–20 μl into the renal arterial cannula. In some experiments that examined the renal vasodilatory response of arachidonic acid, kidneys were perfused with Krebs-bicarbonate buffer containing indomethacin (1 μg/ml) to inhibit cyclooxygenase. Each injection of agonist was separated by a 15-minute interval and no more than two agonists were studied in each kidney. Because the kidneys were perfused at a constant rate, the changes in perfusion pressure are indicative of changes in renal vascular resistance.

In some experiments, examining vasodilatory responses to arachidonic acid in kidneys from control and dexamethasone-treated rabbits, the renal effluent was collected at 1-minute intervals before and up to 6 minutes after injection of a 10 μg bolus of arachidonic acid. PGE₂ and 6-keto-PGF₁α, the stable hydrolysis product of PGI₂, were measured in the renal effluent using the second antibody, solid-phase, enzyme-linked immunoassay described by Pradelles et al. Cross reactivities of antisera are as previously stated.

**Drugs**

PGE₂ and PGI₂ were purchased from Cayman Chemical, Ann Arbor, Michigan. PGE₂ was dissolved in ethanol (1 mg/ml), and aliquots were evaporated under nitrogen and resuspended in Krebs buffer. PGI₂ was dissolved (1 mg/ml) in 50 mM Tris buffer, pH 9.0, and diluted with the same buffer on the day of the experiments. Arachidonic acid purchased from NuChek (Elysian, Minnesota) was dissolved (1 mg/ml) in 50 mM sodium carbonate, pH 7.4, and diluted with Krebs buffer. Phenylephrine, sodium nitroprusside, forskolin, and indomethacin were purchased from Sigma Chemical Co., St. Louis, Missouri. Phenylephrine and sodium nitroprusside were dissolved in distilled deionized water, and forskolin was dissolved in ethanol (all at concentrations of 1 mg/ml). Indomethacin was dissolved in 50 mM sodium carbonate (1 mg/ml).

**Statistical Analysis**

Results are expressed as mean±SEM. The data were analyzed by two-way analysis of variance. If differences were noted, the data were analyzed by a Duncan multiple range test. The data on arachidonic acid-induced changes in renal perfusion pressure and eicosanoid output were analyzed by a paired Student's t test. Differences in responses between control and dexamethasone-treated rabbits were determined by an unpaired Student's t test. The null hypothesis was rejected if the p value was less than 0.05.

**Results**

Figure 1 illustrates the changes in perfusion pressure elicited by bolus arterial injections of PGI₂ and PGE₂ in isolated perfused kidneys from vehicle- and dexamethasone-treated rabbits. PGI₂ caused a dose-dependent reduction of perfusion pressure indicative of vasodilation in kidneys from both groups of rabbits. The vasodilatory response induced by PGI₂ in kidneys from dexamethasone-treated rabbits, however, was only 40–50% of that induced by PGI₂ in kidneys from vehicle-treated rabbits. PGE₂ also caused dose-dependent vasodilation in perfused kidneys from control rabbits. In perfused kidneys from rabbits treated with dexamethasone, however, the injection of PGE₂ caused dose-dependent elevation of perfusion pressure, which is indicative of renal vasoconstriction. Contrasting with the effectiveness of dexamethasone in reducing PGI₂-induced renal vasodilation, and in reversing the effect of PGE₂ on the renal vasculature from vasodilation to vasoconstriction, the steroid did not affect the renal vasodilatory effect of forskolin and of sodium nitroprusside, activators of adenylate cyclase, and of soluble guanylate cyclase, respectively (Figure 2).

Shown in Table 1 are the data on the effects of arachidonic acid on perfusion pressure and output
of 6-keto-PGF₁α and PGE₂ in kidneys from vehicle- and dexamethasone-treated rabbits. In experiments without indomethacin in the perfusate, perfusion pressure was maximally reduced ($p<0.05$) 1 minute after close arterial injection of a 10 μg bolus of arachidonic acid in kidneys from both control and dexamethasone-treated rabbits. The arachidonic acid-induced reduction of perfusion pressure in kidneys from dexamethasone-treated rabbits (49.0±5.6) exceeded ($p<0.05$) the reduction of perfusion pressure elicited by the fatty acid in kidneys from vehicle-treated rabbits (24.8±54). In experiments with indomethacin in the perfusate to inhibit cyclooxygenase, arachidonic acid failed to change perfusion pressure in kidneys from vehicle-treated rabbits but reduced perfusion pressure by $10.5±3.5$ mm Hg in kidneys from dexamethasone-treated rabbits. In kidneys perfused in the absence of indomethacin, arachidonic acid injected close-arterially increased the output of 6-keto-PGF₁α and PGE₂ to the same extent in kidneys from vehicle- and dexamethasone-treated rabbits. In kidneys perfused with indomethacin, both in vehicle- and dexamethasone-treated rabbits, the arachidonic acid induced renal output of 6-keto-PGF₁α and PGE₂ was either suppressed or greatly inhibited.

**Table 1.** Effects of a Bolus Injection of Arachidonic Acid on Perfusion Pressure and Output of Eicosanoids in Isolated Kidneys From Vehicle- and Dexamethasone-Treated Rabbits Perfused in the Absence and Presence of Indomethacin

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Without indomethacin</th>
<th>n</th>
<th>Basal</th>
<th>AA</th>
<th>$a$</th>
<th>n</th>
<th>Basal</th>
<th>AA</th>
<th>$a$</th>
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<tbody>
<tr>
<td>V</td>
<td></td>
<td>10</td>
<td>127.4±7.1</td>
<td>102.7±7.6*</td>
<td>-24.8±5.4</td>
<td>7</td>
<td>110.0±9.6</td>
<td>107.9±9.8</td>
<td>-2.1±1.8</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>10</td>
<td>121.9±4.7</td>
<td>72.9±4.5†</td>
<td>-49.0±5.6†</td>
<td>7</td>
<td>114.0±6.4</td>
<td>103.5±5.7*</td>
<td>-10.5±3.3†</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>5</td>
<td>2.2±0.6</td>
<td>8.4±2.0*</td>
<td>6.2±2.1</td>
<td>6</td>
<td>0.4±0.1</td>
<td>0.9±0.1*</td>
<td>0.5±0.2</td>
</tr>
<tr>
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<td>5</td>
<td>1.2±0.3</td>
<td>5.9±0.9*</td>
<td>4.7±1.1</td>
<td>6</td>
<td>0.2±0.1</td>
<td>0.7±0.4</td>
<td>0.5±0.3</td>
</tr>
<tr>
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<td></td>
<td>5</td>
<td>7.9±1.6</td>
<td>17.3±1.2*</td>
<td>9.4±2.0</td>
<td>6</td>
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</tr>
<tr>
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<td>5</td>
<td>11.4±4.6</td>
<td>20.1±3.2*</td>
<td>8.7±3.4</td>
<td>6</td>
<td>0.7±0.2</td>
<td>1.9±0.5*</td>
<td>1.2±0.5</td>
</tr>
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</table>

*Values are presented as mean±SEM. Indomethacin in Krebs-bicarbonate buffer (1 μg/ml); arachidonic acid bolus injection (10 μg). n, number of rabbits studied; AA, arachidonic acid; $a$, difference between peak values obtained after the bolus administration of AA compared with basal values; V, vehicle-treated rabbits; D, dexamethasone-treated rabbits.

*p<0.05, relative to basal values.

†p<0.05, relative to corresponding value in vehicle-treated rabbits.
Discussion

The present study demonstrates that treatment of rabbits with dexamethasone decreases the expression of PGF2α-induced vasodilation in the isolated perfused kidney, and reverses the effect of PGE2 from vasodilation to vasoconstriction. These effects of dexamethasone are not attributable to nonspecific inhibition of vasodilator mechanisms because the glucocorticoid did not inhibit the renal vasodilatory effect of forskolin and sodium nitroprusside. A priori, the dexamethasone-induced alteration in renal vascular responses to PGE2 and PGF2α can be the result of possible actions of the steroid increasing the renal degradation of PGF2α, enhancing the conversion of PGE2 to a metabolite with vasoconstrictor activity, or impairing the stimulus-receptor coupling of events linked to prostanoid mediated vasodilation. We cannot exclude any of these possibilities as yet. There are reports, however, that dexamethasone reduces renal 15-PG-dehydrogenase activity, an effect that would tend to increase levels of PGI2 and PGE2.10 Furthermore, dexamethasone does not affect the renal activity of the enzyme 9-ketoreductase, thus, not influencing the conversion of PGE2 to PGF2α.11

In our study, treatment of rabbits with dexamethasone resulted in augmentation of the renal vasodilatory effect of arachidonic acid without further enhancement in the renal output of PGE2 and 6-keto-PGF1α, a metabolite of PGI2. This finding is surprising because the renal vasodilatory effect of arachidonic acid is thought to be mediated by PGE2 and PGF2α, which have an impaired ability to produce vasodilation in kidneys from dexamethasone-treated rabbits. Dexamethasone treatment of rabbits also interferes with the ability of PGE2 and PGF2α to produce renal vasoconstriction. As the renal vasodilatory response to arachidonic acid is accompanied by an increased renal production of PGE2 and PGI2, augmentation by dexamethasone of arachidonic acid-induced renal vasodilation might be in part related to diminished effectiveness of endogenous PGE2 to elicit vasoconstriction.

Additionally, enhancement of arachidonic acid-induced renal vasodilation in dexamethasone-treated animals might be the consequence of increases in the metabolism of arachidonic acid by noncyclooxygenase pathways. Indeed, dexamethasone has been shown to enhance the formation of nitric oxide-dependent noncyclooxygenase products of arachidonic acid by renal cortical structures, and there is evidence that some of the products are further metabolized by cyclooxygenase to metabolites with biologic activity.13,14 The possibility that metabolites of arachidonic acid by a noncyclooxygenase pathway contribute to the vasodilatory effect of the fatty acid in kidneys from dexamethasone-treated rabbits is in agreement with the observation that the vasodilatory effect is not completely eliminated by inhibition of cyclooxygenase with indomethacin. Although it can be argued that the residual vasodilatory response to arachidonic acid in kidneys from dexamethasone-treated rabbits results from the enhanced release of endothelium-derived relaxing factors, there is no evidence that dexamethasone produces such an effect.15

This study demonstrates that dexamethasone treatment of rabbits interferes with PGI2- and PGE2-induced renal vasodilation while increasing the renal vasodilatory effect of arachidonic acid. The reciprocal effects of dexamethasone on the renal vascular responses to arachidonic acid and vasodilatory PGs are indicative of a previously unrecognized influence of glucocorticoids on the renal arachidonate-prostaglandin system.

References


Key words: dexamethasone • prostaglandins • vasodilation • arachidonic acid
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