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Previews

Rats, Salt, and History

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The Dahl salt-sensitive rat is a 50-year-old enigma in hypertension research. How does salt increase blood pressure? One hypothesis put forward is the involvement of reactive oxygen species produced in the renal outer medulla. A novel rat gene-deletion model in this issue of *Cell Metabolism* supports this argument.

Half a century ago, Dahl and associates (1962) fed rats an 8% (by weight) salt diet, measured blood pressure, and crossed animals with the highest blood pressures and with the lowest blood pressures. The salt-sensitive (DS) rats developed severe hypertension after several generations, while the resistant (DR) strain did not. Dahl and many other investigators worked diligently to pursue the mechanisms involved (Rapp, 1982). The Guyton laboratory developed the idea that the long-term ''infinite gain'' relationship between blood pressure and salt excretion (pressure natriuresis) is the ultimate blood pressure regulatory mechanism (Coleman et al., 1971). Further implicating the DS kidney is the fact that the DS strain exhibits albuminuric renal disease even when given a low-salt diet, a state of affairs that is greatly potentiated by high salt (Sterzel et al., 1988). Now, Feng et al. (2012) draw attention to a potential molecular renal mechanism.

The control strain in this report is not the DR rat strain, but rather the Brown Norway (BN) strain. The authors probably selected the salt-resistant BN strain because the BN rat underwent total genome sequencing and serves as the reference strain. The group presents evidence that increased nicotinamide adenine dinucleotide phosphate (NADPH) activity in the renal outer medulla is responsible for the salt-sensitive genetic hypertension. The NADPH oxidase is an enzyme complex found on the plasma membrane and in the phagosomic membranes of many cells and is responsible for reactive oxygen species (ROS) production. The complex consists of five phagocytic oxidase (phox) subunits, including the heme-containing Nox, $p22^{phox}$, $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$, each encoded by its own gene. Feng et al. (2012) raise some strong arguments supporting an increase in renal outer medullary ROS production as the hypertensive mechanism in DS rats.

Moreno et al. (2007), from the authors' laboratory, had previously shown that a congenic DS strain containing a 12.2 Mb genomic region from the BN strain was no longer salt sensitive. The finding implies that the segment, which contains the gene encoding $p67^{phox}$, necessarily contains genes critical for the salt-sensitive phenotype. The authors discovered a promoter deletion in the $p67^{phox}$ gene of DS rats that was not present in BN rats. Feng et al. (2012) then used reporter assays in transfected renal medullary cells to show that the mutant promoter produced increased luciferase activity. They postulate that the deletion could contain a not-yet-identified repressor element. The investigators also showed an increase in $p67^{phox}$ mRNA, an increase in protein expression by western blotting, an increase in (microdialysis-determined) medullary ROS production, and a salt-sensitive increase in blood pressure that they documented by radiotelemetry in DS rats, all compared to BN control rats.

The authors then used the zinc finger nuclease technology to produce a 5 bp deletion in the genomic sequence of the DS $p67^{phox}$ gene. The $p67^{phox}$ gene-deleted DS rats no longer had an increase in NADPH oxidase activity, ROS production was reduced, and most importantly, the telemetric blood pressure increase with high-salt diet was reduced to BN rat levels. Finally, the renal target organ findings mentioned earlier in DS rats were diminished in the $p67^{phox}$ gene-deleted rats. These observations would surely have interested Dahl and his supporters and detractors alike. However, are these findings of any clinical relevance?

Intersalt was a most exacting epidemiological study of human salt intake in 52 centers of 200 subjects each worldwide (Intersalt Cooperative Research Group, 1988). In 48 ''industrialized'' centers, the subjects consumed about 160 mmol/day Na^+. Four centers were outside the norm of salt intake. In these centers, the Intersalt investigators found very low sodium excretion, lower blood pressures, and little or no upward slope of blood pressure with increasing age. Across the other 48 (industrialized) centers, sodium excretion was significantly related to the slope of blood pressure with age, but not to median blood pressure or prevalence of high blood pressure. Across centers there was no consistent association between electrolyte excretion and blood pressure. A 250 g rat has about 1% of the human body surface area and about 0.3% of the body weight. Assuming a 20 g chow consumption per day, rats ingesting the 8% salt diet receive 1.6 g salt (28 mmol/day Na^+) daily, compared to animals receiving 0.4% salt diet ingesting 0.1 g salt (1.8 mmol/day). The Na^+ increase is 15-fold.

The Institute of Medicine (http://www.iom.edu/Reports/2010/Strategies-to-Reduce-Sodium-Intake-in-the-United-States.aspx) has advocated a reduction in U.S. adult salt consumption from the current (Intersalt) intake level to about 100 mmol/day Na^+ (0.3-fold reduction). Therefore, the relevance of an 8% salt diet in the rat to salt-sensitive human hypertension is a matter of conjecture.

So why would increased ROS in the outer renal medulla make DS rats hypertensive? Blood pressure is a function of flow and resistance; all hypertension features an increase in peripheral vascular resistance. The authors argue that increased ROS at this site could increase

Na^+ reabsorption via the $Na^+K^+2Cl^-$ cotransporter in the ascending limb of Henle's loop (NKCC2). They further suggest that an influx of T cells into the kidney could perhaps contribute directly; indeed, immunosuppressive treatment can ameliorate salt-sensitive hypertension (Harrison et al., 2011). In DS rats, the pressure natriuresis curve is shifted rightward (Figure 1). In addition to outer medullary ROS, we can add neural inflammatory mechanisms and an elaborate macrophage-driven proteoglycan-Na^+ storage compartment (Machnik et al., 2009). We can debate the role of Na^+, Cl^-, and both ions and the importance of volume-related effects, if any. However, the mechanisms shifting pressure natriuresis are still not solved, and the Guyton laboratory's notion of total body autoregulation remains imperfectly explained.

What are the therapeutic ramifications from this study? The authors argue that NADPH in the kidney could be a therapeutic target. However, the entire chapter of antioxidant treatments for cardiovascular disease does not inspire confidence (Halliwell, 2000). Suffice it to say, much work remains to be done. It would be important to sequence the $p67^{phox}$ gene promoter in the DR strain to determine whether or not this strain (which has been used in hundreds of papers) is similar to the BN control. Furthermore, the postulated regulatory element within the deletion remains to be defined. Dahl and his followers would be intrigued.

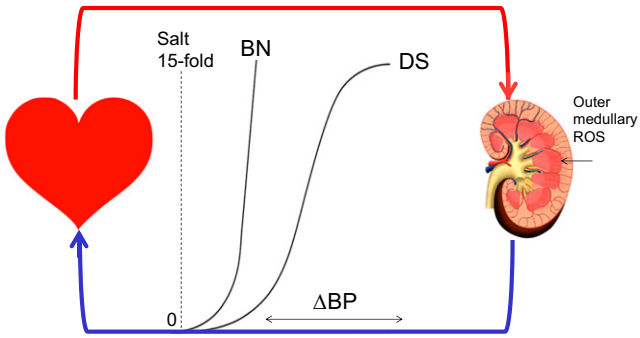

Figure 1. Arterial Blood Pressure Is a Function of Flow and Resistance

Arterial blood pressure is a function of flow and resistance. Increased peripheral resistance must be reflected throughout the entire arterial tree. The infinite-gain controller is the kidney that regulates salt and water in the body by determining the pressure natriuresis relationship. Pressure natriuresis relationships are conventionally shown with salt intake/excretion on the ordinate, although salt intake is the independent and blood pressure the dependent variable. In the BN/DS rat model, salt intake was increased from 0.5% to 8% chow weight. Blood pressure increased about 30 mm Hg in DS rats, resulting in a rightward-shifted flatter sigmoid curve in DS compared to BN. Responsible for the shift in pressure natriuresis was an increase in reactive oxygen produced in the outer medulla of the kidney.

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