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The Pump, the Exchanger and Endogenous Ouabain: Signaling Mechanisms that Link Salt Retention to Hypertension

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The central roles of salt (NaCl) and the kidneys in the pathogenesis of most forms of hypertension are well established.[1, 2] The linkage between salt retention and blood pressure (BP) elevation is often referred to as whole body autoregulation.[3, 4] Surprisingly, however, the precise mechanisms that underlie this linkage (i.e., the signaling pathway) have escaped elucidation. Here, we examine the evidence that endogenous ouabain (EO), Na^+ pumps (Na,K-ATPase) and the Na/Ca exchanger (NCX) are critical molecular mechanisms in this pathway.

Ca^{2+} and the Control of Myogenic Tone

At constant cardiac output (CO), mean arterial BP ≈ CO × TPR (where TPR = total peripheral vascular resistance).[5] In most (chronic) hypertension, in humans and animals, the CO is relatively normal, and the high BP is maintained by an elevated TPR.[1, 4] TPR is controlled dynamically by vasoconstriction/dilation in small "resistance" arteries which exhibit tonic constriction ("tone"). This can be studied in isolated, cannulated small arteries which develop spontaneous (myogenic) tone, MT,[6] under constant or increasing intralumenal pressure. Indeed, the level of tone in isolated arteries "is often comparable to that observed in the same vessels in vivo",[6] and may even be used to predict BP changes[7] (see below).

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MT is triggered by Ca^{2+} entry, primarily through voltage-gated Ca^{2+} channels in arterial smooth muscle (ASM) cells,[6] and contraction is activated by the rise in cytosolic Ca^{2+} concentration, $[Ca^{2+}]_{CYT}$.[8] In salt-dependent hypertension, the enhanced vasoconstriction, and increased tone and TPR are, at least in part, functional and reversible phenomena.[9] Numerous mechanisms contribute to the regulation of myocyte $[Ca^{2+}]_{CYT}$ and vasoconstriction, but the plasma membrane (PM) NCX provides an unique, direct link between Na^+ and $[Ca^{2+}]_{CYT}$ and, thus, Ca^{2+} signaling and contraction in ASM cells.[10] NCX-mediated Ca^{2+} transport is governed by the Na^+ electrochemical gradient generated by the PM Na^+ pump.

We proposed that an endogenous Na^+ pump inhibitor, i.e., a ouabain-like compound, with vasotonic action might be secreted in response to salt retention.[11] In other words, this substance might be a missing hormonal link between salt retention, and the increased TPR and hypertension. Conservation of the high affinity ouabain binding site amino acid sequence in mammalian evolution (see below) implies that there must be an endogenous ligand for this site. Partial Na^+ pump inhibition by the endogenous inhibitor should promote the net gain of Ca^{2+} via the myocyte NCX, and thereby augment Ca^{2+} signaling and vasoconstriction.[10, 11]

Endogenous Ouabain

These ideas triggered an intense international search for the postulated endogenous Na^+ pump inhibitor, a ligand for the pump's ouabain/digoxin binding site, that might mediate the vascular response. In 1991, our group purified EO from human plasma; the substance was identified as ouabain by mass spectroscopy.[12] It is now possible to quantify EO by liquid chromatography-tandem mass spectroscopy (LC-MS-MS) starting from small (1 ml) samples of human or animal plasma.[13] The LC-MS-MS spectra from human and rodent plasma extracts exhibit a major product ion at a mass:charge ratio (m/z) of 445 (Supplementary Figures 1-3; please see http://hyper.ahajournals.org); this is the lithiated aglycone of EO (i.e., lithiated ouabagenin). The possibility that EO might be the 11β isomer of ouabain[14] is excluded because the 11-epimers of ouabain have different chromatographic behavior.[15]

Rat adrenal cortex is highly enriched with EO, and human and cow adrenals also contain very high levels.[12] Bilateral adrenalectomy causes a large decline in rat plasma EO, while treatment of uni-nephrectomized rats with DOCA (deoxycorticosterone acetate) + salt increases plasma EO more than 10-fold, and significantly elevates BP.[12] These data imply that EO is primarily an adrenocortical hormone, although it may also be synthesized in, and secreted by, the hypothalamus.[16]

Studies of humans and intact animals, and of adrenocortical cell cultures, reveal that EO is synthesized in the adrenal cortex, and that its synthesis and secretion are stimulated by adrenocorticotropic hormone (ACTH)[12, 17-19] In humans[19] and animals,[18] ACTH-induced hypertension is associated with elevation of EO. Indeed, a preliminary report indicates that certain rare adrenocortical tumors, which are associated with severe hypertension, may produce prodigious amounts of EO.[20]

About 50% of humans with untreated essential hypertension and a majority of patients with adrenocortical adenomas and hypertension have significantly elevated plasma EO; moreover, BP correlates directly with plasma EO.[21] Even in normal human subjects, a high salt diet elevates plasma EO,[22] and a 10 min infusion of low dose ouabain increases vascular resistance and elevates BP for >60 min.[23]

Plasma EO levels are elevated in several rodent salt-sensitive hypertension models,[12, 24, 25] and chronic administration of low dose ouabain to normal rodents usually induces hypertension in 1-3 weeks.[26, 27] Furthermore, sub-pressor doses of ouabain and DOCA act synergistically to induce hypertension.[28] Ouabain-induced BP elevation in rodents is counteracted by the

ouabain antagonist, PST-2238 ("Rostafuroxin").[29] Also, in ACTH,[18, 30] DOCA+salt,[31] or reduced renal mass[25] hypertension, Digibind (digoxin-selective Fab fragments that bind ouabain with high affinity)[32] lowers BP.

Interestingly, low-dose ouabain increases TPR in dogs, but doesn't raise BP, presumably because heart rate and CO are markedly reduced.[33] Ouabain also doesn't induce hypertension in sheep[34] or in mineralocorticoid-resistant[35] Long-Evans rats.[36] These apparent exceptions may, however, yield novel information to help clarify the relationship between EO and hypertension.

Many of the findings cited above provide strong evidence that circulating EO has a key role in the pathogenesis of salt-sensitive hypertension. Other studies suggest, however, that brain, not plasma, EO,[16] or even marinobufagenin,[37] may be important.

Surprisingly, digoxin, another cardiotonic steroid and Na,K-ATPase inhibitor, does not induce hypertension in rodents.[26, 38] Also, Digitalis glycosides do not elevate BP in patients treated for congestive heart failure or cardiac arrhythmias.[39] Remarkably, digoxin actually lowers BP in ouabain-hypertensive rats[26, 38] and in many patients with essential hypertension.[40] Thus, Strophanthus glycosides such as ouabain may interact differently with Na^+ pumps than do the structurally distinct Digitalis glycosides. Moreover, many observations now indicate that EO is a growth hormone, and that it may participate in a variety of kinase-mediated and other signaling pathways independent of its effects on Na^+ pump-mediated Na^+ transport.[41, 42] EO may therefore contribute to vascular remodeling and target organ damage in hypertension. Clearly, there is much that we do not yet understand about the physiology and pharmacology of these agents.

Membrane Microdomains: a Structural Basis for Ouabain's Action

Na^+ pumps are αβ heterodimers. The catalytic subunit, α, contains the Na^+, K^+, MgATP and ouabain binding sites, and is phosphorylated during each pump cycle.[43] β is essential for pump function; it stabilizes the α subunit conformation and chaperones the αβ complex to the PM.[43, 44] In some tissues, a third subunit, γ, may help to regulate Na^+ pump activity.[44] There are four mammalian α subunit isoforms (α1-α4); they are products of different genes, but have nearly 90% sequence identity, different expression patterns[45] and different kinetics[46], and they are differently regulated.[43, 47] All cells express Na^+ pumps with an α1 subunit and Na^+ pumps with another α isoform.[43, 45] Skeletal, cardiac and smooth muscles, for example, express Na^+ pumps with an α2 subunit as well as pumps with an α1; most neurons express α1 and α3.[48] Renal epithelia express predominantly (>90-95%) Na^+ pumps with α1, which mediate the final step in net transepithelial Na^+ reabsorption.[47]

The functions of the different α subunit isoforms were elucidated by the discovery that, in a variety of cell types, Na^+ pumps with an α2 or α3 subunit are confined to PM microdomains situated adjacent to "junctional" sarco-/endoplasmic reticulum (jS/ER) (Figure 2).[45] Here, these Na^+ pumps co-localize with NCX, which are confined to the same PM microdomains.[45] Na^+ pumps with an α1 subunit are more widely distributed in the PM, but are apparently excluded from these microdomains.[49] Importantly, the PM microdomains are separated by only 12-20 nm from the jS/ER,[50] and these structures form a functional unit, termed the "PLasmERosome".[51] The volume of cytosol in the junctional space (J) between the PM and jS/ER of a single PLasmERosome is only on the order of 10^{-19} to 10^{-18} liters,[51] and diffusion of Na^+ and Ca^{2+} between this space and bulk cytosol is restricted. Thus, standing Na^+ and Ca^{2+} concentration gradients between these compartments and bulk cytosol can be maintained.[52-54]

Differences in Na^+ pump α subunit isoform kinetics play a critical role in PLasmERosome function. The rodent α1 isoform has unusually low affinity for ouabain ($K_{Ouabain} > 100$ μM, vs < 0.05 μM in humans),[55] so that nanomolar ouabain inhibits only the α2 Na^+ pumps in rodent arterial myocyte PLasmERosomes.[7] Even in humans, however, where α1 Na^+ pumps have high affinity for ouabain, partial inhibition of Na^+ pumps by nanomolar ouabain will raise $[Na^+]$ in the junctional space much more than in bulk cytosol. The reason is that the affinity of α2 Na^+ pumps for Na^+ is much lower ($K_{Na} \approx 22$ mM) than is the affinity of α1 Na^+ pumps ($K_{Na} \approx 12$ mM).[46]

The widespread distribution of α1 Na^+ pumps implies that they have a "housekeeping" function: they control, primarily, $[Na^+]$ in bulk cytosol. In contrast, the pumps with an α2 (in smooth muscle, for example) or α3 catalytic subunit regulate local $[Na^+]$ in the junctional space. Thus, these α2/α3 Na^+ pumps control the local Na^+ electrochemical gradient that influences Ca^{2+} transport by NCX. This organizational arrangement (Figure 1) uniquely links cell Ca^{2+} to Na^+ metabolism. The transporters in the PLasmERosome regulate not only $[Ca^{2+}]$ in the junctional space, but S/ER Ca^{2+} stores and global Ca^{2+} signaling in the cells as well.[51] Modulation of α2 Na^+ pumps in arterial myocyte PLasmERosomes by EO can therefore influence arterial tone and BP. Below, we summarize recent studies in which genetic engineering and pharmacological manipulation of mouse Na^+ pumps and NCX (Figure 2) have been used to examine the roles of these transporters in the long-term control of BP.

Downstream Effector Mechanisms

α2 Na^+ Pumps

As already noted, chronic administration of exogenous ouabain induces hypertension in rodents. The questions we now address are: How does ouabain (or EO) elevate BP? Is it due to inhibition of smooth muscle α2 Na^+ pumps, as implied above?

If circulating ouabain (or EO) elevates BP by inhibiting arterial myocyte α2 Na^+ pumps, reduced expression of α2 Na^+ pumps should have a similar effect. Therefore, we studied mice with a null mutation in either the α1 or α2 Na^+ pump.[56] Heterozygous ($\alpha1^{+/-}$ and $\alpha2^{+/-}$), but not homozygous, null mutants survive, and they express ~50% of the normal complement of α1 or α2 pump protein, respectively, in ASM.[7] Isolated mesenteric small arteries from the $\alpha2^{+/-}$, but not $\alpha1^{+/-}$ mice, exhibit augmented myogenic reactivity in response to step-wise increases in intralumenal pressure, and significantly elevated myogenic tone (MT) when pressurized to 70 mm Hg.[7] Nanomolar ouabain also augments myogenic reactivity and increases MT with an EC_{50} of ~1.3 nM.[7] Consistent with these effects in isolated arteries, $\alpha2^{+/-}$, but not $\alpha1^{+/-}$, mice have significantly elevated BP (Figure 3).[7] Moreover, the $\alpha2^{+/-}$ mice are "salt-sensitive": a high salt diet increases BP much more in these mice than in their wild type (WT) littermates (Figure 3).

The $\alpha2^{+/-}$ mice are "global" single allele null mutants, but it is important to determine if the effects are the result of reduced α2 Na^+ pump activity/expression in ASM. Recently, we found that expression of a short N-terminal segment of the α2 Na^+ pump is dominant negative (DN) for expression of full-length α2 pumps.[57] Therefore, we generated mice ($\alpha2^{SM/DN}$) that express the α2 N-terminal segment with a smooth muscle (SM)-specific myosin heavy chain promoter.[58] These mice exhibit greatly reduced α2 Na^+ pump expression in artery smooth muscle (Supplementary Figure 4; please see http://hyper.ahajournals.org) and elevated BP (Figure 3).

In a complimentary approach, α1 and α2 Na^+ pumps were overexpressed, independently, in mice, under the control of an α-actin smooth muscle-specific promoter.[59] The mice that overexpressed α2, but not those that overexpressed α1, Na^+ pumps had, on average, significantly reduced BP compared to WT mice (Figure 3).

The roles of ouabain/EO and α2 Na^+ pumps in elevating BP was also examined in two other ways. One type of study utilized Rostafuroxin, a derivative of digitoxigenin,[60] that antagonizes the inhibitory action of ouabain on Na,K-ATPase.[29] In isolated arteries, Rostafuroxin counteracted the augmentation of MT by nanomolar ouabain, but not the (ouabain-independent) augmenting effect of reduced α2 expression on MT.[7] Rostafuroxin also lowered BP in ouabain-induced hypertension[29] and in about 30% of humans with essential hypertension.[29]

As an alternative, in a knock-in study, two amino acids in the α2 Na^+ pump ouabain binding site were mutated to reduce, markedly, the α2 pump's affinity for ouabain.[18, 48] Mice that expressed ouabain-resistant α2 pumps ($\alpha2^{R/R}$) were resistant to ACTH-induced hypertension (Figure 4)[18] as well as to ouabain-induced hypertension.[48] Moreover, Digibind prevented the ouabain-induced elevation of BP in the wild type mice.[48] Clearly, ACTH-induced hypertension depends upon the existence of a high-affinity cardiotonic steroid binding site on the α2 Na^+ pump, and upon a water-soluble ligand that binds to this site. The plasma level of this ligand (presumably EO) was increased by ACTH and, like ouabain,[32] bound to Digibind with high affinity.[48]

These genetic engineering studies reveal that arterial myocyte α2 Na^+ pumps mediate the effects of EO and play a role in the long-term regulation of BP. Genetically or pharmacologically reduced α2 activity elevates BP, whereas increased α2 activity lowers BP (Figure 2). The next question is: By what specific mechanism does the altered α2 Na^+ pump activity influence BP? The answer appears to lie in Na/Ca exchange.

NCX Type-1

Na/Ca exchange uniquely and directly links Na^+ to Ca^{2+} metabolism and is a distal regulator of cytosolic Ca^{2+}. There are two classes of Na/Ca exchangers, those that co-transport K^+ with Ca^{2+} (NCKX), and those that do not (NCX).[61] The predominant exchanger in arterial myocytes is NCX, even though NCKX has also been detected,[62] There are three mammalian NCX isoforms (NCX1-NCX3), each the product of a different gene.[63] NCX1, which is expressed in ASM, has multiple splice variants; NCX1.3 is the main variant in arterial myocytes.[64]

Inhibition of Na^+ pumps by nanomolar ouabain augments Ca^{2+} signaling without elevating bulk cytosolic Na^+ in primary cultured rat arterial myocytes.[51] Even knockout of α2 Na^+ pumps in cultured cells (astrocytes) has only minimal effect on bulk cytosolic Na^+, but a large effect on Ca^{2+} signaling.[65] These findings are consistent with a functional linkage between α2 (but not α1) Na^+ pumps and NCX1, and local reduction of the trans-PM Na^+ gradient when α2 activity is reduced, as implied by the PLasmERosome model (Figure 1). Moreover, recent pharmacological and genetic engineering studies now reveal that NCX1 influences not only arterial myocyte Ca^{2+} metabolism, but long-term vascular tone and BP as well.

Mice in which NCX1 is overexpressed in smooth muscle with an α-actin promoter ($NCX1^{SM/Tg}$) have elevated BP that is markedly increased by a high salt diet; i.e., the mice are "salt-sensitive" (Figure 3).[66] The elevated BP in the NCX1 overexpressors on high dietary salt is abolished by SEA0400, a selective NCX1 inhibitor,[67] but not if the overexpressed NCX1 contains a G833C mutation,[66] which specifically abrogates the action of SEA0400.[68]

To perform the converse experiment, mice with floxed NCX1 ($NCX1^{flx/flx}$)[69] were crossed with mice containing a Cre recombinase gene under the control of the smooth muscle myosin heavy chain promoter[58] to generate smooth muscle-specific NCX1 knockout ($NCX1^{SM-/-}$) mice. $NCX1^{SM-/-}$ mice have abnormally low blood pressure (Figure 3), and isolated, pressurized small arteries from these mice have abnormally low MT.[70] Indeed, SEA0400 also lowers BP by about 5-10 mm Hg in WT mice[66] and reduces MT by about 10% in isolated

arteries from these mice.[7, 66] Thus, NCX1 activity apparently makes a modest, but direct, contribution to normal MT and BP. SEA0400 also attenuates the increased MT in arteries from $\alpha 2^{+/-}$ mice,[7] indicating that NCX1 mediates effects distal to α2 Na^+ pumps. The BP and MT data from $\alpha 2^{+/-}$ and $NCX1^{SM-/-}$ mice support the view that MT in isolated arteries is an in vitro reflection of BP[6] and, most likely, TPR.

The mice with genetically engineered NCX1 demonstrate that this exchanger contributes to long-term BP regulation: increased NCX1 expression increases BP while knockout of NCX1 reduces BP (Figures 2 and 3). This conclusion is supported by the effects of NCX blockers in several rodent models of salt-dependent or ACTH-induced hypertension. In DOCA+salt hypertensive rats, spontaneously hypertensive rats (SHR) on a high salt diet, and Dahl salt-sensitive rats on high salt, SEA0400 markedly reduces BP.[66] Also, KB-R7943, a less potent NCX blocker, prevents ACTH from elevating BP in mice.[18] Moreover, although a null mutation in one NCX1 allele has negligible effect on BP ($NCX1^{+/-}$ in Figure 3) or MT,[71] it prevents the induction of hypertension by DOCA+salt.[66] Importantly, SEA0400 did not lower BP in several salt-independent rat hypertension models: SHR on a normal salt diet, stroke prone-SHR, and the renin-dependent two-kidney/one-clip rat.[66] The implication is that NCX1 makes an important contribution to the pathogenesis of salt-dependent hypertension, but not to salt-independent hypertension.

“Kalzium? Ja, das ist Alles!” (Calcium is Everything: O. Loewi)

Arterial myocyte contraction depends, ultimately, upon the availability of cytosolic Ca^{2+},[8] and the sensitivity of the contractile apparatus to that Ca^{2+}.[72] Furthermore, NCX1, under the control of the Na^+ gradient generated by the adjacent α2 Na^+ pumps, helps regulate myocyte Ca^{2+} homeostasis (Figure 1). For example, the nanomolar ouabain-induced increase in MT is associated with increased myocyte $[Ca^{2+}]$;[7] conversely, reduction of MT by SEA0400 is associated with reduced myocyte $[Ca^{2+}]$ (Figure 5).[66] Thus, it is apparent that α2 Na^+ pumps and NCX1 are relatively distal mechanisms in the final common path that links salt to vasoconstriction and hypertension (Figure 2). Indeed, all upstream vasoconstrictor and vasodilator mechanisms (neural and humoral) must, inevitably, be influenced by the activity of these two transporters, because they regulate basal $[Ca^{2+}]_{CYT}$.

An alternative suggestion is that activation of Rho/Rho kinase via the G_{12}-G_{13}-mediated G protein-coupled receptor pathway, which modulates the Ca^{2+} sensitivity of the contractile apparatus in ASM,[72] is selective for salt-dependent hypertension.[73] Those authors, however, studied only a salt-dependent (DOCA+salt) mouse model; they did not test whether the G_{12}-G_{13} pathway also operates in salt-independent forms of hypertension.[73] In fact, interference with the G_{12}-G_{13} pathway, whether at the agonist receptor level,[74] or at the level of Rho kinase,[75] lowers BP in salt-independent models such as the stroke-prone spontaneously hypertensive rat[74] and the NO synthase-inhibited rat.[75] The G_{12}-G_{13} pathway is, therefore, downstream, and distinct from the key salt-sensitive steps in Na^+-dependent hypertension. Once salt-sensitive NCX1-mediated Ca^{2+} entry has occurred, the G_{12}-G_{13} pathway helps modulate the increases in vascular tone and BP.

Endgame: Na/Ca Exchange, Ca^{2+} Entry and Myogenic Tone

In the heart, the main role of NCX is to extrude, during diastole, much of the Ca^{2+} that enters through voltage-gated channels (VGCs) during systole.[76] Consequently, reduced cardiac NCX1 function as a result, for example, of α2 Na^+ pump inhibition by cardiotonic steroids, is associated with Ca^{2+} gain and augmented signaling in cardiac myocytes. Therefore, it might at first seem surprising that ASM NCX1 contributes directly to vascular tone, and that reduced expression or pharmacological inhibition of NCX1 in arterial myocytes lowers $[Ca^{2+}]_{CYT}$ and

attenuates Ca^{2+} signaling (Figure 5). Indeed, Ca^{2+} entry via NCX has sometimes been called "reverse mode" exchange, implying, erroneously, that this is the backward or abnormal operation of the exchanger.[77] NCX can transport Ca^{2+} in either direction across the PM,[78] under the control of the local Na^+ electrochemical gradient across the PM (Figure 1), and considerations of the electrical component of this gradient are of paramount importance. In the heart, the driving force on the exchanger, i.e., the difference between the prevailing membrane voltage, V_M, and the NCX1 reversal potential, $E_{Na/Ca}$,[a] which determines the direction of net Ca^{2+} movement, varies during the cardiac cycle. For example, the rapid membrane depolarization during the upstroke of the cardiac action potential rapidly switches NCX1 from the Ca^{2+} exit to Ca^{2+} entry mode, as the driving force, V_M-$E_{Na/Ca}$, becomes positive. Then, as V_M repolarizes, V_M-$E_{Na/Ca}$ again becomes negative and favors Ca^{2+} exit.[78]

A different situation exists in ASM, where changes in V_M are normally quite slow and cells are often partially depolarized for very long periods of time.[79] Here, intralumenal pressure in small arteries depolarizes the myocytes and activates dihydropyridine-sensitive L-type VGCs. Opening of stretch-activated non-selective cation channels[80] may initiate the depolarization. This depolarization is insensitive to dihydropyridines: nifedipine blocks Ca^{2+} entry through L-type VGCs and reduces MT, but has little effect on the pressure-activated depolarization.[79] The Na^+ entry through stretch-activated channels and consequent depolarization, as well as the rise in $[Ca^{2+}]_{CYT}$,[80] should also have another, previously unrecognized consequence: they should promote Ca^{2+} entry via NCX1 and thereby contribute to MT. The reason is that the exchanger is activated by cytosolic Ca^{2+},[81] and the rise in cytosolic $[Na^+]$ and the depolarization augment the Ca^{2+} entry mode of NCX1 by increasing the driving force, V_M-$E_{Na/Ca}$. The implication is that both the L-type VGCs and NCX1 contribute to the maintenance of Ca^{2+} entry, elevated $[Ca^{2+}]_{CYT}$ and arterial tone when the arteries are pressurized.

"In My End is My Beginning" (T.S Eliot)

In this review, we have explored some of the critical steps that link salt retention to the long-term increase in TPR and elevation of BP. Recent results, especially those from chemical analyses of human and rodent plasma samples, and from genetic engineering and pharmacological studies in rodents and rodent arteries, are summarized above. These studies give new insight into some of the molecular events that help regulate cytosolic Ca^{2+} and vascular tone. The data supply compelling evidence that EO, and smooth muscle $\alpha 2$ Na^+ pumps and NCX1, are key mechanisms in the pathway that leads from salt retention to hypertension (Figure 2).

While these findings provide a framework, the story is far from complete. For example, a key area where knowledge is lacking is at the early steps between salt retention and the release of EO, as indicated by the broken vertical lines in Figure 2. Also, Coffman and colleagues recently demonstrated that the renal and extra-renal arteries make apparently independent (and equal) contributions to the long-term regulation of BP.[82] But how the distal mechanisms, discussed above, affect the renal and extra-renal vasculature and renal function, and thereby contribute to BP control, is still unexplored. And, of course, a fundamental question is: What makes us salt-sensitive in the first place? Hopefully, the progress outlined above will clarify new directions for hypertension research to help resolve these issues.

[a]For NCX1, which mediates the exchange of $3Na^+$ for $1Ca^{2+}$, $E_{Na/Ca} = 3E_{Na} - 2E_{Ca}$, where E_{Na} and E_{Ca} are, respectively, the equilibrium potentials for Na^+ and Ca^{2+} [$E_{Na} = (RT/F) \ln ([Na]_o/[Na]_i)$ and $E_{Ca} = (RT/2F) \ln ([Ca]_o/[Ca]_i)$, and R, T and F are the gas constant, temperature (Kelvin) and Faraday's number].[78]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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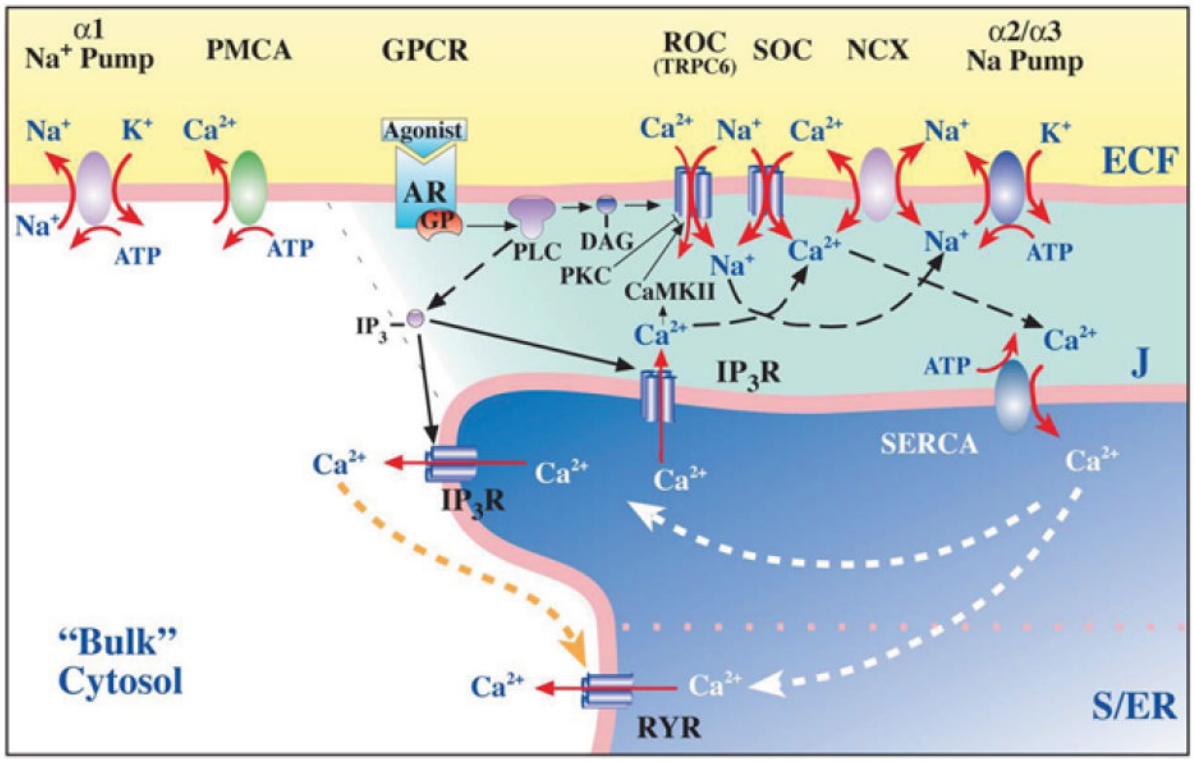

Figure 1.

Model of the plasma membrane-junctional sarco-/endoplasmic reticulum (PM-jS/ER) region, the PLasmERosome, showing the location of key transport proteins involved in local control of jS/ER Ca^{2+} stores and Ca^{2+} signaling. The PLasmERosome consists of a PM microdomain, the adjacent jS/ER, and the intervening 'diffusion-restricted' junctional space (J). The PM microdomain contains α2 (in smooth muscle) or α3 Na^+ pumps, NCX, and receptor and store-operated channels (ROCs and SOCs, composed of various transient receptor potential channel proteins or TRPCs). The jS/ER membrane contains SR Ca^{2+} pumps (SERCA), inositol trisphosphate receptors (IP_3R) and ryanodine receptors (RYR). Other regions of the PM contain α1 Na^+ pumps and Ca $^{2+}$ pumps (PMCA), as well as agonist receptors (ARs, which are G-protein coupled receptors, or GPCRs). Activation of GPCRs and release of G-proteins (GPs) stimulates phospholipase C (PLC) to produce IP_3 and diacylglycerol (DAG). DAG may activate ROCs directly. Na^+ may enter locally, through ROCs, SOCs or stretch-activated channels (not shown) to promote Ca^{2+} entry via NCX. Shading indicates relative Na^+ and/or Ca^{2+} concentrations. (Reprinted with permission).[52]

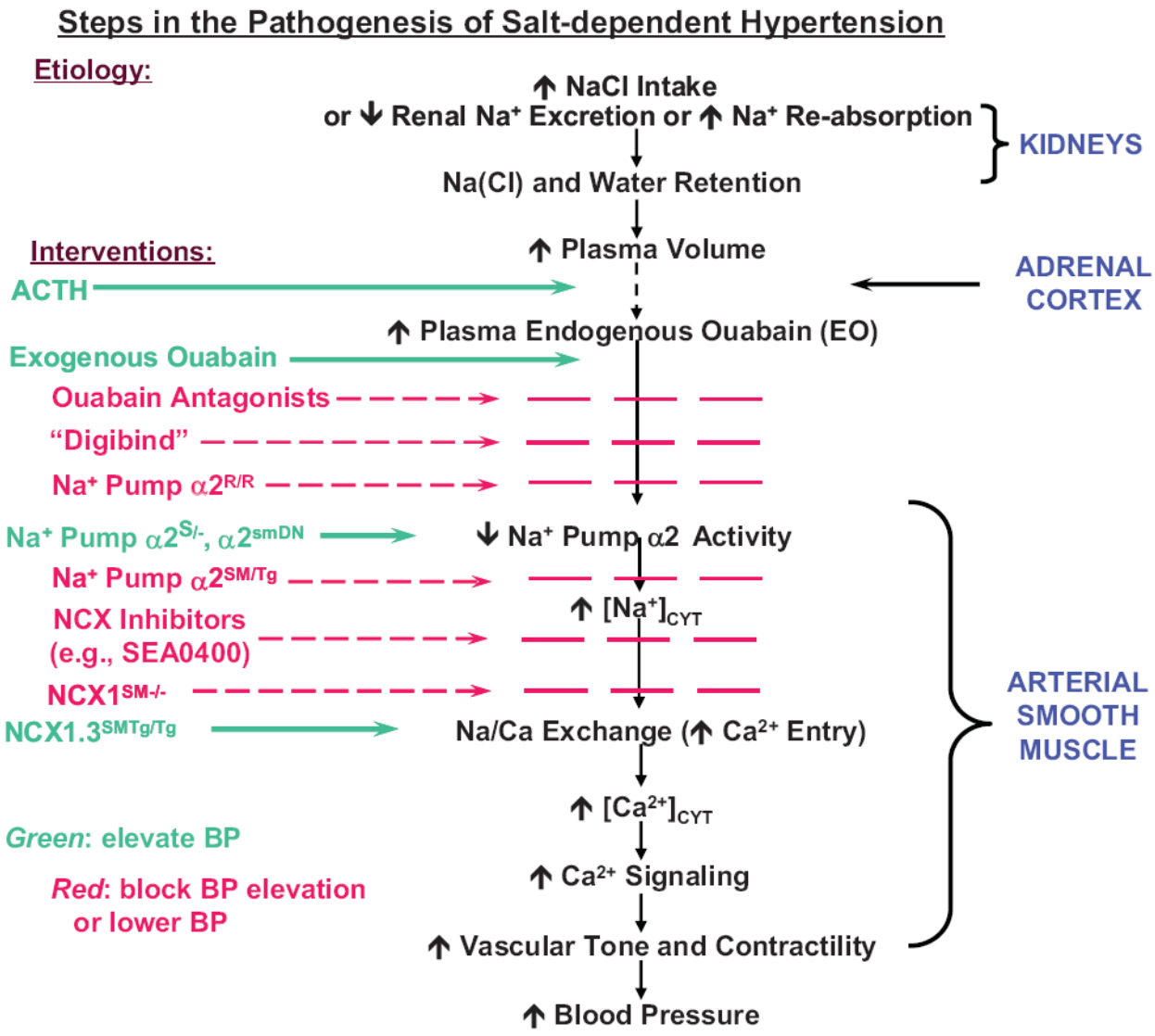

Figure 2.

Proposed sequence of steps in the pathogenesis of salt-dependent hypertension. The "interventions," listed at the left, indicate some of the pharmacologic and genetic manipulations that were used to test the proposed sequence, as discussed in this review. Genotype nomenclature for genetically-engineered mice is given in the text and in Figure 3 and 4 legends. Interventions shown in green increase traffic through the pathway and promote BP elevation; those shown in red block traffic through the pathway and prevent BP elevation or lower BP. Modified from Ref. 7.

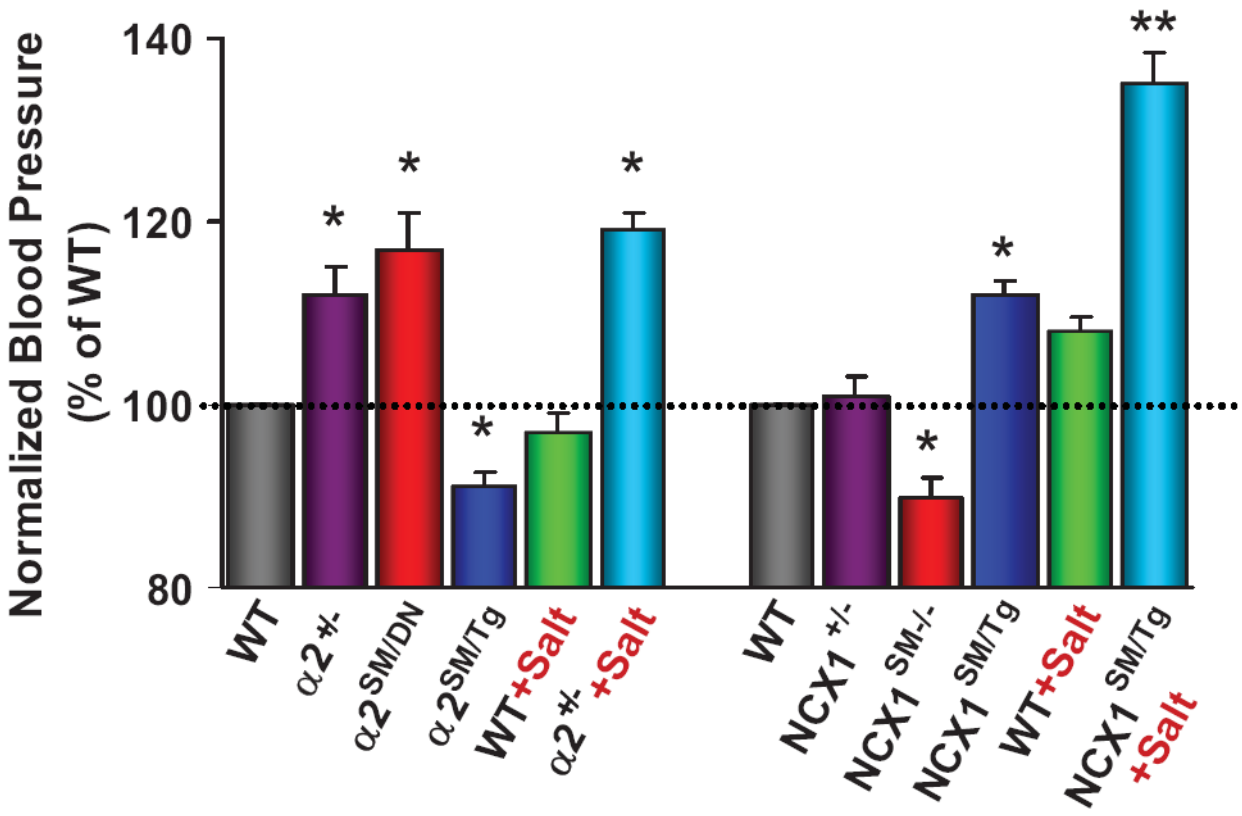

Figure 3.

Relative blood pressures of mice with genetically-engineered α2 Na^+ pumps and NCX1. The data from several sources, are normalized to the BPs of the respective control wild type (WT) mice. Mice with a null mutation in one α2 Na^+ pump allele ($\alpha2^{+/-}$)[7] or smooth muscle-specific α2 knockdown ($\alpha2^{SM/DN}$) (Song, Chen, Kotlikoff and Blaustein, unpublished; see Figure 4 in Supplementary Data), or increased smooth muscle-specific NCX1 overexpression ($NCX1^{SM/Tg}$),[66] had significantly elevated BP. A high salt diet augmented the elevated BP in $\alpha2^{+/-}$ mice (4% NaCl × 2 weeks) and $NCX1^{SM/Tg}$ mice (8% NaCl + 1% NaCl in tap water × 4 weeks). Smooth muscle-specific overexpression of α2 Na^+ pumps ($\alpha2^{SM/Tg}$)[59] or Cre-recombinase knockdown of NCX1 ($NCX1^{SM-/-}$)[70] significantly reduced BP. * = $P < 0.05$, ** = $P < 0.01$ vs WT or the respective genotypes on a normal (0.5%) salt diet.

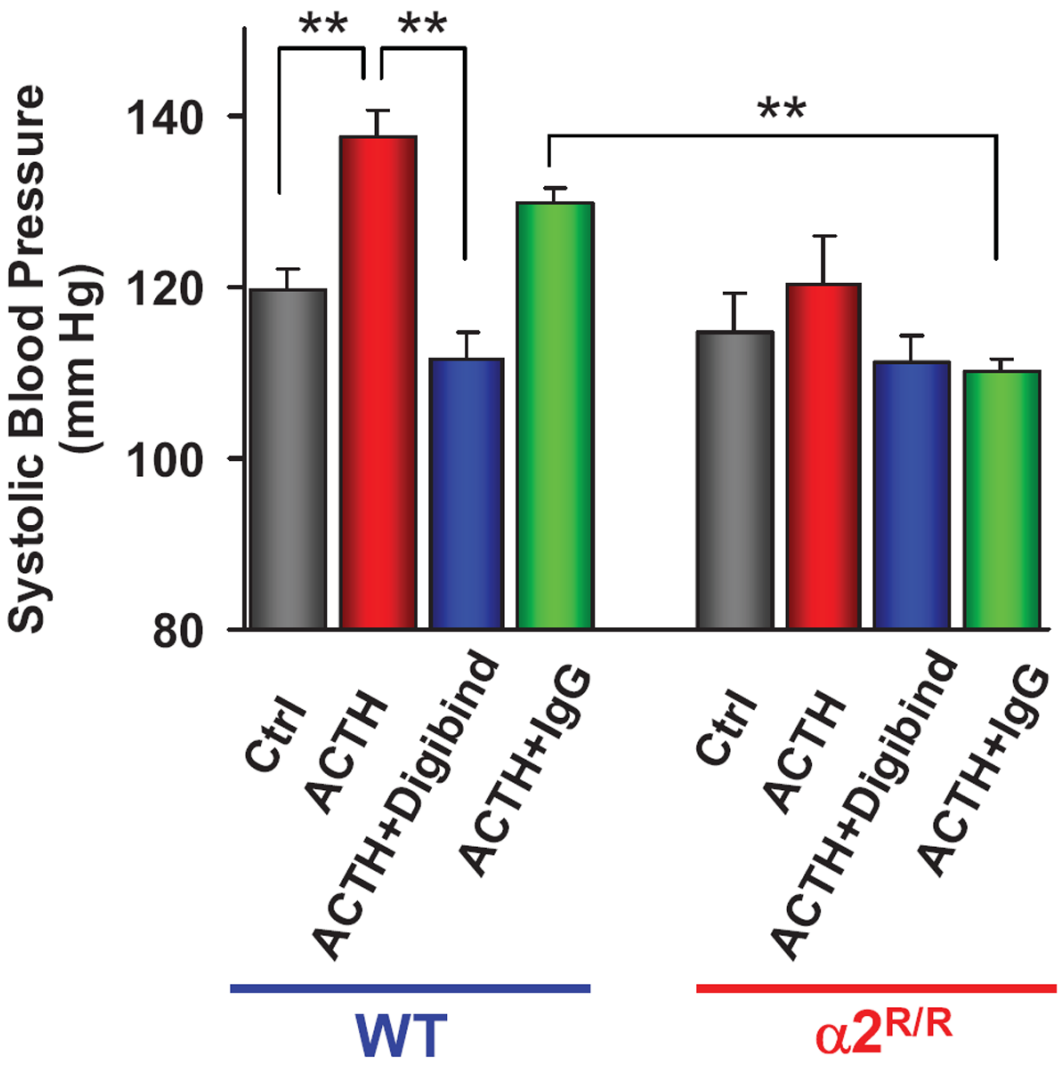

Figure 4.

Effects of ACTH on blood pressure in mice with high (normal) and low ouabain affinity α2 Na^+ pumps. ACTH (500 μg/kg s.c. every 8 hr × 5 days) elevated BP in wild type (WT) mice, but not in mutant mice expressing α2 Na^+ pumps with low affinity for ouabain ($\alpha2^{R/R}$).[18] Treatment with Digibind (30 μg/kg daily, 2 hr before BP measurement), which binds ouabain with high affinity,[32] but not control IgGγ, prevented the ACTH-induced elevation of BP in WT mice. ** = $P < 0.1$ for the pairings indicated. These re-graphed BP data were obtained by tail cuff,[18] but comparable results were recently obtained by telemetry (Lingrel and colleagues, unpublished).

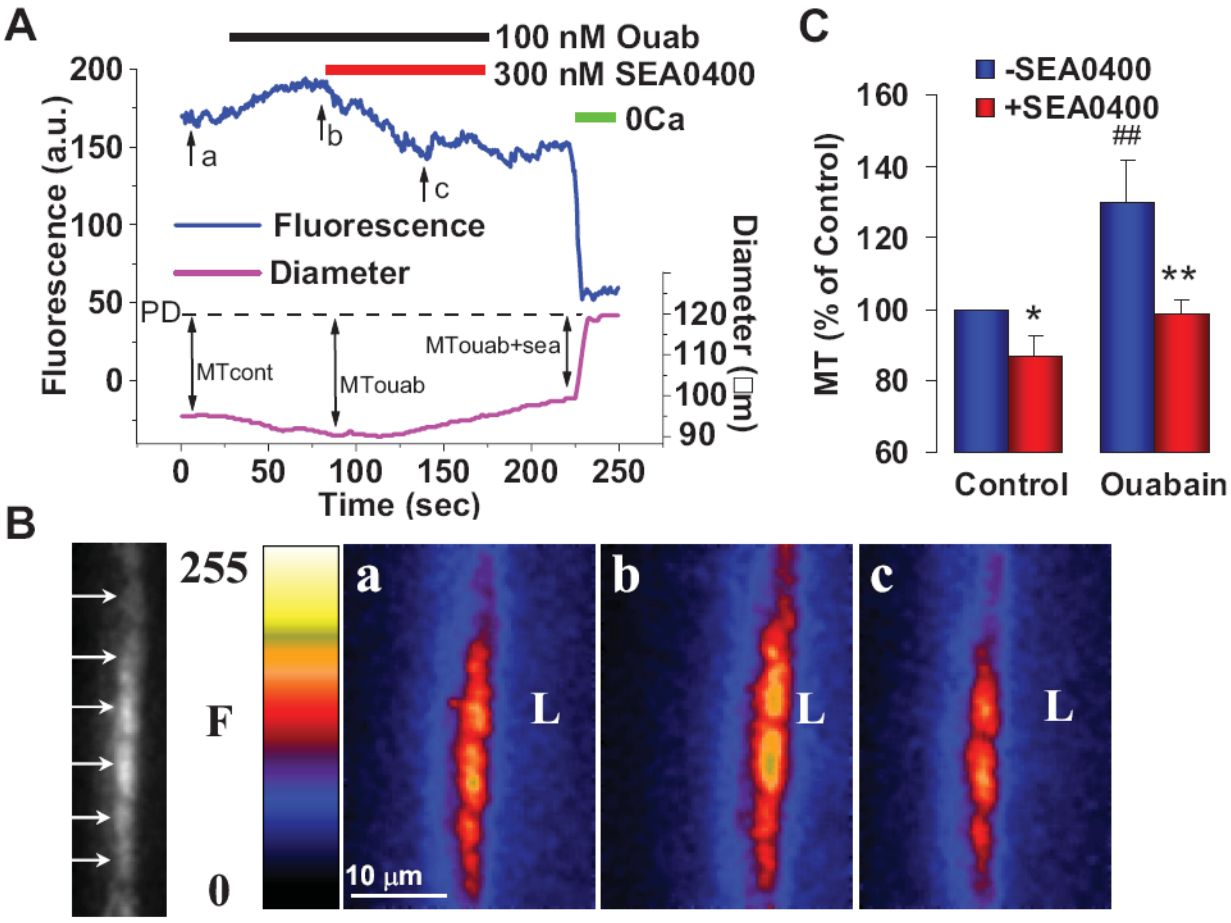

Figure 5.

Effects of low dose ouabain and SEA0400 on $[Ca^{2+}]_{CYT}$ and myogenic tone (MT) in mouse pressurized mesenteric small arteries. A. Simultaneous recording of fluorescence (F, in arbitrary units, a.u., a measure of $[Ca^{2+}]_{CYT}$) and external diameter in a representative fluo-4 loaded normal mouse artery pressurized to 70 mm Hg. Bars at the top indicate periods of exposure to 100 nM ouabain, 300 nM SEA0400 and 0Ca medium (to determine passive diameter, PD). B. Arrows in the black and white spinning disk confocal image at the left indicate fluorescence in individual myocytes of a longitudinal cross-section through one wall of the artery in A. Pseudocolor images of this artery wall were captured at the times indicated by arrows “a” (control MT), “b” (MT with ouabain) and “c” (MT with ouabain + SEA0400) in A; “L” is located in the artery lumen. C. Summary of normalized MT data from this and five other, similar experiments. * = $P < 0.05$, ## = $P < 0.01$ vs untreated control; ** = $P < 0.01$ vs ouabain alone. Corrected from Ref. 66.