

## A NEW BIOASSAY METHOD FOR MEASUREMENT OF ANGIOTENSIN II USING ISOLATED BOVINE ADRENAL CELLS : CLINICAL APPLICATION ON THE PLASMA RENIN ACTIVITY

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### ABSTRACT

A new bioassay method for measurement of angiotensin II was clinically applied to determine the plasma renin activity (PRA) in normal subjects and hypertensive patients. Angiotensin II was extracted by a modified procedure reported by Boucher *et al.* Levels of angiotensin II extracted from 17 blood samples were independently determined by our new bioassay method and the rat pressor assay. The new method could determine the minimum level of PRA of 0.25 ng. According to the new bioassay method, mean PRA levels for 9 normal subjects were  $63.6 \pm 48.7$  ng/dl,  $177.7 \pm 99.3$  ng/dl, and  $172.3 \pm 124.3$  ng/dl at 1 hr after supine position, 1 hr after upright position with administration of 80 mg furosemide, and 2 hrs after upright position, respectively. Correlation coefficient of PRA levels between two bioassay methods was  $r=0.85$  ( $p=0.005$ ). It was concluded that the new method for PRA might be more reliable and much simpler than the rat pressor assay for clinical applications.

PRA, Angiotensin II, Bioassay, Isolated cells.

### INTRODUCTION

In recent years, the radioimmunoassay of angiotensin for plasma renin activity (PRA) has been widely employed. Disadvantages of radioimmunoassay, however, may be the essential requirements of expensive materials and well equipped isotope laboratories. For measurement of angiotensin II, our new bioassay method which uses isolated bovine adrenal cells<sup>1)</sup> was presumed to be more reliable than the rat pressor assay.<sup>2)</sup> In this paper, therefore, the feasibility of clinical application of our new bioassay method was examined.

### MATERIALS AND METHOD

Materials: Nine normotensive and nineteen hypertensive patients were investigated. The hypertensives included 11 patients with essential hypertension, 5 with primary aldosteronism, 2 with renovascular hypertension, and 1 with pheochromocytoma. Blood samples from these 28 subjects were collected in supine position, usually between 8:00 a.m. and 9:00 a.m. The effect of administered furosemide in upright position was compared between normotensive and hypertensive groups. PRA levels were independently determined for 17 blood samples by two methods; usual rat pressor assay and our new bioassay method.

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Measurement of PRA: Angiotensin II was extracted by a modified procedure reported by Boucher *et al.*<sup>3)</sup> Ten ml of blood was cooled, and stirred rapidly in a 40 ml glass tube containing 1 mg of EDTA(NH<sub>4</sub>). After centrifugation at 0–5°C, the plasma was removed,

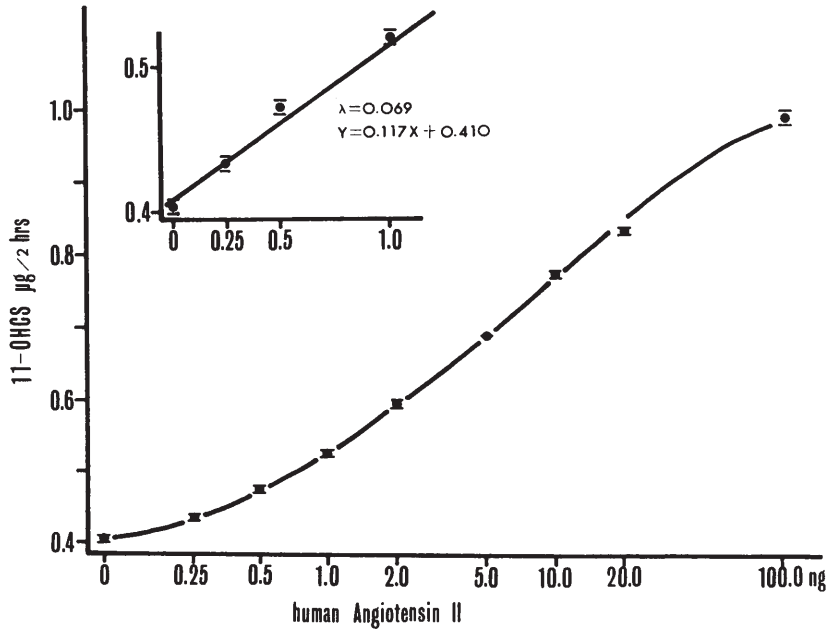


Fig. 1. Result of experiment 3 (see Table 1). The small plot at the upper left illustrates the linear relation between arithmetic values of 11–OHCS production and low concentration of angiotensin II.

Table 1. 11-OHCS production in  $\mu\text{g}$  by isolated bovine adrenal cells in response to various quantities of standard human angiotensin II (from 0.25 to 100 ng)

Ang. II	0	0.25	0.5	1.0	2.0	5.0	10.0	20.0	100.0 ng
Exp. 1	0.51	0.53	0.56	0.61	0.67	0.75	0.79	0.86	
	0.51	0.54	0.56	0.63	0.65	0.75	0.81	0.88	
Exp. 2	0.32	0.34	0.37	0.40	0.47	0.60	0.72	0.79	1.00
	0.30	0.34	0.36	0.42	0.49	0.60	0.74	0.78	1.00
Exp. 3	0.41	0.43	0.47	0.53	0.59	0.69	0.78	0.83	1.00
	0.40	0.44	0.48	0.52	0.60	0.69	0.77	0.84	0.98
Exp. 4	0.61	0.63	0.64	0.72	0.77	0.83	0.91	0.97	
	0.62	0.63	0.65	0.72	0.77	0.80	0.93	0.96	
Exp. 5	0.53	0.57	0.62	0.69	0.74	0.80	0.84	0.88	0.95
	0.53	0.57	0.62	0.67	0.72	0.79	0.84	0.87	0.96
Exp. 6	0.40	0.43	0.46	0.50	0.57	0.64	0.72	0.81	
	0.41	0.43	0.45	0.51	0.55	0.65	0.72	0.79	
Exp. 7	0.45	0.48	0.51	0.53	0.61	0.71	0.78	0.86	
	0.45	0.49	0.51	0.53	0.61	0.71	0.77	0.82	

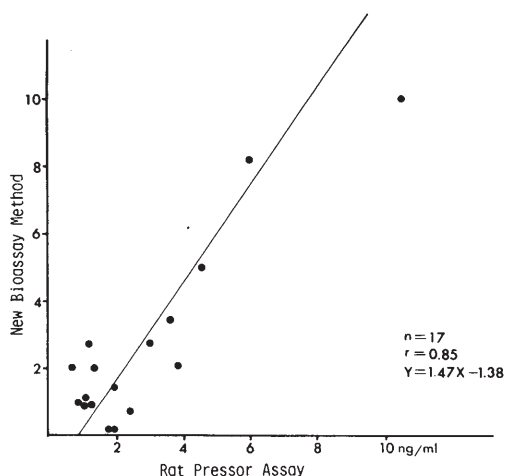


Table 2. Accuracy of 7 experiments

Exp. 1	$\lambda = 0.070$ ( $Y = 0.116X + 0.504$ )
Exp. 2	$\lambda = 0.086$ ( $Y = 0.092X + 0.318$ )
Exp. 3	$\lambda = 0.069$ ( $Y = 0.117X + 0.410$ )
Exp. 4	$\lambda = 0.079$ ( $Y = 0.123X + 0.593$ )
Exp. 5	$\lambda = 0.084$ ( $Y = 0.132X + 0.546$ )
Exp. 6	$\lambda = 0.050$ ( $Y = 0.099X + 0.405$ )
Exp. 7	$\lambda = 0.121$ ( $Y = 0.057X + 0.475$ )

Fig. 2. The levels obtained by the two assay method from the same sample.

and its volume was recorded. The plasma, to which 4 ml of moist Dowex 50WX2(NH<sub>4</sub>) resin equilibrated at pH6 by 0.2N ammonium acetate solution was adjusted to pH5.5 by addition of 1N HCl. The mixture was incubated for 3 hrs at 37°C with a mechanical shaker. Following incubation, the mixture was transferred to a chromatographic column (1 cm x 10 cm) containing 1 ml of Dowex 50WX2 resin, and was maintained at 4°C. The column was washed five times with 10 ml of distilled water, discarding all elute. Angiotensin II was eluted with 7.5 ml of 0.1N diethylamine. The elute was evaporated to dryness in a conical flask connected to a rotary evaporator. The dry residue was dissolved in 0.5 ml of 0.9% saline. Angiotensin II, thus eluted, was assayed by the method previously reported using isolated bovine adrenal cells.<sup>1)</sup>

## RESULTS

1) 11-OHCS production in  $\mu\text{g}$  by isolated bovine adrenal cells, responding to standard human angiotensin II.

Table 1 details the results of 7 assays. 11-OHCS production is related to the log of the concentration of angiotensin II by a sigmoid curve over the range 0.25 to 100 ng (Fig. 1). At low concentration of angiotensin II (0.25–1.0 ng), 11-OHCS production is related to arithmetic values of angiotensin II concentration by a straight line (Fig. 1). Table 2 summarizes the index of accuracy ( $\lambda$ ) for 7 experiments, indicating much smaller values than usually obtained by the rat pressor assay.

2) Comparison of PRA levels obtained by the new bioassay and rat pressor assay.

Figure 2 illustrates the PRA levels for 17 blood samples obtained by two bioassay methods. The PRA levels obtained by the new bioassay correlate well with those by the rat pressor assay ( $r = 0.85$ ;  $p = 0.005$ ).

3) PRA levels obtained by the new bioassay method in normotensive group (Fig. 3).

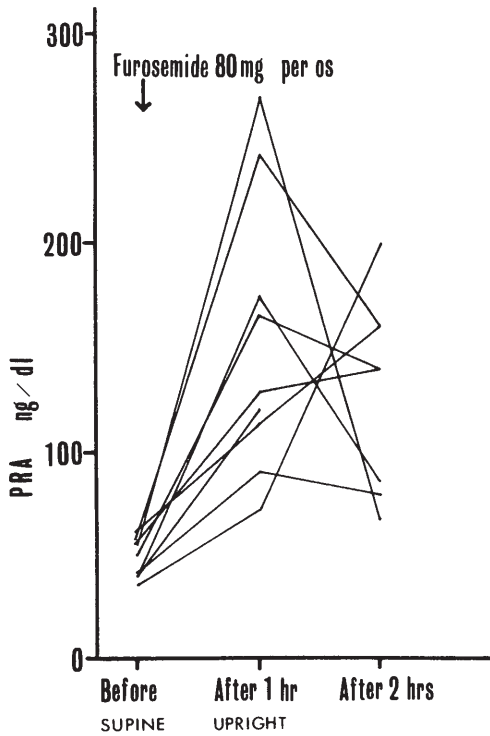


Fig. 3. The effect of administration of furosemide in upright position in normotensive group.

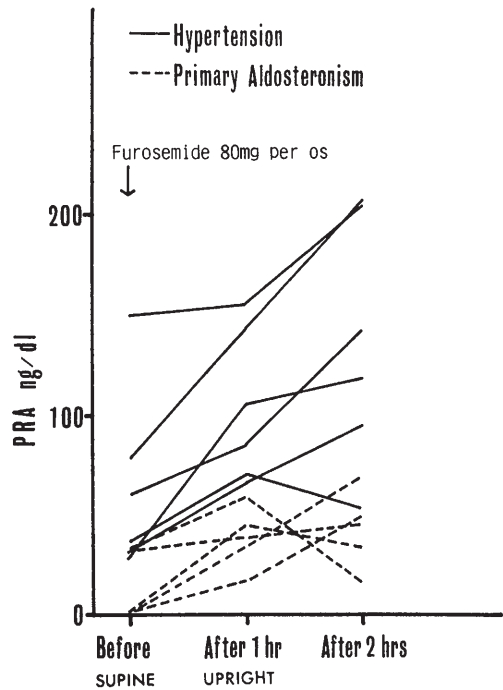


Fig. 4 The effect of administration of furosemide in upright position in essential hypertension and primary aldosteronism.

Table 3 PRA in renovascular hypertension

	case 1	case 2
peripheral vein	250	930
r-renal vein	250	1250
l-renal vein	1200	159 ng/dl
After surgery (2 months)		
	before	after 80 mg of furosemide
case 1	23	119 ng/dl

Mean PRA levels for 9 normal subjects, by our new method, are  $63.6 \pm 48.7$  ng/dl,  $177.7 \pm 99.3$  ng/dl, and  $172.3 \pm 124.3$  ng/dl at 1 hr after supine position, 1 hr after upright position with administration of 80 mg furosemide, and 2 hrs after upright position, respectively.

4) PRA levels obtained by the new bioassay method in hypertensive group (Fig. 4).

The hypertensives, excluding those due to primary aldosteronism, response very similarly to the normotensives when 80 mg furosemide is administered in upright position. Five patients of primary aldosteronism with relatively low levels of PRA do not show the elevated PRA at 2 hrs after upright position.

5) PRA levels obtained by the new bioassay method in renovascular hypertension (Table 3).

Table 3 may possibly suggest the presence of obstructed renal artery at left side in case 1 and at right side in case 2. PRA levels in case 1 recovered to normal level after surgery.

#### DISCUSSION

In endocrinological practice it is well known that the analytical method, such as radioimmunoassay, may produce misleading results. These may be due to the immunological determinants being split off, as biologically inactive fragments, from the hormone molecule, or being contained within either a prohormone or a larger but biologically less active form of the hormone.<sup>4,5</sup>) Another shortcoming of radioimmunoassay is essential requirements of expensive materials and well equipped isotope laboratories. Accordingly, a new biological microassay should be developed which would have the same sensitivity as radioimmunoassay, but eliminates these disadvantages.

The method for measurement of angiotensin II using isolated bovine adrenal cells has been previously reported by authors in 1976, where val-5-angiotensin II was used for standard curve. Human angiotensin II was used for standard curve in this experiment, by which it was shown that human angiotensin II produced more steroids than the same dose of val-5-angiotensin II. The new method was revealed to be more accurate ( $\lambda=0.050-0.121$ ), compared with the rat pressor assay. Further advantage of our method is the lack of the following methodological deficits inherent to the rat pressor assay: difficulty of maintaining the biologically stable conditions of the rats during the experiment and the possibility of including some pressor or hypotensive fragments in extracted angiotensin II. In this report ten ml of blood sample was used for extraction of angiotensin II. When using Arakawa's method reported in 1968,<sup>6</sup>) however, only 1-2 ml of blood sample is sufficient enough for this purpose. This blood volume of 1-2 ml is almost same amount necessary for the radioimmunoassay. In conclusion, therefore our new bioassay method is believed to be equally sensitive and accurate to radioimmunoassay as well as more reliable and much simpler than the rat pressor assay.

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