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 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$_2$) and prostaglandin E$_2$ (PGE$_2$) in isolated Krebs-perfused kidneys constricted with phenylephrine. In kidneys from dexamethasone-treated rabbits, the vasodilatory response to PGI$_2$ was reduced by 57%, whereas that to PGE$_2$ 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$_2$ and PGI$_2$. 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$^2$ as well as systemic and renal hemodynamics.$^3$ For example, glucocorticoids have been shown either to increase or to decrease blood pressure,$^4$ to increase renal blood flow,$^5$ and to reduce vascular smooth muscle contractile responses to prostaglandin (PG) 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$_2$ 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$_2$, PGI$_2$, 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 iso-
lated 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 composi-
tion (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 cou-
pled to a Grass polygraph (model RPS 7C8A, Grass

After a 60-minute equilibration period, the perfu-
sion 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 main-
tained throughout the experiments except during
administration of vasodilatory agonists. Renal perfu-
sion 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 con-
trol 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.8 Cross reactivities of antisera are as
previously stated.9

Drugs

PGE2 and PGI2 were purchased from Cayman
Chemical, Ann Arbor, Michigan. PGE2 was dis-
solved in ethanol (1 mg/ml), and aliquots were evap-
orated 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 pur-
 chased from NuChek (Elysian, Minnesota) was dis-
solved (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 nitro-
prusside were dissolved in distilled deionized water,
and forskolin was dissolved in ethanol (all at concen-
trations 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 Stu-
dent’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 vasodi-
lation in perfused kidneys from control rabbits. In
perfused kidneys from rabbits treated with dexa-
methasone, however, the injection of PGE2 caused
dose-dependent elevation of perfusion pressure,
which is indicative of renal vasoconstriction. Con-
trasting with the effectiveness of dexamethasone in
reducing PGI2-induced renal vasodilation, and in
reversing the effect of PGE2 on the renal vascular-
ture from vasodilation to vasoconstriction, the ste-
ring of changes in renal vascular resistance.

Shown in Table 1 are the data on the effects of
arachidonic acid on perfusion pressure and output
FIGURE 1. Bar graphs showing effect of prostaglandin (PG) I₂ and PGE₂ on renal perfusion pressure in isolated perfused kidneys from vehicle- and dexamethasone-treated rabbits. Kidneys were perfused at 12 ml/min and renal vasculature constricted with phenylephrine infusion (−3.5 μg/min) to observe vasodilatory responses to prostanoids. Results are mean±SEM of six experiments for PGI₂ and five experiments for PGE₂. *Denote significant differences between groups; p<0.05.

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>With indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Basal</td>
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<tr>
<td></td>
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<tr>
<td>Perfusion Pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td>107.4±7.1</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>121.9±4.7</td>
</tr>
<tr>
<td>6-keto-PGF₁α (ng/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>2.2±0.6</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>PGE₂ (ng/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>7.9±1.6</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>11.4±4.6</td>
</tr>
</tbody>
</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 PGJ2-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 PGJ2 can be the result of possible actions of the steroid increasing the renal degradation of PGJ2, 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 PGJ2 and PGE2. Furthermore, dexamethasone does not affect the renal activity of the enzyme 9-keto-reductase, thus, not influencing the conversion of PGE2 to PGF2α.

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 PGJ2. This finding is surprising because the renal vasodilatory effect of arachidonic acid is thought to be mediated by PGE2 and PGJ2, which have an impaired ability to produce vasodilation in kidneys from dexamethasone-treated rabbits. Dexamethasone treatment of rabbits also interferes with the ability of PGF2α to produce renal vasoconstriction. As the renal vasodilatory response to arachidonic acid is accompanied by an increased renal production of PGF2α augmentation by dexamethasone of arachidonic acid-induced renal vasodilation might be in part related to diminished effectiveness of endogenous PGF2α 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 nicotinamide di-nucleotide phosphate (NADPH)-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. 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 indo- methacin. 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.

This study demonstrates that dexamethasone treatment of rabbits interferes with PGJ2- 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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