The Kidney Factors in the Production of Acute Hypertensive Vascular Lesions

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MANY reports show that the kidney plays an important role in the production of the hypertensive vascular disease.¹⁻¹⁰ It has not been settled, however, whether renal pressor material(s) alone or some additional renal factor(s) plays a role in the development of this type of vascular disease.

The present experiment was designed to observe acute vascular changes elicited in anephric rats by the injection of subcellular fraction of renal cortical extract.

MATERIALS AND METHODS

Wistar-King strain female rats were used in this experiment. The step-wise centrifugation method by *Shibko* and *Tappel*¹¹ was used to obtain subcellular fraction of the kidney cortical extract. Nuclear, lysosomal, mitochondrial, mitochondrial-microsomal, microsomal and soluble fraction could be fractionated. Acid-phosphatase activity was the highest in lysosomal fraction, cytochrome c oxidase activity was in microsomal fraction and glucose-6-phosphatase activity was in microsomal fraction.^{1,2} Renin content of each fraction was determined by the method of *Conradi et al.*^{1,3} and expressed by equi-pressor amount of synthetic angiotensin II.²

These subcellular fractions were intraperitoneally injected into 6 hours-nephrectomized rats. Eighteen hours later, they

were sacrificed by exsanguination from the aorta. Vascular lesions in pancreatic arteries, pleural effusion and pancreas edema were examined. For the histological examination, HE, PAS, PTAH staining procedure and the direct method of tissue immunofluorescein antibody technique, using fluorescein conjugated anti-rat serum rabbit gammaglobulin,^{1,4} were used.

As for the control, physiological saline was injected. Neither vascular lesion nor tissue edema was observed.

Mean arterial pressure in the experimental rats was continuously recorded in unanesthetized state.^{1,5} Synthetic angiotensin II(CIBA) was continuously infused through juglar vein.

RESULTS

1) Renin content in subcellular fraction of renal cortical extract obtained from normal kidney: It was highest in Lysosomal fraction (1.10 ± 0.25 $\mu\text{g}/\text{mg}$). It was 0.60 ± 0.12 in mitochondrial fraction and 0.60 ± 0.11 $\mu\text{g}/\text{mg}$ in soluble fraction. Renin activity could be undetectable in microsomal fraction.

2) By the intraperitoneal injection of lysosomal fraction (2.5 mg/kg), the mean arterial pressure in unanesthetized anephric rats was continuously elevated for 6 hours, then gradually returned to the former level within 4 hours. (Fig. 1) Fibrinoid necrosis in pancreatic arteries, pancreas edema and pleural effusion could be induced by this fraction. The pathological changes were more marked when the dose of the fraction injected was increased to 5 and 10 mg/kg.

On the contrary, microsomal fraction could not induce blood pressure elevation and patho-

Key Words:

Kidney extract
Lysosomal fraction
Angiotensin II
Fibrinoid necrosis

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This paper was presented at the III Conference on the Pathogenesis of Hypertension, November 18, 1973, Tokyo.

TABLE I FIBRINOID NECROSIS, PANCREAS EDEMA AND PLEURAL EFFUSION IN ANEPHRIC RATS INJECTED WITH MICROSOMAL FRACTION

Dose injected (mg/kg)	Fibrinoid Necrosis	Pancreas Edema	Pleural Effusion (ml)
2.5	—	—	0
5	—	—	0
10	—	—	0
20	—	—	0.4
30	±	—	0.3
50	±	—	0.3
100	+	±	1.0
200	+	+	2.5

logical changes when it was administered by the dose comparable to that of lysosomal fraction.

3) Synthetic angiotensin II, 0.08 $\mu\text{g/kg/min}$, equi-pressor dose to 2.5 mg/kg of lysosomal fraction, was infused intravenously. Fibrinoid necrosis was definitely more severe in the rats injected with lysosomal fraction than in those with angiotensin II (Fig. 2).

4) Blood pressure did not change in the rats injected with microsomal fraction, even of a much larger dose up to 200 mg/kg. A larger dose of this fraction could, however, produce not only fibrinoid necrosis but also pancreas edema and pleural effusion (Table I).

Any changes could not be produced by the injection of 200 mg/kg of other tissue extract, such as liver, spleen, heart, and brain and bovine serum albumin.

5) The smallest dose of microsomal fraction in this experiment, 2.5 mg/kg, could not produce any pathological changes. However, combined administration of that dose (2.5 mg/kg) of this fraction and synthetic angiotensin II (0.08 $\mu\text{g/kg/min}$) produced a very severe fibrinoid necrosis, pancreas edema and pleural effusion. Microsomal fraction plus saline (i.v.) or saline (i.p.) plus angiotensin II could not cause these pathological changes.

DISCUSSION

It was said that the kidney extract which contained the high renin activity could induce vascular lesion, tissue edema and serous effusion. The pressor effect in the extract was thought to be most effectively working in the production of such lesions. In our present experiment, lysosomal fraction had the highest renin content and produced the pathological changes by the most smallest dose. The result might support the former reports^{2,4,6,16} But the finding that the vascular lesion was more severe in the rats injected with lysosomal fraction than in those with equi-pressor amount of angiotensin II means that the lysosomal fraction contains some other factor(s) to produce vascular changes.

Moreover, a larger dose of microsomal fraction produced vascular lesion, pancreas edema

EFFECTS ON ARTERIAL PRESSURE OF LYLOSOMAL FRACTION IN UNANESTHETIZED ANEPHRIC RAT

Fig.1.

FIBRINOID NECROSIS IN ANEPHRIC RATS INJECTED WITH LYSOSOMAL FRACTION OR SYNTHETIC ANGIOTENSIN II

Fig.2.

and pleural effusion without causing blood pressure elevation. This shows that some factor(s) other than renin exist in the microsomal fraction to produce vascular changes. A factor to increase vascular permeability may be responsible for the changes.

Combined administration of smaller dosis of microsomal fraction and angiotensin II produced the similar pathological changes as lysosomal fraction. This means that the factor(s) in microsomal fraction may produce the pathological changes when it acts together with the pressor materials.

Identity of the factors in the lysosomal fraction and microsomal fraction is not clear at the present.

CONCLUSION

The kidney extract contains some factor(s) other than renin to produce vascular disease. The factor(s) will facilitate the production of vascular lesion as well as tissue edema and serous effusion when it acts together with the pressor materials.

Acknowledgement

This study was supported in part by KANAE Foundation of the New Medicine Research Association.

REFERENCES

1. WINTERNITZ, M. C., MYLON, E., WATERS, L. L., & KATZENSTEIN, R.: Studies on relation of kidney to cardiovascular disease. *Yale J. Biol. Med.* 12: 623, 1940.
2. NAIRN, R. C., MASSON, G. M. C., & CORCORAN, A. C.: The production of serous effusions in nephrectomized animals by the administration of renal extracts and renin. *J. Path. Bact.* 71: 155, 1956.
3. ASSCHER, A. W. & ANSON, S. G.: A vascular permeability factor of renal origin. *Nature* 198: 1097, 1963.
4. GIESE, J.: Pathogenesis of vascular disease caused by acute renal ischaemia. *Acta Path. Microbiol. Scand.* 59: 417, 1963.
5. MASSON, G. M. C., KASHII, C., PANISSET, J., YAGI, S., & PAGE, I. H.: Production of hypertension and vascular disease by kidney extracts. *Circ. Res.* 14: 150, 1964.
6. CUTHBERT, M. F., ASSCHER, A. W., & HENRY JONES, J.: Characterization of a vascular permeability factor of renal origin. *Clin. Sci.* 31: 325, 1966.
7. NAKAO, K., IKEDA, M., FUJII, J., TERASAWA, F., KURIHARA, H., KIMATA, S., MATSUSHITA, S., & YAMAGUCHI, H.: Acute vascular lesions produced by selected non-pressor renal cortical extracts. *Jap. Circ. J.* 30: 539, 1966.
8. ONOYAMA, K., HATTORI, N., OMAE, T., & KATSUKI, S.: Vascular lesions produced in bilaterally nephrectomized rats by injection of fractionated renal cortical extracts. *J. Jap. Coll. Angiol.* 11: 163, 1971.
9. KIRA, J., SAITO, N., MATSUNAGA, M., OGINO, K., & TAKAYASU, M.: Hemoconcentrating substance and vascular permeability factor from rat kidney lysosomes. *Jap. Circ. J.* 32: 1, 1968.
10. NAMBA, S., HIRAMATSU, Y., KUSUNOKI, F., OKUDA, K., & SOKABE, H.: Partial purification of a transcellular-fluid-forming factor from the renal cortex. *Jap. Circ. J.* 32: 1585, 1968.
11. SHIBKO, S. & TAPPEL, A. L.: Rat-kidney lysosomes: Isolation and properties. *Biochem. J.* 95: 731, 1965.
12. ONOYAMA, K., HARA, M., TANAKA, K., & OMAE, T.: Renin content in subcellular fractions of normal rat kidney extract. *Jap. Heart J.* 14(5): 440, 1973.
13. CONRADI, K., JELINEK, J., & GROSS, F.: Strain and sex differences in renin content of rat kidneys. *Proc. Exp. Biol. Med.* 132: 984, 1969.
14. HAMASHIMA, Y. & KYOGOKU, M.: Immunohistology. 1st. Ed., Igaku Shoin, Tokyo, 1965.
15. ONOYAMA, K., FUKIYAMA, K., TANAKA, K., & OMAE, T.: The blood pressure measurement in unanesthetized rat *Igaku no ayumi* 85: 560, 1973 (in Japanese).
16. GIESE, J.: Deposition of serum proteins in vascular walls during acute hypertension. *Acta Path. Microbiol. Scandinav.* 53: 167, 1961.

Discussion:

Chairman: Dr. M. IKEDA, Tokyo Univ.

Dr. N. SAITO (Kyoto Univ.): Was there any difference in the grade of elevating blood pressure between injection of lysosomal fraction and angiotensin II?

Dr. K. ONOYAMA: A high blood pressure has been continued for around 8 hours after injection of both lysosomal fraction and angiotensin II. Thereafter, the blood pressure gradually returned to normal.

Dr. N. SAITO: How was acid phosphatase activity in microsomal fraction? Wasn't there any contamination of lysosome into microsomal fraction?

Dr. K. ONOYAMA: Purification of microsomal fraction was made by centrifuging at 105000G the supernatant which was obtained by centrifuging at 6600G. I think there is little contamination of lysosome.

Dr. K. YAMAMOTO (Osaka City Univ.): Did you examine the renin in tissues or within cells?

Dr. K. ONOYAMA: No. I can not give you any information from our data.

Dr. H. UEDA (Tokyo Univ.): Are there the other physiological activities in angiotensin II, although you used, angiotensin II only as a pressor substance. What do you think about that the same results could be expected in case of applying norepinephrine instead of angiotensin II?

Dr. K. ONOYAMA: We used angiotensin II because the rise of blood pressure following injection of a renal extract was thought to be due to angiotensin II came from renin. However, other investigators use norepinephrine as a control pressor substance as well as angiotensin II. Both substances would be available for the examination of pressor activity.

Dr. S. HAYASE (Gifu Univ.): Can you tell me about fibrin in the acute arterial lesion, fibrinoid necrosis of small arteries?

Dr. T. ETO (Kyushu Univ.): There are electron-microscopic studies on fibrin in the acute arterial lesion, fibrinoid necrosis, by Hütter, Sprio, Wiener, Aikawa, in other countries and Ooneda in Japan. The fibrinoid substance of arterial walls observed in the light microscope consists of stria-

tions having a cristal structure with periodicity of 160 to 220 Å by electronmicroscopy.

Dr. J. FUJII (Asahi Adult Inst.): Were the distribution of acute vascular lesions in the recipient rats following injection of renal extracts the same as that in hypertensive rats?

Dr. K. ONOYAMA: We could find the fibrinoid necrosis in small arteries in pancreas, lung, mesenterium, heart but could not find it in the brain as far as examined.

CHAIRMAN: Acute arterial lesions including fibrinoid necrosis of small arteries were widely distributed in the organs in hypertensive animals. As far as the pathogenesis of the acute vascular lesion concerned, it is considered that the acute vascular lesion develops partly due to a mechanical factor of high blood pressure and partly due to humoral factors such as a certain vasculotoxic substance from ischemic renal cortex. Two kinds of substances have been postulated as humoral factors of renal origin. One is renin and the other is non pressor fractions from ischemic renal cortex. Authors suggested for the first time that both of them would contribute for development of the acute arterial lesion, fibrinoid necrosis referred with high blood pressure. Mechanism of the development of acute arterial lesions by these vasculotoxic substances is not clear yet. These substances seem to accelerate the permeability of plasma component from the blood into vascular walls although these substances might injure directly the tissues of vascular walls.

Further studies should be made concerning with the following points.

1. Identification of a nonpressor vasculotoxic substance, for example, molecular weight, heat stability and dializability etc.

2. Method of quantitative measurement of vasculotoxic activity which induces acute arterial lesions.

3. Specificity in races of animals.

4. Application of this concept to human essential hypertension especially concerned with pathogenesis of cerebral hemorrhage in hypertension.