PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 397–402 (1974)

Effects of Sera and Sera Fractions from Spontaneously Hypertensive Rats on Renal Organic Anion and Cation Transport[1,2] (37818)

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Okamoto and Aoki have bred and studied a strain of Wistar rats which spontaneously develops hypertension (1, 2). Early in life, the blood pressure of the spontaneously hypertensive rat is no different from that of control (C) Wistar rats, but with the passage of time, the blood pressure of SHR's rises steadily. Because of the gradual development of hypertension in the setting of a genetic background, the SHR may be the closest laboratory model of human essential hypertension that we have.

Based upon the speculation that some forms of clinical and experimental hypertension are secondary to deranged kidney function (3, 4), one might easily hypothesize that deranged kidney function also may be, at least in part, a primary cause of hypertension in the SHR. In this investigation, we examined the effects of sera and sera fractions from SHR and C rats on para-aminohippurate (PAH) and tetraethylammonium (TEA) transport in kidney cortical slices from control rats. The purpose of our report is to show that plasma obtained from SHR's affect renal transport of PAH and probably TEA in a different manner from that of C rats. The factor or factors demonstrated in this *in vitro* assay bear in many ways a resemblance to those found in azotemic sera which affect both renal PAH and sodium transport (5, 6).

Methods and Materials. Male Wistar rats, control normotensive (C) and spontaneously hypertensive (SHR), were obtained at 14–20 weeks of age from Purina Laboratories (Purina Laboratory Animals, Vincentown, NJ). The rats were allowed free access to rat chow and drinking water and were housed in pairs (one C and one SHR together) in a room maintained at a temperature of 23° with a light–dark period of 14 and 10 hr, respectively. Blood pressure (BP) was estimated by tail plethysmography (7). At least 3 readings of BP determined on different days were taken for each rat. All C rats had an average BP below 120 mm Hg whereas all SHR's had BP exceeding 145 mm Hg. Rats were lightly anesthetized with ether, and blood was drawn from the lower aorta. The blood was allowed to clot, and the sera were frozen and stored at −20° within 1 hr after drawing. Cortical slices (0.4 mm), cut with a Stadie–Riggs microtome (8), were removed from a normotensive 200-g Sprague–Dawley rat and halved. One half was placed in serum (10% v/v) from C rats and the other half in serum (10% v/v) from SHR's. The sera were paired according to the day they were drawn, and 4–8 slice pairs were studied for each set of sera on any one day (Table I).

Where fractions of serum were studied (Tables II and III), we collected whole blood from the lower aorta of 3 rats (either spontaneously hypertensive or control rats) on a given day and allowed the pooled blood to clot at room temperature. Following the

[1] Supported by a grant from the Washington Heart Association and NIH Grant AM 15458.

[2] Presented in part before the AFCR, New Orleans, Jan. 1973.

[3] Supported by Summer Student Fellowship from the Washington Heart Association.

[4] Supported by High School Summer Fellowship from the Washington Heart Association.

TABLE I. Effects of Sera (10% v/v) from Spontaneously Hypertensive and Control Wistar Rats on PAH and TEA Transport of Control Rat Kidneys.

Expt. No.	PAH S/M			TEA S/M		
	SHR	C rat	Δ	SHR	C rat	Δ
1	13.7	14.8	−1.1	14.5	17.4	−2.9
2	19.6	24.2	−4.6	20.2	27.8	−7.6
3	11.3	14.1	−2.8	12.5	13.7	−1.2
4	14.1	13.3	+0.8	13.3	13.7	−0.4
5	14.1	16.2	−2.1	16.4	21.1	−4.7
6	11.3	12.0	−0.7	14.6	13.9	+0.7
7	8.8	9.7	−0.9	11.0	12.4	−1.4
8	8.6	12.2	−3.6	12.0	14.1	−2.1
9	11.8	11.2	+0.6	15.7	14.9	+0.8
10	13.6	13.6	0	18.0	16.8	+1.2
11	12.8	14.8	−2.0	13.4	15.5	−2.1
12	17.6	17.0	+0.6	20.3	18.5	+1.8
Av.		−1.3 ± 0.50			−1.5 ± 0.78	
		$P < .05$			$0.1 > P > .05$	

methodology described by Bourgoignie, Klahr, and Bricker (6), sera was passed through Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden), eluted with 10 m*M* ammonium acetate, and the fractions collected in small aliquots. The fractions were separated on the basis of the uv absorption pattern into five fractions as shown in Fig. 1. Each pooled fraction was lyophilized and reconstituted later to the original volume by adding isotonic saline. An exception to this was fraction 2 where the original saline peak from the sera was located. This fraction was reconstituted with water. Later, each fraction was tested separately (10% v/v) added to medium against its own half-slice (again using kidney slices from Sprague–Dawley rats) incubated in medium to which was added isotonic saline (10% v/v).

The basic incubation medium used was that of Cross and Taggart (9)—phosphate-buffered sodium, potassium, and chloride solution gassed with 100% O_2. To this we added ^{14}C-tetraethylammonium bromide (TEA) New England Nuclear, Inc. and ^{3}H-paraaminohippurate (PAH) Amersham Radiochemical Center), both to a concentration of 10^{-5} *M*. The final medium pH was 7.4. Following 90 min of incubation at 24° in a Dubnoff shaker, slices were removed, blotted, and weighed. Tissue weights ranged between 40 and 80 mg. Slices were placed in 5% trichloroacetic acid, homogenized, centrifuged, and the supernatant used for β counting. The medium in which incubation took place also was added to trichloroacetic acid, centrifuged, and the supernatant counted. The basic scintillation mixture was composed of Triton X-100 (1/3 volume) (Packard Instrument Co.), toluene (2/3 volume), 2,5-diphenyloxazole (5.5 g) and 1,4-bis-[2-(4 methyl-5-phenyloxazole)]benzene (125 mg). Double-isotope β counting and quench correction were performed utilizing a Packard Counter, Model 2420. Results are expressed as S/M ratios, i.e., the ratio of counts per minute per gram of tissue weight to the counts per minute per milliliter of incubation medium (Tables I–III).

Due to the great variation reported in this type of transport study (10, 11), we calculate statistics by paired analysis—the transport in the test half-slice compared to transport in the control half-slice. The Student's *t* test was used; statistical significance was set at $P < 0.05$.

Results. In the presence of sera (10% v/v) from SHR's (av BP = 151 mm Hg), PAH S/M ratios in kidney slices were depressed significantly when compared to paired halves incubated in sera (10% v/v) from C rats (av BP = 111 mm Hg, $P <$ .05) (Table I). In contrast, the decrease in TEA transport was not statistically significant ($0.1 > P > .05$).

Each fraction of pooled sera from 3 rats was tested as to its effect on PAH and TEA transport in kidney slices from Sprague–Dawley rats (Tables II and III). A total of six experiments were performed, one with pooled sera from normotensive Sprague–Dawley rats, two from normotensive Wistar rats, and three from SHR's. Compared to slices incubated in the medium alone, all sera fractions (C or SHR's) with the exception of No. 4 affected PAH transport in a similar manner, i.e., a slight stimulation of transport was induced by fractions 1 and 2, but no consistent change in PAH transport was obtained with fractions 3 and 5 (Table II). However, while fraction 4 from "normotensive sera" did not appear to change PAH transport, fraction 4 from "SHR sera" sig-

TABLE II. PAH Transport in Rat Renal Slices Incubating in Plasma Fractions from Control and Spontaneously Hypertensive Rats.

	Fractions														
	1			2			3			4			5		
	C	T	Δ[a] ± SEM	C	T	Δ ± SEM	C	T	Δ ± SEM	C	T	Δ ± SEM	C	T	Δ ± SEM
Spontaneous Hypertensive Wistars (14–20 weeks)															
Expt 1—SHR[b]	11.3	12.5	1.2 ± 0.34*	10.8	17.4	6.6 ± 1.3***	10.5	10.4	−0.1 ± 0.71	11.6	10.2	−1.4 ± 0.50**	12.5	11.8	−0.7 ± 0.50
BP = 146[c]		(4)[d]			(4)			(12)			(12)			(4)	
Expt 3—SHR	9.2	11.2	2.0 ± 0.35***	7.9	14.2	6.3 ± 1.2***				9.8	8.2	−1.6 ± 0.29***	7.8	8.9	1.1 ± 0.94
BP = 160		(6)			(6)						(8)			(6)	
Expt 5—SHR	8.0	10.1	2.1 ± 0.87	8.0	13.7	5.7 ± 1.1***	8.2	9.9	1.7 ± 0.93	8.5	7.2	−1.3 ± 0.34***	7.4	7.7	0.3 ± 0.48
BP = 166		(6)			(6)			(6)			(9)			(6)	
Control Rats (14–20 weeks)															
Expt 2—SD	9.4	9.6	0.2 ± 0.43	9.6	15.7	6.1 ± 0.62***	10.6	11.5	0.9 ± 1.19	11.4	12.5	1.1 ± 1.5	9.6	9.3	−0.3 ± 0.52
BP = 114		(4)			(4)			(4)			(4)			(4)	
Expt 4—W	9.9	11.1	1.2 ± 0.45	8.6	14.2	5.6 ± 1.6*	10.0	10.1	0.1 ± 0.52	9.8	10.0	0.2 ± 0.33	10.4	11.1	0.7 ± 0.91
BP = 104		(4)			(4)			(4)			(4)			(4)	
Expt 6—W	7.2	7.4	0.2 ± 0.73	8.2	13.5	5.3 ± 0.77***	7.6	7.5	−0.1 ± 0.29	7.7	8.0	0.3 ± 0.32	8.2	7.3	−0.9 ± 1.06
BP = 116		(6)			(6)			(6)			(6)			(6)	

[a] Δ = T–C.
[b] SHR = hypertensive, W = Wistar, SD = Sprague–Dawley.
[c] Average of 3 rats.
[d] No. of slice pairs studied for each fraction.
* $P < .05$.
** $P < .02$.
*** $P < .01$.

TABLE III. TEA Transport in Rat Renal Slices Incubating in Plasma Fractions from Control and Spontaneously Hypertensive Rats.

	Fractions														
	1			2			3			4			5		
	C	T	Δ[a] ± SEM	C	T	Δ ± SEM	C	T	Δ ± SEM	C	T	Δ ± SEM	C	T	Δ ± SEM
Spontaneous Hypertensive Wistars (14–20 weeks)															
Expt 1—SHR[b]	14.5	14.0	−0.5 ± 1.0	9.3	16.1	+6.8 ± 1.8*	12.7	14.4	+1.7 ± 1.3	14.3	13.0	−1.3 ± 1.0	15.4	13.9	−1.5 ± 1.0
BP = 196[c]		(4)[d]			(4)			(4)			(14)			(4)	
Expt 3—SHR	12.8	12.8	0 ± 0.9	10.0	13.9	+3.9 ± 1.6				12.1	9.4	−2.7 ± 1.4	11.6	12.8	+1.2 ± 1.0
BP = 160		(6)			(6)						(6)			(6)	
Expt 5—SHR	11.3	11.6	+0.3 ± 1.0	13.7	15.4	+1.7 ± 1.1	12.3	12.9	+0.6 ± 2.6	12.4	10.8	−1.6 ± 0.8	10.6	12.3	+1.7 ± 0.7
BP = 166		(4)			(4)			(4)			(9)			(4)	
Control Rats (14–20 weeks)															
Expt 2—SD	12.6	10.8	−1.8 ± 1.3	13.3	18.7	+5.4 ± 1.7	13.2	16.0	+2.8 ± 1.1	15.7	16.7	+1.0 ± 1.9	15.1	15.8	+0.7 ± 1.4
BP = 114		(4)			(4)			(4)			(4)			(4)	
Expt 4—W	15.3	13.1	−2.2 ± 0.8	12.9	13.6	+0.7 ± 1.6	15.4	14.2	−1.2 ± 1.0	15.3	14.6	−0.7 ± 1.5	14.5	14.0	−0.5 ± 1.3
BP = 104		(4)			(4)			(4)			(4)			(4)	
Expt 6—W	10.9	10.4	−0.5 ± 1.6	13.5	14.6	+1.1 ± 1.6	13.5	12.2	−1.3 ± 1.0	11.1	12.2	+1.1 ± 1.0	12.9	12.4	−0.5 ± 1.5
BP = 116		(6)			(6)			(6)			(6)			(6)	

[a] Δ = T–C.
[b] SHR = hypertensive, W = Wistar, SD = Sprague–Dawley.
[c] Average of 3 rats.
[d] No. of slice pairs studied for each fraction.
* $P < .05$.

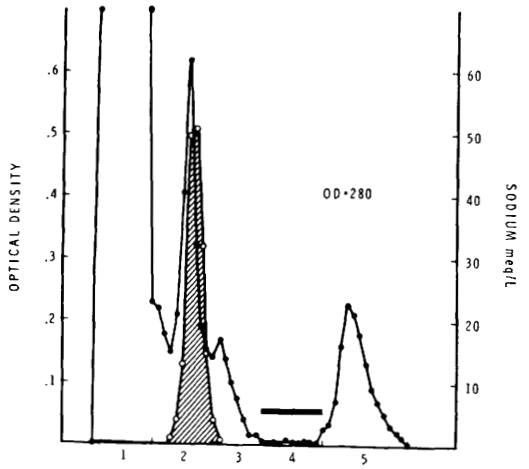

FIG. 1. Pattern of elution of protein off Sephadex column from sera of a rat with spontaneous hypertension. Salt peak is shown in dark.

nificantly depressed transport in all 3 studies.

The data for TEA transport are more difficult to interpret. No consistent effect was seen when each individual fraction from the sera pools was tested (Table III). However, when the observations from the 3 experiments in each group were combined (sera from normotensive and spontaneously hypertensive rats), fraction 2 ($n = 14$) each) stimulated TEA transport (C = 2.2 ± 0.9 SEM, $P < .05$; SHR = 3.3 ± 0.9 SEM, $P < .01$), and fraction 4 from SHR's ($n = 29$) significantly depressed TEA transport (-1.7 ± 0.6 SEM, $P < .01$) while C ($n = 14$) did not (1.1 ± 0.8 SEM).

Discussion. Organic anion (PAH) and organic cation (TEA) transport were studied in kidney slices incubated in sera and sera fractions from Wistar rats of a normotensive (C) and hypertensive strain (SHR). When sera from C and SHR's were compared as to their effect on PAH and TEA transport in kidney slices, a small but statistically significant depression in PAH transport and a decrease in TEA transport were noted in slice halves incubating in "SHR" sera. To further characterize these phenomena, we fractionated the sera on Sephadex columns and found that the relative depression in PAH transport by SHR serum was related to a specific fraction. Although in individual experiments no significant effects of these sera fractions could be seen in TEA transport, pooling the data resulted in findings similar to the PAH studies, i.e., fraction 2 from both C and SHR's stimulated TEA transport while fraction 4 from SHR's proved depressive. The finding that a substance or substances present in fraction 2 is the major contributor to the PAH stimulation observed with nonazotemic serum is new (12).

While these are preliminary observations, we feel that they deserve some attention and even some speculation since the pathogenesis of essential hypertension is still not known. Based upon previous reports that factors in azotemic sera suppress organic anion transport *in vitro* in rabbit renal tubules (13), rat kidney slices (12) and *in vivo* in rat kidneys and liver (14), Bricker *et al.* (5, 6) isolated by Sephadex gel filtration a fraction (fraction 4) from azotemic sera which contained a suppressor to PAH transport. We might add that this sera fraction probably plays only a role in the suppression of PAH transport by azotemic sera based on the finding that (a) at equal volumes azotemic sera have far more suppressive effect than fraction 4 (5, 13), (b) azotemic sera obtained from acutely azotemic animals suppress organic anion transport while the fraction 4 inhibitor is found only in chronic azotemia (6), and (c) the stimulation noted in fraction 2 here in C a SHR's is also present in sera from normal humans but absent in sera obtained from chronic uremics (unpublished data). More important, however, Bourgoignie *et al.* (6) found that fraction 4 not only inhibited PAH transport in rabbit renal cortical slices but sodium transport in frog skin and toad bladders as well. Because PAH uptake by kidney slices is a sodium-dependent function, they hypothesized that an inhibitor to PAH transport in azotemic sera also might be involved with altered rate of sodium excretion per nephron noted in uremia (6). The relationship of sodium metabolism and hypertension is well-known. It is of interest that in human essential hypertension, the kidneys handle sodium abnormally; they excrete a load of sodium more rapidly than normal (15) and may respond more slowly to a restriction of

sodium (16). Therefore, it remains to be seen whether our suppressor to PAH transport which resides in the same fraction as the azotemic suppressor also affects sodium transport and how this might participate in sodium handling by hypertensives.

Less is known about TEA transport. These two transport systems (PAH and TEA) seem to be independent of each other as evidenced by the following: (a) accumulation of organic anions and cations occurs simultaneously without competitive interference, (b) metabolic inhibitors affect transport in each system differently, and (c) the optimum temperature and pH differ for accumulation of PAH or TEA in rat kidney slices (17, 18). The majority of studies concerned with organic base transport have been carried out *in vitro* because many of these agents bring about hemodynamic changes *in vivo* (17, 18). While there appears to be a component of sera which depresses or competes with TEA transport, more data are needed to elucidate this problem.

Summary. Sera taken from spontaneously hypertensive Wistar rats compared to sera from normotensive Wistar rats depressed both PAH and TEA systems in kidney slices from normotensive Sprague–Dawley rats. This depression was found to reside in a specific fraction of sera separated on Sephadex columns, the same fraction which in azotemic sera suppresses both PAH transport and sodium transport *in vitro*. The observation that factors in sera affect organic anion and cation transport early in the development of hypertension may have implications in the pathogenesis of hypertension.

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Received Sept. 6, 1973. P.S.E.B.M., 1974, Vol. 145.