Effect of Variations of Plasma Sodium Concentration on the Adrenal Response to Angiotensin II


The aldosterone-stimulating action of systemic infusion of angiotensin II in a number of species has been reported.1-5 Further, it has been demonstrated that an angiotensin II infusion into the adrenal arterial blood supply promotes aldosterone secretion.6-9

In 1959, Denton et al.10 demonstrated that a decrease of sodium and increase of potassium in the adrenal arterial blood of a sodium-replete sheep with a cervical adrenal autotransplant11,12 caused an alteration in the composition of parotid saliva which indicated an increased secretion of aldosterone. Subsequently, assay of adrenal venous plasma by a double isotope dilution derivative method13,14 demonstrated a large increase in aldosterone output during such an infusion.7 Recent studies, in which the plasma sodium and potassium concentrations were altered independently, have shown that either a decrease of sodium concentration by 5 to 20 meq/liter or an increase of potassium concentration by 0.5 to 2.0 meq/liter in adrenal arterial plasma increased the secretion of aldosterone.5 Davis et al.15 have confirmed these findings in experiments with the isolated adrenal preparation in the dog.

During the onset of sodium depletion in the sheep, the plasma concentrations of sodium and potassium remain within the normal range until the sodium deficit exceeds approximately 200 meq.5 As the sodium deficit increases beyond this point the plasma sodium concentration falls and the potassium concentration rises to levels which would be sufficient to contribute to stimulation of aldosterone by direct action on the adrenal cortex. If the sodium concentration of adrenal arterial plasma is increased 5 to 20 meq/liter by infusion of concentrated NaCl into the adrenal arterial blood supply of sodium-depleted sheep the aldosterone secretion rate is reduced, though the effect may only be transient over 30 to 60 min.10,16 It appears that a high concentration of sodium in adrenal arterial plasma antagonizes the action of aldosterone stimulating factors by a local action on the adrenal cortex.

The present studies were done to examine the effect of increased plasma sodium concentration on the aldosterone hypersecretion during adrenal arterial infusion of angiotensin II.

Methods

Experiments were performed on six trained Merino sheep with cervical adrenal gland autotransplants.11,12 Sheep with a parotid gland fistula17 were given sufficient NaHCO3 solution via a tube passed into the rumen before the experiment to ensure that each animal was sodium-replete.

All infusions were made directly into the arterial supply to the transplanted adrenal gland. The infusion method has been described in detail previously.5,10 The adrenal blood flow in the

*And unpublished observations also.

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sheep studied was 10 to 20 ml/min. Under these circumstances a high concentration of infused substances can be achieved in the adrenal vasculature without significant effect in the systemic circulation when the adrenal venous effluent is diluted in the cardiac output.

During the control period isotonic NaCl (150 meq/liter) was infused into the adrenal arterial blood supply. Then the infusion was changed to valine-5-angiotensin II amide (Ciba) with isotonic saline for approximately 20 minutes and then changed to angiotensin II with concentrated NaCl (500 to 1000 meq/liter) for a further 20 minutes. After an interval of one to two hours the procedure was repeated but the order of the second and third steps of the infusion procedure was reversed.

Angiotensin II was infused at a constant rate during each experiment. The rate of infusion was between 0.25 and 0.50 μg/hr and adjusted in accordance with the measured adrenal blood flow to achieve approximately equal concentration of angiotensin II in the adrenal arterial blood in all experiments. The infusion of concentrated NaCl was adjusted so that the sodium concentration of the adrenal arterial plasma was increased by approximately 10 meq/liter.

The method of collection of adrenal venous blood from sheep with cervical adrenal transplants has been described previously. Samples were drawn during the control period and approximately 20 minutes after the start of each of the phases of angiotensin II infusion. The specimens were centrifuged and the plasma analysed for aldosterone, cortisol, and corticosterone by a double isotope dilution derivative procedure. Sodium and potassium concentrations in saliva and plasma were determined with a Technicon autoanalyzer.

**Results**

The method and the results of two experiments are shown in figure 1. In the sheep designated Cyrano, angiotensin II was infused at 0.25 μg/hr. In the first stage angiotensin with physiological saline increased aldosterone secretion from 0.8 to 3.2 μg/hr. Twenty minutes after changing the infusion to angiotensin with 500 meq/liter NaCl, the aldosterone output was 1.8 μg/hr, and the plasma sodium concentration was increased by 7 meq/liter without significant change in potassium con-
centration. With the reversed order in the second stage, infusion of angiotensin with 500 meq/liter NaCl increased the aldosterone output from 1.1 to 2.8 μg/hr and the plasma sodium by 6 meq/liter. After 20 minutes' infusion of angiotensin in physiological saline the secretion rate of aldosterone was 3.2 meq/liter. Infusion of angiotensin in physiological saline at 0.50 μg/hr caused the aldosterone secretion rate to reach 5.0 μg/hr.

The initial control cortisol secretion was 800 μg/hr, but during the experiment cortisol output fell to low levels. Both cortisol and corticosterone secretion rates were unaffected by infusions of angiotensin at 0.25 μg/hr.

Results in the animal designated TP 9 (fig. 1) were essentially similar. Angiotensin was infused at 0.33 μg/hr with physiological saline or with 500 meq/liter NaCl in the order described for Cyrano. Angiotensin II with physiological saline increased the secretion rate of aldosterone to 7.6 and 9.1 μg/hr. When the infusion was given at the same rate with 500 meq/liter NaCl, which increased plasma sodium concentration by 12 meq/liter, the aldosterone output was 2.0 and 3.9 μg/hr. Secretion rates of cortisol and corticosterone were not affected in a regular manner.

The aldosterone secretion rates in the six experiments are grouped in figure 2. The effect of angiotensin with physiological saline and no change of plasma sodium concentration exceeded the effect of angiotensin with hypertonic saline and plasma sodium increases of 7 to 13 meq/liter, in 11 cases out of 12, although the differences were not always large. On simple 50:50 chance considerations the probability of this being due to chance alone is 12/212 = 0.00293. The mean outputs of aldosterone during angiotensin infusion under isotonic and hypertonic conditions are shown on the right-hand side of figure 2. To some extent the difference between means is influenced unduly by the large differences in the experiments on TP 9 and Figaro. However, a treatment of the results by paired t-test shows that the differential is highly significant (P < 0.01). The results were treated also in separate groups of six. The differential for each group is significant at the 0.05 level when the reduction associated with high sodium is treated as a percentage of the aldosterone output during angiotensin infusion with isotonic saline.

There is considerable variability in the dimensions of aldosterone secretion caused by

![Graph showing aldosterone secretion rates](https://example.com/graph.png)

**FIGURE 2**

Rate of aldosterone secretion in six sodium-replete conscious sheep with adrenal transplants. Upper section: a) under control conditions, b) when angiotensin II was infused concurrently with isotonic saline, c) when angiotensin II was infused concurrently with hypertonic saline. Lower section: order of saline infusion was reversed.

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infusions of angiotensin II which were intended to produce similar concentrations of angiotensin in adrenal arterial blood. Part of this difference is probably due to differences in the plasma sodium concentration under control conditions. The largest effect of angiotensin was observed in sheep TP 9. This animal had the lowest initial plasma sodium, 140 meq/liter. The smallest effect of angiotensin infusion occurred in the TP 12 experiments and on this day the plasma sodium was 149 meq/liter, which was the highest in the group.

It has been demonstrated that during prolonged infusion of angiotensin II, prepared in isotonic saline, into sodium-replete sheep, there is typically an initial rise in aldosterone secretion and then a large decline. Such a decay during the infusion period of the present experiments might have made interpretation difficult. Therefore, the order of infusion of angiotensin in isotonic or hypertonic saline was reversed in the second run. The alteration of sequence of infusion did not alter the result. Furthermore, in each experiment, there was close quantitative agreement between the aldosterone outputs during angiotensin II infusion under isotonic conditions, irrespective of whether the angiotensin infusion was started under isotonic or hypertonic conditions. The dimensions of the aldosterone response to angiotensin when the plasma sodium concentration was elevated was not influenced by the order of infusion. The results suggest that there was no significant time effect on aldosterone secretion during angiotensin infusion for a period of 40 to 50 minutes and this finding is in agreement with previous results. The results indicate also that 20 minutes of infusion with isotonic saline was sufficient to abolish the decrease in sensitivity to angiotensin caused by increased sodium concentration in adrenal arterial blood.

The consistent effect of angiotensin II infusion on aldosterone secretion was not associated with increased secretion of cortisol or corticosterone (figs. 3 and 4). Cortisol secretion rates (fig. 3) were not altered in a regular manner through the infusion of isotonic NaCl, angiotensin II with isotonic NaCl and angiotensin II with hypertonic NaCl. The lower section of figure 3 shows the results of the second phase of each experiment. Some cortisol outputs which were initially high were lower in this run, probably because the animals were inadvertently disturbed in the initial stages of the experiment. Corticosterone secretion rates (fig. 4) followed the same pattern as cortisol. There was no evidence of a significant increase in corticosterone secretion during angiotensin infusion.

![Graph of cortisol secretion rates](attachment://Cortisol_Secretion_Rates.png)

**FIGURE 3**

Rate of cortisol secretion in six sodium-replete conscious sheep in the same experiments described in figure 2.
Discussion

The potent aldosterone-stimulating action of angiotensin \(^{1-9}\) has suggested an important role for the renin-angiotensin system in the regulation of aldosterone secretion. It has been demonstrated that angiotensin \(\text{II}\) stimulates aldosterone secretion at concentrations as low as \(0.3 \mu g/\text{liter}\) of plasma in the sheep and that higher concentrations, \(0.8 \mu g/\text{liter}\), evoke rates of secretion of aldosterone equal to those observed in moderate sodium depletion. \(^5\) Since increase of adrenal arterial plasma sodium concentration causes a reduction, usually evanescent, in the secretion of aldosterone in sodium depleted sheep, \(^10\,10^*\) a similar antagonism between plasma sodium concentration and angiotensin \(\text{II}\) concentration would be in agreement with the thesis that angiotensin \(\text{II}\) is the aldosterone-stimulating hormone.

The present experiments demonstrated that the dimensions of the aldosterone secretion evoked by angiotensin \(\text{II}\) infusion were modified by the sodium concentration of the adrenal arterial blood. Raising the sodium concentration \(8\text{ to }13\text{ meq/liter}\) reduced the aldosterone hypersecretion evoked by angiotensin \(\text{II}\). Experiments to determine the effect of reducing plasma sodium concentration during angiotensin infusion have not been done yet. The largest aldosterone response to angiotensin was seen in the animal with the lowest control plasma sodium; the smallest effect was observed in the animal with the highest plasma sodium concentration. The results suggest that the aldosterone response to angiotensin \(\text{II}\) may be inversely proportional to the plasma sodium concentration. This effect may have considerable importance with sodium deficits large enough to cause fall of plasma sodium concentration, if angiotensin is, in fact, the normal humoral stimulus which causes aldosterone secretion.

The failure of angiotensin \(\text{II}\) to cause increased secretion of cortisol and corticosterone when it was infused into the adrenal arterial supply agrees with findings of previous studies in conscious sheep. \(^5\,7\) By contrast, aldosterone secretion was increased in each experiment and by an average factor of four during angiotensin infusion in isotonic saline and by an average factor of two when angiotensin was infused with hypertonic saline. This specificity for aldosterone secretion conflicts with the conclusions of Slater et al. \(^{19}\) but their experimental conditions and results were not comparable with those reported here. In

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*And unpublished observations also.

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![Graph](image-url)
The present study angiotensin II was infused at 0.25 to 0.5 µg/hr into the adrenal arterial blood supply of conscious trained sheep with a steady base line for aldosterone. In the experiments of Slater et al., the dogs were anaesthetized, hypophysectomized, and nephrectomized, and showed a variable control level for each dog. The possibility of species difference between dog and sheep in relation to cortisol and corticosterone response to angiotensin cannot, however, be excluded.

The secretion rates of cortisol and corticosterone in all experiments were low by comparison with the outputs observed when ACTH is infused at rates sufficient to stimulate aldosterone secretion in the sheep. In most experiments the glucocorticoid outputs were in the normal range for conscious trained sheep with adrenal transplants. With the sheep Cyrano, which had the highest control secretion rate of cortisol, the simultaneous aldosterone output was 0.8 µg/hr. This value is not high by comparison with the control aldosterone secretion rates in the other animals. The evidence is against changes of ACTH release initiating or modifying the aldosterone secretion rate in these experiments.

These experiments do not indicate the mechanism by which local change of sodium concentration alters the adrenal response to angiotensin II. The major possibilities appear to be that the shift in extracellular sodium concentration may affect reactions at the cell membrane, e.g., permeability to angiotensin II, or at the intracellular level. With respect to the latter possibility the changes in aldosterone secretion occurred within 20 minutes after increasing the plasma sodium concentration or restoring it to normal level. This period is long relative to the half-time of equilibration of radio-sodium across the membrane of smooth muscle and skeletal muscle but in the brain it is a matter of hours. The possibility has to be considered also that the effect of high sodium concentration was determined by increase of osmotic pressure of adrenal blood. This was not investigated in this series of experiments. However, the aldosterone-stimulating effect of an isosmotic reduction in the sodium concentration of adrenal arterial plasma in sodium-replete sheep suggests that ambient sodium concentration has a direct effect on aldosterone production. The finding that change of sodium concentration during angiotensin infusion acts on aldosterone secretion only and not on glucocorticoid secretion suggests that the effect occurs at a late step in the chain of conversions leading to the synthesis of aldosterone.

Studies in man, sheep, and dog demonstrated that the pressor action of infused angiotensin II is directly related to the sodium status of the whole animal but it does not appear to be directly related to aldosterone secretion rate or the peripheral level of aldosterone. The rapid transcellular flux of sodium ion in smooth muscle, the fact that shifts of environmental sodium concentration cause at least transient changes in the contractile response of smooth muscle in in vitro systems and the observations of Friedman and Friedman, that peripheral vascular resistance varies directly with intravascular sodium concentration, suggest that a factor determining the vascular responsiveness to angiotensin II, when the sodium status is altered, may be parallel changes in the sodium content of vascular smooth muscle.

It is noteworthy that in the sodium-replete sheep the thresholds for pressor effect and aldosterone stimulation by angiotensin II infusion are approximately equal, 5 to 10 µg/hr. A wide separation of these thresholds is essential to explain hyperaldosteronism without hypertension in sodium depletion in terms of the renin-angiotensin thesis of aldosterone stimulation. The present experiments suggest that the aldosterone-stimulating action of angiotensin II varies inversely with the sodium concentration in the environment of the adrenal glomerulosa cells. Other studies show that the pressor action of infused angiotensin II varies directly with the balance of the body sodium. These apparently contrasting effects may help to explain the variability of vascular and adrenocortical response in various states with raised renin or angiotensin blood levels.
Summary

The secretion rate of aldosterone was increased by infusion of valine-5-angiotensin II into the adrenal arterial blood supply of sheep. The dimensions of the increase in aldosterone output were inversely related to the control plasma concentration. This high aldosterone secretion was reduced substantially by concurrent adrenal infusion of concentrated NaCl solution which increased plasma sodium concentration by approximately 10 meq/liter. The reduction of aldosterone secretion occurred within 20 minutes. Angiotensin II infusion did not increase the secretion rates of cortisol or corticosterone.

The significance of the finding that environmental sodium concentration has different effects on the aldosterone-stimulating action and the pressor action of angiotensin II is discussed.

References


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