Dexamethasone Selectively Attenuates Prostanoid-Induced Vasoconstrictor Responses In Vitro

William C. Sessa and Alberto Nasjletti

Glucocorticoids bind to specific vascular receptors resulting in a variety of functional consequences that may affect vascular smooth muscle behavior. We, therefore, examined in rabbits the effect of treatment with dexamethasone (2.5 mg/kg) for 6 days on vascular responses to pressor prostanoids in aortic and carotid arterial rings and in the isolated perfused kidney. Isometric tension development to prostaglandin F2α and U46619, a thromboxane/prostaglandin endoperoxide mimetic, was markedly reduced in vessels from dexamethasone-treated rabbits. The inhibitory effect of dexamethasone on vascular reactivity was manifested by an increase in the concentration of agonist for threshold tension development and a reduction in the maximal response to prostaglandin F2α and U46619. In contrast, reactivity to phenylephrine, potassium, histamine, or endothelin was not affected by dexamethasone treatment. In addition, pressor responses to prostaglandin F2α and U46619 in Krebs'-perfused kidneys from dexamethasone-treated rabbits were also diminished. These data suggest that dexamethasone selectively interferes with the expression of receptor-mediated contractile responses to eicosanoids.


The presence of high affinity, low capacity, saturable glucocorticoid receptors in the vasculature suggests a role for glucocorticoids in the regulation of cardiovascular function.1,2 Such a role is consistent with reports that glucocorticoid treatment increases blood pressure in rats, sheep, and humans and lowers blood pressure in dogs.3–6 Furthermore, glucocorticoids exert a modulatory influence on vascular tone because they can affect vascular responses to catecholamines, angiotensin II, vasopressin, histamine, and bradykinin.7–9

That glucocorticoid treatment attenuates hormonally induced release of prostaglandins (PGs) from blood vessels and other tissues in vitro raises the possibility that the cardiovascular actions of the steroids are the consequence of diminished synthesis of vasoactive PGs involved in circulatory homeostasis.10–12 This hypothesis has been challenged by in vivo studies demonstrating that glucocorticoids do not produce a generalized deficit of PGs.13–15

Another interaction of glucocorticoids and PGs that pertains to the vascular actions of glucocorticoids was unexpectedly uncovered while investigating the effect of dexamethasone on endothelium-dependent relaxations of rabbit aortic rings precontracted with PGF2α. We noticed that the effectiveness of PGF2α to elicit a contraction of aortic smooth muscle was diminished in dexamethasone-treated rabbits, suggesting a regulatory influence of glucocorticoids on the vascular actions of prostanoids. This article characterizes the inhibitory actions of chronic dexamethasone treatment of rabbits on aortic and carotid arterial reactivity and renal vascular responses to pressor prostanoids in vitro.

Materials and Methods

Materials

PGF2α tromethamine (PGF2α) and U46619 (15S-hydroxy-11α, 9α [epoxy-methano] prosta-5Z-dienoic acid), a prostaglandin endoperoxide analogue, were purchased from Cayman Chemical, Ann Arbor, Michigan. Phenylephrine and histamine were purchased from Sigma Chemical, St. Louis, Missouri. Endothelin was obtained from the Peptide Institute, Osaka, Japan.

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 sesame oil injections only (0.5 ml) during the treat-
ment period. Rabbits treated with vehicle gained weight over the 6-day period (0.20±0.02 kg, n=20), while dexamethasone-treated rabbits did not (0.03±0.02 kg, n=20, p<0.05). On day 6, one control and one dexamethasone-treated rabbit were anesthetized by an intramuscular injection of ketamine hydrochloride (Ketaset, 50 mg/kg) and xylazine (Rompun, 8 mg/kg). All experiments were conducted in vehicle and in dexamethasone-treated rabbits in parallel.

Isometric Tension Measurements in Rabbit Thoracic Aortic and Carotid Arterial Rings

Rabbit thoracic aortae and carotid arteries were carefully excised and placed into cold Krebs' bicarbonate buffer (pH 7.4), the periadventitial fat was removed, and the vessels were cut into 2–3-mm wide rings. A maximum of four aortic rings from each control and dexamethasone-treated rabbit was studied. The composition (mmol/l) of the Krebs' bicarbonate solution was NaCl 118.5, KCl 4.7, CaCl2 2.5, KH2PO4 1.2, MgSO4·7H2O 1.1, NaHCO3 25.0, and dextrose 5.6. Arterial rings were mounted in 5-ml water-jacketed organ baths containing Krebs' bicarbonate buffer maintained at 37°C and continuously gassed with 95% O2-5% CO2. The vascular rings were equilibrated under 2 g of resting tension for 1.5-2.0 hours. The buffer was changed at 15-minute intervals, and basal tension was adjusted as required during the equilibration period. Two grams of basal tone was optimal for concentration-response curves to agonists in both aortic and carotid arterial rings. Changes in tension were measured using force transducers (model FTO3C, Grass Instruments, Quincy, Massachusetts) coupled to a polygraph (model RPS 7C8A, Grass Instruments). Cumulative dose-response curves to phenylephrine, potassium chloride, histamine, endothelin, PGF2α, and U46619 were performed simultaneously in aortic rings from control and dexamethasone-treated rabbits. In carotid arterial rings, only responses to phenylephrine, potassium, PGF2α, and U46619 were examined. The responsiveness of a maximum of three agonists was randomly assessed in any one set of arterial rings.

In some studies, aortic rings from vehicle and dexamethasone-treated rabbits were precontracted with phenylephrine (3×10^{-7} M) and endothelium-dependent relaxations to acetylcholine were assessed.

In additional studies, carotid arterial rings from control rabbits were incubated with dexamethasone phosphate (100 μM) or indomethacin (5 μM), and concentration-response curves to PGF2α and U46619 were performed.

Isolated Perfused Kidney

After anesthesia, the left kidney was exposed, the left renal artery was cannulated, the renal vein and ureter were cut, and the kidney was 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 Krebs' bicarbonate buffer. Perfusion pressure was monitored by a pressure transducer (Statham P23) coupled to a polygraph (model RPS 7C8A, Grass Instruments). After a 60-minute equilibration period, pressor responses to renal arterial injections of PGF2α, U46619, and phenylephrine were examined in kidneys from vehicle- and dexamethasone-treated rabbits. Each dose of agonist was given as a 5–20-μl volume bolus injection into the renal arterial cannula at 10-minute intervals. Changes in perfusion pressure were used as an index of changes in renovascular resistance.

Statistical Analysis

Results are expressed as mean±SEM. Dose-response curves were analyzed by a two-way analysis of variance (ANOVA). If differences were noted, the data were analyzed by a Duncan multiple range test. EC50 values and maximal responses to agonists were evaluated by the Student’s t test for unpaired samples. The null hypothesis was rejected at values of p<0.05.

Results

As shown in Figures 1 and 2, PGF2α caused concentration-dependent contractions of aortic and carotid arterial rings from vehicle-treated rabbits, which were markedly reduced in vascular rings from dexamethasone-treated animals. In both aortae and carotid arteries from dexamethasone-treated rabbits, isometric tension development to U46619 was also greatly reduced (Figure 2). The diminished vasoactivity was manifested by an increase in the concentration of agonist necessary for threshold tension development and a reduction in the maximal response. As shown in Table 1, in the rabbit aorta, maximal responses to PGF2α and U46619 were reduced by 32% and 45%, respectively, and were accompanied by significant increase in the EC50 values. In the carotid artery (Table 2), maximal
responses were reduced by 51% and 70%, respectively, with no significant change in the EC50 values. Dexamethasone treatment also significantly inhibited PGE2-induced maximal contractions of the rabbit aorta but did not influence the EC50 values: maximal responses were 4.15±0.31 and 2.43±0.86 g of tension, EC50 values were 1.52±0.81×10⁻⁵ and 1.28±0.44×10⁻⁶ M for control and dexamethasone-treated rabbits, respectively (n=7).

Contrasting with the effectiveness of the steroid in diminishing vascular responsiveness to prostanoids, aortic responsiveness to phenylephrine, depolarizing concentrations of potassium chloride, histamine, and endothelin were not affected by dexamethasone treatment (Figure 3). Similarly, in the carotid artery, contractile responses to phenylephrine and potassium chloride were not influenced by dexamethasone treatment (Figure 4).

To investigate whether dexamethasone directly affects contractile responses to PGF2α, carotid arterial rings were incubated with dexamethasone (100 µM for 30 minutes) and dose-response curves were performed. The presence of dexamethasone in the organ baths did not affect vascular responses to PGF2α. For control and dexamethasone-incubated vessels, respectively, EC50 values were 1.95±0.15×10⁻⁶ and 2.10±0.09×10⁻⁶ M and maximal responses were 3.41±0.32 and 3.35±0.23 g of tension (n=6). In addition, indomethacin treatment (5 µM) of carotid arterial rings did not interfere with U46619-induced contractions. EC50 values were 1.25±0.09×10⁻⁸ and 1.34±0.11×10⁻⁸ M and maximal responses were 3.81±0.12 and 4.07±0.20 g of tension for control and indomethacin-treated vessels, respectively (n=6). Dexamethasone treatment did not affect endothelial-dependent relaxations to acetylcholine. For acetylcho-

**Figure 2.** Dose-response curves to prostaglandin F2α (PGF2α) and U46619 in aortic and carotid arterial rings from control (●) and dexamethasone-treated rabbits (○). Results are expressed as mean±SEM; the number of vascular rings and animals is found in Tables 1 and 2. *p<0.05 (analysis of variance followed by Duncan’s multiple range test).

<p>| Table 1. EC50 and Maximal Response Values to Vasoconstrictor Agonists in Aortic Rings From Vehicle- and Dexamethasone-Treated Rabbits |
|------------------|--------|-------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Agonist</th>
<th>n</th>
<th>EC50 (M)</th>
<th>Maximal response (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF2α</td>
<td>V</td>
<td>40(10)</td>
<td>2.12±0.24×10⁻⁶</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>42(10)</td>
<td>1.05±0.33×10⁻⁵*</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>27(8)</td>
<td>1.03±0.28×10⁻⁸</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>28(8)</td>
<td>2.40±0.90×10⁻⁸**</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>V</td>
<td>19(5)</td>
<td>2.98±0.52×10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>19(5)</td>
<td>3.13±0.50×10⁻⁷</td>
</tr>
<tr>
<td>KCl</td>
<td>V</td>
<td>21(7)</td>
<td>4.21±1.00×10⁻²</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>24(7)</td>
<td>3.90±0.84×10⁻²</td>
</tr>
<tr>
<td>Histamine</td>
<td>V</td>
<td>8(2)</td>
<td>6.01±0.26×10⁻⁶</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>8(2)</td>
<td>5.69±0.40×10⁻⁶</td>
</tr>
<tr>
<td>Endothelin</td>
<td>V</td>
<td>8(2)</td>
<td>1.29±0.28×10⁻⁸</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>8(2)</td>
<td>1.53±0.34×10⁻⁸</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PGF2α, prostaglandin F2α. Rabbits received subcutaneous injections of dexamethasone (D) (2.5 mg/kg) or sesame oil vehicle (V) for 6 days. n, number of vascular rings; number between parentheses indicates the number of animals from which rings were obtained.

*Significantly different from control (*p<0.05), using an unpaired t test.
line-induced relaxations, the EC₅₀ values were 1.51±0.1×10⁻⁷ and 1.45±0.9×10⁻⁷ M and the percent maximal relaxations were 98.2±4.0 and 97.3±3.6 in vessels from control and treated rabbits, respectively (n=9).

Figure 5 illustrates the effect of dexamethasone treatment on pressor responsiveness to PGF₂α, U46619, and phenylephrine in the isolated Krebs'-perfused rabbit kidney. Dexamethasone treatment did not affect basal renal perfusion pressure (i.e., 53.3±7.92 vs. 48.3±5.6 mm Hg in kidneys from control and dexamethasone-treated rabbits, respectively [n=6 in each group]) but greatly reduced pressor responses to PGF₂α and U46619. However, vascular responses to phenylephrine were not different in kidneys from control and dexamethasone-treated rabbits.

**Discussion**

The present study demonstrates that dexamethasone attenuates the PGF₂α- and U46619-induced renal vasoconstriction and contraction of aortic and carotid arterial smooth muscle. This effect of dexamethasone is not attributable to a nonspecific depression of vascular function, since the glucocorticoid did not attenuate the contractile responses of the aorta and the carotid artery to potassium, phenylephrine, histamine, and endothelin or the renal vasoconstrictor response to phenylephrine. Additionally, it is unlikely that the effect of dexamethasone in inhibiting the vasoconstrictor effects of PGF₂α and U46619 is the consequence of enhanced acute production of endothelium-derived relaxing factors, since the glucocorticoid did not increase the expression of endothelium-derived relaxing activity in aortic rings stimulated with acetylcholine. Hence, to the extent that dexamethasone inhibits prostanoid-induced vasoconstriction only, the possibility arises that the glucocorticoid may reduce the number and/or the affinity of vascular receptors for PGF₂α and U46619 or that it can interfere specifically with the postrecep-
Potassium revealed no significant differences between curves. Results are expressed as mean±SEM. The number of vascular rings and rabbits are found in Table 2.

The physiological significance of dexamethasone-induced inhibition of vascular responsiveness to PGF₂α and U46619 is unknown. There is evidence that an endogenous prostanoid(s) that interacts with the thromboxane A₂ and PG endoperoxide receptor contributes to endothelial-dependent contractions of vascular smooth muscle and to the pathogenesis of severe angiotensin II/salt hypertension in rats. Our present study urges consideration of the possibility that the vascular actions of such pressor prostanoid(s) are inhibited by glucocorticoids.

In summary, our study demonstrates that dexamethasone treatment of rabbits for 6 days interferes with the expression of PGF₂α and U46619-induced renal vasoconstriction and aortic and carotid arterial contractile responses. The inhibitory effect of dexamethasone is specific for PGF₂α and U46619 in that the contractile responses to other agonists are not affected. The effect of dexamethasone in reducing

**Figure 4.** Dose-response curves to phenylephrine and potassium chloride in carotid arterial rings from control (●) and dexamethasone-treated rabbits (○). Analysis of variance revealed no significant differences between curves. Results are expressed as mean±SEM. The number of vascular rings and rabbits are found in Table 2.

**Figure 5.** Pressor responses to prostaglandin F₂α (PGF₂α), U46619, and phenylephrine in Krebs'-perfused kidneys from control and dexamethasone-treated rabbits. Results are expressed as mean±SEM, in six experiments in kidneys obtained from six vehicle and six dexamethasone-treated rabbits; *p<0.05 (analysis of variance followed by Duncan’s multiple range test).
responsiveness of the vasculature to PGF$_{2\alpha}$ and U46619 is not the consequence of suppression of prostanoid release or of enhanced production of endothelium-derived relaxing factor(s).

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