The Depressor Principle in the Kidney of the New-born Rat*

I. SOME BIOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF THE KIDNEY EXTRACT OF THE NEW-BORN RAT

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THERE IS MUCH evidence on the moderate decrease in blood pressure during the last several days of pregnancy in experimentally hypertensive animals.¹⁾²⁾³⁾⁴⁾⁵⁾⁶⁾⁷⁾⁸⁾⁹⁾¹⁰⁾¹¹⁾ The presence of depressor substance in the fetus kidney⁵⁾⁷⁾ or in placenta³⁾⁴⁾¹¹⁾, or endocrine changes²⁾⁸⁾ or hemodynamic changes¹⁾³⁾⁵⁾⁶⁾⁸⁾¹¹⁾ during pregnancy has been suggested as the cause of this phenomenon.

In our laboratory this decrease in blood pressure has also been confirmed with the several experimentally hypertensive and spontaneously hypertensive rats¹²⁾, and our experimental studies have suggested that the fetus kidney might play an important role in this phenomenon¹³⁾. We already reported the existence of the antihypertensive principle in new-born rat kidney as Depressin (Okamoto and Takeda, 1962)¹⁴⁾. This communication reports some pharmacological and physicochemical studies on the boiled extract of new-born kidney.

MATERIALS AND METHODS

New-born Wistar strain rats bred in our laboratory were used for preparation of the antihypertensive principle(s). Recipient rats for administration of tissue extract were supplied by the Animal Center of Kyoto University. These rats were fed with boiled wheat and fish seasoned with salt (0.3% Na) and vegetables. Tap water was given them ad libitum. Hypertension in recipient rat was induced by renal infarction¹⁵⁾.

Preparation of tissue extract

The kidney, liver or lung of new-born was removed without anesthesia within 24 hours after birth and homogenized in saline (0.5 ml per gram of wet tissue) with Potter-Elvehjem-type glass plunger. The kidney of adult rat was removed immediately after sacrifice by a blow on the head and homogenized similarly as above. After centrifugation at 5,000 r.p.m. for 5 min., the supernatant was heated at 100°C in boiling water for 5 min.. The decanted supernatant was used in this study. pH was not adjusted unless otherwise stated.

Biological test

1) Blood pressure: Under nembutal anesthesia (35 mg/kg intraperitoneally), arterial blood pressure was measured manometrically from the carotid artery of hypertensive rat with renal infarction or of normotensive rat with nephrectomy 20 hours before the experiment. The boiled extract prepared as above was infused through the polyethylene tubing cannulated into the femoral vein.

2) Muscle strip preparation: The following preparations were used to assay the tissue extract; guinea pig ileum, rat stomach fundus, estrogenized rat uterus and rabbit intestine. Guinea pig ileum and rat stomach fundus prepared as reported by Vane¹⁶⁾ were suspended in tyrode's solution at 36–38°C. Estrogenized rat uterus was gained by subcutaneous injection of estradiol (0.2 mg/kg.) on each of the 2 days before the assay and dipped in de jalon's solution at 32–35°C. Rabbit intestine was suspended in MgCl₂-free Tyrode's solution at 36–38°C. Atropine sulfate (10⁻⁷ g/ml) and pyribenzamine (10⁻⁶ g/ml) were added to the perfusate to prevent the contraction induced by acetylcholine and/or histamine, if present, respectively. In some

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case, to test whether the active principle might be tryptamine or serotonin, large dose of such a drug (10^{-4} g/ml) was administered to the bath 30 seconds before each test.

Physicochemical tests

1) Stability: The boiled extract was heated at 100°C with or without HCl or NaOH (final conc. 0.3N or 1N) in a sealed ampule for 30–60 min.. The samples were then neutralized.

2) Fractionation by Sephadex G-50: Sephadex G-50 (Pharmacia Uppsala, Sweden) in a short column (15 x 1.5 cm) was used to estimate the approximate molecular size of the depressor. Before applying the extract, 2 cc of 1% bovine albumin solution and 2 cc of 0.47% ammonium sulfate were filtered separately as indicators. Each 1 cc of the filtrated solution was collected in a small tube, then albumin and ammonium sulfate content of each fraction were determined. The column was then washed with distilled water and then 2 ml of the new-born kidney extract (equivalent to 4.97 g of new-born kidney) was applied to it, and fractions of 1 ml were collected. One fifth portion of each fraction was used to determine the ultraviolet absorption at $260\text{m}\mu$. Then, the rest of each fraction was divided into two portions for the assay of depressive activity on the 2 rats with renal infarction hypertension.

3) Charcoal treatment: The extract was stirred with appropriate amount of charcoal for 10 min., and then centrifuged. The clear supernatant was used for assay of depressor activity.

4) Ultraviolet absorption: The extract of new-born rat kidney was diluted 160-fold. The ultraviolet spectrum of its diluted extract was recorded in the Cary's recording spectrophotometer.

5) Enzymatic degradation: Histaminase was kindly prepared by Dr. Y. Nishizuka (Dept. of Biochemistry, Kyoto University School of Medicine) according to Tabor's method¹⁷⁾. Chymotrypsin, Trypsin and snake (Habu) venom were also used. The boiled extract was incubated with these enzymes for 60 min. and the reaction was stopped by heating the mixture at 100°C for 10 min..

RESULTS

Physiological and Pharmacological Properties

Blood pressure: When the boiled extract of the kidney of new-born rat was injected intravenously into the hypertensive rat with renal infarction, the high blood pressure began to fall immediately after the infusion of the extract to reach the nearly normal level in

Fig. 1 A Depression of high blood pressure by the new-born kidney extract.

The extract from 0.23 g of new-born kidney per 100 g body weight of the hypertensive recipient animal (R 76) was administered. The decrease in blood pressure was 54 mm Hg.

Fig. 1 B Comparison of the depressive activity of the adult kidney with that of the new-born kidney.

Arrow 1 shows the administration of the extract from 0.30 g of adult kidney per 100 g body weight of the hypertensive animal (R 132) and arrow 2 shows the administration of the extract from 0.28 g of new-born kidney per 100 g body weight of the animal.

The decrease in blood pressure in the former (22 mm Hg) was less than that in the latter (56 mm Hg).

Fig. 1 C Effect of the intravenous injection of the new-born kidney extract on the normal blood pressure of the nephrectomized rat (N 2).

The extract from a 0.3 g of new-born kidney per 100 g body weight of the test animal was administered. No definite change in blood pressure was observed.

0.5–1.0 min. and rose towards initial level in the same time interval (Fig. 1 A). As shown in Table I, the mean value of blood pressure fall 58 ± 6 mm Hg was evoked by 0.22 ± 0.02 g (mean) wet new-born kidney per 100 g body weight of test object. But the extent of depressant activity of the adult kidney extract (-23 ± 4 mm Hg) was less than that of the new-born (Fig. 1 B).

The action of this new-born kidney extract on the normotensive nephrectomized rat was also studied. However, no definite effect on the normal blood pressure was obtained by using the extract from 0.27 ± 0.03 g wet kidney per 100 g body weight of these test animals (Table I) (Fig. 1 C). On the other hand, the new-born liver extract (0.17 ± 0.02 g wet liver per 100 g body weight of hypertensive rat) showed a similar depressor response (31 ± 9 mm Hg). But the lung extract did not show any significant effect on the high blood pressure (Table I).

Motility of isolated smooth muscle preparation: The extract contracted guinea pig ileum, rat stomach fundus and rat estrogenized uterus, but inhibited the motility of the isolated rabbit intestine (Table II). These actions of extract on guinea pig ileum, rat stomach fundus and rat estrogenized uterus were not antagonized by atropine, but its action on guinea pig ileum was antagonized by pyribenzamine. The desensitizing effect by a large dose of serotonin could not be observed in guinea pig ileum, rat stomach fundus or rat estrogenized uterus. The same effect by a large dose of tryptamine was not convinced in guinea pig ileum preparation.

Physicochemical Nature of Kidney Extract

Stability: The data presented in Table III illustrate the stability of kidney extracts to heat and acid or alkali. The depressive activity of new-born kidney extract was stable to heating at 100°C for 60 min. or to heating at 100°C in 0.3N HCl for 60 min.. But the depressive activity, the rat stomach fundus-stimulating activity and the rabbit intestine-inhibiting activity of the kidney extract were lost by heating the extract at 100°C in 1N HCl for 30 min.. The rabbit intestine rather increased its touns by such procedure.

Ultraviolet absorption: The maximum absorption (λ max) was observed at $256 \text{ m}\mu$. The minimum one was at $234 \text{ m}\mu$ (Fig. 2). After charcoal treatment this characteristic absorption disappeared.

Fractionation by Sephadex G-50: As shown in Fig. 3, the depressive activity appeared in tubes 8, 9, 10 and 11, in which ammonium ion had already appeared. The content of tube 10 had strong ultraviolet absorption at $260 \text{ m}\mu$.

Charcoal treatment: After charcoal treatment of the boiled kidney extract, the depressive activity and rat stomach fundus-stimulating or rabbit intestine-inhibiting activity almost disappeared.

Enzymatic degradation: As shown in Table III the depressive activity of the new-born rat kidney extract did not diminish by treatment with chymotrypsin, trypsin, histaminase or habu venom treatment. On the

TABLE I INFLUENCES OF INTRAVENOUSLY INJECTED TISSUE EXTRACT ON BLOOD PRESSURE OF HYPERTENSIVE RATS WITH RENAL INFARCTION OR OF NEPHRECTOMIZED NORMOTENSIVE RATS

Kinds of Extract	Assay Animals		Doses of Extract (g) (Wet Tissue Weights per 100 g Body Weight)	Blood Pressure (mm Hg)		Changes of Blood Pressure (mm Hg)	Sites of Cannulation	
	Animal Numbers	Body Weights (g)		Before Injection	After Injection			
New-born Kidney	Rats with Renal Infarction Hypertension	R 76	195	0.23	160	106	- 54	C. A.
		R 81	200	0.31	204	141	- 63	C. A.
		R 92	200	0.28	155	111	- 44	C. A.
		R106	260	0.21	163	92	- 71	F. A.
		R106	260	0.22	181	115	- 66	F. A.
		R119	285	0.20	161	119	- 42	F. A.
		R131	250	0.11	200	146	- 54	C. A.
		R132	240	0.28	225	115	-110	F. A.
		R132	240	0.28	175	119	- 56	F. A.
		R133	280	0.12	167	144	- 23	F. A.
		R133	280	0.19	163	134	- 29	F. A.
		R135	250	0.24	188	117	- 71	F. A.
		Average	245 ± 28	0.22 ± 2.02	179 ± 6	121 ± 4	- 58 ± 6	
	Nephrectomized Normotensive Rats	N × 1	185	0.18	109	90	- 19	C. A.
N × 2		195	0.30	117	117	0	C. A.	
N × 3		210	0.31	120	104	- 16	C. A.	
N × 4		185	0.31	125	140	+ 15	C. A.	
N × 5		180	0.34	111	131	+ 20	C. A.	
N × 6		190	0.17	149	138	- 11	C. A.	
Average	191 ± 4	0.27 ± 0.03	122 ± 5	120 ± 7	- 2 ± 6			
Adult Kidney	Rats with Renal Infarction Hypertension	R 92	200	0.31	155	121	- 34	C. A.
		R106	260	0.21	169	157	- 12	F. A.
		R129	280	0.26	160	134	- 26	F. A.
		R132	240	0.30	181	159	- 22	F. A.
		R135	250	0.24	196	163	- 33	F. A.
		R135	250	0.28	200	190	- 10	F. A.
	Average	247 ± 10	0.27 ± 0.01	177 ± 7	154 ± 9	- 23 ± 4		
New-born Liver	Rats with Renal Infarction Hypertension	R 61	320	0.14	170	99	- 71	F. A.
		R 76	195	0.21	160	127	- 33	C. A.
		R 76	195	0.10	150	106	- 44	C. A.
		R 92	200	0.15	155	155	0	C. A.
		R 93	200	0.20	187	162	- 25	F. A.
		R133	280	0.23	165	152	- 15	F. A.
	Average	232 ± 20	0.17 ± 0.02	165 ± 5	134 ± 10	- 31 ± 9		
	Nephrectomized Normotensive Rats	N × 1	185	0.22	109	95	- 14	C. A.
		N × 2	195	0.32	117	117	0	C. A.
		N × 3	210	0.10	120	131	+ 11	C. A.
		N × 4	185	0.33	125	125	0	C. A.
		N × 4	185	0.33	115	115	0	C. A.
		N × 6	190	0.11	149	132	- 17	C. A.
		N × 7	180	0.16	141	135	- 6	C. A.
N × 9		190	0.21	145	130	- 15	C. A.	
Average	190 ± 3	0.22 ± 0.03	128 ± 5	123 ± 4	- 5 ± 3			
New-born Lung	Rats with Renal Infarction Hypertension	R 92	200	0.41	155	155	0	C. A.
		R133	280	0.45	163	163	0	F. A.
		R135	250	0.35	196	207	+ 11	F. A.
		R135	250	0.40	196	196	0	F. A.
	Average	245 ± 14	0.36 ± 0.04	177 ± 8	179 ± 8	2 ± 2		

Notes: C. A. ... Carotid Artery, F. A. ... Femoral Artery, ± ... Standard Error.

other hand, most of the depressive activity of new-born liver was lost by histaminase incubation. The guinea pig ileum- or rat stomach fundus-stimulating activity of new-born rat kidney was not affected by chymotrypsin or trypsin treatment, but the guinea pig ileum-contracting activity was lost by histaminase incubation (Table II, III)

DISCUSSION

The concept that the kidney exerts an antihypertensive function has been supported by the following observations. 1) After bilateral nephrectomy¹⁸⁾¹⁹⁾²⁰⁾ or bilateral corticectomy²¹⁾, hypertension ensues. Intact renal tissue without an excretory function (such as

TABLE II EFFECTS OF NEW-BORN KIDNEY EXTRACT ON SEVERAL PREPARATIONS OF SMOOTH MUSCLE

	Guinea Pig Ileum	Rat Stomach Fundus	Rat Estrogenized Uterus	Rabbit Intestine
Control	Stimulating	Stimulating	Stimulating	Inhibiting
Boiling in 1N HCl for 30 min		+		+
Absorbed with Charcoal		+		+
Antagonized with Atropine	—	—	—	—
Pyribenzamine	+	—	—	—
Digested with Histaminase	+			
Chymotrypsin	—	—		
Trypsin	—	—		
Desensitized with large doses of Serotonin	—	—	—	
Trypamine	—			

Note: — Not Affected, + Antagonized or Inactivated.

Fig. 2 The Ultraviolet Absorption of the Diluted Extract (160-fold) of New-born Rat Kidney.

after uretero-caval anastomosis) is not associated with hypertension¹⁹⁾. 2) Grafting a normal kidney to the hypertensive animal^{22) 23) 24)} as well as renal perfusion arrangement²⁵⁾ promptly lowers the high blood pressure. 3) Autoexplantation of whole kidney or renal medulla as cell suspension prevent the development of renoprival hypertension²⁶⁾. 4) In parabiotic rats, two intact kidneys, in the presence of two ischemic ones, prevent the development of hypertension in both animals²⁷⁾.

TABLE III INFLUENCE OF ENZYMATIC INACTIVATION OR PHYSICOCHEMICAL TREATMENT ON DEPRESSOR ACTION OF NEW-BORN KIDNEY OR LIVER EXTRACT

Procedure	Extract	
	New-born Kidney	New-born Liver
Boiling at pH=7 for 1 hour	—	
Boiling in 0.3N HCl for 1 hour	—	
Boiling in 1N HCl for 30 minutes	+	
Boiling in 0.3N NaOH for 1 hour	—*	
Boiling in 1N NaOH for 1 hour	—*	
Histaminase	—	+
Chymotrypsin	—	
Trypsin	—	
Snake (Habu) Venom	—	
Charcoal Absorption	+	—

Note: — Not Affected, + Inactivated,
* Not Affected or Rather Strengthened and Prolonged.

Fig. 3 Fractionation of Depressor Principle by Column Chromatography (Sephadex G-50). Each fraction was divided into two portions. Each portion was assayed on the 2 rats with renal infarction hypertension.

*: The administered volume of this fraction 10 was one half the other fractions.

Now, it is an important problem to ascertain which active principle in the renal tissue is responsible for the antihypertensive function. It has been known for a long time that kidney tissue extracts contain substances which cause blood pressure elevation. In 1898, Tigerstedt and Bergman²⁸⁾ found a pressor protein in renal extract and named it renin. In 1903, Vincent and Sheen²⁹⁾ reported for the first time the presence of a heatstable and nonprotein depressor substance in kidney tissue. Pearce³⁰⁾ and Hartwich³¹⁾ also investigated this kind of depressor substance but the nature of it was not conclusively determined. Felix et al.³²⁾ and Lange^{33,34)} extracted a new depressor substance besides choline, acetylcholine, histamine and adenosine compounds from the several organs including kidney. However, its chemical nature was not proven. Zipf³⁵⁾ reported that fresh defibrinated blood, serum, or boiled- or alcohol extracts of several organs including kidney reduced the blood pressure and ascribed this action to adenosine or adenylic acid. In these experiments the active principles administered parenterally reduced the normal blood pressure. And these active principles seemed to be by no means unique in renal tissue.

Grollman, Williams and Harrison³⁶⁾ reported that the extracts from pork- beef- or dog-kidney administered per os were active in lowering the high blood pressure of experimental animals and of human patients. The active principle in this extract was considered non-protein, water soluble but insoluble in organic solvent. Page et al.³⁷⁾ reported that the extract of pork kidney was capable of lowering the high blood pressure of experimental animal or of man by parenteral administration. The active principle in this extract was non-dialyzable and slow-reacting. Yoshimura and Negishi³⁸⁾ observed that the alcohol extract of rabbit kidney administered intravenously reduced the normal blood pressure of renoprival rabbit. The active principle acts for a short duration of 1–2 min. Hamilton and Grollman³⁹⁾ prepared a pork kidney extract effective by oral administration in lowering the high blood pressure of experimental animals and studied the physicochemical nature of this principle.

The active principle was alkali-labile, and unstable to heating and dialyzable through collodion membrane. Preliminary studies by paper-chromatography or countercurrent distribution of the extract revealed that the active principle was a smaller polypeptide. Gordon⁴⁰⁾ reported that the boiled extract of alcohol-insoluble fraction of rabbit kidney reduced the normal blood pressure. He ascribed its depressive action to A.T.P., A.D.P., A.M.P., and other adenosine nucleotides, but not to histamine, choline, acetylcholine or kallikrein by precise pharmacological or physicochemical study. Muirhead, Jones and Stirman⁴¹⁾ observed that the intravenous administration of extract from dog kidney, especially medulla, protected against or reduced the renoprival hypertension. According to the recent studies^{42,43)}, this active principle, presumably of low molecular weight, was slightly watersoluble, not retained on Dowex-50 resin and not likely to be an amino acid or peptide. Milliez, Meyer, Lagrue and Boivin^{44,45)} prepared acetone- or butanol extracts of pig or rabbit kidney effective in lowering the high blood pressure of experimental animals by intraperitoneal injection. Sokabe and Grollman²¹⁾ recently stated that the extract of renal cortex, not of medulla, was capable of lowering the high blood pressure by oral administration. Summarizing these reports, there are substances in kidney extract which reduce the high blood pressure or the normal blood pressure by parenteral or oral administration. But the chemical nature of the active principle has not been fully investigated except the report by Gordon⁴⁰⁾.

Judging from the data in this study, the depressor substance in new-born rat kidney extract might be a relatively small molecule. It is not choline, acetylcholine, histamine, serotonin, or biologically active polypeptides such as substance P, kallidin or bradykinin. Above results on stability and ultraviolet absorption might suggest that the active principle in new born rat kidney responsible for the depressor action is chiefly adenosine compounds as reported in adult rabbit kidney by Gordon. But further investigation are required for its final identification of the depressor principle in

the new-born kidney.

As shown in above results, the extracts of new-born kidney stimulate the guinea pig ileum, rat stomach fundus or rat estrogenized uterus and inhibited the rabbit intestine. But the rat stomach fundus-stimulating and rabbit intestine-inhibiting activities as well as the depressor activity are lost by boiling the extract in 1N HCl for 30 min. or charcoal absorption. This result presumably indicates that the active principle responsible for the depressor action is the same as one responsible for the rat stomach fundus-stimulating and rabbit intestine-inhibiting activities.

The extract of new-born kidney contains enough histamine to stimulate guinea pig ileum, but this can be excluded as a possible factor of depressor activity in new-born kidney extract because the depressor activity is not destroyed after incubation with histaminase. On the other hand, the new-born liver extract contains enough histamine to depress the blood pressure as well as stimulate guinea pig ileum and the depressor activity is diminished to a great extent by incubation with histaminase. These results seem to agree with those of Kahlson that fetus liver is the principal site for histamine formation in the fetus⁴⁶⁾.

The hypertensive and the normotensive animals respond differently to similar doses of kidney extract. In the former the blood pressure is reduced to nearly normal level but in the latter the response is indefinite as described above. This difference might be due to the susceptibility of recipient animal to the kidney extract; that is, the hypertensive animal seemed to respond to a relatively small dose of extract but the normotensive animal only to large dose of extract.

Further, a difference of response between extract from the new-born and from the adult kidney was also observed in this study. Although this difference may have some interesting biological implications, its final clarification requires the further studies.

SUMMARY

1. The boiled extract of new-born rat kidney reduced the high blood pressure induced

by renal infarction to nearly normal level. This activity of the new-born rat kidney was more pronounced than that of adult rat kidney.

2. This extract also stimulated guinea pig ileum, rat stomach fundus and rat estrogenized uterus but inhibited rabbit intestine.

3. The depressor activity as well as rat stomach fundus-stimulating or rabbit intestine-inhibiting activity of new-born rat kidney extract disappeared on boiling in 1N HCl for 30 min. or by charcoal treatment. But the depressor activity was not inactivated by incubation with trypsin, chymotrypsin, histaminase or habu venom.

4. By Sephadex G-50 column chromatography, it was evident that the depressor substance was a relatively small molecule. The activity and the ultraviolet absorption at 260 m μ seemed correlated. Ultraviolet absorption maximum of the extract was at 256 m μ .

5. The boiled extract of new-born rat liver reduced the high blood pressure but most of the activity was destroyed after incubation with histaminase. The extract of new-born lung did not show any depressive activity.

6. From above results, the active principle responsible for the depressor action in the new-born kidney extract was not choline, acetylcholine, histamine, serotonin or biologically active polypeptides such as bradykinin, substance P or kallidin. It is possible that the depressor substance may be related to adenosine compounds.

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Addendum

Recently Lee et al. reported that the active substance—not nucleotides—with sustained depressor effect on the normotensive rat was extracted from rabbit renal medulla (*Circulat. Res.* 13 : 359-366, 1963). But in our laboratory, as shown in above results, the boiled extract of new-born rat whole kidney did not show any sustained depressor effect on the normotensive rat as well as hypertensive rat.

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