The role of endogenous cardiotonic steroids in pathogenesis of cardiovascular and renal complications of arterial hypertension

Znaczenie endogennych steroidów kardiotonicznych w patogenezie sercowo-naczyniowych i nerkowych powikłań nadciśnienia tętniczego

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Summary

Endogenous cardiotonic steroids (CTS), also called digitalis-like factors, are a group of steroid hormones linking high salt intake and elevated blood pressure and in part responsible for target organ damage in arterial hypertension. CTS act primarily through their ability to inhibit the ubiquitous transport enzyme sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase). A portion of Na^+/K^+-ATPase does not seem to actively "pump" sodium and potassium but is closely associated with other key signaling proteins. Plasma concentration and urine excretion of CTS are increased in experimental models with volume expansion and on a high salt diet. Elevated plasma concentration of marinobufagenin has been shown in volume-expanded states such as essential hypertension, primary aldosteronism, chronic renal failure, congestive heart failure and pregnancy. In experimental models marinobufagenin induces heart and kidney fibrosis to the same extent as observed in uremia. Neutralization of marinobufagenin with antibodies prevents such heart remodeling. Expanding our understanding of this new class of hormones may lead to development of novel and effective therapeutic strategies in hypertensive patients with renal and cardiovascular complications.

Keywords: Marinobufagenin • ouabain • hypertension • heart failure • chronic kidney disease

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Abbreviations: **ADPKD** – autosomal-dominant polycystic kidney disease, **CHF** – congestive heart failure, **CKD** – chronic kidney disease, **CTS** – cardiotonic steroids, **MBG** – marinobufagenin, **Na^+/K^+-ATPase** – sodium-potassium adenosine triphosphatase, **RAS** – renin-angiotensin system.

Introduction

Endogenous cardiotonic steroids (CTS), also called digitalis-like factors, are produced by the adrenal cortex, placenta and hypothalamus. By the chemical structure they belong to two groups: cardenolides (e.g. ouabain) and bufadienolides (e.g. marinobufagenin) (Fig. 1). CTS act primarily through their ability to inhibit the ubiquitous transport enzyme sodium-potassium adenosine triphosphatase (Na$^+$/K$^+$-ATPase).

There are four α and three β isoforms of the Na$^+$/K$^+$-ATPase known, thus allowing numerous combinations of αβ complexes among tissues with different characteristics including different sensitivity to different cardiotonic steroids. The α1β1 complex is found in nearly every tissue and is recognized as the major form of the enzyme. The α2 isoform is expressed in adult heart, smooth muscle, skeletal muscle, brain, adipocytes, cartilage, and bone. The α3 isoform is expressed in the central and peripheral nervous tissues and in the conductive system of the heart. The α4 isoform seems to be testis-specific. The expression of β1 is also ubiquitous. The β2 and β3 isoforms are expressed in the brain, cartilage and erythrocytes, whereas β2 can also be found in cardiac tissue and β3 in lungs.

The cardenolides have been determined to have a predilection for the α2 and α3 isoforms, whereas the bufadienolides act primarily on the α1 isoform, the predominant form of the enzyme in the kidney (among other things determining sodium ion reabsorption from the glomerular filtrate).

The "classic" function of Na$^+$/K$^+$-ATPase is maintaining the gradient of sodium and potassium concentrations across the plasmalemmal barrier. In recent years an alternative or "signaling" function for Na$^+$/K$^+$-ATPase has been described. This model proposes that a certain portion of plasmalemmal Na$^+$/K$^+$-ATPase resides in the caveolae of the cells and does not seem to actively "pump" sodium and potassium but is closely associated with other key signaling proteins [1] (Fig. 2).

Upon binding to Na$^+$/K$^+$-ATPase, ouabain induces internalization and degeneration of the enzyme [3]. In physiological conditions, internalization may serve to eliminate pumps that have been blocked by CTS. This mechanism may be required due to the very slow dissociation of the CTS-Na$^+$/K$^+$-ATPase complex.

Regulation of blood pressure

Prevalence of hypertension is greater in populations with higher salt intake [5]. Moreover, reduction of dietary salt intake allows lowering of blood pressure [4]. Although an increase in blood pressure with increased salt intake is observed in almost all individuals, the magnitude of the increase is highly variable. Epidemiological studies indicate that 51% of hypertensive and 26% of

normotensive individuals are salt-sensitive (≥10 mmHg difference in systolic blood pressure between high and low salt intake) [39]. The mechanism linking high salt intake and hypertension is complex and far from being fully understood. The concept that circulating vasoactive substances might be involved etiologically and mechanistically in the development of hypertension was first proposed by Dahl [6]. Later, based on observations in humans and in experimental animals under the conditions of salt loading and volume expansion, de Wardener and others postulated that the humoral factor implicated in the pathogenesis of hypertension is an endogenous natriuretic [7]. They postulated that Na$^+$/K$^+$-ATPase inhibition results, via the sodium-calcium co-transporter in vascular smooth muscle, in increased intracellular calcium concentration and increased vascular tone, producing elevated blood pressure.

Laragh et al. [23] postulated that two forms of essential hypertension can be described: one related to vasoconstriction (largely the result of RAS activation) and the other due to volume expansion (excess salt and water), in which renin activity is suppressed.

Abnormalities in renal sodium handling have been proposed as a major cause of essential hypertension. The handling of body sodium and blood pressure is regulated by the interaction of several hormones (angiotensin II, aldosterone, vasopressin, ouabain, dopamine, and others) and the genetically determined constitutive capacity of the renal tubules to reabsorb sodium. The prohypertensive effect of endogenous ouabain involves a mutation of the gene encoding the cytoskeletal protein adducin. In a strain of Milan hypertensive rats, both increased plasma concentration of ouabain and adducin mutation are associated with elevated expression and activity of the Na$^+$/K$^+$-ATPase in the renal tubular epithelium. This is apparently due to an increase in the resident time of the sodium pump in the cellular membrane. In these animals ouabain causes further upregulation of renal Na$^+$/K$^+$-ATPase expression, renal sodium retention and, in effect, hypertension. Administration of rostafuroxin, an ouabain antagonist selectively displacing ouabain from Na$^+$/K$^+$-ATPase, reduces blood pressure in these Milan hypertensive rats. Disappointingly, the Ouabain and Adducin for Specific Intervention on Sodium in Hypertension Trial (OASIS-HT) published in 2011 showed that rostafuroxin did not reduce blood pressure in humans with essential hypertension [34].

There is evidence supporting the role of bufadienolides in the pathogenesis of the forms of hypertension related to excessive salt and fluid accumulation. The amount of circulating bufadienolides in blood and excreted in urine is increased in experimental animals in states of volume expansion and on a high salt diet. Normotensive human subjects on a high salt diet are characterized by elevation in plasma levels and renal excretion of marinobufagenin. In normotensive rats and dogs, plasma marinobufagenin concentration was elevated by acute
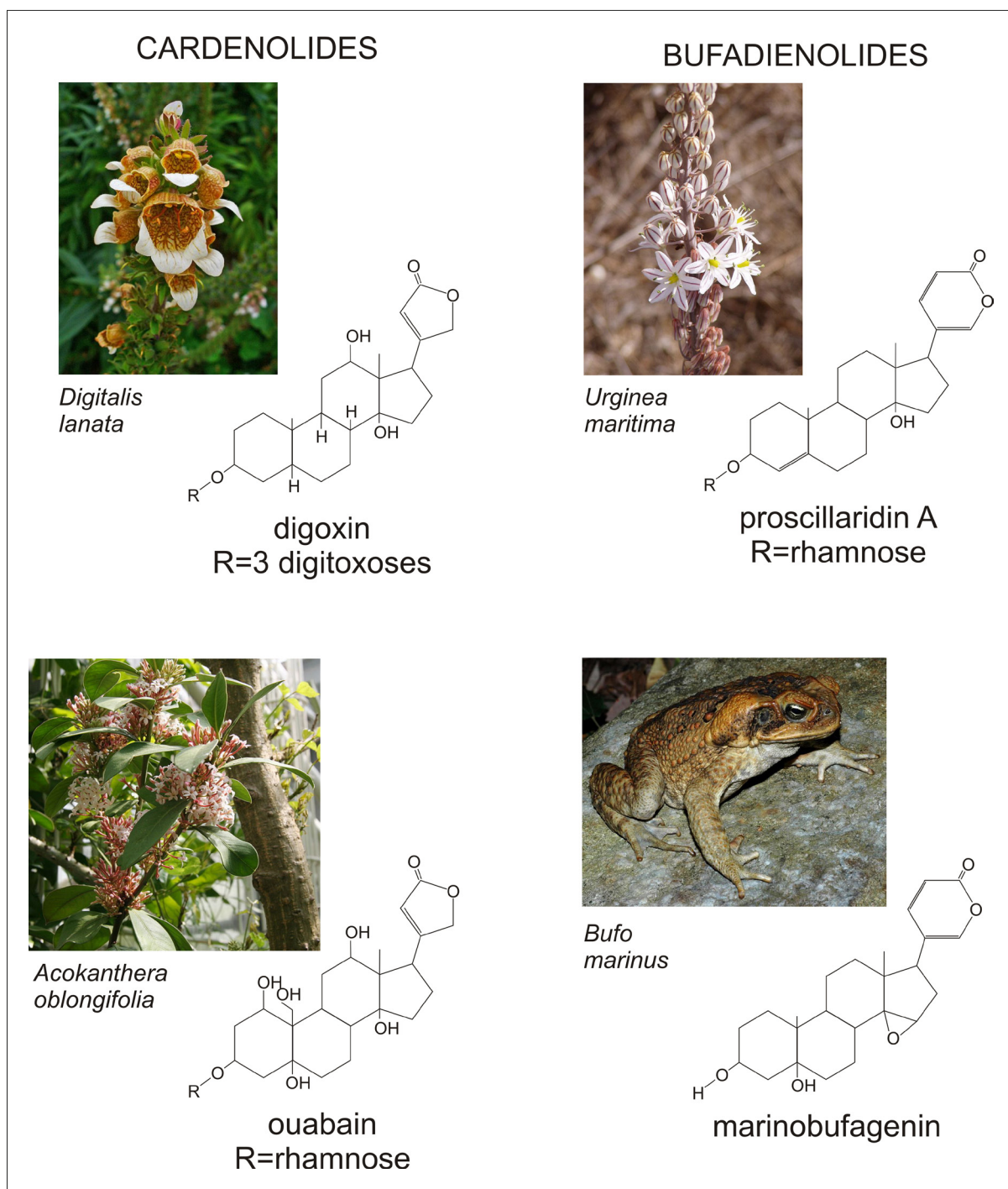
Fig. 1. Chemical structures and main natural sources of cardiotonic steroids: cardenolides (ouabain and digoxin) and bufadienolides (marinobufagenin and proscillaridin A). Photographs from Wikimedia Commons licensed under the Creative Commons Attribution-Share Alike 3.0 unported license. *Digitalis lanata* by H. Zell; *Bufo marinus* by M. Linnenbach; *Acokanthera oblongifolia* by M. Wolf; *Urginea maritime* by J. Reis

plasma volume expansion and by chronic administration of a high NaCl diet. Increased secretion of marinobufagenin has been documented in volume-expanded states such as essential hypertension, primary aldosteronism, chronic renal failure, congestive heart failure and pregnancy [1]. Interestingly, both in patients and experimental animals with impaired kidney function, plasma levels of marinobufagenin but not ouabain are increased [22]. Moreover, in hypertensive Dahl rats and in pregnant rats with hypertension induced by NaCl supplementation, monoclonal anti-marinobufagenin antibody reduced blood pressure and increased activity of the sodium pump in the vasculature.
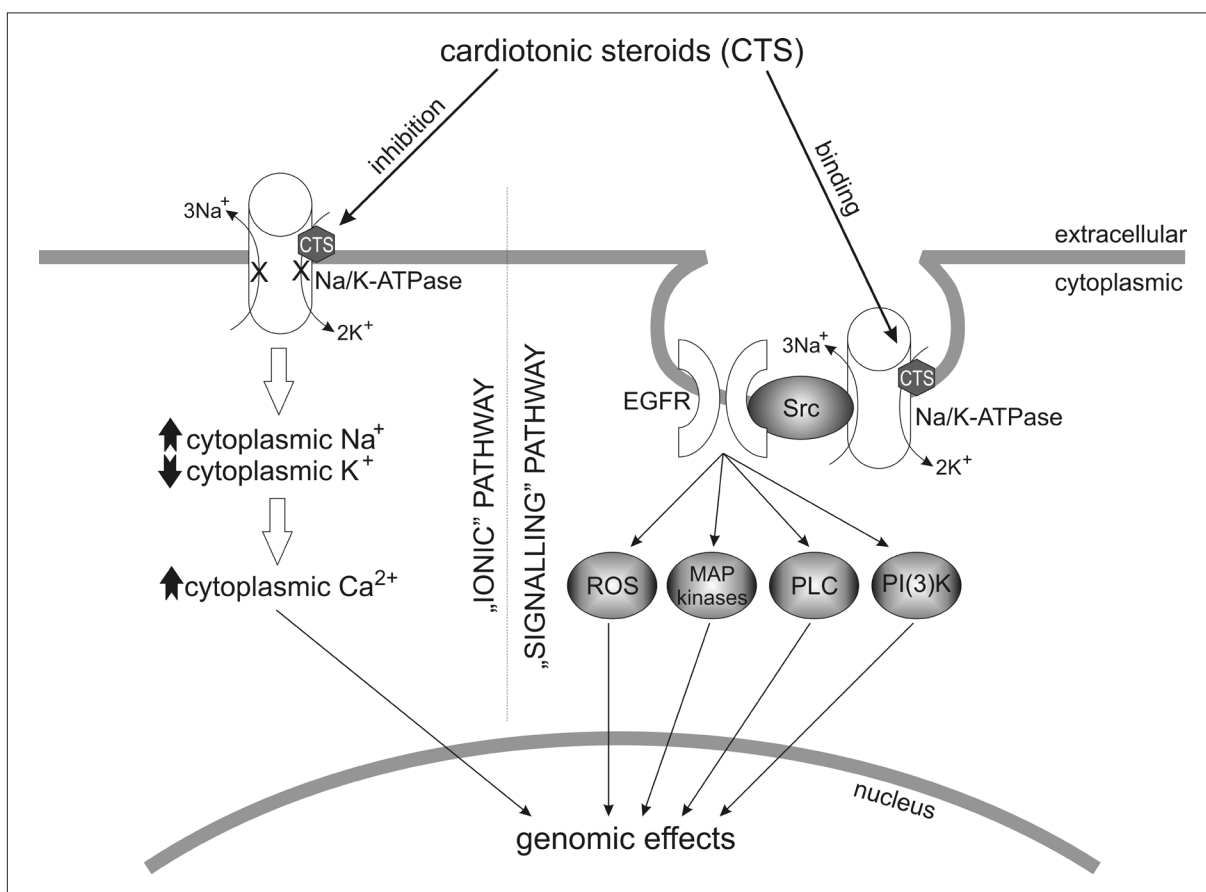
Fig. 2. Schematic diagram of "classic" and "signaling" pathways for CTS effects. In the classic pathway the Na$^+$/K$^+$-ATPase pumping activity is inhibited, which in turn is accompanied by changes in cytosolic [Na$^+$] and [K$^+$]. The transient increases in [Na$^+$] and decreases in [K$^+$] induce an increase in cytosolic [Ca^{2+}], activating a variety of pathways that have combinations of genomic and non-genomic effects. In contrast, the signaling pathway involves the association of signaling proteins with the Na$^+$/K$^+$-ATPase in a caveolar domain. Binding of CTS to Na$^+$/K$^+$-ATPase activates Src and transactivates the EGF receptor and phospholipase C (PLC). This leads to a cascade that involves generation of reactive oxygen species (ROS), activation of mitogen-activated protein kinase (MAPK), activation of PI(3) kinase, stimulation of endocytosis and activation of Akt as well as activation of protein kinase C, finally resulting in combinations of genomic and non-genomic effects. (Own figure)

In humans, dietary sodium restriction reduces urinary marinobufagenin excretion and in parallel lowers blood pressure and aortic stiffness [16]. Moreover Tian et al. [35] demonstrated that renal ischemia due to renal artery stenosis causes elevation of MBG levels and successful stenting of the stenosis reduced MBG plasma concentration.

HEART REMODELING

Congestive heart failure (CHF) is associated with fluid retention and plasma volume expansion, so one could expect that cardiotonic steroids may play an important role through the regulation of sodium transport and arterial pressure. More recent work implicates these hormones in regulation of cell growth, differentiation, apoptosis and fibrosis. The relationship between CTS, cardiac geometry and hemodynamic parameters has been analyzed in several studies. Gottlieb et al. [13] found that plasma levels of endogenous ouabain were elevated in patients with a severely dysfunctional left

ventricle (ejection fraction less than 21%). Manunta et al. [25] demonstrated that plasma concentration of ouabain positively correlated with left ventricle mass index. Moreover, the plasma ouabain concentration was substantially higher in patients with eccentric remodeling compared with those subjects who had normal left ventricle geometry or concentric hypertrophy. Experimental data suggest that CTS signaling may lead to fibrosis. Kennedy et al. [19] noted that experimental renal failure produced a tremendous amount of cardiac fibrosis in rats and mice. Similarly, infusion of MBG to animals with normal kidney function produced cardiac fibrosis as well. Active immunization against marinobufagenin as well as reduction of circulating levels of MBG by adrenalectomy prevented cardiac hypertrophy and fibrosis seen in these animals. Active immunization against MBG also reduced levels of oxidative stress. Additional studies showed that MBG causes increased collagen production by fibroblasts and fibrosis in experimental uremic cardiomyopathy [9]. The authors noted that marinobufagenin did not influence the expression of the potent

pro-fibrogenic cytokine TGF-β, but induced decreases in expression of Fli-1 (Friend leukemia integration-1), which is a negative regulator of collagen synthesis in several types of fibroblasts: cardiac, renal and dermal.

Infusion of an anti-MBG antibody in rats after 5/6 nephrectomy reduces cardiac fibrosis by increasing Fli-1 expression and reducing oxidative stress [15]. This observation demonstrates that interference with the CTS-Na$^+$/K$^+$-ATPase signaling pathway is a possible target for novel treatment methods in patients with heart failure.

It is well known that men are at greater risk for cardiovascular and renal disease compared to premenopausal women at the same age [30]. Drummond et al. [8] recently showed that progesterone inhibits ouabain binding to Na$^+$/K$^+$-ATPase and ameliorates cardiac fibroblast synthesis induced by MBG.

CTS can influence cell survival and growth in different tissues [32]. There is evidence that these effects of CTS depends on the content of the Na$^+$/K$^+$-ATPase in the affected cells [36]. There is also evidence for reduction of Na$^+$/K$^+$-ATPase in patients and experimental animals with congestive heart failure [28,33], aging [11,18], diabetes, and hypertension [38]. This is related to the deficiency in cardiac contractility due to cell growth inhibition and apoptosis. Myocyte apoptosis may play an important role in the development of myocardium dilatation and heart failure. Liu and co-workers [24] studied whether reduction of Na$^+$/K$^+$-ATPase potentiates CTS-induced myocyte apoptosis and cardiac dysfunction. The authors examined heart function in Na$^+$/K$^+$-ATPase α1 heterozygote knock-out mice in comparison to wild type (WT) animals after infusion of MBG. The results demonstrated that MBG infusion increased myocyte apoptosis and induced left ventricle dilatation in α1$^{+/-}$ mice, but not in WT. It was found that in WT myocytes MBG activated the Src/Akt/mTOR signaling pathway, which increased phosphorylation of ribosome S6 kinase and BAD (Bcl-2-associated death promoter) and protected cells from apoptosis, but this mechanism failed in α1$^{+/-}$ mice. Taken together, these results indicate that reduction of Na$^+$/K$^+$-ATPase enables MBG to induce apoptosis in cardiac myocytes, leading to heart failure. Taking into account that disease states correlating with decreased Na$^+$/K$^+$-ATPase activity also correlate with increased CTS concentrations, this mechanism may be one of the pathways responsible for the development of heart failure in renal disease, diabetes and hypertension.

Spironolactone is known to reduce arterial stiffness, left ventricular mass, and heart fibrosis. However, spironolactone and its major metabolite, canrenone, also interact with the plasmalemmal Na$^+$/K$^+$-ATPase [2]. Tian et al. [37] demonstrated that spironolactone is able to attenuate heart remodeling and failure induced by MBG infusion as well as by renal failure.

While the pro-fibrotic effects of aldosterone are clearly visible with volume expansion and salt loading [12,31], one can speculate that these effects are in fact attributable to the coexisting increases in MBG levels.

Arterial stiffness contributes to the increased cardiovascular risk in arterial hypertension. Of note, high MBG levels lead to vascular remodeling (wall thickening and fibrosis) in offspring of rats on a high-salt diet [29]. Interestingly, this vascular remodeling developed before the increase of blood pressure in these animals and also occurs in low-pressure pulmonary circulation, pointing to a potential causal role of cardiotonic steroids. This is further supported by the observation in humans with preeclampsia: this syndrome is associated with a rise in plasma and placental levels of MBG, and elevated levels of MBG induce vascular fibrosis via a Fli-1-dependent mechanism which leads to an impairment of vasorelaxation [27].

KIDNEY DISEASE

In addition to the effect of bufadienolides on the heart and vascular system, these hormones have renal effects. First, increased plasma MBG concentration accompanies decreases in renal function [22]. More importantly, it has been demonstrated that MBG induces endocytosis of the proximal tubular Na$^+$/K$^+$-ATPase and reduces renal sodium reabsorption, increasing sodium excretion. Administration of anti-MBG antibodies alters the endocytosis and reduces urinary sodium excretion in Sprague-Dawley rats. The relationship between salt intake, total body sodium, fluid balance and blood pressure is based largely on the fundamental role of the kidney. Relative to sodium intake, any prolonged tendency of the kidney to reabsorb additional Na$^+$ results in fluid retention. The salt and fluid retention eventually elevates blood pressure. So cardiotonic steroids also act as physiological regulators of sodium pump activity and are implicated in regulation of natriuresis and vascular tone. More recently, CTS have been shown to contribute to pro-hypertrophic and pro-fibrotic cell signaling.

In the study by Fedorova et al. [10] marinobufagenin infusion induced periglomerular and peritubular accumulation of collagen type I in the renal cortex. Additionally, the pro-fibrotic transcription factor Snail, a key regulator of epithelial-mesenchymal transition (EMT), was found to be up-regulated by MBG infusion. So MBG may be an important target for the prevention of kidney disease progression.

Infusion of antibodies to MBG not only reduced kidney fibrosis in 5/6 nephrectomized rats but also improved creatinine clearance and lowered proteinuria [14].

Experimental data suggest that MBG may be implicated in fetal programming. Low birth weight due to various reasons is associated with a lower nephron number and development of hypertension later in life [20]. Offspring
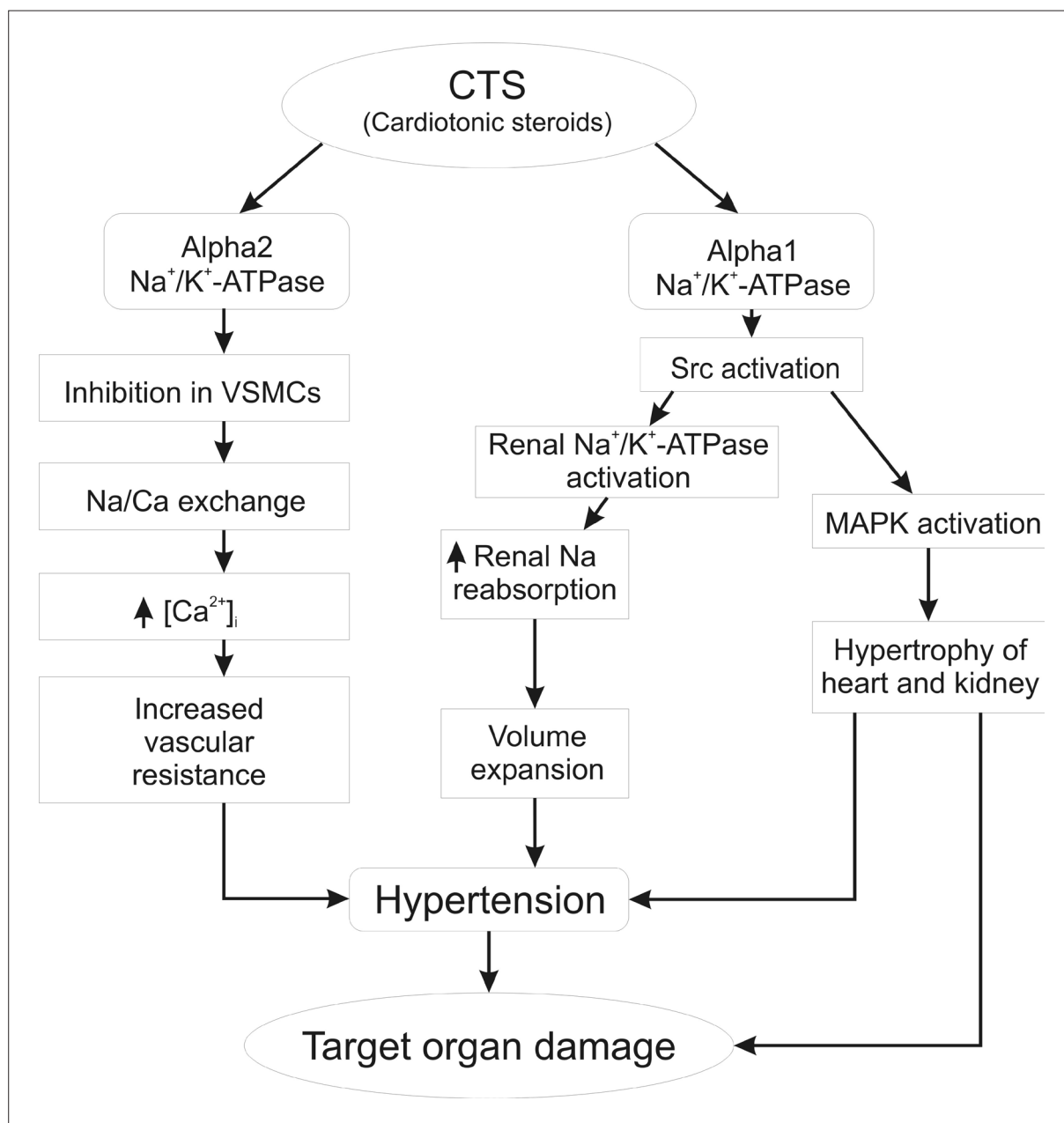
Fig. 3. Pathways linking cardiotonic steroids (CTS), hypertension, and target organ damage. Direct inhibition of Na+/K+-ATPase in vascular smooth muscle cells (left) increases the intracellular Ca2+ concentration, causing their contraction and increase of peripheral arterial resistance. Inhibition of Na+/K+-ATPase in renal tubules (middle) increases renal sodium reabsorption, leading to fluid retention, and activation of the signaling cascade (right) activates hypertrophic signals, contributing to both elevated blood pressure and target organ damage in hypertension. (Own figure)

of rats fed a high-sodium diet in pregnancy despite normal birth weight also have a low nephron number and develop hypertension, and this is associated with high MBG levels in amniotic fluid [21].

Recent studies also show the role of ouabain in autosomal-dominant polycystic kidney disease (ADPKD) where the renal cysts develop by aberrant epithelial cell proliferation and transepithelial fluid secretion. ADPKD is a common inherited disorder characterized by formation and progressive growth of numerous fluid-filled cysts in kidney and other organs with ductal structures. Auto-

somal-dominant polycystic kidney disease is caused by mutation in Pkd1 or Pkd2, genes that encode polycystin-1 and -2, respectively. The cyst growth within the kidney disrupts the renal architecture and compromises organ function, resulting ultimately in renal failure.

Notwithstanding the genetic origin, cyst growth can be accelerated by a variety of factors including cAMP agonists, EGF, and insulin-like growth factor. Recently ouabain in concentrations similar to those circulating in blood was shown to stimulate proliferation of human ADPKD cyst-lining epithelial cells but had no significant effect on

normal human kidney cells [26]. Ouabain binding to Na$^+$/K$^+$-ATPase activates the EGF receptor, the tyrosine kinase Src, and the MEK-ERK pathway, representing a novel agent that has the ability to promote ADPKD cell growth and fluid secretion [17]. These data provide important evidence for the role of ouabain as an endogenous hormone exacerbating ADPKD progression. It could be potentially used to develop therapeutic strategies to inhibit ouabain-dependent Na$^+$/K$^+$-ATPase signaling to delay ADPKD progression.

Conclusions

Our understanding of the role of endogenous cardiotonic steroids has evolved. Regulation of blood pressure by CTS is achieved not only by inhibition of Na$^+$/K$^+$-ATPase followed by increase in cytosolic Ca^{2+}, but also by hypertrophic signaling via the Src and MAP kinase pathway (Fig. 3). Nowadays we know that CTS exhibit physiological functions that go far beyond regulation of sodium transport, natriuresis and blood pressure, including regulation of cell growth, differentiation, apoptosis and proliferation, fibrosis, glucose metabolism and central nervous function. Dysregulation of these hormones seems to play an important role in pathogenesis of many diseases. Expanding our understanding of this class of hormones may lead to novel and effective therapeutic strategies especially for renal and cardiovascular diseases.

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