

Na⁺ homeostasis by epithelial Na⁺ channel (ENaC) and Na_x channel (Na_x): cooperation of ENaC and Na_x

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The body fluid (extracellular fluid: ECF) volume is mainly regulated by Na⁺ uptake (absorption) in the colon (1-3) and Na⁺ reabsorption in the kidney (4-7), and plays various important roles in the body functions such as regulation of blood pressure. Na⁺ uptake (absorption) in the colon (1-3) and Na⁺ reabsorption in the kidney (4-7) are conducted by epithelial Na⁺ transport (Na⁺ transport across epithelial cells) via epithelial Na⁺ channel (ENaC) (8), which was cloned from rat distal colon (1,2). The epithelial Na⁺ transport via ENaC is one of the most important factors controlling the adequate volume of fluids covering the apical surface of alveolar epithelial cells of the lung, which is essentially required to keep normal gas exchange across alveolar epithelium (9-11) and prevent the body from viral and bacterial infection (9-11). ENaC also plays an important role in sensing taste (12,13). However, if the ENaC-mediated Na⁺ transport is abnormally up-regulated, over-volume of body fluid occurs developing hypertension, and dryness of airway surface also appears like patients of cystic fibrosis (CF) leading to infectious diseases in the lung (14-17). In the latter case, ENaC is one of the therapeutic targets for CF patients whose lung is dry due to a lack or little of Cl⁻ secretion (18,19) caused by functional deficiency of cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel (20): i.e., as mentioned above, functional ENaCs contribute to decrease the amount of fluids covering the airway surface of epithelial cells of the lung by reabsorbing Na⁺, therefore partial blockade of functional ENaCs with some ENaC blockers prevents the airway surface from dryness. Thus,

the Na⁺ homeostasis based on regulation of epithelial Na⁺ transport via ENaCs shows essentially important physiological action on various body functions. Further, partial blockade of functional ENaCs with some ENaC blockers can show antihypertensive action by diminishing Na⁺ reabsorption in cortical collecting ducts of the kidney. Indeed, spironolactone, an aldosterone antagonist, is used for anti-hypertensive drug (21-23) keeping K⁺ unlike loop diuretic drugs such as furosemide (24).

The epithelial Na⁺ transport consists of two steps: (I) the entry step of Na⁺ from the luminal (air) space into the intracellular space via ENaC located on the apical membrane (1,2,25), and (II) the extrusion step of Na⁺ from the intracellular space to the interstitial space (facing blood vessels) via the Na⁺,K⁺-ATPase located on the basolateral membrane (26,27). The ENaC-mediated Na⁺ entry step is recognized to be the rate-limiting step of the epithelial Na⁺ transport (27). Based on this fact, the body has many intrinsic factors such as aldosterone, vasopressin (antidiuretic hormone), insulin, growth factors and osmotic stress that regulate synthesis, localization and activity of ENaCs (25,26,28-38). Although ENaC is one of the most essential targets for control of blood pressure, the Na⁺,K⁺-ATPase is also an important target for control of blood pressure: e.g., an inhibitor of the Na⁺,K⁺-ATPase, triamterene, shows a diuretic action by diminishing the epithelial Na⁺ transport (renal Na⁺ reabsorption) via blockade of the Na⁺,K⁺-ATPase in the collecting duct of the kidney (39,40).

We could not maintain homeostasis of body Na⁺ contents without any sensors detecting the body Na⁺

content, although ENaCs play various important roles in homeostasis of body Na^+ contents. The mechanisms sensing the body Na^+ content are considered to exist in the kidney and the brain. The kidney detects the body Na^+ content via the Na^+ concentration in the early distal nephron via the Na^+ - K^+ - 2Cl^- cotransporter (NKCC2) (41-45), while the brain detects the body Na^+ content via the Na_x channel (Na_x) (46-53) in addition to an osmotic sensor located at hypothalamus (54,55) as follows.

In the kidney, juxtaglomerular apparatus located at the glomerular pole of the nephron senses the NaCl concentration in the early distal nephron coming from its own glomerulus (56,57). When glomerular filtration rate (GFR) becomes lower, the concentration of NaCl in the early distal nephron becomes lower. This low NaCl concentration decreases NaCl uptake into the intracellular space of juxtaglomerular cells via NKCC2, releasing renin. As well known, renin stimulates the renin-angiotensin-aldosterone system elevating the serum aldosterone level. The renin-induced elevated aldosterone increases ENaC production and the apical surface expression of ENaCs mediated by SGK1 (58,59) via a decrease in endocytotic rate of ENaC (37). Thus, the low GFR due to a decrease in the circulating blood caused by low body Na^+ content increases renin release, leading to elevation of body Na^+ content due to an increase in Na^+ reabsorption via aldosterone-induced increases of ENaC production and surface expression in the collecting duct.

Further, recently Na_x has been reported to be a Na^+ concentration-sensitive Na^+ channel acting as a Na^+ sensor (46-53,60,61). Na_x was found in the brain as an atypical Na^+ channel, poorly homologous to the voltage-gated Na^+ channels (62). Interestingly, Na_x knock-out mice do not stop taking salt even at dehydrated (high ECF Na^+ concentration in the body), while wild-type mice avoid salt intake (60,61). This observation suggests that Na_x acts as a sensor detecting the ECF Na^+ concentration in the body. Later, Na_x is reported to require α_1 -isoform of the Na^+ / K^+ -ATPase to act as a sensor detecting Na^+ (47,48). The functional linkage of Na_x and α_1 -isoform of Na^+ / K^+ -ATPase is performed by lactate production: i.e., high Na^+ in the intracellular space caused by high Na^+ influx via Na_x dependent on high extracellular Na^+ activates the Na^+ / K^+ -ATPase, leading to metabolic enhancement resulting in extensive lactate production (52). Further, the linkage between detection of high ECF Na^+ concentration via Na_x and water intake is mediated by TRPV4 (51): knock-out of TRPV4 in mice induces no water intake even at high Na^+

concentration in cerebrospinal fluid (CSF) (51), in which CSF-contacting nucleus (CSF-CN) plays an important role in sensing the Na^+ concentration of CSF and satiating Na^+ appetite (53). Na_x has the cation selectivity of $\text{Na}^+ \approx \text{Li}^+ > \text{Rb}^+ > \text{Cs}^+$ and is bound to postsynaptic density protein 95 (PSD95) via its PSD95/Disc-large/ZO-1 (PDZ)-binding motif at the C-terminus in neurons, suggesting involvement of this complex in the surface expression of Na_x (49). Thus, these observations clearly indicate the role of Na_x in the oral Na^+ intake at high Na^+ concentration in CSF by sensing the Na^+ concentration and its mechanism.

A further observation indicates that Na_x regulates ENaC activity (46). As mentioned above, Na_x acts as a sensor detecting the extracellular Na^+ concentration in the brain. In addition to the brain, Na_x is expressed in multiple epithelial tissues and up-regulates its downstream genes in hypertrophic scars (46). When Na_x detects an increased extracellular Na^+ concentration, Na_x up-regulates prostatic release into the extracellular space (46), which activates ENaC by cleaving the extracellular loop of γ ENaC subunit (23,63), increasing Na^+ influx via ENaC associated with elevation of downstream mRNA synthesis of inflammatory mediators (46). Further, blockade of Na_x expression improves scarring and atopic dermatitis (46). These findings strongly indicate that Na_x plays an important role in maintaining epithelial homeostasis via control of ENaC activity.

In summary, this article provides the following points regarding Na^+ homeostasis in the body. ENaC determines the amount of Na^+ uptake (reabsorption) into the body by performing the epithelial Na^+ transport in the colon, the kidney and the lung, while Na_x acts as a sensor detecting the extracellular Na^+ concentration, controlling the amount of oral Na^+ intake. Nevertheless, little knowledge on the cooperation of these channels (ENaC and Na_x) was available: i.e., even though Na_x controlled the oral Na^+ intake by sensing the extracellular Na^+ concentration in CSF, it was unknown if ENaC activity would be affected by the extracellular Na^+ -dependent activity of Na_x . Recently, it becomes clarified that activation of Na_x by an increased extracellular Na^+ concentration in the wounded skin stimulates secretion of prostatic, a protease, which activates ENaCs, reducing the osmolality of the surface fluid of the wounded skin by elevating Na^+ reabsorption from skin surface into the intracellular space (46). Thus, Na_x and ENaC cooperatively function for Na^+ homeostasis in the body. This study (46) is the first report indicating the cooperatively functional linkage of ENaC and Na_x .

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Footnote

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