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Salt sensitivity, endogenous ouabain and hypertension

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Abstract

Purpose of review—Endogenous cardiotonic steroids (CTS) exert long-term effects on salt and blood pressure homeostasis. Here we discuss recent observations on mechanisms of salt sensitivity that involve endogenous ouabain and novel pathways in the brain and discuss their possible relationship to arterial and renal function in hypertension.

Recent findings—Chronic elevation of brain sodium promotes sustained hypertension mediated by central endogenous ouabain and the Na⁺ pump α -2 catalytic subunit. The intermediary pressor mechanism in the brain involves aldosterone biosynthesis, activation of mineralocorticoid receptors and increased epithelial sodium channel activity. In the periphery, elevated plasma CTS raise contractility and blood pressure by augmentation of sympathetic nerve responses, increasing arterial Ca²⁺ signaling and blunting nitric oxide production in the renal medulla and collecting ducts.

Summary—Endogenous ouabain in the brain appears to play a critical role in salt sensitivity and hypertension. In the periphery, the J-shaped relationship of plasma endogenous ouabain in response to short-term changes in salt balance in humans raises the possibility that endogenous ouabain contributes to the increased risk of adverse cardiovascular events associated with both low and high salt intakes.

Keywords

aldosterone; blood pressure; brain; kidney; nitric oxide; sodium

INTRODUCTION

Virtually all terrestrial creatures actively conserve sodium and water, and all mammals must also maintain blood pressure (BP). Thus, cardiac and vascular mechanisms, in addition to those that support salt and water homeostasis, must be highly regulated and must work in an integrative fashion. With respect to salt intake, modern day humans evolved consuming approximately 1 g salt/day [1]. In many Western acculturated societies, life-long consumption of salt ranges from approximately 4 to 10 g/day, which far exceeds need, and has been linked with the incidence of hypertension across populations [2]. The molecular mechanisms by which salt raises BP are unknown. Moreover, the sensitivity of BP to short-term changes in salt balance among individuals is variable, and the mechanisms of acute salt sensitivity both in normotensive and hypertensive individuals and their longterm significance remain

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Conflicts of interest

There are no conflicts of interest.

unclear [3]. In this brief review, we offer our interpretation of recent advances in the interrelationships between endogenous cardiotoxic steroids (CTS) focusing on endogenous ouabain, vascular function and mechanisms involved in salt-sensitive BP.

THE KIDNEY

The classical causal inter-relationship between salt intake, total body Na^+ , fluid balance and BP is based largely upon a fundamental role for the kidney [4]. Relative to sodium intake, any prolonged tendency of the kidney to reabsorb additional Na^+ results in fluid volume retention. The salt retention leads eventually, via mechanisms that are unclear, to sustained hypertension that may or may not be preceded by a high cardiac output phase [5,6]. It is widely believed that a high cardiac output stage invariably mediates the early rise in BP in salt-sensitive hypertension. In young humans with borderline hypertension, increased cardiac output has been documented, although the salt sensitivity was undetermined [7]. However, it is not widely recognized that total peripheral resistance also was elevated in these individuals relative to controls. This is evident from the fact that following the acute normalization of cardiac output by pharmacological means, the BP remained elevated. The only reasonable conclusion is that total peripheral resistance was significantly elevated in the borderline individuals. Thus, the early elevated BP in these individuals was a mix of elevated cardiac output and total peripheral resistance driven by the sympathetic nervous system. In this regard, these individuals fall into the acute pressor pathway diagrammed in Fig. 1, which is dependent in part on brain endogenous ouabain. With time, as the slow pathway takes over, these individuals would be expected to transition to sustained hypertension mediated entirely by high total peripheral resistance. Irrespective of the early hemodynamic events, the underlying importance of salt retention by the kidney is underscored by rare monogenetic lesions that enhance sodium reabsorption and that induce hypertension [8]. For the typical patient with essential hypertension, including those with overt salt sensitivity of their BP, augmented proximal tubular reabsorption [9,10], reduced dopamine signaling [11] and altered function of the renal medulla [12,13] have been proposed. Some renal transplant studies favor the idea of a fundamental causative role for the kidney in salt-sensitive hypertension, whereas other studies show that the kidney from the salt-sensitive animal is not causative [14]. The latter studies make it clear that salt sensitivity and salt sensing can occur outside the kidney. In addition to angiotensin II, aldosterone, the atrial peptides and nitric oxide, circulating endogenous ouabain has been linked with variations of electrolyte balance, renal function and hypertension [15,16].

WHERE IS THE SALT SENSOR?

Prolonged salt retention invariably leads to increased peripheral vascular resistance and hypertension. But where and how is the excess salt sensed? Important molecular links must exist between sodium and the dynamic function of the arteries and veins to explain the pressor and depressor effects of high and low-salt diets. One link is the renin–angiotensin–aldosterone system (RAAS). As salt intake is reduced, RAAS activity augments expression of Na^+ channels and Na^+ pumps [17,18], and increases renal Na^+ reabsorption as well as vascular and central mechanisms that augment arterial tone and thirst. Nevertheless, during high-salt diets, the possibility that increased pressor signaling arises from the kidney to the vasculature seems remote because the circulating RAAS is usually suppressed.

The brain is involved in osmoregulation and appears most feasible as the likely site of salt sensing and, by inference, salt sensitivity. High-salt diets raise plasma Na^+ and many patients with essential hypertension have small but significant increases in plasma Na^+ [19–21]. Plasma Na^+ can enter the brain via the fenestrated vasculature of the circumventricular organs and, along with cerebrospinal fluid (CSF) Na^+ , stimulates

magnocellular neurons. Further, raising brain Na^+ and Cl^- acutely by infusion has a well recognized short-term pressor effect that is associated with increased sympathetic outflow [22]. Most important to understanding the role of excess salt in long-term BP is the first demonstration that the prolonged administration of sodium chloride into the brain leads to sustained hypertension [23]. Work with Dahl salt-sensitive rats had already shown that brain Na^+ is increased by high-salt diets, and that Cl^- also has an essential pressor role [24]. Remarkably, the relative significance of Na^+ and Cl^- -dependent mechanisms in the acute and chronic phases of the BP elevation is unexplored. In humans, CSF Na^+ increased in both salt-sensitive and salt-resistant hypertensive patients [25]. The issue of increases in CSF Na^+ *per se* may be moot; even in the absence of increased CSF Na^+ , salt-sensitive rats have an exaggerated centrally mediated BP response [26]. Thus, CSF Na^+ need not be increased if the central pathways in Fig. 1 are already, or become, activated. Nevertheless, recent studies reveal that the pressor effect of adding salt into the brain depends on a high-affinity ouabain-binding site on the α -2 subunit isoform of the Na^+ pump [27]. This same Na^+ pump isoform has been shown to influence long-term BP in other models [28,29] and underscores the critical importance of the endogenous CTS.

In addition to its role as a salt sensor, an accumulating body of evidence clearly shows that the brain plays a critical role in many forms of salt-sensitive hypertension. The blood of volume-expanded rodents contains elevated amounts of a sodium pump inhibitor which is absent in animals with electrolytic lesions of the anteroventral third ventricular (AV3V) area of the hypothalamus [30]. Further, neither hypertension nor the humoral Na^+ pump inhibitor is detected in many animal models of hypertension with AV3V lesions [31]. Such experiments make it clear that the kidney need not invariably have the leading or dominant role in hypertension. Whereas lesioned animals can be criticized because they may have significant volume deficits that cannot be compensated by the renal tubular and vascular actions of mineralocorticoids, other work is definitive concerning a key role for the brain in salt sensitivity and hypertension. For example, central mineralocorticoid receptors are involved in Dahl salt-sensitive hypertension, and tiny, but functionally significant, amounts of aldosterone appear to be synthesized in the brain [32-35] where they act via specific mineralocorticoid receptors. The occupation of central mineralocorticoid receptors by mineralocorticoids (notably aldosterone) activates epithelial sodium channels (ENaCs), stimulates brain endogenous ouabain and raises BP via a pathway that involves central angiotensin II receptors [35]. Specific overactivation of renal ENaCs had been assumed to be the key molecular disturbance underlying the salt-sensitive hypertension in Liddle's syndrome. New and striking results show that overactivity of ENaCs in the central nervous system (CNS) leads to salt-sensitive hypertension associated with increased CSF Na^+ and hyper-responsiveness to CSF Na^+ [23]. Thus, there is a functionally important central mineralocorticoid pathway, independent from that in the periphery, which contributes to the regulation of brain Na^+ [35]. The activity of this central mineralocorticoid pathway is overactive, as judged by elevated brain levels of aldosterone [36] and the response to mineralocorticoid receptor blockers mentioned above. Thus, the activity of the brain mineralocorticoid pathway, in contrast to its peripheral counterpart, is increased, and not decreased, by salt; it involves additional key upstream and downstream components not evident in the kidney. Further, it is noteworthy that central pharmacological or immunological blockade of any of the above-mentioned steps from aldosterone synthase to angiotensin II type 1 receptors (AT1Rs) prevents the sympathetic hyperactivity and salt-sensitive hypertension irrespective of the presence of a salt-sensitive kidney.

As might be predicted from this sequence, animals infused peripherally with maximally effective doses of ouabain that induce hypertension in rodents [15,37,38] are largely insensitive to diuretics or high-salt diets [38]. This important, but generally overlooked, observation indicates that the key salt-sensitive step is upstream of circulating endogenous

ouabain and that the salt-sensitive step is not involved to any great extent in the subsequent events by which endogenous ouabain controls arterial Ca^{2+} signaling or renal function. Additionally, although the participation of the CNS had been previously demonstrated in the control of plasma endogenous ouabain [39-41], its significance and broad implication were not appreciated prior to crucial new studies from the Leenen laboratory [35]. When taken together, the available evidence indicates that the brain aldosterone–mineralocorticoid receptor–ENaC–AT1R cascade (Fig. 1) may explain the role of the CNS as a bidirectional regulator of peripheral endogenous ouabain [39-41]. Furthermore, this pathway may be especially relevant; once peripheral endogenous ouabain is elevated, it has a long-term impact on arterial Ca^{2+} signaling that raises BP. Further, ouabain augments endothelial nitric oxide generation in peripheral arteries that may oppose myogenic tone [42], whereas prolonged in-vivo ouabain severely blunts nitric oxide production in the endothelium of the vasa recta and epithelial cells of the renal medulla and blunts natriuresis [13,43]. As medullary function depends critically upon nitric oxide [12,13], long-term deficits in nitric oxide production at this site may be expected to shift the pressure–natriuresis relationship and maintain ouabain-induced hypertension [44]. More importantly, the combination of these ideas provides a fascinating new way to consider salt sensitivity as a reflection of the interaction of several distinct organ systems that previously have been investigated independently.

WHO IS SALT-SENSITIVE?

There are numerous definitions of salt sensitivity. The minimally reliable method may involve following BP for 5–7 day periods on normal salt, followed by low, and then a high, sodium diet [45]; the results from shorter, more acute protocols correlate poorly. At the other extreme, and based upon the well known biphasic BP response to diuretics, we would suggest that each dietary intervention period should be of 3 months duration to properly capture the true BP response. Salt sensitivity is associated with sympathetic overactivity and diminished suppression of circulating renin activity in patients with essential hypertension [46]. Based largely upon the imposition of an acute high or low-salt challenge either with or without diuretics, the index of salt sensitivity is approximately 10–15% among normotensive individuals vs. approximately 25% among patients with essential hypertension. However, we suspect that current salt-sensitive measures underestimate the prevalence of the phenomenon in both groups. In the Intersalt study, the rate of rise of population BP with age was strongly associated with dietary salt intake [2]. Our interpretation of this observation is that virtually all individuals in a population can be viewed as being salt-sensitive, albeit to variable degrees; the clear implication is that with sufficient time, salt ‘floats most boats’. Hence, relatively acute tests for salt sensitivity in humans may underestimate the phenomenon; they miss slow salt-sensitive responders and, by definition, label them as salt-resistant. The impact of this type of misclassification is incalculable.

SALT, BLOOD PRESSURE, ENDOGENOUS OUABAIN AND THE J CURVE

Population-wide reduction in sodium intake has been suggested based upon its significant impact on BP in many but not all individuals. Even in responders, the depressor effects of a low-sodium diet are offset to a variable degree by reflex increases in many variables including plasma renin and aldosterone levels, sympathetic nerve activity, plasma insulin and glucose, and serum lipids. In individuals with average sodium intakes of less than 4 g/day, there is generally an inverse association of intake with adverse outcome [47]. In contrast, a direct relationship with BP is more likely with average intakes of above 5 g/day. Thus, among individuals chronically adapted to their diets, the relationship of subsequent changes in salt intake with cardiovascular outcomes is ‘J-shaped’. This type of relationship

is not unique. A J curve exists in type II diabetes; moderate lowering of blood sugar is beneficial, whereas the most aggressive correction of blood sugar with target values near normal increases mortality [48]. And, in pregnancy, a J curve relates maternal salt intake with low nephron number in the offspring [49].

The BP response of individuals to changes in dietary salt depends upon physiological mechanisms in the CNS and periphery and is consistent with the 'set point' hypothesis [50]. A consequence of a defended set point is that the short-term raising or lowering of salt intake can and will have paradoxical effects. For example, in response to 30 days of a low-salt diet, individual BP can drop, remain unchanged or even increase [51]. The significance of the latter group, which we term 'inverse salt sensitives', has been largely ignored in the debate concerning the relationship between salt and BP and especially in the drive to make a single recommendation concerning the value of a low-salt diet for the public at large [52]. This raises the question: What mechanisms might explain the J curve that relates dietary salt with increased risk of adverse events? Among the two most studied CTS (endogenous ouabain and marinobufagenin), both respond to changes in salt balance. In Dahl S rats with high salt, the urinary excretion of endogenous ouabain increased transiently, whereas that for marinobufagenin rose progressively [53]. In normal humans, a small but carefully done study found a J-shaped relationship of sodium balance with plasma endogenous ouabain, whereas BP was unchanged [54]. Relative to the plasma endogenous ouabain levels on the diet to which the study participants were chronically adapted, the circulating levels of endogenous ouabain rose during periods of high dietary Na^+ and during periods of Na^+ depletion. This is reminiscent of a bi-directional stress response. With salt depletion, endogenous ouabain, like aldosterone, behaves as if it were a teleologically important hormone helping to maintain vascular tone and BP. This also has been suggested from epidemiological studies of endogenous ouabain in the Belgian population [55]. By itself, the observation of a J-shaped relation of endogenous ouabain with salt balance is interesting but hardly noteworthy. However, in virtually all clinical studies in which endogenous ouabain is chronically elevated, outcome is universally poor [15,56,57■■■]. This raises the possibility that high endogenous ouabain *per se* either is a biomarker and/or a functional mediator of adverse cardiovascular outcomes.

MECHANISMS AND RELEVANCE OF THE SHORT AND LONG-TERM VASOPRESSOR ACTIONS OF ENDOGENOUS OUABAIN

Thirty-five years ago, one of us proposed a hypothesis linking the high salt intakes of western acculturated societies with hypertension. The links involved the binding of a circulating inhibitor of the Na^+ pump to the vascular myocyte Na^+ pump, diminished Ca^{2+} efflux via the vascular sodium calcium exchanger (NCX1.3) and increased tone of isolated arteries [58]. The increased peripheral vascular resistance in essential hypertension was a consequence of elevated circulating levels of an unidentified Na^+ pump inhibitor that were evoked by high salt. With the exception of the identification of the human circulating Na^+ pump inhibitor as an endogenous ouabain [59], the primary elements of the hypothesis have been confirmed only recently. Key roles of the vascular myocyte α -2 isoform of the Na^+ pump and NCX1.3 in ouabain, ACTH-, and salt-sensitive hypertension were recently demonstrated in transgenic mice [28,29,60]. Unforeseen by the original hypothesis, there are at least two additional signaling components of relevance. The first involves Na^+ pumps that may exist in a nonpumping state [61]. Binding of ouabain and endogenous ouabain to these pumps activates the non-receptor tyrosine kinase c-Src and stimulates pathways [62] that may help support increases in long-term myogenic tone and reactivity [63-65]. The second component is a transient receptor operated channel (TRPC) of which the type 6 protein appears to be most relevant [63]. The overall importance of NCX1.3 and TRPC6 in regulating vascular tone [63,66], when taken together with the ability of ouabain to up-

regulate their expression [63,67], enhance Ca^{2+} signaling [68■■], amplify ganglionic sympathetic nerve traffic [69] and augment the impact of normal sympathetic tone in the artery [70], indicates their critical role in sustaining hypertension. Clinically, the observation that approximately 40–50% of patients with mild to moderate essential hypertension have elevated circulating endogenous ouabain [15], together with the defined mechanisms by which ouabain induces hypertension in rodents, suggests that endogenous ouabain should be considered as a major contributor to increased BP and overall cardiovascular risk. Specific instances of adverse outcomes that have been associated with elevated endogenous ouabain include hypertension, cardiac enlargement, cardiac and renal failure, and a variety of terminal disorders [15]. Most recently, elevated preoperative plasma levels of endogenous ouabain, when combined with other clinical parameters, were shown to better predict adverse outcomes resulting from acute kidney injury [57■■]. It was suggested that the elevated preoperative levels of endogenous ouabain led to subtle subclinical podocyte damage that was exacerbated by subsequent cardiac surgery. Thus, endogenous ouabain appears to be an especially valuable functional marker for acute kidney injury.

CONCLUSION

The CNS, the peripheral vasculature and the renal medulla link salt sensing with the salt-sensitive control of BP and kidney function. Short-term changes in dietary salt and CNS infusions stimulate salt-sensing pathways within the brain that initially mediate large increases in sympathetic nervous system activity that constrict the peripheral vasculature and raise BP. The more clinically relevant phenomenon of long-term salt sensitivity involves central sodium sensors that also stimulate a pathway that has a slow pressor action. The slow pathway involves the activation of central aldosterone, mineralocorticoid receptors, ENaCs, endogenous ouabain and AT1 receptors and, in the periphery, raises circulating CTS. Chronic elevation of circulating endogenous ouabain (and possibly other CTS) amplifies existing sympathetic tone, augments the contractile function of the peripheral vasculature, suppresses nitric oxide-related signaling in the renal medulla and resets kidney function to help maintain the elevated BP. Whereas our hypothesis primarily concerns salt-sensitive hypertension, the activation of the molecular sequence distal to the brain mechanism should induce a salt-resistant hypertension. Specific targeting of the salt-sensitive pathway in the brain may be therapeutically useful.

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 131).

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KEY POINTS

- The brain is salt-sensitive. It has specific and discrete short-term rapid-acting and long-term slow-acting pathways that affect blood pressure.
- The central long-term slow pathway involves aldosterone, mineralocorticoid receptors, epithelial sodium channels and endogenous ouabain.
- Blocking of any component in this central pathway prevents salt-sensitive hypertension in experimental animals.

FIGURE 1.

Proposed sequence of events linking dietary, plasma and CSF NaCl with salt sensitivity and sodium-sensitive hypertension. In this scheme, short-term increases in CSF [NaCl] are sensed primarily by magnocellular neurons. This activates an AT1R-dependent pathway that raises sympathetic nerve activity and BP. Long-term increase in CSF [NaCl] or angiotensin II activates a slow CNS sequence comprised of aldosterone–MR–ENaCs–EO. Maintained activation of this sequence chronically augments AT1R-mediated signaling and, via mechanisms that are not clear, stimulates peripheral CTS including EO and EO isomers. Maintained elevation of plasma EO increases the arterial expression of NCX1.3, TRPC6, and raises cytosolic Ca^{2+} , myogenic tone, vascular reactivity and BP. In addition, chronic EO suppresses NO-mediated signaling in the renal medulla and helps to reset the pressure–

natriuresis relationship. In this model, salt sensitivity originates in the CNS; the blockade of central aldosterone synthesis, MR, ENaC or central EO prevents or abrogates chronic salt-sensitive hypertension. Thus, hypertension will arise as the result of any primary abnormality in any component of the CNS pathway alone, or can be triggered by renal-vascular or renal-tubular abnormalities that lead to net salt retention and increased CSF NaCl. +, amplifying effect;?, uncertain relationship. EO, endogenous ouabain; icv, intracerebroventricular; Ang II, angiotensin II; CNS, central nervous system; CSF, cerebroventricular fluid; PVN, paraventricular nucleus; MR, mineralocorticoid receptor; AT1R, angiotensin II type 1 receptor; LTP, long-term potentiation; ACTH, adrenocorticotrophic hormone; AVP, arginine vasopressin; NO, nitric oxide (see text for details and other definitions).