Dexamethasone Hypertension in Rats: Role of Prostaglandins and Pressor Sensitivity to Norepinephrine

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SUMMARY Glucocorticoid hypertension was induced by oral administration of dexamethasone (DX) in male Wistar rats. The mechanism of hypertension was studied by observing the changes in plasma renin activity, urinary excretion of prostaglandin E2 (PGE$_2$), and the pressor response to norepinephrine. Following administration of DX (0.1 mg/day), the blood pressure began to rise within 3 days and reached a plateau on the 5th day (from 108 ± 2 to 162 ± 7 mm Hg, mean ± SE). On the other hand, urine volume and urinary excretion of sodium were increased. In spite of the marked natriuresis and diuresis, the administration of DX resulted in a marked decrease in the urinary excretion of PGE$_2$. This decrease in PGE$_2$ excretion appeared before the blood pressure rose and continued throughout the experiment. Plasma renin activity did not change. The pressor response to norepinephrine was enhanced on the 2nd day of DX treatment, at which time the blood pressure was not yet elevated, and it was further augmented on the 6th day. In the DX-treated rats, the pressor response to norepinephrine was not enhanced by administration of indomethacin, whereas the pressor response was significantly potentiated by indomethacin in control rats. These results suggest that DX-induced hypertension in rats is associated with inhibition of prostaglandin synthesis leading to increased sensitivity in the vascular response to norepinephrine.

(Hypertension 6: 236-241, 1984)

KEY WORDS • Cushing's syndrome • renin-angiotensin system • prostaglandin • vascular reactivity • indomethacin

Several mechanisms have been proposed to explain the pathogenesis of glucocorticoid hypertension. As we reported recently, the renin-angiotensin system may play some role in the elevation of blood pressure of this type of hypertension. However, in our study blockade of the renin-angiotensin system by the angiotensin analog (saralasin) or the angiotensin I converting-enzyme inhibitor (SQ 14,225) could not reduce the elevated blood pressure to normal levels, which indicated some other mechanisms may be involved in the development of glucocorticoid hypertension.

Increased vascular reactivity to pressor substance may be involved in the development of glucocorticoid hypertension. However, the precise mechanism underlying this change in vascular reactivity remains controversial. Previous studies have not fully examined the temporal relationship between the development of hypertension and the changes in pressor response to vasoactive substances, although such studies are necessary to clarify whether alterations in vascular reactivity underlie the development of glucocorticoid hypertension.

On the other hand, since many in vitro studies have revealed that glucocorticoids affect the biosynthesis of prostaglandins, changes in endogenous prostaglandin levels might be observed in glucocorticoid hypertension. Prostaglandins, especially prostaglandin E$_2$ (PGE$_2$) and I$_1$ (PGI$_1$), have been suggested as modulators of pressor systems that operate within the vascular wall to attenuate constriction induced by pressor substances. Therefore, it is conceivable that changes in prostaglandins may be involved in glucocorticoid hypertension.

In the present study, glucocorticoid hypertension was induced by administering to rats dexamethasone (DX), a potent synthetic glucocorticoid, as previously reported by us. The pressor response to infused norepinephrine was examined before and after the rise in blood pressure during acute and chronic administration of DX.
Materials and Methods

Male Wistar rats weighing around 200 g were housed separately in humidity- and temperature-controlled metabolic cages. All animals were fed the same stock chow diet (Na, 0.23 g/100 g; K, 0.77 g/100 g).

Experiment 1
The rats were divided into two groups. Group 1 rats (n = 18) were used as controls and given tap water to drink. Group 2 rats (n = 18) were given tap water containing 0.3 mg/dl of DX (Decadron, Nippon Merck-Banyu Company, Tokyo, Japan). Each took about 0.1 mg of DX per day.

In one-third of the rats of both groups, the systolic blood pressure, urine volume, and urinary excretions of sodium and PGE₂ were determined every other day (on Days 1, 3, 5, 7), and the body weight was measured on the 3rd and 7th days. In another one-third of the rats of both groups, the animals were decapitated on the 2nd or 6th day, at which time blood samples were obtained for assay of plasma renin activity and for determination of plasma sodium and potassium concentrations. The systolic blood pressure was measured by a tail plethysmographic method while the animals were conscious.¹² Plasma renin activity was measured by the bioassay method of Skinner,¹³ and urinary and plasma electrolytes were measured with a flame photometer (Instrumentation Laboratory, Lexington, Massachusetts). Urinary PGE₂ was measured by radioimmunoassay according to the method of Dray et al.¹⁴ after extraction and partial purification by silicic acid chromatography. The antibody was supplied by Ono Pharmaceutical Company (Osaka, Japan). The intra- and interassay coefficients of variance for this radioimmunoassay in our laboratory were less than 10%.

Experiment 2
The rats were divided into two groups. Group 1 rats (n = 12) were used as control, and Group 2 rats (n = 12) were treated with DX as described in Experiment 1. The experiment was carried out on the 2nd day in half of the rats of each group, and in the remaining half on the 6th day. Cannulation of the right femoral artery and vein was performed under ether anesthesia 6 hours before the experiment. The experiments were performed on conscious and unrestricted rats. Arterial pressure was recorded directly through a catheter implanted in the femoral artery with a Nihon Koden MPU-0.5 pressure transducer coupled to a Nihon Koden RN-25 recorder. Fifty, 100, and then 200 ng of norepinephrine (L-arterenol bitartrate, Sigma Chemical Company, St. Louis, Missouri) dissolved in 0.3 ml of DX (on Days 1, 3, 5, 7), and the body weight was measured on the 3rd and 7th days. In another one-third of the rats of both groups, the animals were decapitated on the 2nd or 6th day, at which time blood samples were obtained for assay of plasma renin activity and for determination of plasma sodium and potassium concentrations. The systolic blood pressure was measured by a tail plethysmographic method while the animals were conscious.¹² Plasma renin activity was measured by the bioassay method of Skinner,¹³ and urinary and plasma electrolytes were measured with a flame photometer (Instrumentation Laboratory, Lexington, Massachusetts). Urinary PGE₂ was measured by radioimmunoassay according to the method of Dray et al.¹⁴ after extraction and partial purification by silicic acid chromatography. The antibody was supplied by Ono Pharmaceutical Company (Osaka, Japan). The intra- and interassay coefficients of variance for this radioimmunoassay in our laboratory were less than 10%.

On the 6th day, we studied the effects of prostaglandin synthesis inhibition by indomethacin on the vascular response to norepinephrine in both controls and DX-treated rats. Indomethacin (Sigma) was dissolved in 100 mM of sodium phosphate buffer just prior to administration. We had confirmed¹⁵ that the vehicle alone did not alter the blood pressure or pressor response to norepinephrine. After initial test doses of 50, 100, and 200 ng of norepinephrine, indomethacin (5 mg/kg) was injected into the femoral vein. Ten minutes after the administration of indomethacin, the same doses of norepinephrine were again injected and their pressor effects studied.

Experiment 3
In six untreated rats under ether anesthesia, cannulation of the right femoral artery and bilateral femoral veins was performed 6 hours prior to the experiment. The arterial pressure was recorded directly through a catheter implanted in the femoral artery, as described in Experiment 2. At the beginning of the experiment, 50, 100, and 200 ng doses of norepinephrine dissolved in 5% glucose were injected into the right femoral vein at 5-minute intervals. Each dose of norepinephrine was injected twice. Then DX (0.01 mg/min) was infused into the left femoral vein by a Harvard infusion pump, and 10 minutes after the beginning of this infusion, 50, 100, and 200 ng doses of norepinephrine were again injected and their pressor effects examined.

Results are expressed as the means ± SE. Data were analyzed statistically using Student’s t test and analysis of variance. A p value of < 0.05 was considered statistically significant.

Experiment 1
Administration of DX caused a marked increase in blood pressure (Figure 1). The blood pressure began to rise within 3 days and reached a plateau on the 5th day (from 108 ± 2 to 162 ± 7 mm Hg)(p < 0.005). In the DX-treated rats, the urine volume and urinary sodium excretion were increased (Figure 2) and the plasma sodium concentration was slightly decreased, but this change was not statistically significant. The plasma renin activity and potassium concentration were not significantly changed (Table 1). In spite of the marked natriuresis and diuresis, the administration of DX resulted in a marked decrease in urinary PGE₂ excretion. This decrease in urinary PGE₂ excretion appeared prior to the elevation of blood pressure and continued throughout the experiment (Figure 3).

Experiment 2
On the 2nd day of the experiment, the basal mean blood pressure in DX-treated rats was not significantly different from that of control rats (92 ± 2 and 88 ± 3 mm Hg, respectively) (Table 1). However, at this time, the pressor response to norepinephrine was already significantly (p < 0.01) potentiated in the DX-
treated rats (Figure 4). On the 6th day of the experiment, the basal mean arterial pressure of the DX-treated rats was significantly elevated (143 ± 3 mm Hg, p < 0.005) (Table 1). The pressor response to norepinephrine at this time was further potentiated (Figure 4).

Intravenously administered indomethacin potentiated the pressor response to norepinephrine in control rats without changing the basal pressure. However, in DX-treated rats, indomethacin did not significantly affect the pressor response to norepinephrine (Figure 5).

**Table 1. Changes in Mean Arterial Pressure (MAP), Plasma Renin Activity (PRA), Plasma Sodium and Potassium Concentrations in Dexamethasone (DX)-Treated Rats**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>MAP (mm Hg)</th>
<th>PRA (ng/ml/hr)</th>
<th>Sodium (mEq/liter)</th>
<th>Potassium (mEq/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88 ± 3</td>
<td>2.9 ± 0.2</td>
<td>141 ± 1</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>DX-2 (n = 6)</td>
<td>92 ± 2</td>
<td>3.3 ± 0.2</td>
<td>138 ± 2</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>DX-6 (n = 6)</td>
<td>142 ± 3*</td>
<td>3.1 ± 0.3</td>
<td>139 ± 1</td>
<td>4.6 ± 0.2</td>
</tr>
</tbody>
</table>

DX-2 = 2nd day of dexamethasone treatment; DX-6 = 6th day of dexamethasone treatment. n = number of the rats. Values are expressed as means ± se.

* p < 0.005, significant difference from control.
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Experiment 3

Intravenous infusion of DX at the doses employed in this experiment did not change the pressor response to norepinephrine (Figure 6) or the basal pressure.

Figure 4. Dose response curve for norepinephrine in conscious rats. ○ = control rats (n = 12); ● = 2nd day of dexamethasone treatment (n = 6); △ = 6th day of dexamethasone treatment (n = 6). The values are means ± se. The difference from control was significant at **p < 0.01.

Figure 5. Effect of indomethacin on the pressor response to norepinephrine in conscious rats (n = 6). Doses of 50, 100, and 200 ng of norepinephrine were used in each experiment. ○ = before indomethacin administration; ● = 10 minutes after indomethacin (5 mg/kg) injection. The values are means ± se. The difference between responses with and without indomethacin was significant at **p < 0.01.

Figure 6. Effect of acute dexamethasone (DX) administration on the pressor response to norepinephrine in conscious rats (n = 6). ○ = before DX; ● = during intravenous DX infusion (0.01 mg/min). The values are means ± se.
Discussion

In the present study, we found that in DX-induced hypertension the pressor response to norepinephrine was enhanced prior to the elevation of blood pressure. This result suggests that the increased pressor response may participate in the pathogenesis of DX hypertension.

There have been many reports on the vascular reactivity in glucocorticoid hypertension, but the results are somewhat conflicting. The pressor response to norepinephrine was found to be decreased in patients with adrenal insufficiency and to be potentiated in normotensive subjects within 24 hours after administration of cortisone or adrenocorticotropic hormone (ACTH). These results, in addition to those of Fritz and Levine and Mendlowitz et al., are consistent with our findings. However, Krakoff et al. and Kohlmann et al. reported that in methylprednisolone-treated rats, the pressor response to norepinephrine was not significantly enhanced. It is considered that the mineralocorticoid action of natural glucocorticoids might be related to the enhanced pressor response. However, in the present study, we confirmed that DX, which is devoid of mineralocorticoid activity, also enhanced the pressor response to norepinephrine. The discrepancy in the results between the methylprednisolone-treated rats and the DX-treated rats may be related to the differences in the glucocorticoids used and the dosage. Compared to our dose of DX, Krakoff et al. and Kohlmann et al. used a relatively small amount of methylprednisolone.

In contrast to the effects of chronically administered DX, acute intravenous administration of DX did not change the pressor response to norepinephrine. This result is in accord with previous studies. Ross and Schömig et al. reported that pressor response to norepinephrine was not changed by acute intravenous administration of glucocorticoids, while it was potentiated by treatment with glucocorticoids for several days. It would appear, therefore, that the enhancement of the pressor response in DX-treated rats was brought about by modulation of some intrinsic factors.

Although enhancement of the pressor response to norepinephrine could be due to changes in baroreceptor reflexes or the other systemic blood pressure regulatory systems, the enhanced pressor response observed in the present study is most likely due to increased vascular sensitivity, as observed by many other investigators. In some of these in vitro studies, the hyperresponsiveness to norepinephrine was explained either on the basis of changes in the extraneuronal uptake or metabolism of norepinephrine. However, since acute intravenous administration of DX did not alter the dose response curve for norepinephrine in the present in vivo study, the increased pressor response caused by DX could not be explained by changes in either the extraneuronal uptake or metabolism of norepinephrine.

Other possible explanations include structural changes of the vascular wall, and electrolyte and hormonal changes induced by DX. Structural changes are unlikely to have occurred after 2 days of administration of DX, at which time the pressor response to norepinephrine was already enhanced in the DX-treated rats. The slight reduction in plasma sodium concentration and negative sodium balance observed in DX-treated rats would tend to cause a decrease in pressor response. There was no difference in plasma potassium between controls and DX-treated rats, although the values we obtained by decapitation may be spurious relative to samples collected via an indwelling catheter. In addition, hypokalemia, which might have been expected with DX treatment, is generally associated with increased PG synthesis and vascular insensitivity to pressors, as in the situation of psychogenic vomiting or Bartter’s syndrome. Hormonal changes such as activation of the renin-angiotensin system should be considered since angiotensin II is known to potentiate the vascular response to norepinephrine. However, in this case, such a possibility is unlikely because the plasma renin activity was not significantly changed by DX treatment.

Prostaglandins are known to be modulators of vascular reactivity, and possible involvement of the prostaglandin system in glucocorticoid hypertension was suggested recently by Eliovich and Krakoff. In vitro studies showed that glucocorticoids affected prostaglandin biosynthesis by inhibiting the release of arachidonic acid from phospholipids. In our present study we found that the administration of DX resulted in a marked decrease in urinary excretion of PGE; this decrease appeared before the blood pressure became elevated and continued throughout the experiment. Since a marked diuresis and natriuresis were observed in DX-treated rats in spite of a marked decrease in the urinary excretion of PGE, the change in urinary PGE excretion does not seem to be a result of the changes in urinary sodium and water excretion.

Prostaglandins have been shown to be released from a variety of tissues in response to the administration of norepinephrine, and this may blunt the vasoconstrictor activity of this agent. In fact, as previously reported by us, inhibition of prostaglandin biosynthesis by indomethacin potentiated, while PGE, and PGI, attenuated, the pressor response to norepinephrine in untreated rats. In DX-treated rats, in contrast to control rats. indomethacin did not significantly potentiate the pressor response to norepinephrine. If indomethacin and DX had caused enhancement of the pressor response by different mechanisms, their effects should have been additive. It is likely, therefore, that DX also caused enhancement of the pressor response to norepinephrine by inhibiting prostaglandin biosynthesis. This hypothesis is not inconsistent with the finding that acute intravenous administration of DX had no effect on the pressor response, since reportedly several hours are required after the administration of the glucocorticoid before inhibition of prostaglandins becomes evident.

Our in vivo results agree with the in vitro observations of Rascher et al. who reported enhanced vascular reactivity to norepinephrine in isolated perfused
hindlimb preparations of rats treated with corticosterone, which was reversed by perfusion with arachidonic acid and PGI₂. In our study, we could not determine whether the enhanced pressor response to infused norepinephrine was caused by the reduction of prostaglandins in the kidney or in other tissues, such as vascular beds. However, since glucocorticoid hormone reduces the capacity of tissues to produce prostaglandins, the results suggest that the reduction of urinary PGE₂ may reflect the decreased synthesis of prostaglandin in vascular beds as well as in the renal medulla. Although PGI₂ and thromboxane A₂ (TXA₂) are considered to be more important prostaglandins in the vascular tissues compared to PGE₂, they were not quantitated in this study since methods for the determination of PGI₂ and TXA₂ are unavailable to us.

In summary, our study revealed that treatment of rats with DX resulted in a decrease in urinary PGE₂ excretion and an increase in the pressor response to norepinephrine, both of which preceded the rise in blood pressure. Indomethacin potentiated the pressor response to norepinephrine in control rats but not in DX-treated rats. Therefore, it is suggested that the increased sensitivity of the vascular smooth muscle to norepinephrine, subsequent to inhibition of prostaglandin biosynthesis, may be one of the factors contributing to the development of glucocorticoid hypertension.

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Hypertension. 1984;6:236-241
doi: 10.1161/01.HYP.6.2.236

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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