Protective Effects in Ambulatory Blood Pressure and Centralized Injuries in Hydrocephalic Dahl Rats on High and Low NaCl Diets

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Our previous studies in hydrocephalic Dahl rats indicated that the NaCl signal is perceived in the structures around the third brain ventricle. In the present study, we assessed 24-h ambulatory blood pressures (ABPs), weight (WT) gains, and the kidney and brain injuries in induced hydrocephalic and control group Dahl S rats on 0.3% and 6% NaCl diets. Constant WT gains were observed among low NaCl fed and high NaCl fed aqueduct blocked groups, whereas high NaCl-fed sham group rats were losing WT and dying off after 8 weeks. A circadian BP curve in the high NaCl sham group was very distinct from that in the other three groups with markedly increased peak pressure during the light cycle with very high amplitude as compared with all the other three groups (both $P < .0001$). There was not only a very unstable fluctuating curve present, but a day–night rhythm shift was also prominent in the high NaCl sham group.

In a kidney cross-section, 54 rats on a 0.3% low NaCl diet for 14 weeks averaged 156 glomeruli. A total of 34 high NaCl fed, aqueduct blocked rats averaged 141 glomeruli, whereas 23 sham blocked rats averaged 102 glomeruli ($-28\%$, $P < .0001$). A brain cross-section showed many small lacunae in high NaCl-fed sham rats as compared with the truly blocked rats ($193 \pm 14$ v $77 \pm 10$ lacunae, a 2.5-fold reduction, $P < .0001$).

The changes in the blocked group may be due to volume and electrolyte rebalance with reduced pressures in the brain volume-controlling center. Am J Hypertens 2003;16:307–311 © 2003 American Journal of Hypertension, Ltd.

Key Words: Hydrocephalus, ambulatory blood pressure, mortality, lacunae, renal injuries.

Materials and Methods

The cerebral aqueduct was blocked stereotaxically by silicone and epoxy materials to produce hydrocephalus in 4-week-old Dahl S rats. Either 6% or 0.3% NaCl chow was introduced (Zeigler Co., Gardners, PA) on the day of surgery to all rats. After 6 weeks, intra-arterial blood pressures (BPs) were measured under Brevital anesthesia, 50 mg/kg (Eli Lilly and Co., Indianapolis, IN). Immediately after the BPs were measured, a heparinized, 5% dextrose filled, polyethylene catheter was inserted in the femoral artery and then tunneled subcutaneously to exit through the dorsal part of the neck. The end of the catheter from the neck was connected to a Statham pressure transducer attached to the EEG Polygraph Data Recording System (model 79D; Grass Instruments, Quincy, MA) through a long, stainless steel, hollow tube with a swivel.
end that was attached to a modified metabolic cage. This system allowed rats to move around freely. The 6% or 0.3% NaCl diet was continued, and rats were allowed 4 days to recover from the surgery before monitoring ABP. After the recovery period, BPs were recorded every 10 min continuously for 3 consecutive days. Body weights were measured every week. Plasma and urine Na concentrations and hematocrit were measured at 6 weeks post-surgery. Rats that survived until 14 weeks were killed under pentobarbital anesthesia (50 mg/kg). Fresh kidneys were taken out and fixed immediately in Zenker solution for renal tissue slides. Blood vessels were washed out with 100 mmol/L phosphate buffer, and brains were fixed by perfusion with 5% neutral formalin through the carotid artery under a perfusion pressure with the same level as in each rat’s BP. The fixation was completed in 10% neutral formalin for 2 days, and hematoxylin eosin stained, cross-sectioned renal and brain tissue slides were made for blinded microscopic examinations for the number of kidney glomeruli and brain lacunae. Each rat was examined thoroughly for pathologic changes, and aqueduct blocks were verified histologically using the same methods as in our previous study.2

Results were analyzed by the least squares method and the ChronoLab software package analysis (Bioengineering and Chronobiology Laboratories, E.T.S.I. Telecommunication, Vigo, Spain).11 Data are expressed as mean ± SEM. A P value of < .05 was considered to be significant.

FIG. 1. Twenty-four-hour ambulatory blood pressure in Dahl salt sensitive rats on a high NaCl Diet. The hourly data curve represents a 24-h blood pressure curve of either sham or truly blocked rats on a 6% NaCl diet. These values were originally measured at 10-min intervals and plotted into hourly values. The sham group clearly shows the circadian rhythm change with much higher blood pressure during the light cycle among these nocturnal rats as compared with the blocked group. Horizontal bars on the graph indicate ± SE. MESOR = midline-estimating statistic of rhythm.

<table>
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<tbody>
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<td>ns</td>
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<td>Sham (n=139)</td>
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n = number of time points; LL: lesion, 0.3% NaCl Diet; LS: sham, 0.3% NaCl Diet; HL: lesion, 6% NaCl Diet; HS: sham, 6% NaCl Diet. NS = not significant.
Table 2. Renal injury observed in induced hydrocephalic Dahl salt-sensitive rats on either a 0.3% or 6% high NaCl diet

| Group          | Glomeruli Number | Cast Number | P-value
|----------------|------------------|-------------|--------
| 0.3% NaCl diet | 156 ± 4          | 48 ± 13     |         |
| 6% NaCl diet   | 102 ± 6          | 488 ± 67    | P < .0001 |
|                |                  |             | 35%    |
|                |                  |             | 28%    |

Values are expressed as mean ± 1 SEM.

Results

We examined the effects of induced-hydrocephalic rats on 24-h ABPs and morbidity along with cell/tissue injuries of the brain and kidney on a 0.3% or 6% NaCl diet. Group BPs averaged 130 in 0.3% NaCl fed rats; 149 in 6% NaCl fed, truly blocked rats; and 175 mm Hg in sham blocked rats. The truly blocked aqueduct of Sylvius abolished 58% of the NaCl induced BP rise as compared with that in sham blocked rats, and reduced mortality 82% with a 6% high NaCl diet. In weight gain monitoring, sham blocked rats, and reduced mortality 82% with a 6% high NaCl diet was similar to those of the low NaCl fed groups, however, the BP level was somewhat higher than those of the low NaCl diet groups. Parameters of the 24-h ABP in the aqueductal sham and truly blocked groups on both 0.3% and 6% NaCl diets are summarized in Table 1. As indicated, the high NaCl fed sham group had a markedly increased circadian BP, with a day–night shifted curve (13:24 v 06:20) and more than doubled the amplitude as compared with the truly blocked group (39 v 17 mm Hg).

Table 2 shows the results of cross-sectional microscopic examination on renal tissues. Glomeruli numbers were markedly reduced in the 6% NaCl fed sham group, with dramatically increased cast numbers as compared with those in the truly blocked group, both P < .0001 (102 ± 6 v 141 ± 5 glomeruli, a 28% reduction; 488 ± 67 v 223 ± 38 cast numbers, a twofold increase). On a 0.3% low NaCl diet, both sham and truly blocked groups averaged 156 glomeruli with 48 casts; thus, 6% high NaCl fed sham group rats had 35% reduced glomeruli with 10-fold increased casts, both P < .0001. Urinary albumin excretion was significantly lower in both sham and truly blocked groups on the 0.3% low NaCl diet than those values in both groups on the 6% high NaCl diet, P < .001 (1536 ± 182.5, 1226 ± 199.5, 3577 ± 305.6, and 2857 ± 272.2 μg/h, respectively). The high NaCl fed sham blocked group had still significantly higher albumin excretion as compared to that in the truly blocked group (P < .02).

Fig. 2 summarizes a comparison of BPs and numbers on the cross-sectional brain lacunae on a 6% high NaCl diet. There is a linear relationship with increased BP and the number of the lacunae formation, P < .001. The brain sections showed many small lacunae with the high NaCl diet, mainly in the cerebral white matter and brain stem. The 6% high NaCl fed, sham blocked rats averaged significantly increased lacunae over those of truly blocked rats (193 ± 14 v 77 ± 10, 2.5-fold increased lacunae, P < .0001). When BP levels were adjusted, 11 sham and 10 truly blocked rats on the high NaCl diet had BPs between 150 and 175 mm Hg, averaging 162 and 160, respectively. Brain lacunae averaged 199 ± 16 in the sham group and 73 ± 16 in the blocked rats. Even after BP adjustment with equal BPs, the blocked group had 63% fewer brain lesions.
indicating far fewer vascular lesions. Therefore, the induced hydrocephalus by an aqueduct block protects vascular lesions.

Furthermore, cardiac and renal hypertrophy was prominent in the 6% high NaCl (Table 3). The 6% high NaCl fed sham rats averaged significantly heavier heart weights than those of the truly blocked rats, 2.45 ± 0.09 vs 2.28 ± 0.06 g for wet hearts, P < .01; 0.41 ± 0.01 vs 0.37 ± 0.01 g for dry hearts, P < .005. Kidney weights of the 6% NaCl fed sham group averaged 2.22 ± 0.10 g, whereas the truly blocked rats had much reduced wet weight, 1.91 ± 0.05 g, P < .001, and 0.40 ± 0.01 vs 0.38 ± 0.01 in dry weight. Overt nephrotic syndrome was noticed in all of the sham group rats, with severe ascites, pleural effusions, edematous body, and albuminuria as compared with the blocked rats. These results indicate that kidneys were injured by a high NaCl diet, which extended to the hearts in the sham rats; however, it appears to be the systemic effect that the injured organs are protected from further injury in the truly blocked rats.

In conclusion, the aqueduct blocked group showed markedly reduced BP, mortality rates, cardiac hypertrophy, and vascular and kidney injuries. As shown in the high NaCl fed sham group rats, the shifted peak hours, markedly increased amplitude, and unstable fluctuations with significantly increased BP may be important.

### Discussion

Normal central neural integration of the CNS may be critical in NaCl dependent hypertension. In our previous studies in borderline hypertensive individuals from Minnesota, BPs were much higher during the active daytime hours and much lower during sleep, showing clearly a diurnal rhythm. Changes in circadian rhythms and depth of amplitudes were noticed among the patients as compared to these values in normotensive individuals. Diurnal activity levels and cardiovascular patterns were shown along with elevated BPs during active night/light-off hours in nocturnal animals. The circadian rhythm change may be very important with physiologic regulations in BP and the cardiovascular system.

In the present study, glomeruli in a kidney cross-section showed conservative numbers among the truly blocked group of rats on the 6% high NaCl diet, whereas the same high NaCl fed sham group averaged significantly decreased glomeruli and increased renal cast numbers, P < .001. The number of lacunae detected on magnetic resonance imaging, computed tomography, or autopsy was correlated with ABP, and most lacunae are asymptomatic among most patients as seen in increased BPs. Ischemic and intracerebral hemorrhage are common in hypertension, especially in the basal ganglia, pons, thalamus, cerebellum, and deep hemispheric white matter. In our study, almost all of the lacunae were found in the cerebral and cerebella white matter as well as brain stem and thalamic regions, which agree with other studies conducted mostly among hypertensive patients. Some studies suggested that hematocrit (Hct) is correlated with BP, however, our study did not find the correlation: at 6 weeks 46% in all four groups; at 9 weeks, 6% NaCl-fed sham rats averaged 38%, whereas the other three group had 45%, P < .01. Intraarterial BP averaged 130 in low NaCl fed sham and blocked groups, and 149 in high NaCl fed truly blocked and 175 mm Hg in the sham group. The 45–mm Hg higher BP in the high NaCl sham blocked group could partially explain the reduction of glomeruli, increases in tubular casts, and increased brain lacunae. In addition, the high NaCl fed sham group had a prominent rhythm change, with much higher BP during the light cycle, along with markedly increased amplitude and very unstable BP fluctuations throughout the 24-h period.

Sympathetic overactivity can cause an increased heart rate that directly affects development of hypertension, atherosclerosis, and increased cardiovascular events. Investigators observed swollen brains accompanied by flattened gyri with compressed ventricle during the hypertensive crisis. In hydrocephalic brains induced by the aqueduct block, the fourfold widened, slitlike, third brain ventricle prevents one wall from touching another. This widening may be responsible for blocking neural/neurohumoral signals. This study supports...
the CNS involvement both in regulating BP and in cardiovascular morbidity.

Acknowledgments
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References