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Are endogenous cardenolides controlled by atrial natriuretic peptide

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ABSTRACT

Endogenous cardenolides are digoxin-like substances and ouabain-like substances that have been implicated in the pathogenesis of hypertension and mood disorders in clinical and pre-clinical studies. Regulatory signals for endogenous cardenolides are still unknown. These endogenous compounds are believed to be produced by the adrenal gland in the periphery and the hypothalamus in the central nervous system, and constitute part of an hormonal axis that may regulate the catalytic activity of the α subunit of Na^+/K^+-ATPase. A review of literature suggests that there is great overlap in physiological environments that are associated with either elevations or reductions in the levels of atrial natriuretic peptide (ANP) and endogenous cardenolides. This suggests that these two factors may share a common regulatory signal or perhaps that ANP may be involved in the regulation of endogenous cardenolides.

Introduction

The discovery of the sodium- and potassium-activated adenosine triphosphatase (Na^+/K^+-ATPase or sodium pump) by the Danish researcher, Jens Christian Skou, in 1957, was a big leap in better understanding of ion and fluid homeostasis in nearly all animals. Since then, regulation of Na^+/K^+-ATPase has been an active topic of research. Exogenous Na^+/K^+-ATPase inhibitors of plant and animal origin have been used for various purposes for centuries. Due to their effects on cardiac functioning, these Na^+/K^+-ATPase inhibitors often referred as cardiotonic steroids (CTS). Exogenous cardiotonic steroids of plant origin, like plants of genus *Digitalis*, bark of *Acokanthera ouabaio*, ripe seeds of *Strophanthus* (renamed *Roupellina*), e.g.: ouabain and digoxin, were named as cardenolides. Exogenous CTS derived mostly from amphibians, like *Bufo* toad were named as bufadienolides e.g.: marinobufagin derived from the giant toad, *Bufo marinus*. The first endogenous CTS to be characterized, ouabain-like factor (OLF), was so named because of its close structural resemblance to ouabain [1]. Since then several other CTSs have been isolated from various human tissues and fluids. Their exact physiological role and regulation in human body is still controversial, but they are thought to play important roles in sodium and fluid homeostasis (via modulation of ion transport) as well as cellular signaling pathways (mechanism of which is thought to be independent of ion transport) [1–4]. Digitalis glycosides are implicated in positive inotropic effects in the heart. At low concentrations, CTSs activate Na^+/K^+-ATPase activity [5,6] and may activate multiple signaling cascades via low frequency intracellular calcium (Ca^{2+}) oscillations, causing activation of several transcription factors, like NF-κB and modulating cell functioning [7,8].

Cardenolide receptor

The Na^+/K^+-ATPase is an integral cell membrane protein. The energy stored in one molecule of adenosine triphosphate (ATP) can be utilized to translocate 3 sodium ions (Na^+) out, and 2 potassium ions (K^+), into the cell. This establishes the electrochemical gradient across the cell membrane, which drives many cellular functions, like volume maintenance, cotransport of many nutrients, muscle contraction, impulse transmission, and Na^+ resorption in renal tubular epithelial cells. Each Na^+/K^+-ATPase comprises of α and β subunits. A third subunit, γ, has also been described which belongs to FXYD family of proteins, and is believed to modulate the function of the sodium pump [9–11]. The α subunit comprises a group of seven transmembrane sequences. It has the ATP binding site and catalytic activity, and contains the binding sites for Na^+ and K^+. It also binds to the regulatory factors such as the CTSs and contains the phosphorylation sites for the regulatory proteins, protein kinase A and protein kinase C. The β subunit serves as an anchoring protein and as such determines where on the cell

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membrane the Na^+/K^+-ATPase will reside, and may have additional regulatory function [12–15].

The α subunit has 4 isoforms, while β subunit has 3 isoforms. This allows various combinations with differing characteristics and differing sensitivity to CTSs [16]. Each isoform has varying affinity to CTSs, so that the affinity for α3 > α2 > α1, across all the CSTs [17–20]. Expression of the various isoforms is tissue-specific. The expression of α1 isoform is ubiquitous; α2 is expressed in heart, vasculature, brain, adipocytes, skeletal muscles, cartilage, and bone; α3 is expressed mostly excitable tissue, like brain, spinal cord, peripheral nervous tissues, and cardiac conductive tissue; and α4 isoform is specifically expressed in testes. The β1 isoform is expressed ubiquitously; β2 and β3 isoforms in brain, heart, cartilage, bone, and erythrocytes; and β3 in lungs.

Endogenous cardenolides and exogenous CTSs

The cardiotonic steroid family shares a common steroid nucleus with a 5-member lactone ring (cardenolides), or 6-member lactone ring (bufadienolides), and contains a variety of combinations of hydroxyl, sulfate or carbohydrate groups [21,22]. Endogenous CTS are classified similar to exogenous compounds. Endogenous cardenolides consist of ouabain [1,23,24], digoxin [25] and their metabolites – dihydro-ouabain and dihydro-digoxin. Endogenous bufadienolides consist of marinobufagenin (MBG), telcinobufagin, proscillaridin A [26–29]. Additionally, another endogenous bufadienolide – 19 norbufalin – was isolated from cataractous lens [30].

To date endogenous cardenolides have been isolated from various tissues and body fluids, like human plasma [1], human breast cyst fluid [31], human pleural fluid [32], bovine adrenal gland [33,34], hypothalamus [35,36], cerebrospinal fluid [37], and urine [25]. Biosynthesis of endogenous CTS is poorly understood, but these compounds are thought to be synthesized in adrenal gland [38,39], and hypothalamus [40]. After an adrenalectomy in patients who had an adrenal tumor, there is a marked decrease in cardenolide levels [41]. In bovine adrenocortical cells, endogenous ouabain was released after stimulation with adrenocorticotropic hormone (ACTH) [42,43], angiotensin-II via the AT type 2 receptor [44], and α1 adrenergic receptor agonists [45]. In human adrenocortical cells, ACTH and angiotensin-II did not stimulated endogenous ouabain release but rather secretion was stimulated by cAMP [39], ADH and phenylephrine [44]. Stimulation of nicotinic receptors in rat adrenocortical cells was seen to release endogenous ouabain [46]. It has also been hypothesized that the heart may also be a possible site of production of endogenous cardenolides, where its concentration was found to be 2 orders of magnitude higher than the plasma and increased after myocardial ischemia [47].

Pregnenolone and progesterone might be the precursors of endogenous CTS [38]. For example, OLF production is inhibited in bovine adrenocortical cell culture after adding trilostane, a selective 3β-hydroxysteroid dehydrogenase inhibitor [48], and mammalian digoxin-like factor synthesis from radiolabeled acetate and cholesterol has been demonstrated [49]. Despite these finding, it remains unclear how endogenous CTS are regulated.

Mechanism of action of endogenous cardenolides

The mechanism of action of CTS is counterintuitive for people aware of the inhibitory effect of exogenous CTS. At physiologic concentrations, endogenous cardenolides stimulate the Na^+/K^+-ATPase [5]. Pharmacologic, or toxic, concentrations of CTS, will inhibit the pump. Consequently, the role of endogenous cardenolides is to *increase* sodium pump activity and increase the clearance of intracellular sodium, and indirectly, increase the concentrations of extracellular sodium. The body is able to target the effect in specific tissues by the distribution of the α subunit, so that, as endogenous cardenolide concentrations increase, very high affinity α3 in the conducting heart tissue or brain might be stimulated prior to lower affinity α2 in muscles or glia. And both α2 and α3 get stimulated prior to the ubiquitous α1. Stated differently, the most electrically active tissues are the first to respond to endogenous cardenolides (i.e., at lower concentrations), and progressively less electrically active tissues become stimulated as the concentrations of endogenous cardenolides increase. It is important to remember that pharmacologic concentrations of exogenous CTS are intended by the organisms that produce them to be toxic. That is the reason that grazers avoid eating plants that produce CTS, or birds vomit after eating a Monarch butterfly that has fed on milkweed. Thus, the sodium pump inhibitory action of these agents is best understood as toxicity.

Atrial natriuretic peptide

Natriuresis and fluid regulation was previously thought to be primarily under control of renin-angiotensin-aldosterone system. But these systems were inadequate to explain physiology associated with volume depletion. Another factor, called the "third factor", was postulated to play an important role. It was noticed that supraventricular dysrhythmias induced natriuresis, suggesting link between "third factor" and heart. In the 1980 s, atrial natriuretic peptide (ANP) was discovered when researchers noticed rapid diuresis and natriuretic response with injection of atrial tissue extracts in rats [50,51]. The natriuretic peptide family mainly consists of ANP, Brain Natriuretic Peptide (BNP), and C-type Natriuretic Peptide (CNP). Each natriuretic peptide in this family appears to induce diuresis, natriuresis, vasodilation, and inhibition of the renin-angiotensin-aldosterone system and the sympathetic nervous system [51–56]. In the past 3 decades since the discovery of ANP extensive studies have been done to understand their role in various medical conditions, most notably in cardiac dysfunction (Table 1).

Three types of NP receptors have been characterized: NPR-1 or NPR-A or Guanylyl cyclase-A (GC-A); NPR-2 or NPR-B or Guanylyl cyclase-B (GC-B); and NPR-3 or NPR-C. In the CNS of man and rodents NPR-A is found mainly in cortex and hippocampus, and is selectively responsive to ANP > BNP > CNP (essentially no physiologic activity to CNP). NPR-B is expressed highly in the brain, kidney, heart and vascular tissue [57,58]. In the CNS of man and rodents NPR-B is present in the amygdala and several brainstem regulatory sites. CNP selectively activates the receptor at physiological concentrations (pM range) and has a binding affinity some 50–500 fold higher than the other natriuretic peptides (CNP > ANP ⩾ BNP) [59,60]. In the cardiovascular system, NPR-B is found predominantly on veins, but also in arteries. The vasodilator activity of CNP in larger conduit vessels is dependent on NPR-B-triggered cGMP generation, whereas in resistance arteries CNP responses are mediated predominantly via NPR-C (see below) [61]. NPR-C is the most abundantly expressed and widely distributed NPR and is found in major endocrine glands, lungs, kidneys and the vascular wall [62] (e.g. vascular endothelial cells). All natriuretic peptides bind to NPR-C with high affinity, but it has the following selectivity profile: ANP > CNP > BNP [59].

Endogenous cardenolides and ANP

In the past 3 decades since the discovery of NPs extensive studies have been done to understand their role in various medical conditions, most notably in cardiac dysfunction (Table 1). Recent research has shown increasing evidence for multiple interactions