How NaCl raises blood pressure: a new paradigm for the pathogenesis of salt-dependent hypertension

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Submitted 12 September 2011; accepted in final form 2 November 2011

Blaustein MP, Leenen FH, Chen L, Golovina VA, Hamlyn JM, Pallone TL, Van Huysse JW, Zhang J, Wier WG. How NaCl raises blood pressure: a new paradigm for the pathogenesis of salt-dependent hypertension. Am J Physiol Heart Circ Physiol 302: H1031–H1049, 2012. First published November 4, 2011; doi:10.1152/ajpheart.00899.2011.—Excess dietary salt is a major cause of hypertension. Nevertheless, the specific mechanisms by which salt increases arterial constriction and peripheral vascular resistance, and thereby raises blood pressure (BP), are poorly understood. Here we summarize recent evidence that defines specific molecular links between Na+ and the elevated vascular resistance that directly produces high BP. In this new paradigm, high dietary salt raises cerebrospinal fluid [Na+]i. This leads, via the Na+-sensing circumventricular organs of the brain, to increased sympathetic nerve activity (SNA), a major trigger of vasoconstriction. Plasma levels of endogenous ouabain (EO), the Na+ pump ligand, also become elevated. Remarkably, high cerebrospinal fluid [Na+]i-evoked, locally secreted (hypothalamic) EO participates in a pathway that mediates the sustained increase in SNA. This hypothalamic signaling chain includes aldosterone, epithelial Na+ channels, EO, ouabain-sensitive α2 Na+ pumps, and angiotensin II (ANG II). The EO increases (e.g.) hypothalamic ANG-II type-1 receptor and NADPH oxidase and decreases neuronal nitric oxide synthase protein expression. The aldosterone-epithelial Na+ channel-EO-α2 Na+ pump-ANG-II pathway modulates the activity of brain cardiovascular control centers that regulate the BP set point and induce sustained changes in SNA. In the periphery, the EO secreted by the adrenal cortex directly enhances vasconstriction via an EO-α2 Na+ pump-ANG-II-exchanger-Ca2+ signaling pathway. Circulating EO also activates an EO-α2 Na+ pump-Src kinase signaling cascade. This increases the expression of the Na+/Ca2+ exchanger/transient receptor potential cation channel Ca2+ signaling pathway in arterial smooth muscle but decreases the expression of endothelial vasodilator mechanisms. Additionally, EO is a growth factor and may directly participate in the arterial structural remodeling and lumen narrowing that is frequently observed in established hypertension. These several central and peripheral mechanisms are coordinated, in part by EO, to effect and maintain the salt-induced elevation of BP.

endogenous ouabain; epithelial sodium channel; α2-sodium pump; transient receptor potential cation channel; calcium signaling; protein kinase

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Introduction

Primary (essential) hypertension is a major public health problem. About 65 million Americans and more than a billion people worldwide are hypertensive (132, 136). Hypertension is a major risk factor for cardiovascular, cerebrovascular, and renal diseases, and the control of blood pressure (BP) in hypertensive patients can markedly reduce morbidity and mortality from these complications (102, 132).

There is broad agreement that excess dietary salt (NaCl) is the single most important controllable factor responsible for the rise in BP with advancing age in our culture and, thus, for the high incidence of essential hypertension (132, 185). Despite extensive research, however, the specific mechanisms by which high dietary salt actually leads to the elevation of BP are poorly understood and generally ignored. Most investigations have focused on 1) the mechanisms by which the kidneys retain salt (as the primary defect) and 2) the manifestations associated with the salt-dependent, sustained elevation of BP.
The latter include 1) increased sympathetic nerve activity (SNA) (2, 6, 46, 83, 167, 228) with resetting of the brain and baroreflex barostat mechanisms that control the steady-state BP level (34, 199, 278); 2) augmented arterial myocyte Ca\(^{2+}\) signaling and constriction in response to vasoconstrictors (16, 27) and increased myogenic tone in resistance arteries (42); 3) attenuated endothelial vasodilator responses (203, 227); and 4) arterial structural remodeling, including inward eutrophic changes (lumen narrowing due solely to increased cellular overlap) (177, 192), increased arterial oxidative stress (251), collagen deposition, and wall thickening with lumen narrowing (105, 177, 191, 192, 227). All of these changes, in various combinations, can contribute to the development and/or maintenance of the increased arterial tone and total peripheral vascular resistance (TPR) and, thus, to the elevated BP. They do not, however, answer the key question: How does a high-salt intake induce these changes in SNA and arterial function and structure? Our goal, therefore, is to elucidate the underlying mechanisms.

**Salt, plasma volume, and BP.** According to widely accepted dogma, salt and, consequently, water retention by the kidneys is a major factor in the pathogenesis of salt-induced hypertension (35, 91, 132, 183, 220). Slightly elevated plasma [Na\(^+\)] is a major factor in the pathogenesis of salt-induced hypertension, salt and, consequently, water retention by the kidneys are key factors in the pathogenesis of salt-induced hypertension. About 35 years ago, we (21) and others (94) hypothesized that an endogenous Na\(^+\) pump (Na\(^+\),K\(^+\)-ATPase) inhibitor, a ouabain-like compound (OLC), might directly inhibit renal Na\(^+\) reabsorption to promote natriuresis. It was also suggested that this OLC might directly enhance vascular tone and elevate BP (21) and, hence, indirectly promote saluresis through pressure natriuresis (91, 93). This hypothesis focused on the primary active Na\(^+\) transport system, the Na\(^+\) pumps (18, 131) that 1) maintain low cytosolic [Na\(^+\)] and high [K\(^+\)] in virtually all cells, 2) play a pivotal role in renal Na\(^+\) reabsorption, and 3) power numerous Na\(^+\)-coupled secondary-active transport systems including Na\(^+\)/Ca\(^{2+}\) exchangers (NCXs) (21, 24). In conjunction with functionally coupled NCX, Na\(^+\) pumps help regulate Ca\(^{2+}\) homeostasis and Ca\(^{2+}\) signaling in most types of cells including arterial myocytes, endothelial cells, and neurons (24). Although they predated the discovery of Na\(^+\) pump isofoms (155), these ideas emphasized the possible physiological and pathophysiological role of the Na\(^+\) pump’s highly conserved high-affinity cardioactive steroid binding site (154). Indeed, an endogenous ligand for this site had long been postulated (24).

**Endogenous Ouabain, a Key Player**

An endogenous OLC was subsequently purified from human plasma (97) and from bovine adrenals (229, 244) and hypothalamus (134). Structural analysis by both mass spectroscopy and nuclear magnetic resonance (NMR) revealed that this endogenous OLC was identical to that of the exogenous cardiac glycosides. But, it raised another obvious question: Can ouabain, per se, induce hypertension? Indeed, prolonged subcutaneous administration of ouabain does elevate BP in normal rats and mice (ouabain hypertension), albeit with a delay of 1 to 2 wk (37, 175, 277) (Fig. 2; and see Long-Term Effects of Ouabain/EO: the Concept of Functional Remodeling). Chronic intracerebroventricular (icv) infusion of ouabain, in amounts...
too low to exert direct effects on peripheral tissues, also elevates BP (37, 111, 255). All pressor effects of EO and ouabain appear to be mediated by a common Na\(^+\)/H\(^+\) pump receptor in the central nervous system and the periphery. Arterial myocytes, endothelial cells, and glia express both $\alpha_1$ and $\alpha_2$ Na\(^+\) pumps\(^1\) in a ratio of about 4:1 (75, 235, 283). Most neurons express $\alpha_1$ and $\alpha_3$ Na\(^+\) pumps, although some express $\alpha_1$ and $\alpha_2$ pumps, especially in the neonate (190), but most of the $\alpha_2$ pumps expressed in the adult brain are in glia (18, 184). In rodents, but not humans and most other mammals, $\alpha_1$ Na\(^+\) pumps are ouabain resistant, whereas high ouabain affinity is conserved in the $\alpha_2$ and $\alpha_3$ Na\(^+\) pumps (18, 154). The ouabain-induced elevation of BP in rodents is mediated by the high-affinity ouabain binding site on $\alpha_2$ Na\(^+\) pumps: ouabain-induced hypertension is prevented by mutations that markedly reduce the affinity of the site for ouabain (52, 255). This raises the question of whether glia play a role in the pathogenesis of hypertension.

The $\alpha_2$ Na\(^+\) pump-mediated pharmacological effects of ouabain on BP are paralleled by studies on mice with genetically reduced Na\(^+\) pump expression. Mice with a null mutation in one Na\(^+\) pump $\alpha_2$ allele ($\alpha_2^+/−$ mice, with ~50% reduction in $\alpha_2$-subunit expression) and transgenic mice in which $\alpha_2$ Na\(^+\) pump expression is reduced by the smooth muscle-specific expression of a nonfunctional, dominant negative NH\(_2\)-terminal segment have elevated BP (26, 283). Conversely, mice that overexpress $\alpha_2$ are hypotensive (209); i.e., BP appears to be inversely related to the $\alpha_2$ gene dose. In contrast, mice with a null mutation in one Na\(^+\) pump $\alpha_1$ allele (and the much more prevalent low ouabain affinity $\alpha_1$ Na\(^+\) pumps), and mice that overexpress $\alpha_1$, all have normal BP (209, 283). The implication is that the hypertension is caused by either functionally or genetically reduced $\alpha_2$ Na\(^+\) pump activity. In humans, however, whether the $\alpha_1$ Na\(^+\) pumps, which are ouabain sensitive, also play a role in the pathogenesis of hypertension, has not been determined.

Other cardiotonic steroid/Na\(^+\) pump inhibitors, most notably bufadienolides such as marinobufagenin (MBG) and proscil-
laridin A, have also been identified in mammals (14, 152, 229, 230). MBG has been implicated in the pathogenesis of essential hypertension (14, 63) and pregnancy-induced hypertension (212, 257). One suggestion is that MBG and EO cooperatively act to raise BP in salt-dependent hypertension (13). Unfortunately, MBG is not commercially available; thus, the number of laboratories using MBG and the number of physiological studies of MBG action have been very limited. Moreover, almost all in vivo and clinical studies have relied on immunoassay and/or immune neutralization where the nature of the cross-reacting species has often been assumed.

Surprisingly, in contrast to ouabain (52, 123), MBG augments cardiac contraction and elevates BP in mice with ouabain-resistant α2 and α1 Na+ pumps, but not in normal (wild-type) mice with ouabain-sensitive α2 Na+ pumps (263). Indeed, it appears that MBG preferentially inhibits α1 Na+ pumps in vivo (61). These MBG data seem inconsistent with the aforementioned results from the genetically engineered mice, and they raise the possibility that EO and MBG may have different physiological (and pathophysiological) effects. Given these disparate findings and the more extensive documentation of the role of ouabain/EO in the hypertension literature, the ensuing discussion will focus on the role of ouabain/EO. As will become clear, EO, like aldosterone (see next section), is both a hypothalamic and an adrenal hormone, with a critical role in both Na+ metabolism and BP regulation. We will begin with the brain and then focus on circulating ouabain/EO and its role in the periphery.

**The Hypothalamus and Enhanced Sympathetic Drive**

As already noted, a high-salt diet elevates plasma [Na+] by a few millimolar, but the rise is not different in salt-sensitive and salt-resistant or hypertensive and normotensive subjects. A high-salt diet also elevates CSF [Na+] in salt-sensitive Dahl S rats and spontaneously hypertensive rats (SHRs) but not in their salt-resistant or normotensive counterparts (115, 237). The rise in CSF [Na+] stimulates the hypothalamic circumbroventricular organs, such as the subfornical organ (108, 265), and activates a central sympathoexcitatory pathway. This induces a rapid, short-term, ANG II-mediated increase in peripheral SNA and elevation of BP (187, 231, 265) that is prevented by central ANG-II type-1 receptor (AT1R) blockade (181) (Fig. 3).

Prolonged (days to weeks) icv administration of Na+-rich CSF (34, 116, 255), aldosterone (76, 274), or ANG II (70, 116, 274) also acts via the circumbroventricular organs to augment central sympathoexcitatory mechanisms, but apparently by activation of another brain pathway (72, 148) (Fig. 3). This is a slowly activated neuromodulatory pathway that involves increased local (hypothalamic) synthesis and secretion of aldosterone and EO, as well as ANG II (118, 256). This proposed Aldo-EO-ANG II pathway appears to be crucial for the development of chronic, salt-dependent hypertension. As previously noted (148), this distinction between the direct, rapid signaling and a slower neuromodulatory pathway is analogous to the distinction between rapid, classic chemical synaptic transmission and the much slower modulation of synaptic transmission elucidated by Greengard (85). The latter depends on protein phosphorylation and changes in protein expression. Some examples are increases in angiotensin-converting enzyme (290), AT1Rs, and NADPH oxidase and a decrease in neuronal nitric oxide (NO) synthase (nNOS) in the hypothalamus (119). These mechanisms may all contribute to the Aldo-EO-ANG II-mediated activation of the central sympathoexcitatory pathways.

Here, and in the ensuing discussion, we refer to Na+-rich rather than NaCl-rich CSF, and we ignore the anionic component. The Na+-rich CSF-induced effects in the rat are apparently not due to the increased osmolarity because equiosmolar mannitol does not have these effects (72). The anionic component, however, does appear to be important, perhaps because substitutes such as nitrate, bicarbonate, phosphate, and amino acids (204, 232, 267, 291) can be metabolized and cannot be retained in body fluids. Both Na+ and Cl− ions are required for the acute pressor effect of centrally infused artificial CSF (232), as well as for the chronic effect of dietary salt on BP in salt-sensitive models of hypertension (141, 204, 267, 291).

The roles of aldosterone and epithelial Na+ channels in the brain. The effects of prolonged icv infusion of Na+-rich CSF, which include the secretion of EO in the brain, the increase of SNA, and the elevation of BP, are all inhibited by local (icv) application of a steroid synthesis inhibitor or by mineralocorticoid receptor (MR) blockade (77, 118, 261). Conversely, icv infusion of aldosterone raises the EO content in the hypothalamus, increases SNA, and induces hypertension (258, 289). Indeed, a systemic administration of aldosterone also appears to act largely via the brain MRs because systemic aldosterone plus salt-induced hypertension is antagonized by icv administration of MR blockers and MR silencer RNA (274). This mineralocorticoid hypertension also has a distinct salt-dependent component, however, because thiazide diuretics prevent or attenuate the BP elevation even though the brain MRs may be flooded with aldosterone (15, 88, 252, 253). The specific role of the salt in this situation is, however, unresolved.
Importantly, a high-salt diet increases hypothalamic aldosterone content in Dahl salt-sensitive S rats; this increase is blocked by icv infusion of aldosterone synthase inhibitors, which also greatly attenuate the hypertension (79, 80, 118). Central infusion of an MR blocker also prevents the salt-induced hypertension (77, 118). These findings indicate that central MR activation, mainly via aldosterone, mediates the salt-induced hypertension in Dahl S rats. Changes in the corticosterone level, however, may also contribute to the hypertension because trilostane, a steroid synthesis inhibitor that blocks both aldosterone and corticosterone synthesis, is even more effective than an aldosterone synthase inhibitor in preventing the hypertension (77).

The downstream effects of chronic icv infusion of Na⁺-rich CSF or aldosterone on hypothalamic EO and on BP in Wistar and Dahl S rats can also be prevented by icv infusion of benznamid, which blocks epithelial Na⁺ channels (ENaCs) (194, 258, 259). DOCA-salt hypertension and hypertension in Dahl S rats on a high-salt diet are also prevented by icv benznamid (3, 78, 137). ENaCs are members of the degenerin/ENaC superfamily of channels (161, 262), all of which are blocked by subnanomolar concentrations of amiloride and its analog, benznamid (32). Degenerin/ENaCs are expressed in many cell types, including epithelia, neurons, glia, and vascular smooth muscle, and some members of this superfamily are important for baroreceptor and autonomic control of the circulation (161) and for arterial myogenic constriction (124). Aldosterone modulates ENaC activity, among other effects, by regulating the ubiquitin ligase, neural precursor expressed, developmentally downregulated protein 4-2 (Nedd4-2), which ubiquinates ENaCs and thereby marks the channels for retrieval from the plasma membrane (PM) and for degradation.

Liddle’s syndrome (pseudoaldosteronism with hypertension) results from gain-of-function mutations in ENaC (153). The Nedd4-2-deficient mouse is a model of Liddle’s syndrome since ENaC function is upregulated because of reduced degradation (236). Preliminary data indicate that Nedd4-2-deficient mice develop hypertension in response to either a high-salt diet or icv Na⁺-rich CSF, that is blockable by icv benznamid (254). ENaCs in the choroid plexus may be important for the elevation of CSF [Na⁺] in salt-sensitive hypertension. The aforementioned data suggest, however, that Na⁺ entry through benzamil-sensitive ENaCs in magnocellular neurons (e.g., in the supraoptic or paraventricular nuclei) (3, 5, 260) may be crucial for the salt- and aldosterone-dependent elevation of BP. Thus, much of the hypertension in these primarily renal models may actually be attributable to brain mechanisms. Indeed, one possible mechanism for salt sensitivity is altered Na⁺ transport, not just in renal tubules but also in the brain (neurons and/or choroid plexus), as exemplified by this Nedd4-2-deficient model. In addition, the central sympathoexcitatory pathway appears to be less suppressed and, thus, more sensitive to the rise in CSF [Na⁺] in salt-sensitive animals (117, 254) and humans (135).

The roles of EO and ANG II in the brain. The effects of Na⁺-rich CSF on SNA, heart rate (HR, often taken as a measure of SNA), and BP are also mimicked by icv infusion of ouabain and blocked by the conjugation of EO with icv Digibind (111, 114) [antibody fragments that bind ouabain with high affinity (211)]. Intravenous infusion of the same total amount of Na⁺ or ouabain used in the icv infusions does not affect SNA, HR, or BP. Like icv Na⁺-rich CSF or icv aldosterone, a high-salt diet increases hypothalamic EO content in SHRs and Dahl S rats (149, 150). Thus it seems that Na⁺-rich...
CSF stimulates the synthesis of aldosterone in the brain and that this, in turn, triggers the local synthesis and secretion of EO. Furthermore, the aldosterone-induced alteration of AT1,R, NADPH oxidase, and nNOS protein expression may be mediated by an EO-α2 Na+ pump-protein kinase signaling cascade (see The ouabain/EO-Na+ pump-protein kinase signaling cascade) because it is blocked by Digibind (119).

The increases in SNA, HR, and BP, induced by both acute and chronic ivc infusion of ouabain and Na+–rich CSF in rodents, are all apparently mediated by AT1,Rs because the effects are blocked by central infusion of AT1,R antagonists (72, 113, 114, 256, 274). These effects are also markedly attenuated in rats with a genetically reduced brain renin-angiotensin system (110). Furthermore, in mice, these effects depend on the integrity of the high ouabain-affinity binding site on Na+ pumps with an α2 catalytic subunit (255), and they are abolished by ivc infusion of Digibind (256). Conversely, the effects of ivc Na+-rich CSF are augmented in α2-−/− mice (109), perhaps because the hemizygous null mutation is the equivalent of ∼50% inhibition of α2 Na+ pumps by EO. Digibind, however, does not affect the increase in HR or BP induced by ivc ANG II (256). The implication is that brain EO activates the brain renin-angiotensin system, which then increases peripheral SNA. This, in turn, promotes arterial vasoconstriction and thereby elevates BP.

The brain mechanisms that control the BP set point (the central barostat) (199, 278) are also likely regulated by these BP neuromodulatory mechanisms. The nucleus tractus solitarius in the medulla, which receives input from other medullary cardiovascular control centers as well as from peripheral baroreceptors, serves as a comparator to detect the offset between the set point and baroreceptor signals. The nucleus tractus solitarius projects to the paraventricular nucleus and hypothalamic circumventricular organs, such as the subfornical organ, that are directly involved in sympathoexcitation (278) and, as just described, are modulated by the Aldo-EO-ANG II pathway (117, 148).

In sum, the aforementioned data indicate that the Aldo-EO-ANG II pathway plays a crucial role in the sympathoexcitation and hypertension induced by Na+-rich CSF as well as by a high-salt diet in SHRs and Dahl S rats. Figure 3 illustrates some of the key steps in this proposed neuromodulatory pathway; additional details and references are provided in Leenen (148).

We now turn our attention to the targets of the sympathoexcitation, and, in particular, to the arteries that are responsible for the augmented vascular tone and increased peripheral vascular resistance, the hallmark of most chronic hypertension.

Peripheral sympathetic innervation and the consequences of increased sympathetic drive. Tonic sympathetic drive is the predominant mechanism for maintaining vascular tone, and a block of α1-adrenoceptors in vivo rapidly lowers arterial myocyte [Ca2+]s, dilates arteries (280), and reduces BP (214). Thus augmented SNA should elevate BP directly. This sympathetic nerve-mediated vasoconstriction and elevation of BP must surely come into operation when the central sympathetic drive increases, as in the case of a sudden, short-term rise in CSF [Na+]. Compensatory mechanisms, including the baroreflex response and endothelium-mediated vasodilation, should, however, counter the tendency for a sustained, large elevation of BP unless they are downregulated (34, 36), a subject we address below.

The topic of increased SNA usually focuses on augmented norepinephrine release and rapid activation of vasoconstriction; the roles of the cotransmitters, ATP and neuropeptide Y (NPY), are often ignored. Release of the cotransmitters is greatly influenced by the pattern of SNA and increases with bursts of activity; moreover, ATP and NPY both modulate the effects of norepinephrine on smooth muscle (268). Of particular interest in the present context is the fact that NPY, especially, is a mitogenic agent and growth regulator that may make an important contribution to vascular remodeling (59, 247).

The preceding discussion has implied that the increase in sympathetic activity is generalized, involving all vascular beds, but this may not necessarily be the case, and region-specific increases in SNA (e.g., in the splanchnic bed) has been observed in some forms of hypertension (130, 197). Enhanced renal SNA can be expected to promote NaCl retention (46, 47) and thus serve as a positive feedback mechanism for maintaining the augmented SNA. Although still somewhat controversial, much evidence indicates that the activation of renal afferent nerves, too, may increase sympathetic drive and thus promote or sustain vasoconstriction and hypertension (38, 240). Indeed, renal denervation can prevent or alleviate hypertension in several rodent models (46) and lower BP in drug-resistant humans (47). Renal denervation does not, however, prevent the development of DOCA-salt hypertension in rats (54, 130; but see 122, 133). Finally, because the adrenal cortex is innervated by sympathetic nerves (45, 250) and adrenal EO secretion is stimulated by catecholamines (174), we speculate that the enhanced SNA might also help to elevate plasma EO, the effects of which are discussed next.

Circulating EO and Its Actions

Salt, circulating EO, and hypertension. The preceding discussion indicates that an increase in brain ouabain is sufficient to raise BP. Nevertheless, in many patients with essential hypertension and in many salt-sensitive experimental models, the ouabain/EO level in the circulation is elevated; moreover, in essential hypertensive patients, plasma EO is directly correlated with BP (172, 179). Indeed, both peripheral and central mechanisms have been suggested to contribute to the ability of dietary salt to raise BP (100), and we next address the role of EO in the periphery.

High plasma EO levels are observed in about 45% of patients with essential hypertension (172, 206, 219) and in many patients with aldosterone-producing adenomas (180, 219). In the latter cases, excision of the adenomas normalized the EO level (180). Plasma EO levels are also elevated in several rodent hypertension models, including DOCA-salt, reduced renal mass, the Milan hypertensive strain, Dahl S rats on a high-salt diet, and adrenocorticotropin hormone (ACTH)-induced hypertension (50, 66, 67, 97, 129, 142, 149, 242).

Indeed, in high-EO hypertension models (ACTH, DOCA-salt, and reduced renal mass), systemic administration of Digibind lowers BP or prevents the hypertension (50, 129, 142). Also, in the Milan hypertensive strain, the systemic administration of the ouabain antagonist PST-2238 (rostafuroxin) lowers BP (168). Moreover, as already mentioned, chronic subcutaneous administration of ouabain, per se, elevates the circulating ouabain level and induces hypertension (175, 277). In ouabain-
treated rats, high ouabain levels are observed not only in the circulation but also in the kidneys, hypothalamus, and posterior pituitary; the levels in these tissues often exceed those in the circulation but correlate with the plasma level and with BP (175). The implication is that key tissues involved in cardiovascular regulation and electrolyte homeostasis specifically accumulate ouabain. A notable exception is the adrenal gland, whose high EO content is independent of the circulating ouabain level (175).

Importantly, for the ensuing discussion, neither ACTH nor ouabain induces hypertension in mice with mutant, ouabain-resistant α2 Na+ pumps (α2R mice) even though ACTH increases plasma EO in these mice (50, 52, 160). The latter results emphasize the importance of α2 Na+ pumps and their high-affinity ouabain binding site in the pathogenesis of some forms of hypertension.

In both normal men and hypertensive subjects, the acute intravenous infusion of saline has negligible effect on plasma EO (172, 173), but a sustained increase in dietary salt does increase plasma EO (101, 169). In the hypertensive subjects, the dietary salt-induced rise in EO was directly correlated with the rise in BP (101). Salt depletion with a thiazide diuretic also increases plasma EO, however, indicating that the relationship between (dietary) salt and EO is V-shaped in the short term (99, 169); when salt is restricted, EO may be needed, along with activation of the renin-angiotensin system, to help sustain the BP. The salt restriction-induced elevation of plasma EO might suggest that EO is not natriuretic, but chronic administration of anti-ouabain antibodies to normal rats, to bind up circulating EO, induces transient salt retention, implying that the EO normally is natriuretic (193). The mechanism(s) by which high dietary salt and salt depletion induce the adrenals to secrete EO and whether this involves the brain and increased SNA, or direct action on the adrenals, are unknown.

**Adrenal EO secretion and circulating EO.** The adrenals contain large amounts of EO (97, 229, 244), and the plasma EO level is greatly reduced by the removal of the adrenal cortex (97, 162, 175). Moreover, humans with adrenocortical insufficiency have very low EO levels that do not respond to ACTH (238). Conversely, plasma EO levels are elevated in mineralocorticoid hypertension (82, 97, 140, 219). These data all indicate that most of the circulating EO is derived from the adrenals, although a hypothalamic source, which likely mediates the local effects in the brain, has also been suggested (150).

Both in vivo data and data from primary cultured bovine adrenocortical cells reveal that EO secretion is stimulated by ACTH, ANG II, and catecholamines (50, 169, 174, 234, 238). De novo EO biosynthesis by primary cultured adrenal glomerulosa cells is well documented (28, 144, 233); i.e., adrenal stimulation increases the EO concentration in the medium without affecting the content in the adrenocortical cells (144). Furthermore, femoral artery and adrenal vein catheterization studies demonstrate that EO is secreted by the adrenals in awake dogs (28); the secretory rate is unaffected by acute plasma volume expansion. Interestingly, secreted EO appears to suppress its own secretion; i.e., there is feedback inhibition (144). Removal of this inhibition by binding up secreted EO might explain why the chronic administration of anti-ouabain antibodies causes the adrenal cortex to hypertrophy (193).

While details of the EO biosynthetic pathway remain to be elucidated, some information is available. Preliminary data show that the steroid moiety in EO, unlike that in MBG, is derived from the side-chain cleavage of cholesterol and progesterone by a pathway that shares early intermediates (e.g., pregnenolone and progesterone) with aldosterone biosynthesis (98). The enzymes that mediate formation of the lactone ring, the inversions at carbons 5 and 14, and the numerous hydroxylation have not yet been identified. Mass spectroscopy and NMR studies show that the sugar in EO is a 6-deoxypentose (134, 182). The sugar in plant ouabain is l-rhamnose, but despite extensive NMR analysis (134), the epimeric form of rhamnose in EO has not been resolved. Interestingly, l-rhamnose in mammals is metabolized primarily in the adrenals and hypothalamus (218).

Does circulating EO help maintain normal BP? When circulating (free) EO was reduced by ~50% by infusion of normal rats with anti-ouabain antibodies, mean BP (tail cuff) was not affected, although the adrenal cortex hypertrophied, presumably to help increase EO production (193). Severe hypotension may occur in adrenocortical insufficiency because of the loss of aldosterone and cortisol, but perhaps also in part because of the lack of adrenal EO production and very low plasma EO levels (238). Also, as already noted, the EO level rises in normal human subjects when a diuretic is administered to deplete body Na+ (169); this response suggests that EO may help prevent a drop in BP in this case. Finally, BP normally declines modestly during the first two trimesters of pregnancy in normal women and rodent dams and then rises to or slightly above the prepregnancy level during the third trimester. In pregnant α2R mice, however, the third trimester rise in BP is significantly attenuated (200). This implies that the α2 Na+ pump high-affinity ouabain binding site and its ligand EO play a role in helping to maintain normal physiological BP in pregnancy.

**Acute effects of ouabain and EO: augmentation of Ca2+ signaling and vasoconstriction.** We now consider the direct effects of low-dose ouabain (and EO) on arteries. Almost all of the studies described in this section were performed with plant ouabain because sufficient purified EO is not available. A single study, however, performed with human EO indicates that ouabain and EO have virtually identical acute effects on arteries and on cardiac muscle (29). Here we summarize the evidence that many of the observed functional and structural alterations in arteries from hypertensive humans and animals (16, 27, 74, 210, 239, 292) may be consequences of the elevated plasma EO. Indeed, the increased SNA and altered arterial function should be synergistic in driving sustained BP elevation.

The cardiotonic effects of low-dose cardiotonic steroids are widely recognized: inhibition of α2 Na+ pumps raises sub-PM [Na+]*. This leads to an NCX-mediated gain of [Ca2+] and augmentation of cardiac contraction (23, 51). Similarly, low-dose ouabain (and EO (29)) augments Ca2+ signaling, vascular myogenic tone, and agonist-induced arterial constriction in rodents (Fig. 4); the ouabain EC50 is on the order of 10–9 M (10, 215, 266, 283). Infusion of low-dose ouabain in humans, too, leads to acute vasoconstriction and increased TPR (178).

Low-dose ouabain usually does not, per se, initiate contractions (e.g., it does not constrict arteries without tone), but it does modulate contractions induced by other means such as increased intraluminal pressure (215, 283) and SNA (215). It
seems apparent that the critical effect of ouabain is, indirectly, via NCX, to elevate cytosolic \([\text{Ca}^{2+}]\) and activate myosin light chain (MLC) kinase (104). Phosphorylation of MLC by the kinase is required for tonic arterial constriction and for the regulation of BP and maintenance of salt-induced hypertension (104). Following activation of contraction, the arterial constriction may be sustained by the activation of the RhoA/Rho kinase (ROCK) pathway and consequent phosphorylation and inactivation of MLC phosphatase (166). The implication is that the augmented vasoconstriction in salt-dependent hypertension is unlikely to be initiated by the activation of the RhoA/ROCK pathway [contrast (269)].

We stress the term “low-dose ouabain” (or EO), by which we mean subnanomolar or low-nanomolar concentrations. In rodents, these concentrations block only \(\alpha_2\) Na\(^{+}\) pumps (195), which comprise only about 20% of the expressed Na\(^{+}\) pumps in most cells (75, 235, 283). These low concentrations have negligible effect on the membrane potential but do enhance NCX-mediated Ca\(^{2+}\) signaling (283). Even the knockout of \(\alpha_2\) Na\(^{+}\) pumps may induce only a small (~1 to 2 mM) increase in cytosolic \([\text{Na}^{+}]\) (75). The lack of depolarization when only a small fraction of the total electrogenic Na\(^{+}\) pump population is inhibited is consistent with theoretical considerations (19). In contrast, higher (micromolar) ouabain concentrations inhibit low-ouabain affinity (in rodents) \(\alpha_1\) Na\(^{+}\) pumps, the majority of expressed pumps in most cells, including arterial myocytes. This depolarizes the cells and, in myocytes, opens voltage-gated Ca\(^{2+}\) channels and triggers arterial constriction. The underlying mechanisms seem to be very similar in humans and rodents, but human \(\alpha_1\) Na\(^{+}\) pumps have high affinity for ouabain (18). The functional significance of this ouabain affinity difference in \(\alpha_1\) needs to be determined.

In this review, we focus on arterial function, but veins, too, must be mentioned. Venous smooth muscle contraction is governed by similar mechanisms, including Na\(^{+}\) transport and NCX (146). Moreover, there is abundant evidence that veins, too, are constricted in systemic hypertension, so that venous compliance and capacitance are reduced and blood volume is translocated to the arterial side of the circulation (198, 225). Indeed, all blood vessels appear to be involved, including pulmonary vessels (89), and even Ca\(^{2+}\) signaling in platelets.
may be augmented in essential hypertension (43). Such broad effects are strong circumstantial evidence that humoral mechanisms are involved (20), although neural mechanisms are also implicated in the widespread vascular changes (198).

In arteries, as in the heart, the effects of low-dose ouabain on Ca$^{2+}$ signaling and augmentation of constriction are mediated by α2 Na$^+$ pumps and NCX (Fig. 4) (123, 216). The effects of ouabain on arteries are largely attenuated in mice with mutant, ouabain-resistant α2 Na$^+$ pumps (52); 2 mimicked by reduced α2 Na$^+$ pump expression (equivalent to pump inhibition) (26, 282) and by transgenic expression of a tumor protein that inhibits α2 Na$^+$ pumps (128) and induces hypertension in mice (138); 3 blocked by the NCX antagonists, SEA0400 and KB-R7943 (120, 282); and, 4 greatly attenuated in arteries from mice with smooth muscle-specific knockout of NCX type 1 (NCX1) (284). Also, as in the heart, comparable Ca$^{2+}$ signaling and cardio-/vasotonic effects are observed with a variety of other cardiotonic steroid/Na$^+$ pump inhibitors including ouabagenin, digoxin, and digitoxin (10, 30, 33, 73).

Significantly, even though a prolonged administration of ouabain-like Strophantus steroids such as ouabain and ouabagenin induces hypertension in rats, the digoxin-like Digitalis steroids, digoxin and digitoxin, do not (Fig. 2) (139, 170). Indeed, digoxin and digitoxin antagonize the hypertensinogenic effect of ouabain (Fig. 2) and high dietary salt in rats (112, 171). Thus the acute vasotonic effects of ouabain cannot alone explain the hypertensinogenic action of prolonged ouabain administration or long-term exposure to elevated plasma EO.

**Long-Term Effects of Ouabain/EO: the Concept of Functional Remodeling**

We have already alluded to the distinction between the rapid, direct stimulation of central sympathoexcitatory drive and a slower, sustained neuromodulatory mechanism that is mediated by the Aldo-EO-ANG II pathway in the brain. An analogous situation prevails in the vasculature. Specifically, EO induces alterations in protein expression that modulate Ca$^{2+}$ homeostasis and Ca$^{2+}$ signaling in arterial myocytes and endothelial cells; this gives rise to many of the functional and structural modifications in the vasculature that are observed in hypertension. It seems appropriate to characterize this EO-induced sequence of events as “functional remodeling,” a term that is also applicable to the brain mechanisms.

In arterial smooth muscle as well as in neurons and some other types of cells, Ca$^{2+}$ signaling is regulated by several PM proteins that localize to PM microdomains which orchestrate functional (subsurface) sarcoplasmic reticulum. These proteins include the ouabain-sensitive α1 (α1 in neurons) Na$^+$ pumps, NCX1, and various transient receptor potential cation channel (TRPC) proteins (126, 127, 147, 164, 292). The latter are components of receptor- and store-operated, cation-selective channels (ROCs and SOCs, respectively) (48, 107, 264) (Fig. 4). These proteins are functionally coupled; they uniquely link Na$^+$ and Ca$^{2+}$ homeostasis and play important roles in controlling arterial tone (25). It is noteworthy that the much more prevalent α1 Na$^+$ pumps, which are ouabain resistant in rodents, may be excluded from the PM-junctional sarcoplasmic reticulum regions in the myocytes (Fig. 4) (147).

One suggestion is that ROCs, which are relatively nonsel ective cation channels, may admit primarily Na$^+$ ions that, in turn, promote Ca$^{2+}$ entry by NCX (9, 208). Moreover, it is possible that other Na$^+$-selective channels that have been identified in vascular smooth muscle but not yet tested in this context might also contribute to the Na$^+$ entry that feeds NCX-mediated Ca$^{2+}$ entry. Some examples are voltage-gated Na$^+$ channels (17, 145, 226, 288), TRPM4 channels (55, 56), and mechanosensitive vascular ENaC protein channels (124, 125). Interestingly, aldosterone directly constricts arteries (87, 243) and may also induce arterial remodeling (243), but whether or not these effects are mediated by the ENaC channels is unknown.

**NCX1 and TRPC protein upregulation in arterial myocytes.** Arterial smooth muscle NCX1, and TRPC6 and/or TRPC3 (ROC), proteins are upregulated in ouabain-hypertensive rats (210) and in a number of other rodent hypertension models (12, 74, 292) and in pulmonary artery myocytes in human primary pulmonary hypertension (276, 286) (Table 1). This protein upregulation is a clue to the origin of the augmented arterial responsiveness in hypertension (74). One functional consequence of NCX1 and TRPC6 upregulation is the enhanced Ca$^{2+}$ signaling in freshly isolated arterial myocytes from hypertension models including ouabain hypertension (210, 292). The fact that several other models listed in Table 1, NCX1SM/Tg/Tg, smooth muscle-specific Na$^+$/Ca$^{2+}$ exchanger-1 (NCX1) knockout mouse (284); ND, not determined.

### Table 1. Expression of NCX1 and some TRPC protein components of ROCs is increased in several hypertensive animal models and in human primary pulmonary hypertension

<table>
<thead>
<tr>
<th>Artery Smooth Muscle</th>
<th>Hypertension</th>
<th>ROC</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NCX1</td>
<td>TRPC3</td>
<td>TRPC6</td>
</tr>
<tr>
<td>1 Ouabain (vs. vehicle and digoxin)</td>
<td>↑</td>
<td>↑</td>
<td>(210, 293)</td>
</tr>
<tr>
<td>2 DOCA-salt*</td>
<td>ND</td>
<td>↑</td>
<td>(12)</td>
</tr>
<tr>
<td>3 Milan hypertension* (vs. Milan NT)</td>
<td>↑</td>
<td>↑</td>
<td>(292)</td>
</tr>
<tr>
<td>4 SHR (vs. WKY)</td>
<td>↑</td>
<td>↑</td>
<td>(156, 245)</td>
</tr>
<tr>
<td>5 Dahl salt-sensitive/high (vs. low) salt*</td>
<td>↑</td>
<td>↑</td>
<td>V. Golovina (unpublished)</td>
</tr>
<tr>
<td>6 NCX1SM/Tg/Tg</td>
<td>↑</td>
<td>ND</td>
<td>L. Chen, M. Li, M. Blaustein (unpublished)</td>
</tr>
<tr>
<td>7 Angiotensin II</td>
<td>↑</td>
<td>↑</td>
<td>(276, 286, 287)</td>
</tr>
<tr>
<td>8 Human primary pulmonary hypertension</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

*Hypertension associated with elevated plasma ouabain levels. TRPC, transient receptor potential cation channel; ROCs, receptor-operated channels; NT, normotensive control; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto normotensive control for SHR; NCX1SM/Tg/Tg, smooth muscle-specific Na$^+$/Ca$^{2+}$ exchanger-1 (NCX1) knockout mouse (284); ND, not determined.
including DOCA-salt, Dahl S, and Milan hypertensive rats, have elevated plasma EO levels (62, 68, 95, 97, 149) fits the view that this protein upregulation is triggered by EO. These observations contradict the statement that “functional changes in the vasculature (smooth muscle)... have... not been found (in hypertension)” (192).

The ouabain/EO-Na$^+$ pump-protein kinase signaling cascade. Importantly, $\alpha_2$ Na$^+$ pump and, especially, NCX1 and TRPC6, protein expression and function are augmented in primary cultured normal rat arterial myocytes treated with 100 nM ouabain for 48–96 h in vitro (210). The implication is that upregulation of these proteins, which may well account for the enhanced vascular responsiveness in hypertension (16, 27), is not a consequence of either the augmented SNA or the elevated BP. The ouabain-induced enhancement of protein expression might be due to a ouabain/Na$^+$ pump-activated protein kinase signaling cascade, mechanisms that have been receiving increasing attention (151, 158). There is strong evidence for an ouabain-activated, Na$^+$ pump-mediated Src kinase signaling cascade (the Na$^+$ pump signalosome). This cascade acts through MAPKs, including ERK1/2 and P38, and other kinases (Fig. 5), to effect alterations in protein expression and phosphorylation in a cell type-specific manner (8, 151, 157, 273, 285). Another Na$^+$ pump-linked protein kinase cascade involves phosphatidylinositol 3-kinase, PKD, and Akt (159). These mechanisms may affect not only the NCX1 and TRPC protein expression in the arteries but the protein expression in the brain as well (see The Hypothalamic and Enhanced Sympathetic Drive), and they may also contribute to arterial structural remodeling.

As the aforementioned observations indicate, many of the downstream effects of ouabain are mediated by NCX. Indeed, the upregulation of TRPC6 by ouabain is mediated through NCX1 and is abolished by knockout of NCX1, but the effect of ouabain on NCX1 is unaffected by knockout of TRPC6 (210). Thus NCX might be a good target for novel antihypertensive agents (121). In fact, the NCX antagonist, SEA0400, effectively lowers BP specifically in salt-dependent forms of experimental hypertension (120).

Ouabain-digoxin antagonism. The augmented expression of $\alpha_2$ Na$^+$ pumps, NCX1, and TRPC6 is not simply due to Na$^+$ pump inhibition. Digoxin, like ouabain, inhibits Na$^+$ pumps (170) and augments myocyte Ca$^{2+}$ signaling (10) and arterial constriction (73). Yet, not only does digoxin not enhance the expression of these proteins in arterial myocytes in vivo or in vitro, but, instead, it blocks the NCX1 and TRPC6 protein upregulation induced by ouabain in vitro (293). This cardioactive steroid specificity correlates with the ability of ouabain, but not digoxin, to induce hypertension in the rat and the ability of digoxin and digitoxin to antagonize ouabain-induced and salt-dependent hypertension (Fig. 2) (112, 171). Even in humans, a small uncontrolled study has suggested that digoxin lowers BP in some essential hypertensive patients (1). This implies that the ouabain binding site on $\alpha_2$ Na$^+$ pumps may be a prime target for the development of novel, specific antihypertensive therapies. One such compound, the ouabain antagonist, rostafuroxin, a derivative of digitoxigenin (213), is currently in phase 2 clinical trials (69, 143).

Regulation of endothelial function. Endothelial cells also express $\alpha_2$ Na$^+$ pumps, NCX1, and some TRPC proteins (36, 48, 64, 249). Arterial constriction activates an endothelial cell Ca$^{2+}$ signaling cascade that enhances endothelial NO synthase (eNOS) activity and NO production; this limits vasoconstriction because NO is a vasodilator (58, 279). In addition, endothelial cell activation releases another vasodilator, endothelium-derived hyperpolarizing factor (EDHF) (65). These vasodilator mechanisms, which are also activated by agonists such as acetylcholine and bradykinin (186), may be mediated by TRPC4-containing channels (71). The ability of acute low-dose ouabain to stimulate endothelial Ca$^{2+}$ signaling and endothelium-dependent vasodilation has been documented in a number of studies (36, 215). This may occur through both the NO-dependent (272) and NO-independent (81, 221–223) mechanisms. Acute exposure of cultured aortic endothelial cells or hand-dissected descending vasa recta (DVR) endothelium to nanomolar ouabain increases bradykinin-induced cytosolic Ca$^{2+}$ transients and enhances the release of NO (36, 49, 207).
Endothelial dysfunction, with reduced release of endothelium-derived relaxing factors, has been observed in essential hypertension (202, 205) as well as in various hypertension models, including Dahl S and DOCA-salt rats (39, 163). The consequences of chronic ouabain infusion on endothelial modulation of vasoactivity has also been frequently investigated in both cultured cells and acutely harvested macro- and microvascular preparations, but the findings have not been consistent. Rings derived from the main renal artery of ouabain-hypertensive rats retain NO-dependent relaxation (139). In aortic and caudal arteries, however, chronic ouabain exposure blunts phenylephrine-induced contraction by amplifying the vasodilatory influence of the endothelium through both EDHF and NO (224). Electric field stimulation of mesenteric arteries induces vasoconstriction that is highly sensitive to inhibition of nNOS, suggesting that vascular nNOS activity is amplified in ouabain-hypertensive animals (271). Similarly, chronic exposure to ouabain enhances endothelium-dependent NO- and prostaglandin-mediated vasodilation in mesenteric vessels, while attenuating the role of EDHF (201, 270). In contrast to these observations, chronic infusion of ouabain attenuated the acetylcholine and bradykinin-induced endothelial cytosolic Ca\(^{2+}\) transients as well as NO release in the microvascular DVR that perfuse the renal medulla (36). The latter is of particular interest because DVR endothelium has been predicted to be a major source of NO in the renal outer medulla (57) wherein NO inhibits Na\(^+\) reabsorption by adjacent nephrons (106). Attenuation of NO release by the DVR endothelium in ouabain-hypertensive rats may therefore contribute to Na\(^+\) retention as well as intramedullary vasoconstriction.

We postulate that this attenuation of NO release is due to downregulation eNOS activity as a consequence of prolonged exposure to plasma EO. Thus, as in neurons (84), the same signal (in this case, EO) can apparently induce opposite changes in the Ca\(^{2+}\) signaling mechanisms in different cell types, so that the integrated behavior of the target system (here, the artery) is modulated in a coherent fashion. In this case, the functional remodeling results in augmented Ca\(^{2+}\) signaling and contraction of arterial smooth muscle and attenuated endothelium-mediated vasodilation. The net effect is enhanced vasoconstriction in response to input signals, especially the SNA. In this way, arteries can sustain the vasoconstriction without excessive SNA activity and with reduced feedback inhibition (i.e., vasodilation) by the endothelium.

Effects of ouabain/EO on the peripheral sympathetic nervous system. In addition to its effects on the brain, the kidneys, and the vasculature, it is not surprising that ouabain/EO also apparently affects peripheral sympathetic neurons. Indeed, the augmentation of myogenic constriction of isolated small arteries by nonnular ouabain (see Acute effects of ouabain and EO) (215, 281, 283) is due, in part, to an ouabain-evoked increase in spontaneous neurotransmitter release from sympathetic nerve terminals. This component is blocked by the \(\alpha\)-adrenergic antagonist, prazosin (215). Furthermore, prolonged in vivo exposure to ouabain increases both short- and long-term potentiation of synaptic transmission in sympathetic ganglia (4); thus postganglionic SNA should be augmented for any given level of presynaptic input. It is not yet known whether similar mechanisms contribute to the elevation of BP in salt-dependent models of hypertension, but reduced accommodation and augmented synaptic transmission has also been observed in sympathetic ganglia from SHRs (165, 275).

In sum, both central and peripheral pressor mechanisms contribute to the elevation of BP in hypertensive humans and animals, and EO plays a key role in these processes. The relative contributions of the central and peripheral mechanisms, however, remain to be determined. These effects are synergistic, and BP cannot be elevated without a direct contribution from the arteries. Thus it will be important to develop new models and tools to distinguish the roles with certainty; moreover, the relative roles may vary depending on the model being studied.

**Does ouabain/EO also promote arterial structural remodeling?**

The structural remodeling of arteries in salt-sensitive hypertension must also be initiated by functional alterations linked to the salt, but the mechanisms are unresolved. One possibility is that the elevation of BP and resultant increase in arterial wall tension, induced by the mechanisms described in the preceding sections, directly triggers the arterial remodeling that produces wall thickening and lumen narrowing. Vascular wall remodeling has also been attributed to ANG II-triggered inflammation, oxidative stress, and the generation of reactive oxygen species (53, 176, 251), although the renin-angiotensin system should be suppressed in salt-dependent hypertension. Ouabain, per se, can promote cell growth and proliferation (7, 11, 217, 230), perhaps in part as a consequence of elevated cell [Na\(^+\)] and/or [Ca\(^{2+}\)]. Moreover, as already noted, ouabain can activate protein kinase signaling cascades (151, 157, 248). All of these mechanisms might directly contribute to the wall remodeling. Alternatively, or in addition, the enhanced sympathetic drive and/or augmented Ca\(^{2+}\) signaling in the arterial myocytes, described above, might cause oxidative stress and thereby induce arterial wall remodeling. If the remodeling and other target organ (heart, kidneys) damage in salt-dependent hypertension are consequences of EO action, it should be possible to antagonize or prevent these effects by selectively blocking the action of EO with agents such as ronofurox (see Ouabain-digoxin antagonism).

**Summary and Conclusions**

We have reviewed the evidence that, in response to a high-salt intake, EO is secreted by both the brain and adrenals and that it acts both centrally and peripherally to promote the elevation of BP and the excretion of salt. In addition to ouabain’s well-documented, acute augmentation of Ca\(^{2+}\) signaling and its consequent cardiotoxic and vasotonic effects, ouabain/EO mediates crucial, slow, modulatory pathways in both the brain and the periphery. In the hypothalamus, Na\(^+\) activates the Aldo-EO-ANG II pathway that enhances sympathoexcitatory mechanisms and sustains increased SNA. In the periphery, EO, whose plasma level is elevated by unresolved mechanisms, activates a Src-mediated protein kinase cascade that modulates Na\(^+\) and Ca\(^{2+}\) transporter protein expression and protein phosphorylation. This upregulates Ca\(^{2+}\) signaling mechanisms and contractile responses in arterial smooth muscle but downregulates Ca\(^{2+}\) signaling and vasodilatory responses in endothelial cells. There is a striking parallelism in the brain and the periphery: in both, EO-mediated signaling through \(\alpha_2\) Na\(^+\) pumps and the protein kinase cascade play a key role in the modulation, even though the signals appear to

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**Review**

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alter the expression of different proteins in the brain and periphery (119, 210, 292). These central and peripheral effects of EO, especially the slow, modulatory effects, are synergistic. The net result is enhanced vasoconstriction and thus increased TPR and maintained elevation of BP. These functional changes may also initiate the vascular structural remodeling often observed in chronic hypertension.

The genetic engineering experiments on the α2 Na\(^+\) pump and the actions of ouabain/EO summarized in this review provide compelling evidence that these Na\(^+\) pumps and their natural ligand, EO, play a fundamental role in the pathogenesis of salt-dependent hypertension. EO is emerging as one of a trio of key endocrine/paracrine agents that, along with aldosterone and ANG II, help regulate salt homeostasis, blood volume, and BP.

This concept of EO and its receptor provides a unifying explanation that ties together many disparate observations and fuses two seemingly divergent views of how salt leads to the elevation of BP (22, 148). It elucidates the way in which the four key organs/tissues, i.e., the brain, kidneys, adrenals, and arteries, function in a coordinated fashion to elevate BP and promote (pressure) natriuresis to defend against plasma volume expansion in the face of an excess salt load. Guyton and colleagues (93) focused on the kidneys, which may be responsible for salt retention and is the organ that mediates the natriuresis to maintain salt balance. That model largely ignored other important contributors to BP elevation and the long-term control of BP (90, 189), especially the brain and central sympathetic drive (196). Moreover, defective Na\(^+\) transport mechanisms usually linked to renal salt retention (153) may also be expressed in the brain (148). Here, they may circumvent the kidneys and directly influence central sympathoexcitatory control of SNA and BP (see The roles of aldosterone and ENaC in the brain).

A key feature of the Guyton-Coleman model is the evidence that acute plasma volume expansion initially elevates BP by increasing CO without affecting TPR (92). The delayed rise in TPR and concomitant decline in CO (whole body autoregulation) was attributed to vascular readjustment needed to avoid tissue overperfusion at high flow rates (92), but no mechanisms were identified. The elevated TPR with normal CO accounts for the elevated BP in chronic hypertension (41). We suggest that the elevated TPR can be explained by the activation of the brain Aldo-ENaC-EO-α2 Na\(^+\) pump-ANG II pathway (Fig. 3) and the arterial EO-α2 Na\(^+\) pump-Src kinase signaling cascade (Fig. 5). This helps us to understand the differing views of the critical changes that actually generate and sustain the elevated TPR and high BP, i.e., increased SNA, augmented arterial myocyte Ca\(^{2+}\) signaling and contraction, attenuated endothelial function, and perhaps even structural remodeling of the arterial wall. We suggest that all or most of these manifestations of hypertension are linked to excess dietary salt through EO (Fig. 1). Additionally, because EO is also a growth factor, it may even directly contribute to the target organ damage that contributes to the morbidity and mortality from hypertension (132).

Admittedly, some aspects of our proposal are speculative, but the general framework is strongly supported by the experimental and clinical data cited in this and other reviews (22, 26, 148, 154, 230). Furthermore, by its very nature, our proposal provides ideas for direct and explicit tests of some of the more speculative aspects. It will be essential to develop a more user-friendly, well-standardized EO assay, so that data misinterpretation arising from poorly standardized methods (e.g., use of antibodies of questionable cross-reactivity) can be avoided. Our proposal also highlights the α2 Na\(^+\) pump ouabain binding site and NCX1 as targets for the logical development of new antihypertensive therapies.

ACKNOWLEDGMENTS

We thank the members of our laboratories who contributed to the original studies that led to the hypothesis described in this article.

GRANTS

This work was supported by National Institutes of Health Grants R01-HL-45215 (to M. P. Blaustein and J. M. Hamlyn), R01-HL-78870 (to M. P. Blaustein, with subprojects and cores directed by M. P. Blaustein, W. G. Wier, V. A. Golovina, T. L. Pallone, and J. M. Hamlyn), R01-DK-67651 and R37-DK-42495 (to T. L. Pallone), R01-HL-09196 (to W. G. Wier), and R01-HL-107654 (to J. Zhang); by Canadian Institutes of Health Research Grants FRN:MOP-74432 and FRN:MOP-13182 (to F. H. H. Leenen); and by a Heart and Stroke Foundation of Ontario Grant-in-Aid NA-6324 (to J. W. Van Huysse). F. H. H. Leenen holds the Pfizer Chair in Hypertension Research, which is supported by Pfizer Canada, the University of Ottawa Heart Institute Foundation, and the Canadian Institutes of Health Research.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


AUTHOR CONTRIBUTIONS


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