Cardiac glycosides are drugs which have been used successfully for over 200 years to treat patients having dropsy or heart failure (1). The most widely used cardiac steroids nowadays are digoxin and proscillaridin A.

Cardiac glycosides encompass a group of substances (endo- and exogenous) that share the capacity to bind to the membrane-inserted protein, the Na+, K+-ATPase (the sodium pump). This pump is responsible for maintaining of the membrane potential of living cells, and for regulating cell volume as well as intracellular pH and Ca2+ concentration.

50 years ago Schatzman (2) found that cardiotonic steroids were specific inhibitors of plasma membrane sodium pump. The discovery that the digitalis receptor is the Na+, K+-ATPase (2,3) marked the beginning of understanding of cardiac glycosides therapy at the molecular level. The discovery of the Na+/Ca2+ exchanger in mammalian cardiac muscle led to the hypothesis that the inhibition of the sodium pump by cardiotonic steroids leads to an increase in the concentration of intracellular Ca2+ as a secondary event. This, in turn, results in a positive inotropic effect on cardiac muscle (4). The Na+/Ca2+ exchanger is expressed in the plasma membrane of all animal cells including vascular smooth muscle cells (5). This exchanger catalyzes electrogenic exchange of three Na+ and one Ca2+ through the plasma membrane in either the Ca2+-efflux (regularly) or Ca2+-influx (at high intracellular Na+ concentrations) which depends on the electrochemical gradients of the substrate ions. The extrusion of Ca2+ from cytosol during the relaxation phase (Figure 1), which balances Ca2+ entry via L-type and ryanodine-sensitive calcium channels, is the primary function of the Na+/Ca2+ exchanger in the heart and in vascular smooth muscle cells. Na+/Ca2+ extrudes about 30% of the cytosolic Ca2+ which is required to activate the myofilaments in rabbit and human ventricles (8). Sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA) removes most of the remaining Ca2+. Under pathological conditions such as: cardiac ischemic reperfusion or digitalis intoxication, the rise in the cytosolic concentration of Na+ results in the inhibition of calcium efflux via Na+/Ca2+ exchanger or even leads to Ca2+ uptake when exchanger operates in reverse mode (exchange of intracellular Na+ for extracellular Ca2+) (5,8). This way the inhibition of Ca2+ efflux via Na+/Ca2+ exchanger causes slight increase in the concentration of free cytosolic Ca2+. This, in turn, may result in sequestration of higher Ca2+ quantities in sarcoplasmic/endoplasmic reticulum due to the operation of SERCA. Then this extra stored Ca2+ is available for mobilization whenever the cells are activated and substantially improves contracting function of cardiomyocytes (positive inotropic effect) or vascular smooth muscle cells.

Cardiac glycosides exert their effect by inhibiting the cardiomyocyte sodium pump, which, in turn, increases intracellular Na+. The rise in the concentration of Na+ results in the Ca2+ influx due to the Na+/Ca2+ exchanger operating in a reverse mode (4). This mechanism is over-simplified and still needs refinement. More recently it was found that the Na+, K+-ATPase might also play a role in the regulation of cell growth and expression of various genes (9).

PST 2238 AS AN ANTIHYPERTENSIVE COMPOUND THAT ANTAGONIZES THE EFFECT OF ENDOGENOUS CARDIAC GLYCOSIDES

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Abstract: This review examines the role of endogenous cardiac glycosides (OLF – ouabain like factors) in the pathogenesis of hypertension. The discovery of ouabain as a new adrenal hormone affecting salt and water homeostasis has initiated research on a new group of compounds. OLF may provide new insights into the mechanisms and therapy of common cardiovascular diseases. PST 2238 (17β-(3-furyl)-5β-androstane-3β-14β-17α-triol), an antagonist of endogenous ouabain, might open new possibilities for the therapy of hypertension and congestive heart failure.

Keywords: endogenous cardiac glycosides; endogenous ouabain; ouabain antagonist; hypertension; PST 2238

GENERAL

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Cardiac glycosides exert their effect by inhibiting the cardiomyocyte sodium pump, which, in turn, increases intracellular Na+. The rise in the concentration of Na+ results in the Ca2+ influx due to the Na+/Ca2+ exchanger operating in a reverse mode (4). This mechanism is over-simplified and still needs refinement. More recently it was found that the Na+, K+-ATPase might also play a role in the regulation of cell growth and expression of various genes (9).
It was only newly discovered that cardiac glycosides might affect cells at the concentrations lower than that required for the inhibition of the sodium pump. Dmitrieva (10) suggested that the Na\textsuperscript{+}, K\textsuperscript{+}-ATPase might act as a cell signalling receptor activated by a cardiac glycosides binding. It is thought that this signalling may influence cytoskeletal reorganization as well as cell survival, its growth and differentiation (9,10). However, this pathway is still unresolved form of cardiac glycosides action.

Since the cardiac glycosides binding site of the sodium pump has been conserved over the millennia, it has been suggested that endogenous cardiac glycosides might exist. The search for endogenous cardiac glycosides has led to the isolation of several cardiotoxic steroids of the cardenolide and bufadie-nolide type from blood, adrenals and hypothalamus (11). The main component of endogenous sodium pump inhibitors seems to be ouabain (12,13,14). Ouabain-like factors (OLF) have been found in almost all tissues, including plasma. Structural and functional characteristics of OLF, which are mammalian cardiac glycosides, are similar to the plant-derived ouabain. Beside ouabain, other mammalian
endogenous cardiac glycosides such as digoxin, dihydrouaibain, 19-norbufalin, marinobufagenin and proscillaridin A have been identified (15,16). These endogenous compounds are thought to be produced by the adrenals (4,11). They also are believed to constitute a part of hormonal axis that may regulate the catalytic activity of the α-subunit of the Na⁺, K⁺-ATPase. Now OLF are recognized as a novel group of mammalian adrenocortical hormones (11). The hormonal control of the release of ouabain from adrenals and the interaction of ouabain with other hormones participating in salt and water metabolism or in heart functions remain unknown so far.

**OLF and its role in the pathogenesis of hypertension**

There is growing evidence for OLF involvement in the regulation of blood pressure and renal function (4,10,17). Endogenous cardiac glycosides are supposed to participate in several forms of hypertension and congestive heart failure. The inhibition of the sodium pump in the kidney by OLF may result in renal sodium excretion. OLF-evoked inhibition of the Na⁺, K⁺-ATPases in the vascular system may contribute to increased vascular contraction and elevated blood pressure (10).

The concentrations of OLF are known to correlate with the blood pressure. It was found that the blood concentrations of OLF were significantly elevated in hypertension and in patients with heart failure (17,18,19,20,21). Among Caucasians with essential hypertension 30-45% have elevated circulating levels of OLF (20).

The elevated levels of OLF are also observed in preeclampsia. Preeclampsia is an example of a rapidly developing, volume-dependent and sodium-sensitive hypertension. Both clinical and experimental studies have demonstrated that pregnancy is associated with dysregulation of the Na⁺-, K⁺-ATPase function. Interestingly, Digibind, an antibody Fab fragment used to treat digitalis poisoning, has been found to lower blood pressure in patients with pre eclampsia (10).

Chronic administration of exogenous ouabain for 10 days or more (peripherally or centrally) induces hypertension in normotensive rats (22,23,24). In contrast to ouabain, digoxin does not induce hypertension in rats (25). Because ouabain and digoxin have comparable potency as inhibitors of the sodium pump, the long-term pressor activity of ouabain might be independent of its ability to inhibit this enzyme.

The mechanisms responsible for the ouabain-induced hypertension are still unclear. Initially, it was proposed that volume expansion and increase in total body sodium might be a primary stimuli for the release of the Na⁺, K⁺-ATPase inhibitors involved in the onset of low-renin hypertension (4,26). However, the findings in humans have provided no support for such a relationship. Although increased dietary salt might raise plasma OLF levels in humans, other observations suggest that hypervolemia *per se* is not correlated to production of OLF (18,19).

The aforementioned data constitute the background for a new hypothesis linking OLF with the pathogenesis of hypertension. This hypothesis assumes that elevated OLF primarily affect the central nervous system and activate central angiotensin II-dependent pathways that mediate sympathetic nerve activity. This assumption is based on the observation made by Leenen (23) who has demonstrated that intracerebroventricular administration of fragments of antibodies, which bind ouabain and related steroids, prevent the hypertension induced by subcu-

![Figure 2. Structure of ouabain (G-Strophanthin, Acocantherine), the main component of OLF; 1β, 3β, 5β, 11α, 14, 19-hexahydroxydioxycard-20(22)-enolide3-(6-deoxy-α-L-mannopyranoside).](image2)

![Figure 3. Structure of PST 2238 (17β-(3-furyl)-5β-androstane-3β, 14β, 17α-triol) – an ouabain antagonist (32).](image3)
taneous ouabain. He also found that neurons in the ventral anteroventral third ventricle region partly mediate both pressor and tachycardic responses to the administration of ouabain in rats (possibly via sympathetic activation) (27).

Hamlyn (28) proposed that the induction of ouabain-induced hypertension in rats might be independent of the Na⁺, K⁺-ATPase inhibition. He concluded that rat adrenocortical cells express ouabain receptors that are not coupled to the sodium pump. He also suggested that those receptors might be involved in the regulation and/or the secretion of endogenous ouabain (28). However, the existence of novel binding sites for ouabain, the relations of OLF with hypertension and congestive heart failure needs further investigations.

PST 2238 as the prototype of a new class of antihypertensive compounds

PST 2238 (17β-(3-furyl)-5β-androstan-3β-14β-17α-triol) is a new antihypertensive compound able to selectively antagonize the pressor effect of ouabain (or OLF) (Figure 2) and devoid of typical for digitalis side effects (29,30,31.32).

PST 2238 is derived from digitoxigenin (Figure 3).

In vitro, PST 2238 displaced ouabain from its binding sites on purified Na⁺, K⁺-ATPase (IC₅₀ 1.7×10⁻⁶M) without interacting with other receptors involved in blood pressure regulation (33). It lowers blood pressure by normalizing the expression and activity of the renal sodium pump in the rat models with increased OLF levels. In cultured renal cells incubated with 10⁻⁹M ouabain, PST 2238 normalizes the Na⁺, K⁺-ATPase activity. PST 2238 (1 µg/kg) prevented both ventricle and renal hypertrophy in rats (31). This effect was associated with the ability of this compound to antagonize the ouabain-dependent activation of growth-controlling genes (31). In rats, made hypertensive by chronic infusion of 50 µg/kg/day of ouabain, PST 2238 given per os at very low doses (0.1-1 µg/kg/day for 4 weeks) abolished the increase in blood pressure and renal Na⁺, K⁺-ATPase inhibition. These results indicate that the chronic exposure to low concentrations of ouabain consequences in an upregulation of the Na⁺, K⁺-ATPase and this effect may be antagonized by low concentrations of PST 2238. It is of importance to note that PST 2238 did not affect blood pressure and renal Na⁺, K⁺-ATPase activity in normotensive rats (31).

To sum up, PST 2238 antagonizes selectively the pressor effect of OLF and corrects the ion transport defect. It seems to be the first new, and orally active, antihypertensive compound which selectively displaces ouabain from the purified sodium pump receptor in vitro at micromolar concentrations. Despite its steroidal structure, PST 2238 does not affect receptors for steroidal hormones such as estrogens, androgens or mineralocorticoids. Therefore, there is a hope that PST 2238 might be devoid of those side effects typical for the antimineralocorticoids such as spironolactone, which is very popular in Poland. It may prevent cardiovascular complications associated with hypertension through the selective modulation of the very little known so far Na⁺, K⁺-ATPase function.

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