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Renal response to acute saline loading in Sabra hypertension-prone and -resistant rats

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Summary. The renal handling of an oral isotonic saline load was studied in hypertension-prone (SBH), hypertension-resistant (SBN) and the parental Sabra (SB) rats. The diuretic and natriuretic response of SBH rats was unequivocally diminished, thus lending further support to the concept of impaired salt handling in hypertension.
Key words. Hypertension-prone rats; hypertension-resistant rats; oral saline load; impaired salt handling; renal response.

The role of the kidney in the pathogenesis of hypertension has attracted considerable interest. A reduced capacity of the kidney to excrete salt and water has been proposed as a major factor in the development of hypertension[2]. In support of this hypothesis, several studies have demonstrated an impaired natriuretic response to acute salt loading in four different models of genetic hypertension[3-6].

In the present study, we compared the renal response to an acute isotonic oral saline load in the Sabra hypertension-prone (SBH), hypertension-resistant (SBN) and the Sabra (SB) rats from which the two substrains had been derived[7]. The results show an unequivocally reduced diuretic and natriuretic response in the hypertension-prone animal and lend further support to the concept of impaired renal sodium handling in genetically hypertensive rats.

Materials and methods. The study was performed in female SBH, SBN and SB rats aged 15 weeks. The animals were housed in air-conditioned quarters and maintained on standard purina chow (AMROD 931, Ambar Food Mills, Hadera, Israel) with free access to tap water. The experiment was carried out in the morning, following an overnight fast during which access to water was permitted. The urinary bladder was voided by compression of the lower abdomen and the urine discarded. A load of 0.9% NaCl, 4 ml/100 g b.wt (616 μmoles Na/100 g b.wt) was administered through a gastric tube. The rats were then placed in individual metabolic cages fitted with plastic bags for urine collection and allowed to void spontaneously. At the end of 2 h, complete voiding was obtained by abdominal compression. Urine electrolytes and osmolality were measured by standard laboratory techniques. Systolic BP was measured on the day prior to the experiment in conscious prewarmed animals by tail-cuff plethysmography, using an automatic BP recorder (Ueda Electronic Works). Values are expressed as mean ± SEM and corrected per 100 g b.wt. For statistical analysis, Student's t-test was used.,

Results. The BP of SBH rats was 143 ± 1 mm Hg and significantly higher than that of SBN; 123 ± 1.5 mm Hg ($p < 0.01$) or of SB; 123 ± 1 mm Hg ($p < 0.01$). SBH rats weighed 222 ± 2 g and were heavier than SBN; 181 ± 2 g ($p < 0.01$) or SB; 211 ± 3 g ($p < 0.01$).

Following the acute salt load, SBH rats excreted significantly less urine, sodium and potassium during 2 h than SBN or SB (fig. 1). Figure 2 illustrates the percent excretion of the administered load. The reduced Na excretion in SBH rats was mainly due to a lower urine output, as the urine concentration of Na was significantly higher in these animals as compared to SBN or SB. The excretion of the load was comparable in SBN and SB rats.

Discussion. The major findings in this study were the diminished renal sodium and water excretion by SBH rats in response to acute saline loading, as compared with SBN or SB. Since the pattern of response in SBN and the parental SB strain was comparable, the results suggest an inherent abnormality in sodium handling in SBH rats. In another set of experiments (not included) urine was collected for 4 h and the pattern of response was similar to that observed after 2 h. Moreover, in a long term study on regular laboratory chow, 24-h urinary sodium excretion tended to be lower in SBH as compared with SBN (in preparation). These observations suggest an alteration in sodium and water handling in the hypertension-prone strain. This tendency to retain sodium became clearly evident when the rats were challenged with an acute salt load.

Under various experimental conditions, previous saline loading studies in hypertensive rats have yielded conflicting results. The findings in the Sabra rats are in agreement with earlier studies where an impaired natriuretic response to acute salt loading was observed[3-6]. The results are, however, at variance with data obtained in Dahl's strain, where using a similar load protocol, an exaggerated diuresis and natriuresis were observed in DS rats as compared with DR[8]. The dissimilar response of SBH and DS rats to the salt load is particularly intriguing in

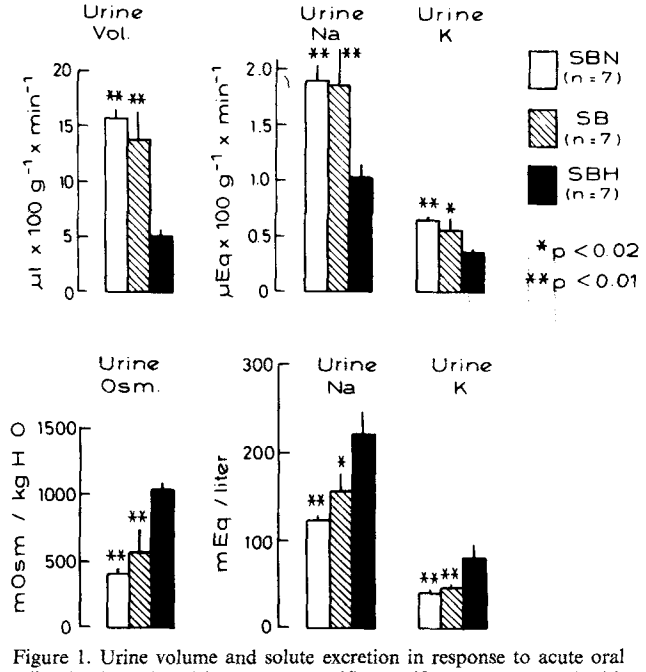

Figure 1. Urine volume and solute excretion in response to acute oral saline loading. Asterisks indicate significant difference compared with SBH.

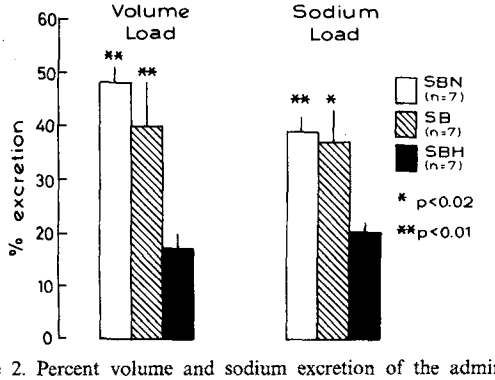

Figure 2. Percent volume and sodium excretion of the administered load. Symbols as in figure 1.

view of the similarity of these strains with respect to salt sensitivity, baroreceptor function and cardiac catecholamine turnover[9].

The mechanism for the impaired handling of an acute salt load by SBH rats remains to be clarified. Differences in GFR during acute volume expansion are unlikely, as renal function of SBH and SBN rats is comparable under steady state conditions (in preparation). Other proposed mechanisms for sodium retention by SBH are increased proximal tubular reabsorption mediated by activation of alpha adrenergic receptors[10], increased fractional reabsorption along the thick ascending limb of the loop of Henle mediated by ADH[11] or prostaglandin[12] and increased distal tubular sodium reabsorption[13].

The significance of the impaired response to acute saline loading in the Sabra hypertension-prone rats remains unknown. Future attempts to elucidate the mechanism of this phenomenon may shed more light on its relevance to the development of hypertension.

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Regional distribution of thiamin pyrophosphokinase in rat brain

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Summary. The highest specific activity of thiamin pyrophosphokinase was found in the cerebellum, and lower activity in cerebral cortex and midbrain. The regional difference in the enzyme activity was similar to that in thiamin content and the influx rate in rat brain, suggesting that the enzyme is involved in the thiamin transport.
Key words. Thiamin; thiamin pyrophosphokinase; cerebellum.

Thiamin pyrophosphokinase is an enzyme responsible for synthesis of thiamin pyrophosphate which acts as a coenzyme, and the brain enzyme has been purified from rat[1,2] and pig[3-5]. It is thought to be involved in membrane transport of thiamin in brain[6] and in intestine[7,8], though some studies[9-11] are against this idea. Rindi et al.[12,13] have recently reported that there is a significant difference in total thiamin content and the turnover rate among various regions of rat brain. In this paper, we determined the activity of thiamin pyrophosphokinase in different regions of rat brain in order to speculate about the possible involvement of the enzyme in the transport of thiamin in brain.

Materials and methods. Sprague-Dawley rats weighing about 250 g were decapitated and the brains were dissected according to the method of Glowinski and Iversen[14]. The tissue was homogenized in 10 volumes of 0.02 M Tris-HCl (pH 7.4)/2 mM 2-mercaptoethanol/1 mM EDTA and the homogenate was centrifuged at 100,000 × g for 1 h. The supernatant was used as the enzyme source. Thiamin pyrophosphokinase activity was determined by the method of Deus[15] with minor modification as follows. 1) 0.1 M Tris-HCl buffer (pH 7.5) was used instead of 0.1 M glycylglycine buffer (pH 7.3). 2) After the reaction, thiamin and thiamin pyrophosphate were separated by paper electrophoresis as described previously[16]. Under the conditions used, the activity was linear with respect to protein concentration and reaction time. Protein was determined by the method of Lowry et al.[17].

Results and discussion. The specific activity of thiamin pyrophosphokinase in different regions of rat brain is shown in the table. The highest activity was observed in the cerebellum, and lower activity in midbrain and cerebral cortex. The finding correlates well with the in vivo analysis of thiamin metabolism of rat brain reported by Rindi et al.[12,13]. They[12] demonstrated that there were differences in total thiamin content and the influx fractional rate constant among the regions of rat brain: the order of total thiamin content was cerebellum > striatum, pons, midbrain, medulla and hypothalamus > cerebral cortex > spinal cord, and that of the influx fractional rate constant was cerebellum > hypothalamus, pons and medulla > striatum, spinal cord and midbrain > cerebral cortex. Furthermore, they[13] have more recently found that rat cerebellum exhibits the highest level of thiamin pyrophosphate and the shortest turnover time for thiamin pyrophosphate. On the whole, the regional distribution of thiamin pyrophosphokinase activitiy described here is similar to that of total thiamin content and the influx rate constant. These

Thiamin pyrophosphokinase activity in different regions of rat brain

Regions	Thiamin pyrophosphokinase activity (nmol/mg protein/h)
Cerebellum	2.00 ± 0.06 (9)
Striatum	1.42 ± 0.19 (4)
Medulla oblongata	1.27 ± 0.09 (4)
Spinal cord	1.26 ± 0.17 (4)
Hippocampus	0.93 ± 0.19 (4)
Hypothalamus	0.87 ± 0.06 (4)
Cerebral cortex	0.79 ± 0.06 (9)
Midbrain	0.71 ± 0.11 (4)

Each value is mean ± SEM of the number of experiments shown in brackets.