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STUDIES ON THE CONTROL OF ALDOSTERONE SECRETION IN MAN

V. H. T. JAMES, R. D. G. TUNBRIDGE and G. A. WILSON
Department of Chemical Pathology, St. Mary's Hospital Medical School, London, W2 1PG, England

SUMMARY

Plasma cortisol, plasma aldosterone and in some cases plasma renin activity (PRA) were measured in blood samples taken every 20 or 30 min overnight from recumbent sleeping subjects. In 9 control subjects peaks of aldosterone were matched by peaks of cortisol on all of 21 occasions and in 8 out of the 9 studies the plasma levels of the two steroids were significantly correlated. Of 11 peaks of PRA, only three were matched by aldosterone. Dexamethasone abolished cortisol secretion, but aldosterone secretion was unchanged. Long-term administration of propanolol or oxprenolol failed to suppress the episodic changes in PRA, and PRA levels were not suppressed, and neither was the episodic secretion of cortisol or aldosterone in two patients with high cervical spinal cord transection. PRA changes and aldosterone secretion continued in a hypophysectomised patient. In a patient studied after renal transplantation, changes occurred in PRA levels, but aldosterone secretion was low and fixed. It is concluded that cortisol secretion is mediated by ACTH, and that some other factor which is not the renin-angiotensin system, ACTH, sodium or potassium is responsible for mediating the episodic release of aldosterone and that it acts synchronously with, but independently of ACTH.

INTRODUCTION

Aldosterone is a major secretory product of the human adrenal cortex and is an essential factor in the control of sodium and potassium metabolism. In some circumstances, inappropriate secretion of aldosterone is clearly a causal factor in the genesis and maintenance of hypertension. Many investigators have also concluded that in a substantial number of hypertensive patients in whom no clear-cut cause for the elevated blood pressure can be demonstrated, some abnormality of aldosterone metabolism exists. A clear and detailed understanding of the factors involved in the control of aldosterone secretion is therefore important not only for a fuller knowledge of normal physiology, but also for a proper understanding of the pathology of hypertension.

A number of factors have been shown to be able to influence aldosterone secretion. These include the renin-angiotensin system, corticotrophin (ACTH) and changes in plasma levels of sodium and potassium. Although these are well-established stimuli to aldosterone secretion, and have been documented extensively both in man and other species, almost invariably the experimental conditions employed have been unphysiological, or at least, markedly abnormal. In consequence, the extent to which these factors are of significance in the day-to-day control of aldosterone secretion, either in normal subjects or in hypertensive patients, is not clear. More recently, attention has been turning towards this aspect of the problem, and the improvements in biochemical assay techniques has made such studies feasible.

In this investigation, we have examined the short-term changes in the plasma level of aldosterone, cortisol and in some cases, plasma renin activity in a variety of experimental conditions in an attempt to discover how far angiotensin and ACTH are the major regulators of basal aldsoterone secretion. To permit more rigorous control of the experimental situation, we have specifically examined the hormone changes which occur in sleeping recumbent subjects, using the technique of frequent sequential blood sampling.

METHODS AND SUBJECTS

The experimental subjects were healthy student volunteers, members of the medical staff or patients. In each case, informed consent for the study was obtained, and the subject was admitted to a metabolic ward on the evening before the study commenced. No attempt was made to control diet. Blood samples (10 ml) were taken at 20 or 30 min intervals through an indwelling catheter which was inserted at the beginning of the study into a forearm vein. The subjects remained recumbent in bed, usually until 8 a.m. Sampling could be carried out without apparently disturbing sleep. The plasma was separated immediately and stored at $-20°$ until analysis was performed.

Cortisol was measured by radioimmunoassay after extraction of the plasma with dichloromethane and evaporation of an aliquot of the extract. The precision (C.V) is 7%. Aldosterone was also measured by radioimmunoassay. In the initial studies, separation by paper chromatography was employed, but this was later omitted since it was found to be unnecessary. The methods for aldosterone have been described in detail elsewhere [1]. The precision of the method is 7%. Plasma renin activity was measured by the method of Boyd *et al.* [2]. The precision is 10%.

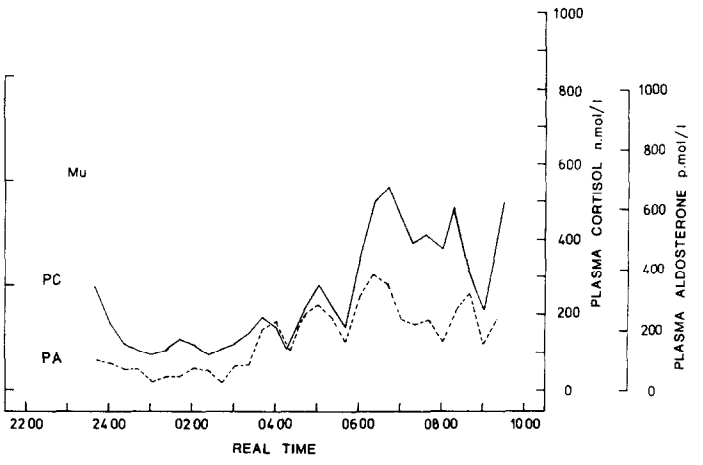

Fig. 1. Plasma aldosterone (PA) and plasma cortisol levels (PC) in control subject Mu.

Nine control subjects were studied, three women (Wh, St, Be) and six men (Do, Mu, Sc, Ro, La, Tu). None had any relevant abnormality and none were receiving any medication. Two control subjects were re-investigated after receiving a single oral dose of 2 mg of dexamethasone at 2200 on the evening of the study. Two other control subjects received propanolol for 2 weeks before the study in a total daily dose of 160 mg. Subject La received the drug twice daily and Tu, every 6 h. Patient Ha, a 43 year old male with essential hypertension, had been receiving 120 mg propanolol twice daily for one year. Subject Bui, age 34 years, was receiving oxypranolol, 80 mg three times daily, for 3 months for minimal anginal pain. Two patients, Ma and Bu, with traumatic cervical cord transection were studied at Stoke Mandeville Hospital. Both these subjects were males, aged 30 and 35 with complete tetraplegia and known interruption of their cervical sympathetic outflow. Patient Wa is a 57 year old female who had a transfrontal hypophysectomy in 1962 for removal of a chromophobe adenoma. She was receiving 37.5 mg of cortisol and 0.1 mg of thyroxine daily as replacement therapy.

Patient Ma is an 18 year old male, studied three weeks after receiving a renal transplant. He was receiving 75 mg of prednisolone and 150 mg of azathioprine daily.

RESULTS

Figures 1 and 2 are typical examples of the results obtained for the nine control subjects. In each case a number of episodes of cortisol secretion occurred, reaching maximum levels of plasma cortisol at some time between 0600 and 0800. Aldosterone levels also fluctuated sharply showing a pattern which visually, in many of the subjects, appeared to correlate well with the plasma cortisol changes. Thus, on every one of 21 occasions when secretory episodes of aldosterone occurred, there was a synchronous peak in plasma cortisol levels. However, cortisol peaks occurred on seven occasions without a concomitant increase in plasma aldosterone. A total of 11 peaks of plasma renin activity (PRA) occurred, of which only three were matched by peaks of aldosterone, whereas six occurred simultaneously with cortisol peaks.

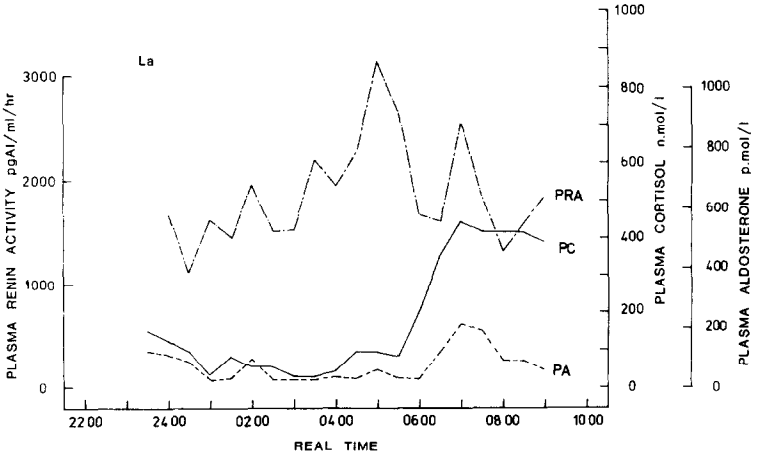

Fig. 2. Plasma aldosterone, plasma cortisol and PRA levels in control subject La.

Table 1. Correlation co-efficients between plasma aldosterone (PA), plasma cortisol (PC) and plasma renin activity (PRA)

Subject	PA:PC	PRA:PA	PRA:PC
Wh	NS		
St	0.70*		
Be	0.55*		
Do	0.53*		
Mu	0.79**		
Sc	0.88**		
Ro	0.82**	0.64*	0.88**
La	0.65*	NS	NS
Tu	0.87**	0.51*	0.72**
La$^+$	NS	0.46*	NS
Tu$^+$	0.80**	0.58*	0.59*
Ha$^+$	NS	NS	NS
Bui$^+$	0.55*		
Mc	0.66*	NS	NS
Bu	NS	NS	0.5*
Wa		NS	

Subjects receiving beta-blocking drugs.
* $P < 0.01$ ** $P < 0.001$ NS = not significant.

Table 1 gives the correlation co-efficients calculated for the correlations between plasma aldosterone, plasma cortisol and PRA respectively.

Figure 3 shows the results for one subject studied after receiving dexamethasone. The secretory episodes of cortisol secretion have been abolished but aldosterone continued to be secreted in a pulsatile fashion. The other subject showed similar results.

Four subjects were receiving a beta-blocking drug prior to the study and the results for one of them is shown in Fig. 4. Two of these were control subjects who had been studied before taking the drug and there was no clear-cut difference when pre-(Fig. 2) and post-(Fig. 4) treatment patterns were compared. Table 1 shows that in subject La, the good correlation between plasma cortisol and plasma aldosterone was lost after treatment, but remained in the other subject (Tu). In the other two subjects receiving a beta-blocking drug, the pattern of cortisol and aldosterone appeared normal. PRA was not suppressed and several marked secretory episodes appeared in all four subjects. PRA and plasma aldosterone were significantly correlated in La and Tu during drug administration, but several secretory episodes of PRA occurred which were not accompanied by an increase in plasma aldosterone levels.

Figures 5 and 6 show the results for the two patients with spinal cord transection. Both subjects showed a number of secretory episodes of PRA, and also peaks of plasma cortisol and aldosterone. Secretory episodes of PRA occurred in both subjects without any concomitant change in plasma aldosterone levels. In patient Mc, the pattern of plasma cortisol and aldosterone was much flatter than in the control subjects, and the single late rises of cortisol and aldosterone occurred together. In Bu, simultaneous peaks occurred at 0300, but after that, no clear association was seen.

Figure 7 shows the results in the patient studied three weeks after renal transplantation. PRA showed a marked increase starting at 0700 and reaching a peak at 0800, but no significant change in plasma aldosterone levels occurred.

Figure 8 shows the results for the hypophysectomised patient. Four secretory episodes of PRA occurred during the period of study, and three of these coincided with increases in the level of plasma aldosterone. Nevertheless, the PRA and aldosterone levels did not correlate significantly overall (Table 1).

DISCUSSION

In all the nine control subjects who were studied in this investigation, the nocturnal episodes of cortisol secretion which occurred were similar to those which have been described and well-documented by others [3, 4]. In addition, a series of secretory episodes of aldosterone were observed. A number of earlier studies, [5–8], including our own [9], have shown

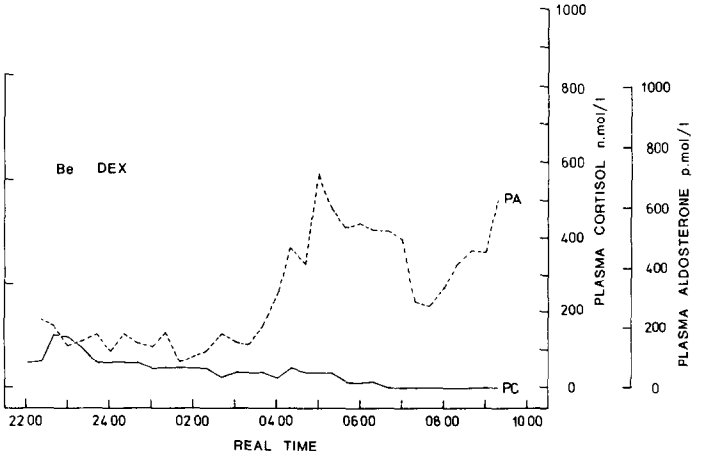

Fig. 3. Plasma aldosterone and plasma cortisol levels in control subject Be, after dexamethasone.

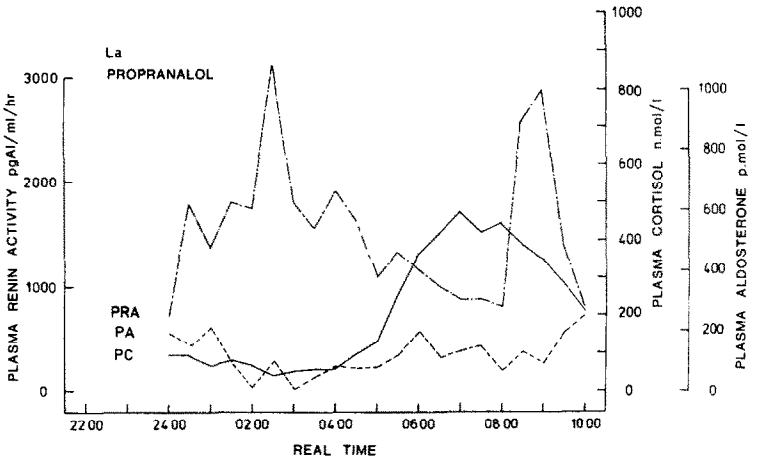

Fig. 4. Plasma aldosterone, plasma cortisol and PRA levels in control subject La after receiving propanolol.

that cortisol and aldosterone appear to be secreted synchronously in resting salt-replete control subjects at night. In the present study this observation was confirmed, and with only one exception, plasma levels of cortisol and aldosterone were significantly correlated when examined statistically. In four subjects in whom PRA was also measured, episodic secretion of PRA also occurred but the relationship of PRA to plasma aldosterone was less clear cut than was that of aldosterone to cortisol. Although the late morning rise of plasma aldosterone appeared to be matched by a concomitant rise in PRA, several major episodes of PRA secretion occurred without any significant change in aldosterone levels. In contrast, every episode of aldosterone secretion was matched by a secretory episode of cortisol.

The remarkable synchrony between cortisol and aldosterone in normal subjects under these controlled conditions has led some investigators [5, 8] to conclude that there is a common controlling mechanism for the secretion of these two hormones, and that this common factor is corticotrophin (ACTH). In support of this view, there are numerous reports which confirm that the administration of ACTH to normal subjects will increase the plasma level of aldosterone. However, there are a number of observations which are inconsistent with this conclusion. Thus, it has been amply documented here and elsewhere that when ACTH secretion is blocked by dexamethasone, episodic release of aldosterone continued although cortisol secretion is abolished [6, 7, 9–11]. Further, in one of the subjects studied here, plasma aldosterone levels were considerably increased, and showed more marked secretory episodes during dexamethasone suppression as compared with the control study. In several subjects, marked secretory episodes of cortisol occurred which were not accompanied by changes in plasma aldosterone levels. Lastly, there is the question of whether the concentrations of ACTH which occur physiologically are capable of influencing aldosterone secretion. Although recent reports [12, 13] suggest that infusions of administered ACTH which produce

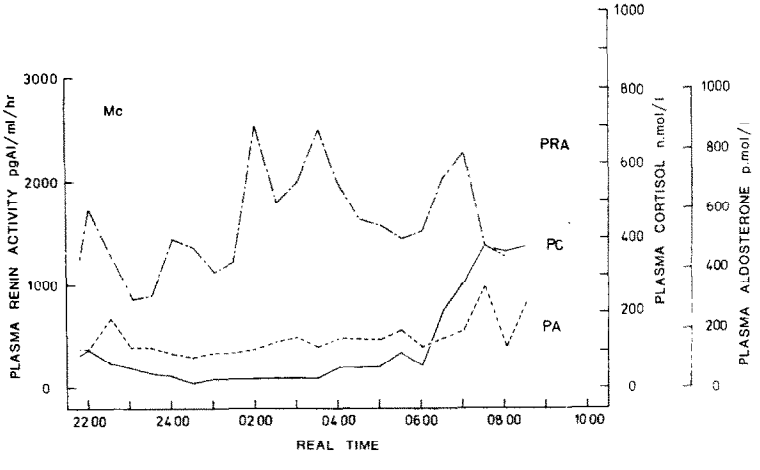

Fig. 5. Plasma cortisol, plasma aldosterone and PRA levels in patient Mc, with spinal cord transection.

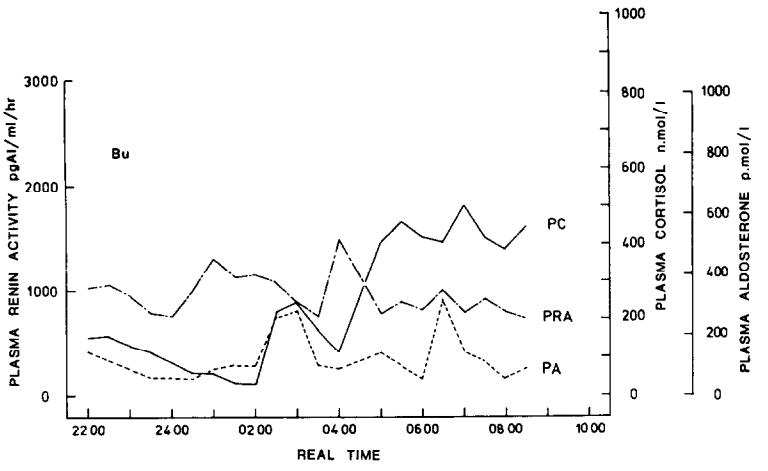

Fig. 6. Plasma cortisol, plasma aldosterone and PRA levels in patient Bu, with spinal cord transection.

physiological increases in plasma ACTH levels are capable of stimulating both aldosterone and cortisol secretion, there are also conflicting data. We have shown elsewhere [14] that in subjects on a normal diet, ACTH release provoked by insulin-induced hypoglycaemia, whilst sufficient to produce maximal secretion of cortisol, causes no change in plasma aldosterone levels. When a similar study was made in salt-deplete subjects, a marked increase in aldosterone levels occurred. Thus, the total evidence appears to us to be against the view that the secretory episodes of aldosterone are mediated by ACTH and

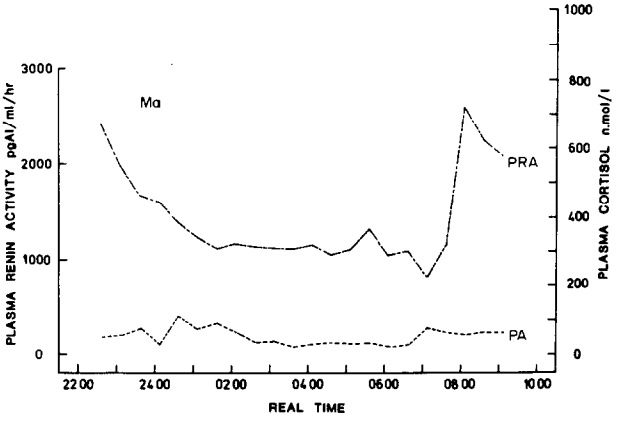

Fig. 7. Plasma aldosterone and PRA levels in patient Ma, studied three weeks after renal transplantation.

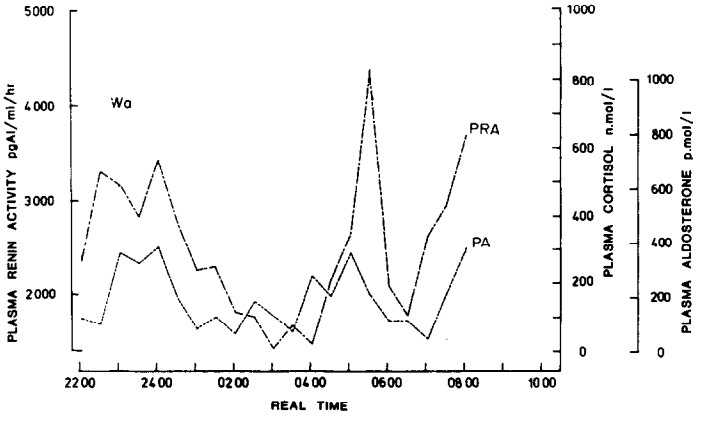

Fig. 8. Plasma aldosterone and PRA levels in hypophysectomised patient Wa.

another mechanism or mechanisms must be involved, which acts in many cases synchronously with, but independently of ACTH.

The extent to which angiotensin is involved in the episodic secretion of aldosterone is also debatable. In this study, several eposides of PRA secretion occurred without provoking any change in plasma aldosterone levels; conversely, secretion of aldosterone occurred independently of any change in PRA levels. Armbruster *et al.* [8] concluded that in the absence of ACTH (as in the dexamethasone suppressed subjects) the renin-angiotensin system was the major determinant of aldosterone secretion. To avoid the possible complications of effects other than ACTH suppression of a large dose of dexamethasone, we have studied a hypophysectomised subject receiving only a replacement dose of cortisol. In this patient, with presumed absence of circulating ACTH, marked episodic secretion of aldosterone, and several increases of PRA occurred. However, the relationship between PRA and aldosterone was not entirely convincing, and was not statistically significant. Thus, if neither ACTH nor the renin-angiotensin system are responsible for the control of aldosterone secretion, whatever factor is involved would seem to be independent of the pituitary. In an attempt to study this problem further, studies were made of two groups of subjects in whom it was anticipated that renin release would be minimised or absent. It has been reported that administration of beta-blocking drugs such as propanolol will suppress the secretion of renin [14], although diurnal studies have not to our knowledge appeared. Four subjects receiving a beta-blocking drug were therefore included in the present study. However, although the dose of drug was substantial, all four subjects continued to show episodic changes in PRA which appeared little different from those seen in the control period. In particular, episodic changes in PRA levels also occurred in these subjects independently of aldosterone secretion.

Two subjects were investigated who had suffered traumatic spinal cord transection. Both these patients showed a number of peaks of PRA and also secretory episodes of cortisol and aldosterone similar to those seen in the control subjects. In these patients therefore, episodic changes in PRA still occur, which suggests that it is unlikely that the sympathetic nervous system plays a dominant role in controlling the nocturnal changes in PRA and plasma aldosterone.

Studies of the changes in plasma concentrations of PRA and aldosterone in the renal transplant recipient were made in the expectation that complete denervation of the kidneys would remove any neural stimulus to renin release. In addition, administration of substantial amounts of prednisolone should virtually ensure that plasma ACTH levels are minimal. Under these conditions, plasma aldosterone levels were low and showed no significant changes through the period of study. PRA levels also tended to be constant, but a marked rise occurred towards the end of the study. The interpretation of these findings is not straightforward. It seems unlikely that in such a short time regeneration of the renal nerve supply could have occurred and so it is reasonable to suppose that any change in renin levels would occur independently of neural stimulation. In the absence of any marked change in plasma electrolyte levels, the late rise seen in PRA is presumably due to changes in renal blood perfusion pressure, which may also be the cause of the changes in PRA in the patients with cervical transection. It may be significant that in the transplant patient, there were no marked and regular peaks of PRA as occurred in most of the control subjects. In spite of the substantial increase in PRA, no change occurred in plasma aldosterone levels. Since we were unable to test directly the responsiveness in this patient of the adrenal cortex to angiotensin, it is possible that the fixed pattern of aldosterone secretion simply represents an inability to respond to stimulation, and this may be an effect of the large dose of prednisolone given to this patient. However, we have shown in other similar patients that after transplantation, responsiveness to exogenous ACTH and to angiotensin which is reduced in the absence of the kidney, soon returns, [16] and so this study suggests that if there is no direct suppressive effect of prednisolone on the adrenal cortex, the kidney may be involved, other than through the production of renin, in the control of aldosterone secretion.

Finally, some consideration of plasma sodium and potassium levels is necessary. In this study, electrolyte levels were examined in three subjects, but only minor changes occurred which were unrelated to plasma aldosterone levels and in common with other investigators [7, 8] we conclude that changes in sodium or potassium levels are unlikely to be sufficient to explain the marked secretory episodes observed.

From the results of this study, and others quoted above, it seems well established that in normal supine sleeping subjects, episodic secretion of cortisol and aldosterone occurs with a characteristic synchrony. Although ACTH is undoubtedly the stimulus to cortisol secretion in this situation, the evidence is inadequate to conclude that this factor also mediates aldosterone secretion. In addition, neither the renin-angiotensin system, nor changes in sodium or potassium appear to be involved. Some other, non-pituitary factor would therefore seem to be implicated, and if so, in these particular conditions, this factor acts synchronously with, but independently of ACTH. Such a factor has been implicated in the response of aldosterone to sodium depletion [17], but may also be important in day to day control of aldosterone secretion.

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DISCUSSION

Fraser. The frequent lack of effect of inhibitors of angiotensin II, such as Saralasin, on plasma aldosterone concentration in normal, sodium replete subjects would seem to agree with your conclusion that the renin-angiotensin system may not be all important in maintaining basal aldosterone secretion, although removal of the source of renin by nephrectomy does lead to a fall in aldosterone secretion to low levels. However, in normal subjects with raised plasma aldosterone concentrations caused by sodium depletion, Saralasin infusion does reduce this level, probably indicating an important role for angiotensin II in this situation. Do you have any evidence of periodicity in plasma aldosterone levels sodium depleted subjects and, if so, is the correlation with renin improved in these subjects? Since there would be a delay between renin release and alteration of aldosterone secretion, would the renin-aldosterone correlation be improved by taking this into account during statistical analyses?

James. We have made no studies in which sodium balance has been altered. All our subjects were permitted unrestricted sodium intake. However, Vagnucci and his colleagues (*J. clin. Endocr. Metab.* **38** (1974) 761) have done this; they studied four subjects on a low salt diet and examined the changes through 24 h in plasma levels of aldosterone and renin activity in a protocol similar to that which we used. Under these conditions, they found evidence for episodic secretion of both these substances and that the patterns of plasma renin activity and aldosterone were essentially identical. They concluded from these data that in these salt-depleted subjects, the renin angiotensin system is the main regulator of aldosterone production. In a similar study, Armbruster *et al.* (1975) reached the same conclusion. We would not dissent from their views, but it appears that the situation is different in subjects on an unrestricted salt intake. It is also interesting that in the study of Vagnucci *et al.*, they also found several instances in which plasma renin and aldosterone levels were dissociated.

Your second point is valid; one should take note of the time delay between the stimulus and the response. We did look at the results with this in mind, but it did not improve the correlations to any extent.

Adlercreutz. We did a study on hard training male athletes and compared them to normal sedentary individuals and we found that during the most active competitive period during the summer the plasma renin activity and angiotensin II was significantly higher than in the controls. Plasma aldosterone was almost unchanged and slightly but not significantly lower than in the controls during the same period. Thus the aldosterone values did not react to the elevated angiotensin II values.

Crabbé. Dr. Adlercreutz, is it not so that one also can have a reverse situation, unexplained to my knowledge, that fasting subjects are characterized by an increase in plasma aldosterone levels without significant changes in plasma renin activity?

Adlercreutz. I am not aware of that study.

James. I wonder if you are referring to the paper by the Group in Glasgow (*Clin. Sci.* **39** (1970) 437). They showed that during a period of total fasting following sodium deprivation in obese subjects, although plasma renin concentration decreased, plasma aldosterone increased quite markedly. This type of dissociation has been demonstrated in a number of situations, although the conditions were usually rather abnormal.

Jones. Do your results in hypophysectomised subjects totally exclude pituitary factors? I would have thought that they exclude anterior pituitary factors but the pituitary stalk can continue as a functional neurohypophysis. So it seems to me you have not excluded ADH as a possible factor.

James. That is quite true. The study only eliminates involvement of anterior pituitary factors.

Morris. Have you seen any interaction between the episodic secretion of aldosterone and prolactin levels which other people have reported?

James. No, we have not looked at prolactin levels.

Morris. A group in Montreal have shown that you get systematic secretion of prolactin at the same time as increases in plama aldosterone.

James. That's interesting, I was unaware of that work.

Fraser. We have recently followed the effect on plasma prolactin levels of dietary and diuretic-induced sodium depletion in normal human subjects. While plasma aldosterone and angiotensin II levels rose markedly and consistently, plasma prolactin, measured by Drs. Alan Craig and Fred Rutherford (Searle Inc., England) remained unchanged.