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RADIOIMMUNOASSAY OF ALDOSTERONE IN ADRENAL VENOUS EFFLUENT IN A CASE OF CONN'S SYNDROME

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SUMMARY

In a case of Conn's syndrome samples were obtained from the venous effluent of both adrenals and from peripheral veins during venography. The aldosterone concentration was measured by means of radioimmunoassay. The sensitivity of the aldosterone assay was 27 pg ($P<0.05$), the parallelism between the standard and the serum dilutions was excellent and there was no cross-reaction with cortisol, cortisone, 21-desoxycortisol, dexamethasone or spironolactone in amounts up to 1 μg per incubation. The aldosterone concentrations measured in peripheral venous blood were 220–250 ng/100 ml serum. In the effluent of the left adrenal, in which an aldosterone producing tumour was localized, an aldosterone level of 8480 ng/100 ml serum was estimated.

INTRODUCTION

An aldosterone radioimmunoassay was recently developed by us. A case of Conn's syndrome (adrenal aldosterone secreting adenoma leading to aldosterone excess) proved the usefulness of this new technique in combination with sampling during venography. Samples were drawn from the venous effluent of the affected adrenal during venography. The values found could be compared with those in peripheral venous blood under various known conditions. Cross-reactivity of spironolactone and contrast-material used in venography had to be excluded. The technique of the aldosterone radioimmunoassay is described and its application in a case of Conn's syndrome is discussed.

CASE REPORT

A 52-year-old woman (patient S.) suffered from hypertension. The hypertension was associated with episodes of marked weakness of the muscles, generalized fatigue, constipation, and nycturia. Laboratory data showed a hypokalaemic metabolic alkalosis, a hypernatraemia, and an elevated urinary aldosterone secretion. She was

diagnosed as having aldosteronism, possibly primary. Symptoms and signs reacted favourably to the administration of spironolactone (a competitive inhibitor of aldosterone), 400 mg daily. The influence of a daily intake of 160 mEquiv Na^+ during 2 days was studied since the levels of aldosterone produced by tumours are generally little affected by sodium intake. Venography with sampling of adrenal venous blood was undertaken after spironolactone withdrawal in order to identify the adrenal lesion. The venography was done twice, since during the first examination only the right adrenal gland, which was normal, was visualized, and because no slective adrenal blood samples had been taken. The second time, a left adrenal tumour was demonstrated and blood—probably to some extent diluted with renal venous blood—was obtained from the left adrenal vein. No sample was available from the right adrenal venous blood (Fig. 3). At operation, a 30-g adrenal adenoma was resected. The peripheral aldosterone level was measured again 9 days after surgery. The patient recovered very well but retained a slight hypertension.

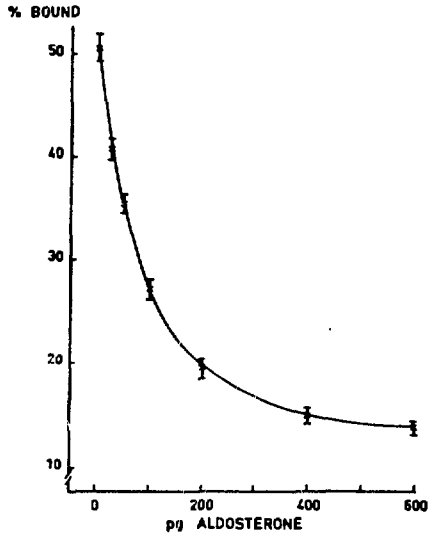
METHODS

Antibodies against aldosterone were raised in rabbits by intracutaneous injection with aldosterone-18,21-diacetate-3-carboxymethyloxim (Bayard *et al.*[1]) coupled to bovine serum albumin (BSA) (Erlanger *et al.*[2]). To 1 mole BSA 36 moles aldosterone-18,21-diacetate were attached. The final antiserum dilution we used was 1/10000. With this dilution the sensitivity of the assay was 27 pg ($P<0.05$) and the association constant was 3.2×10^{12} l/mole. Bound and free aldosterone were separated with dextran-coated charcoal (Herbert *et al.*[3]). The incubation medium was a 0.1 M phosphate buffer, pH 7.6, to which was added 0.05% Tween-20 and 0.02% BSA. Serum samples (1 to 5 ml) were extracted with dichloromethane, after addition of an internal standard of 10 pg aldosterone-1,2-^{3}H (specific activity 51 Ci/mmole, RCC, Amersham). The aldosterone fraction was isolated by chromatography on Whatmann-1-paper in a Bush system [*i.e.* petroleum ether 80–100–toluene–methanol–water (5:5:7:3)] in a 5-h run at 28°. After elution with dichloromethane–methanol (1:1) the recovery of aldosterone ranged from 60 to 85%. Aldosterone was localized by scanning aldosterone-1,2-^{3}H running in parallel. Using this procedure the blank value was <27 pg.

RESULTS

In Fig. 1 a typical aldosterone standard curve is presented. The slope of the semilogarithmic plotted standard line is in good agreement with those of six dilutions of the serum from the patient with Conn's syndrome and three dilutions of a normal serum as shown in Fig. 2. In our chromatography system aldosterone runs between cortisol and cortisone. The contamination of the aldosterone spot with cortisol was less than 0.3%. In amounts up to 1 μg per incubation no cross-reaction with cortisol or cortisone was detected. The same results were obtained with dexamethasone, 21-desoxycortisol and spironolactone. Overmore, spironolactone was separated from aldosterone during the chromatography; it almost moved with the solvent front.

Angiografin® (Schering A.G.), which was used as contrast material in venography, was removed from the serum during the extraction and purification proce-



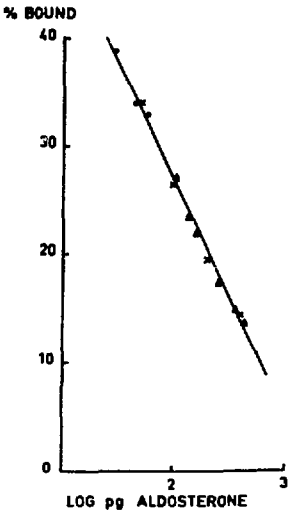


Fig. 1. Aldosterone standard curve. $\frac{1}{2}$ = S.D. (n = 18). Sensitivity 27 pg ($P < 0.05$).

Fig. 2. Parallelism of aldosterone (×——×), serum from a patient with Conn's syndrome (Δ——Δ) and from a normal female (●——●).

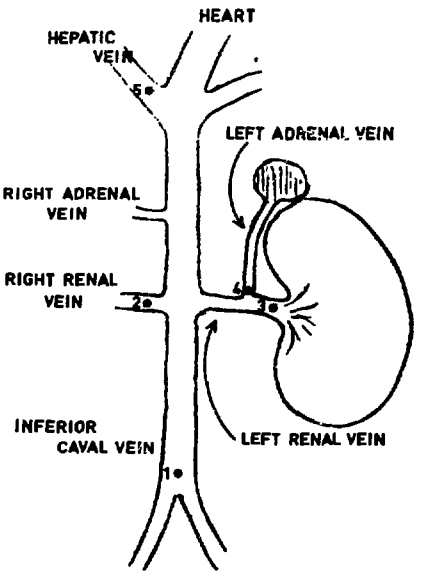


Fig. 3. Sites of blood sampling during venography in Patient S.

dure. If 100 μg of undiluted Angiografin was added, the binding of the tracer to the antiserum was not diminished. This excluded the possibility that traces of free iodine in Angiografin (if present) damaged aldosterone-1,2-^{3}H, which was added as an internal standard to the serum samples.

Normals. Peripheral venous blood was taken from normal individuals at 5 p.m.

TABLE I

ALDOSTERONE LEVELS IN SERUM SAMPLES DRAWN DURING TWO VENOGRAPHIC EXAMINATIONS
Sample numbers refer to Fig. 3.

Site of the catheter tip	*No.*	*First venography*		*Second venography*	
		Aldosterone (ng/100 ml)	*Range*	*Aldosterone (ng/100 ml)*	*Range*
Inferior caval vein	1	0.49×10^3	$0.45-0.52 \times 10^3$ ($n = 2$)	0.54×10^3	$0.51-0.60 \times 10^3$ ($n = 5$)
Right renal vein	2	0.28×10^3		0.17×10^3	$0.15-0.21 \times 10^3$ ($n = 4$)
Left renal vein	3	5.52×10^3	$5.22-5.82 \times 10^3$ ($n = 2$)		
Left adrenal vein	4			8.48×10^3	$8.21-8.76 \times 10^3$ ($n = 3$)
Hepatic vein	5	16.2	14.2–17.8 ($n = 3$)		

TABLE II

ALDOSTERONE CONCENTRATIONS IN SERUM FROM PERIPHERAL VEINS OF A PATIENT WITH CONN'S SYNDROME UNDER DIFFERENT CONDITIONS

Cubital vein	*Aldosterone (ng/100 ml)*	*Range*
Value prior to first venography	0.22×10^3	$0.16-0.25 \times 10^3$ ($n = 4$)
Value prior to second venography	0.25×10^3	$0.21-0.29 \times 10^3$ ($n = 3$)
After daily intake of 160 mEquiv Na^+ during 2 days	0.06×10^3	$0.05-0.06 \times 10^3$ ($n = 3$)
Nine days after removal of the tumour	22	21–23 ($n = 2$)

in upright position and on *ad libitum* salt intake. The mean aldosterone level was 9.5 ng/100 ml serum (range: 4.9–16.0 ng/100 ml, $n = 6$).

Patient S. In Fig. 3 the site of the catheter tip during sampling is demonstrated. Aldosterone concentrations in serum obtained during venography 3 and 8 days after spironolactone withdrawal are presented in Table I. The aldosterone levels in the cubital vein under basal conditions, during salt load and after removal of the tumour, are shown in Table II.

DISCUSSION

Our normal values are in good agreement with those reported by Williams *et al.*[4] and Underwood *et al.*[5]. Basal aldosterone concentration in peripheral venous blood of patient S. was 220 ng/100 ml serum, which is about 25 times the mean normal aldosterone value. Spironolactone was completely extracted from serum with dichloromethane, but in our chromatography system spironolactone moves nearly with the solvent front, while aldosterone is located between cortisol and cortisone. Moreover, the possibility of disturbance of our assay by spironolactone was checked. No cross-reactivity was detected with spironolactone in amounts up to 1 μg per incubation. Interference by spironolactone metabolites is unlikely because of the equal slopes of the aldosterone standard curve and the curve representing the dilutions of

the serum obtained from patient S. (Fig. 2). The aldosterone levels found in the patient's right renal vein and cubital vein are in the same range. In the left renal vein a level of 5520 ng aldosterone/100 ml serum (sample No. 3, Fig. 3) was estimated. In this sample the level of the left adrenal vein of 8480 ng/100 ml serum (sample No. 4, Fig. 3) is lowered by the left renal effluent in which the aldosterone level is supposed to be in the range of the right renal level of 280 ng/ml serum (sample No. 2, Fig. 3). This confirmed the localization of the tumour in the left adrenal, as was shown during venography. As was expected, aldosterone concentration in hepatic venous effluent was proportionally very low as a result of clearance by the liver. We were surprised by the low peripheral venous aldosterone levels during salt load. Even if the output of the right adrenal was normal, it is impossible that this diminution is caused by suppression of the non-tumourous aldosterone excretion. This means that in this case of Conn's syndrome the aldosterone production of the tumour was dependent on salt load. So we can say that in a case of aldosteronism, a tumour cannot be excluded if the aldosterone level lowers on salt load. Nine days after the removal of the tumour a slightly elevated aldosterone level of 22 ng/100 ml serum was measured in peripheral venous blood. At this moment the patient still revealed a slight hypertension, probably due to secondary renal damage from longstanding hypertension.

ADDENDUM

Aldosterone: 11β,21-dihydroxy-3,20-dioxo-4-pregnene-18-al.

Angiografin®: Methylglucamine salt of *N,N'*-diacetyl-3,5-diamino-2,4,6-triiodobenzoic acid (6.5 g/10 ml per ampoule).

Dexamethasone: 9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione.

Cortisol: 11β,17α,21-trihydroxy-4-pregnene-3,20-dione.

Cortisone: 17α,21-dihydroxy-4-pregnene-3,11,20-trione.

Spironolactone: 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androstene-17α-yl)propionic acid-γ-lactone.

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REFERENCES

1 F. BAYARD, I. Z. BEITINS, A. KOWARSKY AND C. J. MIGEON, *J. Clin. Endocrinol.*, 31 (1970) 1.
2 B. F. ERLANGER, F. BOREK, S. M. BEISER AND S. LIEBERMAN, *J. Biol. Chem.*, 234 (1958) 1090.
3 V. HERBERT, K. S. LAU, C. W. GOTTLIEB AND S. J. BLEICHER, *J. Clin. Endocrinol.*, 25 (1965) 1375.
4 G. H. WILLIAMS, J. P. CAIN, R. G. GLUHY AND R. H. UNDERWOOD, *J. Clin. Invest.*, 51 (1972) 1731.
5 R. H. UNDERWOOD AND G. H. WILLIAMS, *J. Lab. Clin. Med.*, 79 (1972) 848.