PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 350-354 (1976)

Acid Excretion in Spontaneously Hypertensive Rats[1] (39543)

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Spontaneously hypertensive rats (SHR) (1) offer a unique opportunity to study the pathogenesis of genetic hypertension in an animal model. Since it has been proposed that human essential hypertension may be related to altered renal function, it occurred to us that genetic hypertension might be associated with renal malfunction as well (2, 3). Recent evidence suggests that kidneys are involved, at least in part, in the pathogenesis of the genetic hypertension in rats (4). Our initial interests have been to compare renal function in SHR with normotensive Wistar rats (NWR). Toward this end, we have examined PAH transport and oxygen consumption in kidney slices from SHR and NWR and found differences in these functions (5). The purpose of the present investigation was to extend our knowledge of renal function in SHR by assessing acid excretion. We found that, compared to two normotensive strains of rats, SHR showed decreased acid excretion secondary to depressed ammonium excretion.

Methods. Experiments were initiated on 11 normotensive male Sprague-Dawley rats with an average weight of 237 g ± 15.0 (SEM) (Flow Laboratories, Dublin, Va.), on 50 normotensive male Wistar rats with an average weight of 455 g ± 19.6 (SEM) (Microbiological Associates, Walkersville, Md.), and on 56 spontaneously hypertensive male Wistar rats with an average weight of 347 g ± 9.2 (SEM) (Taconic Laboratories, Germantown, N.Y.). All rats were housed in a constant-temperature room with a light-dark phase of 14 and 10 hr, respectively, and given Purina rat chow and water *ad libitum*. To study acid excretion under normal acid-base conditions, rats were given 2 ml/100 g BW of water by stomach tube, and urine was collected over the ensuing 4 hr. In other studies, rats were made acutely acidotic by giving them 1 mmole/100 g BW of 0.5 *M* ammonium chloride solution administered by stomach tube. After this, the rats were placed in metabolic cages and timed 4-hr collections of urine were obtained. To produce chronic acidosis, rats received 1 mmole/100 g BW of 0.5 *M* ammonium chloride b.i.d. by stomach tube. On the third day, urine was collected over the 4 hr following the morning dose of NH_4Cl.

Some of the rats were anesthetized lightly with ether, blood was drawn from the aorta, and the kidneys were removed and immediately placed in cold saline either following the collection of urine after water load or after the urine collection following chronic acid challenge. The decapsulated kidneys were weighed to the nearest milligram.

Using a Radiometer pH meter equipped with an automatic titrator, we estimated pH and determined titratable acidity by titrating the urine to pH 7.4 and 0.01 *M* NaOH. Ammonia was measured by the method of Preuss *et al.* (6). Carbon dioxide content was determined and calculated as described by Natelson (7). No blood pH's were determined; and so, for our purposes, we assumed that CO_2 content in millimoles per liter = millequivalents of HCO_3^- per liter (8). Blood pressure (BP) was estimated by tail plethysmography (9). At least three readings of BP from different days were taken for each rat.

Kidney slices, cut to approximately 0.4-mm thickness with a Stadie-Riggs microtome (10), were trimmed so as to weigh 50 mg and were placed in 5 ml of incubation medium containing NaCl, 110 m*M*; KCl, 5 m*M*; $MgSO_4$, 1.2 m*M*; $CaCl_2$, 1 m*M*; $NaHCO_3$, 25 m*M*; and NaH_2PO_4, 1.2 m*M*. Glutamine (2 m*M*) was added to the me-

[1] Supported by NIH Grant 15458 and Public Health Service Grant RR5360 (Medical).

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dium in some studies. The medium was gassed with 95% O_2 and 5% CO_2 at 37°. Initial pH was 7.4 and after incubation was below 7.5. After an incubation of 90 min, media from the flasks were deproteinized with cold 1.2 *N* perchloric acid. Following centrifugation, the perchloric acid was precipitated by the addition of KOH, phosphate buffer mixture (final pH 7.0), and ammonia determinations were made on the supernates.

Statistics were performed by Student's *t* test with statistical significance set at $P < 0.05$.

Results. In Table I are summarized results of acid excretion in normotensive Sprague-Dawley rats (SD), BP < 125 mm Hg; normotensive Wistar rats (NWR), BP < 125 mm Hg; and hypertensive Wistar rats (SHR), BP > 150 mm Hg. Following water loading, the pH of the SHR was higher than in either normotensive species of rat. In addition, ammonium excretion was less. After acute acid challenge, the volume excretion of SHR was lower than the NWR but not lower than the normotensive SD. While the urine pH and titratable acid were no different among the groups, ammonium excretion was significantly lower in the SHR. Following chronic acid challenge, the urine volume excretion was lower in the SHR than the other two groups, the urine pH's were similar, the titratable acids were higher in SD than the other two groups, and, again, ammonium excretion was lower in the SHR. Serum HCO_3^- concentrations (CO_2 content) were estimated in some of the rats. The serum HCO_3^- in nine SHR on the third day of acidosis (15.2 μequiv/liter ± 1.2 (SEM)) was lower than that in nine normotensive Wistar rats (18.7 μequiv/liter ± 1.2 (SEM)). These differences just miss statistical significance, $0.05 < P < 0.1$.

Figure 1 depicts the relationship between kidney size and body weight in 50 normotensive Wistar rats and 56 spontaneously hypertensive Wistar rats. No obvious difference between the two groups is noticeable.

Figure 2 depicts results obtained by studying ammoniagenesis in kidney slices from SHR and NWR. Ammonia production was not different between slices from either group whether no substrate was present in the medium (endogenous production) or in the presence of 2 m*M* glutamine. However, when slices were removed from rats on the third day of acidosis, SHR slices showed less "endogenous" and "glutamine" ammoniagenesis.

Discussion. Following acute acid challenge, normal kidneys excrete more H^+ as titratable acid and ammonium (11). If the challenge continues, urinary ammonium increases more and accounts for the majority

TABLE I. ACID EXCRETION IN NORMOTENSIVE AND HYPERTENSIVE RATS.[a]

Rat	Number	Urine volume (ml/4 hr)	Urine pH	Titratable acid (μequiv/hr/100 g BW)	Ammonium (μequiv/hr/100 g BW)
Water load					
SD	11	4.8 ± 0.6	6.9 ± 0.2		9.4 ± 1.7
NWR	22	8.5 ± 0.4	7.2 ± 0.1		5.8 ± 0.6
SHR	25	6.2 ± 0.3[b]	7.8 ± 0.1[c]		4.6 ± 0.6[c]
Acute acid challenge					
SD	7	6.3 ± 1.1	5.9 ± 0.9	6.6 ± 2.0	38.7 ± 5.1
NWR	30	9.9 ± 0.4	6.0 ± 0.1	7.8 ± 1.3	39.6 ± 1.4
SHR	29	5.8 ± 0.5[b]	5.9 ± 0.1	6.8 ± 0.8	28.5 ± 1.6[c]
Chronic acid challenge					
SD	7	6.4 ± 0.7	5.7 ± 0.6	11.7 ± 1.3	70.2 ± 8.3
NWR	26	6.7 ± 0.4	5.9 ± 0.1	6.8 ± 0.1	72.4 ± 3.8
SHR	25	4.6 ± 0.4[c]	5.9 ± 0.1	5.6 ± 0.6[d]	54.9 ± 4.1[c]

[a] SD = normotensive Sprague-Dawley rats, NWR = normotensive Wistar rats, SHR = spontaneously hypertensive Wistar rats. Values are means ± SEM.
[b] $P < 0.05$ compared to NWR.
[c] $P < 0.05$ compared to SD and NWR.
[d] $P < 0.05$ compared to SD.

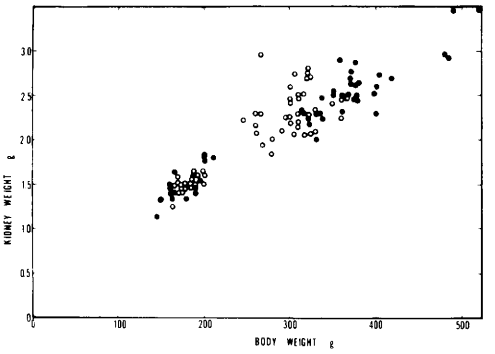

FIG. 1. Correlation between kidney weight (KW) and body weight (BW) in normotensive Wistar rats (NWR) (●) and spontaneously hypertensive Wistar rats (SHR) (○).

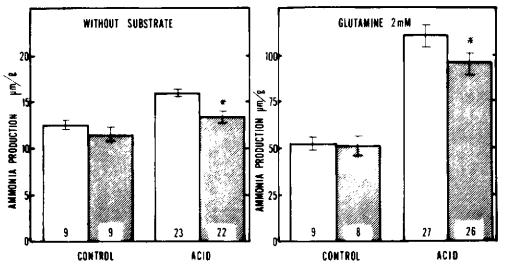

FIG. 2. Histograms depicting ammoniagenesis by kidney slices from normal (control) and acidotic normotensive Wistar rats □ and spontaneously hypertensive Wistar rats ■. Slices were incubated without substrate or in the presence of 2 m*M* glutamine. The scales for these two situations differ. Numbers at the base of the columns indicate the number of rats studied. (*) $P < 0.05$. SEMs are shown by the vertical lines.

of H^+ excreted (11). Van Slyke *et al.* first reported that the source of renal ammonia is the amide nitrogen of glutamine (12). Subsequently, other studies verified this and added that the amino nitrogen of glutamine also contributes to the formed ammonia (13–15), and that the remaining carbon skeleton becomes a major renal fuel during acidosis (16, 17).

In the present study, we assessed the response of SHR to acid challenge. Compared to normotensive Wistar and Sprague–Dawley rats, hypertensive Wistar rats showed a similar ability to lower urine pH and excrete titratable acid but less ability to increase ammonium excretion following acute or chronic acid challenge. The extent of acid challenge among the groups appeared to be similar. When serum HCO_3^- concentrations were checked in a few chronically acidotic rats, systemic acidosis, if anything, was more severe in SHR than NWR.

Why the decrease in ammonium excretion in SHR? Ammonium excretion can be affected, for the most part, by three factors: urine volume, urine pH, and renal ammonia production. The first two are factors because ammonia (NH_3) is excreted into the tubular lumen by nonionic diffusion as a gas (where it reacts with H^+) to form ammonium (NH_4^+) (18, 19). Both increased urine flow and decreased urine pH lower urine pNH_3 and allow more diffusion of free base NH_3 from the renal cells and the peritubular blood stream. Richterich (20) has performed extensive studies relating urine flow to urine ammonium excretion in humans and dogs. At higher urine pH, flow can enhance excretion but becomes less of a factor at lower pH's, e.g., below pH 6.5 (21). In our studies, there was no consistent correlation between urine flow and ammonium excretion at high or low urine pH's. Sprague–Dawley rats, after water loads, put out a lower urine volume and still excreted more ammonium. During acute acid challenge, the differences in urine flow between Sprague–Dawley rats and SHR were not significantly different. Because we could find no consistent relationship between urine flow and ammonium excretion, we believe that changes in urine flow could not be the only factor in bringing about differences in ammonium excretion here. In addition, urine pH could not be a major factor; for during acute and chronic acid challenge, urine pH's were similar.

The above reasoning suggests that lesser renal ammonia production is the basis for the lowered urinary excretion of ammonium. Could kidney size, i.e., the amount of ammonia producing mass, play a role (22)? No. If anything, the younger Sprague–Dawley rats were smaller rats when studied and had smaller kidneys. Additionally, although NWR are larger than SHR of the same age (23), Fig. 1 shows that the kidney weight to body weight ratio was similar in the two groups of Wistar rats. It follows that, when we report ammonium excretion per 100 g BW (see Table I), we are, in essence, taking

kidney size into consideration. Therefore, kidney mass differences between groups are not responsible for the differences in ammonium excretion (Table I).

We have no definitive answer as to how renal ammoniagenesis is different in SHR, but our *in vitro* studies suggest that some of this can be attributed to intrinsic renal metabolic differences. Ammonia production by kidney slices from NWR and SHR in normal acid–base balance was not different in the presence or absence of glutamine (2 m*M*). This seems strange as slices from SHR have a higher oxygen consumption (5), and this is usually associated with a greater capacity to produce ammonia in normotensive SD (24). In addition, slices from chronically acidotic SHR compared to slices from chronically acidotic NWR produce less ammonia in either medium. This 12% decrease in production is considerably less than the decrease in ammonium excretion in SHR compared to NWR following chronic acid challenge (−24%). Therefore, differences in intrinsic renal metabolism during chronic acidosis may be partially responsible for the differences in excretion but probably cannot explain the entire difference.

We believe that additional factors are affecting ammonia production *in vivo*. Circulating factors affect the metabolism of glutamine by renal cells (25, 26). Any changes in renal substrate concentrations (27) or stimulators (28) to ammonia production could bring about changes that would not necessarily be fully mirrored in slice studies. In addition, if acidosis markedly affects renal blood flow or distribution, this might result in less glutamine being presented to ammonia-producing cells in kidneys. This is a strong possibility that should be checked out since we know that the vascular system of the SHR tends to be hyperreactive (29).

Whether this finding relates directly or indirectly to the pathogenesis of hypertension and whether this defect is present in patients with essential hypertension is unknown. That SHR and patients with essential hypertension both have an exaggerated natriuretic response is known (30), but we are unaware of any conclusive studies on acid handling in patients with essential hypertension.

Summary. SHR excrete less ammonium than NWR and SD following water load and acute and chronic acidosis. Thus, their ability to handle acute and chronic acid challenge is impaired because of the lesser ammonium excretion. Since these differences in ammonium excretion cannot be related in entirety to urine flow, urine pH, and kidney mass, basic differences *in vivo* in renal metabolism of glutamine, the major precursor of renal ammonia, seem likely. Slices from chronically acidotic SHR compared to slices from chronically acidotic NWR produce less ammonia, suggesting some basic intrinsic difference in renal glutamine metabolism during persistent acidosis. Because these differences in slice ammoniagenesis are smaller than the differences in ammonium excretion, and because slices from nonacidotic SHR and NWR show no differences in ammoniagenesis, we feel that other *in vivo* factors, probably extrarenal, additionally decrease renal ammonia production in SHR.

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Received October 8, 1975. P.S.E.B.M. 1976, Vol. 153.