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 several 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 manner [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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**References**

1. Kometiani P, Li J, Gnudi L, Kahn BB, Askari A, Xie Z. Multiple Signal Transduction Pathways Link Na+/K+-ATPase to growth-related genes in cardiac myocytes: the roles of Ras and mitogen-ACTIVATED protein kinases. Journal of Biological Chemistry. 1998 Jun 12;273(24):15249-56.

2. Xie Z. Molecular mechanisms of Na/K-ATPase-mediated signal transduction. Annals of the New York Academy of Sciences. 2003 Apr;986(1):497-503.

3. Aizman O, Aperia AN. Na, K-ATPase as a signal transducer. Annals of the New York Academy of Sciences. 2003 Apr;986(1):489-96.

4. Lopatin DA, Alamazian EK, Dmitrieva RI, Shpen VM, Fedorova OV, Doris PA, Bagrov AY. Circulating bufodienolide and cardenolide sodium pump inhibitors in preeclampsia. Journal of hypertension. 1999 Aug 12;273(24):15249-56.

5. Bagrov AY, Shapiro JI, Fedorova OV. Endogenous cardiotonic steroids: physiology, pharmacology, and novel therapeutic targets. Pharmacological reviews. 2009 Mar 1;61(1):9-38.

6. Nikitina ER, Mikhailov AV, Nikandrova ES, Frolova EV, Fadeev AV, Shman VY, Shilova YV, Tapilskaya NI, Shapiro JI, Fedorova OV, Bagrov AY. In preeclampsia endogenous cardiotonic steroids induce vascular fibrosis and impair relaxation of umbilical arteries. Journal of hypertension. 2011 Apr;29(4):769-75.
marinobufagenin attenuates renal fibrosis and improves renal function in experimental renal disease. American journal of hypertension. 2014 Apr 1;27(4):603-9.

8. Fedorova OV, Doris PA, Bagrov AY. Endogenous marinobufagenin-like factor in acute plasma volume expansion. Clinical and experimental hypertension. 1998 Jan 1;20(5-6):581-91.

9. Kennedy DJ, Vetteth S, Periyasamy SM, Kanj M, Fedorova L, Khouri S, Kahaleh MB, Xie Z, Malhotra D, Kolodkin NI, Lakatta EG. Central role for the cardiotonic steroid marinobufagenin in the pathogenesis of experimental uremic cardiomyopathy. Hypertension. 2006 Mar 1;47(3):488-95.

10. Elkareh J, Kennedy DJ, Yashaswi B, Vetteth S, Shidyak A, Kim EG, Smaili S, Periyasamy SM, Hariri IM, Fedorova L, Liu J. Marinobufagenin stimulates fibroblast collagen production and causes fibrosis in experimental uremic cardiomyopathy. Hypertension. 2007 Jan 1;49(1):215-24.

11. Fedorova OV, Zernetkina VI, Shilova VY, Grigorova YN, Juhasz O, Wei W, Marshall CA, Lakatta EG, Bagrov AY. Synthesis of an endogenous steroidal Na pump inhibitor marinobufagenin, implicated in human cardiovascular diseases, is initiated by CYP27A1 via bile acid pathway. Circulation: Cardiovascular Genetics. 2015 Oct;8(5):736-45.

12. Haller ST, Yan Y, Drummond CA, Xie J, Tian J, Kennedy DJ, Shilova VY, Xie Z, Liu J, Cooper CJ, Malhotra D. Rapamycin attenuates cardiac fibrosis in experimental uremic cardiomyopathy by reducing marinobufagenin levels and inhibiting downstream pro-fibrotic signaling. Journal of the American Heart Association. 2016 Sep 30;5(10):e004106.

13. Zhang Y, Wei W, Shilova V, Petrashevskaya NN, Zernetkina VI, Grigorova YN, Marshall CA, Fenner RC, Lehrmann E, Wood III WH, Becker KG. Monoclonal Antibody to Marinobufagenin Downregulates TGF-β Profibrotic Signaling in Left Ventricle and Kidney and Reduces Tissue Remodeling in Salt-Sensitive Hypertension. Journal of the American Heart Association. 2019 Oct 15;8(20):e012138.

14. Ling J, Yan Y, Nie Y, Shapiro JI. Na/K-ATPase Signaling and Sodium Transport in Renal Distal Tubular Cells. Antioxidants. 2017 Mar;6(1):18.

15. Yan Y, Shapiro AP, Mopidevi BR, Chaudhry MA, Maxwell K, Haller ST, Drummond CA, Kennedy DJ, Tian J, Malhotra D, Xie ZJ. Protein Carbonylation of an Amino Acid Residue of the Na/K-ATPase α1 Subunit Determines Na/K-ATPase Signaling and Sodium Transport in Renal Proximal Tubular Cells. Journal of the American Heart Association. 2016 Sep 9;5(9):e003675.

16. Khalaf FK, Dube P, Mohamed A, Tian J, Malhotra D, Haller ST, Kennedy DJ. Cardiotonic steroids and the sodium trade balance: new insights into trade-off mechanisms mediated by the Na+/K+-ATPase. International journal of molecular sciences. 2018 Sep;19(9):2576.

17. Grigorova YN, Wei W, Petrashevskaya N, Zernetkina V, Juhasz O, Fenner R, Gilbert C, Lakatta EG, Shapiro JI, Bagrov AY, Fedorova OV. Dietary sodium restriction reduces arterial stiffness, vascular TGF-β-dependent fibrosis and marinobufagenin in young normotensive rats. International journal of molecular sciences. 2018 Oct;19(10):3168.

18. Xie Z. Ouabain interaction with cardiac Na/K-ATPase reveals that the enzyme can act as a pump and as a signal transducer. Cellular and molecular biology (Noisy-le-Grand, France). 2001 Mar;47(2):383-90.

19. Aperia A, Akkuratov EE, Fontana JM, Brismar H. Na+/K+-ATPase, a new class of plasma membrane receptors. American Journal of Physiology-Cell Physiology. 2016 Apr;310(7):C491-5.

20. Morth JP, Pedersen BP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG, Nissen P. A structural overview of the plasma membrane Na+, K+-ATPase and H+-ATPase ion pumps. Nature Reviews Molecular Cell Biology. 2011 Jan;12(1):60-70.

21. Jørgensen PL. Structure, function and regulation of Na,K-ATPase in the kidney. Kidney international. 1986 Jun;30(1):10-20.

22. Xie JX, Zhang S, Cui X, Zhang J, Yu H, Khalaf FK, Malhotra D, Kennedy DJ, Shapiro JI, Tian J, Haller ST. Na/K-ATPase/src complex mediates regulation of CD40 in renal parenchyma. Nephrology Dialysis Transplantation. 2018 Jul;13(7):1138-49.

23. Tian J, Cai T, Yuan Z, Wang H, Liu L, Haas M, Maksimova E, Huang XY, Xie ZJ. Binding of Src to Na+/K+-ATPase forms a functional signaling complex. Molecular biology of the cell. 2006 Jan;17(1):26-37.

24. Periyasamy SM, Liu J, Tanta F, Kabak B, Wakefield B, Malhotra D, Kennedy DJ, Nadoor A, Fedorova OV, Gunning W, Xie Z. Salt loading induces redistribution of the plasmalemmal Na/K-ATPase in proximal tubule cells. Kidney international. 2005 May;67(5):1868-77.
25. Liu J, Liang M, Liu L, Malhotra D, Xie Z, Shapiro JI. Ouabain-induced endocytosis of the plasmalemmal Na/K-ATPase in LLC-PK1 cells requires caveolin-1. Kidney international. 2005 May 1;67(5):1844-54.

26. Liu J, Kesiry R, Periyasamy SM, Malhotra D, Xie Z, Shapiro JI. Ouabain induces endocytosis of plasmalemmal Na/K-ATPase in LLC-PK1 cells by a clathrin-dependent mechanism. Kidney international. 2004 Jul 1;66(1):227-41.

27. Kolmakova EV, Haller ST, Kennedy DJ, Isachkina AN, Budny GV, Frolova EV, Piecha G, Nikitina ER, Malhotra D, Fedorova OV, Shapiro JI. Endogenous cardiotonic steroids in chronic renal failure. Nephrology Dialysis Transplantation. 2011 Sep 1;26(9):2912-9.

28. Komiyama Y, Dong XH, Nishimura N, Masaki H, Yoshika M, Masuda M, Takahashi H. A novel endogenous digitalis, telocinobufagin, exhibits elevated plasma levels in patients with terminal renal failure. Clinical biochemistry. 2005 Jan 1;38(1):36-45.

29. Haller ST, Kennedy DJ, Shidyak A, Budny GV, Malhotra D, Fedorova OV, Shapiro JI, Bagrov AY. Monoclonal antibody against marinobufagenin reverses cardiac fibrosis in rats with chronic renal failure. American journal of hypertension. 2012 Jun 1;25(6):690-6.

30. Jinnin M, Ihn H, Yamane K, Mimura Y, Asano Y, Tamaki K. Alpha2 (I) collagen gene regulation by protein kinase C signaling in human dermal fibroblasts. Nucleic acids research. 2005 Jan 1;33(4):1337-51.

31. Elkareh J, Periyasamy SM, Shidyak A, Vetteth S, Schroeder J, Raju V, Hariri IM, El-Okdi N, Gupta S, Fedorova L, Liu J. Marinobufagenin induces increases in procollagen expression in a process involving protein kinase C and Fli-1: implications for uremic cardiomyopathy. American Journal of Physiology-Renal Physiology. 2009 May;296(5):F1219-26.