Sequential Changes of Cerebrospinal Fluid Sodium During the Development of Hypertension in Dahl Rats

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The role of sodium retention and consequent changes in cerebrospinal fluid sodium concentration in the genesis of hypertension in Dahl rats was evaluated. Dahl salt-sensitive (DS, n=7), Dahl salt-resistant (DR, n=7), and Sprague-Dawley (n=6) rats were housed in metabolic cages and instrumented with a stainless steel cannula in the cisterna magna and a femoral arterial catheter. A blood sample was drawn daily (200 μl), and cerebrospinal fluid was collected by continuous 24-hour withdrawal (200 μl/day). Daily sodium, potassium, and water balances were also determined. Rats were studied sequentially on 0.4%, 4%, and 8% sodium diets (7 days per sodium level). Mean arterial pressure increased with 4% NaCl from 107 to 120 mm Hg (p<0.05) over 24 hours in DS rats and remained at about that level until the NaCl was increased to 8%, which resulted in a gradual rise of mean arterial pressure over the next 7 days to 135 mm Hg. Cerebrospinal fluid sodium was unchanged in DR and Sprague-Dawley rats fed 4% or 8% sodium, but in DS rats rose from 152.3 to 155.2±0.6 meq/l on the third day at 4% sodium and remained elevated over the next 2 weeks of study. Blood sodium was unchanged throughout the study in all groups. On the first day only of the 4% and 8% sodium diets, both DS and DR rats exhibited a similar net retention of sodium, which was greater than the Sprague-Dawley rats (p<0.05). There was no evidence for greater retention of sodium or water, however, in DS rats compared with DR or Sprague-Dawley rats when subjected to a high sodium diet. In summary, since mean arterial pressure rose in DS rats on the first day of the 4% sodium diet while cerebrospinal fluid sodium did not change until the third or fourth day, we conclude that these central changes were not the stimulus for the initial rise of arterial pressure but might contribute to the maintenance of the hypertension. (Hypertension 1989; 13:243-249)

Dahl salt-sensitive (DS) and salt-resistant (DR) rats have been studied by many investigators interested in the phenomenon of sodium-dependent hypertension.1,2 These studies have yielded useful information related to the cardiovascular factors and renovascular abnormalities associated with the established phase of hypertension after 6–8 weeks of a high sodium diet.2 It has been shown that DS rats have an impaired renal capacity to excrete sodium, even before exposure to a high salt diet and subsequent hypertension.3,4 This renal impairment appears to be related to an intrinsic alteration of renal excretory function since cross-transplantation of kidneys from DS rats to DR rats transfers the salt-sensitive characteristic to the normotensive strain.5 It has therefore been suggested that a reduced ability to excrete sodium could lead to a greater retention of sodium and water in DS rats during transition from a low to a high sodium diet and could lead to the development of hypertension.

Based on the presumption that DS rats first underwent exaggerated retention of sodium and water, three hypotheses have been proposed to explain the consequent development of hypertension. One is based on the concept of “whole-body autoregulation”, whereby an increase in cardiac output secondary to blood volume expansion could lead to elevation of systemic vascular resistance via local tissue and vascular responses to increased blood flow and arterial pressure.6,7 A second hypothesis proposes that excess sodium and water retention releases an inhibitor of ouabain-sensitive sodium-potassium adenosine triphosphatase (Na⁺,K⁺-ATPase) that increases vascular smooth muscle contractility.8,9 The third theory proposes that the central nervous system detects increases of sodium...
intake and consequently increases the activity of the sympathetic nervous system.1,2,4,10-12

There is little information, however, regarding the early developmental stages of this form of hypertension. There has been only one previous report of daily fluid and electrolyte measurements during the development of hypertension in Dahl rats.13

Elevation of cerebrospinal fluid (CSF) sodium or osmolality activates the sympathetic nervous system and raises arterial blood pressure.14-18 It has also been shown that chronic cerebroventricular infusion of hypertonic sodium chloride in rats elevates arterial pressure.18 It was also observed that, in salt-sensitive subjects with essential hypertension, increased sodium intake resulted in elevation of CSF sodium concentrations, changes that were not observed in salt-resistant hypertensive subjects.19 The present study was designed to examine whether DS rats are predisposed to respond to a high salt intake with a greater elevation of CSF sodium that could trigger or contribute to the hypertensive state. Sequential measurements of blood and CSF sodium levels have not previously been made during the development of this form of hypertension.

DS, DR, and Sprague-Dawley rats were surgically prepared with a stainless steel cannula in the cisterna magna and a femoral artery catheter and were housed in metabolic cages. This allowed collection of CSF by continuous 24-hour withdrawal of 200 µl/day for analysis and daily measurement of arterial blood pressure. Daily electrolyte and water balances were determined and 250 µl of blood was collected daily for sodium measurements.

Materials and Methods

Female DS (n=7) and DR (n=7) rats (Brookhaven National Laboratories, Brookhaven, New York) were used in this experiment. All results were compared with a group of male Sprague-Dawley rats (n=6; King Animal Supplier, Madison, Wisconsin). All of the Dahl rats used in these studies were born during the same week. They were delivered to our laboratory 1 week after weaning and were maintained on a 0.4% NaCl diet with drinking water provided ad libitum until the start of the experiment. At the time of study, all rats were 10–12 weeks of age and weighed 250–300 g.

Surgical Preparation of Rats

All rats were surgically prepared with a left femoral artery catheter (Polyvinyl SV, 31, Dural Plastics, Dural, Australia), and a stainless steel cannula was implanted into the cisterna magna. Surgery was performed on Sprague-Dawley rats anesthetized with ketamine (80 mg/kg) and acepromazine (2 mg/kg). The Dahl rats were considerably more sensitive to anesthetics and received only half of this dosage. For placement of the cisterna magna cannula, rats were mounted in a stereotaxic instru-

ment with ear bars and mouthpiece (David Kopf Instruments, Tujunga, California). The tissue was cleared to expose the skull over the lambda. A hole was drilled in the midline of the skull 1 mm posterior to the lambda, with a 70° angle to the surface of the skull, at a depth of 7.5 mm below the dura. A 23-gauge stainless steel cannula was then inserted into the cisterna magna through this opening, as described by Lai et al.20 This cannula was connected to polyvinyl tubing that was attached to the syringe withdrawal pump as described below.

A catheter was placed in the abdominal aorta via the left femoral artery for measurement of arterial pressure by a method previously described by our laboratory.21 The arterial line was tunneled subcutaneously to the head along with the tubing from the cisterna magna cannula and was secured to the skull surface by two stainless steel retaining screws and dental acrylic. The lines were then threaded through a lightweight flexible spring connected to a swivel. These arterial catheters remained patent throughout the study and were used for the daily collection of arterial blood for analysis of plasma sodium levels.

All incisions were swabbed with Betadine (Purdue Frederick Company, Norwalk, Connecticut) and closed. Penicillin G (200,000 units/kg body wt) was given intramuscularly for 3 days after surgery. Rats were then placed in a stainless steel metabolic cage with the swivels mounted over each cage. This setup allowed the rats complete freedom of movement about the cage and permitted the handling of the catheters without disturbing the animals.

Experimental Protocol

After surgery, the rats were allowed to recover for 3–5 days before study. Each animal was housed in a separate cage in an environmentally controlled room where temperature (24° C), humidity (60%), and 12-hour light/dark cycle could be maintained constant. During the surgical recovery period, the rats were maintained on 0.4% NaCl diet with water available ad libitum. At the end of this period, rats were studied at the 0.4% level of sodium intake for 3 days, then the diet of all rats was changed to 4% NaCl for 7 days, and then switched to 8% NaCl for the following 7 days. In six rats the diet was then returned to a control level of 0.4%, and the water was restricted to test the CSF sodium response to elevation of plasma sodium.

CSF was withdrawn continuously for the entire 17–19-day experimental period. This was accomplished by using a precision, low-speed, infusion-withdrawal pump (EDCO Scientific Inc., Chapel Hill, North Carolina) set to withdraw at a very slow rate of 200 µl/day. Precautions were taken to maintain the sterile conditions of this collection system to prevent possible retrograde contamination of the CSF.

Each day between 8 and 11 AM, CSF samples were collected from the withdrawal syringe, arterial blood samples were withdrawn (200–250 µl), and a
1-hour recording of mean arterial pressure (MAP) obtained. Blood was replaced by transfusion every other day with blood obtained from the same strain of rat, which were maintained on the same diet as the experimental group. Sodium, potassium, and water excretion were determined daily from 24-hour urine samples collected from the metabolic cages. The amount of sodium and potassium intake was determined by daily weighing of the food. Daily water intake was also measured. Sodium, potassium, and water balances were determined daily as the absolute difference between intake and excretion.

Analytical Measurements

Sodium concentration in CSF, urine, and whole blood, and potassium concentration in CSF and urine were determined by using ion-specific electrodes (Nova Biomedical, Boston, Massachusetts).

Statistics

Data are presented as mean±SEM. Significant differences within each rat group were determined by a repeated-measures analysis of variance followed by a Duncan's test for multiple comparisons. Differences between DS and DR rat responses were determined by using a two-way analysis of variance repeated on one dimension.

Results

Relations Between Blood and Cerebrospinal Fluid Sodium Concentrations and Arterial Pressure

Figure 1 summarizes the average daily responses of blood and CSF sodium concentration and mean arterial pressure before and during two levels of high sodium intake. Sodium concentration in the blood (blood sodium) remained unchanged throughout the study, averaging 147.0±0.5 in Sprague-Dawley rats, 145.3±0.7 in DS and 146.5±0.8 in DR rats. CSF sodium concentrations, however, became significantly elevated by nearly 4 meq/l in DS rats by the third day of the 4% diet and remained significantly above control levels throughout both periods of high sodium intake. Control levels of CSF sodium averaged 152.3±0.8 in Sprague-Dawley rats, 152.2±0.4 in DR rats, and 152.3±0.5 in DS rats. That is, CSF sodium concentration during the control periods averaged about 6 meq/l higher than the blood sodium concentration. CSF potassium concentration averaged 2.81±0.03 meq/l in Sprague-Dawley rats during the control period, 2.70±0.04 meq/l in DR rats, and 2.77±0.02 meq/l in DS rats. No significant changes were observed in any of the groups throughout the study.

Mean arterial pressure averaged 103±2 mm Hg in Sprague-Dawley rats, 103±2 mm Hg in DR rats, and 107±2 mm Hg in DS rats. Only DS rats exhibited significant elevations of blood pressure with increased sodium intake. DS rats were already significantly hypertensive (120±3 mm Hg) after only 1 day of receiving the 4% NaCl diet. Furthermore, this elevation of mean arterial pressure preceded the elevation of CSF sodium by several days, indicating that a rise of CSF sodium was not the initiating factor for the rise of blood pressure. On switching to the 8% sodium diet, arterial pressure rose even further to an average of 135±5 mm Hg by the seventh day.

Daily Sodium, Potassium, and Water Intake, Excretion, and Balance

Figure 2 summarizes the daily measurements of sodium intake, sodium excretion, and the calculated sodium balance throughout the study. There was a tendency for Sprague-Dawley rats to consume and excrete more sodium when presented with an 8% diet of NaCl. All three groups exhibited significant retention of sodium during the period of
Figure 2. Line graphs summarizing changes in daily sodium intake (Na Intake), urinary sodium excretion (Na Excretion), and sodium balance (Na Balance) as dietary NaCl was increased from 0.4% to 8.0%. Significant increases of both sodium intake and excretion occurred at both 4% and 8% level of sodium diets in all groups. Significant retention of sodium (Na Balance) occurred during the 8% period in all groups, but statistical significance was not achieved at the 4% level when compared with control levels. No significant intergroup differences were observed. Solid lines represent Sprague-Dawley rats (n=6), dashed lines represent Dahl salt-resistant (DR) rats (n=7), and dotted lines represent Dahl salt-sensitive (DS) rats (n=7).

Figure 3. Line graphs summarizing changes in daily potassium intake (K Intake), urinary potassium excretion (K Excretion), and potassium balance (K Balance) as dietary NaCl was increased from 0.4% to 8%. DR rats lost a slight (0.4 meq) but significant amount of K on day 1 of 4% sodium intake. No other significant differences were observed. Solid lines represent Sprague-Dawley rats (n=6), dashed lines represent Dahl salt-resistant (DR) rats (n=7), and dotted lines represent Dahl salt-sensitive (DS) rats (n=7).

highest NaCl intake but intergroup differences were not observed. Measurements of potassium intake, excretion, and balance are summarized in Figure 3. Daily determination of potassium balance indicated that DR rats lost an average of 0.4 meq potassium the first day of 4% sodium intake, and this value was statistically significant. DR and Sprague-Dawley rats did not show any significant change in potassium balance throughout the study. No other changes in potassium balance were observed throughout the experimental period at either level of sodium intake in any of the three groups. Daily potassium intake ranged from 0.8 to 1.2 meq/day throughout the studies in all groups.

Figure 4 summarizes the daily measurements of water intake, urine volume, and water balance in the three groups of rats when fed 0.4%, 4%, and 8% NaCl diet. As seen with the sodium balance measurements, no differences were observed between the groups of rats studied. Sprague-Dawley rats, however, did exhibit a positive water balance compared with the control periods on all but 1 day of the 8% high salt diet. This was not observed in either the DS or the DR rats.

After the administration of 8% NaCl in the diet for 7 days, six rats were returned to 0.4% NaCl for 2 days and water intake was restricted for this period. Four other rats were water restricted simultaneously on return to the 0.4% NaCl diet. These studies were performed to test the reliability of the methods being used to collect and measure CSF sodium and to determine the relation of change between whole blood and CSF sodium. The overall response obtained with water restriction was an equivalent rise of blood and CSF sodium concentration of 7–10 meq/l.
FIGURE 4. Line graphs summarizing changes in water intake, urine volume, and water balance as dietary NaCl was increased from 0.4% to 8%. Water intake at 8% sodium intake was significantly elevated in both DR and DS rats when compared with control levels and 4% levels of sodium intake. Water intake of Sprague-Dawley rats was significantly increased at both the 4% and 8% levels. Urine volume was significantly elevated only in DR rats at 4% sodium intake and in all three groups at 8% sodium intake level. Significant differences were observed between any of the groups. Water balance was significantly positive only in Sprague-Dawley rats at all but one of the days of 8% sodium intake. No significant differences between groups were observed. Solid lines represent Sprague-Dawley rats (n=6), dashed lines represent Dahl salt-resistant (DR) rats (n=7), and dotted lines represent Dahl salt-sensitive (DS) rats (n=7).

Discussion

The present studies examined the possibility that retention of sodium and water in DS rats could lead to greater elevations of blood or CSF sodium concentration, or both, and thereby contribute to salt-induced hypertension. Daily determinations of sodium, potassium, and water balances, blood sodium, and CSF sodium and potassium levels were made to evaluate this possibility. A continuous slow withdrawal of CSF was used after it was observed that a single daily sample of 150–200 μl resulted in a blunting of the thirst mechanism. Rats fed a high salt diet became hypernatremic and remained so throughout the study. As seen in Figure 1, however, blood sodium levels remained unchanged when the CSF was withdrawn at a low continuous rate. These rats maintained normal drinking responses such that the increased water intake expected with the high sodium diets maintained plasma electrolytes at a normal level.

Previous studies have indicated that some form of renal dysfunction may be involved in the development of hypertension in DS rats. Studies have demonstrated that transplantation of kidneys from DS rats to DR rats confers the salt sensitivity to the DR rats. Tobian et al found that blood-perfused isolated kidneys of DS rats require a higher perfusion pressure to excrete amounts of sodium and water equivalent to DR rats. Roman recently reported that both prehypertensive and hypertensive conscious DS rats exhibit a blunting of the pressure-natriuresis relation when compared with DR rats.

It has been assumed that renal dysfunction in the DS rats would lead to a greater retention of sodium and water when subjected to a high salt diet. The results of the present study indicate, however, that both DR and DS rats retained similar amounts of sodium and water when fed a high salt diet. Furthermore, blood sodium concentrations were not measurably changed in either group of rats. Our observations are similar to the only other study that has determined the time course of changes in fluid and electrolyte balance in these animals. Observations also concur with several other studies that reported that plasma volume, plasma sodium concentration, and total body sodium content are similar in DS and DR rats fed a high salt diet. The results, therefore, indicate that the major difference between DR and DS rats in response to a high salt diet is not in the relative amounts of retained sodium and water, but rather the difference in the response of these rats to the retention of similar amounts of sodium and water.

Three general hypotheses have been put forward to explain how fluid volume expansion could lead to elevation of peripheral vascular resistance. One, the whole-body autoregulatory hypothesis, proposes that the fluid volume expansion results in an initial elevation of cardiac output and arterial pressure that, through local tissue metabolic or myogenic responses, results in an elevation of vascular resistance. It is possible that DS rats respond to volume expansion with exaggerated autoregulatory responses. Two, fluid volume expansion has been claimed to release a natriuretic hormone from the hypothalamus that is a potent inhibitor of ouabain-sensitive Na+,K+-ATPase, which would lead to vascular smooth muscle depolarization and an increase in peripheral vascular resistance. Recent observations of Overbeck et al, however, indicate that the Na+-K+ pump activity of tail arteries of hypertensive DS rats was...
elevated rather than reduced in comparison with DR rats, which is opposite to results predicated by this second hypothesis.

A third theory has emphasized the role of the sympathetic nervous system in the development of hypertension. It has been suggested that sodium retention in DS rats increases the activity of the sympathetic nervous system, thereby increasing blood pressure. There is also evidence that an abnormality in the baroreceptor reflex control of heart rate exists in DS compared with DR rats. These observations suggest that there could be a defect in baroreceptor reflex sensitivity of Dahl rats whereby a similar retention of sodium and water in the DS rat could be less effectively blunted, contributing to the hypertension.

It has also been suggested that the central nervous system in some manner detects sodium retention that either initiates the release of the natriuretic hormone or stimulates sympathetic nerve activity. There is evidence indicating that central osmotic receptors could be responsible for both the release of vasopressin and the natriuretic hormone. Brody and colleagues have suggested that alterations in CSF sodium may play an important role in the development of many forms of experimental hypertension. It has been suggested that the primary stimulus for the development of low renin, sodium-dependent models of hypertension may be the elevation of CSF sodium levels and not necessarily fluid volume expansion. Haywood et al. observed a significant increase in CSF sodium (5%) and osmolality (9%) in one-kidney, figure-8 hypertensive rats within 3 days after surgery. Plasma sodium and osmolality, however, did not rise even after 28 days in these rats. These investigators proposed that these alterations in CSF osmolality may provide the primary stimulus for the development of hypertension.

The present studies determined whether hypertension in DS rats could occur via exaggerated elevations of CSF sodium concentration. Salt-sensitive subjects with essential hypertension receiving a high sodium intake, have demonstrated elevated CSF sodium concentration (+8 meq/l). It is well known that acute infusions of hypertonic sodium solutions into the cerebral ventricles of experimental animals results in a number of responses that are similar to those observed in the Dahl salt-sensitive model of hypertension and in other low renin models of hypertension. These include reduction in the plasma renin levels, elevation of plasma catecholamines and vasopressin, increase in central sympathetic discharge, and the release of a putative sodium pump inhibitor into the circulation. Furthermore, it has been reported that the pressor responses of DS rats are slightly greater than DR rats in response to intracerebroventricular administration of angiotensin or hypertonic saline. Such observations have suggested that if there was an inherent difference in the ability of DS rats to regulate CSF sodium concentrations, the greater elevations of CSF sodium in response to increased sodium intake could initiate, or contribute to the hypertensive state in the DS rat.

There was no evidence in the present study to indicate that the onset of hypertension was due to an elevation of CSF sodium in DS rats. Mean arterial pressure was elevated 2–3 days before the observed elevation of CSF sodium in DS rats. However, it is interesting that CSF sodium concentration was elevated only in the DS hypertensive rats. These results suggest that this elevation of CSF sodium could have been secondary to an elevation of MAP. It is possible that the choroid plexus of DS rats exhibits alterations in ion transport capacity in response to sodium and water retention or in response to elevations of arterial pressure. It is also possible that, although the change in CSF sodium was not the stimulus for the hypertension, the elevated CSF sodium participated in the maintenance of the hypertension.

It should be recognized that CSF sodium concentration was nearly 6 meq/l higher than plasma sodium levels in all of our rats. Most previous investigators have found CSF sodium concentrations to be from 5 to 6 meq/l higher than plasma levels. This has been most recently reported in both conscious rabbits and dogs. The mechanisms responsible for these differences are not well understood.

The rise of arterial blood pressure was much more rapid in DS rats exposed to a high sodium diet than would be predicted from most of the previously reported studies. The reason for this discrepancy is probably because blood pressure was measured with tail-cuff plethysmography in previous studies, whereas blood pressure was measured directly with chronic indwelling arterial catheters in the present study. Studies by Dahl and others suggested that the hypertension develops over a 2–6-week period after DS rats are placed on a high salt diet. The results of the present studies, however, conform with recent observations by Roman and Osborn in conscious Dahl rats with pressure recorded from indwelling catheters.

In summary, the results indicate that the hypertensive response of DS rats fed a high salt diet cannot be explained by a greater retention of sodium and water in DS rats compared with DR rats. The difference between these two strains of rats appears to be related to the mechanisms whereby the DS rat responds to a normally expected retention of sodium and water. The present studies indicate that hypertension in DS rats is not initiated by elevations of plasma or CSF sodium concentrations. The small elevations of CSF sodium that develop during the second to third day of high salt intake in DS rats could be secondary to elevations of arterial pressure and could participate in the chronic maintenance of this form of hypertension. It remains to be
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determined whether the small elevations observed in CSF sodium of DS rats would be a sufficient stimulus to be of pathological importance.

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