PRELIMINARY COMMUNICATION ON THE DISCREPANCY
BETWEEN RENIN ACTIVITY AND HYPERTENSION-INDUCING
POTENCY IN RAT KIDNEY EXTRACT
DUAL RENAL PRESSOR SUBSTANCE HYPOTHESIS

JUN KIRA, KATSUYA OHNISHI,
JIN YAMAMOTO, NOBUYA KONISHI,
KANAME YAMATORI,* AND TAKASHI KAMIGUCHI**

DURING the last thirty years, detailed investigations have accumulated on the pathogenetic role of the renin-angiotensin system for renal hypertension. It has yet to be ascertained whether or not the renin-angiotensin system plays a primary role in both initiation and maintenance of experimental renal hypertension, although humoral mechanism of renal origin is generally considered responsible for the elevation of blood pressure in this type of hypertension. Therefore, it can be presumed that the kidney has at least one pressor substance other than renin, this playing a more important pathogenetic role than renin. Since 1968 efforts have been made to determine this presumed factor¹ and although experiencing some disappointing results, of interest is the fact that hypertension-inducing potency in kidney extract does not run parallel with the renin activity.

MATERIALS AND METHODS

Kidney Extract

Three kinds of kidney extract were prepared from Wistar strain rats weighing 180 to 250 g.

Extract I: Male rats were adrenalectomized bilaterally under thiopental anesthesia. Following surgery they were fed regular food and tap water for six days, then bled under ether anesthe-

sia. The kidneys were removed and homogenized in equal amounts of saline (g/ml). The homogenate was freeze-thawed ten times, centrifuged at 10^4 G for 30 mins and supernatant obtained.

Extract II: Male and female rats were fed on rice and deionized water for 7 days in order to restrict salt intake, sacrificed and kidneys removed. Supernatant, prepared as described above was acidified to a pH 3 by dropping 5 N HCl solution, then neutralized with 5 N NaOH. Denatured protein was sedimented by centrifugation and supernatant was obtained.

Extract III: Kidney extract of normal female rats was prepared in the same way as Extract II.

Determination of Renin Activity

A test tube contained 1 ml of phosphate buffer (0.15 M, pH 6.5) which contained 0.002% thimerosal, 0.5 ml of one hundredfold diluted kidney extract, 1 ml of plasma from bilaterally nephrectomized rats containing 5 mM EDTA- Na_2 , 0.5 ml of 0.01 M EDTA- Na_2 solution and one drop of 5% DFP in a total volume of 3 ml. After incubation at 25°C from 15–30 minutes, the reaction was stopped by heating the test tube in boiling water for 10 mins, the denatured protein then being removed by centrifugation. Angiotensin formed was bioassayed by the bracket method in the rat, using synthesized angiotensin II (Ciba-Geigy) as a standard. Renin activity was expressed in micrograms of angiotensin formed in the test tube.

Key Word: Renal Hypertension
Renin-Angiotensin System

(Received for publication, December 13, 1971)

* Department of Internal Medicine, National Himeji Hospital

** Department of Research and Clinical Laboratory, National Himeji Hospital, Himeji

Address reprint requests to: Dr. Jun Kira, National Himeji Hospital 68 Hommachi, Himeji, Japan.

Injection of Kidney Extract and Determination of Blood Pressure

Female Wistar rats weighing about 100 g were divided into four groups and uninephrectomized. One milliliter of kidney extract was injected subcutaneously at 12 hr intervals for 10 days. Arterial pressure was measured by tail sphygmography once a day prior to injection. At the end of experiment the animals were sacrificed by bleeding under ether anesthesia. Organs were weighed, and fixed in 10% formalin solution for histological examination.

RESULTS

Fig.1 shows the elevation courses of arterial pressure in the four groups, number of animals in each group, and heart, body wt. ratios. Blood pressure of the treated rats began to rise after a latent period of 4 to 6 days, was most marked in Extract I group, slightest in Extract III group and intermediate in Extract II group. No elevation in arterial pressure was found in the control group injected with saline. Ratio of heart to body wt. in each group to which the kidney extract was administered significantly increased as compared to the control group ($P < 0.005$). Further, ratios in Extract I and II groups were significantly higher than the Extract III group ($P < 0.02$). The grade of cardiac hypertrophy was therefore in parallel with that of the blood pressure elevation. Histological examination on the various organs of hypertensive animals revealed cardiac microinfarction, medial hypertrophy in the arterial wall and plasmatic vasculosis in arterioles. Renin activity of the three kinds of kidney extract is

Fig.1. Curves of the mean arterial pressure of rats injected with extracts of rat-kidneys. adrenalex.: Extract I, Na restricted: Extract II, normal: Extract III, 0.9% NaCl: physiological saline. Number in parenthesis indicates number of experimental animals in each group. Heart wt. to body wt. ratios are also shown (mean $\pm$ S.E.M.).

shown in Fig.2. Enzyme activity was highest in Extract II and lowest in Extract I.

DISCUSSION

The course of blood pressure elevation as well as vascular lesions in the experimental rats following injection of kidney extract was very similar to that seen in the acute phase of renal-clip hypertension. Therefore, a diagnosis of hypertensive cardiovascular disease can be made and may well be a model of renovascular hypertension. No correlation between hypertension-inducing potency and renin activity in the kidney was found. This fact indicates that hypertension following injection of the kidney extract cannot be explained in terms of the renin-angiotensin system alone. It is considered that an undetected factor(s) in the kidney extract influences blood pressure. Concerning the mode of action of this factor(s), there are two possibilities. Firstly, if the renin-angiotensin system plays a primary role in the development of hypertension following injection of kidney extract, then an antagonist to the system is more abundant in Extract III than in Extract I so as to suppress the hypertensinogenic effect of the renin-angiotensin system. Secondly, an unknown pressor substance(s) in

Fig.2. Renin activity of the three kidney extracts is shown. adrenalectomized: Extract I, Na restricted, Extract II, normal: Extract III.

Fig.3. Synthesis on humoral mechanism of renal hypertension.

the extract plays a more important role than the renin-angiotensin system in the development of hypertension. Recently, very interesting papers²⁻⁴ have been published where rabbits immunized against angiotensin II developed renal hypertension. This fact favours the latter consideration. We propose a "Dual Renal Pressor Substance Hypothesis" wherein an unknown renal pressor substance(s) other than renin is released from the kidney into the circulation and plays a primary role in initiating and maintaining renal hypertension. This factor(s) would not exert the immediate vasopressor effect as renin but elevate blood pressure after a latent period, mediated by a complex system (Fig.3). Because of the delayed action, this presumed factor has been hitherto unnoticed and awaits discovery.

SUMMARY

Three kinds of kidney extract namely, adre-

nalectomized, sodium restricted and normal rat-kidney extract were prepared. Hypertension-inducing potency as well as renin activity in each extract was determined and discrepancy between the two activities was found. This experiment suggests the occurrence of at least one pressor substance apart from renin in the kidney which effects blood pressure elevation after a latent period. A "dual renal pressor substance" hypothesis is proposed concerning the humoral mechanism of renal hypertension.

Acknowledgement

We are grateful to Drs. M. Matsunaga and K. Ogino for kind advice and also to Miss M. Ohara for assistance with the manuscript.

REFERENCES

1. KIRA, J., OHNISHI, K., KONISHI, N., & YAMATORI, K.: High blood pressure following injection of renal extracts from rats under various experimental conditions and a hypothesis: dual renal pressor substance theory (reinvestigation of renal pressor substance, report No.1), *Jap. Circul. J.* 34: 851, 1970.
2. EIDE, I. & AARS, H.: Renal hypertension in rabbits immunized with angiotensin-II, *Scand. J. clin. Lab. Invest.* 25: 119, 1970.
3. MacDONALD, G. J., LOUIS, W. J., RENZINI, V., BOYD, G. W., & PEART, W. S.: Renal-clip hypertension in rabbits immunized against angiotensin II, *Circulation Res.* 27: 197, 1970.
4. AARS, H., EIDE, I., & AKRE, S.: Abolished sympathetic stimulating effect of angiotensin in rabbits immunized with angiotensin II, *Scand. J. clin. Lab. Invest.* 27: 47, 1971.