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From salt sensitivity to hypertension – what do we know about endogenous cardiotonic steroids?**

Od sodowrażliwości do nadciśnienia tętniczego – co wiemy na temat endogennych steroidów kardiotonicznych?

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Słowa kluczowe

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Summary

Dietary sodium intake is one of the major risk factors for developing high blood pressure. Endogenous cardiotonic steroids (CTS) have been proposed to play a significant role in salt-sensitive hypertension. They are a class of specific ligands for one of the most important membrane proteins, Na/K ATPase that play a central role in salt and water transport. Binding of cardiotonic steroids to the NA/K ATPase not only inhibits the enzyme activity but also activates intracellular signaling pathways. These mechanisms are known to underlie a number of cellular functions, but also contribute to impaired renal sodium excretion and vasoconstriction in response to an excessive dietary salt intake. A significant relationship has been shown between circulating levels of CTS and mean arterial pressure in humans. Moreover, recent studies implicates these hormones in the pathogenesis of cardiovascular and renal fibrosis.

Streszczenie

Nadmierna podaż sodu w diecie jest jednym z głównych czynników sprzyjających rozwojowi nadciśnienia tętniczego. Endogenne steroidy kardiotoniczne (CTS) wydają się pełnić znaczącą rolę w patogenezie sodowrażliwego nadciśnienia tętniczego. Związki te pełnią rolę specyficznych cząsteczek sygnałowych (ligandów), mających zdolność wiązania się z pompą sodowo-potasową (Na/K ATPazą), jednym z ważniejszych enzymów białkowych odpowiedzialnych za przezbłonowy transport jonów sodu i potasu. Endogenne steroidy kardiotoniczne nie tylko hamują aktywność Na/K ATPazy, ale także uczestniczą w aktywacji szlaków przekaźnictwa wewnątrzkomórkowego. Mechanizmy te leżą u podstaw licznych procesów komórkowych, ale także, w odpowiedzi na nadmierne spożycie soli, prowadzą do nieprawidłowego nerkowego wydalania sodu oraz obkurczenia naczyń. Istnieje bezpośrednia zależność pomiędzy stężeniem krążących steroidów kardiotonicznych w osoczu a średnim ciśnieniem tętniczym u ludzi. Ponadto, ostatnie badania sugerują udział endogennych steroidów kardiotonicznych w patogenezie włóknienia w układzie sercowo-naczyniowym i nerkach.

INTRODUCTION

Dietary salt intake is an important factor implicated in the pathogenesis of hypertension. Both epidemiologic and observational studies have provided evidence that dietary sodium intake as well as urinary sodium excretion are closely associated with the prevalence of hypertension (1-3). There is also strong evidence that reduction in sodium intake can decrease blood pressure (4). In 1997, the Dietary Approaches to Stop Hypertension (DASH) trial has proven, that a low-sodium diet, rich in fruits and vegetables, can both prevent and treat hypertension (5). It has been demonstrated by Weinberger that increased salt loading causes an increase in blood pressure in all individuals (6). However, there is a substantial heterogeneity among-individuals in blood pressure responses to alterations in sodium and extracellular volume balance. The magnitude of this salt sensitivity is associated with a variety of demographic, physiological and genetic characteristics. It is possible to identify two main groups in the general population: salt-sensitive and salt-resistant. A variety of techniques and criteria

**This paper is dedicated to Professor Franciszek Kokot – our outstanding teacher and mentor.

have been proposed to assess the blood pressure response to changes in sodium and extracellular fluid balance, including specific maneuvers, such as intravenous infusion of saline, rapid sodium and volume depletion by diuretic administration or longer periods of dietary sodium manipulation. The largest epidemiological study conducted so far with 378 normotensive volunteers and 198 patients with essential hypertension, found 26% normotensive and 51% hypertensive subjects to be salt-sensitive (6).

KIDNEY AND SALT-SENSITIVE HYPERTENSION

The cardiovascular system and the kidneys play an indispensable role in the regulation of arterial blood pressure. The kidneys play a central role in both development and maintenance of arterial hypertension through a direct control of sodium and water homeostasis. Evidence from a variety of studies in humans suggests an abnormality in salt handling by the kidneys as an underlying factor causing salt-sensitive hypertension (7-10). These alterations in renal function, may contribute to the etiology of salt-sensitive hypertension and are mediated by both genetic and environmental factors (11). Experimental studies have clearly shown that the central nervous system, alongside kidneys, plays a critical role in many forms of salt-sensitive hypertension (12-16).

Molecular mechanisms linking salt intake and blood pressure elevation are complex, multifactorial and remain unresolved. Recent studies have demonstrated that endogenous cardiotonic steroids (CTS) are important regulators of renal sodium excretion as well as blood pressure and may play a key role in the pathogenesis of salt-induced hypertension (17-19).

FROM A CONCEPT OF NATRIURETIC HORMONE TO FIRST CTS IDENTIFICATION

The concept of circulating "humoral factor" hypothesized to induce salt-sensitive hypertension, came from the study performed by Dahl et al. in 1969 (20). In 1974, it was shown this pro-hypertensive "humoral factor" reduced activity of the sodium pump, the Na/K ATPase (21). A relationship between circulating Na/K ATPase inhibitors and blood pressure was first identified in humans in 1982. Moreover, there is a significant correlation between the level of these circulating inhibitors and mean arterial pressure in hypertensive patients (22). Based on numerous clinical studies and observations in volume expanded experimental animals, de Wardener and Clarkson suggested that a mysterious "humoral factor" implicated in pathogenesis of salt-sensitive hypertension is an endogenous natriuretic (23). Subsequent studies have shown that this presumptive "natriuretic hormone" has digitalis-like properties. Digitalis glycosides are specific ligands of Na/K ATPase and Na/K ATPase plays a major role in the renal tubular sodium transport. Therefore, a hypothesis was proposed that the essential role of endogenous digitalis is to promote natriuresis via inhibition of Na/K ATPase and sodium reabsorption in the renal proximal tubules (23). Moreover, endogenous digitalis-like factors could also contribute to vasoconstriction via inhibition of Na/K ATPase activity in vascular smooth muscle cells (24). In 1991 Hamlyn et al. suggested that their highly concentrated samples comprised a compound indistinguishable from plant-derived ouabain. Thus, endogenous ouabain (EO) was the first CTS to be identified in human plasma (25). Subsequent works by Bagrov et al. in patients after acute myocardial infarction have identified another widely studied member of the endogenous cardiotonic steroids, marinobufagenin (MBG) (26). Shortly thereafter, MBG has been detected in human plasma (27, 28).

ENDOGENOUS CARDIOTONIC STEROIDS (CTS) AND NA/K ATPASE

Cardiotonic steroids were first found in plants, most notably digitalis in the foxglove plant, and then in the skin of toads like the *Bufo marinus* (29). They have been used in traditional ancient medicine to treat congestive heart failure (30). CTS, or digitalis-like substances, are divided into two distinct groups related by structure: cardenolides, represented by digoxin and ouabain, and bufadienolides, represented by marinobufagenin and telocinobufagin. Bufadienolides differ from cardenolides in having a double-unsaturated six-atom lactone ring. Considering that, the amphibian skin participates in water and electrolyte homeostasis and the concentration of bufadienolides in toad skin is regulated by the salt content and its environment, it was hypothesized that the sodium pump (Na/K ATPase) and bufadienolides work together as a regulatory system serving as a basic stimulus-response coupling mechanism to maintain water and electrolyte balance (31, 32). Extremely important contributions were made by several groups which indicated that human fluids contain material that cross-react with antibodies against one of the bufadienolides, bufalin (33, 34). In the 1990s, endogenous ouabain and marinobufagenin were purified from human plasma.

Na/K ATPase is an active transport mechanism moving sodium and potassium ions across the cell membrane. This process is responsible for maintaining both an electrical and chemical gradient that is essential for maintaining a number of vital cell functions, such as communication, excitation, muscle contraction, and many other cellular functions. Na/K ATPase is a membrane-spanning enzyme expressed in virtually all cells of higher organisms. It structurally consists of two subunits, a large catalytic subunit α and a smaller glycoprotein subunit β (35).

Endogenous cardiotonic steroids bind to a specific site within the α subunit of Na/K ATPase and inhibit its activity. There are four isoforms of the α-subunit that have been identified in various tissues (36). The $\alpha 1$ isoform is ubiquitous and it is the main isoenzyme expressed in the renotubular epithelium (35). The $\alpha 2$ isoform is predominant in heart, vascular smooth muscle, skeletal muscle, adipocytes and brain. The $\alpha 3$ isoform

is mostly found in neurons and in the cardiac tissue. The α4 isoform is expressed in the testis (37). The renal α1 isoform of Na/K ATPase plays a key role in the sodium reabsorption along the nephron. The α1 and α2 isoforms are a major determinant of smooth muscle contraction and vasoconstriction.

Various types of cardiotonic steroids have different binding affinities to each type of α isoforms of Na/K ATPase, thus allowing diverse effects in different tissues. Rodent renal epithelial α1 isoform has low affinity to ouabain compared with the α2 and α3 (38). Many studies were performed to identify the mechanism that leads to vasoconstriction due to Na/K ATPase inhibition by cardiac glycosides. The most likely theory is based on Na/Ca- exchanger (NCX) and the plasmerosome region. Blaustein has described plasmerosome as a region of plasma membrane that is closely located to the sarcoplasmic reticulum. Only α2 and α3 isoforms appear to be placed in the plasma membrane alongside the sarcoplasmic reticulum. Moreover, α2 and α3 isoforms of Na/K ATPase form a specific complex with NCX, the so-called microdomain (39).

According to the Blaustein theory, inhibition of Na/K ATPase, coupled to the NCX, may lead to increase in intracellular sodium levels, which in turn results in increased cytosolic calcium levels and stimulates vascular smooth muscle contraction and hypertension (40). Interaction between the Na/K ATPase and NCX promotes specific intracellular Ca- signaling, initiated by ouabain binding. Further experimental investigations on the phenomenon of ouabain response supported the role of NCX in the regulation of myogenic tone (41). Moreover, endogenous ouabain can also raise blood pressure through alterations of gene expression in a pathway involved in intracellular Ca- signaling (42). This evidence suggests that endogenous ouabain and α2 isoform have a role in the pathogenesis of arterial hypertension.

In rodents, smooth muscle plasmerosome, associated with endoplasmic reticulum, contains sodium pump isoforms α2 and α3, which have higher ouabain affinity than the α1 isoform. In humans, however, ouabain seems to have almost the same affinity to all 4 isoforms (43). In short, above mentioned data suggest that in humans, endogenous ouabain can exert a much greater pro-hypertensive effect through inhibition both α2/α3 and α1 isoforms.

Marinobufagenin has been shown to significantly inhibit the α1 Na/K ATPase activity in rat renal tissue (44). Inhibition of renal tubular Na/K ATPase by marinobufagenin seem to prevent the reabsorption of filtered sodium and promote natriuresis.

ENDOGENOUS CTS AND DAHL SALT-SENSITIVE HYPERTENSION

The most studied rodent model of salt-sensitive hypertension is Dahl salt-sensitive rat which manifests an impaired sodium excretion response to increases in renal perfusion pressure. Dahl salt-sensitive rats develop hypertension after exposure to high salt diet or NaCl loading (45).

Majority of experimental studies have shown that in the normotensive Sprague-Dawley rats and in Dahl salt-sensitive (salt-susceptible) rats, after acute and chronic NaCl loading, the natriuretic effect was significantly correlated with elevated plasma levels and renal excretion of MBG. Moreover, the administration of anti-MBG antibody abolished the natriuretic response and increased renal sodium pump activity (46, 47). Marinobufagenin would therefore be expected as a putative natriuretic hormone. On the other hand, marinobufagenin, a selective inhibitor of the vascular smooth muscle Na/K pump, induces vasoconstriction in rat aorta and contributes to blood pressure elevation in Dahl salt-sensitive rats (48, 49). Different Na/K ATPase inhibitory effects of marinobufagenin among two strains of rats were studied by Bagrov et al. They have examined MBG response to sodium loading in normotensive and salt-sensitive rats and they found that MBG preferentially inhibits the Na/K ATPase in the kidney and in vascular smooth muscle cells. Thus, in normotensive Sprague-Dawley rats, marinobufagenin acts as a natriuretic hormone, promoting an adaptive natriuresis, but elicits vasoconstriction and sodium retention in salt-sensitive rats (50). These findings were extremely important for understanding the pathophysiological role of marinobufagenin in the development of salt-induced hypertension.

The ability of cardiotonic steroids to cause natriuresis may not only be due to an inhibition of sodium reabsorption, but also internalization of the sodium pump in the proximal tubule and decreased expression of the transport protein, Na/H exchanger (NHE3) in apical membrane of the renal proximal tubule (51, 52). Additional studies have reported that CTS may play an important role in regulation of renal sodium handling and blood pressure through the activation of a Src-EGFR signaling cascade via caveolar Na/K ATPase (53). All things considered, recent studies have revealed that the Na/K ATPase is not only an ion pump, but also an important transmembrane receptor and the CTS serve as specific messenger molecules (ligands) capable of activating the intracellular signaling pathways. These findings suggest that endogenous CTS in physiological conditions, play a significant role and regulate a number of cellular functions, such as growth, development, and apoptosis through alterations in target gene expression (54, 55).

MECHANISMS OF CTS STIMULATION AND THE ROLE OF RENIN-ANGIOTENSIN SYSTEM (RAAS)

Aside from marinobufagenin and ouabain, other endogenous digitalis-like factors have been identified in human plasma and/or urine, such as telocinobufagenin and bufalin (34, 56). Plasma concentrations of MBG and ouabain in humans seem to be in the range of 200 to 1500 pmol/l in health and disease (29). Evidence suggests that endogenous ouabain is produced in

mammalian adrenal cortex and hypothalamus (57-59). Marinobufagenin seems to be secreted by the adrenal glands and brain. The renin-angiotensin aldosterone system (RAAS) is thought to be a key factor linking central EO and peripheral MBG. A sequence of events underlying the pathophysiology of salt-induced hypertension was clearly demonstrated in NaCl loaded Dahl salt-sensitive rats and a possible scenario for the pathogenesis of human hypertension is overall illustrated in figure 1 (47). NaCl loading leads to transient elevations in EO in the hippocampus, hypothalamus and pituitary. Brain endogenous ouabain acts like a neurohormone activating central renin-angiotensin system (RAS) and triggers the sympathetic nervous system (SNS) transmission. This, in turn, stimulates the renin-angiotensin system in adrenal cortex and causes local production and release of marinobufagenin. These events contribute to decreased renal tubular Na/K ATPase activity as well as in vascular smooth muscle cells. It is believed that primarily marinobufagenin excretion is a compensatory response to salt loading and impaired renal sodium transport, which makes it able to promote natriuresis. However, an excessive rise in plasma MBG concentrations results in a maladaptive vasoconstrictor effect and, ultimately, arterial pressure increase and hypertension. Interestingly, administration of an anti-ouabain and anti-MBG antibodies prior to salt load prevented pressor and natriuretic response. The role of RAAS activation as a factor linking brain and adrenal CTS secretion was demonstrated, when centrally administered ouabain to DS elicits adrenal MBG production and its peripheral reaction (60).

Experimental animal studies have shown that during both chronic (administration of 8% NaCl diet) and acute NaCl loading of Dahl salt-sensitive rats, the central and peripheral levels of CTS have the same tissue concentration and urinary excretion pattern, that is a transient increase in EO but sustained increase in MBG levels (46, 49). Thus, a sustained increase in MBG production leads to chronic BP elevation induced by sodium loading.

CLINICAL TRIALS OF SALT-SENSITIVE HYPERTENSION

Few clinical studies on the pathogenesis of salt-induced hypertension have been performed in humans. During acutely elevated dietary NaCl, normotensive subjects presented similar urinary marinobufagenin and ouabain excretion patterns, as observed in animal models and renal MBG excretion was positively correlated with systolic blood pressure. Furthermore, researchers found that a sustained increase in MBG in response to NaCl intake is inversely linked to salt sensitivity (61). Another study has shown a significant increase in EO levels following chronic salt intake and a direct correlation between the change in EO and blood pressure in normotensive men (62). These findings strongly support the hypothesis that in humans cardiotonic steroids are the linking factor between salt

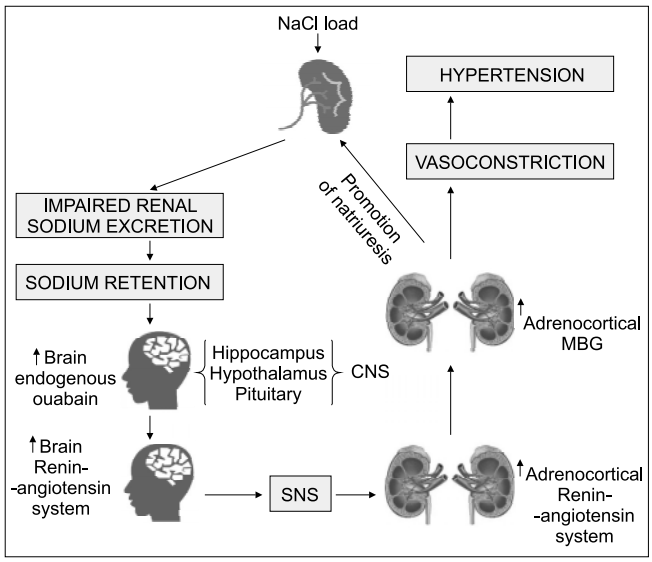

Fig. 1. A possible scenario for the pathogenesis of salt-induced hypertension in humans. In case of impaired renal sodium excretion, NaCl loading leads to sodium retention and stimulates brain endogenous ouabain. Endogenous ouabain in the brain appears to play a critical role and activates the local renin-angiotensin system as well as sympathetic nervous system (SNS). These actions stimulate renin--angiotensin system in adrenal cortex and adrenocortical marinobufagenin (MBG) release. MBG is secreted with the primarily adaptive aim of promoting natriuresis, but excessive MBG production induces vasoconstriction and ultimately leads to arterial hypertension

intake and hypertension. Unfortunately a trial involving rostafuroxin – an ouabain antagonist, failed to show any significant blood pressure lowering in humans with hypertension (63).

There is little research on cardiotonic steroids in hypertensive patients. Manunta et al. demonstrated in essential hypertension that plasma level of EO increased during sodium restriction (both acute and chronic). These results indicate that circulating levels of EO elevate, as an adaptive mechanism to total body sodium depletion and that EO does not fulfill the criteria for a putative natriuretic hormone (64). According to a recent study, dietary sodium restriction reduces urinary MBG excretion, which, in turn, is positively correlated with systolic blood pressure in hypertensive individuals (65).

Circulating CTS concentrations are increased in patients with essential hypertension and an important relationship between the level of these endogenous factors and mean arterial pressure has been shown in both hypertensive and normotensive individuals (28, 65, 66). Significantly elevated levels of plasma MBG has been observed in patients with primary aldosteronism, suggesting a possible role of MBG in the pathogenesis of hyperaldosteronism related hypertension (67).

Numerous observations indicate that CTS relevance in the pathogenesis of arterial hypertension is not confined to vasoconstriction and impaired renal sodium excretion. Endogenous cardiotonic steroids, via activation of signal transduction pathways, seem to be important contributors to cardiovascular dis-

eases and kidney failure. It has been demonstrated that MBG is involved in the pathophysiology of aortic stiffness, cardiac and renal fibrosis (65, 68, 69). High levels of endogenous ouabain induce and attenuate pathological cardiac hypertrophy, vascular remodeling and increase total peripheral vascular resistance (70-73).

CONCLUSIONS

During several last decades, the significance of dietary NaCl intake for the development of hypertension has become of great interest to hundreds of scientists from all over the world. Due to their huge efforts, a mechanism of salt-induced hypertension matured to a well-established phenomenon. Endogenous digitalis-like factors, also known as cardiotonic steroids are intimately involved in sodium and blood pressure homeostasis. The only known target for CTS is Na/K ATPase. Specific inhibition of Na/K ATPase by CTS results in natriuretic response, but also in an increase in systemic vascular resistance as well as in a rise in blood pressure. Recent studies have indicated that Na/K ATPase also functions as a classical receptor and many of cellular functions are regulated by low concentrations of CTS. This substantial discovery has widespread the physiologic and pathophysiologic implications and has given a hope to identify therapeutic targets for new drugs and more successful pharmacological treatments in the years to come.

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