Changes of Plasma Renin Substrate Concentration in Rats Under Various Experimental Conditions*

K. Hiwada, K. Nishimura, and T. Kokubu

It is well known that the concentration of renin substrate in plasma increases following nephrectomy, renal damage or estrogen treatment. The mechanism responsible for the increase in plasma renin substrate might be due to increased rate of renin substrate synthesis by the liver.

In this presentation regulatory mechanism of renin substrate concentration in plasma was studied under various experimental conditions in rats with special reference to plasma renin concentration.

The female Wister rats, weighing 200 to 230 g, received a commercial rat diet and tap water ad libitum. Heparinized plasma was collected under light pentobarbital anesthesia from the inferior vena cava after the ligation of bilateral renal arteries and veins or the remaining renal artery and vein to avoid the further release of renin during bleeding. Rat renin and renin substrate were prepared by the methods described previously (Kokubu, Hiwada and Yamamura. Pflügers Archiv 358: 303, 1975). The renin preparation had a pressor activity equivalent to 550 ng of synthetic Val(5)-angiotensin II amide (Hypertensin CIBA) per mg of protein when assayed directly in the rat anesthetized with pentobarbital, treated with pentolinium and vagotomized. The renin substrate preparation contained 120 ng of angiotensin content per mg of protein. The plasma renin substrate concentration was estimated from the release of angiotensin after incubation of plasma in the presence of an excess of rat renin (Kokubu, Hiwada and Yamamura. Pflügers Archiv 358: 303, 1975). Plasma renin concentration was determined according to the modified method of Boucher et al. (Boucher, Ménard and Genest. Canad. J. Physiol. Pharmacol. 45: 881, 1967). In the incubation mixtures for the estimation of plasma renin concentration, the renin substrate was added to provide an excess of substrate (more than 200 ng of angiotensin content per ml). Angiotensin I formed in the reaction mixtures was assayed by bioassay using rat. The pressor substance was identified as angiotensin I by radioimmunoassay for angiotensin I. Each experimental group consisted of 10 rats. Results obtained were as follows.

(1) In normal rats a mean plasma renin substrate concentration was 374 ± 6.6 (S.E.) ng angiotensin II per ml and a mean plasma renin concentration was 24.55 ± 2.41 (S.E.) ng angiotensin II per ml per hour.

(2) In unilateral nephrectomized rats the concentration of renin substrate in plasma increased maximally 3.5-fold within 24 hours after the operation and on the third day it decreased to about half of the maximum level. Twenty four hours after unilateral nephrectomy the plasma renin concentration decreased to about one third of normal level. The decreased concentration of plasma renin, however, returned to normal 3 days after uninephrectomy.

(3) The maximum increase in plasma renin substrate concentration after bilateral nephrectomy was more than 17 times higher than the preoperative level 24 hours later. However, when one kidney was removed and then 10 days later the remaining kidney was removed, the maximum increase of renin substrate was found only 6 times higher than the normal concentration. While plasma renin concentration in each group was similar, being about one hundredth of normal rats.

(4) Twenty four hours after bilateral ureteral ligation the increase of plasma renin substrate

The 2nd Department of Internal Medicine, Ehime University School of Medicine, Shigenobu, Onsen-gun, 791-02 Ehime, Japan

* This work was supported in part by a grant No.077267 from Ministry of Education.

This paper was presented at the V Conference on the Pathogenesis of Hypertension, December 7, 1975, Osaka.

Japanese Circulation Journal Vol. 40, August 1976 945
concentration was about 6 times of normal level. When one kidney was removed and the ureter of the remaining kidney was ligated at the same time, the increase of renin substrate in plasma was similar to that of the simultaneous bilateral ligation of the ureter 24 hours after the operation. In rats subjected to unilateral nephrectomy and then ligated the ureter of the remaining kidney 10 days after the first operation, the renin substrate in plasma increased equally as above two experimental groups. On the other hand, the plasma renin concentration in each group was changed quite differently. In simultaneous bilateral ligated rats, the plasma renin concentration was within normal level. In rats which were removed one kidney and ligated the ureter of the remaining kidney simultaneously, the renin concentration in plasma decreased about one fifth of the normal level. When one kidney was removed and 10 days later the ureter of the remaining kidney was ligated, the level of plasma renin concentration was three fifth of intact rats.

(5) Rats were received on injection of 17 β-estradiol (0.1, 0.5, 1.0 or 2.0 mg) and their blood samples were taken 24 hours later. Increase of plasma renin substrate by 17 β-estradiol was dose related and was reached maximunly at the dose of 1.0 mg of 17 β-estradiol. In normal rats received 1.0 mg of 17 β-estradiol, plasma renin substrate increased about 4 times 24 hours later. In (10 days previously) unilaterally nephrectomized rats, 10 mg of 17 β-estradiol injection caused about 4 times increase of renin substrate in plasma 24 hours later as well. Rats which were nephrectomized unilaterally (10 days previously) were given 1.0 mg of 17 β-estradiol injection and removed the remaining kidney simultaneously. After 24 hours the plasma renin substrate concentration reached 10 times higher level than normal, showing the summation of the separate effects of nephrectomy and estrogen treatment. In above two groups of rats which had intact kindneys or were nephrectomized unilaterally, the plasma renin concentration was supressed to half of normal level by 17 β-estradiol.

In conclusion these results indicate that the renin in plasma might not be a major factor regulating the renin substrate concentration in plasma. Level of renin substrate in plasma is regulated in large part by corticosteroids, mostly glucocorticoids released by ACTH (Hasegawa et al. Am. J. Physiol. 225: 1, 1973). By pathologic stimulations such as high dose of estrogen treatment or renal damage due to ureteral ligation the biosynthesis of renin substrate in liver might be accelarlated to increase renin substrate in plasma like other acute phase reactive proteins. Furthermore, in acute anephric state biosynthesis of renin substrate in liver might be out of normal control. From the point of view of inter-relation between liver and kidney this phenomenon is very interesting.

Discussion:


Dr. T. TAKEDA: It has been suggested that the level of circulating angiotensin II might be a stimulating factor for the production of renin substrate. Is there any possibility that the substrate-elevating response could depend on the difference of endogenous angiotensin levels between the two types of operative procedures?

Dr. K. HIWADA: I don't think that there is any difference in endogenous angiotensin levels between the two types of operative procedures. Because we have reported that on the 10th day after unilateral nephrectomy, the plasma renin concentration was within normal level.

Dr. T. SARUTA: Is there any difference in protein components between renin substrate increased by estrogen and that by nephrectomy?

Dr. K. HIWADA: We are studying it.

Dr. K. YAMAMOTO: My question is the mechanism of the increasing of renin substrate level in the plasma after nephrectomy. You pointed out that the plasma renin dose not influence on the synthesis of renin substrate. Do you think the kidney contains any substance which suppress the synthesis of renin substrate in the liver?

Dr. K. HIWADA: The answer to your question is "Yes". Our results can be explained if we hypothesize the existence of any renal suppressor against the synthesis of renin substrate in the liver.

Dr. K. YAMAMOTO: Is there any kind of plasma protein which is stimulated their synthesis as well as renin substrate following the procedure of nephrectomy or estrogen injection?

Dr. K. HIWADA: It has been reported that corticosteroid-binding protein, ceruloplasmin, α1- antitrypsin, fibrinogen, haptoglobin, orosomucoid, C-reactive protein, etc. were increased by estrogen treatment. I don't have data about these plasma protein after nephrectomy.

Japanese Circulation Journal Vol. 40, August 1976