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Short Communication

# Increased number of aldosterone-sensitive NTS neurons in Dahl salt-sensitive rats

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**Abstract**

Dahl salt-sensitive rats develop severe hypertension during a high-sodium diet, but the basis of their salt-sensitive phenotype is not completely understood. A subset of neurons in the nucleus tractus solitarius (NTS) are uniquely sensitive to the adrenal steroid hormone aldosterone, which is critically involved in sodium homeostasis, due to their expression of the enzyme 11-β-hydroxysteroid dehydrogenase type 2 (HSD2). The number of HSD2 neurons in the NTS was counted in prehypertensive 7-week-old Dahl salt-sensitive rats and compared with two control strains: Dahl salt-resistant and Sprague–Dawley rats. Dahl salt-sensitive rats had more HSD2 neurons than age-matched Dahl salt-resistant and Sprague–Dawley rats (24% and 21%, respectively). Cell counts were also made in spontaneously hypertensive rats (SHR) and Wistar–Kyoto (WKY) rats; the number of HSD2 neurons in both of these strains was similar to the values obtained for Sprague–Dawley rats. The increased number of HSD2-immunoreactive neurons counted in Dahl salt-sensitive rats suggests that they may have a greater number of aldosterone-sensitive NTS neurons. Alternatively, an increase in HSD2 expression in Dahl salt-sensitive rats could increase the overall immunoreactivity, permitting detection of more of these neurons. In either case, the roughly 20% increase in HSD2 neurons in the NTS of prehypertensive Dahl salt-sensitive rats is a novel factor associated with their salt-sensitive phenotype. These neurons may play a role in regulating sodium appetite, which is abnormally suppressed in Dahl salt-sensitive rats.


*Theme:* Endocrine and autonomic regulation
*Topic:* Cardiovascular regulation

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Dahl salt-sensitive rats become extremely hypertensive during a high-sodium diet [5,22], whereas dietary sodium has only a minor influence on blood pressure in normal rats and humans. However, specific individuals in genetically heterogeneous populations show varying sensitivities to salt possibly related to varying degrees of hypertension [5]. The specific genetic mutations responsible for salt-sensitive hypertension in the Dahl salt-sensitive rat remain unknown, despite decades of investigation [23]. Moreover, there remains some controversy over the specific physiologic changes underlying the unusual salt sensitivity in this strain [13,19,21,33]. A large body of evidence suggests that the cardiovascular and renal deficits in Dahl salt-sensitive rats are caused by a deficiency in nitric oxide-mediated vascular relaxation [2,13,15] (particularly in the kidney [18,19]), which is an important mechanism for preventing a rise in blood pressure when blood volume is elevated by increased sodium ingestion [32]. The sympathetic nervous system plays at least a permissive role in Dahl salt-sensitive hypertension because a high-salt diet no longer increases blood pressure in these rats after chemical ablation of the peripheral sympathetic innervation of the blood vessels and heart [7,29]. Baroreflex regulation of blood pressure is abnormal in these rats, even before severe hypertension develops [6,11,12]. Adrenal steroids have also been

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implicated because a high-salt diet fails to produce hypertension in Dahl salt-sensitive rats after adrenalectomy [14].

A small subset of neurons within the nucleus tractus solitarius (NTS) is uniquely sensitive to the adrenal steroid aldosterone, a vital hormone for sodium conservation. These neurons express the mineralocorticoid receptor (MR) as well as the enzyme 11-β-hydroxysteroid dehydrogenase type 2 (HSD2) [9]. HSD2 is required for aldosterone sensitivity [8,20] because glucocorticoids (corticosterone in rodents, cortisol in humans) otherwise saturate MR due to their 100–1000× higher concentrations and equivalent MR binding affinity [28]. The HSD2 neurons may play a role in salt appetite [9]. We analyzed the distribution of HSD2 neurons, which are uniquely sensitive to aldosterone, in Dahl salt-sensitive rats, which are uniquely sensitive to dietary sodium.

Experiments were performed in inbred Dahl salt-sensitive rats and salt-resistant rats (SS/JR and SR/JR; Harlan Laboratories, Indianapolis, IN) and outbred Sprague–Dawley rats (Harlan). All rats were male, 7 weeks old and weighed 170–255 g. Rats were fed standard PicoLab rodent diet (#20, PMI, Brentwood, MO), which contains 0.33% sodium, and were provided tap water to drink. No alterations in dietary sodium content were made because the purpose of this study was to test for an intrinsic difference in the HSD2 neurons prior to the onset of salt-induced hypertension.

Pentobarbital-anesthetized (50 mg/kg) rats were perfused transcardially with isotonic saline followed by 4% paraformaldehyde (Sigma, St. Louis, MO) in 0.1 M sodium phosphate buffer, pH 7.4. Immunofluorescence staining was performed on a 1-in-5 series of frozen sections (50 μm thick) through the caudal NTS from each rat in order to detect HSD2 or neuropeptide FF (NPFF) and tyrosine hydroxylase (TH), which were stained as independent controls. Seven rats of each strain were stained with a sheep antibody raised against a synthetic peptide unique to rat HSD2 [10] (1:40,000; Chemicon, Temecula, CA) and five of each strain with a rabbit anti-TH antibody (1:10,000; Chemicon), followed by a Cy3-donkey anti-sheep or anti-rabbit (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA). Immunohistochemistry for NPFF (1:5000; Chemicon) was performed in the NTS from seven rats of each strain.

In a set of preliminary experiments, immunostaining for HSD2 had produced results similar to those described below (18% elevation in Dahl salt-sensitive HSD2 neurons vs. Dahl salt-resistant, $n = 7$ from each strain). In these prior experiments, age-matched groups of male spontaneously hypertensive rats (SHR, 7 weeks old, Harlan, $n = 7$) and Wistar–Kyoto rats (WKY, 7 weeks old, Harlan, $n = 7$) had been processed for HSD2 immunohistochemistry in a similar manner, and the numbers of HSD2 neurons in the NTS were not significantly different from control Sprague–Dawley male rats (7 weeks, $n = 7$, Harlan). However, the HSD2 data from these earlier experiments were replicated (as presented below) because the background cross-reactivity was judged to be too high since a higher concentration of the HSD2 antibody (1:12,000) had labeled not only HSD2 cell bodies but also neuronal nuclei throughout the brain. Later, we found that a 1:40,000 dilution stained the HSD2 neurons without nuclear cross-reactivity [9]. As described below, these counts were repeated in Dahl salt-sensitive, Dahl salt-resistant and Sprague–Dawley rats, but not SHR or WKY rats, because these two strains had not shown a difference in the number of HSD2 neurons (relative to Dahl salt-resistant or Sprague–Dawley rats) in our original tests.

An observer who was blinded to the strain of rat counted neuronal cell bodies stained with HSD2, TH, or NPFF, using a digital X–Y microscope plotter (Accustage, Minneapolis, MN). All cases were plotted before data were decoded and analyzed. Groups were compared by Student's two-tailed $t$ test with a significance level of $P < 0.05$, and data are presented as mean ± SEM.

Fig. 1 shows images of the HSD2 neurons, obtained using an Olympus FV 500 confocal microscope. A series of approximately 20 sequential images was acquired through the $z$-axis of a 50 μm section of the HSD2 neurons from representative animals of each strain. These images were z-frame compressed using MetaMorph software (Molecular Devices, Sunnyvale, CA). Pseudocoloration of these images and uniform brightness and contrast adjustments were performed in Adobe Photoshop (Adobe, San Jose, CA).

As shown in Fig. 2A, Dahl salt-sensitive rats were found to have 24% more HSD2-immunoreactive cells than their salt-resistant counterparts (187 ± 7.0 versus 151 ± 3.6 HSD2 cells, for Dahl salt-sensitive and salt-resistant respectively; $P = 0.0006$). Dahl salt-sensitive rats also had 21% more than Sprague–Dawley rats (155 ± 7.0, $P = 0.007$). There was no significant difference between Dahl salt-resistant and Sprague–Dawley rats ($P = 0.61$).

In each strain of rats, control counts were performed for two other medial NTS neuronal phenotypes besides the HSD2 neurons—the A2 noradrenergic neurons and neuropeptide FF (NPFF)-immunoreactive neurons. The number of NTS cells expressing TH, a catecholamine-synthetic enzyme that marks the A2 group, was not significantly different between Dahl salt-sensitive and Dahl salt-resistant rats (TH cells 779 ± 21.6 and 694 ± 33.8, respectively; $P = 0.07$, see Fig. 2B) or between Dahl salt-sensitive and Sprague–Dawley rats (837 ± 24.5, $P = 0.11$). Even though the main focus of this study was a comparison between salt-sensitive versus salt-resistant rats, we also found that Dahl salt-resistant rats had a statistically significant 17% reduction in TH neurons versus Sprague–Dawley rats (694 ± 33.8 and 837 ± 24.5; $P = 0.009$).

Dahl salt-sensitive and salt-resistant rats showed no difference in the number of NTS cells immunoreactive for NPFF (222 ± 11.3 and 227 ± 10.8, respectively; $P = 0.75$, see Fig. 2C), although a significant reduction was found in the number of NPFF cells in Sprague–Dawley rats (148 ± 6.7) in comparison to Dahl salt-sensitive ($P = 0.0001$) or Dahl salt-resistant rats ($P = 0.00005$).