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Trade-off and Cardiotonic Steroid Signaling: Natriuresis Maintains Sodium Balance at The Expense of Cardiac Fibrosis

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The pivotal work by Zijian Xie provided groundbreaking insights describing that the Na/K-ATPase (NKA), in addition to being an essential ion pump, also functions as a signal transducer with the capability to interact with multiple signaling partners [1-3]. As an extension of this, our work along with a large body of work from the laboratories of Blaustein and Hamlyn as well as Bagrov and Fedorova, demonstrated that there were two major classes of endogenous NKA ligands or cardiotonic steroids (CTS), those from the cardenolide class such as ouabain and those which were bufadienolide such as marinobufagenin [4]. Although there has been some debate as to whether the cardenilide CTS serve primarily as neurohormones and the bufadienalides serve as peripheral effectors [5], there is little debate that the circulating concentrations of both chemical classes are elevated in volume-expanded states such as salt-sensitive hypertensive renal disease, preeclampsia, and uremic cardiomyopathy [6-13]. In addition to having effects on vascular reactivity and renal sodium handling [5,6,14-16], CTS signaling through the NKA/Src kinase pathway appears to induce both cardiac and renal fibrosis [6-13]. The recent report by Grigorova et al. represents an essential contribution to the field of NKA pro-fibrotic signaling by demonstrating that in a normotensive animal model, high salt diet-induced aortic stiffness and fibrosis is associated with significantly elevated levels of the CTS marinobufagenin (MBG) in a pathway involving TGF-β and SMAD signaling [17]. Importantly, Grigorova et al. further demonstrate that reduced sodium intake significantly reduced MBG levels and the accompanying aortic fibrosis indicating that reduced dietary sodium intake improves vascular stiffness by reducing MBG levels [17]. These results have important implications for the development and progression of cardiovascular disease as reduced sodium intake improves aortic stiffness and fibrosis by diminishing pro-fibrotic CTS/NKA signaling.

Originally discovered by Jens Skou as an ion pump, the NKA (a P-type ATPase) has also been well described to have important cellular signaling capabilities [18]. The NKA provides the essential function of Na^+ reabsorption in the kidneys and is intimately involved in the regulation of extracellular volume and blood pressure [19-21]. The main structural components of the NKA are composed of a catalytic α subunit, a β subunit, and in some tissues, a γ subunit [20]. The ATP and ligand binding sites are located within the α subunit, which is also the site of ATP hydrolysis responsible for maintaining an ionic gradient by transporting Na^+ and K^+ across cell membranes [20]. The α subunit consists of four isoforms (α1-α4), of which, in mammalian species, the α1 subunit is capable of forming a signaling complex with the tyrosine kinase, Src resulting in the activation of several downstream signaling cascades [16,22]. Apparently, when CTS bind to the NKA, the E2 state becomes preferred. As the α1 subunit binds the Src kinase domain only in the E1 state [15], these CTS effectively activate Src kinase [23]. Activated Src then transactivates the epidermal growth factor receptor (EGFR) which results in the activation of phospholipase C (PLC), phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), and the generation of reactive oxygen species (ROS) and ERK (extracellular-signal-regulated kinase) [2,5].

Importantly, CTS binding to the NKA is also heavily involved in natriuresis [24-26]. Volume expanded states

such as chronic kidney disease and high dietary sodium result in elevated circulating levels of CTS which decrease proximal tubular sodium reabsorption and effect natriuresis [16]. However, there appears to be a "trade off" for this natriuresis.

CTS signaling through the NKA/Src also facilitates the development of fibrosis. Elevated circulating levels of endogenous CTS have been reported in patients with chronic kidney disease [27,28]. In the 5/6 partial nephrectomy model of uremic cardiomyopathy, we reported significantly elevated circulating MBG levels, cardiac hypertrophy, cardiac fibrosis, and ROS generation with activation of NKA/Src/EGFR/ERK signaling in left ventricular tissue [9]. Similar results were reported following infusion of MBG at a concentration similar to levels reported in the partial nephrectomy model [9,10]. Importantly, both active and passive immunization against MBG has been show to attenuate the pro-fibrotic effects of CTS/NKA signaling [9,10,29]. In cardiac fibroblasts, treatment with physiologically relevant concentrations of MBG induced collagen production [10]. This MBG-induced increase in collagen was attenuated following inhibition of Src, EGFR translocation, and treatment with the antioxidant *N*-acetyl cysteine providing further evidence that MBG induces cardiac fibrosis acting through the NKA/Src/EGFR/ROS signaling complex [10]. Further experiments in cardiac fibroblasts were conducted to determine the extent of TGF-β and Smad signaling in MBG-induced collagen production. Here, we demonstrated that although no increase in TGF-β or Smad signaling proteins were observed, treatment with a TGF-β antagonist prevented MBG-induced collagen production [10]. We have also shown that the transcription factor and negative regulator of collagen production, Friend leukemia integration-1 (Fli-1) is involved in MBG induced fibrosis. The δ-isoform of PKC has been shown to phosphorylate Fli-1 leading to collagen synthesis [30]. Fli-1 knockdown mice subjected to 5/6 partial nephrectomy demonstrated significantly elevated left ventricular fibrosis [31]. In cardiac, renal, and dermal fibroblasts, MBG was shown to reduce nuclear Fli-1 expression and increase procollagen expression [31]. Furthermore, MBG treatment resulted in PKCδ translocation into the nucleus in a PLC dependent manor [31]. Taken together, these results indicate that MBG induced signaling through the NKA/Src/EGFR cascade activates PKCδ translocation to the nuclease in a process involving PLC. Once in the nucleus, PKCδ phosphorylates Fli-1 preventing Fli-1 inhibition of the collagen promoter resulting in elevated collagen expression [31]. In addition, we have demonstrated that activation of the serine/threonine mammalian target of rapamycin (mTOR) system is involved in MBG-induced cardiac fibrosis [12]. Here, we show that treatment with the mTOR inhibitor, rapamycin significantly reduced circulating MBG levels and attenuated cardiac fibrosis in the 5/6 partial nephrectomy model [12].

As we and others have extensively reported and in direct relevance to the current work by Grigorova et al. [17], volume expanded states induce elevated circulating levels of CTS which serve an essential function in natriuresis to maintain sodium balance. However, when CTS levels are chronically elevated natriuresis is accompanied by adverse CTS signaling through the NKA/Src complex leading to cardiac fibrosis. Thus, CTS induced natriuresis is intimately linked to the trade-off of fibrosis.

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