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Vascular Na⁺/Ca²⁺ exchanger: implications for the pathogenesis and therapy of salt-dependent hypertension

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Iwamoto, Takahiro, Vascular Na⁺/Ca²⁺ exchanger: implications for the pathogenesis and therapy of salt-dependent hypertension. Am J Physiol Regul Integr Comp Physiol 290: R536–R545, 2006; doi:10.1152/ajpregu.00592.2005.—The Na⁺/Ca²⁺ exchanger is an ion transporter that exchanges Na⁺ and Ca²⁺ in either Ca²⁺ efflux or Ca²⁺ influx mode, depending on membrane potential and transmembrane ion gradients. In arterial smooth muscle cells, the Na⁺/Ca²⁺ exchanger is thought to participate in the maintenance of vascular tone by regulating cytosolic Ca²⁺ concentration. Recent pharmacological and genetic engineering studies have revealed that the Ca²⁺ influx mode of vascular Na⁺/Ca²⁺ exchanger type-I (NCX1) is involved in the pathogenesis of salt-dependent hypertension. SEA0400, a specific Na⁺/Ca²⁺ exchange inhibitor that preferentially blocks the Ca²⁺ influx mode, lowers arterial blood pressure in salt-dependent hypertensive models, but not in normotensive rats or other types of hypertensive rats. Furthermore, heterozygous mice with reduced expression of NCX1 are resistant to development of salt-dependent hypertension, whereas transgenic mice with vascular smooth muscle-specific overexpression of NCX1 readily develop hypertension after high-salt loading. SEA0400 reverses the cytosolic Ca²⁺ elevation and vasoconstriction induced by nanomolar ouabain, as well as humoral factors in salt-loaded animals. One possibility is that circulating endogenous cardiotonic steroids may be necessary for NCX1-mediated hypertension. These findings help to explain how arterial smooth muscle cells in blood vessels contribute to salt-elicited blood pressure elevation and suggest that NCX1 inhibitors might be therapeutically useful for salt-dependent hypertension.

hypertension; salt intake; Na⁺-K⁺-ATPase; vascular smooth muscle

Hypertension is the most common chronic disease worldwide. Untreated hypertension can result in disability or death due to stroke, myocardial infarction, or end-stage renal failure. Hypertension is a multifactorial disease, in which genetic and environmental factors are intricately involved. Understanding the mechanism of hypertension is thus very difficult in individual cases. Nevertheless, clinical and experimental studies have provided critical insights on the relations between salt intake, renal salt handling, and arterial blood pressure. There are many studies showing that high-salt intake elevates arterial blood pressure in humans and animals (23, 61, 99). Large-scale epidemiological studies, such as the International Study of Salt and Blood Pressure (INTERSALT) (15) and the International Cooperative Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study (100), have examined the relationship between 24-h urinary sodium excretion and blood pressure in 10,079 and 7,334 individuals (from 52 and 45 centers), respectively, worldwide. In the INTERSALT Study, initial calculations indicate that urinary sodium excretion is significantly associated with blood pressure in individuals. On the other hand, cross-center analysis shows that urinary sodium excretion is positively related to the linear slope of blood pressure with age. In the CARDIAC Study, cross-center analysis indicates positive correlations between urinary sodium excretion and systolic or diastolic blood pressure in both males and females, but the correlation is significant only in the males (100). Thus the evidence points to a critical link between dietary high salt intake and the development of hypertension. However, the mechanisms by which dietary salt elevates arterial blood pressure are not fully understood.

The kidneys seem to play a central role in the functional disturbances that link salt intake to the arterial blood pressure (21, 61). Indeed, renal cross-transplantation studies between hereditary hypertensive and normotensive rats show that the abnormal kidney is ultimately responsible for the rise in arterial blood pressure (11, 63). Consistently, when terminal nephro-sclerosis patients with hypertension are transplanted with a kidney from a normotensive donor, the blood pressure drops to the normal range (10). Furthermore, numerous genetic analyses of families featuring severe hypertension or hypotension identify several mutations in single genes that cause Mendelian syndromes in humans (52, 61). Significantly, these genes encode proteins that are involved in the control of renal salt handling, such as ion channels and transporters. Mutations...
enhancing renal salt reabsorption raise blood pressure, whereas those reducing salt reabsorption lower blood pressure. A recent review published by Johnson et al. (41) emphasizes that subtle, acquired renal dysfunction (i.e., renal microvascular or tubulointerstitial injury) becomes the most likely mechanism to link salt sensitivity to blood pressure. Thus a defect in renal sodium handling may be responsible for the development of salt-dependent hypertension.

Most hypotheses on the pathological link between salt handling in kidneys and blood pressure maintain that the rise in arterial blood pressure is associated with an increase in extracellular fluid volume. Indeed, high-salt intake in normal animals and humans causes an increase in extracellular fluid volume with a gain in body weight (23, 94). Salt-mediated hypertension, 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 blood pressure (22). On the other hand, others have proposed that volume expansion leads to the rise in arterial blood pressure by the autoregulated constriction of resistance vessels, which accompanies an initial increase in cardiac output (54). However, there are several reports showing that cardiac output does not regulate blood pressure (see Ref. 61). Therefore, other chronic mechanisms may be required to explain salt-dependent hypertension with elevated peripheral resistance.

Experimental animal models are useful in the research of the mechanism, pathophysiology, and medical treatment of hypertension. Classical hypertensive models mimicking known human pathology are generated in rats or larger animals by several surgical manipulations. The experimental model of renovascular hypertension is generated by the two-kidney, one-clip maneuver (73). In this model, renal artery stenosis caused by a clip leads to renal ischemia, resulting in the development of hypertension. In addition, deoxycorticosterone acetate (DOCA)-salt hypertensive model is created by uninephrectomy (reduction of renal mass) and excess administration of mineralocorticoid and high salt (73). This model is defined as a volume-dependent (salt-dependent) hypertension. In addition, genetic models of hypertension such as spontaneously hypertensive rats (SHRs) and Dahl salt-sensitive rats are developed by selective inbreeding of normotensive Wistar-Kyoto (WKY) rats and Sprague-Dawley rats, respectively (73). SHR exhibit hypertension and end-organ damages, including left ventricular hypertrophy, stroke, and renal failure, without further treatment of high salt. Dahl salt-sensitive rats develop hypertension when they are fed a high-salt diet (11). These genetic models are suitable for studying the factors involved in the development of human-inherited and salt-dependent hypertension.

EARLY STUDIES ON THE ROLE OF ENDOGENOUS CARDIOTONIC STEROIDS IN SALT-DEPENDENT HYPERTENSION

In 1982, the presence of a circulating Na\(^+\)-K\(^+\)-ATPase inhibitor was first demonstrated in animals, and a significant correlation between the arterial blood pressure and the activity of Na\(^+\)-K\(^+\)-ATPase inhibitor was found (26). Since then, several endogenous cardiotoxic steroids, such as endogenous ouabain (24) and other steroids (2, 83, 84), including marinobufagenin, proscillaridin A, and bufalin, have been proposed as candidate intermediaries in the pathogenesis of salt-dependent hypertension. In humans, a chronic high-salt diet causes the levels of cardiotonic steroids to rise in the plasma (25–27). Moreover, ~50% of patients with essential hypertension have substantially elevated levels of endogenous ouabain (20, 56). Plasma levels of cardiotonic steroids are also high in several kinds of salt-dependent hypertensive animals (16, 24, 25). Indeed, PST2238, a ouabain antagonist, lowers blood pressure in salt-dependent hypertensive rats and in certain patients with essential hypertension (17, 18, 91). Thus these cardiotonic steroids may be involved in the etiology of salt-dependent hypertension. Generally, it is believed that cardiotonic steroids inhibit the plasma membrane Na\(^+\)-K\(^+\)-ATPase, the “sodium pump,” and lead to an increase in cytosolic Na\(^+\) concentration ([Na\(^+\)]\(_{cyt}\)). Cell Na\(^+\) accumulation raises the cytosolic Ca\(^2+\) concentration ([Ca\(^2+\)]\(_{cyt}\)) through the involvement of the Na\(^+\)/Ca\(^2+\) exchanger and thereby increases contraction in vascular smooth muscle or heart muscle. This sequence of events may lead to hypertension (3), but the hypothesis has not yet been critically tested, because little is understood of the function of the Na\(^+\)/Ca\(^2+\) exchanger in these processes.

FUNCTIONAL PROPERTIES OF Na\(^+\)/Ca\(^2+\) EXCHANGERS

The Na\(^+\)/Ca\(^2+\) exchanger exists in the plasma membrane and transports Ca\(^2+\) either out of cells (the forward mode) or into cells (the reverse mode) in exchange for 3Na\(^+\). The Na\(^+\)/Ca\(^2+\) exchanger is driven by membrane potential, as well as Na\(^+\) and Ca\(^2+\) concentration gradients (4, 77, 86). In cardiac muscle, the Na\(^+\)/Ca\(^2+\) exchanger primarily extrudes Ca\(^2+\) from inside to outside the cell during repolarization and diastole, which balances Ca\(^2+\) entry via L-type Ca\(^2+\) channels during cardiac excitation. The Na\(^+\)/Ca\(^2+\) exchanger also mediates Ca\(^2+\) influx during the action potential upstroke and helps maintain elevated [Ca\(^2+\)]\(_{cyt}\) during the action potential plateau and systole. In vascular smooth muscle, the Na\(^+\)/Ca\(^2+\) exchanger may be mainly responsible for extruding Ca\(^2+\) to maintain Ca\(^2+\) homeostasis, but there is little information about the vascular Na\(^+\)/Ca\(^2+\) exchanger compared with the cardiac Na\(^+\)/Ca\(^2+\) exchanger.

The Na\(^+\)/Ca\(^2+\) exchanger forms a multigene family comprising three isoforms, Na\(^+\)/Ca\(^2+\) exchanger type 1 (NCX1), NCX2, and NCX3 (77). NCX1 is widely expressed in the heart, arteries, kidney, brain, and other organs, whereas NCX2 and NCX3 expression is limited mainly to the brain and skeletal muscle (76). Furthermore, extensive alternative splicing of NCX1 generates at least 12 tissue-specific isoforms; the heart expresses NCX1.1 exclusively, and vascular tissue expresses predominantly NCX1.3 and NCX1.7 (65, 76). Recent topological analyses suggest that the NCX1 protein consists of nine transmembrane segments and a large central cytoplasmic loop, as illustrated in Fig. 1 (35, 38, 67, 75). The former part, particularly the α-repeat regions (α1, α2) with two oppositely oriented reentrant loops (35, 38), may participate in ion transport (38, 66, 68); the latter part, possessing the exchanger inhibitory peptide (XIP) region (51, 59), regulatory Ca\(^2+\) binding sites (60), and phosphorylation sites (36, 39, 85), is primarily involved in various regulatory properties. NCX1 has been shown to be secondarily regulated by the transport substrates Na\(^+\) and Ca\(^2+\). Intracellular Ca\(^2+\) at the submicromolar level activates Na\(^+\)/Ca\(^2+\) exchange activity by promoting the
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EARLY STUDIES ON THE ROLE OF Na⁺/Ca²⁺ EXCHANGE IN VASCULAR SMOOTH MUSCLE

The contraction of vascular smooth muscle cells is initiated by a rise in [Ca²⁺]cyt through voltage-gated and/or receptor-operated Ca²⁺ channels in the plasma membrane or through Ca²⁺ release channels in the sarcoplasmic reticulum (SR) membrane (4, 45, 74). It is thought that the Na⁺/Ca²⁺ exchanger, like the plasma membrane or SR Ca²⁺-ATPases, may contribute to Ca²⁺ extrusion from the cytosol in the relaxation process.

Early studies by Reuter et al. (79) described the functional evidence for Na⁺/Ca²⁺ exchange in aortic smooth muscle. Since then, several experiments observed that lowering extracellular Na⁺ concentration or increasing [Na⁺]cyt elevate [Ca²⁺]cyt through the reverse mode of Na⁺/Ca²⁺ exchange, resulting in enhancing Ca²⁺ entry and producing tonic contractions in various smooth muscle cells (30, 46, 57, 89). Furthermore, such reductions in plasma membrane Na⁺ gradients decelerate Ca²⁺ extrusion through the forward mode of Na⁺/Ca²⁺ exchange, thereby leading to enhanced or prolonged agonist-elicited vasoconstrictions (6, 69, 71). Nevertheless, some other studies suggest that Na⁺/Ca²⁺ exchange plays little role in cellular Ca²⁺ homeostasis because of the relatively low activity of the vascular Na⁺/Ca²⁺ exchanger compared with other electrically excitable tissues (7, 13, 64). These negative results could be explained by the particular experimental conditions, which were used to investigate the function of Na⁺/Ca²⁺ exchange (4, 45).

This controversy was addressed by recent studies using biochemical and molecular biological techniques and genetically engineered mice. It was demonstrated that vascular smooth muscle cells express the mRNA and protein of the Na⁺/Ca²⁺ exchanger (42, 65, 76). Furthermore, the vascular Na⁺/Ca²⁺ exchanger was cloned from rat aortas (65). Immunocytochemical staining indicated that the Na⁺/Ca²⁺ exchanger is localized in the plasma membrane regions that are adjacent to junctional SR (42, 62). This particular localization suggests that the exchanger may play a role in regulating the Ca²⁺ content of the SR stores, thereby modulating Ca²⁺ handling and vasoconstriction. Recent data obtained using antisense oligonucleotides indicate that NCX1 knockdown prolongs agonist responses by delaying the return of [Ca²⁺]cyt to the resting level and also inhibits ouabain-induced augmentation of agonist responses in [Ca²⁺]cyt in cultured vascular smooth muscle cells (87, 88). Furthermore, reduced expression of NCX1 in aortas from NCX1 heterozygous mice decelerated Na⁺-dependent relaxation and contraction (96). These findings
suggest that the plasma membrane Na\(^+/\)Ca\(^{2+}\) exchanger is involved in regulating Ca\(^{2+}\) homeostasis of blood vessels.

**DEVELOPMENT OF BENZYLXOPHENYL Na\(^+/\)Ca\(^{2+}\) EXCHANGE INHIBITORS**

A potent and selective Na\(^+/\)Ca\(^{2+}\) exchange inhibitor would be very useful for analyzing the physiological and pathological roles of the Na\(^+/\)Ca\(^{2+}\) exchanger. So far, Na\(^+/\)Ca\(^{2+}\) exchange inhibitors have long been targeted as a new therapeutic drug (44, 78, 86), although they are not clinically used yet. As the Na\(^+/\)Ca\(^{2+}\) exchanger transports Ca\(^{2+}\) bidirectionally, the pharmacological effects of Na\(^+/\)Ca\(^{2+}\) exchange inhibitors depend on the particular transport modes active at a given time. When the Na\(^+/\)Ca\(^{2+}\) exchanger extrudes Ca\(^{2+}\) from the cell (the forward mode), the Na\(^+/\)Ca\(^{2+}\) exchange inhibitor is expected to increase [Ca\(^{2+}\)]\(_{cyt}\). This will induce cardioactive and hypertensive effects in the circulatory system. On the other hand, when the Na\(^+/\)Ca\(^{2+}\) exchanger works as a pathway of Ca\(^{2+}\) entry (the reverse mode) under pathological conditions, such as ischemia/reperfusion injury and digitalis toxicity, the Na\(^+/\)Ca\(^{2+}\) exchange inhibitor is expected to guard against Ca\(^{2+}\) overloading. Thus it is possible that Na\(^+/\)Ca\(^{2+}\) exchange inhibitors will be an efficient remedy for such conditions.

In 1996, KB-R7943 (Fig. 2) was developed as a prototypical selective Na\(^+/\)Ca\(^{2+}\) exchange inhibitor (40, 97). This inhibitor was fairly specific to the Na\(^+/\)Ca\(^{2+}\) exchanger, because at up to 10 \(\mu\)M, it exerted little influence on other ion transporters, such as the Na\(^+/\)H\(^+\) exchanger, Na\(^+/\)K\(^+\) ATPase, and Ca\(^{2+}\)-ATPases (40). It is now being widely used to study the physiological and pathological roles of the exchanger at the cellular and organ levels. Recently, however, KB-R7943 has been reported to possess nonspecific actions against ion channels, neuronal nicotinic ACh receptors, the N-methyl-D-aspartate receptor, and the norepinephrine transporter (58, 72, 90, 97). In 2001, Matsuda et al. (58) reported on SEA0400 (Fig. 2), a newly developed, more potent, and selective Na\(^+/\)Ca\(^{2+}\) exchange inhibitor. SEA0400 is more specific to the Na\(^+/\)Ca\(^{2+}\) exchanger, because it hardly inhibits other channels, receptors, and transporters (58, 93); recently, however, nonspecific actions of this drug have been reported (80).

In 2002, SN-6, which is more specific than KB-R7943, was found from KB-R7943 derivatives (31). Quite recently, YM-244769, a potent Na\(^+/\)Ca\(^{2+}\) exchange inhibitor with lower cell toxicity, was introduced (49), although its detailed profile is not yet known. As shown in Fig. 2, all these Na\(^+/\)Ca\(^{2+}\) exchange inhibitors possess a benzyloxyphenyl structure, suggesting that this portion may be essential for their affinity to the Na\(^+/\)Ca\(^{2+}\) exchanger.

KB-R7943, SEA0400, and SN-6 inhibit the Ca\(^{2+}\) uptake dose-dependently via the reverse mode of NCX1 in NCX1-transfected fibroblasts and cardiac or vascular myocytes (31–33, 40). These inhibitors have different isoform selectivities: KB-R7943 is more effective on NCX3 than on NCX1 and NCX2 (37), whereas SEA0400 predominantly blocks NCX1, only mildly blocks NCX2, and exerts almost no influence upon NCX3 (32). Furthermore, SEA0400 most potently blocks NCX1.3, a major splicing isoform in vascular vessels, among other splicing isoforms of NCX1 (34). SN-6 is more inhibitory to NCX1 than to NCX2 and NCX3 (31). Recent site-directed mutageneses reveal the important amino acids, Phe213, Val227, Tyr228, Gly833, and Asn839, responsible for inhibition by benzyloxyphenyl derivatives (31–33) (see Fig. 1). These inhibitors probably interact with a specific receptor site, leading to blocking ion pore(s) formed within the membrane regions.

Interestingly, benzyloxyphenyl Na\(^+/\)Ca\(^{2+}\) exchange inhibitors block the reverse mode of NCX1 much more effectively than the forward mode under unidirectional ionic conditions (14, 31, 32, 40, 50, 82, 97). These inhibitors also block both the inward and outward currents via NCX1 (i.e., the forward and reverse modes) induced by ramp pulses with similar efficacy under bidirectional ionic conditions (47, 93). The latter result seems reasonable for blockers of a bidirectional transporter. The former result, however, appears to be consistent with in vivo or in vitro pharmacological profiles that Na\(^+/\)Ca\(^{2+}\) exchange inhibitors preferentially protect pathological Ca\(^{2+}\) overload despite having minimal effects on normal Ca\(^{2+}\) metabolism (40, 82, 92). Recent mutational and electrophysiological analyses provide an explanation for the reverse-mode selectivity of benzyloxyphenyl derivatives (5, 31, 32, 50).

Interestingly, 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 unidirectional ionic conditions, the reverse mode is induced when [Na\(^+\)]\(_{cyt}\) is high, whereas the forward mode is generated when [Na\(^+\)]\(_{cyt}\) is reduced. NCX1 molecules thus tend to undergo \(I_1\) inactivation in experimental conditions for the reverse mode, suggesting an apparent, but not substantial, reverse-mode selectivity. These inhibitors likely stabilize the \(I_1\) inactive state or accelerate the rate of \(I_1\) inactivation. This proposed model of action suggests that benzyloxyphenyl derivatives may be relatively dormant under normal conditions (low [Na\(^+\)]\(_{cyt}\)) but become effective under pathological conditions (high [Na\(^+\)]\(_{cyt}\)). This should be an ideal profile for therapeutic agents against...
intracellular Na⁺-dependent cardiovascular diseases, such as myocardial ischemia-reperfusion injury and salt-dependent hypertension (see below).

EVALUATING THE ROLE OF NCX1 IN SALT-DEPENDENT HYPERTENSION: STUDIES USING Na⁺/Ca²⁺ EXCHANGE INHIBITORS AND GENETICALLY ENGINEERED MICE

Early studies hypothesized that the Na⁺/Ca²⁺ exchanger might be important for the regulation of blood pressure and might play a role in the pathogenesis of essential hypertension (especially, salt-dependent hypertension) (3). However, this hypothesis has not been critically tested because convenient tools for studying the Na⁺/Ca²⁺ exchanger, such as specific inhibitors or genetically engineered mice, were not previously available.

**Benzylxophenyl Na⁺/Ca²⁺ exchange inhibitors.** To explore the role of the Na⁺/Ca²⁺ exchanger in the pathogenesis of salt-dependent hypertension, we examined the effects of SEA0400, a specific Na⁺/Ca²⁺ exchange inhibitor, on various hypertensive animal models. As shown in Fig. 3A, a single oral dose of SEA0400 (1–10 mg/kg) caused a dose-dependent and long-lasting decrease in arterial blood pressure in DOCA-salt hypertensive rats (34). Furthermore, SEA0400 significantly decreased blood pressure in Dahl salt-sensitive rats and SHR when they were chronically loaded with high salt (Fig. 3B). Notably, however, SEA0400 had no effect on blood pressure in normotensive WKY rats, SHR, stroke-prone SHR, salt-loaded or salt-unloaded Dahl salt-resistant rats, salt-unloaded Dahl salt-sensitive rats, and two-kidney, one-clip renal hypertensive rats (34). These findings suggest that the Na⁺/Ca²⁺ exchange inhibitor specifically suppresses salt-dependent hypertension. This antihypertensive profile is quite unique and differs from that of Ca²⁺ channel blockers, such as nifedipine and its derivatives, which lower blood pressure in most hypertensive models.

**Genetically engineered mice.** To determine the involvement of the NCX1 gene in salt-dependent hypertension, we generated transgenic mice overexpressing canine NCX1.3, a major splicing isoform in blood vessels, under the smooth muscle α-actin promoter (34). Notably, the basal systolic blood pressure was slightly but significantly elevated (by about 10 mmHg) in NCX1.3-transgenic mice compared with that of wild-type mice (Fig. 4A). Furthermore, the blood pressure in NCX1.3-transgenic mice was salt-sensitive, and the animals readily developed hypertension after high salt intake. Oral administration of SEA0400 reduced the elevated basal blood pressure of NCX1.3-transgenic mice and lowered the blood pressure of salt-loaded transgenic mice (Fig. 4B). Very importantly, SEA0400 did not affect blood pressure in transgenic mice expressing a NCX1.3 mutant (G833C), which lacked the affinity to SEA0400, showing that SEA0400 specifically acts on the overexpressed NCX1.3 in vascular smooth muscle cells.

NCX1 heterozygous mice, in which NCX1 function is reduced by ∼50% (mild suppression), are also a very useful animal model for evaluating the physiological and pathological

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**Fig. 3.** Antihypertensive effects of SEA0400 in various types of hypertensive rats. **A**: recoding of systolic blood pressure (SBP) after oral administration of vehicle (5% gum Arabic) or SEA0400 (1–10 mg/kg) in DOCA-salt hypertensive rats. **B**: peak change in SBP in mmHg for the respective rats is indicated. SHR, Dahl salt-resistant rats (Dahl-R), and Dahl salt-sensitive rats (Dahl-S) were fed a normal diet (0.3% NaCl) or a high-salt diet (8% NaCl; +salt) for 4–6 wk. SBP was measured by tail cuff. Bars represent means ± SE. (n = 4–6). *P < 0.05; **P < 0.01 compared with each vehicle group. DOCA-Salt, deoxycorticosterone acetate-salt; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat; SHRSP, stroke-prone SHR; 2K, 1C, two-kidney, one-clip renal hypertensive rats. [From Iwamoto et al. (34), Nature Publishing Company, www.nature.com.]
roles of NCX1 (96). Under resting conditions, these mice preserve normal cardiovascular functions (i.e., blood pressure, heart rate, and cardiac contractility), and expression levels of other Ca\textsuperscript{2+}-handling proteins, such as Ca\textsuperscript{2+} channel, SR Ca\textsuperscript{2+}-ATPase, and Ca\textsuperscript{2+} release channel, in heart and blood vessels (96). Intriguingly, DOCA-salt treatment did not significantly alter the blood pressure of NCX1 heterozygous mice, whereas the same treatment produced a progressive elevation in blood pressure in wild-type mice (34). On the other hand, hypertensive responses to chronic ANG II infusion were similar in NCX1 heterozygous mice and wild-type mice (34). These results suggest that NCX1 heterozygous mice are preferentially resistant to salt-dependent hypertension. Taken together, our data provide compelling evidence that vascular NCX1 is a key mediator in the development of salt-dependent hypertension. Notably, recent human genome-wide linkage analysis of genes that affect blood pressure has identified four regions, one of which includes NCX1, as loci containing candidate genes that influence blood pressure (48).

**LINKING HIGH SALT INTAKE TO VASCULAR RESPONSE**

To analyze the mechanism of NCX1-mediated (salt-dependent) hypertension, we infused SEA0400 into the femoral artery of anesthetized DOCA-salt hypertensive rats. Intrafemoral infusion of SEA0400 (10 μg·kg\textsuperscript{-1}·min\textsuperscript{-1}) markedly increased the femoral blood flow in DOCA-salt hypertensive rats (34), indicating that SEA0400 causes peripheral vasodilation. A similar infusion did not affect femoral blood flow in normotensive rats. On the other hand, when the femoral artery of the normal rat (recipient) was cross-perfused with aortic blood from DOCA-salt hypertensive rats (donor), the intrafemoral infusion of SEA0400 significantly increased the femoral blood flow (34). These results suggest that humoral vasoconstrictors, which can be antagonized by SEA0400, participate in DOCA-salt hypertension.

As described above, cardiotonic steroids, including endogenous ouabain, seem to contribute to the pathogenesis of salt-dependent hypertension in patients and animals (16, 20, 21).
24–27, 56). In fact, long-term administration of ouabain to rats caused hypertension (55), which was suppressed by SEA0400 (34). Furthermore, to check the antagonistic interaction between ouabain and SEA0400, either ouabain or SEA0400, or both, were infused into the femoral arteries of anesthetized beagles (34). As shown in Fig. 5, intrafemoral infusion of ouabain (0.5 μg·kg⁻¹·min⁻¹) reduced the femoral blood flow by ~50%, and then the additional infusion of SEA0400 restored it to the basal level. Infusion of SEA0400 alone did not affect the femoral blood flow. Taken together, these results suggest that high-salt diets increase endogenous cardiotonic steroids, and the latter may contract peripheral blood vessels via vascular Na⁺/Ca²⁺ exchanger and thereby result in hypertension.

In vascular smooth muscle cells, inhibition of Na⁺+/K⁺-ATPase by cardiotonic steroids should elevate local [Na⁺] just under the plasma membrane (Fig. 6). The restricted [Na⁺] accumulation facilitates Ca²⁺ entry through the vascular NCX1.3 isoform; this enhances arterial tone and causes hypertension. Notably, in vascular smooth muscle cells, the NCX1 is colocalized with Na⁺+/K⁺-ATPase α₂ and α₃ isoforms, which have high affinity for ouabain, in plasma membrane microdomains (“plasmcoresomes”) adjacent to the SR (43, 62). The concept of intracellularly linked Ca²⁺ and Na⁺ transport at plasma membrane-SR junctions in vascular smooth muscle cells is also known as the “superficial buffer barrier” function (74). Indeed, functional coupling between the Na⁺/Ca²⁺ exchanger and Na⁺+/K⁺-ATPase has been reported in vascular smooth muscle cells (1) and cardiomyocytes (19, 81). Quite recently, it has been demonstrated using genetically engineered mice, expressing a ouabain-insensitive α₂ isoform of Na⁺+/K⁺-ATPase that the α₂ isoform mediates the ouabain-induced vasconstriction (12). We found that nanomolar ouabain increases both [Ca²⁺]cyt and myogenic tone in pressurized small mesenteric arteries of mice, and SEA0400 completely abolishes these effects (34). In addition, the ouabain-induced [Ca²⁺]cyt rise in arterial strips from NCX1.3-transgenic mice was greater than in those from wild-type mice (34). SEA0400 blocked these [Ca²⁺]cyt rises in NCX1.3-transgenic mice and wild-type mice but not in drug-insensitive G833C-transgenic mice. These findings provide evidence that endogenous cardiotonic steroids trigger Ca²⁺ entry through NCX1 in vascular smooth muscle cells by inhibiting the high ouabain affinity Na⁺+/K⁺-ATPase α₂ isoform and elevating submembrane Na⁺ (Fig. 6).

**THERAPEUTIC POTENTIAL OF Na⁺+/Ca²⁺ EXCHANGE INHIBITORS IN SALT-DEPENDENT HYPERTENSION**

The links between high salt intake, endogenous cardiotonic steroids, vascular Na⁺+/K⁺-ATPase, and vascular NCX1 may lead to the elevation of [Ca²⁺]cyt and contractility in arterial smooth muscle cells, thereby resulting in hypertension (see Fig. 6). Vascular NCX1 is a key component in this pathway. In our experiments, long-term treatment with SEA0400, the most potent selective Na⁺+/Ca²⁺ exchange inhibitor, overcomes the development of salt-dependent hypertension, vascular hypertrophy, and renal dysfunction in animal models (34). Importantly, benzoyloxyphenyl Na⁺/Ca²⁺ exchange inhibitors block the reverse mode of NCX1 much more effectively than the forward mode (31, 32, 40, 50). This profile should be advantageous to therapeutic agents against intracellular Na⁺/Ca²⁺ exchange inhibitors, including salt-dependent hyper-


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70. Sobolevsky AI and Khodorov BI. Blockade of NMDA channels in rat hippocampal neurons by the Na\(^+\)/Ca\(^2+\) exchange inhibitor KB-R7943. Neuropearmacology 38: 1235–1242, 1999.


