Effect of angiotensin II on aldosterone and its precursor steroid production in adrenal zona glomerulosa cells from heparin-treated rats


Division of Endocrinology, Department of Internal Medicine, Kanazawa Medical University, Uchinada, Ishikawa 920-02, Japan

Abstract. To assess the nature of the heparin-induced aldosterone deficiency, we investigated the stimulatory effect of angiotensin II (AII) on aldosterone and its precursor steroids in adrenal zona glomerulosa cells from heparin-treated rats compared with those in the cells from vehicle-treated rats. Heparin-treated rats had low plasma aldosterone levels, high plasma renin activity and plasma AII levels, and normal plasma corticosterone level 6 weeks after the treatment (1500 IU/kg, twice daily). Basal aldosterone production, when corrected to a uniform number of cells per group, was similar in the cells from heparin- and vehicle-treated rats. The cells from heparin-treated rats had a less sensitive and lower response of aldosterone production to AII; an increase by 4 orders of magnitude in the threshold dose for AII and a decrease in the maximum AII-stimulated level. The maximum AII-stimulated levels, but not the basal levels, of pregnenolone, corticosterone and 18-OHB production were low in the cells from heparin-treated rats. ACTH caused a similar stimulatory effect on aldosterone production in the cells from heparin- and vehicle-treated rats. The cells from heparin-treated rats had a less sensitive and lower response of aldosterone production to potassium; an increase by one order of magnitude in the threshold dose for potassium and a decrease in the maximum potassium-stimulated level, presumably because of the glomerulosa hyporesponsiveness to AII.

These results suggest that our heparin-treated rats have selective impairment of adrenal zona glomerulosa cells, involving the specific receptors and the aldosterone biosynthesis, to AII.

Heparin and heparinoids are well known to selectively reduce aldosterone production without affecting glucocorticoid function in man (Wilson & Goetz 1964; Abbott et al. 1966a; Conn et al. 1966; Bailey & Ford 1969; Kloppenborg et al. 1975; Phelps et al. 1980; Leehey et al. 1981; O’Kelly et al. 1983) and experimental animals (Gláz & Sugar 1964; Sharma et al. 1967; Levine et al. 1972). The aldosterone deficiency with or without hyperkalaemia in patients receiving hepaterin or heparinoid is usually accompanied by a rise in plasma renin activity (PRA) (Bailey & Ford 1969; Phelps et al. 1980; Leehey et al. 1981; O’Kelly et al. 1983) probably because of sodium diuresis (Baily & Ford 1969). Thus, the primary lesion caused by heparin and heparinoids is thought to be at the adrenal zona glomerulosa (Wilson & Goetz 1964; Bailey & Ford 1969; Phelps et al. 1980; Leehey et al. 1981; O’Kelly et al. 1983). However, the nature of the adrenal abnormality caused by heparin and heparinoids is uncertain, but has been attributed to selective inhibition of the action of angiotensin II (AII) in the zona glomerulosa (Kloppenborg et al. 1975) or enzyme defects in aldosterone biosynthesis (Conn et al. 1966).

To further assess the nature of the heparin-induced adrenal abnormality, we investigated the stimulatory effect of AII on aldosterone and its precursor steroids using isolated adrenal zona glomerulosa cells from rats receiving heparin.

Requests for reprints: Dr. Shinpei Morimoto, Division of Endocrinology, Department of Internal Medicine, Kanazawa Medical University, Uchinada, Ishikawa 920-02, Japan.
Materials and Methods

Male Wistar rats weighing about 150 g at the start of the study were used. They were maintained on standard laboratory chow (Sanyo Lab., Tokyo, Japan) and tap water ad libitum throughout the experimental period. The animals were divided into two groups; one was given heparin sodium im at a dosage of 1500 IU/kg body weight daily for 6 weeks and the other received the same volume of vehicle for the same period. The animals were weighed once weekly and the systolic blood pressure was measured every 2 weeks by tail plethysmography without anaesthesia.

At the end of the 6th week, many of these rats were decapitated and the adrenals were excised. Some were decapitated and the trunk blood was immediately collected for PRA, AII, aldosterone, corticosterone and electrolyte measurements. The excised adrenals were bisected and decapsulated, and the capsules were minced by the method of Giroud et al. (1956). The adrenal zona glomerulosa cells were prepared by a collagenase dispersion technique, as previously described (Kim & Morimoto 1979). They were resuspended in fresh Krebs-Ringer bicarbonate buffer with 2 mg/ml glucose, 1 mg/ml bovine serum albumin (BSA) and 5.6 mM potassium at a concentration of 1 x 10^6 cells/ml. One ml aliquots were then incubated at 37°C, in the presence or absence of stimulants (AII, ACTH and potassium), on a metabolic shaker in a water bath under 95% oxygen and 5% carbon dioxide for 2 h. The cells were then sedimented by centrifugation at 1500 x g for 10 min, and the media were stored at -20°C for corticosteroid measurements. All experiments presented here were repeated on five different occasions.

PRA was measured by direct radioimmunoassay (RIA) using kits from Dinabot Radioisotope Institute (Tokyo, Japan). The intra- and inter-assay variabilities were 7.4 and 10.8%, respectively. Plasma AII was determined by RIA using specific anti-AII antiserum in Kitazato Bio-chem. Lab. (Sagamihara, Japan). The intra- and inter-assay coefficients of variation were 4.5 and 9.7%, respectively. Corticosterone, 18-hydroxycorticosterone (18-OHB) and aldosterone levels in plasma or incubation medium were measured by RIAs after the separation of these steroids from cross-reacting steroids by means of high performance liquid chromatography (HPLC) as described elsewhere (Imaiizumi et al. 1984). The separation column used Zorbas ODS (4.6 mm i.d. x 250 mm) instead of Finepack SIL C18-5. The retention times of corticosterone, 18-OHB and aldosterone were 11.1, 7.3 and 6.3 min, respectively. Cross-reacting steroids such as 18-hydroxydeoxycorticosterone and deoxycorticosterone had the retention times of 13.7 and 20.2 min, respectively. The fraction of corticosterone, 18-OHB or aldosterone was evaporated and assayed by RIA. The intra-assay variabilities of corticosterone, 18-OHB and aldosterone were 5.2, 14.1 and 13.1%, respectively. The inter-assay variabilities of corticosterone, 18-OHB and aldosterone were 7.9, 14.6 and 15.2%, respectively. Pregnenolone in incubation medium was determined by RIA in Kitazato Biochem. Lab. The intra- and inter-assay coefficients of variation were 7.5 and 10.0%, respectively. Plasma sodium and potassium were measured by flame photometry.

Results are expressed as the mean ± SEM. Statistical analysis of the results was performed by Student’s paired or unpaired t-test.

Results

In vivo experiments

The characteristics of heparin- and vehicle-treated rats are shown in Table 1. Body weight, blood pressure and plasma sodium were significantly lower in heparin-treated rats than in vehicle-treated rat (P < 0.05). Plasma potassium was similar in the two groups. Plasma corticosterone tended

<table>
<thead>
<tr>
<th>Table 1. Characteristics of heparin- and vehicle-treated rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
</tr>
<tr>
<td>Plasma sodium (mEq/l)</td>
</tr>
<tr>
<td>Plasma potassium (mEq/l)</td>
</tr>
<tr>
<td>Plasma corticosterone (µg/dl)</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
</tr>
<tr>
<td>Plasma angiotensin II (pg/ml)</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/dl)</td>
</tr>
</tbody>
</table>

* P < 0.01 vs corresponding value for vehicle-treated rats.
** P < 0.001 vs corresponding value for vehicle-treated rats.
to be lower in heparin-treated than in vehicle-treated rats, but the difference was not significantly different. PRA and plasma AII were significantly higher ($P < 0.001$), whereas plasma aldosterone was significantly lower ($P < 0.001$), in heparin-treated than in vehicle-treated rats.

**In vitro experiments**

Basal aldosterone production was similar in heparin- and vehicle-treated rats (2.5 ± 0.3 and 3.0 ± 0.2 ng/10^5 cells, respectively).

The responses of aldosterone production to AII in adrenal zona glomerulosa cells from heparin- and vehicle-treated rats are shown in Fig. 1. All caused a significant increase in aldosterone production at $4.8 \times 10^{-8}$ M in the cells from heparin-treated rats ($P < 0.01$) and $4.8 \times 10^{-12}$ M in the cells from vehicle-treated rats ($P < 0.05$). Thus, the threshold dose for AII in the cells from heparin-treated rats was increased by 4 orders of magnitude compared with that in the cells from vehicle-treated rats, indicating a less sensitive aldosterone response to AII. The maximum AII-stimulated aldosterone production was 3.4 ± 0.4 ng/10^5 cells at $4.8 \times 10^{-8}$ M in the cells from heparin-treated rats and 10.9 ± 0.9 ng/10^5 cells at $4.8 \times 10^{-8}$ M in the cells from vehicle-treated rats. Thus, the maximum AII-stimulated level in the cells from heparin-treated rats was significantly lower than that in the cells from vehicle-treated rats ($P < 0.001$), indicating a lower aldosterone response to AII. The maximum AII-stimulated levels of the precursor steroid production of aldosterone are shown in Table 2. Basal levels of pregnenolone, corticosterone and 18-OHB production were similar in the cells from heparin- and vehicle-treated rats. The maximum AII-stimulated levels of these steroids, however, were significantly lower in the cells from heparin-treated rats than in the cells from vehicle-treated rats ($P < 0.05, 0.005$ and 0.001, respectively).

The responses of aldosterone production to ACTH in adrenal zona glomerulosa cells from heparin- and vehicle-treated rats are shown in Fig. 2. ACTH caused a similar stimulatory effect on aldosterone production in the cells from heparin- and vehicle-treated rats: the threshold dose for

<table>
<thead>
<tr>
<th>Steroid production (ng/10^5 cells)</th>
<th>Basal</th>
<th>Maximum AII-stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnenolone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle-treated rats</td>
<td>0.9 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Heparin-treated rats</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.1*</td>
</tr>
<tr>
<td>Corticosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle-treated rats</td>
<td>14.6 ± 0.9</td>
<td>52.2 ± 2.1</td>
</tr>
<tr>
<td>Heparin-treated rats</td>
<td>11.7 ± 0.3</td>
<td>14.2 ± 0.9**</td>
</tr>
<tr>
<td>18-hydroxy corticosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle-treated rats</td>
<td>8.1 ± 0.4</td>
<td>53.7 ± 2.2</td>
</tr>
<tr>
<td>Heparin-treated rats</td>
<td>8.0 ± 0.5</td>
<td>6.7 ± 0.4***</td>
</tr>
</tbody>
</table>

Results are the mean ± SEM of values obtained in 5 experiments.

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$ vs corresponding maximum AII-stimulated value for vehicle-treated rats.

### Fig. 1

Responses of aldosterone production to AII in adrenal zona glomerulosa cells from heparin- and vehicle-treated rats. B, baseline condition. $* P < 0.05$ vs corresponding basal value. ** $P < 0.01$ vs corresponding basal value. Values are the mean ± SEM of results obtained in 5 experiments.
ACTH and the maximum ACTH-stimulated level were similar in the cells from the two groups.

The responses of aldosterone production to potassium in adrenal zona glomerulosa cells from heparin- and vehicle-treated rats are shown in Fig. 3. Potassium increased aldosterone production significantly at 6.0 mM in the cells from heparin-treated rats ($P < 0.05$) and at 5.0 mM in the cells from vehicle-treated rats ($P < 0.05$). The maximum potassium-stimulated aldosterone production (13.6 ± 2.7 ng/10^5 cells) in the cells from heparin-treated rats was significantly lower than that (30.8 ± 1.8 ng/10^5 cells) in the cells from vehicle-treated rats ($P < 0.001$). Thus, heparin-treated rats had a less sensitive and lower aldosterone response to potassium than that in the cells from vehicle-treated rats.

The effect of direct addition of heparin to adrenal zona glomerulosa cells from intact rats on a maximum AII-stimulated aldosterone production is shown in Table 3. To investigate direct effect of heparin on maximum AII-stimulated aldosterone

**Fig. 2.**

Responses of aldosterone production to ACTH in adrenal zona glomerulosa cells from heparin- and vehicle-treated rats. B, baseline condition. *** $P < 0.001$ vs corresponding basal value. Values are the mean ± SEM of results obtained in 5 experiments.

**Fig. 3.**

Responses of aldosterone production to potassium in adrenal zona glomerulosa cells from heparin- and vehicle-treated rats. B, baseline condition. * $P < 0.05$ vs corresponding basal value. Values are the mean ± SEM of results obtained in 5 experiments.

**Table 3.**

Effect of direct addition of heparin to adrenal glomerulosa cells from intact rats on maximum AII-stimulated aldosterone production.

<table>
<thead>
<tr>
<th>Added heparin dose (IU/ml)</th>
<th>Aldosterone production (ng/10^5 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>0</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>0.3*</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

Results are the mean ± SEM of values obtained in 5 experiments.

* This dose is equal to the maximal plasma level (0.55 IU/ml) after administration of 1500 IU/kg body weight heparin in vivo.

** $P < 0.001$ vs corresponding maximum AII-stimulated value without heparin.
production. 0.3 IU heparin, equal to the calculated maximal plasma level after an injection of 1500 IU/kg heparin, was added to zona glomerulosa cells from intact rats in the incubation medium. Direct addition of heparin to the cells from intact rats produced a significant decrease in the maximum AII-stimulated aldosterone production ($P < 0.001$), whereas this did not affect the basal aldosterone production.

**Discussion**

Selective aldosterone deficiency with or without hyperkalaemia in patients receiving heparin or heparinoid is manifested by high PRA level and low plasma aldosterone level without affecting glucocorticoid secretion (Bailey & Ford 1969; Phelps et al. 1980; Lheehey et al. 1981; O’Kelly et al. 1983). The present data also demonstrate that prolonged heparin treatment in rats produced an increase in both PRA and plasma AII and a decrease in plasma aldosterone. Plasma corticosterone tended to be low in heparin-treated rats. Corticosterone is produced in both the zona fasciculata and the zona glomerulosa, predominantly in the zona fasciculata. The somewhat low plasma corticosterone in these rats might reflect a decreased production of this steroid in the zona glomerulosa because the zona fasciculata is not affected by heparin or heparinoid (Gláz & Sugár 1964; Levine et al. 1972). None of our heparin-treated rats, however, developed frank hyperkalaemia. The aldosterone-lowering effect of heparin, therefore, might be at least partly compensated for by activating the renin-angiotensin system. The purpose of the present study was to assess the nature of the heparin-induced adrenal abnormality in rats.

In man (Wilson & Goetz 1964) and rats (Vallent et al. 1964; Abbott et al. 1966b; Levine et al. 1972), prolonged treatment with heparin or heparinoid is shown to decrease the width of the adrenal zona glomerulosa. Such a morphological change could also contribute to lowering basal levels and subsequent responses of aldosterone and its precursor steroids to a given stimulus because of a reduction in the mass of zona glomerulosa cells. This influence of heparin on the zona glomerulosa mass was corrected for in the present study by expressing steroid production per $10^5$ cells prepared both from heparin- and vehicle-treated rats.

AII has been shown to act on adrenal zona glomerulosa cells to stimulate the specific receptors (Douglas et al. 1978) and the early (conversion of cholesterol to pregnenolone) and late (conversion of corticosterone to aldosterone) steps in aldosterone biosynthesis (Aguilera & Catt 1979). In the present study, the glomerulosa cells from heparin-treated rats had a less sensitive and lower maximal response of aldosterone production to AII than the cells from vehicle-treated rats. In addition, the AII-induced maximal levels of pregnenolone, corticosterone and 18-OHB production were lower in the cells from heparin-treated rats than in the cells from vehicle-treated rats. The failure of AII to stimulate the responses of corticosteroids in the cells from heparin-treated rats suggests that our heparin-treated rats have an impairment of adrenal zona glomerulosa cells, involving the specific receptors and the aldosterone biosynthesis, to AII.

On the other hand, the cells from heparin- and vehicle-treated rats had similar responses of aldosterone production to ACTH: the threshold dose for ACTH and the maximal response were similar. Since ACTH receptors (McIlhinney & Schulster 1975), which activate adenylate cyclase (Lefkowitz et al. 1970), are different from specific AII receptors, it is most likely that our heparin-treated rats have selective impairment of glomerulosa cells to AII but not to ACTH. Of interest, the cells from heparin-treated rats had a less sensitive and lower maximal response of aldosterone production to potassium than the cells from vehicle-treated rats. Potassium is known to stimulate not merely the several steps in aldosterone biosynthesis (Haning et al. 1970; Bauman & Müller 1972), but the AII receptors (Douglas & Müller 1976). Furthermore, potassium and AII interact synergistically in stimulating aldosterone production (Fredlund et al. 1977; Parkinson et al. 1984). Thus, the stimulatory effect of potassium on aldosterone in vitro has been shown to be attenuated in the pre-existing in vivo absence of AII (Parkinson et al. 1984). Our heparin-treated rats with normokalaemia were in abnormally low aldosterone states despite high PRA and plasma AII levels, which were attributable to an impairment of adrenal zona glomerulosa cells to AII. It is likely, therefore, that the reduced glomerulosa response to potassium in heparin-treated rats is secondary to the impairment of the AII receptor and postreceptor sites. In this regard, further works including AII binding studies will be required.

Although an iv administration of heparin or
heparinoid to rats reduced basal aldosterone production within several hours (Gláz & Sugár 1964), these compounds do not suppress basal aldosterone production when added directly to rat adrenal slices (Gláz & Sugár 1964; Levine et al. 1972). Thus, heparin and heparinoids are thought to act indirectly on the adrenal zona glomerulosa by forming some active substance. In the present study, heparin reduced the maximum ADH-stimulated aldosterone production, but not the basal production, when added directly to incubating zona glomerulosa cells from intact rats. It is likely that heparin acts directly on adrenal zona glomerulosa cells to inhibit the action of ADH.

References

Received on April 29th, 1985.