Methylprednisolone-Induced Hypertension
Role for the Autonomic and Renin Angiotensin Systems

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SUMMARY The role of the autonomic nervous system (ANS) in the pathogenesis of hypertension induced by methylprednisolone (20 mg/kg/week subcutaneously) was studied in rats before and during chronic renin angiotensin system (RAS) blockade with captopril (20 mg/kg/every 8 hrs by mouth). Sympathetic nervous system (SNS) blockade was accomplished by the intravenous (i.v.) administration of propranolol (0.20 mg/100g) plus phentolamine (1.25 mg/100g/i.v.) and ganglionlc (G) blockade by the use of pentolnium tar-tarate (0.5 mg/100g/i.v.). After 4 weeks, methylprednisolone-treated animals showed significant decreases in mean arterial pressure (MAP) with both SNS (-34 ± 2 mm Hg) and G (-56 ± 3 mm Hg) blockades; during chronic RAS blockade, even greater falls in MAP were observed (SNS = -43 ± 2 mm Hg and G = -75 ± 3 mm Hg). Nevertheless, for both groups the levels of MAP obtained during SNS and G blockades were higher than those observed in their control groups. At the end of second week, however, in captopril-treated hypertensive rats the values of MAP obtained during ANS blockade were lower than those observed in the control group. An increased responsiveness to exogenous administration of norepinephrine (NE) was observed in animals receiving methylprednisolone and captopril. It is concluded that methylprednisolone hypertension in the rat may be initially explained by activation of RAS and ANS. At later phases, a third mechanism has to be postulated to explain the hypertensive state. (Hypertension 3 (suppl II): II-107–II-111, 1981)

KEY WORDS • glucocorticoid-hypertension • autonomic nervous system • renin angiotensin system • norepinephrine responsiveness • captopril • saralasin

SEVERAL mechanisms have been proposed to explain the pathogenesis of excess glucocorticoid hypertension. They include: salt retention,1 activation of the renin angiotensin system,2 increased plasma and extracellular volumes,4 and alterations in various components of the autonomic nervous system (ANS).5,7 We have recently confirmed that hypertension induced by methylprednisolone in the rat is accompanied by activation of the renin-angiotensin system.9 However, this hypertension may occur even during chronic renin angiotensin system blockade with captopril,9 leading to the conclusion that other mechanism(s) may participate in the pathogenesis of this hypertension. In this study we tested the role of the ANS in hypertension induced by methylprednisolone in the rat. Special emphasis is placed upon the interaction of the autonomic and renin angiotensin systems in this type of experimental hypertension.

Methods

Induction of the Hypertensive State

Male Wistar rats receiving regular rat chow and tap water were used. Four groups of rats were prepared: 1) Group M (n = 20), receiving a long-acting preparation of soluble methylprednisolone (Depo-Medrol, Upjohn) at a dose of 20 mg/kg/week subcutaneously; 2) Group M + C (n = 20), receiving methylprednisolone as in Group M plus concomitant captopril (Squibb and Sons') at a dose of 20 mg/kg/every 8 hours by gavage; 3) Group N (n = 20), receiving distilled water by the same route and same frequency as Group M; 4) Group N + C (n = 20), receiving distilled water as Group N plus concomitant daily captopril given as in Group M + C. All groups were followed for 4 weeks, and tail arterial pressure was registered every week using a tail microphonic method5 in unanesthetized animals.

Pharmacological Blockade of the Sympathetic Nervous System (SNS)

At the end of the fourth week, 10 animals of each experimental group (M; M + C; N, and N + C) had catheters placed into the carotid artery and jugular
vein under ether anesthesia. On awakening, the rats' mean arterial pressure (MAP) was continuously recorded with a Beckman physiograph, and the animals were kept unrestrained with free access to water and food. After a 2-hour control period, blockade of sympathetic nervous system (SNS) was induced by intravenous administration of propranolol (Inderal, Ayerst Laboratories) at a dose of 0.20 mg/100 g body weight, followed by phentolamine (Regitine, Ciba Geigy Corporation) at a dose of 1.25 mg/100 g body weight i.v. The MAP was recorded for 60 minutes after the initial injection. Results are expressed by differences in MAP values before propranolol and 30 minutes after phentolamine administration. Identical procedures as described above were used in animals of Groups M + C (n = 12) and N + C (n = 12) studied at the second week.

Ganglionic Blockade and Vascular Responsiveness to Norepinephrine (NE)

Four weeks after the initiation of methylprednisolone administration, 10 animals of each group (M, M + C, N, and N + C) were prepared, as described above. After a 30-minute control period, norepinephrine (NE) (Noradrenaline, Byk Laboratories) was administered at increasing doses of 50, 100, and 200 ng/100 g body weight, i.v. Each injection was given after the MAP had stabilized following the previous injection, and each dose was given twice. The effect of NE upon MAP is represented by the mean of the two maximal elevations observed for each dose used. Following a recovery period from the last dose of NE, all animals underwent ganglionic blockade by the administration of pentolinium tartarate (Ansolysen, Wyeth Laboratories) given as a single intravenous dose of 0.5 mg/100 g body weight. The MAP was registered continuously, and after stabilization, responses to NE were evaluated as before pentolinium. Vascular responsiveness to NE was also tested in eight animals of Group M + C and N + C studied at the second week.

Results

Table 1 shows the values of tail arterial pressure (TAP) observed in the four groups studied. As can be seen, in animals receiving only methylprednisolone TAP was significantly elevated by 1 week, and reached a maximal elevation of 41 ± 1 mm Hg at 4 weeks. In the group receiving captopril methylprednisolone (M + C), a progressive rise in TAP was observed only after the first week, and a maximal change of 24 ± 1 mm Hg was observed at the fourth week. These values were significantly lower than those observed in Group M (p < 0.01). No significant changes in TAP were noted in Group N, whereas a small but statistically significant decrease (ΔTAP = −9 ± 1 mm Hg, p < 0.01) was observed in Group N + C at the fourth week.

Table 1. Tail Arterial Pressure (in mm Hg) in groups M, M + C, N and N + C

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Groups</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>114 ± 1</td>
<td>126 ± 1</td>
<td>138 ± 1</td>
<td>147 ± 1</td>
<td>155 ± 1</td>
<td></td>
</tr>
<tr>
<td>M + C</td>
<td>116 ± 1</td>
<td>119 ± 1</td>
<td>122 ± 1</td>
<td>134 ± 1</td>
<td>140 ± 1</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>114 ± 1</td>
<td>116 ± 1</td>
<td>117 ± 1</td>
<td>117 ± 1</td>
<td>116 ± 1</td>
<td></td>
</tr>
<tr>
<td>N + C</td>
<td>116 ± 1</td>
<td>114 ± 1</td>
<td>110 ± 1</td>
<td>108 ± 1</td>
<td>107 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.01 compared to groups M + C, N and N + C.
**p < 0.01 compared to group N + C.
†p < 0.01 compared to group N.

Absolute values of direct MAP, as well as changes in MAP induced by SNS and ganglionic blockade, are summarized in table 1 and figure 1 respectively. It is apparent that both SNS and ganglionic blockade evoked significant drops in MAP in all groups. During SNS blockade, reductions in MAP of methylprednisolone-treated groups (M = −34 ± 2 mm Hg and M + C = −43 ± 2 mm Hg) were significantly greater (p < 0.01) than those observed in the control groups (N = −15 ± 2 mm Hg and N + C = −24 ± 1 mm Hg). Similar but greater decreases in MAP were observed during ganglionic blockade: M = −56 ± 3 mm Hg; M + C = −75 ± 3 mm Hg; N = −34 ± 2 mm Hg; and N + C = −48 ± 2 mm Hg. It is also noteworthy that in the captopril-treated groups (M + C and N + C) changes in MAP induced by both blockades were significantly greater than those observed in the groups that did not receive that drug (see figure 1). Furthermore, during autonomic nervous system blockade MAP values in the M + C group

Table 2. Mean Arterial Pressure (MAP) in mm Hg Before and During Sympathetic Nervous System (SNS, Propranolol + Phentolamine) and Ganglionic (Pentolinium Tartarate) Blockade in Groups M, M + C, N and N + C Studied at the 4th week (X ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before</th>
<th>SNS</th>
<th>Ganglionic</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>154 ± 2*</td>
<td>118 ± 2*</td>
<td>100 ± 4*</td>
</tr>
<tr>
<td>M + C</td>
<td>136 ± 1†</td>
<td>93 ± 2†</td>
<td>60 ± 3†</td>
</tr>
<tr>
<td>N</td>
<td>115 ± 3†</td>
<td>100 ± 3†</td>
<td>81 ± 2†</td>
</tr>
<tr>
<td>N + C</td>
<td>98 ± 1</td>
<td>73 ± 2</td>
<td>50 ± 1</td>
</tr>
</tbody>
</table>

*p < 0.01 compared to groups M + C, N and N + C.
†p < 0.01 compared to group N + C.
††p < 0.01 compared to group N.

Table 3. Mean Arterial Pressure (MAP) in mm Hg Before and During Sympathetic Nervous System (SNS, Propranolol + Phentolamine) and Ganglionic (Pentolinium Tartarate) Blockade in Groups M + C, N and N + C Studied at the 2nd Week (X ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before</th>
<th>SNS</th>
<th>Ganglionic</th>
</tr>
</thead>
<tbody>
<tr>
<td>M + C</td>
<td>112 ± 1*</td>
<td>76 ± 2*</td>
<td>50 ± 3*</td>
</tr>
<tr>
<td>N + C</td>
<td>98 ± 2</td>
<td>85 ± 3</td>
<td>61 ± 2</td>
</tr>
</tbody>
</table>

*p < 0.01 compared to group N + C.
reached levels that were significantly lower than those observed in the M group, and similar to those of the N group. However, MAP in the M + C group remained significantly higher than in the N + C group (see table 2).

Values of MAP in the groups studied at the second week (M + C and N + C) before and during SNS and ganglionic blockade are shown in table 3 and figure 2. Basal MAP values in M + C group were significantly higher than those of N + C. Also, both blockade procedures resulted in evident decreases in MAP values for both groups. However, drops in MAP were of greater magnitude in M + C animals (-32 ± 2 and -59 ± 2 mm Hg, figure 2) as compared to those obtained in Group N + C (-13 ± 1 mm Hg and -36 ± 2 mm Hg) (p < 0.01 for both procedures). Thus, during both blockades, MAP was lower in Group M + C as compared to Group N + C.

Figure 3 shows the increments in MAP obtained with NE injections before and during ganglionic blockade. It is evident that the NE-induced increments in MAP were greatly enhanced by ganglionic blockade. Both before and during ganglionic blockade, elevations in MAP induced by NE were greater in Group M + C as compared to the other groups (p < 0.05, M + C vs M or N or N + C).

Figure 4 shows elevations of MAP induced by NE before and during ganglionic blockade in Groups M + C and N + C studied at the end of second week. For all but the first NE dose tested, elevations in MAP for both groups were significantly greater during ganglionic blockade. Again, greater elevations in Group M + C were observed before and during ganglionic blockade for all three NE doses tested.
Thus, methylprednisolone hypertension cannot be explained only by hyperactivity of the ANS. These results agree with a previous report from our laboratory which documented a role for the RAS in the maintenance of this type of hypertension. In the group receiving captopril and methylprednisolone (M + C), ANS blockade caused decreases in MAP that were greater than those of Group M. Also, the levels of MAP attained during ganglionic and SNS blockades were lower in M + C as compared to M. This fact helps to corroborate the hypothesis of a simultaneous activation of the RAS and SNS in the methylprednisolone-induced hypertension. However, the fact that, during ANS blockade, the blood pressure levels of the captopril-treated group (M + C) were still higher than those of its appropriate control (N + C) suggests that a third factor, in addition to ANS and RAS, must be involved to explain the hypertension following methylprednisolone administration.

In contrast, at the second week of glucocorticoid administration, both SNS and ganglionic blockades completely reversed elevations of MAP observed in Group M + C. Actually, during these blockades, MAP levels were even lower in Group M + C compared to those of Group N + C. Thus, it is apparent that in the earlier phases of methylprednisolone administration the elevations in blood pressure may be explained by simultaneous activation of the RAS and ANS only.

Whereas the role of the renin-angiotensin system in this type of hypertension is well established, the effect of glucocorticoid administration on SNS function remains controversial. Some others have suggested alterations in tissue metabolism of catecholamines and others, an increase in vascular sensitivity to exogenous NE. In our work we observed that methylprednisolone alone did not significantly alter vascular responsiveness to the three doses of exogenous norepinephrine used, since MAP elevations were similar in Group M and N both before and during ganglionic blockade. Comparable results were previously reported in the same species studied at 2 weeks. However, when methylprednisolone was associated with captopril (Group M + C), a significant increase in responsiveness to these doses of NE was observed before and particularly during ganglionic blockade. This was observed already at the second and was still present at the fourth week. This increased responsiveness to NE cannot be attributed to captopril alone since the increments in blood pressure observed in Group N + C are not different from those of Group N (fig. 3). Thus, we could observe an increased responsiveness to three exogenously administered doses of NE only with the association of methylprednisolone and captopril. Although interpretation of these results is difficult, one might speculate that a diminished endogenous liberation of catecholamines induced by the diminution of angiotensin II caused by captopril could unmask the reported hypersensitivity of adrenergic receptors caused by corticosteroid. The enhanced responsiveness observed in the Group

**Discussion**

A role for the renin angiotensin system (RAS) in the pathogenesis of methylprednisolone hypertension in the rat has been previously demonstrated. However, we have reported previously that RAS blockade with captopril does not prevent the development of this type of hypertension. This observation is confirmed in the present work, since elevations in arterial pressure were evident at the fourth week in the group treated with methylprednisolone and captopril (M + C). Thus, other mechanism(s) must have come into play to explain the elevations of MAP during chronic RAS blockade.

Our results clearly show that administration of methylprednisolone for 4 weeks causes hypertension accompanied by an increase in SNS activity. Accordingly with both pharmacological blockade procedures used, significantly greater drops were observed in methylprednisolone-treated rats. In the group of animals receiving only methylprednisolone (M), both blockades elicited significant drops in MAP; however, the levels of MAP attained were still greater than those observed in the appropriate control (Group N).

**FIGURE 4. Mean arterial pressure elevations (ΔMAP) with increasing doses of norepinephrine (NE) before and during ganglionic blockade at the second week.**

* $p < 0.05$ compared to Group N + C before ganglionic blockade. ** $p < 0.03$ compared to Group N + C during ganglionic blockade.*

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M + C after ganglionic blockade could help to support this hypothesis.

In conclusion, it is possible to detect the participation of both the renin-angiotensin system and the autonomic nervous system in the pathogenesis of glucocorticoid hypertension. In the early stages (second week) of hypertension, these two factors seem to be responsible for the elevated blood pressure. Subsequently (fourth week) a third factor must be postulated to explain the raised arterial pressure. The apparent role of the ANS hyperactivity in this type of hypertension may be, at least in part, explained by an increased responsiveness to catecholamines.

References

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