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Aldosterone Regulation in Anephric Patients*

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The response of plasma aldosterone to hemodialysis, 3 h orthostatism, K-loading and angiotensin II and ACTH infusions has been studied. Hemodialysis, orthostatism and angiotensin II infusion do not modify aldosterone levels. By the contrary ACTH and potassium originate a significant increase in plasma aldosterone. They seem to be the main aldosterone secretion regulators in the absence of renin production.

The regulation of aldosterone secretion in anephric patients has been studied by several groups (2, 5, 6, 8, 10, 13 ,15, 21) for the past five years. It is now stablished that potassium has a direct effect on aldosterone secretion while the role of other factors like ACTH, angiotensin II, orthostatism or volume depletion has remained controversial.

The present study was designed in order to clarify the role played by those factors on the regulation of plasma aldosterone levels in anephric patients.

Materials and Methods

Twelve binephrectomized patients were studied. All patients were kept on a diet containing 20 mEq Na, 40 mEq K, 1 g protein/kg body weight and 500-1.000 ml of liquid, daily. They were submited to a minimum of 18 h. hemodialysis weekly in two or three sessions.

Body weight, blood pressure, haematocrit, Na, K, Cl, bicarbonate, cortisol, renin activity and aldosterone concentrations in plasma were controlled before and after the following conditions:

1. A 12 h hemodialysis session performed with a plate dialyser without ultrafiltration.

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2. Three hours of ambulation after 8 h bed-rest.

3. Oral administration of 0.7 mEq K/kg body weight.

4. Angiotensin II (Val-5-hypertensin II-asp-, Ciba) (Angio II) infusion for 1 h at subpressor dose (less than 5 ng/kg/min) followed by an additional 1 h infusion of Angiotensin II at a rate sufficient to increase the diastolic blood pressure by 20 mm Hg over the basal values.

5. A 4 h 1-24 β-ACTH infusion (0.25 mg in 5 % dextrose solution) performed after dexametasone blockade (4×0.5 mg) during the previous 24 h.

All the tests were performed on the day following a 12 h hemodialysis, beginning at 8.00 a.m. after at least 8 h bed-rest.

Haematocrit, plasma Na, K, Cl and bicarbonate were measured by conventional techniques. Plasma renin activity (PRA) and plasma cortisol, by RIA and CPB procedures as previously described (4, 9). Plasma aldosterone was measured by a RIA based on that by GÓMEZ-SÁNCHEZ *et al.* (7) sligthy modified. Briefly, the procedure is as follows: Aproximately 1,500 dpm of 1,2-H^3-aldosterone (Specific activity 50 Ci/mmol) is added to 1 or 2 ml of plasma, which is extracted with 10 volumes of methylene chloride in a rotating extractor. The organic phase is whashed once with 1.0 ml of 0.1 N NaOH and twice with 1.0 ml destilled water and then evaporated to dryness at 37° C under nitrogen. Isolation of aldosterone from other interfering steroids present in the extract is acomplished by incubation for 14-18 h with 1.0 ml anti-aldosterone serum (dil. 1/50.000).

Unbound steroids are absorbed with 0.5 ml of dextrancoated charcoal suspension in 0.05 M borate buffer. After centrifugation the supernatant containing the aldosterone bound fraction in extracted with 10 volumes of methylene chloride, as before, the organic phase evaporated to dryness and the dry residue disolved in 0.5 ml ethanol. Aliquots of 150 μl in duplicate are taken por RIA and for recovery. To each standard and unknown, 3,500 dpm/50 μl ethanol of 1,2-H^3-aldosterone is added and then evaporated to dryness. 0,3 ml of anti-aldosterone serum at 1/750,000 dilution in 0.05 M borate buffer, pH 7.5 is added to the standard and unknown samples and incubated overnight at 2-4° C. Bound and free fractions are separated by addition of 0.3 ml of dextran coated charcoal (0.05 % dextran, 0.5 % charcoal), the tubes placed at 4° C during 15 min then centrifuged at 3,000 rpm for 10 min and the supernatant decanted into counting vials containing 1.0 ml dioxan. 10 ml of scintillation mixture (4 g PPO + 0.1 g dimethyl-POPOP in 5 liter toluene), are added and the radioactivity measured in a Nuclear Chicago spectrophotometer, mod. Mark II, for a time sufficient to accumulate 10,000 cpm each sample.

The final recovery found in 124 plasma samples has been 55±7.2 %. The assay allows to detect 7 pg of aldosterone as distinctive of zero. Interanalysis variation was 14.4 % (variation coefficient) determined in a pool of plasma containing 3.9 ng of aldosterone/100 ml ($n = 10$). Blank was negligible if the antiserum used for inmunological purification was treated with a 1.5 % charcoal suspension in 0.05 M borate buffer containing 0.2 % (Bovine serum albumin) (BSA) and 0.2 % γ-globulin. The recovery of known amounts of aldosterone added to a plasma samole from an adrenalectomized patient was satisfactory. The correlation between added and recovered aldosterone was excelent ($r = 0.97$, $p < 0.001$) and the points were on the regresion line $Y = 0.96$. $x \pm 2.47$.

The specificity of the assay was assessed by competition studies using several steroids, including cortisol, 11 deoxycortisol, progesterone, 17-OH progesterone, estradiol, testosterone, corticosterone, DOC and cortisone. Only DOC and cortisone have relative potencies of 0.135 % as compared to aldosterone. The other ste-

roids tested have potencies relative to aldosterone of 0.05 % or lower. In particular, cortisol shows a cross reaction of 0.0053 %.

Results

PRA was always found below detection limits of the assay (0.3 ng/ml/h) both at the begining and at the end of each experimental conditions, as expected. Fig. 1, shows the obtained results of plasma aldosterone, plasma K and plasma cortisol in each experimental condition.

Table I, summarizes the values of these as well as other relevant parameters studied.

Hemodialysis. In all patients a significant decrease in plasma K and body weight as well as significant increase in plasma bicarbonate was observed. No changes in both plasma aldosterone and cortisol could be detected following hemodialysis.

Orthostatism. No significant modifications in any of the studied parameters were observed after postural change.

K-loading. Two h after oral K-loading the mean increase of plasma potassium was 1.3 mEq/l. Plasma aldosterone significantly increased whereas plasma cortisol decreased.

ACTH infusions. Both plasma cortisol, as expected and plasma aldosterone rose significantly at 2 and 4 h during synthetic ACTH infusion. There was considerable variability between individuals for aldosterone response although all patients showed at each time consistently higher values than baseline values. Other parameters studied, remained unchanged.

Angiotensin II infusions. During Angiotensin II infusion at subpressor dose, all parameters were unchanged except in one patient who showed a marked increase in plasma aldosterone from 4.1 to 10.5 ng/100 ml. At higher infusion rates of Angiotensin II, the blood pressure rose significantly in all patients while other parameters remained unchanged as before except in the same responsive patient whose plasma aldosterone rose further to 22.5 ng/100 ml.

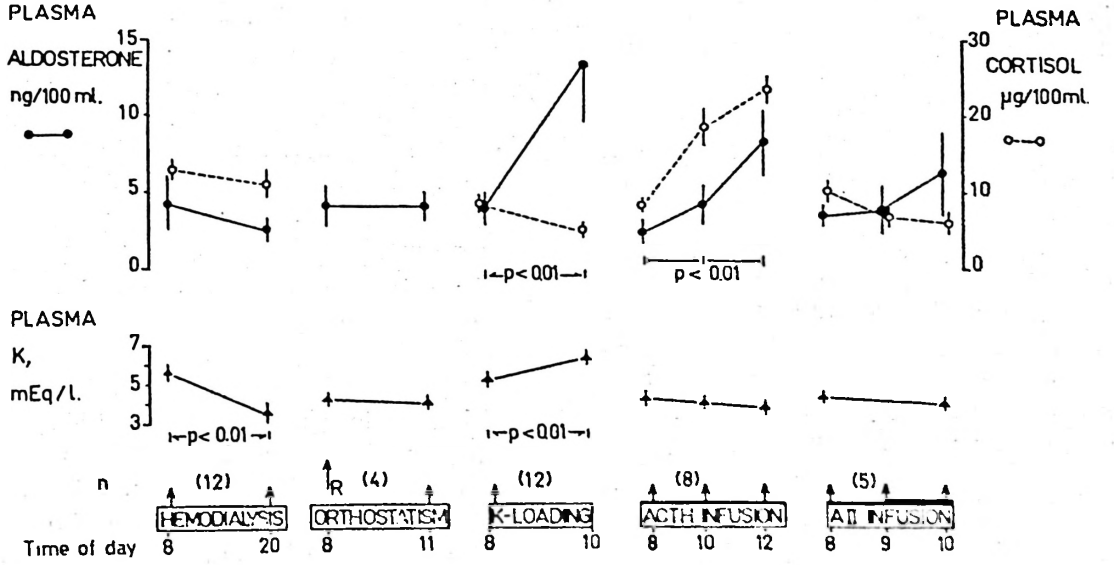

Fig. 1. *Mean plasma aldosterone, cortisol and potassium during the tests performed in the anephric patients.*

Table I. *Values of plasma sodium, potassium, chloride CO_2 content, cortisol and aldosterone bc patier*

Number of ca

	Hemodialysis (12)		Orthostatism (4)		K-Load
	Pre	Post	Pre	Post	Pr
Haematocrit %	16±0.2	16±0.6	19±2	19±1.5	18±
Na (mEq/l)	136±0.5	137±0.6	138±1.5	137±2	136±
K (mEq/l)	5.6±0.1	3.7±0.1*	4.3±0.1	4.2±0.1	5.3±(
Cl (mEq/l)	96±0.8	95±0.8	95±1.5	94±1.5	94±
CO_2 (mEq/l)	21.4±0.4	26.5±0.6*	25.0±0.5	24.5±0.4	24.1±(
Cortisol (ng/100 ml)	13.2±3.3	11.2±5.6	—	—	8.4±
Aldosterone (ng/100 ml)	4.4±1.6	2.5±0.4	4.0±1.1	3.9±0.7	4.3±
Body weight (Kg)	56±2	54±2*	67±3	67±3	62±2
Systolic BP (mm Hg)	148±2	156±7	135±12	130±11	128±(
Diastolic BP (mm Hg)	80±3	85±3	80±8	82±7	75±

* $p < 0.01$ t de student Wilcoxon test.

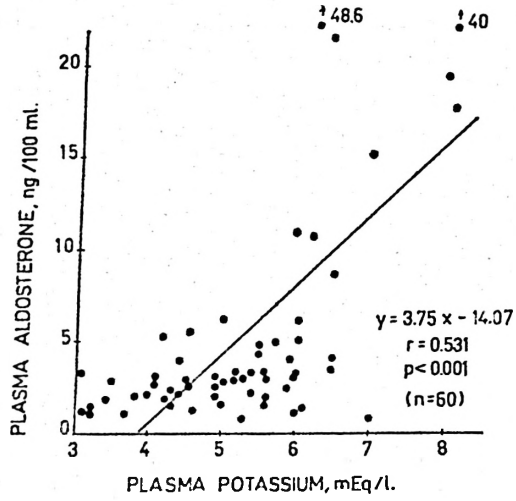

Fig. 2. *Correlation between plasma aldosterone and corresponding potassium concentrations.*

Aldosterone-potassium relationship (figure 2 shows the plasma potassium values and the corresponding plasma aldosterone concentration. A positive and significant correlation was found ($r=0.53$, $p < 0.001$) ($n=60$).

Discussion

It is well established that circulating renin is absent after bilateral nephrectomy (3). Although occasionally as observed in two of our patients, detectable values of generated angiotensin I can be found, most frequently the PRA values are not different from zero. Because of that it is asumed that variation in plasma aldosterone are not mediated through the renin-angiotensin system.

Hemodialysis itself can induce to some extent modifications such as negative potassium balance, volume depletion, correction of the metabolic acidosis and probably increase of ACTH secretion as reflected by the increase in plasma cortisol levels at the end of hemodialysis, although other factors such as distribution volume and endogenous pool of cortisol could account for the increased plasma levels rather than an increased secretion of ACTH. In fact hemodialysis might act on the aldosterone secretion simultaneously through opposite mechanism since the K-deplection decreases its secretion while ACTH has a stimulatory effect. The latter could explain the increased aldosterone plasma levels reported by McCaa *et al.* (11) who did not find the expected diurnal variations in plasma cortisol. On the other hand, Vetter *et al.* (17) reported increased plasma aldosterone values after hemodialysis with normal circadian variations in

/æight, systolic and diastolic blood pressure ($\bar{x}$ ± SEM), during the tests performed in the anephric

ıdlicated between parenthesis.

122)		ACTH Infusion (8)			Angio. II Infusion (5)	
Post	Basal	120 min	270 min	Basal	60 min	120 min
17±0.6	18±0.7	17±1.0	17±0.7	17±1.8	—	16±1.3
1133±1.1	135±1.0	135±2.0	135±2.5	136±0.9	—	135±1.3
16.4±0.1*	4.2±0.1	4.0±0.1	3.9±0.1	4.4±0.4	—	4.1±0.3
92±1.1	90±1.7	90±1.7	89±2.1	95±1.3	—	97±1.3
2:3.0±0.3	26.6±0.7	28.3±0.9	26.0±1.3	24.5±1.0	—	25.5±1.0
!5.3±1.0	8.3±3.9	19.1±5.9*	24.0±7.2*	10.2±2.2	7.1±1.1	6.2±2.8
1:3.4±4.5*	2.5±0.3	4.6±1.2*	8.1±2.8*	3.6±0.5	3.8±1.7	6.6±3.5
62±2	60±3	—	60±3	55±5	—	55±5
1122±4	135±8	—	130±7	128±5	132±5	160±7*
72±6	72±5	—	75±3	76±3	81±5	103±3*

plasma cortisol. Volume depletion seems to play no major role on plasma aldosterone (18, 19). However, McCaa *et al.* (10) found increases in plasma aldosterone levels following volume depletion with hyponatremia. The increase of aldosterone during hemodialysis was due to changes in aldosterone production rate rather than changes in metabolic clearance rate (16). Orthostatism and deambulation did not modify aldosterone levels in the four patients studied. These results are in contrast with those reported by Weidmann *et al.* (18) and Mitra *et al.* (13) who found a significant increase in plasma aldosterone after postural changes. The reason for this increase is as yet unclear. A reduction of the metabolic clearance rate of aldosterone has been suggested.

However, the results by Weidmann *et al.* (18) could not be explained in the light of either and increase or decrease in the metabolic clearance rate following postural changes since the aldosterone plasma levels were only related to the shunt flow. An increased metabolic clearance rate and increased plasma aldosterone levels in response to orthostatism. Another factor to be considered is the size of the potassium pool (18).

The role of potassium on aldosterone secretion has been well established (2, 5, 6, 15, 18). A direct effect for potassium on aldosterone secretion has been shown as observed in our patients. The possibility of a permissive role of potassium which allows others factors to act in a positive or negative way on aldosterone secretion has been suggested and in some way related to the intracelular K^+ concentration (5).

The normal response of aldosterone to angiotensin II infusion both to a subpressor or pressor infusion rates was abolished in all the studied patients but one. This is in agreement with several reports (12, 19, 20). A diminished although evident response to angiotensin infusion has been found by other investigators (8, 18). The lack of response could be related with the high dose of heparin administered during each hemodialysis session. The prolonged absence trophic hormonal stimuli due to the lack of renin seems to be a more likely explanation. In this sense, Aguilera and Marusic (1) have shown that either chronic administration of angiotensin II or homologous renin to hypofisectomized and nephrectomized dogs induce an increased conversion rate of corticosterone to aldosterone, while this response is absent in acute experiments.

Moreover, OELKERS *et al.* (14) demonstrated that repeated angiotensin II administration at low dosis in followed by a higher response in plasma aldosterone to a subsequent angiotensin II infusion.

The response of aldosterone to synthetic ACTH infusion was consistent in all our patients. Individual variations, however are considerable. Increments over basal values ranged from 50 to 400 %. This results are in agreement with those reported by several groups (2, 6, 12, 15, 18) but in contrast with those found by WILLIAMS *et al.* (19).

These discrepancies are difficulty to explain at the moment. Nevertheles it should be kept in mind that factors such as plasma potassium and/or volumen depletion probably play a role in modulating in some way the adrenal response to ACTH.

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Resumen

En un grupo de 12 pacientes anéfricos, se ha estudiado la respuesta de la aldosterona plasmática a la hemodiálisis, deambulación durante 3 horas, sobrecarga oral de potasio, e infusiones de angiotensina II y ACTH. La hemodiálisis, el ortostatismo y la infusión de angiotensina II no se tradujeron en modificaciones de la aldosterona. Sin embargo, la ACTH y el potasio inducen un aumento significativo de la aldosterona plasmática. En ausencia de producción de renina, estos dos últimos factores parecen ser los reguladores fundamentales de la secreción de aldosterona.

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