Effects of Adrenal Steroid Withdrawal on Chronic Renovascular Hypertension in Adrenalectomized Sheep

By J. R. Blair-West, J. P. Coghlan, D. A. Denton, J. W. Funder, B. A. Scoggins, and R. D. Wright

ABSTRACT

Adrenalectomized sheep with a single kidney were maintained chronically hypertensive after renal arterial constriction on a maintenance dosage of desoxycorticosterone acetate and cortisone acetate. This level of steroid support is totally without pressor effects in adrenalectomized animals with normal kidneys. Blood pressure was monitored after steroid withdrawal with (1) no replacement of Na⁺ loss, (2) intravenous replacement of Na⁺ loss, (3) withdrawal of one steroid alone. When cortisone alone was withdrawn, the plasma [Na⁺] and [K⁺] remained within normal limits, no natriuresis occurred, and the blood pressure remained elevated. When both steroids, or DOCA alone, were withdrawn, a profound natriuresis and alteration in plasma ionic concentrations occurred; the blood pressure, however, remained persistently elevated above the values obtained before renal arterial constriction despite clinical and biochemical evidence of adrenoprival status. This pattern of blood pressure response compared with that following release of the constriction is interpreted as evidence against a causative role for the adrenal cortex in the mechanism of renovascular hypertension.

ADDITIONAL KEY WORDS desoxycorticosterone acetate blood pressure experimental renovascular hypertension renal arterial constriction cortisol

Previous work from this laboratory (1) on sheep with intact adrenals, unilateral nephrectomy, and contralateral renal artery constriction demonstrated that plasma renin and aldosterone levels return to basal 5 to 7 days after renal artery constriction; that the animals remain in a state of normal Na balance; and that the persistent hypertension is not associated with elevated plasma levels of renin and aldosterone. The present series of experiments was designed to examine the effect of the withdrawal of basal steroid sup-

Surgical Procedures

Four mature sheep—two wethers (nos. 1, 3) and two ewes (nos. 2, 4)—were used in the study. Surgery was performed with aseptic technique. After thiopentone induction and endotracheal intubation, anesthesia was maintained with halothane and O₂. Initially, we performed a right nephroadrenalectomy and made a cutaneous carotid loop in nos. 1, 2, and 3. Sheep no. 4 had been bilaterally adrenalectomized and had a carotid loop fashioned 18 months before the study; accordingly, she underwent a single operative procedure of unilateral nephrectomy with constriction of the remaining renal artery. The degree of renal artery constriction obtained was subjectively similar in all animals as indicated by a thrill and reduced pulse volume distal to the clip and a wrinkling of the kidney capsule.

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Routine Maintenance and Observations

Each animal was housed in a separate metabolism cage; each day 1 kg of oats/lucerne chaff mixture and 4 liters of tap water were offered, and the amount ingested recorded; the daily urine output was measured and a sample taken for \([\text{Na}^+]\) and \([\text{K}^+]\) determination. Blood samples were taken by carotid artery puncture before clipping and at intervals afterwards for measurement of hematocrit, plasma \([\text{Na}^+]\) and \([\text{K}^+]\) (duplicate determinations, Technicon Autoanalyser), plasma pH (Radiometer), and plasma renin concentration [2]. Systolic blood pressure was measured by carotid loop sphygmomanometry and palpation; recordings were made at least once, and on an average three times a day; each recording represents the mean of several consecutive readings. All blood pressure measurements were made with the animals standing up and with head erect.

Routine Steroid Administration

Fifty milligrams of cortisone acetate (Cortisyl, Roussel) was given intramuscularly before operation to sheep no. 4, and before the second adrenalectomy to the other three sheep, to cover the immediate postoperative period. Thereafter, steroid administration was 12.5 mg cortisone acetate and 2.5 mg desoxycorticosterone acetate (Percorten, Ciba) twice daily by intramuscular injection. This dosage is the usual laboratory maintenance regimen for, and totally without pressor effects in, adrenalectomized sheep with intact kidneys.

Steroid Administration Before Withdrawal

The preparation used for routine steroid maintenance, and the intramuscular route of administration, are specifically designed for slow and sustained release into the circulation over a period of 12 to 24 hours. To establish an accurate and certain cut-off point, the intravenous route of steroid administration was substituted for the intramuscular for a period of 72 hours before withdrawal. In a preliminary trial the intravenous infusion contained 5 mg desoxycorticosterone (Calbiochem), and hydrocortisone hemisuccinate (Efcortelan, Glaxo) equivalent to 20 mg of cortisol, per day in 0.9% NaCl. Due presumably to the more effective route of administration, and to the fact that administration was constant over the whole day, this represented an overdosage relative to intramuscular injections twice daily—an overdosage indicated by uniformly lowered plasma \([\text{K}^+]\) and urinary \([\text{Na}^+]\) at the end of the 3-day period. Accordingly, the dosage of intravenous steroids used in all experiments was arbitrarily fixed at desoxycorticosterone 1.25 mg and cortisol equivalent to 5 mg/day. This commonly represented, in fact, a slight underdosage as reflected by changes in plasma and urinary electrolytes. Since some imprecision, however, was inevitable, it was thought better that withdrawal be from a slightly hypoadrenal state than from one of steroid loading.

Steroid Withdrawal

Steroid withdrawal was effected under four sets of experimental conditions (experiments 1 to 4). Blood pressure recordings were made frequently during the 48-hour period of withdrawal; plasma \([\text{Na}^+]\) and \([\text{K}^+]\) were determined at 0, 24, and 48 hours; and net \([\text{Na}^+]\) deficit (urinary loss minus oral or intravenous intake) was measured at 24 and 48 hours. Plasma renin concentration was determined for one animal in each experiment at 0, 24, and 48 hours.

Experiment 1: Withdrawal of both steroids without any replacement of urinary \([\text{Na}^+]\) loss (sheep 1, 2, and 3).

Experiment 2: Withdrawal of both steroids and concurrent replacement of urinary \([\text{Na}^+]\) loss by the intravenous infusion of titrated volumes of 0.9% NaCl solution (sheep 2, 3, and 4).

Experiment 3: Withdrawal of desoxycorticosterone alone without any replacement of urinary \([\text{Na}^+]\) loss (sheep 3 and 4).

Experiment 4: Withdrawal of cortisol alone, without any replacement of urinary \([\text{Na}^+]\) loss (sheep 2 and 4).

When necessary, sheep were resuscitated after withdrawal with dexamethasone (Decadron, Merck, Sharp and Dohme) 8 mg, im or iv.

Results

Establishment of Renovascular Hypertension in Adrenalectomized Sheep.—Systolic blood pressure levels before and after renal artery constriction are given in Table 1. Values before constriction are the average of recordings taken twice daily over the week before the artery was clipped; values after

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Before</th>
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<tr>
<td>1</td>
<td>98 ± 4</td>
<td>149 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>105 ± 5</td>
<td>156 ± 6</td>
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<tr>
<td>3</td>
<td>108 ± 2</td>
<td>147 ± 5</td>
</tr>
<tr>
<td>4</td>
<td>99 ± 4</td>
<td>147 ± 10</td>
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Values are in mm Hg. The means ± 1 SD of 14 recordings are given.
ADRENAL STEROIDS AND RENAL HYPERTENSION

805

ng/hr/ml
PLASMA RENIN CONCENTRATION

O SHEEP 1
O SHEEP 3
O SHEEP 4
RANGE Na+ REPLETE

DAYS

FIGURE 1
Plasma renin concentration during the period of onset of the hypertension.

constriction are the average of recordings taken twice daily over the third week after renal artery constriction, when the sheep had intramuscular steroid support. Both within and between days, greater variability in blood pressure levels appeared to exist at hypertensive levels than at normotensive. No tendency of this variability, or of the hypertension itself, to become less over the period after renal artery constriction was observed. Plasma renin levels were determined in three animals (Fig. 1) over the period immediately after constriction; these showed a maximum on day 2-3 followed by a return to basal levels. This pattern appears to be essentially the same as that in animals with intact adrenals undergoing renal artery constriction.

Effect of Steroid Withdrawal on Renovascular Hypertension.—Figure 2 shows blood pressure levels, plasma [Na+] and [K+], and Na+ deficit of each sheep in the four experiments. In each experiment plasma renin was measured for one animal.

Na Balance.—In experiment 1, where both steroids were discontinued, urinary Na+ losses of the order of 250 mEq per 24 hours occurred. Where concurrent equivalent replacement of urinary Na+ losses was attempted with intravenous 0.9% NaCl (experiment 2), no gross changes in Na balance were observed. When cortisol was continued after cessation of desoxycorticosterone (experiment 3), urinary losses were of an order similar to those when both steroids were withdrawn. When desoxycorticosterone was continued but cortisol withdrawn, no Na+ deficit was incurred over the 48-hour period.

Plasma Electrolytes.—In the first three experiments, whether or not cortisol was continued or Na+ loss was replaced, over the first 24 hours the plasma [K+] rose to an average of 7 mEq/liter and the plasma [Na+] fell to 137 mEq/liter. In experiment 4, when desoxycorticosterone was continued plasma electrolytes remained at normal levels.

Plasma renin concentration remained within the normal range in all cases except experiment 3, where an elevation from 0.9 to 7.4 ng/hour/ml was associated with a Na+ deficit of 600 mEq.

Blood Pressure.—In no case after withdrawal of either or both steroids did the blood pressure fall to the levels of before constriction. In some cases (e.g., sheep 3, experiment 2), levels remained at or above the prewithdrawal mean. In most, a tendency to fall by 10 to 20 mm Hg was observed over...
### Table 1: SWOT Analysis

<table>
<thead>
<tr>
<th>SWOT</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
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<tr>
<td>Strengths</td>
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<td>Threats</td>
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### Diagram Analysis

- **Sheep 1**
  - **Systolic B.P.**
    - (mm Hg)
  - **Plasma Na⁺/K⁺**
    - 141/5.4 134/7.4 132/8.1
  - **Na⁺ Deficit**
    - 0 100 250

- **Sheep 2**
  - **Systolic B.P.**
    - (mm Hg)
  - **Plasma Na⁺/K⁺**
    - 144/8.2 139/6.7 138/7.2
  - **Na⁺ Deficit**
    - 0 300 650

- **Sheep 3**
  - **Systolic B.P.**
    - (mm Hg)
  - **Plasma Na⁺/K⁺**
    - 148/3.4 137/6.7 138/3.6
  - **Na⁺ Deficit**
    - 0 250 550

- **Sheep 4**
  - **Systolic B.P.**
    - (mm Hg)
  - **Plasma Na⁺/K⁺**
    - 146/4.1 136/7.6
  - **Na⁺ Deficit**
    - 0 150 80

**HOURS**

- **Experiment 1**
  - 16 36 56
- **Experiment 2**
  - 16 36 56
- **Experiment 3**
  - 16 36 56
- **Experiment 4**
  - 16 36 56
the withdrawal period, but even in the presence of an obvious and severe adrenoprival state, the blood pressure remained elevated well above the normal range.

Discussion

The possibility of a role for the adrenal cortex peculiar to and necessary for the development and maintenance of renovascular hypertension was first investigated by Goldblatt 30 years ago (3). His cautious conclusion, "even in the absence of adrenals, provided adequate substitution and supportive treatment is given, some dogs do develop a significant but not great elevation of blood pressure due to renal ischaemia," rules out perhaps only the least likely possibility for an adrenal role peculiar to renovascular hypertension—that of the elaboration of a novel pressor steroid under the stimulus of renal arterial constriction, a compound which cannot be formed from, and whose pressor effects cannot be mimicked by, the range of steroids provided by his extract of normal adrenals.

Subsequent investigators have published conflicting results of adrenalectomy in renovascular hypertensive animals. Collins and Wood (4) reported the persistence of hypertension in dogs with bilateral renal artery constriction, maintained after adrenalectomy without steroid support for up to 9 days. Blalock and Levy (5) claimed that the blood pressure of previously hypertensive dogs was reduced to normal 18 hours after adrenalectomy in all cases, and by 5 hours in those animals in which it was measured at that time. Other workers report findings basically in agreement, but draw quite opposite conclusions from them. Floyer (6) reported that adrenalectomy returned the blood pressure of a previously hypertensive rat to normal if the animal had access to water or very dilute NaCl solutions; restoration of blood pressure to the hypertensive level followed access to 0.9% NaCl solutions. Fregly (7) found no difference in blood pressure levels between two groups of rats, of which one was adrenalectomized, but both made hypertensive by renal encapsulation and allowed access to water and 0.9% NaCl ad libitum. Floyer concluded that the adrenal cortex is necessary for the maintenance of hypertension, since the latter was abolished by adrenalectomy, but that the adrenal effect is a function of its sodium handling capacity, since it can be mimicked by a high sodium intake. Fregly concluded that the adrenal cortex has no particular role in the hypertensive process, since its removal is followed by no change in blood pressure levels in animals fed ad libitum.

More recently, Atwill et al. (8) claimed that adrenalectomy of renal hypertensive dogs and maintenance on hydrocortisone and 9-fluorohydrocortisone (in amounts adequate for normal serum electrolytes, total blood volume and general condition) results in normotension. The authors suggest that adrenal steroid(s) (very possibly aldosterone) other than cortisol or its analogues are necessary for the development and maintenance of renovascular hypertension.

The possibility that adrenal steroids have a long-term role in the maintenance of renovascular hypertension cannot be excluded by the experiments reported in this paper. Whether protracted steroid underdosage plus supplementary Na+ might sustain life but not hypertension, or whether these animals would have shown greater blood pressure

FIGURE 2

Blood pressure, plasma [Na+] and [K+], and Na+ deficit after steroid withdrawal. For each sheep upper and lower hatched areas represent blood pressure ranges after and before renal artery constriction. Experiment 1: Both steroids withdrawn; no replacement of urinary Na+ losses; plasma renin concentration measured in sheep 3. Sheep 1 died at the end of the 48-hour period. Experiment 2: Both steroids withdrawn; attempted replacement of urinary Na+ losses with 0.9% NaCl; plasma renin concentration measured in sheep 4. Experiment 3: Desoxycorticosterone alone withdrawn; no replacement of urinary Na+ losses; plasma renin concentration measured in sheep 3. Sheep 4 successfully resuscitated after cardiac arrest. Experiment 4: Cortisol alone withdrawn; no urinary Na+ losses; plasma renin concentration measured in sheep 4.

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elevations for the same degree of clipping had their adrenals been intact, are questions outside the ambit of the design of this study. However, from the data presented above a number of assertions can be made:

(1). Renovascular hypertension can be elicited in the absence of adrenals provided basal substitution steroid therapy is given.

(2). In view of the known circulating half life of intravenously administered steroids in sheep (9), these animals were without significant levels of circulating steroids for periods of 24 to 48 hours after withdrawal.

(3). When mineralocorticoid was withdrawn, a state of adrenoprival crisis rapidly ensued, as judged by the normal indices of gross changes in plasma \([\text{Na}^+]\) and \([\text{K}^+]\) and profound natriuresis.

(4). Even in the face of such indications of the absence of systemic steroid activity, the blood pressure remained consistently elevated beyond normal limits.

(5). No close correlation seems to exist between blood pressure after withdrawal and changes in \([\text{Na}^+]/[\text{K}^+]\) balance or plasma renin concentration.

One formal possibility consistent with our data is that the adrenal steroids, when present, are responsible for the elevated blood pressure, but after their withdrawal some other mechanism takes over. This seems unlikely in view of the known hypotension accompanying the Addisonian state. In experiment 3, where mineralocorticoid alone was withdrawn, a modest rise in plasma renin was associated with a natriuresis of 600 mEq; in experiments 1, 2, and 4 the plasma renin remained within the normal range throughout the course of the hypertension after withdrawal. Therefore, it seems highly probable that the renin-angiotensin system does not "take over" the maintenance of the elevated blood pressure. The persistently low plasma renin in experiment 1 was a surprising finding in the context of a 500-mEq natriuresis. Adrenalectomized sheep with a single normal kidney on an identical regime of steroid maintenance respond to withdrawal by an elevation of plasma renin concentration commensurate with the degree of their \([\text{Na}^+]\) deficit. This response seems essentially the same as that of sheep with intact adrenals rendered \([\text{Na}^+]\) deficient by uncompensated parotid salivary loss.

The acute (24 to 48 hours) normotensive effect of unclipping a clipped single kidney (10; unpublished observations) is evidence of a mechanism of hypertension that is not only reversible, but in addition rapidly reversible by a procedure such as unclipping. Acute interruption of an infusion of adrenocortical steroid to the adrenalectomized animal with a clipped single kidney does not produce such a normotensive effect. Accordingly, the mechanism of renovascular hypertension appears not to be directly mediated by the steroid secretion of the adrenal cortex.

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