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THE EFFECT OF SODIUM DEPRIVATION AND OF ANGIOTENSIN II INFUSION ON THE PERIPHERAL PLASMA CONCENTRATIONS OF 18-HYDROXYCORTICOSTERONE, ALDOSTERONE AND OTHER CORTICOSTEROIDS IN MAN

P. A. MASON, R. FRASER*, J. J. MORTON, P. F. SEMPLE AND A. WILSON
M.R.C. Blood Pressure Unit, Western Infirmary, Glasgow, G11 6NT, Scotland

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SUMMARY

The effect of angiotensin II infusion on plasma cortisol, 11-deoxycorticosterone, corticosterone, 18-hydroxycorticosterone and aldosterone concentrations was compared in 6 normal subjects before and after different degrees of sodium depletion. Cortisol, 11-deoxycorticosterone and corticosterone levels were unaffected by sodium depletion or by angiotensin II infusion in either replete or deplete states. Plasma 18-hydroxycorticosterone and aldosterone concentrations rose in parallel when sodium replete subjects were infused with angiotensin II. Sodium depletion had a proportionately greater stimulating effect on plasma 18-hydroxycorticosterone than on aldosterone, particularly in the more severely depleted subjects. The rise in plasma aldosterone concentration following angiotensin II infusion was greater in sodium deplete than in sodium replete subjects and this difference increased with the severity of sodium depletion. Basal 18-hydroxycorticosterone concentrations were much higher in the sodium deplete state than when the subjects were replete with sodium, but the subsequent effect of angiotensin II infusion, although further increasing 18-hydroxycorticosterone levels, was less consistent. The effect of angiotensin II was not secondary to a rise in ACTH since, in a seventh subject pretreated with dexamethasone, the response was not abolished. It is concluded that one effect of angiotensin II must occur at or before 18-hydroxylation in the biosynthetic pathway. The availability of a larger pool of aldosterone precursor as a result of sodium depletion may be an explanation of the phenomenon of sensitization of aldosterone production to angiotensin II infusion in this condition. The possibility of a second effect of angiotensin II on the conversion of 18-hydroxycorticosterone to aldosterone is also discussed.

INTRODUCTION

Plasma aldosterone concentration in man rises in response to sodium depletion [1, 2] and also when angiotensin II is infused [1]. The effect of sodium depletion may be mediated by the angiotensin II response, but a direct effect cannot be excluded [3]. The dose-response relationship between peptide and steroid hormone is steeper in sodium deplete than in sodium replete subjects [4, 5] and no satisfactory explanation of this apparent sensitization of the adrenal zona glomerulosa has yet been obtained. It has been suggested that exposure to high circulating levels of the peptide hormone for several days, such as occurs during sodium depletion by means of dietary restriction, may cause the zona glomerulosa to hypertrophy [6], but since a measure of sensitization also occurs in the absence of changes in plasma angiotensin II concentration in anephric subjects depleted of sodium by dialysis [7], this cannot be the whole explanation.

It is possible that the process of sensitization involves increased synthesis or availability of aldosterone precursor, an increase in the activity of a rate-limiting enzyme system or perhaps both of these. Known precursors of aldosterone are 11-deoxycorticosterone (DOC), corticosterone and 18-hydroxycorticosterone. While some previous studies of the effects on plasma DOC and corticosterone levels of sodium depletion [2, 8–10] and angiotensin II infusion [2, 8] have been reported, these have not taken the form of full dose-response studies and the interaction of the two stimuli has not been examined. Although the secretion rate of 18-hydroxycorticosterone is known to rise in response to sodium depletion in man [11] there is as yet no information on the effects of sodium loss and of angiotensin II infusion on plasma 18-hydroxycorticosterone concentration. This was the aim of the following study.

Using a new method for measuring plasma 18-hydroxycorticosterone levels [12, 13] the effects of sodium deprivation and angiotensin II infusion were compared with those on plasma aldosterone, its other precursors, DOC and corticosterone, and also on cortisol.

* To whom correspondence should be sent.

METHODS

Subjects and techniques

Experiments began between 08.00 and 09.00 hours and were carried out on 6 normal male volunteers aged between 24 and 39 years. They were fasting and recumbent throughout the experiments. Intravenous infusions of 1-Asp NH_2-5-Val-angiotensin II (Hypertensin, Ciba) in 5% dextrose were given at rates of 0, 2, 4 and 8 ng/Kg/min via an indwelling catheter into an arm vein, each rate being continued for one hour as previously described [4]. Blood samples were taken from a vein in the contralateral arm immediately before the end of each infusion period, also from an indwelling catheter. Two samples were taken during the control period. Particular care was taken to ensure that samples for angiotensin II analysis were taken rapidly without stasis into a syringe containing enzyme inhibitor solution. Other samples were taken into heparin. Forearm muscle contraction was avoided because of its effect on plasma potassium concentration [14]. Plasma angiotensin II concentration was measured by radioimmunoassay [15], sodium and potassium by flame photometry and individual corticosteroids by gas–liquid chromatography with electron capture detection [12, 13, 16, 17]. Twenty four h urine collections were made throughout the experiments.

Experimental protocol

For 3 days the subjects received a controlled, constant intake of calories, sodium and potassium adjusted to their individual tastes. Daily sodium intake varied from 145 to 200 mEq and potassium from 85 to 108 mEq. The first of two graded series of angiotensin II infusion was given on the morning of the fourth day. Then for a period of 4 days the subjects were depleted of sodium to a variable extent (see below) while keeping calorie and potassium intake as close to control levels as possible. The graded angiotensin II infusion was then repeated. The subjects were divided into three categories:

(a) Subjects 1–3 were subjected to 4 days of reduced sodium intake (13–19 mEq/day). (b) Subject 4, in addition to reduced sodium intake (19 mEq/day), took frequent physical exercise to increase sodium loss in perspiration. (c) Subjects 5 and 6 were given frusemide (40 mg iv) on the first day of the low sodium period to accelerate loss of sodium and were then restricted to a low sodium diet (13 and 11 mEq/day).

Calculated potassium intake was slightly lower during low sodium intake (85 ± 9 mEq/day) than during normal sodium intake (93 ± 10 mEq/day), but this difference was not significant ($P > 0.05$).

Finally, one female subject (subject 7) was treated as described in (c), but both graded angiotensin II infusions were carried out during acute suppression of ACTH secretion by dexamethasone administration (2 mg 10 h before and 2 mg 2 h before the start of the infusion).

RESULTS

Changes in electrolyte balance and plasma electrolyte concentrations

Cumulative sodium balances during the sodium depleting phase of the experiment are listed for individual subjects in Table 1. That for subject 4 is probably an underestimate because no account was taken of sodium loss in the perspiration. Variation of the experimental protocol produced a fairly wide spectrum of sodium loss. There were no significant differences in plasma electrolyte concentrations between the two phases of the experiment (replete subjects: Na 139.2 ± 2.6 (S.D.) mEq l^{-1}; K 4.2 ± 0.2 mEq l^{-1}, deplete subjects: Na 140.7 ± 4.4 mEq l^{-1}; K 4.4 ± 0.5 mEq l^{-1}). Cumulative potassium balance was positive but similar in the sodium replete and deplete

Table 1. Effect of sodium depletion on the plasma concentrations of angiotensin II, aldosterone and 18-hydroxycorticosterone and on the plasma 18-hydroxycorticosterone: aldosterone ratio (values are the mean of two samples)

Subject	Cumulative Na Balance, mEq	Angiotensin II pg ml^{-1}		Aldosterone ng 100 ml^{-1}		18-Hydroxycorticosterone ng 100 ml^{-1}		18-Hydroxycorticosterone: aldosterone	
		+Na	−Na	+Na	−Na	+Na	−Na	+Na	−Na
1	−156	15.5	45.0	8.7	12.7	3.8	16.5	0.44	1.30
2	−175	35.0	34.0	5.8	7.3	12.0	34.6	2.07	4.74
3	−180	20.0	30.0	7.1	17.8	7.5	16.7	1.06	0.94
4	−180	10.0	22.5	8.5	17.1	10.5	32.8	1.24	1.92
5	−220	30.0	54.0	7.4	22.3	14.1	95.2	1.91	4.27
6	−221	24.0	39.0	5.0	23.5	13.0	114.0	2.60	4.85
7	−118	27.5	60.0	3.9	25.4	6.7	54.4	1.72	2.14
Mean		23.1	40.6	6.6	18.0	9.7	52.0	1.58	2.88
S.D.		8.6	13.3	1.8	6.4	3.8	38.5	0.72	1.68
t		3.91		4.09		3.13		3.13	
$P <$		0.01		0.01		0.02		0.02	

states (replete 65 ± 48 mEq, deplete 71 ± 58 mEq, $P > 0.05$). The positive balance was probably due to consistent overestimation of dietary potassium content and to failure to take stool electrolytes into account.

Urine vol. on the final day of the regime (mean vol./24 h ± S.D.) in the sodium replete subjects was 1095 ± 709 ml. and 1029 ± 791 ml. when the subjects were deprived of sodium. Neither corticosteroid concentrations nor angiotensin II concentrations in plasma before commencing angiotensin II infusion (i.e. basal values) correlated with the urine vol.

Changes in plasma cortisol, corticosterone and DOC concentrations

Concentrations of cortisol, corticosterone and DOC were not significantly affected (paired t, $P > 0.05$) by sodium loss. For replete and deplete states respectively the mean concentrations (± S.D.) were as follows: DOC 11.3 ± 5.9 and 16.2 ± 4.7 ng 100 ml^{-1}, corticosterone 0.64 ± 0.23 and 0.50 ± 0.37 μg 100 ml^{-1} and cortisol 6.8 ± 3.0 and 5.8 ± 1.1 μg 100 ml^{-1}. There was no significant correlation between the concentrations of these corticosteroids and the concurrent plasma angiotensin II concentration.

The effect of sodium deprivation on plasma aldosterone and 18-hydroxycorticosterone concentrations (Table 1)

Plasma aldosterone concentration rose in all cases after sodium deprivation although the response in subject 2 was small. The increase in plasma 18-hydroxycorticosterone concentration also occurred in all subjects but was most marked in the more severely depleted subjects 5 and 6. Plasma angiotensin II concentration increased during sodium depletion. The ratio of 18-hydroxycorticosterone to aldosterone in plasma rose in all but one subject. For the group, this change was statistically significant.

The effect of angiotensin II on plasma aldosterone and 18-hydroxycorticosterone concentrations (Fig. 1)

Angiotensin II infusion raised the concentration of plasma aldosterone in all subjects, confirming previous experience [1, 4, 5]. However, plasma 18-hydroxycorticosterone also rose during this treatment, a fact not previously demonstrated. In all subjects, plasma aldosterone and 18-hydroxycorticosterone concentrations were significantly correlated with concurrent plasma angiotensin II concentrations (Table 2). Some of the data for sodium replete subjects have already been published [18].

Effect of sodium deprivation on the response of aldosterone and 18-hydroxycorticosterone to angiotensin II (Fig. 1, Tables 2 and 3)

The angiotensin II-aldosterone dose-response regressions were steeper in the sodium deplete than in the replete state in all cases, but this increase did not achieve statistical significance in subjects 1 and 3 (Table 2). When the slopes of the dose-response curves in sodium depleted subjects were compared

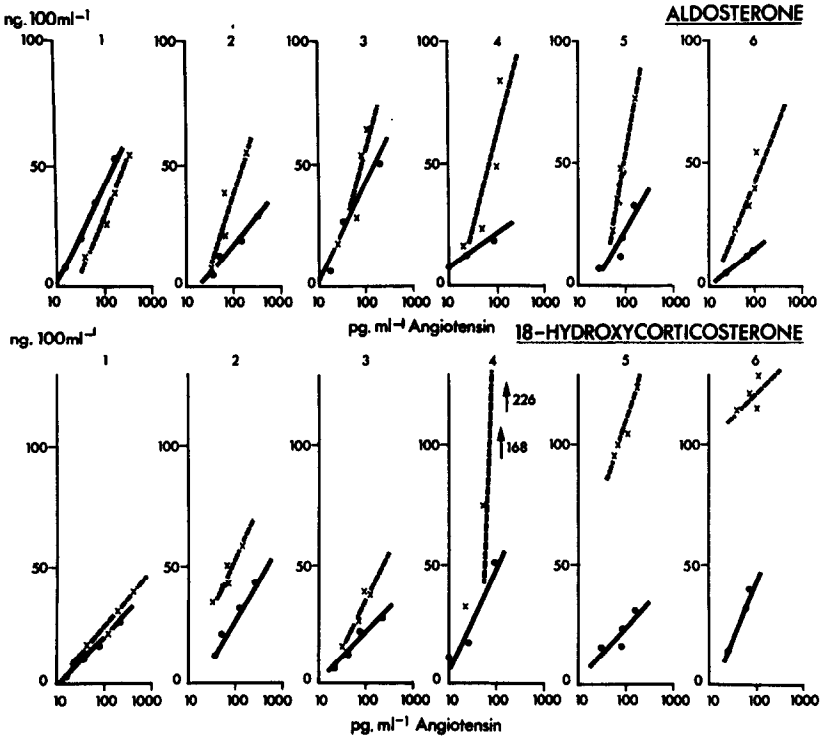

Fig. 1. Dose-response relationships between angiotensin II and aldosterone or 18-hydroxycorticosterone in sodium replete (●——●) and deplete (×----×) subjects. The numbers 1–6 refer to individual subjects (see text). The lines are regressions.

Table 2. Effect of sodium status on the dose-response relationship of angiotensin II and aldosterone or 18-hydroxycorticosterone (18OHB)

Subject	Steroid	Sodium replete			Sodium deplete			Comparison‡ of slope (*P*)
		*a**	*b*	*r*†	*a*	*b*	*r*	
1	Aldosterone	39	−37	0.997	51	−75	0.955	>0.05
2	Aldosterone	23	−28	0.983	61	−84	0.931	0.05
3	Aldosterone	40	−41	0.972	74	−96	0.931	>0.05
4	Aldosterone	11	−2	1.000	72	−89	0.877	<0.05
5	Aldosterone	33	−43	0.900	105	−159	0.999	<0.05
6	Aldosterone	15	−15	0.998	46	−52	0.985	<0.05
7	Aldosterone	31	−37	0.937	134	−194	0.867	>0.05
1	18OHB	21	−21	0.999	28	−33	0.917	>0.05
2	18OHB	20	−18	0.982	37	−39	0.949	>0.05
3	18OHB	34	−38	0.987	44	−33	0.980	>0.05
4	18OHB	45	−38	0.970	228	−293	0.957	<0.05
5	18OHB	21	−18	0.891	58	−6	0.990	<0.05
6	18OHB	52	−59	0.994	19	83	0.812	>0.05
7	18OHB	24	−25	0.942	120	−149	0.803	>0.05

* $y = ax + b$ where y = steroid concentration, x = log angiotensin II concentration, †: Correlation coefficient. ‡: *F* test.

Table 3. Effect of sodium status and angiotensin II infusion on the ratio of 18-hydroxycorticosterone and aldosterone concentrations in plasma

Infusion rate ng Kg^{-1} min^{-1}	+Na		−Na		Comparison	
	Mean	SD	Mean	SD	*t	*p*
0	1.58	0.72	2.88	1.68	3.13	<0.02
2	1.35	0.88	2.09	1.17	3.27	<0.02
4	1.39	0.92	1.83	1.07	1.47	>0.05
8	1.30	0.82	1.48	0.80	1.43	>0.05

* Paired *t*.

with each other, the increase in gradient was related to the cumulative negative sodium balance with the exception of subject 6 and also the dexamethasone treated subject 7.

The slopes of the dose-response curves for angiotensin II and 18-hydroxycorticosterone also steepened following sodium deprivation but the difference was generally less marked, achieving significance only in subjects 4, 5 and 7 (see also Fig. 2). While plasma aldosterone and 18-hydroxycorticosterone concentrations correlated poorly during angiotensin infusion in the sodium replete state ($r = 0.30$, $P > 0.05$), in the deplete state correlation was highly significant ($r = 0.56$, $P < 0.01$). Sodium depletion increased the ratio of 18-hydroxycorticosterone to aldosterone in plasma (Table 2). While angiotensin II infusion did not affect this ratio in replete subjects, in the deplete subjects it had the effect of reducing the ratio to sodium replete levels (Table 3).

The effect of dexamethasone on the corticosteroid response to sodium depletion and angiotensin II infusion (Fig. 2)

Subject 7 had a negative cumulative sodium balance of 118 mEq during the four days of sodium depletion while potassium balance was +26 mEq compared with −2 mEq during the period of normal sodium intake. Plasma electrolyte concentrations were not altered by sodium restriction but plasma angiotensin II levels increased (Table 1). From Fig. 2

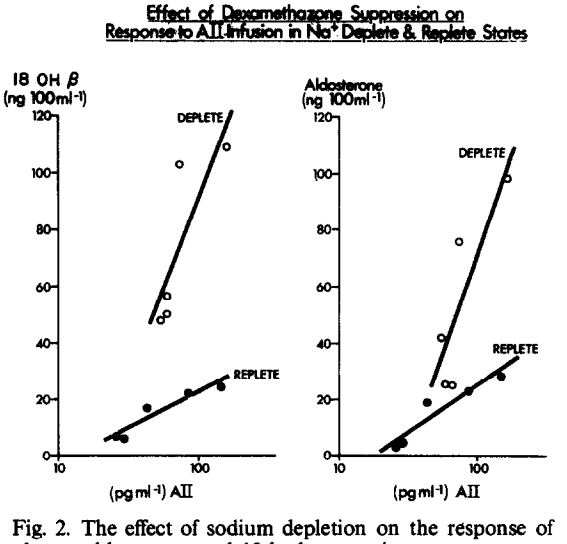

Fig. 2. The effect of sodium depletion on the response of plasma aldosterone and 18-hydroxycorticosterone concentration to angiotensin II infusion in a subject treated with dexamethasone (subject 7).

it is apparent that dexamethasone did not prevent the response of either plasma aldosterone or plasma 18-hydroxycorticosterone to angiotensin II infusion in either sodium replete or deplete states, nor was the response to sodium restriction itself affected. Plasma cortisol concentration was low throughout the experiments (1.9 ± 0.6 S.D. $\mu g\ 100\,ml^{-1}$).

DISCUSSION

Studies of changes in the relative concentrations of individual corticosteroids in plasma may give clues to altered patterns of biosynthesis within the human adrenal cortex while avoiding significant trauma. This study involved graded changes in sodium status and in plasma angiotensin II concentration, other factors being kept constant. Absence of variation in plasma cortisol concentration probably indicates that ACTH secretion was not markedly increased during the experiments. Potassium balance and plasma concentration were also not significantly different between the two phases of the experiment.

The effect of angiotensin II infusion and sodium deprivation on plasma DOC and corticosterone concentrations

The rise in plasma aldosterone concentration following angiotensin II or sodium depletion might possibly occur as a result of stimulation at an early stage in biosynthesis. DOC and corticosterone are precursors of aldosterone in the zona glomerulosa but are also synthesized in the zona fasciculata where no aldosterone is produced. The proportion of the total DOC [19] and probably also of corticosterone produced in the zona glomerulosa is small and any changes in the contribution of this component of total plasma steroid concentration may be difficult to discern. The lack of response of plasma DOC and corticosterone levels to sodium deprivation and to angiotensin II infusion is not therefore conclusive evidence that these stimuli do not affect the zona glomerulosa rate of synthesis of these compounds.

Previous studies [8–10] have failed to show changes in DOC levels following sodium depletion unless ACTH secretion is first suppressed. Very small increases can then be detected [19]. Similarly dietary sodium restriction does not raise plasma corticosterone concentration in normal subjects [20] although severe sodium depletion does [21]. However, corticosterone is more efficiently converted to aldosterone during sodium deprivation in animals [22, 23] and the efficiency is reduced if the animals are treated with ACTH [24]. Plasma corticosterone, cortisol and DOC concentrations in this current study were not measurably altered by angiotensin II infusion. This is in agreement with previous studies [1, 8] even when the infusion was given following suppression of ACTH secretion [20]. However, the dog [25] and isolated preparations of rat adrenal cells [26] may differ from man in this respect.

The effect of sodium depletion on plasma 18-hydroxycorticosterone and aldosterone concentrations

If the assumption is made that 18-hydroxycorticosterone is the main precursor of aldosterone, measurement of the changes in plasma 18-hydroxycorticosterone concentration to sodium depletion and to angiotensin II infusion (see below) and comparison of these with concurrent changes in plasma aldosterone concentration may indicate whether these stimuli affect the biosynthesis of aldosterone before, at or after hydroxylation of the 18-methyl group.

The stimulating effect of sodium loss on aldosterone secretion and plasma concentration is widely accepted but its influence on 18-hydroxycorticosterone plasma levels has not been studied in man, although secretion rate was shown to increase in proportion to that of aldosterone [11]. The range of plasma concentration reported here and previously [12, 13, 18] is similar to that of aldosterone and increases following sodium depletion, the increment usually being greater than that in aldosterone level particularly in the more severely depleted subjects. Thus the overall effect of sodium depletion was to progressively increase the ratio of 18-hydroxycorticosterone to aldosterone in the peripheral plasma. As can be seen from subject 7, the increased ratio was probably not due to a disparate effect of ACTH on 18-hydroxycorticosterone since dexamethasone failed to prevent the response to sodium depletion or to angiotensin II infusion (see below).

The effect of sodium depletion on the response of aldosterone and 18-hydroxycorticosterone to angiotensin II infusion

Some evidence of the mechanism of sensitization of aldosterone secretion to angiotensin II by sodium depletion may be obtained by comparing its dose-response curves in the sodium replete and deplete states with those of its probable immediate precursor, 18-hydroxycorticosterone.

In sodium replete subjects, angiotensin II infusion causes plasma aldosterone concentration to rise in proportion to the rise in the level of plasma angiotensin II [4, 5]. Parallel rises in plasma 18-hydroxycorticosterone concentrations also occur (see also 18). The relative amounts of the two corticosteroids in plasma are therefore unchanged. Sodium deprivation increases the steepness of the angiotensin-aldosterone dose-response curve [4–6] and, from the current experiments, this apparent sensitization may increase with increasing sodium depletion, emphasising the importance of the interaction between the two controlling factors. The effect of sodium depletion on the response of plasma 18-hydroxycorticosterone concentration to angiotensin II infusion is more complex. Mild dietary deprivation (subjects 1–3) caused only small increases in the slope of the dose-response relationship and, although basal levels were markedly raised, little sensitization occurred in subjects 5 and

6 who were more severely depleted. Only in subjects 4 and 7 was there any evidence of sensitization to angiotensin II by sodium depletion. This may indicate either that 18-hydroxycorticosterone is more sensitive to circulating angiotensin II, reaching near-maximum levels at a peptide concentration when aldosterone secretion is by no means maximal or that its secretion is affected by some aspect of electrolyte status unrelated to angiotensin II. The demonstration that 18-hydroxycorticosterone: aldosterone ratios fall in these subjects when angiotensin II is infused may also be important (see below).

Possible implications of changes in plasma aldosterone and 18-hydroxycorticosterone concentrations

If changes in peripheral plasma concentration can be equated with changes in biosynthetic activity within the adrenal cortex, the following tentative conclusions can be drawn. In sodium replete subjects, angiotensin II increases 18-hydroxycorticosterone and aldosterone production in parallel. At least one locus of action of the polypeptide hormone must therefore occur at or before 18-hydroxylation, as has been concluded from *in vitro* studies [27], and the increase in aldosterone production could be explained by the rise in concentration of its immediate precursor, as has been discussed previously [18].

Sodium depletion has a proportionally greater effect on 18-hydroxycorticosterone than on aldosterone production. This, together with the angiotensin II-induced rise of 18-hydroxycorticosterone levels in sodium replete subjects, may indicate that conversion to aldosterone is rate limiting. The rise in 18-hydroxycorticosterone levels during sodium depletion may provide the larger precursor pool required for the enhanced response of aldosterone production to angiotensin II. The fall in the ratio of 18-hydroxycorticosterone to aldosterone during angiotensin infusion to levels found in replete subjects may indicate that angiotensin II has an additional role to play in the conversion of the 18-hydroxy steroid to aldosterone. It would appear that the earlier of these angiotensin II effects is (i) more sensitive to angiotensin II and (ii) more affected by changes in sodium status. The sodium depletion effect may be direct or mediated by angiotensin II or small reciprocal changes in potassium status. Other factors such as ACTH must eventually also be taken into account, although in these experiments acute suppression of ACTH failed to alter steroid responses significantly.

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