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Editorial Open Access

The Tradeoff between Natriuresis and Cardiac and Renal Fibrosis

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Abstract

Cardiotonic steroids play a key role in sodium excretion in response to volume expansion. Their effect is mediated through their binding to the Na/K-ATPase, a membrane ion transporter which has recently been found to serve as scaffolding and signaling protein. These scaffolding and signaling functions not only stimulate natriuresis through the endocytosis of key ion transporters, namely the sodium proton antiporter isoform 3 (NHE3) and itself, but also produces pro-fibrotic effects. The focus of this editorial is to the tradeoff this creates between natriuresis in the short term and progression of cardiac and renal fibrosis in the long term.

Historical Perspective

In 1961, de Wardener et al. introduced a novel concept in renal hemodynamics when he discovered that kidneys were able to increase sodium excretion after saline infusion despite controlling glomerular filtration rate, also considered "factor one" and aldosterone, which was considered "factor two" [1]. Thus, it was proposed that a "Third Factor" existed and that this material was natriuretic in a manner which did not involve GFR or mineralocorticoids. This "Third Factor" was studied by a number of investigators including Schrier et al. [2,3], Kramer and Gonick [4], Bricker et al. [5], and Gruber et al. [6]; the collective wisdom was that this "third factor" was an endogenous digitalis-like substance or Cardiotonic Steroid (CTS) [2-6]. We now know that endogenous CTS include cardenolides such as ouabain and bufadienolides such as marinobufagenin [7-10]. Ouabain and marinobufagenin have been detected in human plasma and urine [7,9,11].

In addition to their role in renal physiology, scientists were also interested in the cardiac effects of these CTS. In 1963, Repke was suggested that the Na/K-ATPase was the receptor for digitalis [12]. Since then, extensive studies from many laboratories revealed that CTS were specific ligands for the Na/K-ATPase, and in fact, produced their physiological effects through binding to the plasmalemmal Na/K-ATPase [13]. For many years, it was assumed that this binding produced inhibition of the enzymatic and ion pumping aspect of the Na/K-ATPase, but more recent data which we will discuss below has cast some doubt upon this assumption.

The Na/K-ATPase is a member of the P-type ATPase family and is responsible for the active transport of Na and K ions across animal cell membranes with energy supplied via the hydrolysis of ATP [14]. The structure and ion pumping function have been extensively studied. The currently accepted (Post-Albers) model proposes that there is are non-covalently linked alpha and beta subunits, of which multiple isoforms in various combinations exist [15] which undergo conformational changes and reversible phosphorylation depending on whether sodium is being pumped out of the cell or potassium is being pumped in. Four alpha isoforms and three beta isoforms have been identified with their expression having tissue specificities, as well as differences in sensitivity to CTS. The alpha-1 isoform which demonstrates considerable species differences in sensitivity to CTS also appears to be the main functional receptor for CTS in the kidney [16-20].

Classically, the mechanism of CTS-induced natriuresis was understood as follows: volume expansion or a salt-heavy diet leads to an increase in circulating CTS, which in turn results in the inhibition of the Na/K-ATPase in the nephron, specifically, its ion pumping ability. Consequently, cytosolic Na+ begins to rise, and eventually this disruption in the Na+ gradient across the cell membrane decreases Na reabsorption in the Renal Proximal Tubules (RPT) leading to increased sodium excretion [21]. Systemically, increased levels of CTS also inhibit the Na/K-ATPase in vasculature, thereby altering intracellular Na gradients in vascular smooth muscle cells. This indirectly leads to the inhibition of the Na/Ca exchanger causing intracellular calcium in these smooth muscle cells to rise as well [21-23]. This pathway has been implicated in the pathogenesis of hypertension [21].

More recently, Xie et al. [24] suggested in the late 1990s that the Na/K-ATPase had an additional signaling function which was related to its function as a scaffolding protein. These workers proposed that CTS also bound to a non-pumping pool of Na/K-ATPase residing in caveolae [24]. This subset of Na/K-ATPase bound the protein Src, a non-receptor tyrosine kinase, and under basal circumstances kept it in an inactive state. With the conformational change induced by CTS binding, Src was activated and a signal cascade involving the Epithelial Growth Factor Receptor (EGFR) and downstream targets such as Ras/Raf/MAPK, PI3 kinase/Akt, phospholipase C/PKC, and the generation of ROS was produced [25-32]. Work by our laboratories demonstrated that this signal cascade was directly linked to Na/K-ATPase and NHE3 endocytosis in renal proximal tubule cells and natriuresis in response to a sodium load [33-37]. Support for this theory was provided by other groups as well. Lingrel et al. first established that ouabain binding to the Na/K-ATPase is crucial in the natriuretic response of the kidney. His laboratory developed ouabain-sensitive mice by incorporating a mutation in the ouabain receptor domain of the mouse alpha-1 Na/K-ATPase and noted that saline infusion increased the natriuretic response in ouabain-sensitive in comparison to ouabain-resistant mice [38]. More recently, Nascimento showed that bufalin, another derivative of the bufadienolides required Na/K-ATPase signaling in order to produce natriuretic effects in isolated rat kidneys [39]. More recently, our group demonstrated that high-salt diets in Dahl salt-

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resistant rats (R) induced the endocytosis of RPT Na/K-ATPase and NHE3 transporters concurrent with increased Src activity. In contrast, Na/K-ATPase signaling was markedly attenuated in Dahl salt-sensitive rats (S) [40].

Natriuresis *vs.* Fibrosis

Using an experimental renal failure model induced by segmental infarction of one kidney and removal of the contralateral kidney (5/6th nephrectomy), we noted that the development of cardiac hypertrophy and fibrosis in both rats and mice appeared to involve an increase in systemic oxidative stress. Interestingly, this oxidant stress appears to depend on increased levels of circulating MBG [41-44]. In other words, the oxidant stress associated with renal failure may be due to the increased signaling of CTS through the Na/K-ATPase, a concept different from prevailing opinions which implicated inflammation as the central cause of this oxidant stress [43-45].

These physiological and morphological findings in the animal models corresponded to evidence of signaling through the Na/K-ATPase as we detected activation of both Src and MAPK phosphorylation in the fibrotic cardiac tissue. These results were similarly demonstrated in rats subjected to MBG infusion. However, after adrenalectomy to lower circulating levels of MBG and either active immunization against an MBG-albumin conjugate or passive immunization using a monoclonal developed against MBG or the ovine antibody fragment, Digibind, cardiac fibrosis was significantly reduced in both partial nephrectomy and MBG-infusion experimental groups [42,43,46-48].

Work from other groups also supports this concept. Wansapura et al. [49] subjected genetically altered ouabain-sensitive mice (originally developed by Lingrel) to aortic banding in order to simulate a pressure overload model. After four weeks, the ouabain-sensitive group was noted to have developed substantially greater cardiac hypertrophy and fibrosis compared to ouabain-resistant (wild-type) mice. Furthermore, the administration of Digibind to the ouabain-sensitive mice diminished these cardiac changes [49]. Using cultures of rat cardiac and renal fibroblasts as well as human dermal fibroblasts, we found that CTS were able to directly increase collagen production and proline incorporation [42,50,51]. By inducing a translocation of PKC to the nucleus, MBG appears to cause the subsequent phosphorylation and degradation of Friend leukemia integration-1 (Fli-1), which Watson et al. [52] have demonstrated is a negative regulator of collagen synthesis in dermal fibroblasts [50,52]. We also observed that MBG infusion stimulates the expression and nuclear translocation of snail, a transcription factor involved in epithelial-mesenchymal transition, which is implicated in renal fibrosis [53].

In addition to the above, we also examined the effects of spironolactone, known to be a competitive antagonist of CTS binding to the Na/K-ATPase, as well as its major metabolite, canrenone on the development and progression of this model of uremic cardiomyopathy. We found that spironolactone significantly attenuated cardiac fibrosis in the renal failure models, and both spironolactone and canrenone reduced collagen production in cardiac fibroblasts [54]. It was further demonstrated that spironolactone blocked MBG-induced Na/K-ATPase signaling both *in vivo* as well as CTS binding to the plasmalemmal Na/K-ATPase [54].

Conclusions

Endogenous circulating CTS are up regulated in the response to volume expansion. The binding of CTS to their receptor, the Na/K-ATPase, leads to decreased proximal tubular sodium reabsorption and increased natriuresis. Our data suggest that this is likely due to the recently discovered scaffolding and signaling functions of the Na/K-ATPase. Unfortunately, this same process in fibroblasts is profibrotic, and this leads to a trade-off between sodium homeostasis in the short term and progressive cardiac and renal fibrosis in the long term. We further speculate that this may explain the long term deleterious effects of a high salt diet on cardiovascular health.

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