4. On the Role of Renin-Angiotensin System with Special Relation to Aldosterone for the Etiology of Hypertension[†]

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There are many studies on the role of renin-angiotensin system to the etiology of renal and essential hypertension, but unfortunately we don't have the definite conclusion so far. On the other hand aldosterone is known as an important factor to the etiology of hypertension and the fact that renin-angiotensin system is a main stimulating factor of aldosterone production has been recognized since the studies of DAVIS et al.¹⁾ was reported.

Therefore, recently the role of above-mentioned two factors became to be discussed earnestly and many studies on this problem were reported from various points of view on the international symposium²⁾ held at Montreal City in 1963, but still the final conclusion could not be obtained.

Recently the methods for measuring renin or angiotensin have become more and more sensitive and accurate by the purification of renin-like substance, the increase of renin-substrate's potency and the progress of the final bioassay methods, and therefore we have become to be able to follow up the detailed variations of renin-angiotensin system.

On the other hand the method for measuring of aldosterone in the small amount of biological samples has been accomplished by our modified method (1965) originated by KLIMAN & PETERSON (1959), with thin layer chromatography and some other characteristics.

In this paper we are going to report on the role of renin-angiotensin and aldosterone to the etiology of hypertension in animal experiments, after introducing our experiments reported on the other papers^{3,4)} already.

METHODS

(1) Method for estimating angiotensin level in plasma is based on the method of BOUCHER et al.^{5,6)} (1961, 1963).

(2) Method for estimating renin level in renal

venous blood plasma is based on the method of SKINNER et al.⁷⁾ (1964).

(3) Method for estimating renin level in kidney tissue of rats

(a) Extraction and purification of renin was based on the method of HAAS, LAMFROM and

TABLE I PREPARATION OF SUBSTRATES

Based on A. F. LEVER, J. I. S. ROBERTSON & M. TREE
(*Biochem. J.* 91. 346. 1964)

TABLE II INCUBATION OF RENIN-ANGIOTENSINOGEN MIXTURE AND RENIN ESTIMATION: OUR MODIFIED METHOD OF SCHAFFENBURG, HAAS & GOLDBLATT.

(*Am. J. physiol.* 199, 788, 1960)

Extracted Renin 0.1 ml + Renin Substrate 5 ml
(Renin/2 ml Saline/Kidney 1g)

↓ for 10 min at 37°C

Acidified to pH 5.2 with HCl

↓

in Boiling water 5 min

↓

Cooling in an ice bath

↓ filtrate

Supernatant
(Containing Angiotensin)

↓

0.25 ml

assayed by Rat pressor activity test

(based on W. S. PEART's method. 1954)

GOLDBLATT⁸⁾ (1954).

(b) Preparing of renin substrate: We have obtained the renin substrate with high potency from dog's plasma by the method of LEVER et al.⁹⁾

[†](Received for Publication, August 3, 1965)

(1964) (Table I).

(c) We have measured angiotensin II level by PEART's bioassay (1954) (rat pressor activities), which was produced following to the method of SCHAFFENBURG, HAAS and GOLDBLATT¹⁰(1960), using the above-mentioned purified renin and renin substrate obtained from dogs (Table II).

Angiotensin II activities produced by this method had pressor activities of 4 to 5 times after 10 minutes' incubation compared with those obtained by using the untreated dog's plasma.

Moreover we have found that our renin substrate could produce the pressor activities with excellent parallelism in high or low concentration of renin.

(4) Method for estimating urinary aldosterone of rats (above mentioned)

RESULTS AND DISCUSSION

(1) The blood plasma angiotensin II levels of patients with secondary aldosteronism are remarkably increased, as well as the dogs with ascites caused by the constriction of inferior vena cava. Urinary aldosterone are also increased apparently in those conditions.

From these phenomena we would like to suggest that first the renin secretion from kidney is

promoted in such conditions, and subsequently the aldosterone secretion from adrenal cortex is increased.

(2) It is statistically significant that the average of the blood angiotensin II levels in patients with essential and renal hypertension are a little higher than normal ones.

Furthermore, it is a very interesting problem that in essential hypertension, the higher the KEITH-WAGENER's grades are, the more the blood angiotensin II levels are increased, while urinary aldosterone and aldosterone secretion has the same increasing tendency as blood angiotensin II levels.

(3) When we compared the pressor response in the persons with secondary aldosteronism with normal ones by a single intravenous injection of 2.5 μ g synthetic angiotensin II, the former induced the remarkable inhibition of the pressor response. And also our experiments of rabbits pretreated with d-aldosterone have showed the

Unilateral Renal Artery Constriction

Fig. 1.

Renin Content in Plasma of Bilateral Adrenectomized Dogs.

Fig. 2.

same response.

(4) We have measured the renin level in the renal venous plasma of the kidney with the unilateral renal artery constriction in dogs.

Within 20 minutes after constriction, we recognized the increase of renin level in most of the dogs and the same level was maintained even after 6 hours (Fig. 1).

Until this time we could not detect the rising of blood pressure and so have found that the increase of renin secretion took place much faster than the rising of blood pressure.

(5) In the bilateral adrenalectomized dogs we have found that the prompt increase of renin level in their renal venous blood was induced (Fig. 2). The blood pressure showed about 50 mmHg fall by adrenalectomy and when the increased renin secretion was blocked by the additional nephrectomy, the blood pressure was finally fallen to 50 mmHg level and maintained this level for a long time until death (Fig. 3).

(6) Finally we have measured the renin level in the kidney tissue with unilateral renal artery constriction and the intact kidney on the other side of GOLDBLATT's hypertensive rats separately. The renin level in the kidney on the constricted side reached to maximum in 1 or 2 weeks and after 3 weeks they fell a little bit gradually and maintained the same level up to 5 weeks (Fig. 4).

On the contrary, the one in the contralateral

intact kidney was almost within normal in 1~2 weeks, but thereafter decreased remarkably. And so we would like to suggest that the urinary aldosterone excretion highly depends on the sum of the renin levels of both kidneys, because the sum of renin levels was quite parallel with the urinary aldosterone excretion.

Moreover, the renin levels of intact kidneys, the urinary aldosterone excretion and blood pressure returned promptly to normal level, when the kidneys with constricted renal arteries were removed.

SUMMARY

From our animal experiments and clinical experiences the conclusion will be as follows.

(1) The increase of both renin activities and aldosterone excretion is parallely recognized in acute stadium of hypertension of GOLDBLATT's rats. However the etiological role of both factors to hypertension is not yet clear.

(2) We must say that the pressor mechanism of renal vascular hypertension in man is still also obscure.

(3) We can not recognize the role of renin-angiotensin system and aldosterone as the etiology of essential hypertension. We suppose that hypersecretion of both factors is merely the secondary phenomenon.

(4) Both factors are increased in secondary aldosteronism. It is still obscure why they don't

Fig. 3.

Following up (each week) of Max blood pressure, renin contents of both kidneys (constricted and intact sides) and urinary aldosterone excretion of Goldblatt's rats and those following the removal of the affected kidneys.

Fig. 4.

cause hypertension.

In conclusion the essential role of renin-angiotensin system is still not clear, but they certainly work as the electrolytes regulator (especially sodium and potassium) to renal tubules directly or by means of aldosterone secretion. According to our experiments, it seems that aldosterone excretion depend entirely on the dynamics of renin-angiotensin system so far.

Acknowledgement

The authors are deeply acknowledged to Drs. Y. Hashimoto, and E. Endo, department of biochemistry and department of physiology, Nihon University Medical School for their valuable advices and all colleagues in our laboratory for their enthusiastic and continuous cooperation.

REFERENCES

1) Davis, J. O., Carpenter, C. C. J., Ayers, C. R., Hol-

- man, J. E. and Bahn, R. C., *J. Clin. Invest.* **40**: 684, 1961.
- 2) Proceedings of the International Symposium on Angiotensin, Sodium and Hypertension, *Canad. Med. Assoc. J.* **90**: No. 4, 1964.
- 3) Oda, T., *Jap. J. Nephrol.* **7**: 9, 1965.
- 4) Oshima, K., Oda, T. et al., Proc. III Asian-Pacific Congress of Cardiology (held on May, 1964, Kyoto, Japan) **1**, 475, 1964.
- 5) Boucher, R., Biron, P. and Genest, J., *Canad. J. Biochem.* **39**: 581, 1961.
- 6) Boucher, R., Veyrat, R., de Champlain, J. and Genest, J., *Canad. Med. Assoc. J.* **90**: 194, 1964.
- 7) Skinner, S. L., McCubbin, J. W., and Page, I. H., *Circulation Res.*, **15**: 64, 1964.
- 8) Haas, E., Lamfrom, H. and Goldblatt, H., *Arch. Biochem. & Biophys.* **43**: 256, 1954.
- 9) Lever, A. F., Robertson, J. I. S., Tree, M., *Biochem. J.* **91**: 346, 1964.
- 10) Schaffenburg, C. A., Haas, E. and Goldblatt, H., *Am. J. Physiol.* **199**: 788, 1960.