Contribution of Vasopressin in Dexamethasone-Induced Hypertension in Rats

FUJIO ILJIMA AND KAFAIT U. MALIK

SUMMARY Our previous finding that dexamethasone-induced hypertension in rats is associated with enhanced reactivity of mesenteric arteries to arginine vasopressin but not to angiotensin II (Ang II) or norepinephrine has led us to postulate that vasopressin contributes to the development or maintenance of glucocorticoid-induced hypertension. To test this view, we investigated the effects of vasopressin, Ang II, norepinephrine, and the vasopressin V$_1$ receptor antagonist d(CH$_2$)$_2$Tyr(Me)AVP on mean arterial blood pressure and heart rate with and without ganglionic blockade with hexamethonium and angiotensin I (Ang I) converting enzyme inhibition with MK 421 in pentobarbital-anesthetized rats made hypertensive by treatment with dexamethasone (1.8 mg/kg/wk for 14 days). Administration of vasopressin, Ang II, or norepinephrine (0.003-3 ng i.v.) produced a dose-related increase in arterial blood pressure. The pressor response to vasopressin, but not to Ang II or norepinephrine, was greater in dexamethasone-treated than in vehicle-treated animals, and this difference became more pronounced in rats that received hexamethonium and MK 421. Administration of the vasopressin V$_1$ receptor antagonist d(CH$_2$)$_2$Tyr(Me)AVP significantly reduced arterial pressure in dexamethasone-treated but not in vehicle-treated animals. Hexamethonium and MK 421 reduced arterial blood pressure in dexamethasone-treated as well as in vehicle-treated rats; however, arterial blood pressure remained higher in the former. Administration of the vasopressin V$_1$ receptor antagonist produced a greater reduction in arterial blood pressure in dexamethasone-treated than in vehicle-treated rats. These data suggest that vasopressin contributes to glucocorticoid-induced hypertension, which is probably due to enhanced vascular reactivity to the peptide.

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KEY WORDS • pressor response • vasopressin • angiotensin II • norepinephrine • ganglionic blockade • angiotensin I converting enzyme inhibition • vasopressin V$_1$, receptor antagonist

HYPERTENSION produced by long-term treatment with glucocorticoids in some animal species or by glucocorticoid excess in patients with Cushing's syndrome has been proposed to be due to increased renin-angiotensin and sympathetic nervous activity and enhanced vascular reactivity to catecholamines. However, reports indicate that blockade of activity of the renin-angiotensin and sympathetic nervous systems does not bring mean arterial pressure (MAP) to normotensive levels in animals made hypertensive by glucocorticoids. Moreover, short- or long-term treatment with glucocorticoids has been reported to have variable effects on the pressor response to norepinephrine (NE). Since glucocorticoids inhibit prostaglandin synthesis in various tissues in vitro, it has been proposed that glucocorticoid-induced hypertension could be due to reduced prostaglandin synthesis, resulting in enhanced vascular reactivity to norepinephrine. However, the increase in MAP produced by glucocorticoids is unlikely to be due to inhibition of prostaglandin synthesis, because glucocorticoids do not reduce but, rather, enhance the plasma levels and urinary output of prostaglandins. Moreover, our recent study indicates that in rats made hypertensive by treatment with dexamethasone, the response of the isolated mesenteric arteries to arginine vasopressin (AVP) but not to NE or Ang II was enhanced by a mechanism independent of prostaglandin synthesis. These observations raise the possibility that AVP may contribute to the development or maintenance of glucocorticoid-induced hypertension in rats.

To test this hypothesis, we investigated 1) the effect of AVP compared with that of Ang II and NE on MAP
and 2) the effect of the AVP V₁ receptor antagonist d(CH₂)₃Tyr(Me)AVP on MAP with and without ganglionic blockade with hexamethonium, and inhibition of the activity of the renin-angiotensin system with the Ang I converting enzyme inhibitor MK 421 (enalapril) in rats made hypertensive with dexamethasone treatment.

Materials and Methods

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN, USA), 10 to 12 weeks old and weighing 350 to 375 g, were used in all the experiments. The animals were kept in a room controlled for temperature (24°C) and humidity (50%), and had free access to water. They were fed ad libitum a standard chow (Purina 5001,Ralston Purina, St. Louis, MO, USA) containing 194 mEq sodium/kg and 282 mEq potassium/kg. Rats were injected subcutaneously with dexamethasone (1.8 mg/kg/wk) or its vehicle, sesame oil, for 14 days. The first injection was given at Day 1 and the second at Day 7; the experiments were performed on Day 14.

Experimental Protocol

The first series of experiments was conducted to investigate the effects of AVP, Ang II, and NE on MAP in rats treated with dexamethasone or its vehicle. The animals were anesthetized with sodium pentobarbital (30 mg/kg i.p.), and catheters were inserted into the right carotid artery for measurement of MAP and into the right jugular vein for administration of drugs. MAP was measured with a Statham P23 Db pressure transducer (Statham, Hato Rey, Puerto Rico) connected to the catheter inserted into the carotid artery.

Animals treated with dexamethasone and its vehicle were subdivided into two groups; one group was given the ganglionic blocking agent hexamethonium (30 mg/kg i.v.) and the Ang I converting enzyme inhibitor MK 421 (3 mg/kg i.v.). The other group received the vehicle of these agents (0.9% NaCl, 0.5 ml) before the administration of pressor agents. AVP (0.003–3.0 μg), Ang II (0.03–3.0 μg), NE (0.01–1 μg), or their vehicle (0.9% NaCl) was injected at random 10 minutes after the administration of hexamethonium and MK 421. The doses of hexamethonium and MK 421 used were able to block the pressor responses produced by dimethylphenylpiperazinium (1–3 mg/kg i.v.) and Ang I (1–3 μg/kg i.v.), respectively. The maximal increase in MAP produced by each dose of AVP, Ang II, and NE in dexamethasone-treated animals was compared with the corresponding values in the vehicle-treated group with and without the administration of hexamethonium and MK 421.

The second series of experiments was performed to determine the contribution of vasopressin to dexamethasone-induced hypertension in pentobarbital-anesthetized rats with and without blockade of the activity of the sympathetic nervous and renin-angiotensin systems. The effect of the V₁ receptor antagonist d(CH₂)₃Tyr(Me)AVP ([1-β-mercaptop-β,β-cyclopentamethy-

lène propionic acid), 2-(O-methyl)tyrosine]AVP) in animals treated with dexamethasone or its vehicle during and without the administration of hexamethonium and MK 421 was investigated. MAP was measured as described above. Heart rate was measured with a tachometer (Grass Instruments, Quincy, MA, USA). Both MAP and heart rate were recorded on a polygraph (Model 7PF4, Grass Instruments). Changes in MAP and heart rate produced by these agents in animals treated with dexamethasone were compared with those obtained in rats treated with the vehicle.

The results are expressed as means ± SEM. The data were analyzed by two-way analysis of variance, and Student’s t test for paired and unpaired observations was used to determine the difference between means. Differences between means were considered significant if the p value was less than 0.05.

Drugs

The following drugs used in this study were purchased: NE as bitartrate (Winthrop-Breon Labs Division of Sterling Drug Inc., New York, NY, USA); AVP, Ang II, and dexamethasone as acetate (Sigma Chemical, St. Louis, MO, USA); and d(CH₂)₃Tyr(Me)AVP (Dr. Maurice Manning, Ohio Medical College, Toledo, OH, USA). Administration was as follows: NE contained in ampules, AVP, and Ang II were dissolved in saline and injected into the catheter leading to the jugular vein in a volume of 0.1 ml; hexamethonium, MK 421, and d(CH₂)₃Tyr(Me)AVP were also dissolved in saline and administered into the jugular vein; and dexamethasone was suspended in sesame oil and given subcutaneously.

Results

Effects of AVP, Ang II, and NE on MAP in Rats Treated with Dexamethasone and Its Vehicle

The MAP in animals treated with dexamethasone for 14 days was significantly higher than in those treated with its vehicle (Table 1). Administration of AVP, Ang II, and NE produced a consistent, dose-related increase in MAP (Figure 1); changes in heart rate were variable (data not shown). The increase in MAP produced by AVP at doses of 0.1 and 0.3 μg was higher in dexamethasone-treated rats (p < 0.05); the increase in MAP produced by Ang II and NE in dexamethasone-treated rats was not different from that in animals treated with the vehicle (see Figure 1A). The increase in the pressor response to AVP in dexamethasone-treated compared to vehicle-treated rats became more pronounced and also significantly higher at doses as low as 0.01 μg after pretreatment with hexamethonium and MK 421 (see Figure 1B). There was an apparent increase in the potency of AVP in dexamethasone-treated compared to vehicle-treated rats (ED₅₀, 0.022 vs 0.076 μg); the efficacy was not altered. The pressor responses to Ang II and NE in dexamethasone-treated rats were not different from those observed in vehicle-treated animals after administration of hexamethionum and MK 421.
TABLE 1. Effect of Vasopressin V1 Antagonist d(CH2)5Tyr(Me)AVP With and Without Hexamethonium and MK 421 on Mean Arterial Blood Pressure in Rats Treated with Dexamethasone or Its Vehicle (Sesame Oil)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle-treated</th>
<th>Dexamethasone-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>n Before</td>
<td>n After Change</td>
</tr>
<tr>
<td>d(CH2)5Tyr(Me)AVP, 30 μg/kg i.v.</td>
<td>6 111 ± 3</td>
<td>107 ± 2</td>
</tr>
<tr>
<td>Hex, 30 mg/kg i.v.</td>
<td>6 110 ± 5</td>
<td>60 ± 4*</td>
</tr>
<tr>
<td>MK 421, 3 mg/kg i.v.</td>
<td>7 102 ± 4</td>
<td>94 ± 3*</td>
</tr>
<tr>
<td>Hex, 30 mg/kg i.v. + MK 421, 3 mg/kg i.v.</td>
<td>6 107 ± 6</td>
<td>46 ± 4*</td>
</tr>
<tr>
<td>+ d(CH2)5Tyr(Me)AVP, 30 μg/kg i.v.</td>
<td>7 102 ± 4</td>
<td>37 ± 3*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Hex = hexamethonium.
*Significantly different from values obtained before drug administration or the change (p<0.05).
†Significantly different from values obtained in vehicle-treated rats (p<0.05).

Effects of d(CH2)5Tyr(Me)AVP on MAP and Heart Rate with and without Pretreatment with Hexamethonium and MK 421 in Rats Made Hypertensive with Dexamethasone

Administration of the AVP V1 receptor antagonist d(CH2)5Tyr(Me)AVP reduced MAP in dexamethasone-treated but not in vehicle-treated rats; heart rate was not altered in these animals. However, MAP remained higher in dexamethasone-treated than in vehicle-treated rats (Tables 1 and 2). Administration of hexamethonium in doses (30 mg/kg i.v.) that blocked the pressor effect of the ganglionic stimulant dimethylphenylpiperazinium produced a decrease in MAP and heart rate in both dexamethasone-treated and vehicle-treated rats. Although the absolute decrease in MAP and heart rate was greater in dexamethasone-treated than in vehicle-treated rats, MAP and heart rate remained higher in the former than in the latter animals (see Tables 1 and 2). Administration of MK 421 in doses of 3 mg/kg, which blocked the pressor response to Ang I, also reduced MAP, but not heart rate, to a similar degree in dexamethasone-treated and vehicle-treated rats; MAP remained higher in the former. Combined administration of hexamethonium and MK 421 produced a greater absolute decrease in MAP and heart rate in dexamethasone-treated than in vehicle-treated rats, but MAP remained higher in the former. When AVP V1 receptor antagonist was administered after treatment with hexamethonium and MK 421, it decreased MAP.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Effect of vasopressin, angiotensin II, and norepinephrine on mean arterial blood pressure in pentobarbital-anesthetized rats treated with dexamethasone or its vehicle without (A) and with (B) pretreatment with hexamethonium and MK 421.
in dexamethasone- and vehicle-treated rats; MAP in dexamethasone-treated rats was reduced to a level that was not different from that obtained in vehicle-treated rats.

**Discussion**

The present study indicates that dexamethasone-induced hypertension in rats is associated with enhanced pressor response to AVP but not to Ang II or NE. The increase in the pressor response to AVP was observed at doses of 0.1 to 0.3 μg but not at lower or higher doses of the peptide. However, after ganglionic blockade with hexamethonium and administration of the converting enzyme inhibitor MK 421, the selective increase in the pressor response to AVP in dexamethasone-treated hypertensive rats was more pronounced and became more evident at doses lower than 0.1 μg. The mechanism by which the sympathetic nervous and renin-angiotensin systems masked the full activity of these pressor systems also does not normalize MAP in glucocorticoid-hypertensive rats. The increase in the pressor response to AVP in the present study was more pronounced and became more evident at doses lower than 0.1 μg.

The selective increase in the pressor response to AVP could contribute to the development or maintenance of glucocorticoid-induced hypertension. Supporting this view is our demonstration that the AVP V₁ receptor antagonist d(CH₂)₅Tyr(Me)AVP significantly reduced MAP in rats made hypertensive by dexamethasone treatment, whereas it produced an insignificant reduction in MAP in vehicle-treated normotensive rats. Since administration of AVP V₁ receptor antagonist failed to bring the MAP of dexamethasone-treated rats to levels observed in normotensive rats, it would appear that other pressor systems also contribute to the maintenance of MAP in dexamethasone-treated rats. Although the sympathetic nervous and renin-angiotensin systems have been reported to contribute to glucocorticoid-induced hypertension, blockade of the activity of these pressor systems also does not normalize MAP in glucocorticoid-hypertensive rats. Similarly, in the present study, administration of either hexamethonium or MK 421 alone or in combination reduced but failed to normalize MAP in dexamethasone-treated rats. Administration of AVP V₁ receptor antagonist during treatment with hexamethonium and MK 421 reduced MAP in these rats, so that the MAP in dexamethasone-treated hypertensive animals was not different from that observed in vehicle-treated normotensive rats. These findings support the view that AVP contributes to glucocorticoid-induced hypertension and that blockade of the activity of all three pressor systems is required to normalize MAP raised by glucocorticoid treatment in rats.

Recently, it was reported that in Brattleboro rats with diabetes insipidus, which lack AVP, administration of methylprednisolone increased systolic blood pressure by 28 mm Hg, indicating that AVP is not involved in the development of glucocorticoid-induced hypertension. However, these findings do not exclude the possibility that increased vascular reactivity to AVP in rats with normal AVP levels can contribute to glucocorticoid-induced hypertension. Since the sympathetic nervous activity in Brattleboro rats with diabetes insipidus has been reported to be increased, it is not known whether it compensates for the lack of AVP in the development or maintenance of glucocorticoid-induced hypertension. Finally, it is not known whether the selective increase in the pressor response to AVP produced by dexamethasone is also caused by other glucocorticoids.

The mechanism by which glucocorticoid treatment enhances vascular reactivity and pressor response to AVP could be due to up-regulation of AVP receptors consequent to alterations in AVP secretion. Supporting this view is some evidence that dexamethasone treatment inhibits neurohypophyseal AVP secretion and increases the number of AVP binding sites in the plasma membranes of mesenteric vessels.

**Table 2. Effect of Vasopressin V₁ Antagonist d(CH₂)₅Tyr(Me)AVP With and Without Hexamethonium and MK 421 on Mean Heart Rate in Rats Treated with Dexamethasone or Its Vehicle (Sesame Oil)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle-treated</td>
</tr>
<tr>
<td></td>
<td>n Before</td>
</tr>
<tr>
<td>d(CH₂)₅Tyr(Me)AVP, 30 μg/kg i.v.</td>
<td>6</td>
</tr>
<tr>
<td>Hex, 30 mg/kg i.v.</td>
<td>6</td>
</tr>
<tr>
<td>MK 421, 3 mg/kg i.v.</td>
<td>7</td>
</tr>
<tr>
<td>Hex, 30 mg/kg i.v. + MK 421, 3 mg/kg i.v.</td>
<td>6</td>
</tr>
<tr>
<td>Hex, 30 mg/kg i.v. + d(CH₂)₅Tyr(Me)AVP, 30 μg/kg i.v.</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Hex = hexamethonium.
*Significantly different from values obtained before drug administration or the change (p<0.05).
†Significantly different from values obtained in vehicle-treated rats (p<0.05).
In conclusion, this study demonstrates that dexamethasone treatment in rats results in a selective increase in pressor response to AVP and that administration of AVP V1 receptor antagonist normalizes MAP during blockade of the activity of the sympathetic nervous and renin-angiotensin systems in rats made hypertensive by treatment with hexamethonium. Although inhibition of the activity of the sympathetic nervous system produces a major drop in MAP, blockade of the activity of all three pressor systems is essential to normalize MAP in dexamethasone-treated rats.

References

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