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ENDOGENOUS CARDIOTONIC STEROIDS IN KIDNEY FAILURE: A REVIEW AND A HYPOTHESIS

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Abstract

In response to progressive nephron loss, volume and humoral signals in the circulation have increasing relevance. These signals, including plasma sodium, angiotensin II and those related to volume status, activate a slow neuromodulatory pathway within the central nervous system (CNS). The slow CNS pathway includes specific receptors for angiotensin II, mineralocorticoids, and endogenous ouabain (EO). Stimulation of the pathway leads to elevated sympathetic nervous system activity (SNA) and increased circulating EO. The sustained elevation of circulating EO (or ouabain) stimulates central and peripheral mechanisms that amplify the impact of SNA on vascular tone. These include: changes in synaptic plasticity in the brain and sympathetic ganglia that increase preganglionic tone and amplify ganglionic transmission, amplification of the impact of SNA on arterial tone in the vascular wall, and the reprogramming of calcium signaling proteins in arterial myocytes. These increase SNA, raise basal and evoked arterial tone, and elevate blood pressure (BP). In the setting of chronic kidney disease, we suggest that sustained elevation of the slow CNS pathway, plasma EO and the cardiotonic steroid marinobufagenin (MBG), comprise a feed-forward system that raises BP and accelerates kidney and cardiac damage. Block of the slow CNS pathway and/or circulating EO and MBG may reduce BP and slow the progression to end stage renal disease.

Keywords

Ouabain; marinobufagenin; brain; hypertension; renal; dialysis; hormones

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Volume Expansion, Natriuretic Hormones and Sodium Pump Inhibitors in Kidney Disease

The kidney is critical to long term salt and water balance. In addition to renal perfusion pressure and an adequate glomerular filtration rate (GFR), salt balance involves well recognized hormones including aldosterone, the atrial peptides and angiotensin II. Moreover, as the number of functional nephrons progressively declines, the behavior of volume regulating hormones and extracellular fluid volume (ECFV) status in general becomes of increasing significance.

A progressive increase in ECFV becomes apparent as the number of filtering nephrons declines to a critically low number and profound adjustments in sodium regulating mechanisms have been suggested. In response to acute or chronic expansion of ECFV, a "third" factor other than aldosterone or GFR (or renal perfusion pressure) is required to explain sodium balance[1]. Numerous studies showed that the third factor was humoral and it induced natriuresis[2]. This so called natriuretic hormone (NH) has long been associated with the efferent natriuretic response to elevated plasma and intracerebroventricular sodium as well as intravascular volume expansion[3]. Hence, a significant portion of the pioneering work in the search for NHs employed human or experimental animals with chronic kidney disease (CKD) in whom ECFV is expanded. Some examples are presented in Table 1. The working assumption was that, in the progression to CKD, circulating factors that inhibit the tubular sodium pump were a compensatory mechanism that might help offset the decline in GFR[29–32]. Further, elevated levels of an NH might, as a side effect, augment vascular tone and raise blood pressure (BP). Indeed, increased circulating levels of a sodium pump inhibitor were readily detected in low renin (i.e., volume-expanded) hypertension[33] and subsequent work demonstrated multiple natriuretic materials in the circulation and urine[3]. Moreover, in the last decade, several natriuretic materials have been isolated and identified. Some inhibit sodium transport and, like the classic cardiotonic steroids (CTS) ouabain and digoxin, inhibit sodium pumps. Yet other identified materials are natriuretic via secondary transport mechanisms[3]. Here, we focus on two endogenous CTS implicated in the pathogenesis of CKD and propose a feed-forward mechanism including specific elements by which one or both CTS become elevated in the circulation, raise BP, and promote renal and cardiac damage.

Endogenous Cardiotonic Steroids Implicated in Kidney Failure

Three endogenous CTS (eCTS) of relevance to ECFV balance and long-term BP control have been isolated and identified by analytical means in human plasma and/or urine[24,34,35]. The identified eCTS are ouabain, marinobufagenin (MBG), and telocinobufagin. In addition, there are strong indications that small amounts of digoxin, in addition to one or more digoxin-immunoreactive materials, are present in the circulation, urine and peritoneal dialysate of humans[18,22,25,28,37]. The presence of these multiple eCTS in mammals implies different signaling systems and functional effects[38–45]. The general evidence supporting the biosynthesis of eCTS is clear[46–51] even though the specific details of the various pathway(s) remain to be elucidated. Nevertheless, as noted below, elevated circulating and or urinary levels of several eCTS are present in conditions associated with acute or chronic increases in

ECFV. Further, these eCTS appear to contribute significantly to the underlying renal and cardiac pathology in renal failure and recent animal studies show that methods to control the negative effects of the eCTS are valuable. Indeed, one or more of these methods may warrant investigation as possible new therapeutic modalities in humans to delay dialysis and/or transplantation.

Endogenous CTS, Kidney Damage and Cardiac Fibrosis

Renal damage

The circulating levels of EO, MBG and telocinobufagin are increased dramatically in virtually all patients undergoing dialysis for kidney disease[15,23,24]. Further, in the rat, partial nephrectomy raises MBG[52] and EO[53]. There is renal fibrosis associated with elevated MBG[54] whereas the sustained elevation of circulating EO or ouabain causes podocyte damage and proteinuria and these effects can be blocked by the ouabain receptor antagonist, rostafuroxin[55,56] which blocks ouabain binding to the Na^+ pump without causing inhibition of ion transport. Thus far, there appear to be no large differences in circulating EO in CKD patients with or without HTN which may be explained by differences in the types and dosages of therapeutic agents. Further, as noted below, the relationship between EO and BP is complicated by lag phases and there are no studies that address these issues.

The mineralocorticoid receptor (MR) antagonists, long recognized for their beneficial effect in heart failure[57] have, together with inhibitors of aldosterone biosynthesis, been suggested as a way to minimize renal fibrosis[58,59]. Indeed, spironolactone attenuates experimental uremic cardiomyopathy where MBG and EO are elevated[60]. It has been suggested that the basis for the beneficial effect of spironolactone is not solely related to MR blockade, but may also involve their ability to compete with MBG and EO and possibly other CTS for binding to the sodium pump[61,62]. Yet other mechanisms have been suggested for the beneficial actions of spironolactone[63]. Further, when given into the CNS in tiny amounts that have no effect when given peripherally, MR antagonists reduce sympathetic outflow, are antihypertensive[64], and have a dramatic beneficial impact on cardiac function in heart failure[65]. Further, as an MR-dependent pathway in the brain controls circulating EO[66], the CNS, not ordinarily recognized as a key factor in CKF, is likely to play an important role in the progression to end-stage renal disease (ESRD). In the latter context, nothing is known about the potential benefits of CNS MR blockade in experimental models of chronic renal failure. However, spironolactone is beneficial for the long-term treatment of resistant hypertension in patients with CKF and may improve kidney function[67,68]. The improved outcomes are reminiscent of the unanticipated beneficial effect of MR blockers in severe heart failure[57]; i.e., a condition where ECFV is often expanded, and there are also elevated levels of circulating EO and other CTS[69–71].

Cardiac fibrosis

Patients with CKF progressively lose cardiac diastolic function and develop ventricular hypertrophy with various geometric manifestations[72]. Systolic dysfunction impacts 10–20% of patients with ESRD. It appears that the diastolic dysfunction and ventricular hypertrophy are not simply secondary to the hypertension and anemia observed in CKF[73]. Partially

nephrectomized rats also develop diastolic dysfunction, ventricular hypertrophy, and cardiac fibrosis[41,74,75]. Further in rats, the prolonged infusion of MBG reproduces many features of uremic cardiomyopathy and MBG *per se* stimulates collagen formation in cardiac fibroblasts in cell culture[41]. More significantly, in partially nephrectomized rats, both active and passive immunization against MBG attenuates most of the cardiomyopathy[75,76].

Recent insights into how EO raises vascular tone and blood pressure

Hypertension is a frequent and early component in CKF. Expansion of ECFV is associated with hypertension in ~75% of patients with chronic renal failure and typically can be controlled with hemodialysis; i.e., benefit reflects removal of fluid and not dialyzable vasopressor agents. Another significant cause of hypertension in uremic patients is hyperreninemia. The hypertension tends to be more severe, unresponsive to volume manipulation, and likely will require bilateral nephrectomy and/or transplant. There is a clear need for better control of hypertension in CRF. But what are the pressor pathway(s) in the volume- and renin-dependent patients?

EO, MBG and telocinobufagin are three known eCTS that circulate in elevated amounts in patients with CKF[23,24,38]. Although elevated EO is often observed in conditions where fluid volume is chronically expanded, it does not explain the acute “salt-sensitive” variations in BP that follow short term changes in salt intake[77]. However, the chronic elevation of EO and MBG typically generate sustained increases in BP in rodents[75,78,79]. It was initially suggested that the long-term pressor effect of EO involved interactions between the brain, arterial vasculature and the kidneys[80]. Subsequent studies in rats and transgenic mice have confirmed this hypothesis and elucidated many key events in the pressor mechanism of EO[81], and also highlight the numerous gaps in knowledge that remain. The vasopressor effect of EO has acute and chronic facets. The acute pressor effect is generally believed to be mediated by inhibition of the Na^+ pump and an indirect action that involves calcium entry mediated via the sodium-calcium exchanger that elevates myogenic and evoked tone[82,83]. The rise in intracellular calcium triggers increased contraction and, when short-term cardiovascular reflexes are blocked, raises BP. In response to sustained elevation of circulating EO, the chronic pressor effect is maintained by activation of a signaling pathway that upregulates expression of several key ion transport proteins in arterial myocytes. These proteins include the sodium calcium exchanger type 1 (NCX1), the sarcoplasmic reticulum calcium ATPase (SERCA) and the transient potential receptor canonical protein 6 (TRPC6). The upregulation of these proteins in arteries requires sustained occupation by circulating EO of the ouabain binding site on the alpha-2 isoform of the Na^+ pump. The long-term binding of EO activates the protein kinase c-SRC and stimulates upregulation of the calcium transport proteins via unknown signaling events.

Further upstream, recent observations show that the CNS can control circulating EO. The CNS has a “slow neuromodulatory pathway”[84] whose long term effects on BP and circulating EO can be blocked centrally by antagonists of aldosterone synthesis as well as MR[66]. The CNS slow pathway is overactive in salt- and volume- as well as angiotensin II-dependent forms of experimental hypertension where EO is elevated, as well as in heart failure[64,65,69,70,85,86]. The slow pathway components appear to constitute a major

mechanism by which BP is elevated in many common disorders. Remarkably, the significance of this CNS pathway in renal failure is unknown. However, because the CNS receives volume, sodium and angiotensin II signals in various phases of CKF, we suggest that this brain pathway is likely to be fundamentally involved in raising BP and affecting progression. The overall pathway is summarized in Figure 1.

Implications for Therapy

Clearance of Cardiac Glycosides in ESRD

The clearance of cardiac glycosides is one significant determinant of their circulating levels. Although digoxin and digitoxin are cleared primarily by the liver, renal clearance is significant, especially for the more polar digoxin[93]. The clearance of the highly polar EO is mediated to a large extent by the kidneys.

Various transport mechanisms recover cardiac glycosides from the tubular fluid. At the basolateral membrane of the proximal tubular cell, an organic anion transporting polypeptide (SLCO4C1) mediates recovery of digoxin and ouabain[94]. In addition, the apical secretion of cardiac glycosides is mediated by the P-glycoprotein (PGP) efflux transporter, a protein encoded by the multidrug resistance 1 gene (MDR1)[95]. Furthermore, among patients with EH, the MDR1 locus is related to elevated circulating EO and may reflect diminished apical secretion of EO[96]. In addition, those hypertensives with elevated circulating EO also exhibit estimated glomerular filtration rates (eGFR) that are reduced[97].

In terms of cardiac glycoside therapy, exogenous ouabain is no longer widely used. Thus, a primary concern is when the declining renal function will impact circulating EO. Plasma EO rises with the progression of CKF and especially in ESRD. The early rise in EO is evident with stages III and IV (Fig 2) although the impact of the lower GFR is surprisingly modest at this level of impairment. One likely reason is that only a small fraction (<1–3%) of the normal filtered load of EO ordinarily appears in the urine[92]. Another reason is that as hyperfiltration develops, the increase in proximal tubular flow rate will diminish tubular recovery of EO and offset a large portion of the decline in the filtered load[53]. However, with the advent of end stage failure and near zero urinary excretion, plasma EO rises substantially into the low nanomolar range[23]. This scenario, analogous to unwanted and uncontrollable digitalization, is worrisome in terms of the potential for undesirable cardiac and vasopressor effects, and because neither hemodialysis nor peritoneal dialysis are effective in removing EO[23,98]. Further, among patients with ESRD in dialysis, plasma EO was strongly associated with left ventricular mass and geometry[23]. This association was independent of arterial pressure and other well-established determinants of left ventricular mass. Thus, in ESRD, left ventricular hypertrophy and high cardiovascular risk, including that from hypertension, represent additional multifactorial problems potentially related to EO.

In addition to raising BP, the elevated EO in kidney failure may directly damage podocytes[55]. In rats, the chronic elevation of circulating ouabain reduced creatinine clearance, increased urinary protein excretion, and reduced the expression of podocyte nephrin, a selective podocyte marker protein. This last finding was replicated *ex vivo* by incubating podocyte primary cell cultures with low-dose ouabain. Notably, the ouabain/EO

antagonist rostafuroxin prevented the podocyte lesions and proteinuria[56]. These results suggest that rostafuroxin should be evaluated in patients that have progressive glomerular disease.

EO as a Biomarker for Acute Kidney Injury

The observation that ouabain foments kidney damage in the rat suggested a possible role of EO in acute kidney injury (AKI). Indeed, circulating EO rises with an interesting time course during the induction of anesthesia in patients about to undergo elective cardiac surgery[98]. In a follow up cross-sectional study, the preoperative plasma levels of EO were measured in 407 patients admitted for cardiac surgery and in a second (validation) population of 219 patients. Among the first group of patients, the incidence of acute kidney injury (2.8%, 8.3%, 20.3%, $p<0.001$) increased with each incremental preoperative EO tertile. Regression analyses revealed that preoperative circulating EO (and not the peak levels achieved during surgery) was the strongest predictor of AKI and this was confirmed also in the validation cohort. Most recently, we demonstrated the power of two clinical risk score models to predict AKI. Strikingly, the predictive power of both models was further and significantly improved by the addition of preoperative plasma EO levels[99]. Thus, preoperative EO appears to be a marker for preexisting renal injury and, as shown by the experimental studies, can be a direct cause of kidney damage. Accordingly, we have suggested that among patients that will need cardiac surgery, those with high EO have preexisting (subclinical) renal injury that is likely to be exacerbated by the stress of anesthesia and/or surgery. It appears that it is the combination of these stressors that augments the probability that the patient will develop AKI following cardiac surgery[55]. Fortunately, methods that lower circulating EO and/or that block its interaction with the Na,K-ATPase are available (see below) for clinical use and these should be tested for their potential to ameliorate or prevent AKI.

Rostafuroxin, a novel antihypertensive compound

Nanomolar concentrations of EO increase renotubular reabsorption of sodium and increase myogenic tone and arterial resistance[81,83]. Structure-activity relationships indicate that the chronic pressor effect is prominent for ouabain and ouabain-like steroids; the chronic infusion of digitalis preparations including digoxin and digitoxin does not lead to sustained increases in BP. Furthermore, in ouabain-dependent hypertension, the infusion of digitalis CTS such as digoxin or digitoxin normalized blood pressure[77] and the effect with digitoxin, a more lipophilic agent likely to penetrate the CNS, was especially marked. Thus, digitalis glycosides have antihypertensive activity under conditions where the circulating levels of EO are elevated. These and other observations[100] led to the development of rostafuroxin (formerly PST2238) (17β-(3-furyl)-5β-androstan-3β,14β,17α-triol), a digitoxigenin derivative that displaces ouabain from its binding sites on the Na^+ pump (Na,K-ATPase) (IC_{50} 1.7×10^{-6} M) without interacting with other receptors involved in blood pressure regulation or hormonal control[101]. In ouabain (and EO)-dependent models of hypertension, rostafuroxin dose-dependently (0.1 to 100 μg/kg p.o.,) lowers BP, and normalizes renal Na,K-ATPase activity[101]. In renal caveolae, subnanomolar ouabain concentrations switch on latent Na,K-ATPase units in the proximal tubular plasma membrane and activate a signaling pathway that involves the tyrosine-phosphorylation of c-Src, transactivation of the epidermal

growth factor receptor[103] and activation of cytosolic p42/44 MAPKl. Rostafuroxin at nanomolar concentrations antagonizes the ouabain-Na^+ pump interaction and normalizes the above signaling events. Moreover, rostafuroxin (PST2238) blocked the ouabain activation of the cSRC-EGFr-ERK pathway and prevented ouabain-induced cardiac and renal hypertrophy[103]. Rostafuroxin is able to correct the abnormal upregulation of renal Na^+ pump number in the plasma membrane without inhibiting renotubular transporters involved in sodium transport, as the diuretics do. The significance of this effect in terms of its ability to reduce BP is not known. It should be recalled that the kidney typically does not affect vascular tone directly. The mechanisms that generate most all of the vascular tone and that are responsible for increases in long-term increases in blood pressure reside in the central nervous system, the adrenal glands and in the vascular wall itself. Further, rostafuroxin blocks the vasopressor effect of ouabain in arteries[104] and ameliorates endothelial dysfunction and oxidative stress in resistance arteries from deoxycorticosterone acetate-salt hypertensive rats[105]. As a digitalis derivative, rostafuroxin is likely (although not yet tested) to be an effective antagonist in the CNS at sites where brain EO augments sympathetic outflow[85].

Clinical studies with Rostafuroxin

More than one-third of patients with essential hypertension (EH), most all patients with chronic renal failure, and a large portion of patients with heart failure have increased circulating levels of EO[23,69,96,97,106,107]. These are primary target populations for rostafuroxin therapy. However, in a trial of unselected patients with EH (i.e., in which ~70% of the patients would not have elevated EO), rostafuroxin had no overall impact on BP[108]. Instead, the BP lowering activity of rostafuroxin depended on genomic variation in factors related to the synthesis and clearance of EO and to cytoskeletal polymorphisms. In rostafuroxin-sensitive patients, the systolic BP declined 14 mm Hg after 4 weeks of treatment relative to controls[108]. The gene variants encode enzymes in steroid biosynthesis, transmembrane EO(ouabain) transport, and the cell cytoskeleton. This combination of gene variants occurred in 23% of the 196 (never treated) hypertensives in the study.

The antihypertensive effect of rostafuroxin was tested in never-treated, recently discovered hypertensive patients. The use of naïve patients may be important as even a 4-week period is not sufficient to wash-out the effects of prior treatment,[107,109,110]. For example, the stimulatory effect of diuretic treatment on the RAAS can last 6 months[109,111]. Thus, different enrollment criteria are required in pharmacogenomics studies. In addition, as one of us has noted elsewhere[104], the maximal dose of rostafuroxin used thus far in clinical studies, even with drug-sensitive patients, may have been suboptimal by at least an order of magnitude or more. Data regarding the impact of rostafuroxin in treating heart failure and the hypertension in patients with end stage renal failure are not available. In the latter settings, circulating EO levels are considerably higher[23,69] than in EH, and dramatically larger doses of rostafuroxin than those used in prior trials may be needed to demonstrate efficacy.

In view of the close structural similarity of rostafuroxin with the digitalis CTS, it is of interest that digoxin is associated with increased mortality in ESRD in one study[112] but not

another[113]. The potential for life-threatening effects of digitalis preparations is readily apparent in the setting of ESRD. However, rostafuroxin has no overt arrhythmogenic activity[101] and thus is worth evaluating for its antihypertensive activity in the setting of CKD and in ESRD.

Volume Management in ESRD, CTS Levels and the Lag Phenomenon

It has been restated persuasively that the effective management of volume to "dry weight" is sufficient to restore normotension in the majority of dialysis patients[114,115] and improve survival[116]. However, overly rapid fluid removal is not invariably synonymous with the restoration of normotension perhaps because it triggers short-term neurohumoral reflexes that attempt to maintain BP. More prolonged dialysis times with less abrupt fluid removal would be expected to minimize short-term reflex sympathetic responses[117,118].

To our knowledge, there are no studies that address the impact of defined changes in volume in ESRD on the circulating levels of eCTS and their relationship with BP. And the design and execution of such studies is not trivial for reasons related to clearance and lag effects. For example, in rodents given steady infusions of ouabain, the elevated plasma ouabain takes nearly a week to begin to elevate BP, and several weeks are needed to achieve the maximal pressor effect[78]. In other words, ouabain (EO) is a slow pressor hormone and, therefore steady-state measurements are needed to reveal the relationship between circulating ouabain (EO) and BP. The slow pressor effect of ouabain involves peripheral and central components. In the periphery, there is functional reprogramming of the sympathetic ganglia and the arterial vasculature (2–3 days in the rat). With regard to the CNS, ouabain enters the brain slowly and is believed to contribute to the EO pool in the slow neuromodulatory pathway (Figure 1); this is thought to drive the sustained increase in sympathetic outflow. When ouabain administration is discontinued, plasma EO normalizes within in hours in the normal rodent[119] while it takes ~3–7 days before BP normalizes[78,79]. Thus, the drop in BP is delayed relative to plasma ouabain and, again, the absence of a steady state obscures the relationship between ouabain (EO) and BP. But how is the delay in the normalization of BP explained, and does it have relevance to the "lag hypothesis" in the setting of the hemodialysis patient?

It is apparent that the slow reversal of ouabain-dependent hypertension is not necessarily explained by the clearance of ouabain from the circulation *per se*. In normal rats, the half time for the clearance of ouabain (EO) is < 5 hours[120], whereas in normal humans it is ~23 hours and ~50 hours in patients with renal failure[120,121]. As dialysis *per se* is unable to effectively remove EO from the circulation, we may ask whether dialysis can remove the volume (or other) stimulus to EO secretion and reduce BP by that mechanism? There are numerous considerations: 1). With short-term rapid dialysis, the answer is probably no. Even if we assume that the chronic volume stimulus to EO secretion could be effectively switched off by a single dry weight dialysis, then in patients with renal failure, the decline in circulating EO would be simply too slow to allow for a significant decline in BP through an EO-dependent pressor mechanism. 2). Even presuming hemodialysis as an effective way to remove EO, it is clear based upon the above half times for clearance, that no significant BP drop would be expected for several days and, by then, the patient's (re)expanded volume

and other stimuli to EO secretion would have returned. In other words, in the CKD setting where EO is chronically elevated, probing of its role in the lag phenomenon does not appear to be easily amenable to investigation by manipulation of dialysis protocols *per se*. 3). In the above context, the ability to specifically and chronically suppress EO secretion and/or physically remove it from the circulation is needed. Much work suggests that the adrenal cortex provides the majority of the circulating EO[49] but currently there are no specific ways to block adrenal EO production that do not affect classical corticosteroid pathways. In experimental studies, MR blockers given into the CNS reduce circulating EO[66] but this is not feasible in patients. However, another approach that may be practical involves the capture of EO by immobilized Fab fragments (Digifab™) during dialysis[122]. A well designed cartridge with Fab fragments inserted into the dialysis chain might also be amenable to regeneration and reusability over several weeks in the same patient. 4). The peripheral, and perhaps CNS, actions of EO on Na^+ pumps might be blocked by rostafuroxin, but this has yet to be tested in CKD. While canrenone, a metabolite of spironolactone, may already perform a similar function, rostafuroxin may have the advantage in that it does not provoke hyperkalemia. 5). Another more radical approach is to actively immunize patients against EO. As EO is produced and secreted slowly (production is <10,000 times lower than angiotensin II), the production of endogenous antibodies would be more than fast enough to neutralize circulating EO. Indeed, in Dahl salt-sensitive rats where there is a renal abnormality driving chronic sodium retention and volume excess, the slow neuromodulatory pathway is overactive[123–127], and active immunity against EO lowers BP significantly[128].

Does circulating EO explain the slow decline in BP that accompanies prolonged dry weight dialysis? Figure 7 of Twardowski[115] indicates that dry weight dialysis requires ~9 months to normalize BP and that the apparent half-time for the decline in BP is approximately one month. For EO to be the mediator of this prolonged BP lag, we suggest there are two potential mechanisms that may be relevant. In the first mechanism, the chronic elevation of plasma EO (ouabain) in CKD, and by analogy with ouabain administration in experimental studies, is expected to lead to prominent accumulation of this steroid in the hypothalamus, anterior pituitary, and especially the kidney[78]. Muscle and many another tissues (with the exception of the adrenal glands) also take up this CTS. Thus, a significant body burden of EO is likely in CKD. Once inside cells, the half times for the turnover of EO (ouabain) are slow, ranging from ~20 hr *ex vivo* to 4 weeks *in vivo*[129,130]. Turnover of body EO “stores” is likely to be especially slow in humans because the volume of distribution for EO (ouabain) is larger than rats. Thus, the BP lag might readily be explained by the slow loss of accumulated intracellular EO to the circulation and its subsequent clearance. As noted in point 3 above, the impact of increasing EO clearance on the lag time could be tested clinically. A second distinct mechanism for the lag phenomenon may involve the plasticity of synaptic connections within the CNS. In response to CKD, the prolonged activation of the electrical elements in the slow neuromodulatory pathway (Figure 1) will likely promote formation of new synaptic connections consistent with Hebbian theory[131]. Thus, in addition to pathway activation by signals from the renal failure, new synaptic connections will likely reinforce downstream signaling events (i.e., increased sympathetic outflow and plasma EO). When the stimulus to the slow neuromodulatory pathway is removed by sustained dry

weight dialysis, electrical activity in the pathway will decline but may not normalize until such time as synaptic pruning has reversed the new connections. Changes in synaptic plasticity occur in key slow pathway nuclei during hypertension[132–134] as well as in the peripheral sympathetic ganglia[86,135]. The full reversal of these changes, and the impact of their electrical consequences on BP, may take several months.

But which of these two possibilities is more likely? Both appear to have general kinetic behavior that is quantitatively relevant to the lag phenomenon. However, there are some paradoxes. In normal humans, reducing total body sodium with diuretics raises EO[92]. This physiological increase in RAAS, EO, and activation of the slow neurohumoral pathway may help to support BP when total body sodium and/or sodium intake is low. Thus, the initial impact of dry weight dialysis seems more likely to us to stimulate the slow pathway, adding to the ongoing chronic CNS activation (stress), synaptic rewiring[136], sympathetic activity, and circulating EO. The predicted time course of these factors would seem to fit the initial changes in total peripheral resistance according to Figure 3 of Twardowski[115].

During chronic dry weight dialysis, removal of the volume stimulus to the slow CNS pathway would permit sympathetic activity and EO to decline secondary to changes in synaptic activity and wiring. If the above-noted ideas are correct, circulating EO and BP (i.e., indices of slow pathway activity) should decline more-or-less in parallel over many months of sustained dry weight dialysis. The importance of the slow neurohumoral pathway and synaptic plasticity is shown by the dramatic impact of hypothalamic lesions. Lesions that impact slow pathway nuclei prevent or reverse renal[137–140], salt and volume-dependent[141–143], and ouabain[144] forms of experimental hypertension. Thus, it is difficult to avoid the conclusion that the CNS exerts a dominant role in determining whether major deficits in renal function, sodium handling and volume excess can raise BP and sustain hypertension. Furthermore, it seems likely that altered synaptic activity and wiring have a fundamental influence on the lag phenomenon in CKD.

ESRD, Resistant Hypertension (rHT), Mineralocorticoid Receptor (MR) Antagonists and Renal Denervation

As defined by the eighth Joint National Committee (JNC 8), rHT is the inability to achieve a BP lower than 140/90 mm Hg despite optimal doses of 3 or more antihypertensive drugs, including a diuretic[109]. The prevalence of rHT varies from 10% to 15% among treated hypertensive patients[145] after exclusion of pseudo-resistant hypertension from non-adherence to medications or from the white coat syndrome. The etiology of rHT typically involves multiple factors, including obesity, older age, high dietary salt, renal artery stenosis, chronic kidney disease, and aldosterone excess[146]. Classical primary aldosteronism and lesser degrees of aldosterone excess, possibly originating from visceral adipocytes, contribute to rHT. Furthermore, many hypertensives with hyperaldosteronism have elevated circulating levels of EO.[104]

MR blockers decrease proteinuria in CKD subjects[147] but their use in patients with low or minimal filtration is limited by the risk of life-threatening hyperkalemia. Novel non-steroidal antagonists with high affinity and selectivity for the MR receptor (BR4628 and SM368229) are being developed [148,149] but it is not yet clear if these drugs will avoid the

negative impact on plasma potassium. MR antagonists are particularly effective for treatment of rHT regardless of circulating aldosterone levels. This raises the obvious question: what is their mechanism of action? Spironolactone and its major bioactive metabolite, canrenone, block aldosterone and cortisol binding to MR yet it is unlikely that their antihypertensive effect is mediated by renal MR in dialysis patients with rHT simply because tubular reabsorption becomes increasingly less relevant in the face of ever diminishing filtration. Therefore, powerful extrarenal sites of action of MR blockers are required. Instillation of MR blockers into the CNS, in amounts too small to have any peripheral effect, lowers sympathetic activity, plasma renin activity, BP, and reduces end organ damage in many experimental models of cardiovascular disease[150–151]. Further, the block of brain ouabain has correspondingly large effects in heart failure[154–156] and appear to be mediated via slow pathway effects that are also amenable to central MR blockers[125]. The slow pathway is chronically activated by a number of signals (plasma angiotensin II, plasma sodium, volume), one or more of which are abnormal in CKD. It is important to note that the slow neuromodulatory pathway differs from the rapidly acting neural networks that govern the sympathetic response to flight or fight in that it is much slower to activate and deactivate, and that it involves a number of neuroendocrine elements (Ang II receptors, aldosterone generation, MR, epithelial sodium channels and EO) that are likely arrayed in a functional sequence in the CNS. Activation of the slow pathway by Ang II leads to sustained increases in circulating EO and BP, and all effects are blocked by central aldosterone antagonists[66]. Thus, the beneficial effect of MR antagonists in many patients with rHT is most likely related to block of slow CNS pathway effects (Figure 1).

In addition, to targeting MR, spironolactone and canrenone compete with EO (ouabain) and eCTS steroids for binding to Na^+ pumps. One of the first clues that canrenone was a CTS receptor antagonist was the ability of therapeutic doses to reduce the effect of digitalis and ouabain on the heart[157–162]. Subsequent work showed canrenone suppressed ouabain binding to the Na^+ pump[163,164] and was antihypertensive in some experimental models of hypertension where ouabain-like factors were implicated[165–168]. In therapeutically relevant concentrations, canrenone blocked the effect of ouabain on basal aortic tone[77] and myogenic tone in pressured mesenteric arteries[169].

MR blockers also reduce the impact of EO in heart failure and severe hypertension.[67–69] and reduce the fibrotic effect of MBG[70]. In other words, the clinical efficacy of MR blockers likely benefits from their polyspecific actions. Based on available dose-response relationships, and the therapeutic circulating levels of MR blockers (1–5 μM), their BP lowering effects in rHT are likely mediated by block of MR and/or competition with central and/or peripheral eCTS for binding to Na^+ pumps. The negative inotropic effects of canrenone on cardiac and vascular contractility are due to block of L-type Ca^{2+} channels[170,171]. These latter effects occur at concentrations ~50–200 fold higher than those achieved in routine therapy and seem unlikely to be of clinical relevance.

There are no reports on the impact of renal denervation on EO or MBG in CKD. Only a minority of patients with apparent rHT may be eligible; the most frequent cause of ineligibility (46.9%) is BP normalization following optimization of therapy that included low-dose spironolactone[172].

Muscle fatigue in CKF and ESRD a role for eCTS?

In both heart failure and CRD, the fatigability of skeletal muscle is increased[173,174]. The underlying mechanisms are unclear but may be common to both conditions. Elevated EO is present in both CKD and heart failure and it is of interest that ouabain induces muscle fatigue[175,176]. In skeletal muscle, the highly ouabain (and EO) sensitive α-2 isoform of the Na^+ pump is expressed in the t-tubular system where it influences Ca^{2+} handling. Reduced expression (activity) of this isoform in the heart or skeletal muscle (i.e., mimicking the functional effects of the high EO state in CKD) leads to hypocontractility and the early onset of fatigue. Although the nephrologist is not ordinarily concerned with skeletal muscle performance in CKD, MR blockers and other agents that block eCTS may have the added benefit of improving exercise performance/tolerance in the kidney disease setting.

Summary

The brain, heart, circulation, and kidneys of patients with CKD are exposed to a potentially devastating mixture of neural and humoral agents, many of which appear to be activated by volume and other signals generated by, or in response to, poor kidney function. In the circulation alone, there is a toxic cocktail comprised of elevated levels of RAAS components, EO, MBG, endothelin, inhibitors of nitric oxide generation, and others. Carefully crafted, as well as bold and imaginative clinical and experimental studies are needed to test fundamental ideas and concepts in CKD, including the roles of the CNS and eCTS, and the potential impact of anti eCTS therapies.

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Clinical Summary

- The failing kidney enhances signals which activate a slow neurohumoral pathway in the brain that, in turn, controls the long term level (i.e., non-reflexive) of sympathetic nerve activity and the circulating level of endogenous ouabain.
- Inappropriate activation of the brain pathway raises sympathetic activity, circulating endogenous ouabain and blood pressure, and all these effects are prevented by agents that block the binding of angiotensin II, mineralocorticoids, and endogenous ouabain with their respective receptors in the brain and periphery.
- In patients with end stage renal failure, two endogenous cardiotonic steroids (endogenous ouabain and marinobufagenin) circulate in elevated and clinically significant amounts and exacerbate cardiac hypertrophy and renal fibrosis. It may be possible to specifically remove these materials during dialysis.
- The “lag phenomenon”, i.e., the delayed return of blood pressure following dialysis to dry weight, may be related to plasticity changes in the brain and periphery, i.e., the slow rewiring of synaptic connections in the CNS and/or sympathetic ganglia that reduce sympathetic outflow to the vasculature.
- Research directed to improving therapy for chronic renal failure should consider the role of the CNS, humoral cardiotonic steroid mediators, and test methods that chronically reduce or neutralize these steroids.

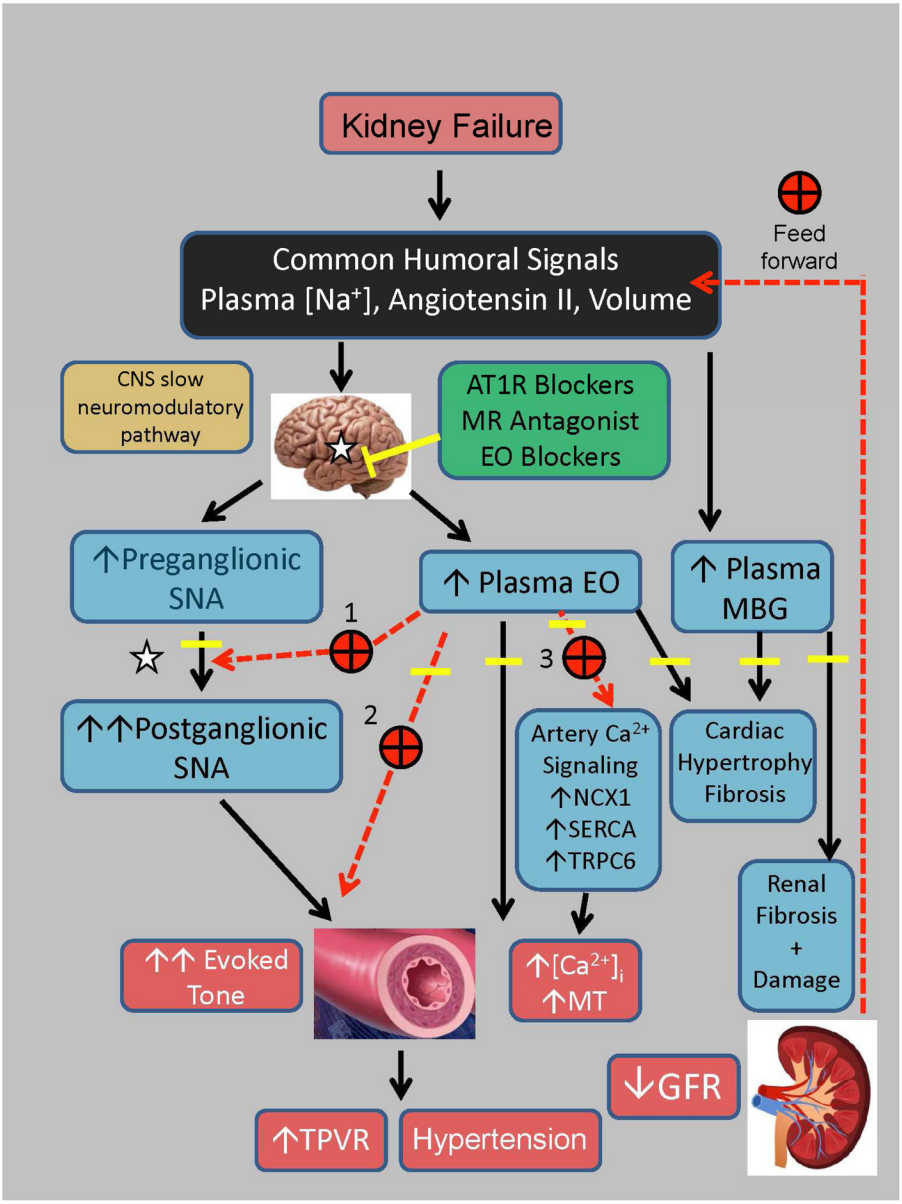

Figure 1. Proposed feed-forward pathway that raises vascular resistance and blood pressure in chronic kidney disease

In CKD, progressive loss of nephrons generates humoral signals that activate a slow neuromodulatory pathway in the brain. The slow pathway raises preganglionic SNA and circulating EO. The sustained elevation of circulating EO (red dashed lines) amplifies ganglionic function **[1]** and raises postganglionic sympathetic nerve activity[86], enhances the effect of sympathetic nerve activity **[2]** in the vascular wall[87], and directly amplifies Ca^{2+} signaling **[3]** in arterial myocytes[88]. Block of angiotensin II receptors, or MR[66], or EO in the brain may normalize downstream events. Elements of the pathway have been demonstrated in rodents and portions have been shown in human tissue[89]. The mechanism by which activation of the CNS slow pathway raises plasma EO may involve ACTH. The relative contribution of adrenal and brain EO to the elevated circulating EO in CKD is not known.

Elevated circulating MBG is shown as secondary to volume expansion (no data demonstrate CNS control of MBG); renal ischemia may trigger MBG[90]. In the scheme presented, sustained increases in MBG and EO elevate BP and also promote renal and cardiac damage that enhances the progression of renal failure. The signaling pathways for the fibrotic effects have been described elsewhere[41,91]. This feed-forward mechanism provides further stimulus to circulating EO and MBG and may accelerate progression to complete failure. Maneuvers that block EO and MBG ameliorate this cycle of events[56,60,75]. Yellow horizontal bars: potential sites for acute antagonism by canrenone. White stars: sites of synaptic plasticity. The same overall pressor mechanism may be active among some patients with essential hypertension and in heart failure, and also where there are sustained increases in angiotensin II (e.g., low salt intake[92]).

Abbreviations: AT1R, angiotensin type I receptor. CKF, chronic kidney failure. CNS, central nervous system. EO, endogenous ouabain, GFR, glomerular filtration rate. MR, mineralocorticoid receptor. MT, myogenic tone. NCX1, sodium calcium exchanger type 1. SERCA, sarcoplasmic reticulum calcium ATPase. SNA, sympathetic nerve activity. TPVR, total peripheral vascular resistance. TRPC6, transient receptor potential cation channel, subfamily C, member 6.

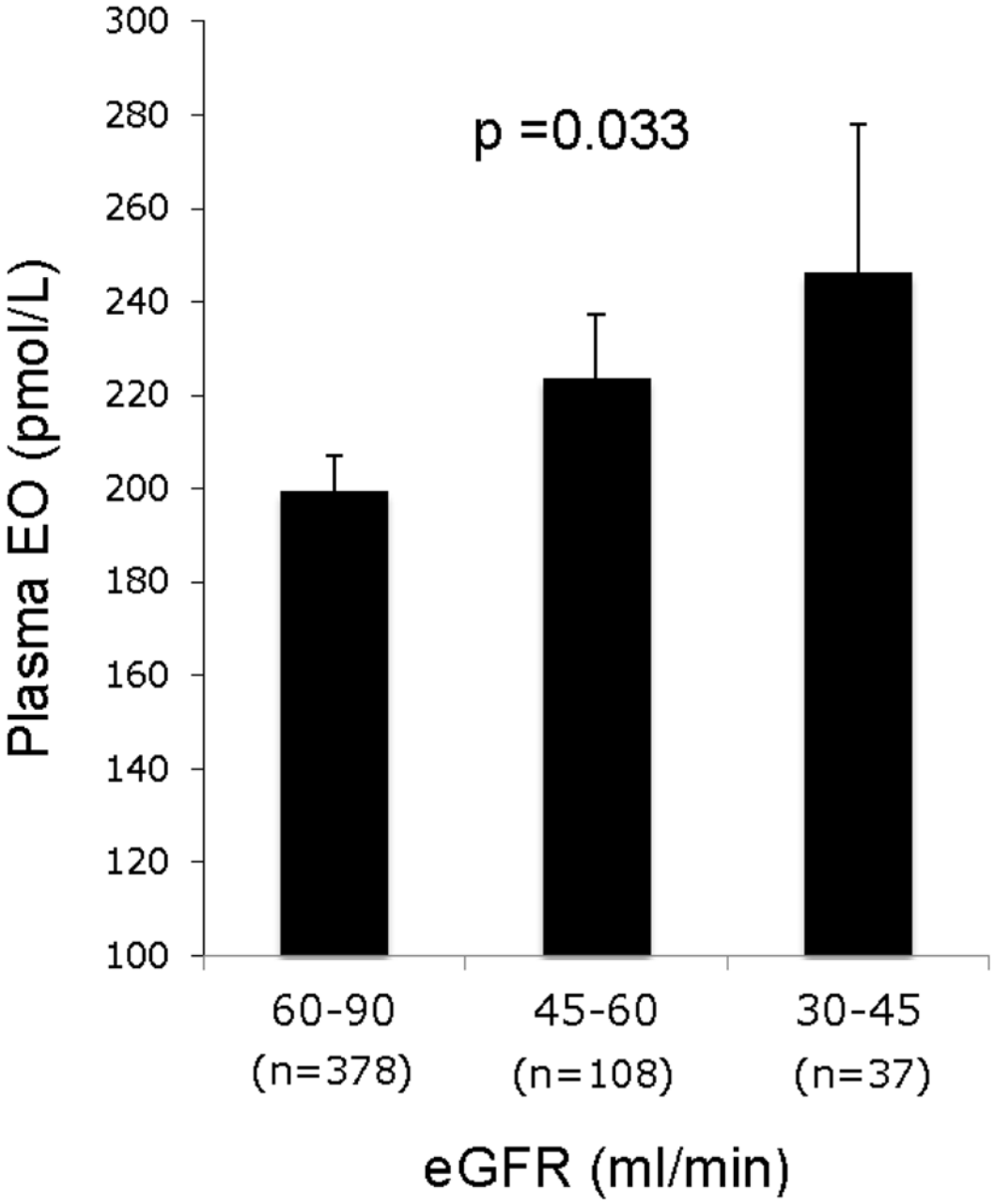

Figure 2. Circulating EO as a function of estimated GFR in CKD (Stage III to V)

The trend to increasing EO as GFR declines is significant. Simonini and Manunta, unpublished observations.

Table 1

An Overview of Humoral CTS-like Factors in Kidney Failure

Assay	Sample	Impact of CKF	Impact of Dialysis	Nature of Humoral Factor	Ref
RBC Na^+ or Rb^+ transport	Plasma or serum	↑Inhibition of sodium pump mediated ion flux	Not tested No effect No effect	Unknown	4,5 6 7
		↓ Inhibition of sodium pump mediated ion flux	↓ pump activity		8
		↓ ouabain-insensitive sodium uptake			9,10
Thymocyte Na^+ Pump Activity		↑ Inhibition	Not tested	Unknown	11
Aortic Sodium Pump Activity	Hemodialysate	↑ Inhibition	Not tested	Unknown, low MW	12
Na,K-ATPase activity	Peritoneal dialysate, Plasma	↑ Inhibition*	↓	Labile DLIF, Unknown	13,14 15
Adipocyte sodium pump activity	Serum	No change	Not tested	Not applicable	16
Skeletal myocyte Na^+ pump activity	Serum	↑ Inhibition	Not tested	NEFA?	16
^{3}H-ouabain binding	Not relevant	↓ Na pump sites adipocytes	Not tested	Not applicable	16
	Plasma	↑ competition	↓	Unknown, not from kidney	17
Digoxin Immunoassay†	Peritoneal dialysate	↑ cross reactivity	Not stated, present in peritoneal dialysate	Labile DLIF, Unknown	13,18
	Plasma	↑ cross reactivity			19
	Serum	↑ cross reactivity	↓		8
	Plasma		Not tested		14,20–22
Ouabain RIA, LC-MSMS	Plasma	↑ in plasma	No major effect on immunoreactivity	ir EO EO	23
MBG Immunoassay, LC	Plasma	↑ in plasma *	Not tested	ir MBG	15
LC-MSMS for MBG and Telocinobufagin	Plasma	↑ in plasma	Not tested	MBG, Telocinobufagin	24

In most studies, CKD increases (worsens) the measured parameter. The identity of the measured humoral agents(s) is determined only in studies after 2005 that employ analytical instruments. Few studies report the impact of dialysis. RBC, red blood cell. NEFA, non-esterified fatty acids. RIA, radioimmunoassay. MBG, marinobufagenin. ir, immunoreactivity. LC, liquid chromatography. MS, mass spectrometry.

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* Effect is reversed by digoxin Fab fragments.

† Not all digoxin immunoassays detect DLIF[22,25–28]. The DLIF signal is correlated with inhibition of Na,K-ATPase activity in some[21] but not other[8,29] studies. The difference likely reflects the reaction conditions of the ATPase assay that dramatically alter its sensitivity.