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Acid excretion in young and adult Wistar Kyoto and spontaneously hypertensive rats[1]

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Summary. Wistar Kyoto rats (WKy), the most widely accepted control for SH rats, show an inability to excrete acid appropriately when compared to another normotensive strain, SD. Coupled with the fact that KWy also develops 'sodium-sensitive' hypertension, this makes them a more complex control than realized. At very young ages (< 10-week-old), neither SH nor WKy show any deficiency in acid excretion.

When Okamoto and Aoki[2] first bred spontaneously hypertensive (SH) rats, they bred simultaneously a control normotensive group, now called Wistar Kyoto rats (WKy). Logically, most investigators have used WKy rats as control for SH rats.

In 1971, Louis et al.[3] pointed out 2 interesting findings from their study on SH rats: 1. young SH (study initiated when rats were prehypertensive) eventually developed hypertension despite consumption of a diet completely devoid of sodium, and 2. when fed excess sodium, SH markedly increased their hypertension[4]. Therefore, SH were 'sodium-sensitive' rats, i.e., hypertension develops or worsens rapidly with heavy sodium ingestion[5,6]. Because of this, they postulated that SH rats inherit 2 groups of autosomal alleles responsible for their hypertension - 1 set that allows sodium sensitivity and 1 set that allows elevated blood pressures in the absence of sodium. Recently, we found that WKy rats also develop hypertension after 1 week of ingesting 1% w/v sodium chloride in their drinking water (average blood pressure 150 mmHg in 10 rats)[7]. Therefore, we postulated that WKy rats inherit at least 1 group of the alleles described for SH, i.e., the 'sodium-sensitive' component.

In 1976, we reported that SH rats when compared to normotensive Wistar rats (American strain) and normotensive Sprague-Dawley SD rats excreted acid poorly in response to both acute and chronic acid loading[8]. We did not perform studies using WKy rats. Our main purpose in the present investigation was to expand these studies on acid excretion to include the WKy rats, the generally accepted control for SH. As a secondary gain, we followed acid excretion in WKy, SH, and SD rats when they were young or less than 10 weeks of age. At this age, SH have not yet become hypertensive.

Methods. Experiments were carried out in 2 stages - first on older adult rats and later on younger rats. The younger rats had an average age of 6-10 weeks, while the older rats were between 20 and 25 weeks of age. The average weights of the younger rats were: SD 182 g ± 6 (SEM); WKy, 185 g ± 18 (SEM); and SH, 181 g ± 12 (SEM). In adult rats, the average weights were: SD, 425 g ± 12.5 (SEM); WKy, 305 g ± 7.6 (SEM); and SH, 332 g ± 6.6 (SEM). Both strains of Wistar rats were obtained from Taconic Labs, Germantown, New York, and the SD, from Flow Labs, Dublin, Virginia. The rats ate rat chow and drank water ad libitum. The procedures for giving acid loads and measuring CO_2

Table 1. Acid excretion in adult WKy, SH and SD rats

Rat	Number	Serum CO_2 content (mEq/l)	Urine Volume (ml/4 h)	pH	Titratable acid (μEq/h/100 g b.wt)	Ammonium (μm/h/100 g b.wt)
Water load						
SD	(17)	28.2 ± 0.6	7.4 ± 0.6	7.1 ± 0.4	-	6.0 ± 0.7
WKy	(29)	28.3 ± 0.8	4.3 ± 0.3[a]	7.1 ± 0.1	-	7.2 ± 0.8
SH	(26)	28.5 ± 0.5	4.9 ± 0.3[a]	7.3 ± 0.1	-	5.3 ± 0.5
Acute acid challenge						
SD	(19)	19.3 ± 0.7	10.3 ± 1.0	5.9 ± 0.04	9.5 ± 0.9	31.3 ± 1.3
WKy	(19)	20.2 ± 1.3	4.9 ± 0.3[a]	5.7 ± 0.03	10.8 ± 0.9	25.9 ± 0.7[a]
SH	(21)	20.9 ± 1.3	5.8 ± 0.7[a]	5.7 ± 0.04	12.6 ± 1.1	24.7 ± 0.7[a]
Chronic acid challenge						
SD	(19)	21.4 ± 0.6	7.6 ± 0.5	5.6 ± 0.04	11.9 ± 2.0	67.6 ± 2.1
WKy	(14)	19.9 ± 1.3	5.1 ± 0.3[a]	5.7 ± 0.03	7.1 ± 0.9[b]	59.7 ± 2.6[b]
SH	(16)	21.7 ± 0.8	5.4 ± 0.4[a]	5.7 ± 0.03	7.3 ± 0.6[b]	52.9 ± 2.0[a]

SD = Sprague-Dawley, WKy = Wistar Kyoto, SH = spontaneous hypertensive, values are means ± SEM.
[a] $p < 0.01$
[b] $p < 0.02$ compared to SD under same conditions.

Table 2. Acid excretion in young WKy, SH and SD rats

Rat	Number	Serum CO_2 content (mEq/l)	Urine Volume (ml/4 h)	pH	Titratable acid (μEq/h/100 g b.wt)	Ammonium (μm/h/100 g b.wt)
Water load						
WKy	(12)	29.6±0.4	4.3±0.5	7.2±0.2	-	6.1±0.8
SH	(12)	28.7±0.8	5.1±0.4	7.4±0.4	-	4.4±0.5
Acute acid challenge						
SD	(6)	20.2±1.0	4.5±0.6	5.8±0.10	9.8±1.9	31.5±3.0
WKy	(12)	22.0±1.3	6.5±1.0	5.9±0.10	10.3±1.1	36.2±4.0
SH	(14)	22.0±0.8	7.2±0.8	6.0±0.04	6.8±0.8*	31.2±1.4
Chronic acid challenge						
SD	(6)	19.8±0.7	5.9±0.3	5.6±0.10	10.5±1.2	62.1±6.4
WKy	(12)	19.0±1.3	6.2±0.3	5.6±0.04	13.0±2.1	68.3±6.4
SH	(10)	18.4±1.0	6.7±0.4	5.6±0.08	10.2±0.8	61.9±5.0

* $p<0.05$. See legend in table 1 for details.

content, urine pH, titratable acid (TA), and ammonium (NH_4+), were outlined in a previous paper[8]. Statistics were performed by Student's t-test with statistical significance set at $p < 0.05$.

Results and discussion. The results of acute and chronic acid loading on adult SH and SD rats did not deviate markedly from those reported earlier[8]. In these new studies (table 1) urinary NH_4+ excretion during acute and chronic acid stress again was significantly lower in SH than SD rats. Urine pH cannot account for these differences as it was the same in both groups[9]. In response to chronic acid loading, TA of SH rats was significantly lower in SH than SD rats. Interestingly, the response of the WKy rats compared to SD rats was significantly lower during acute and chronic acidosis. In response to acute acid stress, NH_4+ excretion by WKy was of a similar magnitude as SH. However, the WKy was able to excrete significantly more NH_4+ in response to chronic acid loading than SH ($p<0.02$) although this value was still significantly lower than that seen in SD rats ($p<0.02$). Therefore, WKy like SH has an inability to excrete comparable quantities of NH_4+ during acid stress compared to SD. Like SH, WKy excretes less TA than SD when chronically acid loaded ($p<0.02$). No differences were brought out by water loading alone.

In looking at these parameters in young rats, (table 2), we found no differences in the 3 strains of rats studied. Water loading was not investigated in the young SD. With 1 exception, values between the 3 strains were no different. Titratable acid excretion in SH during acute acid stress was significantly lower than SD or WKy ($p < 0.05$). Defining the normotensive control for SH presents problems. Although many different substrains or strains of Wistar Rats have served as control, WKy rats have been preferred by the majority. Recently, we found that WKy was a 'sodium-sensitive' rat, i.e., in response to short term sodium loading, WKy rats become hypertensive[5-7]. Therefore, one must consider that WKy has inherited one of the 2 hypertension-producing alleles, i.e. Louis et al.[3], reasoned that SH inherit at least 2 groups of alleles - one that produces hypertension in the absence of sodium and one that creates a worsening of hypertension in the presence of sodium. It is the latter component that WKy appears to inherit. In this respect, WKy does not represent a pure normotensive control. Interestingly, a recent study demonstrates that WKy handles sodium excretion more like SH than a normotensive strain (not Kyoto) of Wistar rats[10]. The majority of studies concerned with renal sodium handling in SH used WKy as control[11,12].

Of interest to us, how do WKy rats compare to SH rats in other respects? Older WKy rats (20–25 weeks old) tended to act more like the SH rat in their acid excreting capabilities. Following acute acid loading, WKy excreted significantly less NH_4^+ than SD. Following chronic acid loading, NH_4+ excretion by WKy rats fell somewhere between that of SD and SH rats, i.e., significantly lower than SD but significantly higher than SH. Thus, our findings with ammonium excretion resemble those reported for sodium handling[10]. We do not know how these 2 separate functions interrelate, if at all.

When we compared acid excreting abilities in the younger rats, we found no difference among the 3 substrains. At 6–10 weeks of age, SH rats are just beginning to develop a higher blood pressure than control. It is approximately beyond 10 weeks of age when they develop a blood pressure of greater than 150 mmHg[13]. Accordingly, we studied younger rats during a period when they are beginning to develop hypertension. Differences in acid excretion between Kyoto Wistar (SH, WKy) and SD develop in a similar time frame as the rise in blood pressure of SH. We are not aware whether studies have been performed relating sodium excretion with the development of hypertension in SH or in WKy placed on high sodium diets.

Thus, adult WKy rats inherit not only the propensity to sodium-sensitive hypertension, but also the inability to excrete ammonium to the same extent as many of their normotensive counterparts. This is not seen in the very young WKy or SH. Renal acid handling by WKy resembles that seen in SH more than in SD although subtle differences do exist.

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