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Endogenous Ouabain: A Link Between Sodium Intake and Hypertension

John M. Hamlyn and

Department of Physiology, School of Medicine, University of Maryland, 655 West Baltimore Street, Baltimore, MD 21201, USA

Paolo Manunta

Nephrology, Dialysis and Hypertension Division, Scientific Institute San Raffaele, Università "Vita-Salute" San Raffaele, Via Olgettina 60, 20132 Milan, Italy manunta.paolo@hsr.it

Abstract

The sodium pump, an ancestral enzyme with conserved ability to bind ouabain, plays a key role in salt conservation and is regulated by aldosterone and endogenous ouabain (EO). Plasma EO is elevated in about 45% of patients with essential hypertension and correlates with blood pressure. The relationship of EO with Na⁺ balance is complex. Na⁺ depletion raises circulating EO, whereas acute saline loads have no effect on EO in essential hypertension, and ambient levels of EO are unrelated to the saline sensitivity of blood pressure. Short-term periods of high dietary salt elevate EO and the relationship with salt balance in normal individuals is V-shaped, whereas the long-term relationship is likely to be L-shaped. Normal individuals suppress the high EO transient triggered by high-salt diets and avoid hypertension. In contrast, patients with elevated EO on normal Na⁺ intakes have hypertension related to poor modulation of EO biosynthesis, clearance, or both.

Keywords

Steroids; Sodium pump; Blood pressure; Diet; Mechanism

Introduction

Virtually all terrestrial creatures, having long ago left the high-salt environment of the oceans, are faced with the often inconvenient and persistent need to actively conserve sodium and water. As a consequence of evolution, modern humans are genetically and physiologically programmed to thrive at a level of salt intake of about 1 g per day [1]. As the addition of salt to food began only about 5,000–10,000 years ago, the now-chronic consumption of about 3–5 g per day for most of one's life is, in evolutionary terms, extraordinarily recent. Prior to the addition of salt to food, hypertension was likely a rare occurrence among humans. In contrast, hypertension is today a major risk factor for cardiovascular disease and, in some countries, has risen to be the main cause of preventable deaths. Experimental and clinical studies suggest that the potential mechanisms by which salt influences blood pressure are multifactorial. The sensitivity of blood pressure to salt

varies among individuals, and the mechanism of salt-sensitivity in both normotensives and hypertensives and its long-term significance remain unclear [2].

A causal interrelationship between salt intake, total body Na^+ , fluid balance, and blood pressure has been proposed [3]. In this model, the kidney plays a key role in the control of total body Na^+ and extracellular fluid volumes. Relative to sodium (Na^+) intake, any prolonged tendency of the kidney to reabsorb additional Na^+ results in fluid volume expansion. The magnitude of the volume excess may be large and sufficient to significantly augment cardiac output, or it may result in no increase in cardiac output [4]. Regardless of whether cardiac output rises, prolonged Na^+ retention invariably leads to increased peripheral vascular resistance and hypertension. Clearly, there must be important molecular links between dietary Na^+ per se and the dynamic function of the arteries and veins to explain such a relationship. One well-known link is the renin-angiotensin-aldosterone system (RAAS). As Na^+ intake is reduced, especially below 2 g per day, the activity of the RAAS increases dramatically to provide a homeostatic response that involves increased renal Na^+ reabsorption as well as vascular and central effects that augment arterial tone and thirst. Many of the effects of the RAAS directly or indirectly involve the plasma membrane Na^+ pump. In the kidney and in the arterial vasculature, aldosterone augments expression of Na^+ channels and the Na^+ pump [5,6]. Though high-salt diets suppress the RAAS, meaningful amounts of aldosterone persist under this dietary condition, especially in the setting of heart failure, in which aldosterone has deleterious actions on the function and structure of the cardiovascular system [7].

Another link between salt and vascular function originates from observations that the blood of volume-expanded rodents contains elevated amounts of an Na^+ pump inhibitor [8]. It has long been known that the Na^+ pump contains a remarkably specific binding site for cardiac glycosides that is highly conserved among species ranging from Hydra to humans. Occupation of this site by digitalis glycosides has been used to treat congestive heart failure, so it was with some surprise that this binding site was implicated in long-term blood pressure control [9,10] and subsequently was proven to have a direct role. This review examines some recent advances in our knowledge of the interrelationships between sodium balance, endogenous ouabain (EO), vascular function, and blood pressure.

Mechanisms of the Short-Term and Long-term Vasopressor Actions of Ouabain

In 1977, Blaustein [11] proposed a link between the high salt intakes of Western-acclimated societies and hypertension. The link involved three molecular entities: a circulating inhibitor of the Na^+ pump, the vascular myocyte Na^+ pump, and the vascular sodium-calcium exchanger (NCX1.3). The basis for the mechanism would prove to be similar to that eventually worked out in heart—namely, that inhibition of the Na^+ pump by cardiotonic steroids raised intracellular sodium and calcium levels and augmented contractile activity [12]. It was proposed that the same mechanism operated in arterial myocytes and, indeed, the acute application of ouabain augmented the tone of isolated arteries [11]. Accordingly, it was suggested that the increased peripheral vascular resistance in essential hypertension (EH) was driven by elevated circulating levels of a sodium pump inhibitor that were evoked by the high salt intake. This humoral factor was proposed to block a small fraction (about 5%–20%) of cellular Na^+ pumps and to cause intracellular sodium to rise globally. One problem with this “cell salt” hypothesis was that the expected increase in total body Na^+ was not detected in patients with EH [13]. This dilemma was subsequently resolved by the discovery that bulk changes in cell Na^+ and thus tissue Na^+ were not needed because the key Na^+ pumps were clustered with the NCX in plasma membrane microdomains adjacent to the underlying sarco-plasmic reticulum [14]. In

response to low, physiologically and pathologically relevant doses of ouabain, there is an increase in cell Na^+ that is largely confined to tiny, diffusion-restricted spaces that represent less than 5% of the bulk cell volume; hence any increase in Na^+ in those regions is simply too small to be detected by exchangeable isotope methods. Another problem with the Blaustein hypothesis was the longstanding uncertainty regarding the nature of the circulating Na^+ pump inhibitor; this concern was answered in 1991 with the identification of a steroidal glycoside identical to ouabain in humans—that is, EO [15]. Thereafter, work in transgenic mice provided direct evidence of the key roles of the vascular myocyte α_2 isoform of the Na^+ pump and NCX1.3 in ouabain-induced, ACTH-induced, and salt-sensitive hypertension [16,17,18]. Thus, the long-term pressor mechanism of ouabain was proven in rodents nearly 30 years after the original hypothesis was proposed. More recent work has shown that whereas the acute vasopressor effect of ouabain is mediated by changes in Na-pump/NCX1.3 activity, as described by Blaustein [11], the chronic vasopressor effect depends upon additional events and molecular entities, including increased vascular myocyte expression of α_2 Na^+ pumps and NCX1.3, as well as the involvement of members of the store-operated channel (SOC) family and receptor-operated channel (ROC) family, including the transient receptor potential channels 1 and 6 (TRPC1/6) [19]. Knockdown of NCX1.3 by siRNA markedly downregulated TRPC6 and eliminated the ouabain-induced augmentation of Ca^{2+} signaling, showing that the expression of these transporters is interrelated [19]. The mechanism by which prolonged ouabain exposure upregulates the expression of α_2 Na^+ pumps, NCX1.3, and TRPC6 is under investigation. In addition to the above-mentioned signaling pathway, some Na^+ pumps are complexed with the tyrosine kinase c-src and are inactive [20]. The binding of ouabain to the pump displaces and activates c-src and leads to the stimulation of a variety of signaling pathways, especially those related to cell growth [21]. It is very likely (although untested) that the abundance of Na^+ -pump c-src complexes will be dramatically reduced by prolonged exposure to ouabain, especially in vivo. Further, prolonged exposure to EO or ouabain may promote long-term increases in c-src activity, and it will be of particular interest to determine the role of this kinase in the ouabain-induced upregulation of the Na^+ pump/NCX1.3/TRPC6 cascade.

The basis for the elevated BP in the ouabain hypertensive rat is primarily increased total peripheral resistance [9]. Recent work suggests that all or part of the increase in total peripheral resistance is due to increased myogenic tone [22], with some contribution from increased sympathetic nerve activity [23]. Another interesting feature of the ouabain hypertensive rat model is the apparent lack of tachyphylaxis to ouabain in vivo. For example, the ouabain-evoked myogenic tone in mesenteric arterioles removed from rats with chronic ouabain-induced hypertension was similar to the tone in normotensive controls [24]. The implication is that BP will be expected to be directly and dynamically related to circulating ouabain or EO in the steady state, with little or no hysteresis. This inference is supported by experimental work, especially clinical studies mentioned below.

Where is the Salt-Sensitive Step in the Pressor Mechanism?

Assuming that salt is related to hypertension via the abovementioned pathway, then it follows that one or more key steps must be responsive to dietary salt. The prolonged administration of ouabain induces hypertension in rodents [9,10,18], but surprisingly, the long-term pressor effect was not affected by treatment with diuretics or high-salt diets [10]. Thus, in otherwise normal rats, it appeared that any salt sensitivity of the pressor mechanism had to be mediated by upstream events that precede EO and/or manifest by salt-evoked changes in the circulating levels of EO itself. Clinical studies show that plasma EO is stimulated by salt restriction, and though high-salt diets can raise plasma EO dramatically, they appear to do so only transiently (see below). Thus, salt intake influences plasma EO but does not explain the link between salt sensitivity and blood pressure in a simple manner.

NCX1.3, which is downstream in the pressor sequence, does play a key and specific role in salt-sensitive experimental hypertension, however, as evidenced by the large decreases in BP evoked by the selective NCX1.3 blocker SEA0400 [16]. Further, mice that overexpress NCX1.3 have elevated baseline BP and are salt-sensitive, whereas underexpressors have low BP and are salt-resistant [16,25]. Thus, NCX1.3 expression per se is a significant determinant of salt sensitivity and blood pressure. Further, as the prolonged administration of ouabain dramatically raises the expression of NCX1.3 in rats, it is not clear why BP does not respond to diuretics or high salt in rats with ouabain-induced hypertension. Clearly, other factors must operate in concert with NCX.

Another emerging feature of ouabain-induced hypertension concerns the role of nitric oxide (NO). In response to acute exposure to ouabain, the generation of endothelial NO initially is augmented [26•]. Increased vascular NO generation may explain the several days of delay in the rise of BP when ouabain is given peripherally [10]. However, during prolonged in vivo exposure to ouabain, the ability to stimulate NO production in the vasa recta of the kidney disappears [26•]; the loss of NO production is striking and, if present in other vascular beds, would be expected to have an important impact on both the onset and level of ouabain-induced hypertension.

Endogenous Ouabain in Hypertension and Renal Sodium Handling

Human kidneys are poised to conserve sodium and excrete potassium. Paleolithic humans, who consumed a sodium-poor and potassium-rich diet, were well served by this mechanism [1]. With such a diet, sodium excretion is negligible and potassium excretion is high, matching potassium intake. With the high intakes of salt common in the contemporary diet, the kidney must meet the obligation to excrete excess salt. Accordingly, it is of considerable interest that the reabsorption of filtered sodium by the renal tubules is often increased in primary hypertension [27]. The augmented reabsorption appears to be mediated by the proximal tubular stimulation of several sodium transporters located at the luminal membrane, as well as the basolateral α_1 Na⁺ pump, and may result from defects in dopamine signaling and mutations in the cytoskeletal protein adducin [28,29].

Several clinical studies mentioned below have shown that circulating EO changes in response to variations in electrolyte balance. Variations in circulating EO would be expected to be more relevant to renal function in humans than in rodents because the dominant α_1 Na⁺ pump isoform is highly ouabain-sensitive in man.

EO as a Blood Pressure Modulating Factor

In the general population, EO behaved as a BP modulating factor that acted to minimize the depressor action of sodium depletion [30]. In subjects with plasma EO less than 140 pmol/L (median), each 50 mmol/day increment in urinary sodium excretion was associated with an increase in systolic BP averaging 2.2 mm Hg (95% CI, 0.7–3.6 mm Hg; $P=0.004$) and an increase in diastolic BP of 1.4 mm Hg (95% CI, 0.3–2.5 mm Hg; $P=0.01$) [30]. This association was not apparent in subjects whose plasma EO was greater than 140 pmol/L and where the association between BP and urinary sodium excretion was not statistically significant. Thus, EO plays a role in the homeostatic regulation of BP in response to a low sodium intake in normotensive subjects.

The Impact of Altered Sodium Balance: Normotensive Subjects

The impact of deliberate alterations in sodium balance on the circulating levels and renal clearance of EO have been described [31]. In 13 normotensive subjects given a high-salt diet, plasma EO increased about 13-fold on the third day of the diet. Conversely, administering hydrochlorothiazide led to declines in body weight and increases in plasma

renin activity, aldosterone, and plasma EO; urinary EO excretion remained within the normal range. The increase in plasma EO evoked by the high salt diet was likely due to increased secretion; the mediators and mechanism of that response are unknown. Nevertheless, in normal men, short-term changes in sodium balance evoked by dietary or pharmacologic means are related to plasma EO by a V-shaped curve (Fig. 1).

The Impact of Altered Sodium Balance: Hypertensive Subjects

Among hypertensive patients (in contrast to long-held hypotheses about the role of sodium pump inhibitors as rapidly acting natriuretic hormones), acute saline loading was not an immediate stimulus to plasma EO. Further, basal levels of plasma EO did not differ among patients with salt-sensitive or salt-resistant hypertension [32,33]. However, among patients with EH who entered a blind, randomized, crossover trial involving chronically controlled Na^+ intake and depletion (170 to 70 mmol/day; 2 weeks each period), the plasma levels of EO were higher only during Na^+ depletion. Thus, the longer-term relationship between sodium balance and EO evoked by dietary sodium appears to be better explained by an L-shaped curve than by a V-shaped curve (Fig. 1) and is similar to that for aldosterone. Taken together, the available evidence [29,34] shows that EO responds specifically to sodium depletion among normal individuals and patients with EH.

Increased proximal tubular reabsorption of Na^+ leading to increased plasma Na^+ has been proposed as an independent determinant of hypertension [35]. Studies of hypertensive patients [29] carrying the mutated alpha-adducin (ADD1) variant reinforce this view; the carriers of the mutant alleles showed increased Na^+ reabsorption that was associated with suppressed plasma renin activity, aldosterone, and plasma EO. Furthermore, patients with high plasma EO also have increased BP and proximal tubular reabsorption [36]. Once again, the behavior of EO tends to argue against the hypothesis that EO is a natriuretic hormone, at least under steady-state dietary conditions.

Renal Effects of EO

The aforementioned view is also supported by direct comparison of the effects of the RAAS and EO among EH patients following acute saline loads. Whereas the RAAS reflected body sodium status and had primarily a compensatory role in the regulation of BP, EO had a biphasic relationship with tubular reabsorption. For example, among most EH patients, those with low to normal plasma levels of EO showed increasing renal Na^+ retention following a saline load, but EH patients with high plasma EO showed augmented ability to excrete Na^+ . We suggest that low to normal circulating values of EO promote renal Na^+ reabsorption by stimulating the basolateral renotubular Na^+ pump and the renovascular Na/Ca exchanger (NCX1)—that is, by a combination of tubular and vascular effects. The mechanism of the renotubular stimulation is unclear; the renovascular action is probably mediated by increased arterial tone secondary to inhibition of the vascular myocyte α_2 Na^+ pumps and increased Ca^{2+} entry via NCX1.3. In contrast, patients with high levels of plasma EO show increased fractional excretion of Na^+ and augmented Na^+ tubular rejection fraction [33]. The cause-effect relationship between the high ambient plasma EO and the augmented natriuresis that follows acute saline loads in those individuals is unclear. One possibility is that the salt load in some way allows high EO to switch from a net Na^+ -retaining effect to one favoring natriuresis. The natriuretic effect in humans is presumably related to inhibition of α_1 Na pumps in the renal tubules; it should not be present in rats, in which the pump is ouabain-resistant. In addition, it is interesting to recall that plasma EO rises dramatically in normal humans during the first few days of a high-salt diet, reaching values 5–10 times normal [31]. During this period, the kidney disposes of the additional sodium and a new balance state is achieved by the end of the third day. Taken together, these two studies raise the possibility that high circulating levels of EO may have some intrinsic natriuretic effect under certain

conditions. More significantly, the possibility that the renal effects of EO might be switched from a Na^+ retentive mode to a Na^+ excretory mode by an acute volume load is most intriguing. Further work is needed in this area.

Genetic Influences

Among patients with EH, and especially among those with more advanced hypertension, circulating EO levels were directly related to both BP and total peripheral resistance and were inversely related to cardiac index [37]. The elevated circulating EO observed in many patients with EH is influenced by their genetic background and renal clearance: *CYP11A1* loci were related to circulating EO, and *MDR1* loci were related to diastolic BP. The basis for these associations remains to be proven, but they seem most likely to involve effects of these loci on EO synthesis and renotubular transmembrane EO transport, respectively [38•].

Biosynthesis and Clearance of Endogenous Ouabain

Since the publication of the original report in 1991 [15], EO has been detected or isolated by a variety of laboratories. High-performance liquid chromatography and immunoassay methods show that EO is present in bovine and human adrenal glands, bovine hypothalamus, rat adrenomedullary cells, and biologic fluids. Mass spectrometry, nuclear magnetic resonance studies, and the co-chromatography of EO with ouabain in all tested chromatography systems confirm the presence of EO beyond doubt and show also that the bovine hypothalamic and adrenal compounds are identical to plant ouabain, as reviewed recently [33].

The adrenal glands are rich in EO in many mammals. Further, EO content in the adrenals remains remarkably constant under different conditions, consistent with a role for these glands as the primary source. Several other pieces of evidence suggest that the adrenal gland is a major source of EO:

- Plasma EO levels declined in adrenalectomized rats but not in rats whose adrenal medulla was removed [39]. Crucially, with prolonged adrenalectomy and corticosterone replacement in rats, plasma EO levels fell to about 5% of their normal levels, near the threshold sensitivity of the radioimmunoassay used in the study. The source of the small residual amount of EO after adrenalectomy is not clear (as with aldosterone), but it is clear that the adrenal cortex is required to maintain normal plasma EO levels, and extra-adrenal sources are not sufficient to maintain circulating EO.
- In an early study in patients with primary aldosteronism, EO levels in mixed inferior vena cava blood were more than fivefold higher than in normal controls [40]. We suggested recently that a step up in adrenal venous plasma of about threefold was likely in that study [34]. Further studies using methods based on mass spectrometry are needed to confirm this impression and to provide crucial proof of the appropriate venous gradients in humans. In work with conscious, afebrile dogs with surgically placed adrenal venous catheters, the EO content of the adrenal venous effluent was about fivefold to sixfold higher than that of arterial blood [41].
- Elevated plasma levels of EO were found in two rare hypertensive patients with nonclassic adrenocortical tumors [42]. Removal of the tumors was associated with a normalization of plasma EO levels and the remission of hypertension. The results were compatible with those in other patients with aldosterone-secreting tumors [43], which also hypersecrete EO and cause hypertension.

- Cultured human and bovine adrenocortical cells secrete EO into the culture fluid [44]. The secretion is augmented by angiotensin II, adrenocorticotrophic hormone (ACTH), and possibly vasopressin, as well as α_1 adrenoceptor agonists [34,44]. With contemporary dietary Na^+ intakes and with plasma renin activity largely suppressed, both plasma K^+ and ACTH appear to be key regulators of circulating EO in humans [30,45].
- The adrenal biosynthesis of EO involves cholesterol side-chain cleavage (*CYP11A1*) and 3β -hydroxysteroid dehydrogenase (HSD3B) with sequential metabolism of pregnenolone and progesterone [46–48].
- The renal excretion of cardiac glycosides is mediated in part by the organic anion transporter (*SLCO4C1*) at the basolateral membrane [49], and in part by the P-glycoprotein (PGP, encoded by *MDR1*) [50] at the apical membrane of the nephron. Accordingly, a single-nucleotide polymorphism (SNP) and haplotype-based association study was performed with a total of 26 informative SNPs in a large cohort of hypertensive patients. In that study, *CYP11A1* and *MDR1* loci were associated with circulating EO and diastolic BP, likely reflecting their influence on EO synthesis and transmembrane transport, respectively [38•].

Conclusions

The persistent and specific association of EO with hypertension indicates that it is primarily a BP regulator in adult humans. EO acts apparently exclusively via the ouabain-binding site on the Na^+ pump. The circulating levels of EO are influenced by dietary Na^+ , and the relationship is most evident with a low Na^+ intake. Thus, EO, like aldosterone, behaves as a teleologically important hormone and may help to maintain overall vascular tone, whereas aldosterone and the mineralocorticoid receptor have evolved to support renotubular Na^+ retention. The combination of incompletely suppressed circulating levels of EO and aldosterone in the setting of contemporary salt intakes is likely to be of major pathologic significance. In this regard, the observation that 40% to 50% of patients with mild to moderate EH have elevated EO with normal dietary salt intakes is noteworthy [32,34,36,37]. The cause of the elevated EO in EH is not certain but appears to be inappropriate secretion and/or impaired clearance. Among EH patients with high EO, dynamic augmentation of myogenic constriction in small arteries likely makes a major contribution to the increased vascular tone and BP. The inability of high- Na^+ diets to fully suppress plasma EO, coupled with the ability of high- Na^+ diets to facilitate pathologic actions of EO and aldosterone, suggests that dietary salt reduction and/or measures that block the biosynthesis or binding of EO may be therapeutically useful.

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Fig. 1.

Idealized relationship of the impact of sodium balance on plasma endogenous ouabain (EO) among normotensive individuals and patients with essential hypertension (EH). Relative to the normal steady state of sodium balance, EO is elevated by negative sodium balance in normotensives (*green line*). EO is transiently elevated by high-salt diets in normotensives (*blue and yellow lines*) but not in the steady state (*green line*). EO is elevated under all conditions in EH with high EO (*red dashed line*). The short-term relationship between sodium balance and circulating EO is V-shaped, whereas the steady-state relationship appears to be L-shaped in normotensive individuals and in most patients with EH. (*Adapted from data in references [29–34].*)