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Sodium balance is not just a renal affair

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Abstract

Purpose of review—The equilibration theory of extracellular body fluids is tightly linked to sodium (Na^+) metabolism. Accepted is the notion that with changes in salt intake, renal sodium elimination will prevent any change in interstitial Na^+ content and concentration. This review summarizes recent anomalous findings in salt and water homeostasis that are inconsistent with current assumptions.

Recent findings—Recent findings from chemical analysis studies of laboratory animals, as well as noninvasive quantitative Na^+ MRI (Na-MRI) studies in patients, have shown that remarkable amounts of Na^+ are stored in muscle and in skin without commensurate water retention. Furthermore, an ultra-long Na^+ balance study in humans suggests the presence of endogenous clocks that generate weekly and monthly infradian rhythmicity of Na^+ storage independent of salt intake. Animal experiments suggest that fluids in the skin interstitium are hypertonic compared with plasma and that interstitial osmotic stress induces local extrarenal immune cell and lymph–capillary driven mechanisms for electrolyte clearance and maintenance of the internal environment.

Summary—Recent quantitative evidence challenges current ideas on salt-and-water homeostasis and suggests that Na^+ homeostasis cannot be maintained without additional hitherto unappreciated extrarenal regulatory mechanisms.

Keywords

hypertonicity; magnetic resonance imaging; Na^+ homeostasis; sodium balance; sodium storage

INTRODUCTION

All bodily cells are bathed in extracellular fluid. The current generally accepted hypothesis is that extracellular bodily fluids readily equilibrate, resulting in constant electrolyte concentrations in the interstitial fluid space between cells. Maintenance of a constant *milieu*

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Conflicts of interest

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intérieur has served since the 19th century to define research methods and problems [1]. The equilibration theory of extracellular body fluids is tightly coupled to Na^+ metabolism (balance). Na^+ is the major extracellular cation and exerts osmotic pressure. Because changes in Na^+ concentrations would change osmotic pressures and thereby lead to fluid shifts and swelling or shrinking of cells with subsequent altered cell function, the accepted theory in electrolyte and fluid physiology is that regulatory systems will, if at all possible, prevent any change in interstitial Na^+ content and concentration. Three explicit rules define the current framework for physiological, pathophysiological, and clinical investigation of disordered salt-and-water balance. Assumption 1 suggests that Na^+ accumulation in the body is almost entirely extracellular and leads to commensurate fluid retention [2]. Assumption 2 holds that any accumulation or loss of Na^+ will be strictly prevented by a variety of regulatory steady-state mechanisms, resulting in constant body Na^+ content that is maintained within narrow limits [3]. Assumption 3 maintains that such regulatory steady state is achieved by the kidneys, which excrete salt and water by a variety of renal clearance mechanisms [4]. These hypotheses regarding the homeostatic regulation of a constant internal environment have resulted in a strong instrumental and methodological focus on molecular control of renal electrolyte and water transport. Study of salt and water metabolism has become a ‘molecular-renal affair’ that focuses on Na^+ reabsorption mechanisms (ion channels, transporters, and their regulators) located in epithelial cells of the renal tubular system. The current above-mentioned research directions, even when coupled with molecular genetic approaches ranging from studies identifying monogenetic disorders [5, 6] to genome-wide associations in populations [7], have not brought us closer to a physiological understanding of primary hypertension and resultant cardiovascular disease. Blood pressure and cardiovascular research remains ‘under pressure’ to solve this scientific puzzle [8]. Recent evidence suggests that current views on Na^+ metabolism are an oversimplification. This review summarizes recent findings from long-term Na^+ balance studies in humans, from Na-MRI measurements of tissue Na^+ content in patients, and animal studies directed at tissue Na^+ storage.

LONG-TERM BALANCE STUDIES IN HUMANS: HOW IS STEADY STATE ORGANIZED, AND WHERE IS THE SALT?

Current understanding of salt homeostasis in humans derives from a series of balance studies in humans carried out in the 20th century, which were of 1–2 weeks duration and evaluated responses to abrupt changes in salt intake [9]. Clinicians’ textbooks suggest that in normal subjects exposed to such challenges, steady state between salt intake and urinary Na^+ excretion is achieved within a few days. For instance, the authors of a popular textbook state: ‘If dietary intake is abruptly increased from a low-sodium diet, only about half is excreted on the first day. The remainder is retained augmenting sodium stores. This state of affairs elevates the plasma osmolality, stimulating both thirst and secretion of antidiuretic hormone. The increments in water intake and renal water reabsorption produce water retention, resulting in increases in effective circulating volume and weight. After 3–4 days, a new steady state is achieved in which renal sodium excretion matches intake. The same sequence occurs in reverse if sodium intake is reduced’ [10]. The steady-state teaching of salt-and-water balance seems perfectly in line with scientific rules on extracellular electrolyte

homeostasis. An imbalance between intake and renal excretion will inevitably lead to fluid retention (equilibrium theory). Therefore, body Na^+ content is maintained within very narrow limits (steady-state theory). A simulated space flight to Mars has recently allowed re-inspection of this theory [11■]. Healthy subjects were confined to an enclosed, restricted environment in two independent studies for 105 and 520 days, which allowed two ultra-long term Na^+ balance studies in humans. The subjects were given diets with scrupulously defined Na^+ content and collected every drop of urine made, day-by-day. The study provided an unprecedented unique and detailed profile of long-term Na^+ balance in humans. Actually, the mock spacemen did not upset the steady-state theory. They ate almost 15 kg of salt during the studies and excreted 90–95% of the dietary salt in their urine. Thus, the Na^+ recovery in this study was superb. However, in contrast to textbook teaching, a steady state was not achieved within a few hours and 24-h Na^+ excretion rarely matched that day's Na^+ intake. When salt intake was fixed for weeks and months, there was considerable day-to-day variability in 24-h Na^+ excretion, accompanied with fluctuations of aldosterone, cortisol, and cortisone that peaked with a periodicity of about 1 week. Regular fluctuations of total body Na^+ content were also observed, but with longer periodicity of approximately 1 month. The subjects thereby rhythmically accumulated and released body Na^+ independent of daily salt intake, presumably regulated by (neuro)-endocrine rhythmical clocks. Endogenous weekly or monthly rhythmical components of Na^+ metabolism have not yet entered the clinical arena, presumably because our traditional clinical knowledge derives from short-term experiments. Getting subjects and patients to collect 24-h urines once is hard enough. Few would be willing to do so for weeks. The resulting amount of weekly and monthly rhythmical variability and the timeframe by which steady state was achieved questions the idea that single 24-h urine samples are valid indicators of salt intake in humans. This new observation is of direct clinical relevance, because 24-h urine Na^+ excretion is considered to be the gold standard for estimating salt intake. Also not in line with the assumption that body Na^+ is maintained constant within very narrow limits, the observed rhythmical release and storage of Na^+ resulted in remarkable variability in body Na^+ content. Large amounts of Na^+ were retained or excreted without commensurate changes in body weight, indicating an uncoupling between Na^+ balance and water balance. Na^+ seemed to be stored in the body without commensurate fluid retention. In summary, these first long-term balance studies do not support the accepted notion that Na^+ retention inevitably leads to volume retention (Assumption 1), and that body Na^+ therefore is to be maintained constant within very narrow limits (Assumption 2). In contrast, this rather 'spooky' Na^+ balance study [12] suggested that Na^+ is stored in the body. The next obvious question therefore is: where is the salt?

Na-MRI STUDIES IN HUMANS: Na^+ ACCUMULATION IN MUSCLE, SKIN, AND BRAIN

The general assumption is that Na^+ content in healthy persons is fairly constant. Developing quantitative measurements to determine the body's Na^+ that have utility in patients is not easy. To that end, special detector coils were developed that sense Na^+ instead of H^+ as in conventional MRI. Na-MRI measurements of Na^+ content in humans suggest that relevant amounts of Na^+ are accumulated in muscle, skin, and brain. Tissue Na^+ storage is associated

with disease [13–15, 16■]. In a patient with hypernatremia, massive Na^+ storage was also observed in muscle [14]. Muscle Na^+ content returned to normal after the serum Na^+ concentration was reduced to normal. This state-of-affairs also coincided with the correction of secondary hyperaldosteronism. In patients with primary hyperaldosteronism, the hypertension was paralleled by Na^+ storage in muscle and skin. Treatment, either by surgical removal of an adenoma or with spironolactone, resulted in rapid mobilization of skin and muscle Na^+ without any changes in body weight [15]. Unpublished observations from our clinical research center suggest that patients with end-stage renal disease feature massive Na^+ storage in their skin and that increased muscle Na^+ content can be reduced within 4h with hemodialysis. In an initial cohort study, we found that Na^+ storage in muscle and in skin increased with age, was more pronounced in men than women, and was directly associated with blood pressure levels [16■]. Patients with refractory hypertension showed increased tissue Na^+ storage and patients treated with spironolactone showed reduced Na^+ storage in muscle. This association raises the hypothesis that tissue Na^+ storage characterizes a disruption of internal environment composition, which could be causally linked with primary hypertension. A straightforward question is whether or not humans with increased Na^+ storage are at risk for developing cardiovascular disease. We have no systematic quantitative information on Na^+ content in the heart. Na-MRI studies revealed that Na^+ accumulation in the brain is linked with autoimmune disease. Patients with multiple sclerosis have increased Na^+ content in the brain [13]. In line with this finding, recent experimental evidence suggests that immune function is closely related to extracellular Na^+ concentration.

ANIMAL STUDIES: ELECTROLYTE METABOLISM AND IMMUNE FUNCTION

The immune cell population of interleukin-17-producing CD4^+ helper T cells (TH17 cells) plays a pivotal role in autoimmune diseases and is critical for the development of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. Two studies report that increasing Na^+ concentration in the cell culture medium boosts the induction of murine and human TH17 cells [17, 18■]. When they were exposed to interstitial osmotic stress, the cells assumed a highly pathogenic phenotype with up-regulation of proinflammatory cytokines. The osmoprotective transcription factor, tonicity enhancer binding protein (TonEBP, *NFAT5*) mediated the pro-inflammatory response by signaling through the serum/glucocorticoid-regulated kinase 1 (SGK1). Gene silencing or chemical inhibition of SGK1 or TonEBP abrogated the high-salt-induced TH17 cell development. Mice fed a high-salt diet developed increased TH17 cell count in the central nervous system, mesenteric lymph nodes, and lamina propria. Furthermore, a high-salt diet aggravated EAE. Increased salt intake might represent an environmental factor for the development of autoimmune diseases through the induction of pathogenic TH17 cells. Although the Na^+ concentration of plasma is approximately 140 mmol/l, concentrations in the interstitium and lymphoid tissue may physiologically range between 160 and 250 mmol/l [19, 20]. Interstitial osmotic stress, especially if enhanced by Na^+ storage with a high-salt diet, may favor an inflammatory response in lymphoid tissues or with migration of cells into tissue with increased electrolyte concentration. These examples may provide evidence that

hypertonicity in the interstitial space modulates immune cell function by boosting anticipated adaptive or innate immune cell reaction patterns [17, 18■, 19].

Besides the concept that osmotic stress boosts adaptive or innate immune responses, an additional emerging idea is that macrophages may also actively regulate and modify the environmental composition of their home, the interstitial fluid matrix. We have found that large amounts of Na^+ are stored in the skin interstitium without commensurate water retention, defining the skin as an extracellular compartment that cannot be controlled by renal blood purification alone. We found that additional unappreciated tissue-specific clearance mechanisms operate in the skin interstitium. Storage of Na^+ in the skin interstitium is paralleled by polymerization of highly sulfated, negatively charged glycosaminoglycans (GAGs). Release of Na^+ from interstitial reservoirs is coupled with reduced negative GAG charge density [20, 21]. The skin microenvironment seems to act like a negatively charged capacitor, which traps cations depending on its negative charge density. The result is an interstitial microenvironment that is highly distinct from the more aqueous blood space. Macrophages are attracted by a hypertonic microenvironment and actively migrate to sites of higher salt concentration [22]. A high-salt diet leads to hypertonic Na^+ accumulation in the skin. Sensing the accumulation of these ions, mononuclear phagocyte system (MPS) cells are recruited to the skin [23, 24, 25■]. In response to osmotic stress with Na^+ storage in the skin, macrophages release vascular endothelial growth factor C (VEGF-C) by a TonEBP/NFAT5-dependent mechanism. VEGF-C binds to VEGFR3 and leads to hyperplasia of the cutaneous lymph capillary network. Blocking this lymphatic response, either by systemic [23, 24] or by skin-specific [25■] VEGF-C depletion, by macrophage-specific deletion of TonEBP [25■], or by specific blockade of VEGF-C/VEGFR3 signaling [25■], results in increased skin electrolyte accumulation and salt-sensitive hypertension. The findings suggest that the interstitial environment of the skin is hypertonic to the plasma compartment. MPS cells outfitted with TonEBP exert homeostatic immune function, acting as extrarenal regulators of interstitial electrolyte homeostasis by inducing local clearance of skin electrolytes. Unclear is how such interstitial electrolyte clearance is achieved mechanistically [25■]. However, the homeostatic effect may be comparable with lymphatic clearance of cholesterol from the arterial intima [26■]. Also unclear is why a reduction in MPS cell-driven lymphatic clearance leads to predominant Cl^- accumulation in the skin, and why this electrolyte accumulation is associated with blood pressure increase. Preliminary work suggests that increased contractility of skin blood vessels could be linked to Na^+ storage in the skin [27].

In summary, textbook concepts suggest that extracellular Na^+ homeostasis is almost exclusively a renal affair. Research generally focuses on mechanisms of renal electrolyte and water excretion for scientific puzzle solving. The idea that interstitial fluid might not readily equilibrate resulted in awareness of Na^+ storage in tissues, novel salt pathways, proteoglycan binding, local 'hypertonicity', immune-cell signaling, infradian rhythms, TH17 cell conversions, and infradian rhythms that point to unknown 'clockworks'. The emerging concept of Na^+ storage opens new questions for basic researchers and clinician-scientists, such as how interstitial electroneutrality is achieved and how osmotic gradients in the fluid matrix are maintained. Also waiting to be tested is the idea that tissue Na^+ storage should be

modified by lifestyle changes or medication. Assessing Na⁺ homeostasis by quantitative measurement is a promising conceptual approach to provide answers.

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KEY POINTS

- Sodium balance in human features circaseptan variations in excretion when dietary intake is fixed, making 24-h urine collections to determine intake of dubious value.
- Total-body sodium also exhibits even longer infradian rhythms independent of blood pressure or body weight.
- Aldosterone, cortisol, and cortisone participate in this regulation controlled by undiscovered ‘clocks.’
- Sodium is stored bound to glycosaminoglycans in skin and in muscle. A novel magnetic resonance imaging tool can assess sodium tissue storage in humans.
- Immune cells control tissue sodium storage. Associated inflammatory responses could be of clinical relevance.