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Tissue sodium storage: evidence for kidney-like extrarenal countercurrent systems?

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Abstract

Recent evidence from chemical analysis of tissue electrolyte and water composition has shown that body Na⁺ content in experimental animals is not constant, does not always readily equilibrate with water, and cannot be exclusively controlled by the renal blood purification process. Instead, large amounts of Na⁺ are stored in the skin and in skeletal muscle. Quantitative non-invasive detection of Na⁺ reservoirs with ²³NaMRI suggests that this mysterious Na⁺ storage is not only an animal research curiosity, but also exists in humans. In clinical studies, tissue Na⁺ storage is closely associated with essential hypertension. In animal experiments, modulation of reservoir tissue Na⁺ content leads to predictable blood pressure changes. The available evidence thus suggests that the patho(?)-physiological process of Na⁺ storage might be even of relevance for human health and disease.

The phenomenon of extrarenal Na⁺ storage has been extensively reviewed recently [29,10,27,30,28]. We feel that another review of the available evidence would plagiarize these summaries. We therefore briefly highlight additional recent evidence in support of the idea that blood pressure is regulated in the skin, and then focus on the still very limited information available on electrolyte gradient formation in the skin.

Blood pressure regulation: only skin deep, and related to skin electrolyte metabolism?

Several recent studies have further substantiated the important role of local physiological-homeostatic regulatory processes in the skin for systemic blood pressure control. Johnson and colleagues have demonstrated that the two hypoxia-inducible factor (HIF) transcription factors HIF-1 α and HIF-2 α critically control the NO equilibrium in the skin, with keratinocyte-HIF-2 α promoting vasoconstriction, while keratinocyte-HIF-1 α mediates vasodilation [5]. Mice with keratinocyte-specific deletion of HIF-1 α therefore had higher blood pressure, while HIF-2 α deletion resulted in low blood pressure levels and body

temperature loss. Weller and colleagues demonstrated that irradiation of the skin with standard erythral doses of UVA increased blood flow and lowered blood pressure by NOS-independent release of NO from a light-sensitive NO pool in the upper epidermis [20]. This finding may explain the seasonal variation of blood pressure with lower pressure in summer than in winter. Karbach et al. showed that keratinocyte-specific overexpression of IL-17 increased reactive oxygen species formation and circulating inflammatory leukocytes in blood, resulting in endothelial dysfunction and increased systolic blood pressure [12]. This finding provides an interesting link between skin immune cell activity and electrolyte homeostasis in hypertension, because salt also polarizes T-cells into a Th-17 pro-inflammatory phenotype [3].

Macrophages seem to control skin electrolyte homeostasis and systemic blood pressure by VEGF-C-driven lymph capillary clearance [22,21,35]. This hypothesis has been recently supported in studies by Graham and colleagues, who studied mice deficient for the inflammatory chemokine-scavenging receptor ACKR2 [17]. Absence of ACKR2 increased the availability of VEGF-C positive macrophages in close proximity to lymph vessels, and increased lymphatic vessel density. Functionally, the mice were characterized by enhanced skin interstitial fluid drainage and low blood pressure. Interestingly, macrophages actively move to sites with higher salt concentration, suggesting that the cells are mobile osmosensors [24]. The idea that macrophage-derived VEGF-C is a clearance factor for lymphatic mobilization of stored tissue sodium has been further corroborated in a first clinical study, where dialysis patients with higher VEGF-C levels showed better removal of reservoir Na^+ during HD treatment. Furthermore, skin Na^+ storage increases in the elderly, and this age-dependent increase is associated with decreased circulating VEGF-C levels [6]. First images of the skin Na^+ content in humans at high magnetic strength (7T) reveal that this age-dependent increase in skin Na^+ content occurs inside or directly under the keratinocyte layer of the skin [19]. Previous studies at 3T have shown that the age-dependent increase in tissue Na^+ is paralleled with increased systemic blood pressure levels [16,15]. Imaging at 7T now shows that even a massive increase in skin Na^+ content is not paralleled by a significant volume increase within the keratinocyte layer (Fig. 1A). This finding suggests that salt is concentrated inside or directly under the keratinocyte layer, an idea which is in line with the concept that keratinocytes create an electric field which guides cell movement in embryogenesis and skin epidermal repair [1,23]. Yang et al have recently reported that this process of “galvanotaxis” is dependent on epithelial Na^+ channel availability in human keratinocytes [37]. This finding suggests that Na^+ is actively pumped from the keratinocyte layer into the cutaneous skin interstitium, which is anatomically characterized by hairpin-like lymphatic and blood capillary structures (Fig. 1B).

Although these details indicate that the skin could function as a “kidney-like” counter current system, physiological studies on electrolyte concentration by counter-currents in skin surprisingly have not been performed. Here, we utilize the available scarce information on the microanatomy of electrolyte distribution in the skin, which to our view supports the hypothesis that the skin acts as a functional counter current system. The counter-current mechanism could enable the skin to differentially control its own microenvironment, creating a hyperosmolal biological barrier designed to prevent interstitial fluid loss, as suggested by Taylor and colleagues earlier [32,33].

Data selection

We screened the literature for available measurements of skin electrolyte concentration inside and under the keratinocyte layer of the skin. Wei et al. studied elemental distribution in guinea-pig skin by x-ray analysis with scanning electron microscopy and provided data on Na^+ , K^+ , and chlorine levels in the epidermis and the dermis. The authors indicated that, “chlorinated hydrocarbons that occur in the microscope column may contaminate specimens during x-ray microanalysis.” [34] We, therefore, did not include chloride data and restricted our model analysis to Na^+ and K^+ . Comparable Na^+ and K^+ gradient formation was observed later in studies of human skin [33,7,1,18]. We thus assume that the selected data may provide a representative sample for skin electrolyte gradient formation in the mammalian skin.

Data presentation, model generation, and limitations

Electron probe analysis of Na^+ and K^+ content in the skin cannot discriminate between intracellular or extracellular microenvironment. In an attempt to address the energetic aspects of potential Na^+ and K^+ gradient formation in the epidermis, we formed three simplified theoretical models of skin electrolyte composition (Fig. 2A). The general assumption is that the five measured skin layers (stratum corneum, stratum granulosum, stratum spinosum, stratum germinativum, and dermis) represent four subsequent binary subsystems separated by theoretical semipermeable membranes (Fig. 2B). Our model assumes that all adjacent layers contain water as an ideal solvent, in which Na^+ and K^+ may readily equilibrate. Wei et al measured the micro anatomical distribution of epidermal electrolyte content in mmol/kg wet tissue weight [34]. To transfer these measurements of electrolyte content into approximations of electrolyte concentration in the epidermal layers, we used three different models.

Model 1 assumes that each measured layer of the skin represents an idealized solvent. We use the original data, which were reported in mmol/kg tissue, and express them as mmol/dm³ fluid in each measured compartment (Fig. 2B). The model cannot discriminate between differences in Na^+ and K^+ concentrations between the intra- and extracellular space within each layer. It does not take into consideration any intracellular or extracellular compartmentalization of fluid spaces, and thereby also neglects that there might be differences in the effectiveness of the solute in generating an osmotic driving force (typically indexed by the reflection coefficient). As this model also neglects the presence of solid matter inside cells and in the extracellular matrix, it will underestimate the actual electrolyte concentrations in the water space. Comparable assumptions have been made earlier by Warner et al. [32,33].

In contrast to Model 1, **Model 2** additionally takes into account that cells and extracellular matrix consist of water and solid matter. It assumes that both layers consist of 50% solid matter, and of 50% water which represents an idealized solvent where all Na^+ and K^+ is dissolved and readily equilibrates. Similar to model 1, there is no discrimination between intracellular and extracellular space within the layers. The model again does not take into account any intracellular or extracellular compartmentalization of fluid spaces, and neglects

potential differences in reflection coefficients. Compared with Model 1, this model is therefore characterized by a 2-fold increase of Na^+ and K^+ concentrations in the idealized solvent (Fig. 2B).

Similar to Model 1, **Model 3** assumes that each of the epidermal keratinocyte layers represents an idealized solvent with no solid matter. Similar to Model 2, the dermis, which is rich in extracellular matrix, would consist of 50% solid matter, and 50% solvent. There is no discrimination between intracellular extracellular space, neglecting water or electrolyte compartmentalization.

Gibbs energy calculation

First, we sought to determine if the concentrations of electrolytes measured by Wei et al could be caused by passive accumulation of electrolytes in the skin, or by an actively established gradient. To do this, we considered that the Gibbs energy (G^0 , equation 1) indicates the stored energy in the system.

$$\Delta G^0_{(K,Na)} = -RT \ln \frac{r_K}{r_{Na}} \quad \text{Equation 1}$$

where c_i is electrolyte molar concentration (molarity) at binary layers 1 (') and 2 (''),

$$r_i = \frac{c_i'}{c_i''}$$

$$R = 8.314 \frac{\text{J}}{\text{K mol}},$$

$$T = 310 \text{ K (at } 37^\circ \text{C body temperature)}$$

Perfectly nonselective membranes will yield an equivalent r_{Na} and r_{K} . This relationship applies to bodily fluid layers in which the ratios of Na^+ and K^+ ions are equivalent and therefore G^0 approaches zero. This condition is known as the ideal Donnan equilibrium and is interpreted to mean that the separation is membrane non-selective. For cells and tissues that perform active selection of Na^+ and K^+ across membranes, the change in Gibbs energy is non-zero. A positive change in G^0 thus indicates a system in a non-equilibrium state, requiring energy input to maintain the difference. As seen here, the electrolyte distribution in the skin results in a positive change in Gibbs energy in all models at the transition from the stratum germinativum to the dermis (Fig. 3–5B). This suggests that the electrolyte gradient at the epidermis/dermis binary system was established by active transport. We observe a G^0 nearly zero in the upper layers of the skin, where initially viable epidermal cells scale off and most likely lose their ability to actively transport electrolytes.

Electrical potential calculation

While the Gibbs energy may be calculated from measurable ratios of ions, the more common practice is to measure an electrical potential. Therefore, we used the Goldman

equation (equation 2) to calculate the theoretical electrical potential difference between layers using the concentrations from models 1–3.

$$E^0 = \frac{RT}{zF} \ln \left(\sum P_i r_i \right) \quad \text{Equation 2}$$

where, $F = 96500 \frac{C}{mol}$ z = charge [C] P = relative permeability

In our models, we assume that the relative permeability of each ion is equivalent, and therefore $P=1$ for both Na^+ and K^+ . Na^+ transport in keratinocytes is relevant for electrolyte gradient generation in the stratum germinativum [37]. We therefore considered the electrical potential caused by sodium distribution alone, as well as the potential caused by sodium and potassium distribution. As seen in the plots (Fig. 3–5C), the stratum germinativum/dermis may generate an electrical potential of around 50 mV, with the negative sign referring to the serum. This result is corroborated by experimental measurements of transcutaneous electrical potentials made by Barker et al in guinea pigs [1]. We take this as an indication that the treatment of the skin electrolyte-to-water ratio in these model systems yields a physiologically relevant result.

Osmotic pressure calculation

To calculate the osmotic pressures, we used the Morse equation for an ideal solution of dissociable electrolytes:

$$\Pi = i c_i R T \quad \text{Equation 3}$$

c_i – electrolyte molar concentration (molarity), R – universal gas constant, T – temperature, i – dimensionless Van't Hoff factor.

The calculations are based on the following additional assumptions: the sum of Na^+ and K^+ approximates the total number of cations present in an ideal solution. Each binary system of two neighboring layers is in a state of equilibrium. Neglecting electrical gradient formation, we also assume that Na^+ and K^+ are accompanied by an equal number of corresponding monovalent anions, resulting in an ideal dissociation of the osmolytes in the following way:

where A^- is any monovalent anion. Hence, we assume that the theoretical Van't Hoff constant value is 2 ($i=2$). The models result in very large osmotic pressure in the lower layers of the skin. These large pressures are again the result of a significant accumulation of electrolytes. However, the theoretical assumptions of Na^+ and K^+ driven osmotic pressure gradients could not be confirmed in the experimental setting. Even with dietary-induced additional salt storage in the skin, interstitial fluid pressures remain negative [35]. We take this as an indication that the treatment of the skin electrolyte-to-water ratio in our model

systems does not represent our experimental results, most presumably because the real biological fluids in our binary compartment models do not exhibit “ideal” behavior. This can be due to the inhomogeneous distribution of electrolytes between the solid and solvent phases of the model, by different reflexion coefficients for the ions, and by water impermeable structural segments within the layers. Similar gradient formation is well known in the kidney [13], where hydrostatic pressures are slightly positive and in the range of 2–10 mmHg [8,36]. In the kidney, investigators agree that the theoretically available osmotic driving force for water in the renal interstitium appears to be partially used for water reabsorption from the primary filtrate [14].

Conclusions

Na^+ storage exists in humans and in experimental animals. Increased Na^+ storage in extrarenal tissue is associated with essential hypertension, autoimmune disease, and host defense against infection, suggesting that extrarenal Na^+ storage might be relevant in health and disease. Skin is an important site for extrarenal regulation of Na^+ metabolism. The mechanisms of Na^+ entry into the skin, and its clearance from interstitial tissue, are largely unknown. Preliminary evidence suggests that the skin is a functional, kidney-like countercurrent system. Dermal vascular counter currents could multiply an electrolyte concentration gradient which is presumably initiated in the keratinocyte layer. Lymph capillaries could serve as a tubular/urether-like drainage system of the hypertonic subepidermal fluid layer. The body seems to invest significant amounts of energy to generate and maintain a hypertonic/hyperosmolal fluid layer under the skin, which may primarily serve to prevent water loss at this large biological barrier between the internal and external environment [32]. Our model analysis of Na^+ and K^+ gradient formation in the skin suggests that active Na^+ transport is involved in Na^+ storage inside or directly under the epidermis. The predicted subepidermal positive potential has been confirmed experimentally long ago by other investigators [1]. Not in line with our model calculations, however, is the absence of significant increases in interstitial fluid pressures in the skin, while corresponding calculations would predict local increases in osmotic pressures by several thousands of mmHg. Similar calculations could be easily performed for electrolyte and osmolyte gradient formation in the kidney [2]. Differences in epithelial reflection coefficients and segments of relative water impermeability may partially explain how such interstitial concentrations processes in the kidney may create a hypertonic barrier designed to prevent renal fluid loss by enhancing water reabsorption from the primary urine. However, the purely integrative question of how the inner medullary interstitium is concentrated remains unanswered [14]. Schmidt-Nielsen emphasized the spongelike properties of the renal interstitial hyaluronan in concentration of the medullary interstitium [26]. Knepper et al. hypothesized that compression of this polyanion may sequester free cations [14]. Such transporter-independent chemical processes could easily reduce the effectiveness of the electrolytes to generate osmotic driving forces for water – and thereby fine-tune water reabsorption in the kidney, and in extrarenal tissue. Cannon concluded much earlier that the skin represents a similar spongelike meshwork which stores and releases Na^+ [4]. Skin Na^+ storage is associated with increased polymerization of sulfated glycosaminoglycans [9,31,25]. The paradox of skin electrolyte accumulation without massive increases in fluid pressures therefore could be due

to the fact that tissues and cells are “highly ordered structures, made up of insoluble and soluble charged macromolecular components, where the criterion for equilibration is that the changes of chemical potential be constant for all ions of the same charge.”[11] *In summary*, evidence suggests that the skin interstitium concentrates electrolytes and thereby may provide with a physiological barrier which induces a continuous solvent drag for water, very much alike the renal medullary interstitium. The (patho)physiological function of this still not well characterized and presumably hypertonic electrolyte/fluid barrier under the skin seems to coincide with essential hypertension in humans. Perhaps it is time for physiologists to unravel the black box of interstitial electrolyte and water homeostasis – inside and outside the kidney. X-ray microprobe analysis of electrolyte gradient formation in mice with genetically targeted, skin specific deletion of electrolyte and urea transporters, water channels, and polyanionic matrix components will provide answers to the question whether or not a kidney-like countercurrent system exists in the skin.

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Abbreviations

²³NaMRI	Sodium magnetic resonance imaging
HIF	hypoxia-inducible factor
VEGF-C	vascular epithelial growth factor C
ACKR2	atypical chemokine receptor 2
HD	hemodialysis
s	stratum

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Figure 1.

Panel A. $^1\text{H}/^{23}\text{Na}$ MR images of human calf skin. Left: ^1H image (top) and ^{23}Na MR image (bottom) of the lower limb of a 25-year old man. Right: same imaging procedure in a 67-year old man. At the strong field strength of 7T, skin (marked by arrows) is very well delineated in the ^{23}Na MRI images, which also show the agarose gel standards with increasing Na^+ content (white). The Na^+ is mainly accumulated in or directly under the epidermis. Quantitative analysis shows that salt storage in this epidermal/subepidermal layer increases with age. Adapted from Linz et al, [Ref 16], with permission. *Panel B.* Proposed electrolyte concentration in the counter-current system of the skin. Keratinocytes pump Na^+ into the dermis, creating increased Na^+ concentration at the top of the dermal papilla, where Langerhans cells and dendritic cells reside. The dermal papilla is infused with a blood vascular counter current system, which will further concentrate interstitial electrolytes in the dermal papilla, and with a draining lymph-capillary system, which will reduce interstitial electrolytes via lymphatic drainage. Immune cells residing within the different layers with different electrolyte concentrations may act as physiological regulators of skin electrolyte homeostasis.

Figure 2.

Panel A - represents the histomorphological entities of the skin, with the corresponding thicknesses of the layers (adapted from reference [25], with permission). *Panel B* - proposed models that largely influence the colligative properties within the skin layers. Model 1 - the two layers are both considered ideal solutions. Model 2 - the solid matter in the layer accounts for 50% of the volume of the layer, with the ideal solution filling the second half. Model 3 - the epidermal layers behave like an ideal solution, whereas the dermis, consist of 50% solid matter and 50% of an ideal solvent. *Panel C* - proposed K⁺ and Na⁺ concentrations deriving from the models shown in the panel B and measurements performed in freeze-dried skin sections. Original measurements are from Wei et al., reference [24].

Figure 3. Colligative and electrical properties of the skin layers compared to plasma, based on the concentrations deriving from the Model 1

(A) shows the approximate molar concentrations of Na⁺ and K⁺ (mmol/dm³) across the epidermal strata (s. corneum, s. granulosum, s. spinosum, and s. germinativum), dermis, and in the plasma. (B) shows the Gibbs energy change (kJ/mol) across the skin layers and plasma. (C) represents the cell battery potentials of [Na⁺] or [Na⁺+K⁺] in mV as calculated from the values presented on graph (A) as derived from our Model 1. (D) represents the calculated osmotic pressures according to the conditions determined by the Model 1.

Figure 4. Colligative and electrical properties of the skin layers compared to plasma, based on the concentrations deriving from the Model 2

(A) shows the approximate molar concentrations of Na^+ and K^+ (mmol/dm³) across the epidermal strata (s. corneum, s. granulosum, s. spinosum, and s. germinativum), dermis, and in the plasma. (B) shows the Gibbs energy change (kJ/mol) across the skin layers and plasma. (C) represents the cell battery potentials of $[\text{Na}^+]$ or $[\text{Na}^+ + \text{K}^+]$ in mV as calculated from the values presented on graph (A) as derived from our Model 2. (D) represents the calculated osmotic pressures according to the conditions determined by the Model 2.

Figure 5. Colligative and electrical properties of the skin layers compared to plasma, based on the concentrations deriving from the Model 3

(A) shows the approximate molar concentrations of Na⁺ and K⁺ (mmol/dm³) across the epidermal strata (s. corneum, s. granulosum, s. spinosum, and s. germinativum), dermis, and in the plasma. (B) shows the Gibbs energy change (kJ/mol) across the skin layers and plasma. (C) represents the cell battery potentials of [Na⁺] or [Na⁺+K⁺] in mV as calculated from the values presented on graph (A) as derived from our Model 3. (D) represents the calculated osmotic pressures according to the conditions determined by the Model 3.