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ROLE OF ACTH, ANGIOTENSIN AND POTASSIUM IN STRESS-INDUCED ALDOSTERONE SECRETION

E. A. ESPINER, S. LUN and D. S. HART
The Medical Unit, The Princess Margaret Hospital and Lincoln College, Christchurch 2, New Zealand

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SUMMARY

The relative roles of ACTH, angiotensin II and potassium concentration in aldosterone secretion have been studied, after acute jugular venous cannulation, in four conscious sheep with cervical adrenal transplants. Samples of adrenal venous blood for assay of cortisol and aldosterone secretion were drawn at 15 min intervals for 6 h, and related to samples drawn simultaneously for measurement of potassium and angiotensin. Cortisol secretion was used as an index of plasma ACTH. To assess the importance of ACTH, identical studies were repeated in each sheep, except for the addition of dexamethasone to suppress endogenous ACTH secretion. Immediately after cannulation, major fluctuations in cortisol and aldosterone secretion usually occurred together, whereas only small changes were observed in plasma potassium and angiotensin. Dexamethasone promptly reduced cortisol to undetectable levels, and appreciable but slower reductions in aldosterone were also seen, without consistent change in angiotensin or potassium concentration. The results suggest that angiotensin and potassium play a minor role in the stress related fluctuations of aldosterone secretion, which is largely determined by ACTH.

INTRODUCTION

Although at least three factors—ACTH, angiotensin II and potassium [1, 2] have been shown to be important in aldosterone secretion, the relative contributions of these and other possible stimuli to natural fluctuations in aldosterone secretion are still disputed [3, 4]. ACTH appears to play a prominent part at least during such major stress as surgical trauma [5, 6] whereas the renin angiotensin system predominates under conditions of ECF volume depletion [7, 8]. Whether these same stimuli continue to regulate aldosterone during less challenging conditions is unknown since studies relating natural fluctuations of aldosterone secretion to simultaneous measurements of angiotensin, potassium and ACTH secretion have not been undertaken. This paper examines the relationship between aldosterone secretion and known regulatory factors (ACTH, angiotensin and potassium) associated with and following the acute stress of jugular venous cannulation in conscious sheep with cervical adrenal transplants. To assess the influence of ACTH secretion under these conditions, identical studies were repeated in each sheep after the administration of dexamethasone.

EXPERIMENTAL

The experiments were carried out on four merino ewes (35–50 kg body weight) with left adrenal autotransplants exteriorized in jugular carotid skin loops [9]. Sheep were housed indoors in individual crates and maintained on a diet of concentrates which provided an electrolyte intake of 120 mmol sodium and 100 mmol potassium per day. The housing, upkeep and methods employed for collection of adrenal venous blood have been described previously [10, 11].

Between 0900 and 0930 hours the sheep was removed from its crate, briefly restrained by a technician, and a French gauge 6 Portex catheter quickly inserted into the left jugular vein using a MacGregor introducer needle. The sheep was then returned to its crate and collection of adrenal venous blood samples was begun immediately. Collection of approximately 15 ml adrenal-venous blood for steroid assay (cortisol and aldosterone) potassium and angiotensin concentration was taken over a one to two min period at intervals of 15 min for a period of six h after venous cannulation. So that blood loss was reduced, the red cells were resuspended in 0.9 g% saline to a vol. of 100 ml and were returned to the sheep at 160 min and 240 min after cannulation. Identical experiments were performed in each of the four sheep, one to four weeks later, except that dexamethasone (16α-methyl-9-α-fluoro Δ^1 cortisol 21-phosphate, Decadron; Merck Sharp and Dohme) 4.0 mg was given intravenously 110 min post cannulation.

Plasma assays

Blood was centrifuged within five min of its collection at 4°C for 5 min and the plasma was stored at −15°C until assayed. Cortisol was measured by a fluorescent technique as previously reported [12]. Aldosterone was measured after paper chromatography by radioimmunoassay [13] using an anti-aldosterone serum donated by the United States National Institute of Health. Standard deviations calculated from results of duplicate pairs were 2.9 ng in the 0–20 ng/100 ml plasma range, 6.8 ng in the 20–50 ng,

sone administration at 110 min, cortisol secretion fell 12.4 in the 50–100 ng and 38.3 ng in the 100–500 ng/100 ml range. The coefficient of variation for replicate aldosterone samples (50 assays of a plasma pool of 7 ng/100 ml) was 22%. Secretion rates for cortisol and aldosterone were calculated from plasma concentration, haematocrit and measured adrenal blood flow.

Plasma angiotensin II (A-II) was measured by radioimmunoassay after an ethanol extraction step as previously described [14]. For this assay 5 ml aliquots of blood were added immediately after collection to EDTA tubes, shaken well and centrifuged at 4°C. Samples left at room temperature for 2 min before addition to EDTA tubes showed levels of A-II 20–30% higher than those where blood was added immediately to EDTA tubes. Standard deviation of duplicate samples measured in the same assay were 3.0 pg in the 10–30 pg/ml plasma range and 4.2 pg in the 30–50 pg/ml range. The coefficient of variation in replicate samples (34 assays of a plasma pool of 42 pg/ml concentration) was 20%. All samples from any one sheep experiment were run together in the same assay so that errors from interassay variation were avoided. The possibility that adrenal arterial concentrations of angiotensin differed from simultaneously drawn adrenal venous samples was assessed in preliminary experiments by drawing blood samples from adrenal arterial and venous vessels simultaneously. Only small differences were found, no greater than the difference between duplicate pairs, and appeared to be quite random.

Plasma potassium was measured on an EEL 227 flame photometer using Lithium as an internal standard.

In these studies, the direct measurement of cortisol secretion has been used as an index of plasma ACTH concentration. Previous work [11] has shown that the two measurements are closely coupled whereas plasma immunoreactive ACTH may not always reflect biologic ACTH activity [15].

RESULTS

Changes in steroid secretion and angiotensin and potassium

A representative example of the fluctuations of aldosterone and cortisol secretion and in plasma A-II and potassium after cannulation is shown in Fig. 1. Cortisol and aldosterone secretion were high immediately following cannulation, then fell to low values in 60 min after which episodic steroid secretion occurred. Plasma A-II and potassium levels showed less variation although there was a trend for plasma A-II to rise and for plasma potassium to fall over the period of sampling. Peaks of cortisol and aldosterone were associated at times (e.g. at 170 min, 250 min and 360 min) but cortisol was secreted independently of aldosterone at other times, e.g. at 58 min and 230 min (Fig. 1). The correlation between aldosterone secretion, plasma A-II, cortisol secretion and potassium are shown for all four sheep in Table 1. Aldosterone and cortisol were positively correlated in all four sheep but correlation between aldosterone secretion and plasma A-II or potassium was significantly positive in only two of four sheep.

Effect of dexamethasone

The pattern of aldosterone and cortisol secretion, plasma A-II and potassium concentration is shown for all four sheep before and after dexamethasone in Fig. 2. Because of the variation between sheep, steroid secretion has been plotted as a percentage of the maximum value observed during the experiment. As observed previously, cortisol and aldosterone secretion were elevated immediately after cannulation. Subsequently (15–110 min) aldosterone and cortisol tended to either rise (Dale and Beryl) or fall (Sheba and Phoebe) together in individual sheep. Plasma A-II and potassium concentration showed less variation over the period although there was a trend for plasma potassium to fall (Fig. 2). After dexametha-

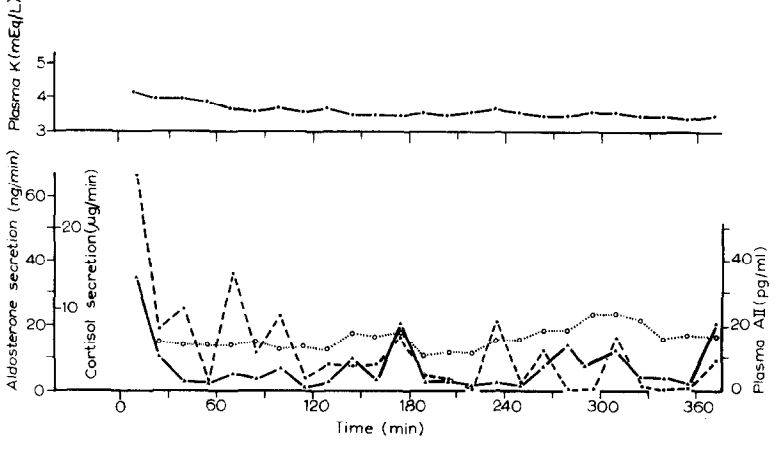

Fig. 1. Fluctuation in cortisol (■---■) and aldosterone (▲——▲) secretion and plasma A-II (○---○) and potassium concentration after venous cannulation (Phoebe).

Table 1. Individual correlation coefficient observed between aldosterone secretion and angiotensin (A-II) cortisol and potassium (K)

	Sheep	A-II	Cortisol	K
Untreated	Dale	−0.22	0.5*	0.42*
	Sheba	−0.4	0.48*	0.78***
	Phoebe	0.45*	0.61*	0.3
	Beryl	0.47*	0.57**	0.37
Dexamethasone treated	Dale	0.53**	0.78***	0.65***
	Sheba	0.03	0.5*	0.59**
	Phoebe	−0.37	0.73***	0.88***
	Beryl	0.65***	0.76***	0.74***
	N = 25		* $P < 0.05$	
			** $P < 0.01$	
			*** $P < 0.001$	

abruptly and was virtually undetectable 60 min later and remained so for the experiment's duration. Aldosterone secretion was also reduced by dexamethasone although the change was less abrupt and suppression was less complete (Fig. 2). In contrast there was no significant change in plasma A-II before and after dexamethasone and plasma potassium, as noted previously in untreated sheep, showed a gradual decline. Occasionally, peaks in aldosterone secretion occurred independently of obvious change in plasma A-II or potassium at a time when cortisol secretion was well suppressed (e.g. 195 min in Dale, 345 min in Beryl, 360 min in Sheba). Correlation coefficients for aldosterone with cortisol and aldosterone with A-II and potassium in dexamethasone-treated sheep are shown

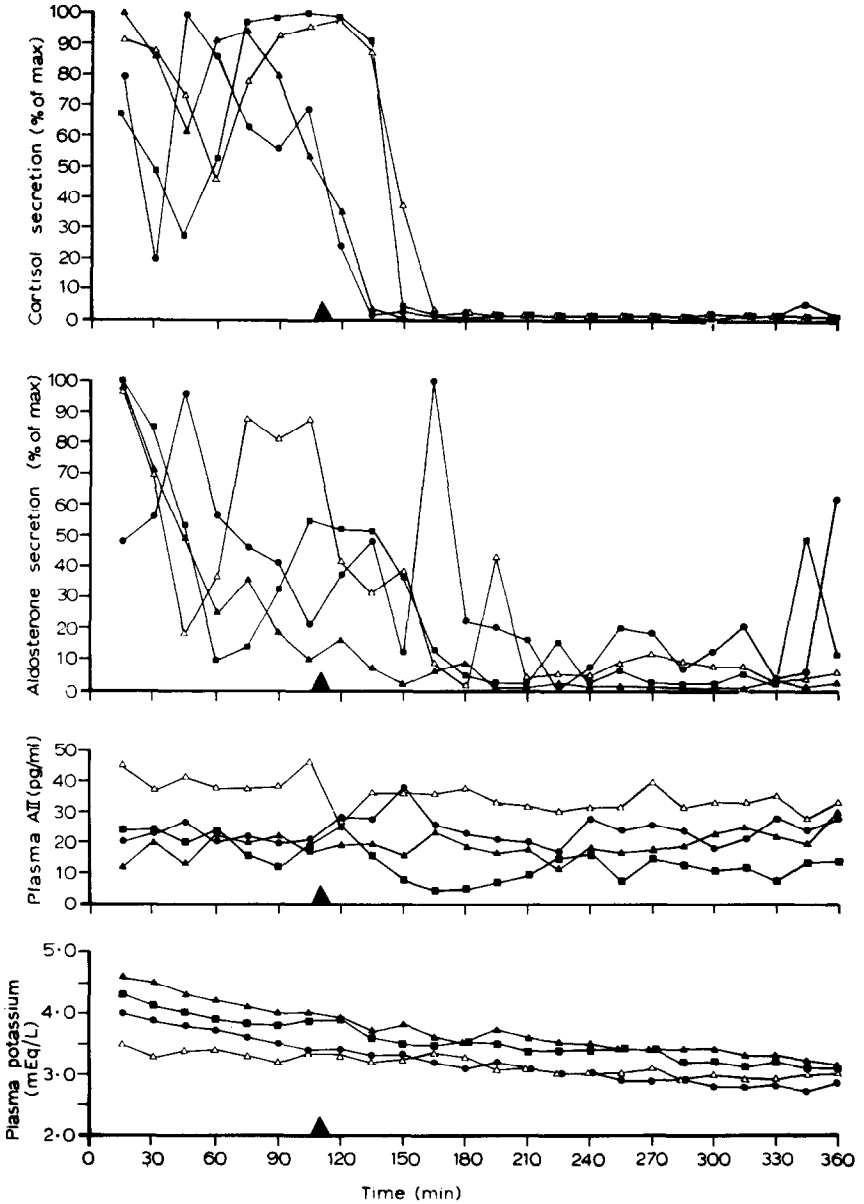

Fig. 2. Change in cortisol and aldosterone secretion and plasma A-II and potassium concentration in four sheep given dexamethasone at 110 min (arrow). Steroid secretion is shown as a percentage of the maximum value observed during the experiment.

● Sheba △ Dale ▲ Phoebe ■ Beryl

in Table 1. There was a significant correlation between aldosterone and cortisol and between aldosterone and plasma potassium in all four sheep but significant correlations between aldosterone and plasma A-II were seen in only two of four sheep. The effect of dexamethasone was further assessed by examining the relationships between these indices in individual sheep for the time period 180–360 min post cannulation. Aldosterone secretion was significantly less on the day dexamethasone was given in all sheep (non-paired t test, $P < 0.05$ in two sheep $P < 0.001$ in two sheep) whereas a significant decrease in plasma potassium ($P < 0.001$) and AII ($P < 0.001$) occurred in one and two sheep respectively. Mean values ($\pm$ S.D.) in untreated sheep for the time period 180–360 min, compared to values in dexamethasone treated sheep (given in brackets) were as follows—cortisol 8.0 $\pm$ 6.6 μg/min (0.13 $\pm$ 0.1); aldosterone 13.1 $\pm$ 8.8 ng/min (3.0 $\pm$ 4.3); A-II 29.4 $\pm$ 11.3 pg/ml (21.9 $\pm$ 8.4); potassium 3.3 $\pm$ 0.3 mEq/l (3.2 $\pm$ 0.2).

DISCUSSION

Previous studies in man, employing peripheral venous blood samples, have shown that the secretion of cortisol and aldosterone is often synchronous [16, 17]—suggesting an important role for ACTH in the normal secretion of aldosterone. Plasma renin activity has also been correlated with oscillations in plasma aldosterone in man and this relationship was strengthened when dexamethasone was given to suppress ACTH secretion [16]. Although a number of studies in experimental animals have examined the role of ACTH, renin and potassium in aldosterone regulation by direct measurements on adrenal venous blood, such studies have usually involved anaesthesia and considerable surgical trauma, —itself associated with major and sustained stimulation of endogenous ACTH secretion [5, 6]. The present studies, however, further suggest that ACTH is a major determinant of aldosterone secretion in the acutely cannulated conscious sheep. Examination of all secretory episodes showed that peaks in aldosterone and cortisol secretion were most often associated when the cortisol secretion rate equalled or exceeded 50% of the functional capacity of the gland's ability to secrete cortisol [12]. These findings are consistent with previous reports on the acute effect of exogenous ACTH in sheep [1] and should be contrasted with the lack of effect [18] or suppression of aldosterone secretion [19] which results from high doses of ACTH given for periods of days. The importance of ACTH in aldosterone secretion was also suggested by the experiments employing dexamethasone. Thus 70–100 min after intravenous dexamethasone significant falls in aldosterone secretion, which did not occur in control experiments in the same sheep, were observed. The possibility that dexamethasone might have a direct adrenal action, inhibiting aldosterone secretion, appears to be unlikely since we have previously shown that the acute aldosterone response to trophic stimulation is unaffected in dexamethasone pretreated sheep [20]. Further, the local infusions of high doses of dexamethasone through the transplanted adrenal does not alter the aldosterone response to ACTH stimulation (unpublished observations).

In contrast to cortisol, plasma A-II was less well correlated with aldosterone secretion in control experiments and did not change significantly during the period of suppression of aldosterone following dexamethasone. Because increments in plasma A-II would be expected to precede aldosterone secretion, the statistical relationship between the two hormones was also examined, using a 15 or 30 min phase shift, but the correlation coefficient was not increased. Although both *in vitro* [21] and *in vivo* [20] studies indicate that the adrenal glomerulosa is very sensitive to exogenous A-II, there are few if any previous studies where aldosterone secretion has been examined along with concurrent plasma A-II concentrations under conditions of frequent sampling. The present results, however, fail to support any close relationship between A-II and aldosterone secretion—at least in sodium replete sheep.

Changes in plasma potassium concentration were small but consistent in that levels tended to drop over the experimental period. The highly significant correlation between aldosterone secretion and plasma potassium observed in all four sheep given dexamethasone, is unlikely to represent a causal relationship, since similar drops in plasma potassium were associated with sustained aldosterone secretion in untreated sheep. The reason for the observed fall in plasma potassium is not known—it is not associated with any change in sodium concentration—but it is a reasonably consistent finding in sheep studied after acute venous cannulation. The fall does not appear to influence the aldosterone response from the adrenal gland which retains normal sensitivity to acute exogenous stimulation by angiotensin, ACTH or the potassium ion itself [20].

While these studies indicate one major determinant of aldosterone secretion under physiologic conditions, it is difficult to be certain of the factors responsible for smaller oscillations of aldosterone and in particular whether these can all be accounted for by concurrent change in plasma angiotensin, potassium or ACTH. Review of all aldosterone secretory episodes in these studies showed several instances where aldosterone secretion occurred in the absence of significant change in ACTH (cortisol), A-II or potassium. Similar observations have been made previously by less direct means [16, 22] and provide some support for evidence that additional factors may be concerned in the secretion of aldosterone [23].

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