Evaluation of the mineralocorticoid activity of 18-hydroxycorticosterone

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Summary

1. The mineralocorticoid activity of 18-hydroxycorticosterone (18-OH-B) has been compared with that of aldosterone by using human bioassay in vivo with measurement of rectal potential difference and urinary log 10 Na+/K+ ratio.

2. A log-linear relationship was found between maximum change in rectal potential difference and increasing doses of aldosterone.

3. No mineralocorticoid activity could be demonstrated after an intravenous bolus and infusion of 18-OH-B.

4. The half-life of clearance of 18-OH-B was measured in three subjects and found to be 28, 48 and 24 min.

Key words: aldosterone, 18-hydroxycorticosterone.

Introduction

18-Hydroxycorticosterone (18-OH-B) is thought to be an intermediate in aldosterone biosynthesis [1] and is produced by the 18-hydroxylation of corticosterone [2]. In man the secretion rate of 18-OH-B parallels that of aldosterone [3]. However, despite this association and the fact that the secretion rate of 18-OH-B is approximately twice that of aldosterone, very little is known of its biological activity.

Feldman & Funder [4] and Baxter, Schambelan, Mattulich, Spindler, Addison & Bartter [5] demonstrated that 18-OH-B has a low affinity for mineralocorticoid or glucocorticoid receptors. In man the low plasma concentrations of 18-OH-B in conjunction with this low affinity would suggest that 18-OH-B has no significant mineralocorticoid or glucocorticoid effects [3, 6]. It has, however, been suggested that the constant hypersecretion of even a weak mineralocorticoid may lead to hypertension [7]. The mineralocorticoid activity of 18-OH-B has not been evaluated in man. These studies were therefore undertaken to determine the effect of infusions of 18-OH-B on transmural rectal potential difference and urinary sodium and potassium excretion and to compare the effect of 18-OH-B with that of aldosterone. In addition, studies were also carried out to determine the half-life of clearance of 18-OH-B from the circulation.

Materials and methods

Rectal potential difference was measured with silver, silver chloride, saline/agar electrodes [8] used in conjunction with an Orion 701 millivoltmeter. An EEL flame photometer was used for urinary electrolyte determination and a Sage (Orion) constant-infusion pump for infusion studies.

Experiments were carried out with five normal male volunteer subjects between the ages of 29 and 55 years to whom the protocol was fully explained and who gave their informed consent. Quantitative mineralocorticoid bioassays were established in subjects nos. 1, 2 and 3; subjects nos. 4 and 5 took part in qualitative studies.
The infused doses of steroid and the parameters measured are shown in Table 1. All subjects were allowed a sodium intake ad libitum. 18-OH-B was bought from Fluka Fluorochem Ltd, Switzerland. Ampoule concentrations and plasma levels of 18-OH-B and 18-hydroxydeoxycorticosterone (18-OH-DOC) were assayed as described previously [6, 9]. Plasma aldosterone was measured by a highly specific direct radioimmunoassay [10]. Plasma cortisol levels were assayed with a commercial kit (Cortipac; The Radiochemical Centre, Amersham, Bucks., U.K.). The infusion rate required to maintain a constant plasma concentration of 18-OH-B or aldosterone after a bolus dose was calculated from the equation: infusion rate = D₀(0.693/τₜ), where τₜ is the biological half-life of the infused steroid and D₀ is the bolus dose of administered steroid [11]. In establishing dose-response curves for aldosterone, bolus doses of between 0.11 to 14.284 pg/kg were used. The infusion calculation was performed with an approximated half-life for aldosterone of 20 min [12]. In the absence of information on the half-life of 18-OH-B the same figure was used in the calculation of the infusion dose for this steroid.

**Experimental protocol**

All experiments were performed on fasting subjects who were hydrated by being given 200 ml of water every 2 h. An interval of at least 1 week was allowed to elapse between infusion experiments. All experiments were commenced at 09.00 hours, the subjects remaining recumbent throughout the experimental period apart from rising to pass urine. The design of the infusion experiments is shown in Fig. 1. Measurements of transmural rectal potential difference were made 7 cm from the anal margin avoiding pressure artifacts; the reference site was an area of skin injected intradermally with sodium chloride solution (150 mmol/l) [13]. Urinary sodium and potassium concentrations were measured by flame photometry.

The bolus injection of either aldosterone or 18-OH-B was given over 1 min in 10 ml of 5% (w/v) glucose solution and was followed by a constant-rate infusion in 45 ml of 5% (w/v) glucose solution over 60 min; a control bolus and infusion of 5% (w/v) glucose solution alone was also given. 18-OH-B or glucose solutions were administered in a non-blind cross-over fashion to subjects nos. 1, 2 and 3.

To assess the half-life of clearance of 18-OH-B, blood samples were taken at intervals up to 150

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### Table 1. Experimental protocol for aldosterone and 18-OH-B bioassays

<table>
<thead>
<tr>
<th>Bolus dose (µg/kg)</th>
<th>Infusions (µg min⁻¹ kg⁻¹)</th>
<th>Aldosterone</th>
<th>18-OH-B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.284</td>
<td>0.494 **</td>
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<tr>
<td>7.142</td>
<td>0.247 *** ***</td>
<td></td>
<td></td>
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<tr>
<td>3.571</td>
<td>0.1235 *** *** ***</td>
<td>+ ***</td>
<td>**</td>
</tr>
<tr>
<td>1.785</td>
<td>0.0617 *** ***</td>
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<tr>
<td>0.892</td>
<td>0.0308 *** ***</td>
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<td>0.446</td>
<td>0.0154 *** ***</td>
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<td>0.223</td>
<td>0.0077 *** ***</td>
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<tr>
<td>0.111</td>
<td>0.0038 *** ***</td>
<td>***</td>
<td>**</td>
</tr>
</tbody>
</table>

Control

*Measurement of rectal potential difference.
**Measurement of urinary Na⁺/K⁺ excretion.
† Subject no. 1 was given two infusions of 18-OH-B at this dose level.
min after the infusion of 18-OH-B was stopped in subjects nos. 1, 2 and 3. In addition to 18-OH-B, plasma levels of aldosterone, cortisol and 18-OH-DOC were also measured. In all subjects urine samples were collected immediately before the bolus injection and at 2 h intervals for 8 h. Further samples were obtained at 12 and 24 h. Blood pressure measurements were made at 15 min intervals for 30 min before delivery of the bolus, at 15 min intervals for 1 h after delivery of the bolus, at 30 min intervals for 2 h and then hourly until 7 h after the end of the infusion. A London School of Hygiene and Tropical Medicine sphygmomanometer [14] was used to measure blood pressure with Korotkoff’s first and fifth sound for each measurement.

Results

Rectal potential difference and urinary log 10 Na⁺/K⁺ ratio

The maximum increase in rectal potential difference in response to intravenous aldosterone occurred 4 to 5 h after the administration of the bolus. A typical time course of response in subject no. 1 is shown in Fig. 2. The relationship between the log-bolus dose of aldosterone and maximum change in rectal potential difference in the same subject is shown in Fig. 3. Plasma aldosterone levels achieved by the bolus and infusion technique in subjects nos. 1 and 2 are shown in Fig. 4. Table 2 includes the maximum changes in rectal potential difference and urinary log 10 Na⁺/K⁺ found in subjects nos. 1, 2 and 3 after aldosterone and Table 3 the maximum changes in these parameters in response to 18-OH-B infusion.

In subjects nos. 4 and 5 changes in only the log 10 Na⁺/K⁺ ratio were measured. Neither of the parameters demonstrated any evidence of mineralocorticoid activity after 18-OH-B bolus and infusion in any subject.

![Fig. 2. Time course of response of rectal potential difference (p.d.) to intravenous aldosterone in subject no. 1 (bolus dose 7.142 μg/kg, infusion dose 0.247 μg min⁻¹ kg⁻¹).](image)

![Fig. 3. Dose–response relationship between maximal change in rectal potential difference (p.d.) and bolus dose of aldosterone in subject no. 1.](image)

![Fig. 4. Plasma aldosterone levels attained by bolus and infusion techniques in subjects nos. 1 and 2. Subject no. 1: ●, bolus 3.571 μg/kg and infusion 0.1235 μg min⁻¹ kg⁻¹; ○, bolus 0.446 μg/kg and infusion 0.0154 μg min⁻¹ kg⁻¹. Subject no. 2: ■, bolus 0.892 μg/kg; infusion 0.0308 μg min⁻¹ kg⁻¹.](image)
Table 2. Maximum changes in rectal potential difference and urinary log 10 Na+/K+ responses to aldosterone bolus and infusion

<table>
<thead>
<tr>
<th>Bolus dose (µg/kg)</th>
<th>Infusion (µg min⁻¹ kg⁻¹)</th>
<th>Subject no. 1</th>
<th>Subject no. 2</th>
<th>Subject no. 3</th>
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<tbody>
<tr>
<td></td>
<td>Max. change in p.d. (mV)</td>
<td>Max. change in log 10 Na⁺/K⁺ of urine</td>
<td>Max. change in p.d. (mV)</td>
<td>Max. change in log 10 Na⁺/K⁺ of urine</td>
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<td>14.284</td>
<td>0.494</td>
<td>-36</td>
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<tr>
<td>0.892</td>
<td>0.0308</td>
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<td>Control</td>
<td>-1</td>
<td>+0.04</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Maximum changes in rectal potential difference and log 10 Na⁺/K⁺ ratio in response to bolus and infusion of 18-OH-B

<table>
<thead>
<tr>
<th>Bolus dose (µg/kg)</th>
<th>Infusion (µg/kg)</th>
<th>Subject no. 1</th>
<th>Subject no. 2</th>
<th>Subject no. 3</th>
<th>Subject no. 4</th>
<th>Subject no. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max. change in p.d. (mV)</td>
<td>Max. change in log 10 Na⁺/K⁺</td>
<td>Max. change in p.d. (mV)</td>
<td>Max. change in log 10 Na⁺/K⁺</td>
<td>Max. change in p.d. (mV)</td>
<td>Max. change in log 10 Na⁺/K⁺</td>
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<tr>
<td>3.571</td>
<td>0.1235</td>
<td>(1) +3</td>
<td>+0.37</td>
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<td>+0.13</td>
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<td>1.785</td>
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<td>-1</td>
<td>+0.25</td>
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<td>0.0308</td>
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<tr>
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<td>-2</td>
<td>+0.08</td>
<td>-3</td>
<td>-0.08</td>
<td>-3</td>
</tr>
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</table>

Blood pressure responses

In subject no. 1 (experiment 1) a blood pressure rise of 40 mmHg systolic and 30 mmHg diastolic was observed 2 h after the bolus injection of 18-OH-B; this was not reproduced when the experiment was repeated after an interval of 3 months or after the infusion of 18-OH-B into any other subject. No subject showed a significant change in pulse rate or other side effects during the infusion. None of the aldosterone infusions produced a change in blood pressure.

Plasma levels of 18-OH-B

Plasma concentrations of 18-OH-B, 18-OH-DOC, aldosterone and cortisol during infusion of 18-OH-B in subjects nos. 1, 2 and 3 are shown in Table 4. Assuming that the distribution of 18-OH-B throughout the tissue was complete during the infusion and that the exponential decay of plasma concentration of 18-OH-B after cessation of the infusion represents only metabolism and excretion, the half-lives of elimination of 18-OH-B from the plasma of subjects nos. 1, 2 and 3 were 28, 48 and 24 min respectively.

Plasma levels of 18-OH-B showed only a minimal rise after infusion of 18-OH-B in subjects nos. 1 and 2; a fivefold rise in plasma 18-OH-DOC concentration was seen at 60 min in subject no. 3. In this subject, in comparison with the other two, plasma cortisol levels rose during the 18-OH-B infusion and it would seem likely that the rise in 18-OH-DOC was secondary to adrenocorticotropic hormone release in response to the experimental stress.

Plasma aldosterone levels before and after 18-OH-B infusions are shown in Table 4. There was no consistent change of plasma aldosterone during or after the 18-OH-B infusion.
A log-linear relationship between maximum change in rectal potential difference and increasing doses of intravenously administered aldosterone has previously been demonstrated [15]. This relationship was confirmed in the present study as was the threshold nature of the urinary electrolyte response to increasing mineralocorticoid activity.

The synchronous use of two mineralocorticoid assay systems was thought appropriate as circumstantial evidence has been obtained for a dissociation of renal and large gut mineralocorticoid response to an unidentified adrenal steroid in a hypertensive patient [16]. A similar dissociation appeared to be present in the responses of normal subjects to carbenoxolone treatment, after which rectal effects predominated [17, 18]. It seemed important therefore to measure both renal and gastrointestinal responses.

It has been demonstrated that supine plasma aldosterone levels and electrical asymmetry across sodium transporting epithelia are almost completely suppressed by a sodium intake of 100–300 mmol/day [19], this being the range of sodium intake encountered in developed countries [20, 21]. In this study, in which no alterations to the subjects' normal diets were made, there was no consistent depression of plasma aldosterone by the 18-OH-B infusion which might have masked a minor mineralocorticoid activity of 18-OH-B.

No mineralocorticoid activity could be demonstrated in response to an intravenous bolus and infusion of 18-OH-B in any subject. As might have been predicted from the findings of Vecsei, Purjesz & Wolff [22] conversion of 18-OH-DOC was minimal and did not produce a measurable mineralocorticoid response. Log 10 Na+/K+ ratio was greater after 18-OH-B bolus and infusion than after control infusion in four of the five subjects. It has been shown that sodium excretion in adrenalectomized rats is increased by the infusion of 18-OH-B [23]. These results, therefore, raise the possibility of a natriuretic effect in man. This requires further investigation and also measurement of titratable H+ and NH4+ excretion which are increased by 18-OH-B in the rat [23].

The hypertensive response observed after the first infusion of 18-OH-B in subject no. 1 was not reproducible and remains unexplained. If 18-OH-B does play a role in the genesis of hypertension in man, the present study would suggest that it is unlikely to be mediated through mineralocorticoid activity.
Acknowledgments

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References


