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PAH and TEA Transport in Kidney Slices from Spontaneously Hypertensive and Normotensive Wistar Rats[1] (38451)

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Based upon speculation that some clinical and experimental hypertension is secondary to deranged kidney metabolism (1, 2), altered kidney function could contribute to the spontaneous hypertension which develops in a strain of Wistar rats (SHR). To compare some aspects of renal function, we followed *para*-aminohippurate (PAH) transport, tetraethylammonium bromide (TEA) transport, and oxygen consumption in cortical slices from normotensive rats (NR) and SHR at a point in time before tissue damage from hypertension could occur in SHR.

Methods and Materials. Male Wistar rats, both of a control normotensive strain (NR) and a spontaneously hypertensive strain (SHR) (Carworth, Vincentown, NJ), were studied at 7–9, 14–20, and 26–28 weeks of age. These rats were allowed free access to rat chow and drinking water, housed in pairs (one NR and one SHR together) in a room maintained at a temperature of 23° with light–dark periods of 14 and 10 hr, respectively. Blood pressures (BP) were obtained by tail plethysmography (3). The BP's of at least three readings determined on different days were averaged to give the results depicted in the tables. BP's on the 7–9 and 14–20 week old rats depicted in Table I were performed on rats lightly anesthetized with ether; the BP's on the 26–28 week old rats were performed on unanesthetized rats.

On each experimental day, an NR and an SHR were sacrificed by a blow to the head; the kidneys were removed rapidly and were placed in cold isotonic saline. Cortical slices (0.4 mm) were cut with a Stadie–Riggs microtome and used in the 7–9 and 14–20 week old rats experiments. In the experiments performed on the 26–28 week old rats, renal fragments were made as previously described (5). Oxygen consumptions were determined on a Gilson submarine respirometer at 37°. The basic incubation medium was that of Cross and Taggart (6)—phosphate-buffered sodium, potassium and chloride solution. To this, we added ^{14}C-tetraethylammonium bromide (TEA) (New England Nuclear, Inc.) and ^{3}H-*para*-aminohippurate (PAH) (Amersham Radiochemical Center), both 10^{-5} *M*. Final medium pH was 7.4. Slices or fragments were removed, and weighed after blotting to estimate PAH and TEA transport following 90 min of incubation at 24° on a Dubnoff shaker. Tissue weights ranged between 40 and 80 mg and a minimum of four flasks was studied from each rat. The results were averaged at the end of the experiment to yield a single observation. Slices and fragments were placed in 10% trichloroacetic acid, homogenized, centrifuged, and the supernatant used for beta counting. The incubation medium also was added to 10% trichloroacetic acid, centrifuged and the supernatant counted. The basic scintillation mixture was composed of Triton X-100 (Packard Inc.) (0.33 liter), toluene (0.67 liter), 2,5-diphenyloxazole (5.5 gm) and 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene (125 mg). Double isotope β counting and quench correction were performed utilizing a Packard Counter, Model 2420. Results are expressed as T/M ratios, *i.e.*, the ratio of counts per minute per g of tissue weight to the counts per min per ml of incubation medium (Table I). Statistics were obtained with Student's *t* test using group analysis;

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TABLE I. PAH and TEA Transport and QO_2 in Kidney Slices from Spontaneously Hypertensive (SHR) and Normotensive (NR) Wistar Rats.[a]

Age weeks	No of experiments	Body (g) wt		Kidney (g) wt		BP (mm Hg)		PAH T/M		TEA T/M		QO_2 (μl/mg/hr)	
		NR	SHR	NR	SHR	NR	SHR	NR	SHR	NR	SHR	NR	SHR
7–9	9	168 ± 4.9	182 ± 3.4 ($P < 0.05$)	1.44 ± .06	1.53 ± .03 (NS)	107 ± 2.2	110 ± 2.9 (NS)	9.5 ± 1.0	10.3 ± 0.7 (NS)	11.5 ± 0.5	10.3 ± 0.6 (NS)	—	—
14–20	11	435 ± 25.3	307 ± 10.2 ($P < 0.01$)	2.88 ± .16	2.24 ± .09 ($P < 0.01$)	116 ± 2.1	144 ± 3.5 ($P < 0.01$)	8.9 ± 0.7	11.8 ± 0.6 ($P < 0.01$)	14.3 ± 1.2	12.8 ± 0.8 (NS)	—	—
26–28	9	486 ± 15.4	342 ± 7.2 ($P < 0.01$)	3.52 ± .19	2.46 ± .05 ($P < 0.01$)	113 ± 2.2	171 ± 3.3 ($P < 0.01$)	5.4 ± 0.1	5.8 ± 0.1 ($P < 0.05$)	9.3 ± 0.5	8.9 ± 0.3 (NS)	2.45 ± .10	3.01 ± .11 ($P < 0.01$)

[a] NS = not statistically significant ($P > 0.05$); SEM is depicted.

statistical significance was set at $P < 0.05$.

Results. Nine experiments were performed on kidney slices obtained from rats between 7 and 9 weeks old (Table I). At this age, SHR had not yet developed a marked difference in systolic blood pressure from their control counterparts, the average of the SHR being 110 mm Hg compared to the NR, 107 mm Hg. In eight of nine experiments, the PAH transport was greater in the slices from these prehypertensive SHR rats (+ 12%). However, by group analysis, this difference in PAH S/M missed statistical significance. TEA transport appeared decreased in these prehypertensive rats (−10%), but again this difference was not statistically significant.

In the group of SHR at 14–20 weeks of age weighing 146 g more than the 7–9 week old rats, an elevation in blood pressure became apparent (Table I). While the BP in these older NR averaged only 9 mm Hg over the NR studied at 7–9 weeks of age, the blood pressure of the SHR averaged 34 mm Hg higher than their younger counterparts. The average blood pressure of the SHR, 144 mm Hg, was statistically higher than the 116 mm Hg of the NR ($P < 0.01$). There was no doubt that over a similar period, NR gained more body weight, 267 g, than SHR, 125 g; and the difference in kidney weight was statistically significant. The average PAH T/M of the 14–20 week old NR was 8.9. The 14–20 week old SHR kidneys had a significant increase in PAH transport, T/M of 11.8, when compared to slices for NR kidneys, T/M of 8.9 ($P < 0.01$); while TEA transport in SHR when compared to NR again was depressed. This depression missed statistical significance.

Data collected on the 26–28 week old rats were obtained in a different manner in an attempt to corroborate our previous findings: (a) Fragments instead of slices were used to estimate PAH and TEA transport, and (b) in addition, O_2 consumption was followed in these fragments. As noted previously, the body weights and kidney weights of the older SHR are less than NR of a comparable age, and SHR show systolic blood pressure in the hypertensive range (150 mm Hg) (Table I). PAH transport remained significantly increased in SHR and QO_2 was also higher in kidney fragments from SHR.

Discussion. The possibility exists that the enhanced PAH transport of SHR kidney slices may not relate to the hypertensive process itself but may be a separate characteristic bred into these

genetically hypertensive rats. At the moment, we have no definitive explanation for all our observations, but, since the pathogenesis of essential hypertension is an important unresolved problem which may relate to altered renal function (2), these observations deserve attention and some speculation.

Organic anion (PAH) and organic cation (TEA) transport are independent of each other based on the findings that (1) accumulation of organic anions and cations occurs simultaneously without mutual interference, (2) metabolic inhibitors have been noted to affect transport of each system differently, and (3) the optimum temperature and pH for accumulation in rat kidney slices differ (7, 8). In light of our results, PAH transport but not TEA transport in SHR differs probably to some extent from that of NR prior to the development of obvious blood pressure elevations, and definitely during the early stages of hypertension much before tissue damage from the elevated pressures could take place. Accordingly, the enhancement of PAH transport without similar change in TEA transport makes it unlikely that the former results from renal damage wrought by elevated blood pressures. The renal tissue from the 26–28 week old rats was studied as fragments to corroborate the results of the slice studies. Previously, this assay has been found to yield more reproducible results in our hands (5). Therefore, the smaller stimulation in the older SHR may be due more to technique than the physiologic effects of age. While the differences are less, the small standard deviation within each group makes the differences statistically significant.

A possible explanation for these transport differences between SHR and NR may be found in a separate group of studies concerning oxygen consumption in renal tissue performed simultaneously with the last set of studies. PAH accumulation *in vitro* by renal tissue is markedly dependent upon oxidative processes. Oxygen consumption was measured on renal fragments from the NR and SHR. As is the usual case in studies on tissue oxygen consumption, we performed the incubation at 37° in contrast to *para*-aminohippurate studies studies which were performed at 25° as suggested by Cross and Taggart (6). Shideman *et al.* noted that four compounds which inhibit oxidation of succinic acid depress transport of PAH but not *N*-methylnicotinamide, a compound requiring the same transport mechanism as TEA (9). Malonate, which blocks oxidative steps in the citric acid cycle, affects PAH transport but has no effect on TEA transport (10). Therefore, the significant increase in QO_2 in fragments from SHR compared to NR may reflect metabolic processes which, in some manner, enhance PAH transport but not TEA transport. Another possibility is that tissue adaptation has occurred in this renal transport to counteract a circulatory inhibitor to PAH transport present in the SHR (11).

Albeit small and whether interrelated or not, the significant differences in PAH transport and oxygen consumption between the normotensive and hypertensive Wistar rats constitute basic differences in renal metabolism between the two groups of rats.

Summary. In comparing renal tissue metabolism between a normotensive and genetically hypertensive strain of Wistar rats, we noted a relative increase in PAH transport and oxygen consumption in kidney slices from the hypertensive rats. No statistically significant difference in TEA transport was observed. The changes in tissue function are present early in the development of hypertension and constitute a physiologic difference in renal function between a normotensive and hypertensive strain of Wistar rats.

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