Sodium and asthma: something borrowed, something new?


Sodium and asthma: something borrowed, something new? Am J Physiol Lung Cell Mol Physiol 293: L1369–L1373, 2007. First published September 28, 2007; doi:10.1152/ajplung.00379.2007.—Some early studies have called attention to the potential contribution of sodium (both dietary and serum levels) in airway-related disease, although the picture was not entirely clear. Two recent developments may now allow a more careful consideration of this: first, the greatly improved understanding of the role of salt in hypertension (particularly the identification of subgroups of salt-sensitive individuals within the general population), and second, the recent discovery of the role of the Na+/Ca2+ exchanger in smooth muscle function. Here, we first review those two developments and then apply them to airway smooth muscle and asthma.

Dietary salt and cardiovascular dysfunction. Dietary salt has been examined as a causative agent in the pathogenesis of a number of conditions, including left ventricular hypertrophy, airway dysfunction, and renal injury resulting in proteinuria (reviewed in Ref. 15). The bulk of the literature surrounding the negative health effects of high dietary salt intake has dealt with its association with hypertension. Early reports from long-term epidemiological studies suggested a strong positive correlation between salt intake and blood pressure (1, 2). However, subsequent analysis and additional studies suggest that this association does not hold true for the entire population (57, 63). It is now apparent that a subsection of the population displays a phenotype that renders them extremely sensitive to changes (i.e., increased and decreased) in dietary salt intake, whereas others display little or no change in mean arterial pressure in response to salt loading and/or salt deprivation protocols (reviewed in Ref. 15). The current opinion is that the hypertensive population is heterogeneous in terms of disease etiology.

Salt-dependent hypertension and sodium homeostasis. It is apparent from studies of both humans and animals that excess salt intake triggers an acute elevation of plasma [Na+] and extracellular fluid volume that leads to an overall increase in blood pressure (28, 39, 51, 61). However, rapid fluid expansion cannot be the sole driving force behind the associated elevations in blood pressure since intravenous infusion of isotonic saline alone does not elevate blood pressure (51). The importance of the kidney in fluid regulation and blood volume is highlighted by transplantation studies, performed in animals, where elevated blood pressure can be normalized after a hypertensive animal receives a kidney from a normotensive donor (18, 48). Similar observations have been made in renal transplants in human hypertensive patients (17). Despite the importance of the kidney in the etiology of salt-sensitive hypertension, the link between increased salt intake and reduced salt excretion resulting in increased blood pressure has remained elusive. Furthermore, the incomplete correlation between increased plasma volume and hypertension evoked by salt loading suggests an extra-renal component to salt-sensitive hypertension.

Recently, there has been a renewed interest in the possibility that serum-borne factors, termed cardiotonic steroids (e.g., endogenous ouabain), may alter intracellular Na+ homeostasis resulting in elevated vascular tone and hypertension. High-salt diets have been associated with elevated plasma concentrations of cardiotonic steroids (24, 25, 29, 53). Elevated levels of endogenous ouabain are observed in animal models of salt-dependent hypertension (25, 29) and in a significant portion of patients with essential hypertension (29, 30). Interestingly, ouabain antagonists can normalize blood pressure in animal models of salt-dependent hypertension and in some humans with essential hypertension (22). Furthermore, nearly one-half of human cases of congestive heart failure exhibit elevated levels of endogenous ouabain with the cardiac index of these patients being negatively correlated with plasma ouabain concentrations (27). These data suggest that the release of endogenous ouabain may be involved in the etiology of heart failure.

Sodium homeostasis and muscle contraction. Although a positive correlation between plasma cardiotonic steroids and blood pressure exists, the mechanism by which ouabain triggers contraction of vascular smooth muscle is still under investigation. In isolated vascular smooth muscle preparations, it is evident that ouabain-induced contractions are dependent on extracellular Ca2+ (34). However, these contractions are only slightly sensitive to blockers of L-type Ca2+ channels but are significantly reduced by inhibition of sodium-calcium exchange processes (34, 49). Recently, a series of reports has suggested that endogenous ouabain may elevate intracellular [Na+], which in turn drives Ca2+ entry via the reverse-mode of the Na+/Ca2+ exchanger (NCX) (7–9, 33, 35, 36, 44).

Although reverse-mode NCX-mediated Ca2+ influx is well documented in cardiac muscle, only recently has it been implicated as a Ca2+ influx pathway in smooth muscle physiology. Along with the plasma membrane Ca2+-ATPase, the NCX was originally thought to contribute to Ca2+ extrusion following agonist-induced increases in intracellular [Ca2+] (7, 8). Harnessing the Na+ concentration gradient across the plasma membrane, the NCX extrudes 1Ca2+ for 3Na+. However, if intracellular [Na+] could be elevated in the regions surrounding the NCX, much as occurs in the cardiac myocyte, the direction of the concentration gradient could be sufficiently altered to trigger Ca2+ influx in exchange for the extrusion of Na+ (i.e., the reverse-mode). Indeed, an early study by Reuter et al. (52) provided evidence for NCX-mediated tone develop-

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ment in aortic smooth muscle. This was followed by a number of reports suggesting that the reverse-mode of the NCX could mediate $\mathrm{Ca}^{2+}$ influx and contraction in vascular smooth muscle (34, 40, 45, 56). Following the identification and characterization of nonselective cation channels expressed in smooth muscle, it was hypothesized that cationic current may elevate intracellular $[\mathrm{Na}^+]$, triggering $\mathrm{Ca}^{2+}$ influx through the reverse-mode of the NCX. The advent of selective inhibitors of the reverse-mode of the NCX has added support to the concept that this process contributes substantial $\mathrm{Ca}^{2+}$ influx in a number of types of smooth muscle (5, 10, 20, 31, 32, 43, 60, 65).

Evidence from animal models of salt-dependent hypertension suggests that the NCX-mediated $\mathrm{Ca}^{2+}$ influx is responsible for the elevations in vascular tone. In a very elegant study, Iwamoto et al. (38) identified a role for NCX1 in the mechanisms of hypertension in a rat-model of salt-dependent hypertension. In this model, selective inhibition of the reverse-mode of the NCX1, through oral dosing with SEA0400, significantly reduced systemic blood pressure of Dahl salt-sensitive rats (38). Indeed, direct intrafemoral arterial infusion of SEA0400 significantly increased femoral blood flow, suggesting its antihypertensive effects were mediated by vasodilation of the peripheral vasculature. Interestingly, SEA0400 had no effect on salt-independent models of hypertension, including the stroke-prone spontaneously hypertensive (SHR) and Dahl salt-resistant rats. However, upon chronic salt loading (i.e., consumption of a diet high in sodium), SEA0400 significantly reduced the systemic blood pressure of stroke-prone SHR animals. Utilizing transgenic mice, Iwamoto et al. revealed that overexpression of NCX1.3 enhances salt-dependent increases in blood pressure, whereas animals with reduced NCX1 expression (~50% of wild-type expression) were resistant to hypertension induced by salt loading (37, 38). Furthermore, animals expressing an SEA0400-insensitive mutant NCX1.3 lost the anti-hypertensive effects of the drug (38). In mesenteric vascular smooth muscle cells, ouabain treatment triggered a significant increase in intracellular $[\mathrm{Ca}^{2+}]$ in NCX1.3 overexpressing animals that was completely blocked by SEA0400, an effect not observed in SEA0400-insensitive NCX1.3 mutants (38).

It is apparent from these data that the NCX plays a pivotal role in animal models of salt-sensitive hypertension. Sufficient evidence exists to support the study of the NCX in human disease (e.g., salt-induced increases in plasma ouabain); however, these findings have yet to be translated into a clinical intervention to target salt-sensitive hypertension in the human population.

**Dietary salt and airway dysfunction.** An examination of the literature pertaining to dietary salt intake and respiratory ailments reveals somewhat conflicting findings. Epidemiological studies have reported that an increase in asthma death rates is associated with high rates of table salt purchasing (12). Additionally, bronchial reactivity, measured using a histamine challenge test, was associated with elevated 24-h urine sodium excretion. However, this association did not hold true for females (12). A subsequent study examining dietary salt intake and bronchial reactivity in men revealed a positive correlation between airway reactivity and 24-h urine sodium excretion. In contrast to the aforementioned studies, a number of large cohort studies have failed to observe any associations between 24-h urinary sodium excretion and airway reactivity (11, 21, 59, 66). Two large cohort studies by Britton et al. (11) and Devereux et al. (21) found no association between airway hyperresponsiveness and sodium excretion. Furthermore, Sparrow et al. (59) observed an association between airway reactivity and urinary potassium, but not sodium secretion.

In terms of the cellular regulation of $\mathrm{Na}^+$, Tribe et al. (62) reported a humoral (i.e., serum-borne) factor in asthmatics that altered sodium transport in an in vitro assay. This finding was supported by a barrage of studies that suggested the existence of a serum-borne inhibitor of the $\mathrm{Na}^+/\mathrm{K}^+$ pump in subjects with allergic rhinitis and/or allergic asthma (3, 13, 23, 54, 55, 64). This unknown factor increased intracellular $[\mathrm{Na}^+]$, decreased uptake of $\mathrm{Rb}^+$, and decreased overall activity of the $\mathrm{Na}^+/\mathrm{K}^+$ pump in isolated human platelets, leukocytes, and peripheral blood mononuclear cells (3, 13, 23, 54, 55, 62, 64). Furthermore, the plasma from allergic individuals could inhibit $\mathrm{Rb}^+$ uptake in leukocytes from control (i.e., nonallergic) individuals. Interestingly, the level of inhibition of the donor plasma correlated well with the degree of airway responsiveness observed in the donating subject (62).

**Dietary salt and allergy.** Together, the data from studies examining airway function in the context of asthma and dietary salt intake are suggestive of a weak association between salt intake and increased airway reactivity. Interestingly, allergy has also been associated with elevated plasma ouabain and altered intracellular $\mathrm{Na}^+$ homeostasis (23, 54, 55, 64). Furthermore, extensive study of the plasma of individuals with allergic asthma and/or allergic rhinitis revealed the existence of a serum-borne factor that leads to the accumulation of $\mathrm{Na}^+$ within platelets, leukocytes, and peripheral blood mononuclear cells (23, 55).

An early hypothesis in the study of allergy suggested a defect in cationic metabolism may be responsible (12, 58). Indeed, reports have described an alteration in intracellular $\mathrm{Na}^+$ homeostasis that appears to exist in those with allergy (23, 54, 55, 64). Skoner et al. (55) reported that the serum of allergic individuals contained a “factor” that reduced the activity of the $\mathrm{Na}^+/\mathrm{K}^+$ pump in isolated platelets in a dose-dependent fashion (i.e., serum dilutions). Interestingly, the degree of inhibition correlated with the severity of allergic symptoms. For example, IgE levels were highest in those whose serum contained greatest inhibitor activity. Following freezing, the platelet $\mathrm{Na}^+/\mathrm{K}^+$ pump activity was restored, even beyond values observed in the normal population before serum-freezing. This suggests that atopic individuals may have increased basal pump activity (since no change in pump density was observed) (55). Collectively, these data suggest that a serum-borne factor could inhibit the $\mathrm{Na}^+/\mathrm{K}^+$ pump through direct binding, which was abolished upon freezing platelets. This finding was recapitulated by Tribe et al. (62) who reported that high levels of $\mathrm{Na}^+$ influx into isolated peripheral blood mononuclear cells were associated with the greatest levels of airway responsiveness (62). Furthermore, serum from allergic individuals conferred elevated $\mathrm{Na}^+$ influx into leukocytes isolated from nonatopic individuals. The magnitude of this effect was well associated with the degree of airway dysfunction in the donor subjects (62). These data were confirmed by two more recent studies that reported an association between $\mathrm{Na}^+/\mathrm{K}^+$ pump inhibition and resulting intracellular $\mathrm{Na}^+$ accumulation and increased severity of airway dysfunction (3, 13). This phenomenon does not seem to be limited to airway dysfunction associated with atopy. Plasma from subjects with...
allergic rhinitis contains a factor that inhibits the Na\(^+/K^+\) pump in isolated peripheral blood mononuclear cells. As reported previously, the inhibition of the pump observed in atopic individuals was removed upon freezing. Furthermore, freezing of peripheral blood mononuclear cells (PBMCs), isolated from atopics, revealed an elevated basal Na\(^+/K^+\) pump activity (64).

The exact nature of the serum-borne factor associated with inhibition of the Na\(^+/K^+\) pump in allergy has yet to be completely elucidated. Some have suggested that lysophosphatidylcholine (LPC) may be a likely candidate (13). Indeed, high-serum LPC levels have been associated with increased asthma severity and increased accumulation of Na\(^+\) in PBMCs and leukocytes (4, 23, 54).

Na\(^+/K^+\) pump and airway smooth muscle. It is apparent from the aforementioned literature that allergy is associated with elevated serum levels of an endogenous inhibitor of the Na\(^+/K^+\) pump, which can lead to an accumulation of intracellular Na\(^+\). In fact, it is possible that this process may be driving the airway dysfunction associated with the Na\(^+\) accumulation in the aforementioned studies: that the inhibition of the Na\(^+/K^+\) pump, observed in platelets, leukocytes, and PBMCs, could also occur in the airway smooth muscle (ASM). Oubainh triggers substantial contractions of human, canine, bovine, guinea pig, rat, and mouse ASM (14, 16, 32, 41). These are dependent on extracellular Ca\(^{2+}\) and Na\(^+\) and are partially inhibited by nonselective inhibitors of Na\(^+/Ca^{2+}\) exchange (14, 16). Recently, a number of papers have suggested that Ca\(^{2+}\) influx via the reverse-mode of the NCX plays a substantial role in excitation-contraction coupling and the refilling of the sarcoplasmic reticulum in ASM (19, 20, 31, 32, 42). Thus, the inhibition of the Na\(^+/K^+\) pump, which appears to be associated with atopy, may contribute to airway dysfunction by elevating cytosolic levels of Na\(^+\), thereby promoting Ca\(^{2+}\) influx via reverse-mode NCX activity and increasing tone.

Dietary Na\(^+\) and inflammation in exercise-induced airway hyperresponsiveness. During ventilation, inhaled air is conditioned (i.e., warmed and humidified) by contact with the inner epithelial layer and its associated mucous coating. When the intensity of ventilation increases, as during exercise, the conditioning capacity of the airways is taxed, resulting in a reduced capacity to humidify inhaled air and an increase in mucosal osmolarity (i.e., mucous dehydration). The net result of airway dehydration is the activation of resident airway inflammatory cells (e.g., mast cells) and the release of inflammatory mediators (e.g., histamine, leukotrienes, and prostaglandins) that trigger contraction of the ASM.

Although the role of dietary sodium in the modulation of asthma and its associated airway hyperresponsiveness is unclear, recent evidence suggests that dietary sodium can potentiate exercise-induced bronchoconstriction (EIB). Gotshall et al. (26) reported that low-salt diets improved and high-salt diets worsened postexercise pulmonary function in individuals with diagnosed EIB (or exercise-induced asthma) as measured by spirometry. However, even the low-salt diet could not normalize the postexercise pulmonary function in the EIB group. In contrast, the control subjects (i.e., subjects without a diagnosis of EIB) displayed no change in postexercise pulmonary function under any dietary intervention (i.e., no change with low-salt and/or high-salt diets). In addition to hindering pulmonary function, Mickleborough et al. (46) reported that high-salt diets decreased arterial blood oxygen saturation during intense exercise (i.e., 90% of predicted maximum heart rate in individuals with EIB). In a well-designed follow-up study (double-blinded placebo-controlled trial), it was revealed that a low-salt diet could attenuate some of the inflammation associated with EIB (e.g., attenuation of increases in sputum levels of eosinophil cation protein, IL-1B, and IL-8 but not cysteinyl leukotrienes C\(_4\)-E\(_4\), leukotriene B\(_4\), or prostaglandin D\(_2\)-methoxime) (47). This was associated with a significantly reduced degree of pulmonary dysfunction (as determined by spirometry following exercise challenge) in the low-salt diet group. In contrast, the high-salt diet group displayed significantly greater airway dysfunction than both the low- and normal-salt groups. This correlated well with a significant increase in the levels of inflammatory cells and cytokines measured in the sputum of individuals following an exercise challenge (47).

Together, it appears that increases in dietary salt may worsen the inflammatory status in the airways of individuals with EIB, leading to the release of mediators that trigger contraction of the ASM, resulting in airway dysfunction. This may act in concert with changes in mucous osmolarity that decrease the ability of the airways to condition inhaled air. Unfortunately, the mechanism by which sodium is increasing inflammation is unknown. Both atopy and high-salt diets are associated with altered cellular Na\(^+\) homeostasis that appears to result from an inhibition of the Na\(^+/K^+\) pump. Mast cells express functional Na\(^+/K^+\) pumps and NCXs, the latter playing an important role in cellular Ca\(^{2+}\) handling through both the forward- (Ca\(^{2+}\) extrusion) and reverse-modes (Ca\(^{2+}\) influx) (6). Since the release of inflammatory mediators from the mast cell is preceded by an increase in intracellular [Ca\(^{2+}\)], it is plausible that Ca\(^{2+}\) influx via the reverse-mode of the NCX might contribute to this process. Indeed, selective inhibition of the reverse-mode of the NCX with KB-R7943 significantly reduced the release of histamine from peritoneal mast cells following stimulation. This was associated with significant reduction in Ca\(^{2+}\) responses within stimulated mast cells (50). Thus, dietary sodium may contribute to the severity of EIB in the following ways: 1) high-salt diets could potentiate the accumulation of Na\(^+\) within mast and ASM cells of atopic individuals, 2) an increase in intracellular Na\(^+\) would trigger Ca\(^{2+}\) influx via the reverse-mode of the NCX, and 3) the resulting increases in intracellular [Ca\(^{2+}\)] would augment mediator release from mast cells and trigger contraction of ASM cells.

Future directions. As summarized above, dietary sodium is well recognized to play a causal role in cardiovascular dysfunction, and the underlying mechanisms are beginning to be elucidated. The latter include Ca\(^{2+}\) loading of the muscle via reverse-mode NCX. Evidence is now accumulating that airway function may also be impacted negatively by dietary sodium. Conflicting data may be resolved in a number of ways. First, future studies examining the effects of dietary sodium should employ protocols that directly increase or decrease sodium intake (i.e., sodium loading or sodium deprivation). This may unmask an effect that is not observed when sodium levels are extremely variable in study populations or sodium intake is estimated by other means. Second, patients with airway dysfunction should not be treated as a homogeneous population. In hypertension, only certain individuals exhibit changes in blood pressure in response to increasing or decreasing sodium intake.
Although further characterization of this heterogeneity is required, animal studies suggest that differential expression of NCX1 isoforms may confer salt sensitivity (38). Future population studies must consider the possibility that the level of arterial dysfunction observed in response to salt loading may vary considerably, just as is observed in salt-sensitive hypertension. Furthermore, linking the specific NCX1 isoform expression with salt-sensitive arterial dysfunction will be essential in elucidating the mechanisms governing this effect.

In terms of a working hypothesis, we propose the following: dietary sodium alters airway function by elevating systemic levels of endogenous ouabain that in turn inhibit the Na+/K+ pump. The resulting accumulation of Na+ within ASM cells triggers membrane depolarization and Ca2+ influx via the reverse-mode of the NCX. The extent of this effect will depend on the relative expression of certain NCX isoforms within the ASM (e.g., NCX1.3). Furthermore, atopic individuals may display arterial dysfunction that is related to elevated plasma ouabain concentrations resulting in aberrant Na+ handling, elevation of intracellular Na+, and triggering Ca2+ influx through the NCX. Indeed, these individuals may represent the ideal population in which to evaluate the efficacy of pharmacological agents targeting the Na+/K+ pump (signaling sites for ouabain) or the NCX (to inhibit reverse mode).

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