

CALL FOR PAPERS | *Molecular Mechanisms Linking Salt to Hypertension*

How does salt retention raise blood pressure?

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Departments of ¹Physiology and ²Medicine and ³Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Maryland School of Medicine; and ⁴Division of Endocrinology, Department of Medicine, Baltimore Veterans Affairs Medical Center and University of Maryland School of Medicine, Baltimore, Maryland

Blaustein, Mordecai P., Jin Zhang, Ling Chen, and Bruce P. Hamilton. How does salt retention raise blood pressure? *Am J Physiol Regul Integr Comp Physiol* 290: R514–R523, 2006; doi:10.1152/ajpregu.00819.2005.—A critical question in hypertension research is: How is long-term blood pressure controlled? Excessive NaCl ingestion or NaCl retention by the kidneys and the consequent tendency toward plasma volume expansion lead to hypertension. Nevertheless, the precise mechanisms linking salt to high blood pressure are unresolved. The discovery of endogenous ouabain, an adrenocortical hormone, provided an important clue. Ouabain, a selective Na⁺ pump inhibitor, has cardiotoxic and vasotonic effects. Plasma endogenous ouabain levels are significantly elevated in ≈40% of patients with essential hypertension and in animals with several forms of salt-dependent hypertension. Also, prolonged ouabain administration induces hypertension in rodents. Mice with mutant Na⁺ pumps or Na/Ca exchangers (NCX) and studies with a ouabain antagonist and an NCX blocker are revealing the missing molecular mechanisms. These data demonstrate that α₂ Na⁺ pumps and NCX1 participate in long-term regulation of vascular tone and blood pressure. Pharmacological agents or mutations in the α₂ Na⁺ pump that interfere with the action of ouabain on the pump, and reduced NCX1 expression or agents that block NCX all impede the development of salt-dependent or ouabain-induced hypertension. Conversely, nanomolar ouabain, reduced α₂ Na⁺ pump expression, and smooth muscle-specific overexpression of NCX1 all induce hypertension. Furthermore, ouabain and reduced α₂ Na⁺ pump expression increase myogenic tone in isolated mesenteric small arteries in vitro, thereby tying these effects directly to the elevation of blood pressure. Thus, endogenous ouabain, and vascular α₂ Na⁺ pumps and NCX1, are critical links between salt and hypertension. New pharmacological agents that act on these molecular links have potential in the clinical management of hypertension.

ouabain; Na⁺ pump; Na/Ca exchanger; Ca²⁺; myogenic tone

HYPERTENSION, DEFINED AS A diastolic blood pressure (BP) ≥ 90 mmHg and/or systolic BP ≥ 140 mmHg, is endemic in Westernized societies. This is a very important public health issue because hypertension is a major risk factor for premature death and disability from heart attack, heart failure, stroke, and many other afflictions (16, 59). In the United States, alone, ≈20% of the population (i.e., ≈50 million individuals) are hypertensive; moreover, more than half of all individuals over the age of 60 years have hypertension. In a small fraction of cases, the hypertension is due to specific causes, such as renal vascular disease or excessive secretion of aldosterone (primary aldosteronism) or catecholamines (pheochromocytoma). The vast majority (≈90%) of patients, however, have elevated BP of unknown cause; hence the terms, primary or essential hypertension. The immediate cause for the elevated BP in nearly all chronic hypertensive persons is excessive narrowing of the small (resistance) arteries. Nevertheless, a key question in any

discussion of hypertension is: What specific mechanisms actually lead to the abnormal arterial constriction and elevation of BP?

SALT, PLASMA VOLUME, AND THE KIDNEYS

The pressure necessary to enable the blood to circulate is provided by the pumping action of the heart [cardiac output (CO)] and the tone of the arteries (peripheral resistance). The contraction of the heart propels blood through the arterial tree. However, it is the dynamic regulation of artery diameter, especially in the smaller branches of the tree, that controls BP and flow in the periphery. Acutely, BP and flow may change under the control of neural and humoral factors that can rapidly constrict or dilate local arterial segments and/or large arterial beds to meet short-term circulatory demands. Baroreceptor reflexes play an important role in rapidly resetting BP following acute changes and may also exert long-term control over sympathetic nerve activity and renal Na⁺ excretion in hypertension (69). Over the long term, however, BP is controlled primarily by salt and water balance because of the infinite gain property of the kidneys to rapidly eliminate excess fluid and

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salt (36, 37). Neither sinoaortic denervation (77) nor renal nerve denervation (21, 52) prevents the generation of salt-dependent forms of hypertension.

When renal function is reduced, a small increase in extracellular fluid (ECF) volume inevitably causes the BP to rise (36). The hypertension that develops as a result of salt retention, as in mineralocorticoid hypertension, is always preceded by increased plasma volume (42, 113). In the chronic state, the elevated BP promotes a pressure natriuresis so that normal ECF volume is restored at the expense of (chronically) elevated pressure (110). This is the typical situation in patients with chronic essential hypertension: normal (or even low-normal) blood volume and elevated BP (17, 59).

The ECF is, to a first approximation, an isotonic salt (primarily NaCl) solution, and the kidneys are the primary regulators of salt and water balance. Thus, it is hardly surprising that renal function and salt balance have been widely recognized as critical factors in the pathogenesis of hypertension for as long as this topic has been studied. This is exemplified by the statement, written about 1000 BC, that "if too much salt is used in food, the pulse hardens" (112), or the seminal studies of Bright demonstrating a link between the kidneys and hypertension (15, 88). The role of dietary salt in the pathogenesis of hypertension has been extensively documented in numerous reviews (e.g., Refs. 59, 80). Moreover, it has long been known that diuretic/natriuretic agents, such as hydrochlorothiazide, which directly counteract the tendency for salt and water retention, are effective antihypertensive agents in a large percentage of humans with essential hypertension (16, 28, 59). Also, renal transplant studies in humans and in animal models have demonstrated that "hypertension goes with the kidneys" (18, 35, 95).

GENETICS AND HYPERTENSION

There is substantial evidence for genetic influences on BP. A number of rare, monogenic defects in renal salt transport have clear effects on BP; those that promote NaCl retention are all associated with hypertension, and those that promote salt wasting are associated with hypotension (68, 81, 86). Indeed, mutation, knockout, or duplication of genes that affect BP all induce either salt-dependent forms of hypertension or hypotension, or unusual forms of salt-independent alterations in BP (109). The salt-independent forms are, in general, associated with genes that affect the synthesis and secretion of humoral vasoconstrictors or vasodilators.

The frequency of hypertension increases with age in Westernized societies (59). This has raised the possibility that the primary defect in the disease may, in most individuals, be a result of subtle renal injury rather than a genetic polymorphism (55). Indeed, many linkage studies have been unrewarding (5). Some recent studies, however, suggest that polymorphisms in G protein-coupled receptor kinase (GRK) and α -adducin genes may be associated with hypertension in subsets of the population (7, 25, 62, 119). GRKs regulate dopamine receptors that are involved in modulating renal proximal tubule Na^+ transport, especially during Na^+ excess (119). Adducins are cytoskeletal proteins, and some α -adducin variants augment the activity of Na^+ pumps (Na^+ - K^+ -ATPase) with α_1 -subunits and enhance Na^+ reabsorption in renal epithelia (25, 111).

In sum, the problem is that essential hypertension is a complex disease with polygenic (5, 7, 109, 119) and environmental contributions to the etiology; elevated BP is simply the common consequence. The central roles of the kidneys and salt and water balance in the pathogenesis of hypertension are readily apparent, but the primary renal defects are still unresolved in most cases. A topic that has generally been ignored, however, is precisely how the tendency toward salt retention and ECF volume expansion actually elevates the BP. This is the focus of our review.

BP-BLOOD VOLUME RELATIONS AND VASCULAR TONE

Mean arterial BP is a function of CO and total peripheral vascular resistance (TPR) (6) and, in mathematical terms, at constant CO, $\text{BP} \approx \text{CO} \times \text{TPR}$. CO, in turn, is directly related to ECF volume and the volume of the venous return to the heart. Indeed, Borst and Borst-de Geus (13) and Guyton and colleagues (36, 37) observed that acute plasma volume expansion elevates the BP by increasing CO. When the plasma volume expansion was maintained for more than 3–4 days, however, the BP remained elevated while CO declined toward normal levels. Thus, the elevation of BP was sustained because of an increase in TPR. Similarly, most patients with chronically elevated BP have a relatively normal CO and significantly elevated TPR (17, 59). Borst (13) and Guyton and their coworkers (36, 37) attributed the switch, from a high CO to an elevated TPR, to whole body autoregulation. According to Guyton, the tissue overperfusion is an abnormal condition, and TPR, therefore, increases until tissue perfusion returns to normal. One suggestion is that this autoregulation is controlled by the metabolic demands of the tissues (17, 36, 54), but this has been disputed (58), and no specific underlying molecular mechanisms have been described. Moreover, the autoregulation concept begs the question: Why is contractility also augmented on the venous side of the circulation (98) and in the pulmonary circulation (3, 34) in systemic essential hypertension? In contrast, the idea of whole body autoregulation (i.e., altered tone in the entire circulatory system) raises the possibility of a circulating agent that can affect all blood vessels. Surprisingly, despite many decades of research and hundreds of reports on the topic, a major challenge in the field of hypertension remains to "identify the key determinants of long-term blood pressure control" (83).

ENDOGENOUS OUABAIN AND OTHER ENDOGENOUS CARDIOTONIC STEROIDS

The idea that a circulating inhibitor of the Na^+ pump/ Na^+ - K^+ -ATPase (i.e., a ouabain-like or digitalis-like compound) might be such an agent, and might augment vascular tone in all blood vessels, was first raised in the mid-1970s (9, 10, 39). One proposal was that, by reducing Na^+ pumping, the inhibitor might depolarize vascular smooth muscle myocytes directly because Na^+ pumps are electrogenic and make a small contribution to the membrane potential (39). The depolarization should activate Ca^{2+} entry (presumably via voltage-gated Ca^{2+} channels), which would be expected to augment vasoconstriction. Such an effect can be true only transiently, however, because, in the steady state, Na^+ efflux must rise to equal the Na^+ influx. This occurs when a larger fraction of the unblocked pumps are activated by the slightly elevated cyto-

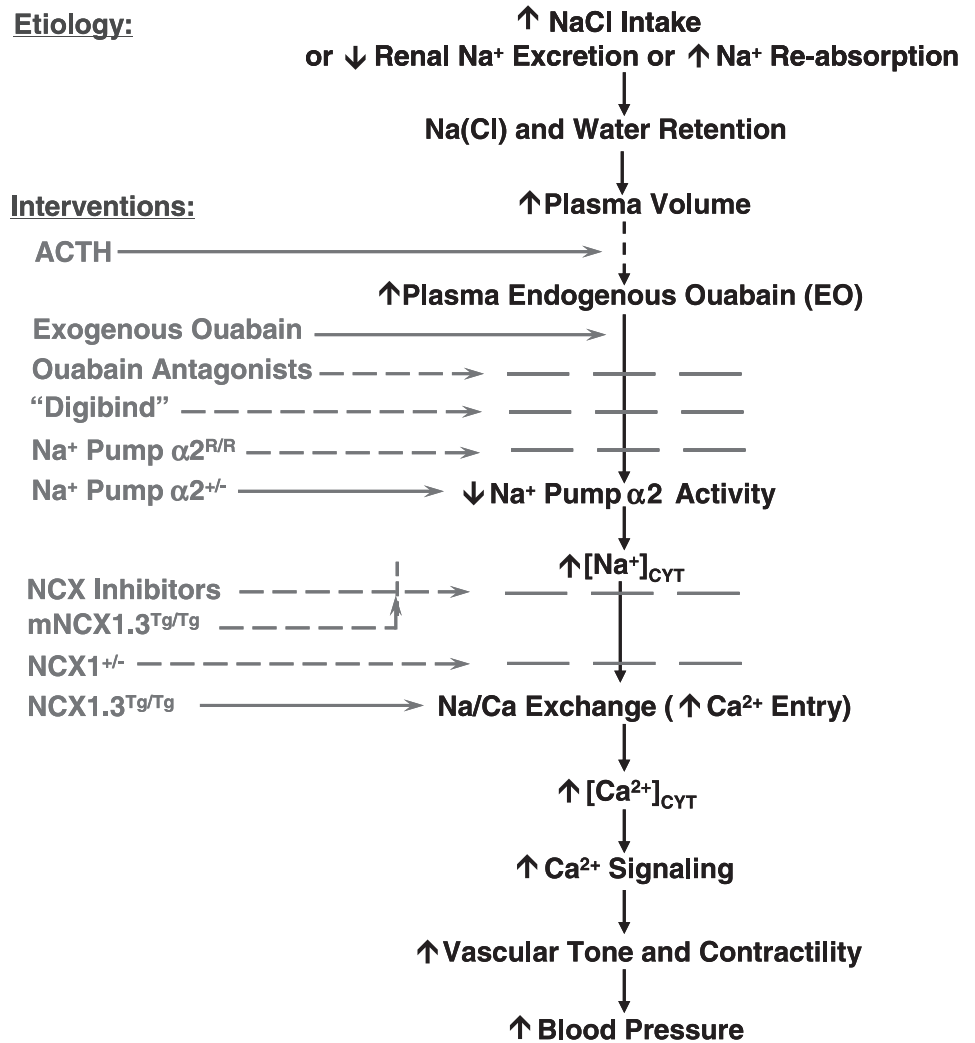


Fig. 1. Proposed sequence of steps leading from salt (NaCl) to hypertension. The sequence usually starts with a renal defect that leads to the retention of salt and water by the kidneys. Interventions discussed in the text include ACTH, ouabain, Digibind (digoxin-specific Fab fragment mixture that neutralizes digoxin and ouabain), ouabain antagonists (Rostafuroxin and canrenone), Na/Ca exchanger (NCX) antagonists (specifically, SEA0400), and mice with various mutations in the α_2 Na⁺ pump and NCX1. The broken vertical line between plasma volume and plasma endogenous ouabain (EO) indicates that the mechanism(s) is(are) not resolved. The broken horizontal lines correspond to the various interventions that inhibit the steps shown.

solic Na⁺ concentration ([Na⁺]_{CYT}) (11). Thus, in the steady state, there should be no reduction in the rate of Na⁺ pumping and in the electrogenic Na⁺ pump current (11).

According to an alternative proposal (see Fig. 1), the small, steady-state elevation of [Na⁺]_{CYT}, due to partial inhibition of the Na⁺ pump, should promote net Ca²⁺ gain due to increased Ca²⁺ entry or decreased Ca²⁺ exit via Na/Ca exchange (NCX) (9, 10). The resulting rise in the cytosolic Ca²⁺ concentration, [Ca²⁺]_{CYT}, should promote vasoconstriction and, in vivo, elevation of BP.

These ideas, and a preliminary report that a circulating Na⁺ pump inhibitor was directly correlated with BP in normotensive and hypertensive subjects (46), led to the intensive search for such a compound. This search culminated in the report, in 1991, that endogenous ouabain (EO), a substance either identical to the plant compound, or a stereoisomer, was the culprit (43). This compound was purified from human plasma and identified by immunoassay and mass spectroscopy (43, 78). EO, like its plant-derived counterpart, is a Na⁺ pump inhibitor that has cardiotoxic and vasotonic actions; indeed, these effects of EO and ouabain are indistinguishable (14, 43).

Initial studies revealed that EO did not come from the diet and that it was synthesized and secreted by the adrenal cortex (43). There are reports that EO also may be produced in the

brain (38) and that elevated cerebrospinal fluid levels (39) are associated with hypertension without an elevation in the circulating EO level (48).

A number of reports indicate that other cardiotoxic steroids are also present in human and animal plasma and tissues. These substances include digoxin (102, 103), proscillaridin A (101), and the bufodienolides (67, 70). Only plasma EO levels, however, have been directly correlated with BP in humans (89, 97) and in several animal models of hypertension (43, 75, 115, 117). Elevated EO levels were observed in about 40% of patients with untreated essential hypertension (97) in patients with primary aldosteronism (97) and in those with ACTH-induced hypertension (32, 33). Elevated EO levels were also observed in rodents with DOCA-salt hypertension (41, 43), ACTH-hypertension (20, 20a, 115), reduced renal mass hypertension (108), and in salt-sensitive Milan strain rats on a high-salt diet (22, 23). Indeed, a critical corollary to these reports is the evidence that prolonged administration of ouabain, itself (a *Strophanthus* steroid), but not digoxin or digitoxin (*Digitalis* steroids), elevates BP in normal rats and mice (19, 60, 72, 74, 75, 116).

The synthesis and secretion of EO, like aldosterone, occurs in adrenal glomerulosa cells (64, 105). It is stimulated by chronic high salt intake (73) and by ACTH (63, 64). Never-

theless, the mechanism(s) by which salt retention and plasma volume expansion promote EO synthesis and secretion are not yet resolved (see Fig. 1). Another unanswered question is: What maintains the elevated plasma EO level when plasma volume returns to normal in the chronic state? A likely possibility is that the plasma volume is still inappropriately high for the level of BP, and that it is this offset in the servocontrol system that maintains the elevated plasma EO (42).

The biosynthesis of EO utilizes cholesterol and progesterone as precursors (44, 45, 61, 87, 92) and follows the same initial steps as in plants and in aldosterone production. The EO biosynthetic pathway diverges from the aldosterone pathway either 1) at corticosterone, in which case there is an 11 β hydroxyl, forming a stereoisomer of ouabain, as suggested by some mass spectrometry and NMR data (44, 45) or 2) at 11-deoxycorticosterone, which allows an 11 α hydroxyl, as in ouabain itself.

HOW DOES LOW-DOSE OUBAIN AUGMENT CONTRACTILITY?

As indicated in the preceding section, the prevalent view of the mechanism of the cardiotoxic effect of cardiotoxic steroids is that these agents inhibit the Na⁺ pump and thereby cause [Na⁺]_{cyt} to rise. This, in turn, via NCX, elevates [Ca²⁺]_{cyt} and augments cardiac contractility (85). A similar mechanism should prevail in vascular smooth muscle, which also has an NCX (56, 96). This simple idea was, however, complicated by the discovery that there are four isoforms of the Na⁺ pump catalytic (α) subunit. The isoforms have different kinetic properties (e.g., different affinities for Na⁺), may have very different affinities for cardiotoxic steroids, and are independently regulated (8).

Functional Na⁺ pumps are $\alpha\beta$ -dimers in which the small β -subunit may be a chaperone that is required for function and that modulates α -subunit activity. The α -subunit contains the cardiotoxic steroid binding site as well as the Na⁺, K⁺ and ATP binding sites (8). All cells express Na⁺ pumps with an

α_1 -subunit. The α_1 -pumps, which are the predominant isoform in most cells, are the housekeepers responsible for maintaining the low bulk [Na⁺]_{cyt} (30). In humans and most other mammals, α_1 has high affinity for ouabain (low nanomolar K_d), but in rodents α_1 has a 100- to 1,000-fold lower ouabain affinity (84). Most cells also express either α_2 or α_3 Na⁺ pumps (sperm express α_4) and, in contrast to α_1 , the high ouabain affinity of α_2 and α_3 (K_d s in the low nanomolar range) (84) has been conserved through mammalian evolution (90).

Another key difference is that α_1 appears to be relatively uniformly distributed in the plasma membrane (PM). In striking contrast, at least in some cell types, including vascular smooth muscle myocytes, neurons, and glia, α_2 and α_3 are confined to PM microdomains closely juxtaposed to the underlying sarco(endo)plasmic reticulum (SER) (56, 57, 106). Indeed, preliminary results suggest that α_1 may be excluded from these junctional microdomains (65). Moreover, the NCX also is confined to the junctional PM microdomains in arterial myocytes, neurons, and glia (56), suggesting that the Na⁺ pumps with the α_2 - or α_3 -subunit and the NCX function cooperatively. This fostered the concept that the PM microdomains and the adjacent, junctional SER (jSER) form a specialized unit, the "PLasmERosome" (Fig. 2) that helps regulate Ca²⁺ homeostasis and mediates the action of cardiotoxic steroids (12). We suggested that diffusion of cations between the tiny junctional space (JS; between the PM and jSER) and bulk cytosol must be restricted to enable α_2 and α_3 , which have much lower affinity for Na⁺ than α_1 (43), to function at normal, low bulk [Na⁺]_{cyt} (4, 30). Thus, the α_2 and α_3 Na⁺ pumps can help indirectly, via NCX, to regulate the Ca⁺ concentration, not only in the tiny JS (i.e., [Ca²⁺]_{JS}) (4, 30), but also within the SER ([Ca²⁺]_{SER}) and even in bulk cytosol (i.e., [Ca²⁺]_{cyt}). This would explain how low concentrations of cardiotoxic steroids can modulate Ca²⁺ homeostasis and contractility in cardiac and arterial myocytes even in rodents, where α_1 has such a low affinity for these agents.

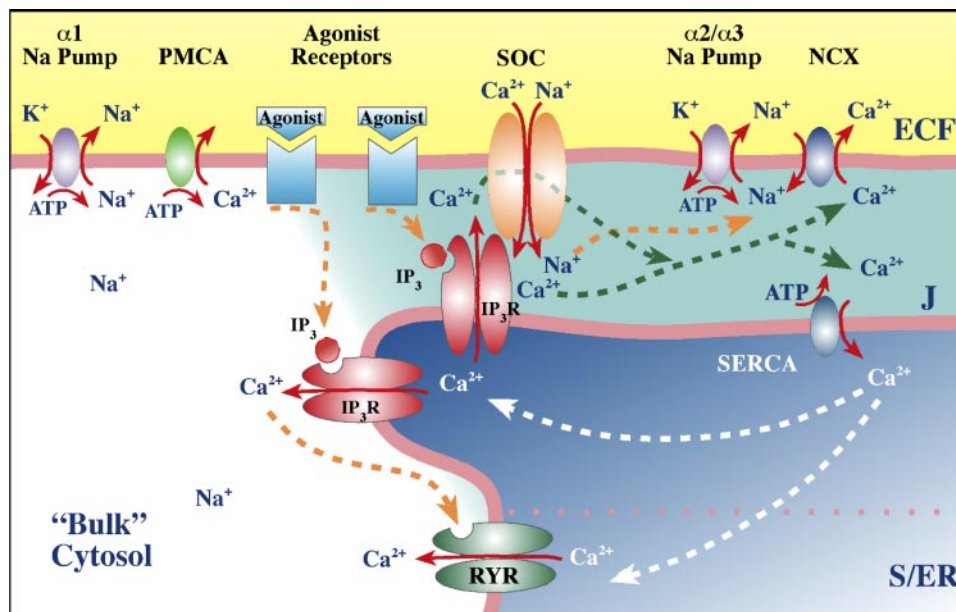
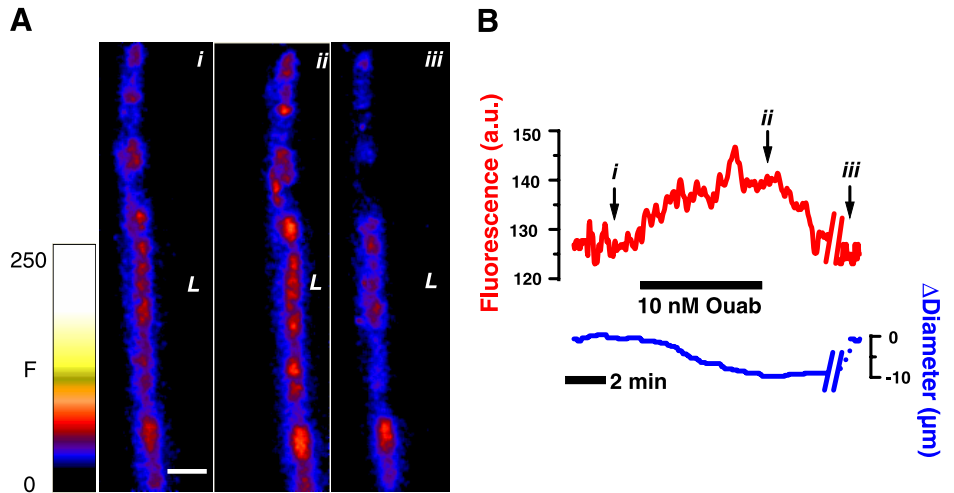


Fig. 2. Model of the PLasmERosome [plasma membrane-junctional sarco(endo)plasmic reticulum; PM-jSER] region showing key transport proteins involved in local control of jSER Ca²⁺ stores and modulation of Ca²⁺ signaling. The PM region shows vasoconstrictor (agonist) receptors and a nearby PM microdomain containing store operated channels (SOCs), α_2/α_3 Na⁺ pumps, the NCX, adjacent jSER with sarco(endo)plasmic reticulum Ca-ATPase (SERCA), inositol 1,4,5-trisphosphate receptors (IP₃R), ryanodine receptors (RYR), and the intervening diffusion-restricted cytosolic space (J). [Na⁺]_J will rise in the restricted (aqua colored) space following inhibition of α_2/α_3 Na⁺ pumps by low-dose ouabain. Initially, the local Ca²⁺ concentration, [Ca²⁺]_J, will rise, but, in the steady state, [Ca²⁺]_{SER} and global [Ca²⁺]_{cyt} will also rise. The α_1 Na⁺ pumps and PM Ca²⁺ pumps (PMCA) are widely distributed in the PM but may be excluded from these PM-jSER microdomains. ECF, extracellular fluid. Broken lines: ion diffusion paths. (Reproduced from Ref. 30 with permission.)

Fig. 3. Effects of 10 nM ouabain (Ouab) on $[Ca^{2+}]_{\text{cyt}}$ and myogenic tone (MT) in a mesenteric small artery. *A*: fluo-4 pseudocolor images from a representative wild-type mouse artery captured at the times (*i-iii*) indicated in *B*. Intraluminal pressure in the artery was raised to 70 mmHg at 35°C. MT then developed [i.e., the artery constricted from 130 μm external passive diameter (PD) to 101 μm external diameter] before these data were obtained. F, fluorescence; L, artery lumen. *B*: simultaneous $[Ca^{2+}]_{\text{cyt}}$ and diameter changes during exposure to 10 nM ouabain in the artery in *A*. Scale bar = 10 μm . a.u., Arbitrary units. Figure reproduced from Ref. 120 with permission.



THE α_2 Na⁺ PUMP: CENTRAL ROLE IN SALT-DEPENDENT HYPERTENSION

With this concept in mind, we return to the question of the linkage between salt retention and hypertension. In view of the evidence (see *Endogenous ouabain and other endogenous cardiotonic steroids*) that EO levels are elevated in many humans with essential hypertension and in all tested animal models of salt-dependent hypertension, we examined the effect of ouabain on myogenic tone in mouse isolated mesenteric small artery segments. Low-dose ouabain elevated $[Ca^{2+}]_{\text{cyt}}$ and increased myogenic tone [the spontaneous constriction evoked by intraluminal pressure (48)] (Fig. 3). This response was unaffected by α -adrenergic blockade and, thus, was not due to catecholamine release from sympathetic neurons in the artery wall. The effect of ouabain also was maintained following endothelium removal (120). The half-maximally effective concentration of ouabain (EC_{50}) was 1.3 nM (120). This ouabain concentration should inhibit just the α_2 Na⁺ pumps in mouse artery myocytes, which express only α_1 and α_2 Na⁺ pumps (106, 120).

Heterozygous mice with a null mutation in either one α_1 or one α_2 gene (i.e., $\alpha_1^{+/-}$ or $\alpha_2^{+/-}$ mice) (53) were also studied.

These mice express only about half the normal complement of the α_1 or α_2 Na⁺ pumps, respectively, in arterial and cardiac myocytes (and in astrocytes and skeletal muscle myocytes, which also express α_1 and α_2). Normally, there are many more α_1 than α_2 pumps in the arterial myocytes. Thus, there is a smaller total number of Na⁺ pumps (perhaps one-third fewer) in the arterial myocytes in $\alpha_1^{+/-}$ than in $\alpha_2^{+/-}$ mice. Interestingly, the isolated arteries from the $\alpha_2^{+/-}$ mice, but not those from the $\alpha_1^{+/-}$ mice, exhibited significantly greater myogenic tone than did arteries from wild-type mice (Fig. 4A) (120). Blood flow in small arteries is governed by Poiseuille's law (6), which states the resistance to flow (R) is inversely proportional to the fourth power of the internal radius, r (i.e., $R \propto 1/r^4$). Small increases in myogenic tone (decreases in r) should, therefore, have a profound effect on R (or TPR). Indeed, the BP in $\alpha_2^{+/-}$ mice was significantly higher than in the wild-type or $\alpha_1^{+/-}$ mice (Fig. 4, B and C) (112). Thus, α_2 Na⁺ pump activity exerts long-term control over myogenic tone and BP.

A corollary to these findings is the preliminary report that BP is reduced in transgenic mice that overexpress α_2 Na⁺ pumps in smooth muscle, compared with wild-type mice and mice that overexpress α_1 in smooth muscle (107). The Paul

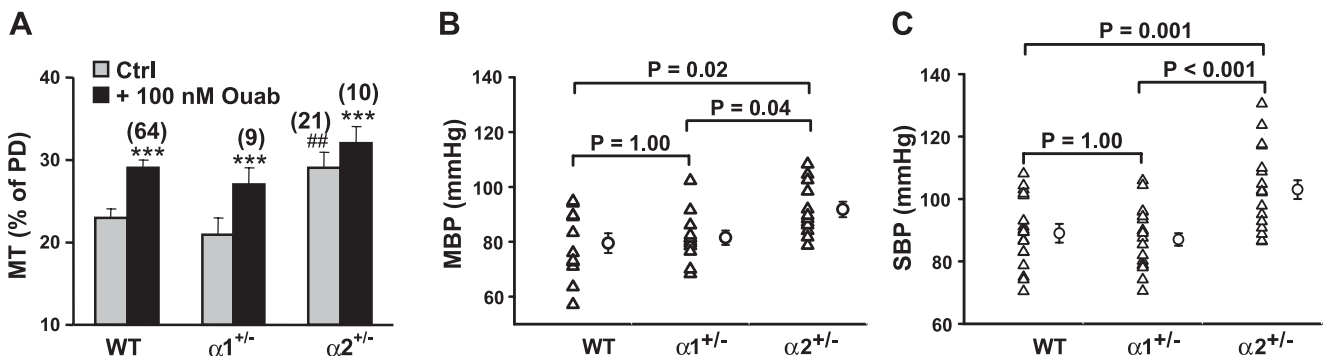


Fig. 4. Effects of reduced Na⁺ pump α_1 - and α_2 -isoform expression on MT and blood pressure. *A*: basal MT and the effects of 100 nM ouabain on MT in wild-type, $\alpha_1^{+/-}$ and $\alpha_2^{+/-}$ mouse small mesenteric arteries. The arteries (125–135 μm PD) were pressurized to 70 mmHg at 35°C to induce the development of MT. MT is shown as a percent of PD \pm SE before (control) and after 4–5 min of treatment with 100 nM ouabain. ## $P < 0.05$ vs. wild-type control; *** $P < 0.001$ vs. genotype control (numbers of arteries in parentheses). *B*: mean femoral artery blood pressure (MBP) in wild-type (WT), $\alpha_1^{+/-}$ and $\alpha_2^{+/-}$ mice under 1.5% isoflurane anesthesia. Triangles are individual measurements for $n = 12$ mice in each group; circles are mean values \pm SE. Mice were age-matched (days): wild type = 113 ± 2 , $\alpha_1^{+/-}$ = 109 ± 4 and $\alpha_2^{+/-}$ = 110 ± 4 . *C*: mean systolic blood pressure (SBP) measured by tail cuff in unanesthetized WT, $\alpha_1^{+/-}$ and $\alpha_2^{+/-}$ mice. Triangles are individual measurements; circles are mean values \pm SE. Mice were age-matched (in days): WT = 116 ± 8 ($n = 18$), $\alpha_1^{+/-}$ = 111 ± 10 ($n = 18$) and $\alpha_2^{+/-}$ = 107 ± 8 ($n = 17$). P values in *B* and *C* were determined by one-way ANOVA. *A* and *B* were modified from Ref. 120.

laboratory, which previously reported that the α_2 Na⁺ pump couples to contractility in the aorta (106), also reported that reduced α_2 expression ($\alpha_2^{+/-}$) accelerated the induction of DOCA-salt hypertension (107).

In related studies, Lingrel and colleagues have shown that transgenic mice with a mutated α_2 Na⁺ pump that renders this isoform resistant to ouabain are resistant to both ouabain-induced hypertension (19) and ACTH-induced hypertension (20, 20a). Moreover, the plasma from mice with ACTH-induced hypertension had significantly elevated levels of a water-soluble substance that cross-reacted with antidigoxin-specific Fab fragments (Digibind) (20). Digibind, which binds ouabain with high affinity (91), abolished the hypertensinogenic effect of ACTH (20, 66). Interestingly, plasma from patients with pregnancy-induced hypertension have elevated levels of a ouabain-like compound and marinobufagenin (70). Digibind, which has much greater affinity for ouabain than for the lipophilic marinobufagenin (91), also is reported to lower BP in patients with pregnancy-induced hypertension (1, 31). In summary, these findings provide convincing evidence that a circulating Na⁺ pump inhibitor (a ouabain-like compound) plays a key role in some forms of hypertension.

The essential point is that reduced α_2 Na⁺ pump activity, whether induced by low-dose endogenous or exogenous ouabain, or by a null mutation that results in reduced α_2 expression (i.e., α_2 heterozygous mice, $\alpha_2^{+/-}$), leads to the elevation of BP. Interactions of ouabain with Na⁺ pumps that do not involve Na⁺ pump inhibition (e.g., 2, 29, 99, 100, 114) cannot explain the striking similarity between the effects of ouabain and of genetically-reduced α_2 activity.

Whereas this role of the α_2 Na⁺ pumps seems straightforward in rodents, the situation in humans is more complex because, as noted above, human α_1 , but not rodent α_1 , has high affinity for ouabain. In transgenic mice with mutated α_1 and α_2 Na⁺ pumps, in which α_1 is rendered ouabain sensitive and α_2 is ouabain resistant, ACTH induces a profound, Digibind-sensitive hypertension within 24 h (20). The implication is that acute inhibition of α_1 , which increases tone and contractility in isolated arteries (120), can elevate BP. However, the evidence that BP is normal in $\alpha_1^{+/-}$ mice, but elevated in $\alpha_2^{+/-}$ mice (Fig. 4, B and C), implies that prolonged partial inhibition of α_1 , but not α_2 , can be compensated. Whether this also is true in humans remains to be determined.

OUABAIN ANTAGONISM AS A THERAPY FOR HYPERTENSION

The aforementioned data imply that inhibition of the (α_2) Na⁺ pumps by EO plays a central role in the pathogenesis of salt-dependent hypertension (Fig. 1). Therefore, it should be possible to lower the elevated BP in salt-dependent hypertension by blocking the effect of EO. Indeed, canrenone, a product of spironolactone metabolism with ouabain antagonist activity (27) has been used clinically as an antihypertensive agent (71, 104).

On the basis of these ideas, a ouabain antagonist, PST-2238 or Rostafuroxin (25), has been synthesized from the cardenolide, digitoxigenin (93). This compound, which antagonizes the effect of ouabain on renal Na⁺-K⁺-ATPase, but does not, itself, inhibit the Na⁺-K⁺-ATPase, lowers BP in ouabain-induced and salt-dependent hypertension in rats (24, 26). In

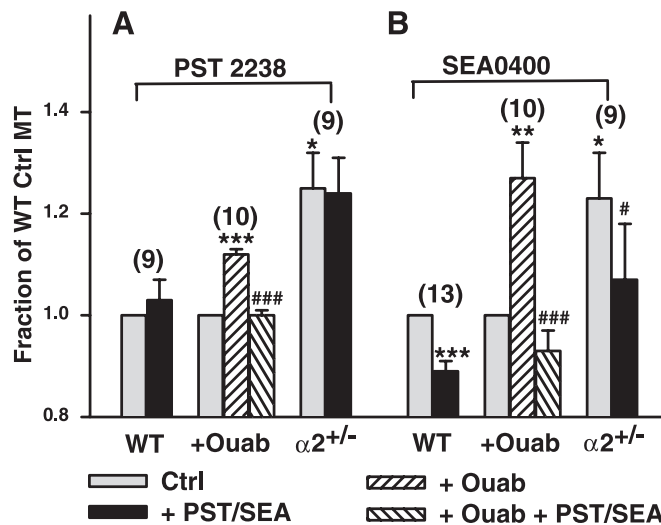


Fig. 5. Effects of a ouabain antagonist and an NCX blocker on MT augmented by 100 nM ouabain or by reduced α_2 -Na⁺ pump expression. A: summary of effects of 5 μ M PST 2238 (Rostafuroxin) on control MT and on MT augmented by 100 nM ouabain and by reduced α_2 expression ($\alpha_2^{+/-}$). B: summary of effects of 1 μ M SEA0400 on control MT and on MT augmented by 100 nM ouabain or by reduced α_2 expression ($\alpha_2^{+/-}$). * P < 0.05; ** P < 0.01; *** P < 0.001 vs. MT_{Ctrl} in wild-type arteries. # P < 0.05; ### P < 0.001 vs. MT_{+Ouab} in wild-type arteries or vs. MT_{Ctrl} in $\alpha_2^{+/-}$ arteries (numbers of arteries are in parentheses) where MT_{Ctrl} is MT in the absence of ouabain. Modified from Ref. 120.

early Phase II clinical trials, Rostafuroxin was observed to lower BP in nearly half of the tested human subjects with essential hypertension (25). Most important, this agent had no detectable effect on normal BP and apparently had negligible side effects (25).

Rostafuroxin antagonized the low-dose ouabain-induced increase in myogenic tone in isolated mesenteric small arteries from wild-type mice, but it had no effect on the augmented myogenic tone in arteries from $\alpha_2^{+/-}$ mice (Fig. 5A) (120). The latter observation is not surprising because the reduced α_2 Na⁺ pump activity in the $\alpha_2^{+/-}$ mice is genetic, and not ouabain induced. These data support the view that Rostafuroxin is a ouabain antagonist that lowers myogenic tone and elevated BP by preventing nanomolar ouabain (or EO) from inhibiting arterial α_2 Na⁺ pumps.

NCX LINKS α_2 Na⁺ PUMP ACTIVITY TO CONTRACTILITY

Ca²⁺, but not Na⁺, binds to calmodulin and initiates the chain of events that activates arterial myocyte contraction. Therefore, there is no reason to expect a rise in local, or even global, [Na⁺]_{cyt} to promote arterial myocyte contraction directly. But the NCX and α_2 Na⁺ pumps are colocalized in PM microdomains at PM-SER junctions (Fig. 2) (56, 106) where these two transport systems appear to function cooperatively (4, 30). Thus, the ouabain or EO-induced elevation of local [Na⁺] in the JS ([Na⁺]_{JS}) and the reduced [Na⁺] gradient across the PM should promote Ca²⁺ entry via NCX (4, 30). As a result, [Ca²⁺]_{SER} and [Ca²⁺]_{cyt} also rise (20), and this increases myogenic tone (50, 120) by a direct effect on the contractile machinery.

There are three NCX genes (NCX1–3); NCX1 is expressed in most tissues while NCX2 and 3 are limited to brain and skeletal muscle (94). The NCX1.3 and 1.7 splice variants are

prevalent in vascular smooth muscle (81). Several recent observations provide additional evidence that NCX in arterial smooth muscle is a critical link in the chain of events leading from salt to an elevation of BP (50, 51).

Mineralocorticoid excess, which causes salt and water retention (42, 113), induces hypertension in humans (59). DOCA-salt hypertension, in which uninephrectomized animals are administered DOCA and placed on a high-salt diet, is a common model of hypertension in mice, rats, and other animals. Mice with a null mutation in the NCX1 gene (NCX1 heterozygous or NCX1^{+/-} mice), which express only half the normal complement of NCX1 in the heart and arteries, are resistant to DOCA-salt hypertension (50). Conversely, mice that overexpress NCX1.3 in smooth muscle (NCX1.3^{Tg/Tg} mice), but not in the heart, have slightly elevated BP and are exceptionally sensitive to salt. For example, a high-salt diet has little effect on BP in wild-type mice, but substantially elevates BP in the NCX1.3^{Tg/Tg} mice (50).

This role of NCX in mineralocorticoid hypertension is supported by the fact that the relatively selective NCX1 inhibitor, SEA0400, lowers BP in rodents with DOCA-salt hypertension (50). SEA0400 also lowers BP in several other forms of salt-dependent hypertension: 1) salt-sensitive Dahl (DS) rats on a high-salt diet, 2) spontaneously hypertensive rats (SHR) on a high salt, and 3) reduced renal mass rats on a high-salt diet (50). The anti-hypertensive effect of SEA0400 is abolished in mice that overexpress a mutated, SEA0400-resistant NCX, mNCX1.3^{Tg/Tg} (50).

SEA0400 not only counteracts the hypertensinogenic effect of ouabain on BP (50), but it also reduces the augmented myogenic tone in arteries from α_2 ^{+/-} mice as well as in ouabain-treated mouse arteries (Fig. 5B) (120). This indicates that the NCX1 acts downstream from the α_2 Na⁺ pump (Fig. 1).

In summary, these recent findings, diagrammed in Fig. 1, provide a clear answer to the major challenge in hypertension research mentioned at the outset, which is that the evidence demonstrates that EO plays a central role, and that arterial myocyte α_2 Na⁺ pumps and NCX1 link salt to hypertension and are key determinants of long-term BP. The data from the mice that overexpress, specifically in smooth muscle, α_2 Na⁺ pumps (107) and NCX1.3 (50), indicate that the transporters in arterial smooth muscle are intimately involved in the control of BP. Moreover, the data from the isolated arteries of α_2 ^{+/-} mice (Fig. 4) support the view that there is a direct relationship between myogenic tone in isolated arteries and BP in intact animals.

These ideas and observations have already led to the development of two new classes of antihypertensive agents, of which Rostafuroxin and SEA0400 are examples. Indeed, in early Phase II clinical trials, Rostafuroxin was found to lower BP in about 40% of patients with essential hypertension. This was essentially the same group of hypertensive patients that also responded to the angiotensin blocker, losartan. Thus, it seems possible that only those patients (the responders) had a salt-sensitive (and volume-dependent) hypertension that involves the sequence of events illustrated in Fig. 1. Therefore, essential hypertension may actually represent a spectrum of diseases that lead to chronic elevation of BP. The identification of a subset of patients in whom this one pathogenetic mechanism (Fig. 1) applies may now make it easier to separate these

patients from those with hypertension that is due to other mechanisms. This may be particularly important for diagnosing and treating the various forms of hypertension that we now lump into the general category of essential hypertension.

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