Hypertension, Na^+/Ca^{2+} exchanger, and Na^+, K^+-ATPase

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Hypertension is the most prevalent risk factor for stroke, myocardial infarction, or end-stage renal failure. The critical importance of excess salt intake in the pathogenesis of hypertension is widely recognized, but the mechanisms whereby salt intake elevates blood pressure have puzzled researchers. Recent studies using Na^+/Ca^{2+} exchange inhibitors and genetically engineered mice provide evidence that vascular Na^+/Ca^{2+} exchanger type 1 (NCX1) is involved in the development of salt-dependent hypertension. Endogenous cardiac glycosides, which may contribute to salt-dependent hypertension, seem to be necessary for NCX1-mediated hypertension. Intriguingly, studies using knock-in mice with modified cardiac glycoside binding affinity of Na^+,K^+-ATPases provide a clear demonstration that this cardiac glycoside-binding site plays an important role in blood pressure regulation. Taken all together: (1) endogenous cardiac glycosides are secreted after high salt intake; (2) these cardiac glycosides inhibit Na^+,K^+-ATPase in vascular smooth muscle cells; (3) this inhibition results in the elevation of local Na^+ on the submembrane area; and (4) this elevation of local Na^+ facilitates Ca^{2+} entry through NCX1, resulting in vasoconstriction. This proposed pathway may have enabled us to explain how to link dietary salt to hypertension.

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Hypertension, commonly referred to as 'high blood pressure (BP)', is the most prevalent risk factor for stroke, myocardial infarction, or end-stage renal failure. Insufficiently treated hypertension can result in the most common causes of disability or death. Epidemiological studies and clinical trials with antihypertensive drugs, as well as studies using experimental hypertensive animal models, have provided critical insights on the relationship between dietary salt intake and high BP.[1–3] Numerous basic and clinical studies have suggested that the mechanism of salt-dependent hypertension is related to the dysfunction of the kidneys, as originally proposed by Guyton.[4] However, the mechanisms by which dietary salt intake elevates arterial BP are not fully understood. Hypertension is a multifactorial disease, in which genetic and environmental factors are intricately involved. Indeed, all patients with essential hypertension are not necessarily salt-sensitive, and are divided into two groups: salt-sensitive and salt-insensitive patients.[5]

Thus far, several reports have shown a correlation between elevated levels of endogenous cardiac glycosides (ECGs), such as ouabain, marinobufagenin, proscillaridin A, and bufalin, and salt-dependent hypertension,[6–9] although the physiological function of ECGs is still uncertain. Recently, we demonstrated by using pharmacological tools and genetically engineered mice that salt-dependent hypertension is triggered by Ca^{2+} entry through Na^+/Ca^{2+} exchanger type-1 (NCX1) in arterial smooth muscle cells.[10] The implications of these findings include the following: (1) Na^+/Ca^{2+} exchange inhibitors lower arterial BP in salt-dependent hypertensive animals, but not in other types of hypertensive animals; (2) heterozygous mice with reduced expression of NCX1 are resistant to developing salt-dependent hypertension, whereas transgenic mice with vascular smooth muscle-specific overexpression of NCX1 readily develop hypertension after high salt-loading; and (3) Na^+/Ca^{2+} exchange inhibitors restore the vasoconstriction induced by nanomolar ouabain. Quite recently, using knock-in mice with modified ECG binding affinity of Na^+,K^+-ATPases, Lingrel's group demonstrated that this ECG-binding site plays an important role in controlling BP, and gave support to the hypothesis that the ECGs have a cardiovascular role.[11,12]

In this review, we discuss the molecular mechanism linking dietary salt to hypertension, and propose that vascular NCX1 as well as Na^+,K^+-ATPase is a new therapeutic or diagnostic target for salt-dependent hypertension.

A LINK BETWEEN DIETARY SALT INTAKE AND ARTERIAL BP

The effect of high salt intake on BP

Epidemiological, genetic, physiological, and biochemical studies provide conclusive evidence of a critical link between dietary salt intake and BP. Dahl's findings on the relationship between excess salt intake and the incidence of hypertension marked a milestone in our understanding of these processes.[13] The large-scale epidemiological study International Study of Salt and BP (INTERSALT) examined the relationship between 24-h urinary salt excretion and BP worldwide.[14] In this study, initial calculations indicated that urinary salt excretion is significantly associated with BP in individuals. In cross-center analysis, the effect of higher median salt excretion (5.7 g/day) over a 30-year period was estimated to be a difference of 10 and 6 mm Hg in systolic and diastolic BP, respectively. Another study, Cardiovascular Diseases and Alimentary Comparison (CARDIAC) also indicated positive correlations between urinary salt excretion and systolic or diastolic BP in males from cross-center analysis (0.98 and 0.68 mm Hg per urinary salt excretion (g/day) for systolic and diastolic BP, respectively).[15]

In experimental animals, the available data verify our current understanding of the relationship between dietary salt intake and BP. When adult baboons were treated with a high-salt diet (4% salt) and drinking water containing 1% salt for 1 year, the systolic and diastolic BP were elevated by 33 and 10 mm Hg, respectively.[16] In African green monkeys, a diet containing 6% salt for 3 months significantly increased the systolic and diastolic BP by 27 and 15 mm Hg, respectively.[17] A colony of chimpanzees given a high-salt diet (5–10 g/day) for 70 weeks developed a highly significant rise in systolic and diastolic BP (33 and 10 mm Hg, respectively).[18] In addition, it is well known that Dahl salt-sensitive rats, a genetic animal model of hypertension, develop hypertension when they are fed a high-salt diet.[19] Spontaneously hypertensive rats are also susceptible to salt-induced hypertension, although they exhibit hypertension and end-organ damage without high salt treatment.[20] Thus, various findings indicate that dietary salt is positively associated with BP.

The effects of high salt intake on plasma sodium and extracellular fluid volume

In humans and experimental animals, excess salt intake acutely increases plasma sodium and extracellular fluid volume, subsequently leading to high BP.[21–24] In normotensive subjects, the acute change of salt intake from 50 to 300 mmol/day for 14 days caused a significant increase in plasma sodium by 1.6 mmol/l with an elevation in systolic and diastolic BP (17 and 6 mm Hg, respectively).[21] In Dahl salt-sensitive rats, a high salt intake for 4 days significantly raised plasma sodium (3 mmol/day) with an increase in mean BP (32 mm Hg).[22] High BP induced by salt-loading, however, does not result directly from an acute increase in extracellular fluid volume, because rapid volume expansion (i.e., intravenous infusion of saline) does not raise BP.[25] On the other hand, others have proposed that volume expansion leads to the rise in arterial BP via the autoregulated constriction of resistance vessels, which accompanies an initial increase in cardiac output.[26] However, there are several reports showing that cardiac output does not regulate BP.[3] Taken together, acute changes in plasma sodium and extracellular fluid volume are potentially, but not strictly, sufficient for controlling BP. Prolonged increases in dietary salt intake or dysfunction of the kidney also cause a persistent increase in plasma sodium, which is closely related to an elevation in BP.

RENAL MECHANISMS RELATED TO SALT-DEPENDENT HYPERTENSION

The evidence for a relationship between renal dysfunction and salt-dependent hypertension

The kidney seems to play a primary role in the functional disturbances that link salt intake to BP.[3,4] Indeed, renal cross-transplantation studies between hereditary hypertensive and normotensive rats show that the abnormal kidney is ultimately responsible for the rise in BP.[19,27] When terminal nephrosclerosis patients with hypertension are transplanted with a kidney from a normotensive donor, the BP consistently drops to the normal range.[28] Furthermore, the primacy of the kidney in controlling BP has been confirmed by Guyton's pressure natriuresis theory.[4] Recently, Johnson *et al*.[21] suggested that subtle, acquired renal dysfunction (i.e., renal microvascular or tubulointerstitial injury) becomes the most likely mechanism to link dietary salt to hypertension. Thus, a defect in renal sodium handling may be responsible for the development of salt-dependent hypertension.

The evidence for genetic effects on renal salt handling

Numerous genetic analyses of severe hypertension or hypotension in families identify several mutations in single genes that cause Mendelian syndromes in humans.[3,29] Importantly, these genes encode proteins which are involved in the control of renal salt handling, such as ion channels and transporters. Mutations enhancing renal salt reabsorption raise BP, whereas those reducing salt reabsorption lower BP. In particular, α-adducin and the epithelial sodium channel (ENaC) seem to play a critical role in regulating urinary excretion of salt, sodium balance, and BP.

α-Adducin is a cytoskeletal protein that modulates Na^+,K^+-ATPase activity in the renal tubular cells.[30] The Gly460Trp variant of the α-adducin gene is related to higher prevalence of hypertension in several populations.[31,32] Hypertensive patients carrying this variant have enhanced Na^+,K^+-ATPase activity, and subsequently exhibit an increased rate of proximal tubule sodium reabsorption.[33] Interestingly, in these patients, increased levels of ECGs have been found to accompany the development of hypertension.[34] In Milan hypertensive rats – a genetic model with two missense mutations of α-adducin (Phe316Tyr) and β-adducin (Gln529Arg) – enhanced tubular sodium reabsorption and increased ECG levels are also observed.[35,36]

Liddle syndrome is associated with mutations in either the β- or γ-subunit of ENaC that truncate their cytoplasmic C termini.[37,38] Normally, these C terminal tails, containing the sequence PPPXY motif, interact with Ndd4-2, an E3 ligase, resulting in ubiquitination and proteasome-mediated degradation of ENaC.[39] The deletion of this motif from the β- or γ-subunit of ENaC leads to prolongation of the ;cell surface half-life of channels in the collecting duct, and consequently an increase in sodium reabsorption. The abnormalities in Liddle syndrome, such as salt-dependent hypertension associated with hypokalemic alkalosis and low plasma renin activity, can be improved by a low-salt diet and amiloride, an ENaC inhibitor.

CARDIOVASCULAR MECHANISMS RELATED TO SALT-DEPENDENT HYPERTENSION

The evidence for a relationship between high salt intake and cardiovascular functions

Excess dietary salt increases stiffness of conduit arteries, thickness and narrowness of resistance arteries, and the mass of the left ventricular wall, as a result of high sodium as well as of salt-induced high BP.[40–42] There is some evidence that a rise in plasma sodium directly causes functional or structural changes in the blood vessels or heart which could contribute to salt-dependent hypertension. Blaustein[43] pointed out that a rise of intracellular Na^+ concentration ($[Na^+]_i$) in vascular smooth muscle cells produces vasoconstriction because of the resultant increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Friedman *et al.*[44] found that the plasma level of sodium is directly related to the systolic and diastolic BP in rats intraperitoneally dialyzed with physiological salt solutions containing variable amounts of sodium. In normotensive and hypertensive subjects, urinary sodium excretion, as used to estimate salt intake, is significantly correlated with the mass of the left ventricular wall.[45,46] Furthermore, in humans and experimental animals, high salt intake stiffens conduit arteries and thickens resistance arteries independent of BP.[41,47] Thus, sodium intake appears to directly induce arterial and cardiac remodeling, although hypertension, induced by high salt, actually leads to shear stress and stretch in cardiovascular organs.

The effect of high salt intake on plasma levels of ECGs

The presence of a circulating Na^+,K^+-ATPase inhibitor was first demonstrated in animals over 20 years ago, and a significant correlation between BP and the activity of Na^+,K^+-ATPase inhibitor has been found.[48] Since then, several ECGs, such as endogenous ouabain[6] and other steroids[7–9] including marinobufagenin, proscillaridin A, and bufalin, have been identified thus far as candidate intermediaries in the pathogenesis of essential hypertension (especially salt-sensitive hypertension). Endogenous ouabain is synthesized and secreted by the adrenal gland[49,50] and hypothalamus.[51] In humans, prolonged increases in dietary salt intake cause the levels of ECGs to rise in the plasma.[48,52,53] Moreover, among patients with essential hypertension, almost half have substantially elevated levels of endogenous ouabain.[54,55] Plasma levels of ECGs are also high in several salt-dependent hypertensive animals.[6,53,56] Thus, these ECGs may be involved in the etiology of salt-dependent hypertension. Furthermore, among patients with congestive heart failure, about half show increased levels of endogenous ouabain, and in these patients the ouabain level is inversely correlated with the cardiac index.[57] This suggests that the secretion of ouabain is enhanced as a compensatory response in heart failure patients. It is generally believed that ECGs inhibit the plasma membrane (PM) Na^+,K^+-ATPase and lead to an increase in $[Na^+]_i$. Cell Na^+ accumulation raises the level of $[Ca^{2+}]_i$ probably through the involvement of the Na^+/Ca^{2+} exchanger, and thereby increases contraction and Ca^{2+}-dependent signaling in vascular smooth muscle or heart muscle, as originally postulated by Blaustein and Hamlyn.[58]

The role of vascular Na^+/Ca^{2+} exchanger in salt-dependent hypertension

The Na^+/Ca^{2+} exchanger is an ion transporter, which transports Ca^{2+} either out of cells or into cells (i.e., the forward mode and reverse mode, respectively) in exchange for $3Na^+$.[59–61] This transporter is controlled by membrane potential and transmembrane gradients of Na^+ and Ca^{2+}. There are three Na^+/Ca^{2+} exchanger isoforms (NCX1, NCX2, and NCX3) in mammals.[60] NCX1 is widely expressed in the heart, kidney, brain, arteries, and other organs, whereas NCX2 and NCX3 expression is limited mainly to the brain.[62] Extensive alternative splicing of NCX1 generates at least 12 tissue-specific variants; the heart expresses exclusively NCX1.1, and vascular tissue predominantly NCX1.3.[62] These exchangers are modulated by at least two inactivation systems: I_1 and I_2 inactivations.[63] High intracellular Na^+ restrains Na^+/Ca^{2+} exchange by facilitating exchanger entry into the I_1 inactivation state.[64] On the other hand, intracellular Ca^{2+} binding to regulatory Ca^{2+} sites in a large cytoplasmic domain stimulates Na^+/Ca^{2+} exchange by promoting exchanger recovery from the I_2 inactivation state.[65]

In vascular smooth muscle cells, the Na^+/Ca^{2+} exchanger, like Ca^{2+}-ATPases, is thought to contribute to Ca^{2+} extrusion from the cytosol in the relaxation process.[59–61] Immunocytochemical staining of vascular smooth muscle cells indicated that the Na^+/Ca^{2+} exchanger is localized in the PM regions that are adjacent to junctional sarcoplasmic reticulum (SR).[66,67] This particular localization suggests that the Na^+/Ca^{2+} exchanger may play a role in regulating the Ca^{2+} content of the SR stores, thereby modulating Ca^{2+} handling and vasocontraction. Recent data obtained using antisense oligonucleotides indicate that NCX1 knockdown prolongs agonist responses by delaying the return of $[Ca^{2+}]_i$ to the resting level, and also inhibits ouabain-induced augmentation of agonist responses in $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells.[68,69] Furthermore, reduced expression of NCX1 in aortas from NCX1 heterozygous mice decelerated Na^+-dependent relaxation and contrac-

tion.[70] These findings suggest that the PM Na^+/Ca^{2+} exchanger is involved in regulating Ca^{2+} homeostasis of blood vessels.

Recently, we found that transgenic mice overexpressing canine NCX1.3, the vascular isoform of NCX1, were hypersensitive to salt, and the animals readily developed hypertension after high salt intake.[10] Oral administration of SEA0400, a specific Na^+/Ca^{2+} exchange inhibitor, consistently reduced the BP in salt-loaded NCX1.3-transgenic mice as well as several salt-dependent hypertensive models.[10] On the other hand, NCX1 heterozygous mice with reduced expression ($\sim$50%) of NCX1 were resistant to salt-dependent hypertension.[10] These findings indicate that vascular NCX1 plays an important role in the development of salt-dependent hypertension.

The pathway from high salt intake to vasoconstriction in salt-dependent hypertension

ECGs are thought to contribute to the pathogenesis of salt-dependent hypertension, primary aldosteronism, and Cushing's syndrome in clinical patients and experimental animals (Figure 1).[52,53,71] Indeed, chronic administration of ouabain to rats caused hypertension, which was suppressed by SEA0400.[10] We further found that the blood from deoxycorticosterone acetate-salt hypertensive rats contained humoral vasoconstrictors. Importantly, arterial infusion of SEA0400 counteracted the vasoconstriction induced by the factor and exogenous ouabain in the femoral arteries of experimental animals.[10] These results indicate that excess dietary salt increases ECGs and the latter may contract peripheral blood vessels via vascular Na^+/Ca^{2+} exchanger and thereby result in hypertension.

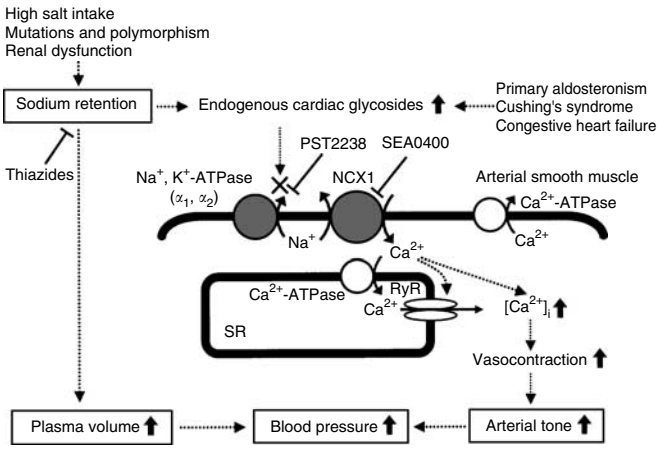

Figure 1 | Proposed pathway responsible for salt-dependent hypertension. High salt intake (or Na^+ retention) and genetic or pathological defects cause the levels of ECGs that inhibit the Na^+,K^+-ATPase α_2-isoform (or α_1-isoform) to rise in the plasma (although Na^+ retention also increases plasma volume, resulting in elevated blood pressure). This results in the increase in subplasma membrane $[Na^+]$ of arterial smooth muscle. The restricted $[Na^+]$ accumulation elevates $[Ca^{2+}]_i$ by vascular NCX1 isoform (NCX1.3)-mediated Ca^{2+} entry. This enhances arterial tone and causes hypertension. SEA0400, as well as thiazides and PST2238, blocks this Ca^{2+} entry and exerts an antihypertensive effect in salt-dependent hypertension. SR; sarcoplasmic reticulum, RyR; ryanodine receptor.

In vascular smooth muscle cells, inhibition of Na^+,K^+-ATPase by ECGs should elevate local $[Na^+]$ just under the PM (Figure 1). The restricted $[Na^+]$ accumulation facilitates Ca^{2+} entry through the vascular NCX1; this enhances arterial tone and causes hypertension. Notably, in vascular smooth muscle cells, the NCX1 is colocalized with the Na^+,K^+-ATPase α_2-isoform, which has high affinity for ouabain in rodents, in PM microdomains ('plasmerosomes') adjacent to the SR.[67,72] On the other hand, the distribution of α_1-isoform in the PM is relatively uniform.[72] The concept of intracellular linked Ca^{2+} and Na^+ transport at PM–SR junctions in vascular smooth muscle cells is also known as the 'superficial buffer barrier' function.[73] Functional coupling between Na^+/Ca^{2+} exchanger and Na^+,K^+-ATPase has been reported in vascular smooth muscle cells and cardiomyocytes.[74,75]

Dostanic-Larson *et al.*[11] and Dostanic *et al.*[12] demonstrated that chronic administration of ouabain and adrenocorticotropic hormone (ACTH), which is an animal model related to the ACTH-dependent Cushing's syndrome, induced hypertension in wild-type mice, but not in knock-in mice with a ouabain-resistant α_2-isoform. These results show that the highly conserved ECG-binding site of the Na^+,K^+-ATPase α_2-isoform plays an important role in the regulation of BP, and also provide a clear demonstration that an endogenous ligand for the Na^+,K^+-ATPase must be present in animals (Figure 1). We found that nanomolar ouabain increases both $[Ca^{2+}]_i$ and myogenic tone in pressurized mouse small mesenteric arteries, and that ouabain antagonists (PST2238 and canrenone) and Na^+/Ca^{2+} exchange inhibitors (SEA0400 and KB-R7943) abolish these effects.[10,76] The ouabain-induced $[Ca^{2+}]_i$ rise in arterial strips from NCX1.3-transgenic mice was greater than in those obtained from wild-type mice.[10] Furthermore, SEA0400 blocked these $[Ca^{2+}]_i$ rises in NCX1.3-transgenic mice and wild-type mice. Interestingly, the isolated mesenteric arteries from heterozygous $\alpha_2^{+/-}$ mice, but not those from heterozygous $\alpha_1^{+/-}$ mice, exhibited significantly greater myogenic tone than those from the wild type.[76] Taken together, these findings provide evidence that ECGs trigger Ca^{2+} entry through NCX1 in vascular smooth muscle cells by inhibiting the Na^+,K^+-ATPase α_2-isoform and elevating submembrane Na^+ (Figure 1).

In humans, the α_1- and α_2-isoforms have high affinity for ouabain, but in rodents the α_1-isoform has low ouabain affinity. Quite recently, Dostanic-Larson *et al.*[11] examined whether the ECG-binding site of the Na^+,K^+-ATPase α_1-isoform can play a role in ACTH-induced hypertension. Interestingly, knock-in mice with both ouabain-sensitive α_1-isoform and ouabain-resistant α_2-isoform, but not mice with both ouabain-resistant α_1- and α_2-isoforms, exhibited ACTH-induced hypertension, suggesting that the α_1-isoform also plays a role in the regulation of BP. However, the basal BP in $\alpha_2^{+/-}$ mice, but not in $\alpha_1^{+/-}$ mice (i.e., normotensive

mice), was significantly higher than in wild-type mice.[76] Therefore, further studies are necessary to determine the physiological implications of the Na^+,K^+-ATPase α_1-isoform in humans.

NEW APPROACHES FOR THE TREATMENT AND PREVENTION OF SALT-DEPENDENT HYPERTENSION

Na^+/Ca^{2+} exchange inhibitors

Vascular Na^+/Ca^{2+} exchanger may play a key role in salt-dependent hypertension, which makes the Na^+/Ca^{2+} exchanger protein a fascinating drug target. Thus far, specific Na^+/Ca^{2+} exchange inhibitors, such as KB-R7943,[77] SN-6,[78] SEA0400,[79,80] and YM-244769,[81] have been developed.[61] These Na^+/Ca^{2+} exchange inhibitors, possessing a common benzyloxyphenyl structure, seem to interact with a specific receptor site in the NCX1 molecule.[78,80,82] Intriguingly, benzyloxyphenyl inhibitors block the reverse mode (i.e., Ca^{2+} influx mode) of NCX1 much more effectively than the forward mode (i.e., Ca^{2+} efflux mode).[78,80,83] Recent mutational and electrophysiological analyses provide an explanation for the reverse mode-selectivity of benzyloxyphenyl derivatives.[78,83] The inhibitory potency of Na^+/Ca^{2+} exchange inhibitors is directly coupled to the rate of I_1 inactivation (i.e., intracellular Na^+-dependent inactivation). Under physiological conditions, the reverse mode is induced when $[Na^+]_i$ is high, whereas the forward mode is generated when $[Na^+]_i$ is reduced. NCX1 molecules thus tend to undergo I_1 inactivation in conditions for the reverse mode, suggesting an apparent, but not substantial, reverse mode-selectivity. These inhibitors likely stabilize the I_1 inactivation state or accelerate the rate of I_1 inactivation. This proposed mechanism suggests that benzyloxyphenyl inhibitors may be relatively dormant under normal conditions (low $[Na^+]_i$), but become effective under pathological conditions (high $[Na^+]_i$). This should be an ideal profile for therapeutic agents against intracellular Na^+-dependent cardiovascular diseases, such as salt-dependent hypertension as well as myocardial ischemia/reperfusion injury.

Indeed, the administration of SEA0400 dose-dependently lowered arterial BP in salt-dependent hypertensive rat models, such as deoxycorticosterone acetate-salt hypertensive rats, salt-loaded Dahl salt-sensitive rats, and salt-loaded spontaneously hypertensive rats, but not in normotensive rats or other salt-independent types of hypertensive rats.[10] This antihypertensive profile is unique, and differs from that of Ca^{2+} channel blockers, which lower BP in almost all hypertensive models. In addition, KB-R7943 abolished the ACTH-induced hypertension, a model of Cushing's syndrome, in mice.[11] Thus, benzyloxyphenyl inhibitors selectively suppress salt-dependent hypertension (that is, ECG-dependent hypertension). Furthermore, long-term treatment with SEA0400 overcomes the development of salt-dependent hypertension, vascular hypertrophy, and renal dysfunction in animal models.[10] Very importantly, SEA0400 lowered the BP in salt-loaded, NCX1.3-transgenic mice, but not in transgenic mice expressing an NCX1.3 mutant (Gly833Cys), which lacked the affinity to SEA0400,[10] showing that SEA0400 specifically acts on the overexpressed NCX1.3 in vascular smooth muscle cells. On the other hand, SEA0400 has little effect on BP (arterial tone) in normal animals, probably owing to the low level of ECGs in the plasma. Interestingly, SEA0400 inhibits the vascular isoform (NCX1.3) more potently than the cardiac and neuronal isoforms,[10,80] indicating the vascular selectivity of SEA0400. Thus, Na^+/Ca^{2+} exchange inhibitors like SEA0400 may have new therapeutic or diagnostic potential for salt-dependent hypertension (Figure 1).

Ouabain antagonists

Increased levels of endogenous ouabain, a major candidate ECG, have been implicated in the development of salt-dependent hypertension and related cardiovascular diseases.[9,51–54] The pathogenic mechanisms of endogenous ouabain involve the inhibition of Na^+,K^+-ATPases in the vascular vessels and kidneys, which are associated with vasoconstriction and renal tubular reabsorption, respectively. Therefore, ouabain antagonists have been targeted as a new class of antihypertensive drugs (Figure 1). PST2238 (Rostafuroxin), a digitoxigenin derivative, is able to antagonize ouabain-specific binding in Na^+,K^+-ATPases.[84] PST2238 lowered BP in ouabain-dependent hypertensive models,[85] subnephrectomized rats drinking saline solution,[86] and Milan hypertensive rats (an adducin mutant model),[87] but not in normotensive rats.[85,87] This drug has no effect on general or hormonal receptors involved in BP regulation.[84,85] In addition to inhibition of the Na^+,K^+-ATPase, ouabain activates a signal transducing function, triggering cell growth and proliferation.[88] Very interestingly, PST2238 is able to block ouabain-induced organ hypertrophy.[89] Thus, PST2238 could be useful for the treatment of essential hypertension, mainly related to Na^+ handling alterations, and cardiac complications which are associated with either increased levels of endogenous ouabain or adducin polymorphism.[84]

CONCLUSION

Recent studies using genetically engineered mice and specific inhibitors or modulators have provided compelling evidence for the molecular mechanism linking salt to hypertension. The links between dietary salt intake, ECGs, Na^+,K^+-ATPase, and vascular NCX1 may lead to the elevation of $[Ca^{2+}]_i$ and contractility in arterial smooth muscle cells, thereby resulting in hypertension (see Figure 1). Notably, recent human genome-wide linkage analysis of genes that affect BP identified four chromosomal regions, one of which includes NCX1, as loci containing candidate genes that influence BP.[90] Furthermore, genetic analyses of severe hypertension in families identified adducin polymorphism, associated with the Na^+,K^+-ATPase activity.[31,32]

Thiazide diuretics are widely used as antihypertensive drugs. So far, thiazides have been thought to lower BP acutely through diuretic action. However, the major antihypertensive mechanism of thiazides during long-term administration

seems to be due to vasodilation rather than to loss of plasma volume by natriuresis.[91,92] Therefore, there is a possibility that thiazides indirectly inhibit vascular NCX1 by normalizing sodium balance and, subsequently, decreasing ECGs (see Figure 1), resulting in suppressing salt-dependent hypertension.

Na^+/Ca^{2+} exchange inhibitors and ouabain antagonists might open new possibilities for the potential treatment of hypertension and related cardiovascular diseases. PST2238 is currently in Phase II trials, whereas Na^+/Ca^{2+} exchange inhibitors are undergoing preclinical development. Recent clinical studies in Europe show that PST2238 effectively lowers BP in essential hypertensive patients.[84] Intriguingly, both PST2238 and SEA0400 lack diuretic activity,[10,84] subsequently resulting in no diuretic-associated side effects. These drugs, if they are developed clinically, might lower BP without the need for a tightly controlled low-salt diet.

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