Electrolyte content of milk differs in normotensive and spontaneously hypertensive rats

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Milk samples were collected from lactating spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) normotensive females 14 days postpartum and were later analyzed for content of calcium (Ca^{++}), sodium (Na^+), potassium (K^+), chloride (Cl^-), magnesium (Mg^{++}), and total protein. Our findings revealed that milk samples from SHR females had significantly higher concentrations of Na^+ and Cl^- and significantly lower concentrations of Ca^{++} , K^+ , and protein when compared with samples from WKY females. In addition, the Na^+/K^+ ratio for milk samples from SHR was significantly higher than that for samples from WKY. Milk levels of Mg^{++} were similar for females of the two strains. These results indicate that preweaning dietary content of several electrolytes implicated in the development of hypertension differs substantially between SHR and WKY pups. Such differences in milk electrolyte levels may constitute an important environmental trigger for age-related increases in arterial pressures of SHRs.

Rats of the inbred spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) normotensive strains have figured prominently in laboratory studies of blood pressure regulation over the past three decades (Geller, 1977; Lovenberg, Simpson, & Yamori, 1986; Okamoto, 1972; Rascher, Clough, & Ganten, 1982). Historically, much of the research with these and other hypertensive animal models has been preoccupied with documenting pathophysiological changes that attend the development of high blood pressure in adult animals or with utilizing these strains in preclinical tests of antihypertensive medications. In contrast, a recent focus of research with rodent models of human essential hypertension has been an examination of neonatal factors that influence the later development of high blood pressure (Blizard, 1992; DiNicolantonio, Imai, Murakami, & Yamori, 1991; Gray, 1984).

In SHR and inbred Dahl hypertension-sensitive (SS/Jr) rats, factors associated with the maternal environment exert an important influence on the expression of the hypertensive phenotype. Specifically, studies from our laboratory and the work of others indicate that genetically hypertensive rats have significantly reduced basal blood pressures in adulthood when they were reared from birth to weaning by normotensive foster mothers. In contrast, the basal blood pressures of adult normotensive rats were

unaffected if these animals were reared from birth to weaning by genetically hypertensive mothers (Azar, Kabat, & Bingham, 1991; Cierpial & McCarty, 1987; DiNicolantonio, 1987; DiNicolantonio, Marshall, Nicolaci, & Doyle, 1986; Murphy & McCarty, 1989).

A significant feature of the interactions between a mammalian female and her developing young is nutrient exchange. Infants must procure milk from the mother's teat by suckling, and mothers must maintain the production and transfer of milk to their dependent young through physiological and behavioral alterations (Brake, 1991). Milk composition may, in fact, be one aspect of the maternal environment that affects the development of high blood pressure in genetically hypertensive rats. A substantial literature indicates that dietary levels of sodium and calcium have important effects on cardiovascular homeostasis in genetically hypertensive laboratory rats and in humans with essential hypertension (Contreras, 1989; Dustan & Kirk, 1989; MacGregor, 1985; McCarron, 1985; Sowers, Zemel, Standley, & Zemel, 1989).

Other electrolytes, such as chloride, magnesium, and potassium, have also been implicated in the etiology of essential hypertension (Dyckner, 1990; Kurtz, Al-Bander, & Morris, 1987; Whelton & Klag, 1989). Finally, it has been suggested that an as yet uncharacterized hypertensinogenic factor in milk might be responsible for the development of high blood pressure in SHRs (McMurty, Wright, & Wexler, 1981).

In the present study, we measured total protein content and concentrations of five electrolytes in milk samples collected from SHR and WKY mothers at 14 days postpartum. We selected this age for milk sampling because it is just prior to the time when rat pups make the transition to the ingestion of solid food (Hall, Cramer, & Blass,

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1977). In addition, milk concentrations of protein and several electrolytes are generally stable from 10 days postpartum through weaning at 21 days of age (Keen, Lonnerdal, Clegg, & Hurley, 1981; Luckey, Mende, & Pleasants, 1954).

METHOD

Breeding colonies of SHR and WKY rats were maintained in a temperature-controlled vivarium room with a 12:12-h light:dark cycle (lights on from 0700–1900 h). The foundation breeding stock for these colonies was originally purchased from Taconic Farms, Germantown, NY. Prior to mating, basal systolic blood pressures were obtained for all SHR and WKY breeders with the use of indirect tail cuff plethysmography (IITC, Inc., Woodland Hills, California). Male and female SHR rats were excluded from the breeding colony if their basal systolic blood pressures were less than 150 mm Hg. Male and female WKYs were excluded from the breeding colony if their basal systolic blood pressures were greater than 130 mm Hg.

After the measurement of blood pressures, 1 male was placed with two multiparous females of the same strain for at least 1 week in a suspended wire cage (65 × 24 × 18 cm) that was provided with Purina laboratory chow (Formulab 5001) and tap water ad lib. After the 1-week mating period, the females were placed into individual plastic maternity cages (46 × 24 × 20 cm) that were provisioned with laboratory chow, tap water, and bedding material. The cages were checked in the early morning and late afternoon for litters, and the day of birth was designated as Day 0. All litters were culled to 8 pups on the day after birth.

On the 14th day after birth, the litters were removed from the maternity cages at 0900 h. The pups from each litter were placed into a single plastic container (15 × 11 × 7 cm); all containers were maintained in a pediatric isolette kept at 33°–35°C and housed in a separate room within the vivarium. At 1500–1600 h, the lactating females received an intraperitoneal injection of oxytocin (Sigma, St. Louis; 0.1 mU in 0.5 ml of 0.9% saline) and were then lightly anesthetized with Metofane. Milk samples (0.3–0.4 ml) were collected by manual expression from the teats. Milk samples were refrigerated for 1–2 days at 4°C and were then analyzed at the Clinical Chemistry Laboratory of the University of Virginia Health Sciences Center.

Milk concentrations (mmol/L) of Na⁺, K⁺, Cl[−], and Mg⁺⁺ were determined with the use of ion-specific electrodes (Hitachi Model 717 Clinical Analyzer; Boehringer-Mannheim, Indianapolis). With the same clinical analyzer, free Ca⁺⁺ was determined by spectrophotometric measurement of cresol-phthalein complexones with EDTA blanking while total protein was determined by a spectrophotometric biuret. Quintuplicate determinations of all parameters in a pooled milk sample yielded values for each electrolyte and total protein that varied by no more than 5%.

Data are presented as means ± SEM. Comparisons between strains were made with the use of two-tailed Student's *t* tests for unpaired data.

RESULTS

Basal systolic blood pressures of male and female SHR rats were significantly greater than corresponding values for WKYs (*p* < .001). The systolic blood pressures of all SHR and WKY rats fell within our selection criteria for inclusion in the breeding colony (Table 1).

Table 2 summarizes the total protein and electrolyte contents of milk samples collected from SHR and WKY females at 14 days postpartum. Total protein content was

Table 1
Basal Systolic Blood Pressures (mm Hg)
of SHR and WKY Breeders

	WKY		SHR	
	<i>M</i> ± <i>SEM</i>	Sample Size	<i>M</i> ± <i>SEM</i>	Sample Size
Males	106 ± 4	6	166 ± 4*	6
Females	100 ± 1	12	167 ± 3*	12

Note—SHR, spontaneously hypertensive; WKY, Wistar-Kyoto. **p* < .001 (two-tailed *t* test).

Table 2
Total Protein (g/dL) and Electrolyte Content (mmol/L)
of Milk Samples Obtained from Lactating WKY
and SHR Females at 14 Days Postpartum

	WKY (<i>n</i> = 10)	SHR (<i>n</i> = 9)
Total protein	8.8 ± 0.1	7.2 ± 0.2†
Mg ⁺⁺	3.4 ± 0.1	3.4 ± 0.1
Ca ⁺⁺	67.7 ± 0.8	58.7 ± 3.0†
Cl [−]	17.0 ± 1.5	29.4 ± 4.4*
Na ⁺	32.3 ± 1.5	47.8 ± 5.9*
K ⁺	45.5 ± 2.2	36.6 ± 2.3*
Na ⁺ /K ⁺	0.7 ± 0.1	1.5 ± 0.4*

Note—SHR, spontaneously hypertensive; WKY, Wistar-Kyoto. Values are presented as means ± SEM. **p* < .05. †*p* < .01. *p* < .001. (Two-tailed *t* test.)

reduced significantly in milk samples of SHR females (*p* < .001). The milk from SHR females also contained significantly less Ca⁺⁺ (*p* < .01) and K⁺ (*p* < .05) than did the milk from WKY females. In contrast, the milk concentrations of Na⁺ and Cl[−] as well as the Na⁺/K⁺ ratio were elevated significantly in SHR females. However, the milk levels of Mg⁺⁺ were similar for SHR and WKY females.

DISCUSSION

The present findings provide important new information regarding the diet available to preweaning genetically hypertensive rat pups. We have demonstrated that SHR females at 14 days postpartum produce milk that contains elevated levels of Na⁺ and Cl[−] and reduced levels of Ca⁺⁺, K⁺, and protein. These strain differences in milk electrolytes may represent an important dietary contribution to the observed increases in arterial pressure of SHR pups from the time between birth and weaning (Gray, 1984). The reductions in milk protein may explain in part the substantial body weight differences between SHR and WKY pups during the preweaning period (Cierpial & McCarty, 1987).

Our data for milk electrolytes are in general agreement with reports by others. For example, DiNicolantonio et al. (1986) noted that the milk [Na⁺]/[K⁺] of SHR females was significantly greater than for normotensive Sprague-Dawley females, although absolute values for the two electrolytes were not reported. In addition, Azar et al. (1991) reported that milk samples collected from SHR females

at 3, 7, 15, and 19 days postpartum had elevated levels of Na^+ when compared with milk samples from matched WKY controls. In contrast to our results, these investigators found no differences in milk content of K^+ at any of the four sample points. Finally, Dene and Rapp (1985) found that milk levels of Na^+ were elevated and milk levels of K^+ were reduced in inbred Dahl salt-sensitive hypertensive rats in comparison with the milk levels in normotensive salt-resistant controls.

It is well known that dietary levels of sodium and potassium have important effects on cardiovascular homeostasis (Dustan & Kirk, 1989; Gavras & Gavras, 1989; McCarron, 1986, 1989; Zicha, Kunes, & Jelinek, 1986). Other electrolytes, such as chloride, potassium, and magnesium have also been shown to have important effects on the cardiovascular system (Dyckner, 1990; Kurtz, Al-Bander, & Morris, 1987; Whelton & Klag, 1989). In addition, increases in dietary sodium exacerbate and increases in dietary calcium ameliorate the development of hypertension in young, postweaned SHR (Gavras & Gavras, 1989; Hatton, Muntzel, McCarron, Pressley, & Bukoski, 1988; Muntzel, Hatton, Metz, & McCarron, 1989).

Studies done with dietary electrolyte manipulations during the preweaning period have limited utility in hypertensive rats because milk electrolyte content is not altered in lactating female rats in response to changes in dietary electrolytes (Azar et al., 1991). In addition, manipulation of maternal dietary electrolytes may influence offspring blood pressure development indirectly because of diet-induced changes in the maternal behavior and/or cardiovascular function. Finally, the test diet could be ingested directly by the hypertensive rat pups prior to weaning.

Cross-fostering studies of hypertensive and normotensive pups provide an indication that the milk provided by hypertensive mothers to their pups represents an important preweaning influence on full expression of the hypertensive phenotype. SHR pups reared from birth to weaning by WKY or Sprague-Dawley normotensive foster mothers have significant reductions in arterial pressure in adulthood when compared with SHR pups reared by their natural mothers or by SHR foster mothers (Cierpial, Konarska, & McCarty, 1990; Cierpial & McCarty, 1987, 1991). Similarly, SS/Jr pups reared by normotensive SR/Jr foster mothers have significant reductions in adult blood pressures when compared with control SS/Jr rats reared by their natural mothers or by SS/Jr foster mothers (Murphy & McCarty, 1989). The influence of the normotensive foster mother appears to be limited to the first 2 postnatal weeks. Our recent studies indicate that cross-fostering SHR pups to a WKY foster mother for Postnatal Days 1-7 or 1-14 has an impact on adult blood pressures that is comparable to the effect observed in SHR pups cross-fostered to WKY mothers for the entire 3-week preweaning period (McCarty, Cierpial, Murphy, Lee, & Fields-Okotcha, 1992). This study indicates that the blood-pressure-lowering effect of cross-fostering corresponds to the time during the preweaning period when pups receive all of their nourishment from the mother.

Much of the literature on electrolytes and blood pressure regulation takes the limited view that a single electrolyte is responsible for the development of hypertension. The present results suggest an alternative view; that is, a combination of electrolyte alterations in milk may provide the stimulus for the development of high blood pressure in genetically susceptible animals. Only by determining a milk electrolyte profile could such relationships be revealed. Additionally, consideration must be given to the occurrence of other hypertensinogenic factors in milk, including peptide hormones that affect the developing cardiovascular system (Hazum, 1983; Koldovsky, 1980).

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