Hypertension-Inducing Potency and Renin Content of Variously Treated Kidney Extract

Jin Yamamoto, M.D.,* Katsuya Ohnishi, M.D.,** Jun Kira, M.D.,* Nobuya Konishi, M.D.,** Kaname Yamatori, M.D.,** Masato Matsunaga, M.D.,* and Koichi Ogino, M.D.*

SUMMARY

Kidney extract from rats which were adrenalectomized and given tap water was dialyzed, salted out, ultrafiltrated or heated. A given dose of each extract or fractionized material was administered to uninephrectomized rats subcutaneously every 12 hours for 10 days. The relationship between renin content of each sample and final blood pressure level following repeated injections as an index of its hypertension-inducing potency was analyzed. There was no apparent discrepancy between the two of each sample. No evidence was obtained for the existence of other renal substance than renin which might be implicated in producing hypertension.

Additional Indexing Words:
Adrenalectomy  Experimental hypertension  Renal pressor substance

AN experimental hypertension, which closely mimics renovascular hypertension, could be induced by repeated injections of some preparations with high renin content.1,2) However, Kira et al3) observed a discrepancy between renin content and hypertension-inducing potency of various kidney extracts. Matsunaga et al4) also demonstrated that the hypertension-inducing effect of kidney extract was not always parallel with its renin content as well as plasma renin level of recipient rat. One possible explanation for this discrepancy would be an involvement of other renal substance than renin in this hypertension. The present study was undertaken to explore the probability of the existence of such a substance by analyzing the relationship between renin content and hypertension-inducing potency of variously treated kidney extract.

MATERIALS AND METHODS

Closely inbred Wistar rats from Animal Center of Kyoto University were used. Recipients of injection experiments were male rats weighing about 100 Gm, which were given a regular diet (Oriental) and tap water. Kidney extract was

* Third Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto.
** Department of Internal Medicine, National Himeji Hospital, Himeji.

Received for publication October 21, 1974.
prepared from adrenalecotorized rats given tap water. This extract has been found to have a potent hypertension-inducing action for its renin content.\textsuperscript{3,4} Methods for preparation of kidney extract and determination of renin content were presented in a previous paper.\textsuperscript{3} Renin content was expressed as microgram of angiotensin II equivalent produced by 1 ml of a sample for 15 min.

\textbf{Dialysis:} Two hundred and forty ml of kidney extract was divided into 2 parts. One part was dialyzed in Visking cellophane tube (20/32) against 5 changes of 4 L amounts of 0.9\% saline.

\textbf{Salting out:} One hundred and twenty Gm of ammonium sulfate was added to 300 ml of kidney extract (final concentration of ammonium sulfate was 40\%(w/v)). The mixture was allowed to stand for 12 hours at pH 7 and centrifuged at 10,000 G for 30 min. The supernatant was retained (Fraction I). The precipitate was suspended in a small amount of 0.9\% saline (Fraction II). Each fraction was dialyzed in Visking cellophane tube (20/32) against 6 changes of 4 L amounts of 0.9\% saline. Heavy precipitates in the material were removed by centrifugation.

\textbf{Ultrafiltration:} Through Diaflo-membrane XM-100 or UM-10 (Amicon, USA), 100 ml of Fraction II solution obtained by salting out was ultrafiltrated in a stirred pressure cell (Model 50 or 401) until the volume of the inside fluid reduced to about 20 ml.

\textbf{Heating:} The kidney extract was heated at 45°C or 55°C at pH 7 in a water bath for 30 min, or at 100°C in boiling water for 10 min. After centrifugation at 10,000 G for 30 min, precipitates were discarded.

Other procedures were performed below 4°C.

\textbf{Protocols of injection experiments:} Rats were uninephrectomized a few days before injection and divided into

![Blood pressure curves of 2 groups of rats receiving the non-dialyzed and dialyzed kidney extract. The symbols represent blood pressure (mean±standard deviation).](image)
several groups. Each fraction or treated kidney extract was adjusted to the volume of the starting material by addition of 0.9% saline. One to 1.15 ml of each sample was administered to a group of 4–6 rats subcutaneously every 12 hours for 10 days. The blood pressure was measured prior to injection every morning by plethysmography. Hypertension-inducing potency of a sample was evaluated on the basis of final blood pressure level following repeated injections. Statistical analyses were performed according to Student’s t-test.

**Results**

*Dialysis:* Courses of blood pressure in 2 groups of rats receiving the injections of the non-dialyzed and dialyzed extract were illustrated in Fig. 1. Blood pressure was gradually increased to a definitive hypertensive level.

![Graph showing blood pressure and renin content](image)

**Fig. 2.** Effect of dialysis on hypertension-inducing potency of the extract. Solid circles indicate the blood pressure levels on the 10th day of treatment. Horizontal lines show the mean of blood pressure. Hatched columns represent the renin content of each extract.
Throughout the experimental period, no significant difference was found in the blood pressure of both groups. Fig. 2 indicates the relationship between hypertension-inducing potency and renin content of 2 extracts. There was no evident difference between the two in either renin content or final blood pressure level attained.

*Salted out:* Results from administration of Fraction I (ammonium sulfate supernatant) and Fraction II (precipitate) were shown in Fig. 3. The renin content of Fractions I and II was 1.47 µg/ml/15 min and 21.8 µg/ml/15 min, respectively. The final blood pressure level attained by Fraction II was 195±15 mmHg (mean±standard deviation), while it was 114±6 mmHg by Fraction I. The difference was highly significant (p<0.001). Namely, hypertension-inducing potency was found only in the fraction with high renin content.

*Ultrafiltration:* The data obtained by XM-100 membrane separation
were shown in Fig. 4. The renin content of the rejected fluid and the filtrate was 30.2 \( \mu g/ml/15\) min and 8.7 \( \mu g/ml/15\) min, respectively. The final blood pressure level was 174±12 mmHg by the rejected fluid, significantly higher (\( p < 0.01 \)) than that by the filtrate (115±6 mmHg). Most of renin—its molecular weight is about 40,000—was retained by XM-100 membrane with cutoff at 100,000 M.W., suggesting that it might have no specific rejection characteristics and be unsuitable for the application to relatively thick solution like kidney extract. The use of UM-10 membrane with cutoff at 10,000 M.W. yielded similar results.

**Heating:** As shown in Fig. 5, the renin content of the extract without treatment and the extract heated at 45°C and 55°C was 20 \( \mu g/ml/15\) min, 18 \( \mu g/ml/15\) min, and 0.4 \( \mu g/ml/15\) min, respectively, and not detectable when heated at 100°C. Final blood pressure level seemed parallel with the renin content of each extract.
DISCUSSION

The observations in this study revealed that final blood pressure levels following repeated injections of the treated or fractionized kidney extract were approximately parallel with its renin content. In other words, there was no evident discrepancy between hypertension-inducing potency and renin content of the materials. Therefore, the hypertension-inducing effect of kidney extract appeared to be attributed to renin, as assumed by Masson et al.\(^1\),\(^2\) Matsunaga et al\(^4\) found that plasma renin concentration of rats receiving the same kind of extract as used in the present study—measured 12 hours after the injection—was significantly higher than that of controls on the 6th and 10th day of treatment. This result also suggests a culpability of renin in the development of hypertension. However, it was observed at the same time that the blood pressure of the recipients receiving 2 other kinds of extracts, which had almost the same renin content, was elevated without any increase in
plasma renin level. Furthermore, considering the impurities of the materials used, it is possible that kidney extract contains multiple active principles which might affect or elicit hypertension. Thus, a causality of renin in this model still remains open to question.

Dahl et al., utilizing parabiosis and, more directly, chronic renal homograft in rats with different predisposition to hypertension, showed that the kidney of a sensitive rat, whose plasma and renal renin levels were lower than those of a resistant one, produced a hypertensinogenic influence. Kawabe et al. observed that hypertension was transferred to nephrectomized rats by transplantation of an infarcted kidney, though its renin content was one-third of that in controls. In addition, it should be noted that renin antibody (its antigen is crude) is more effective than angiotensin inhibitors in remitting hypertension. All these data suggest the implication of renally derived substance other than renin in experimental hypertension. Fasciolo et al. and Grollman et al. reported new acute substances of renal origin. Since either substance is heat-stable and lacking in any evidence for hypertension-inducing action, its involvement in the hypertension produced in this study could be excluded. It is unlikely that angiotensin in the kidney extract, if any, contributes to this hypertension, because the hypertension-inducing potency was unaffected by dialysis and inactivated by heating at 100°C.

In conclusion, no discrepancy between hypertension-inducing potency and renin content of variously treated kidney extract could be recognized in this study. Since the methods for separation used here were relatively simple, the probable existence of renal substance other than renin implicated in this hypertension could not be denied. And further studies using more sophisticated methods are necessary.

REFERENCES


