

Endogenous Cardiac Glycosides: Hormones Using the Sodium Pump as Signal Transducer

Wilhelm Schoner and Georgios Scheiner-Bobis

The search for an endogenous digitalis has led to the identification of the cardenolides ouabain and digoxin and the bufadienolide marinobufagenin in mammalian tissues and biological fluids. Ouabain's release from adrenal glands is under the control of epinephrine and angiotensin II; hence, its blood concentration changes rapidly on physical exercise. It also is controlled by brain areas sensing cerebrospinal Na^+ concentration and apparently the body's K^+ content because urinary K^+ loss leads to an increase in its plasma concentration as well. Long-term treatment of rats with ouabain results in arterial hypertension, and 50% of Caucasians with low-renin hypertension have increased plasma concentrations of this cardenolide. Levels of digoxin, which is synthesized from acetate in adrenal glands, increase slightly in blood on prolonged exercise. It counteracts the hypertensinogenic action of ouabain in rats, as does the ouabain antagonist PST 2238. The plasma concentration of the bufadienolide marinobufagenin is increased after cardiac infarction. It may show natriuretic properties because it inhibits the α_1 isoform of Na^+/K^+ -adenosine triphosphatase (ATPase), the main sodium pump isoform of the kidney, much better than other sodium pump isoforms. These effects of endogenous cardiac glycosides are observed at concentrations that do not inhibit the sodium pump. Apparently, Na^+/K^+ -ATPase is used by these steroids as a signal transducer to activate tissue proliferation, heart contractility, arterial hypertension, and natriuresis via various intracellular signaling pathways. *Semin Nephrol* 25:343-351 © 2005 Elsevier Inc. All rights reserved.

KEYWORDS Endogenous digitals, endogenous ouabain, sodium pump, signal transduction, cardiotonic steroids, congestive heart failure, hypertension, natriuresis

Cardiotonic steroids (CTS) produced by digitalis plants have been used successfully to treat congestive heart failure for more than 200 years.¹ The impressive benefit of this therapy led to the idea that an endogenous digitalis might exist. The search for such a compound gained some credibility despite more than 100 years of failure when Schatzmann² discovered that the sodium pump of plasma membranes is the receptor for CTS in 1953. The discovery of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in the late 1960s in mammalian cardiac muscle led to the view that an inhibition of the sodium pump by CTS may increase the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) as

a secondary event, which in turn results in a positive inotropic action of the cardiac muscle.³ This model has been refined recently (see later). It is clear now that the α_1 isoform of the sodium pump is distributed ubiquitously in plasma membranes of cardiomyocytes. The α_2 and α_3 isoforms, however, reside in specific, tiny cytosolic spaces between the plasmalemma and the adjacent endoplasmic (or sarcoplasmic) reticulum called plasmersome,⁴ which also contain the $\text{Na}^+/\text{Ca}^{2+}$ exchanger protein. Inhibition of the α_2 and α_3 isoforms of Na^+/K^+ -adenosine triphosphatase (ATPase) in such a restricted area leads to a change in cytosolic Na^+ and, indirectly, Ca^{2+} concentrations. This in turn modulates the Ca^{2+} content of the sarcoplasmic reticulum and Ca^{2+} signaling and leads finally to the positive inotropic effect of cardiac glycosides^{4,5} and an altered gene expression of proteins.^{6,7} Progress in the search for endogenous digitalis-like compounds came mainly from the Dahl et al⁸, deWardener and Clarkson,⁹ and Blaustein¹⁰ concept that suggested that natriuresis is caused by an enhanced production of endogenous inhibitor(s) of the sodium pump whose inhibition in renal tubules may decrease the volume of circulating fluid. The increased produc-

From the Institut für Biochemie und Endokrinologie, Justus-Liebig-Universität Giessen, Giessen, Germany.

Supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, Germany; the Fonds der Chemischen Industrie, Frankfurt/Main, Germany; and the Akademie für Tiergesundheit, Bonn, Germany.

Address reprint requests to Dr. Wilhelm Schoner, Institut für Biochemie und Endokrinologie, Fachbereich Veterinärmedizin, Justus-Liebig-Universität Giessen, Frankfurter Str. 100, D-35392 Giessen, Germany. E-mail: wilhelm.schoner@vetmed.uni-giessen.de

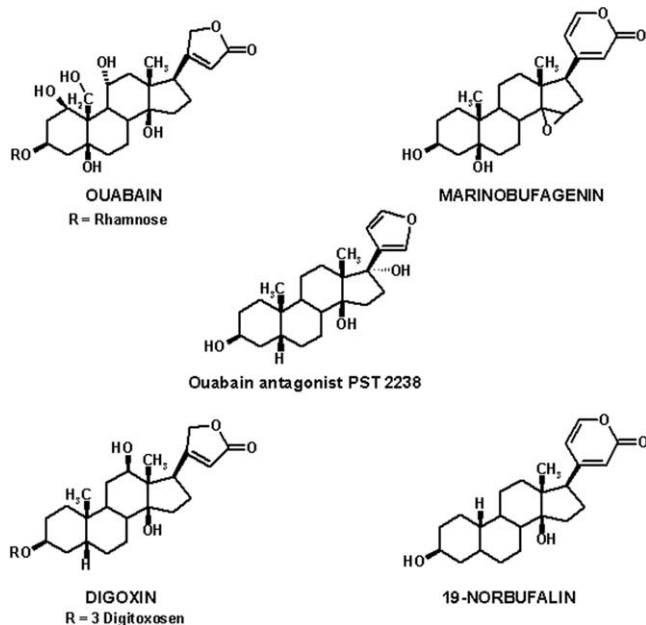


Figure 1 Structures of endogenous cardiotonic steroids that have been isolated in the search for endogenous digitalis. Compounds with an unsaturated 5-membered lactone ring are cardenolides and those with an unsaturated 6-membered lactone ring are bufadienolides. PST 2238 is a ouabain antagonist.^{91,92}

tion of endogenous digitalis-like compounds also would contribute to hypertension by means of inhibition of Na^+/K^+ -ATPase in cardiovascular tissues.⁸⁻¹⁰ Following this concept, Hamlyn et al¹¹ showed that the concentration of a circulating factor in blood plasma inhibiting purified Na^+/K^+ -ATPase correlated with the blood pressure of the donors. This observation paved the way for the identification of endogenous digitalis as a group of cardenolides and bufadienolides (Fig. 1) whose physiologic and pathophysiologic function is only beginning to be understood. Readers who are interested in more detailed information are referred to recent reviews.¹²⁻¹⁵

The Nature of Endogenous Cardiac Glycosides

It long has been known that certain vertebrates such as amphibians synthesize CTS with five 6-member lactone rings.^{16,17} Hence, it is not too astonishing that 4 different CTS have been identified thus far in mammals (Fig. 1).

Endogenous Ouabain

The compound has been isolated from human plasma,^{18,19} bovine adrenals²⁰ and hypothalamus,²¹ and from the supernatant of rat pheochromocytoma (PC-12) cells²² (Figure 1). Endogenous ouabain has been identified by $^1\text{H-NMR}$ ^{20,21} and LC-ESI-MS²² to be identical with the plant-derived steroid. However, one may not exclude that an 11- β -isomer of ouabain exists as well: metyrapone, an inhibitor of the 11- β hydroxylase activity, inhibits the synthesis of endogenous ouabain.²³

The Adrenal Gland as a Source of Ouabain

Orally and parenterally administered ouabain is taken up selectively by adrenal glands,²⁴ but its intestinal uptake accounts for only 3% to 5% of the orally administered substance.²⁵ Hence, it is essential to prove that ouabain isolated from mammalian tissues does not simply represent resorbed ouabain from food. Conscious dogs release ouabain from their adrenal glands.²⁶ Consistent with the assumption that the adrenal gland is a major place of synthesis and/or storage of ouabain, adrenalectomy lead to a decrease of ouabain plasma levels.^{18,27} Most likely the adrenal cortex is the place of storage and/or synthesis. It contains more ouabain than the medulla,²⁸ and medullectomized rats show no decrease in their plasma concentrations of ouabain when compared with sham-operated controls.¹⁴ Cases of adrenal tumors overproducing ouabain have been reported, and their excision lowered the increased blood pressure.^{29,30}

Biosynthesis of Ouabain in Adrenal Cells In Vitro

De novo synthesis of ouabain and dihydro-ouabain has been shown in tissue culture experiments.^{31,32} Bovine adrenocortical cells in vitro secrete up to 10-fold more ouabain than their cell content.³²⁻³⁴ The biosynthesis occurs in zona fasciculata cells. Pregnenolone and progesterone are precursors of endogenous ouabain.^{14,22,32} Inhibition of the pregnenolone's conversion to progesterone by trilostane, an inhibitor of 3β -hydroxysteroid dehydrogenase, inhibits ouabain synthesis.³² When $[7\text{-}^3\text{H}]$ pregnenolone is added to primary rat adrenal cells, radioactivity is found in a fraction with digitalis-like activity but not with ouabain.³⁵ It is possible that the mechanism leading to 5-hydroxylation in ouabain (but not in digoxin) and the A/B conformation of the steroid backbone eliminates the ^3H atom in position 7. The sugar $[^{14}\text{C}]$ rhamnose, which is part of the ouabain molecule, readily enters adrenocortical cells and increases the biosynthesis of endogenous ouabain.³²

Because bovine adrenocortical cells in tissue culture synthesize ouabain, information on its release is necessary to understand its hormonal control. Adrenocorticotrophic hormone (ACTH), α_1 -adrenergic receptor agonists, and angiotensin II stimulate ouabain's release from bovine adrenocortical cells.³⁶⁻³⁸ Yet human CLR7050 cells (an adrenal cortex-derived cell line) are insensitive to ACTH and angiotensin II but sensitive to arginine vasopressin and phenylephrine.³⁷ The phenylephrine-dependent release of ouabain from human CRL7050 and bovine adrenocortical cells in culture is blocked by the α_1 -adrenergic receptor antagonist doxazosin. This was interpreted to indicate that the sympathetic nervous system is involved in regulation of the release of this hormone to the bloodstream.³⁸ In bovine adrenal cortical cells, angiotensin II acts via the angiotensin type 2 (AT_2) receptor because the AT_2 agonist CGP42112 stimulates the release of ouabain and the AT_2 antagonist PD123319 inhibits it.³⁶ However, a signaling pathway involving the brain's O_2 chemoreceptor seems to exist as well because hypoxia triggers a marked

release of the hypothalamic inhibitory factor (ouabain) from midbrain and adrenals in rats.^{39,40}

Endogenous Digoxin

There is much evidence that mammalian cells synthesize digoxin as well: a substance indistinguishable from digoxin was isolated from human urine and identified with FAB-MS, proton NMR, and several different high-performance liquid chromatography systems.⁴¹ Deglycosylated and reduced forms of the digoxin-like immunoreactive factor were identified in bovine adrenals.^{42,43} It was found in blood plasma, urine, adrenal glands, and breast cyst fluid.^{13,15} Because digoxin is taken up at a much higher rate from the gut than ouabain, demonstration of digoxin's biosynthesis is essential to accept a physiologic role of CTS. Qazzaz et al⁴⁴ recently showed that Y-1 murine adrenocortical tumor cells use [1,2-¹⁴C]acetate and [4-¹⁴C]cholesterol as precursors for the synthesis of a [¹⁴C]digoxin-like substance. Its synthesis from acetate was inhibited by the HMG-CoA reductase inhibitor mevastatin. [7-³H]pregnenolone seems to be a precursor for digoxin in bovine adrenal cells.³⁵ Hence, the unsaturated lactone ring in digoxin is not formed from the isoprenoid side chain, a conclusion supported also by the finding that radioactivity from [26,27-³H]-25-cholesterol is not found in the cardiotonic steroid.³⁵ Digitoxose sugars are not known to exist in mammals. However, recent evidence may suggest otherwise.⁴⁵

Endogenous Marinobufagenin and 19-Norbufalin

Marinobufagenin (3 β ,5 β -dihydroxy-14,14-epoxybufadienolide) (Fig. 1) originally was discovered in amphibians and more recently was isolated from the urine of patients with myocardial infarction.⁴⁶ 19-Norbufalin and its Thr-Gly-Ala tripeptide derivative was isolated from human cataractous lenses while searching for the reason why in such lenses immunoreactivities against bufalin and ouabain exceed those of normal lenses.⁴⁷

The Sodium Pump as a Signal Transducer in Various Tissues

Hormones induce cellular and nuclear responses via their specific receptors that influence the cell physiologic state and, possibly, also the physiologic condition of the entire organism. With this in mind, how do ouabain and other cardiotonic steroids fulfill this requirement?

By addressing how the cardiac Na⁺/K⁺-ATPase and its clinically used specific inhibitors, the digitalis drugs, may be involved in cardiac hypertrophy, Xie and Askari⁶ and Huang et al⁴⁸ unveiled in a series of experiments with cardiac myocytes in culture that the same nontoxic concentrations of ouabain that cause partial inhibition of Na⁺/K⁺-ATPase and an increase in cardiac contractility also stimulate myocyte growth and protein synthesis. The signal transduction cascade from the membrane to the nucleus induced by ouabain

may be communicated by the epidermal growth factor receptor (EGFR), which becomes activated by Src, a ouabain-activated tyrosine protein kinase that appears to be the first kinase activated in the signaling cascade.^{49,50} Although it is not known whether Src directly interacts with the Na⁺/K⁺-ATPase or whether other receptor or nonreceptor tyrosine kinases are affected by ouabain, activation of EGFR leads to the recruitment of SH-2 domain-containing protein, Grb2, Sos, and Ras to the plasma membrane.

Ras activation leads to 2 branched signal transduction cascades: one communicating with the mitochondria to increase the generation of mitochondrial reactive oxygen species, resulting in nuclear factor κ B activation, and a second consisting of the Ras/Raf/MAP-kinase-kinase (MEK)/MAPkinase (ERK)1/2 cascade (Fig. 2).^{48,49,51} This latter pathway not only leads to gene activation and proliferation, it also mediates a link between Ca²⁺-dependent protein kinase C (PKC) activation and the cellular signaling pathways induced by the Na⁺/K⁺-ATPase/CTS interactions. Thus, in rat cardiac myocytes, a ouabain-induced increase in [Ca²⁺]_i leads to activation of PKC that in turn activates ERK1/2 via the Raf/MEK cascade,⁵² leading to expression of the transcription factors *c-fos* and *c-jun* activator protein 1 (AP-1) and ultimately resulting in myocyte hypertrophy.⁴⁸ Activation of PKC is most likely caused by activation of phospholipase C- γ , which also is recruited to the ouabain-activated Src/EGFR.⁵² Demonstration of a ouabain-induced activation of PKC is of particular interest because a large body of information exists concerning short-term regulation of [Ca²⁺]_i through activating or inhibitory effects of PKC on voltage-gated Ca²⁺ channels, the sarcoplasmic reticulum Ca²⁺ uptake/release system, and the sarcolemmal Na⁺/Ca²⁺ exchanger. Although a direct action of inositol 1,4,5-trisphosphate (InsP3) generated by ouabain-activated PLC cannot be excluded yet, ouabain-induced increases in [Ca²⁺]_i seem to be caused partially by the structural influence of the endoplasmic reticulum (ER)-localized InsP3 receptor by direct contact with the amino-terminal part of the α subunit of the Na⁺/K⁺-ATPase.⁵³

This ouabain-induced conformational cross-talk with the InsP3 receptor appears to be the initial event that in renal epithelial cells induces slow [Ca²⁺]_i oscillations mediated through the interplay of plasma membrane-localized L-type voltage-gated Ca²⁺ channels and store-operated Ca²⁺ channels (SOCs) that leads to nuclear translocation of nuclear factor κ B, a pleiotropic activator of the expression of various inducible genes.⁵⁴ In primary cultures of human umbilical artery endothelial cells (HUAECs), ouabain also induces slow Ca²⁺ oscillations that might be behind the observed release of endothelin-1,⁵⁵ a peptide hormone that causes vasoconstriction of myocytes in smooth muscle and cardiac muscle cells. Within the same time frame of 5 to 10 minutes, ouabain leads to MAPK activation. After incubation of HUAECs with ouabain for a longer period of time, the glycoside significantly stimulates cell growth and endothelin-1 messenger RNA expression. The results indicate that by increasing endothelin-1 expression and release, ouabain may contribute to the regulation of vascular tone. All of these

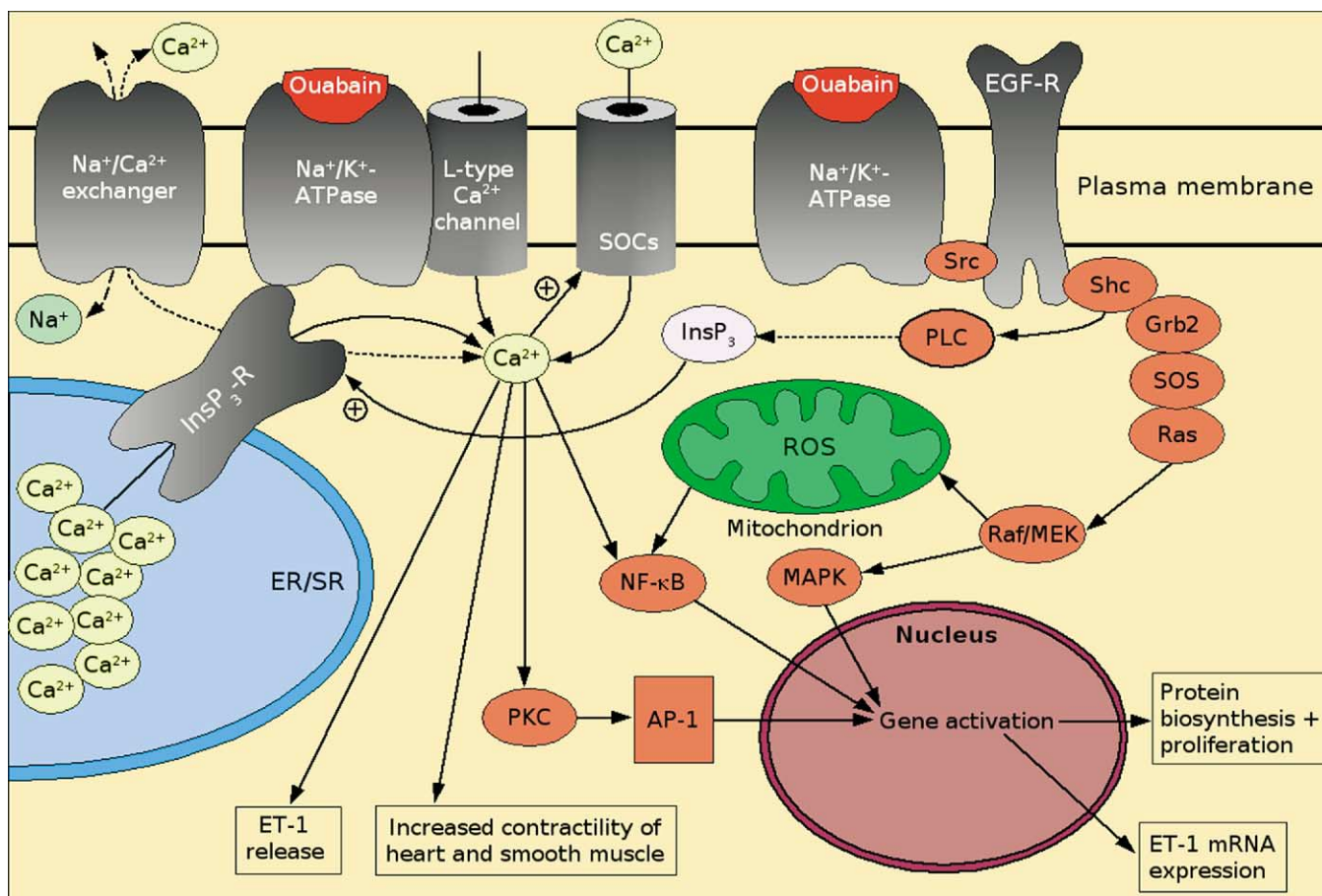


Figure 2 A generalized scheme showing the action of ouabain as a hormone. The figure combines information obtained with various cell systems. Signaling pathways are generated either by Src-transmitted cross-talk between Na^+/K^+ -ATPase and EGFR, or by a Na^+/K^+ -ATPase-transmitted increase in $[\text{Ca}^{2+}]_i$. This in turn is generated by the contribution of plasma membrane-embedded L-type Ca^{2+} channels, the InsP_3 receptor of the endoplasmic (ER)/sarcoplasmic reticulum (SR) membrane, and through the activation of SOCs in the plasma membrane. The $\text{Na}^+/\text{Ca}^{2+}$ exchanger possibly contributes to a local increase in $[\text{Ca}^{2+}]_i$ when the Na^+/K^+ -ATPase is inactivated and the Na^+ gradient over the plasmersome does not efficiently drive the exchanger's activity.

effects were observed at ouabain concentrations ranging from 1 to 10 nmol/L. At this concentration range, no global inhibition of the sodium pump was observed, but rather a stimulation.⁵⁵

In primary cultures of vascular and prostate smooth muscle cells, stimulation of proliferation and activation of mitogen activated protein kinase (MAPK) by ouabain or marinobufagenin also do not correlate with Na^+/K^+ -ATPase inhibition.⁵⁶⁻⁵⁸ This indicates that it is mainly protein-protein interactions that are responsible for the induction of signaling cascades rather than inhibition of the Na^+/K^+ -ATPase, which would have led to an increase in cytosolic $[\text{Na}^+]$ and, consequently caused by the existence of a $\text{Na}^+/\text{Ca}^{2+}$ exchanger, to an increase in intracellular $[\text{Ca}^{2+}]$.

Nevertheless, in pressurized small resistance arteries, it might be that partial inhibition of the ouabain-sensitive α_2 and α_3 isoforms of the sodium pump α subunit by nanomolar ouabain leads to Ca^{2+} transients evoked by a local increase of $[\text{Ca}^{2+}]$ within a plasmersome.⁴ A local ouabain-dependent increase in $[\text{Na}^+]_i$ and $[\text{Ca}^{2+}]_i$ within this space via SOCs and

eventually via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is considered to augment the release of Ca^{2+} from intracellular stores.⁵⁹ The secondary increase in the bulk concentration of $[\text{Ca}^{2+}]_i$ then may lead to the increased contraction. However, the fact that the action of ouabain on HeLa cells,⁵⁰ where little or no $\text{Na}^+/\text{Ca}^{2+}$ exchanger is expressed, also causes reactive oxygen species generation and MAPK activation would appear to contradict this hypothesis. Furthermore, experiments showing that inhibition of the pump is not a prerequisite for the positive inotropic effect,⁶⁰ the fact that HUAECs lack α_2 and α_3 isoforms of the sodium pump α subunit,⁵⁵ and the induction of Ca^{2+} transients in arterial smooth muscle by ouabain without an increase in cytosolic $[\text{Na}^+]$,⁶¹ all point to the possibility that ouabain and CTS might act in a multiplicity of ways depending on species, tissue, and cell type.

In summary, ouabain and CTS fulfill the criteria to be viewed as a new class of hormones. Unveiling the cell-specific primary signal transduction events they induce will help not only to understand how they affect contractility, growth rate, and differentiation, but also will provide important information for the

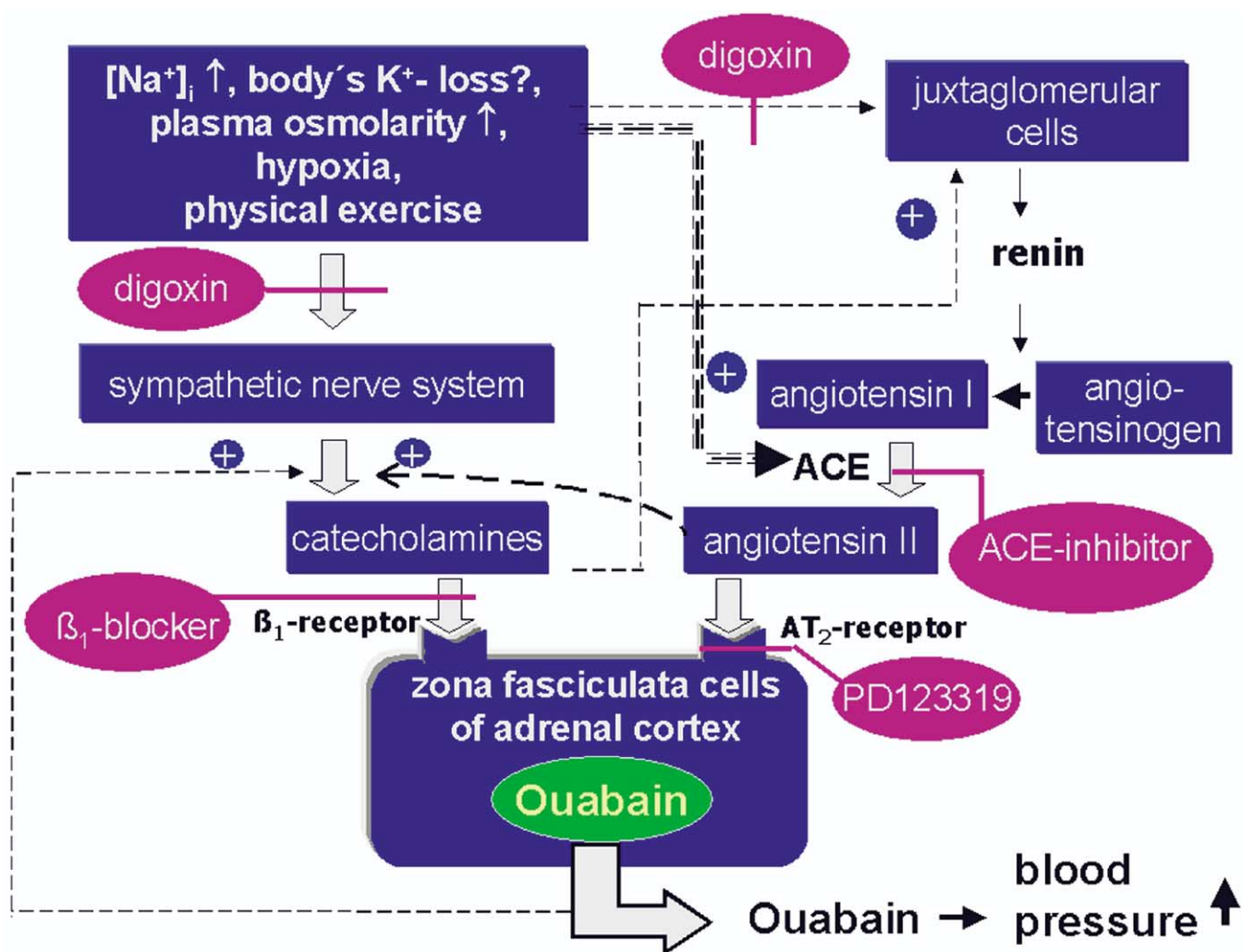


Figure 3 Scheme of the hormonal control of the release of endogenous ouabain from zona fasciculata cells of the adrenal cortex.

better understanding of cardiac failure and hypertension and, more generally, of the control of salt and water metabolism.

Physiology and Pathophysiology of Endogenous Cardiac Glycosides

We are only beginning to get information on the role of the different cardiotoxic steroids in physiology and pathophysiology. The data obtained thus far indicate that the various endogenous compounds act differently on salt and mineral metabolism and, hence, on kidney and heart function, as well as on the circulatory system.⁶² This may be because of differences in intracellular signaling cascades starting from the sodium pump as a receptor in the various target cells.

Endogenous Ouabain, a Blood Pressure–Modulating Factor

Although endogenous ouabain^{18–22} was isolated in the search for a natriuretic hormone,^{8–10} the interrelation between salt

and endogenous ouabain in the homeostatic regulation of blood pressure is not understood.⁶² Ouabain's plasma concentrations change quickly in stress situations, as seen in humans and dogs running on a treadmill⁶³ or in rats swimming.⁶⁴ Ouabain increases rapidly and concomitantly with epinephrine when physical exercise starts, and decreases quickly on rest. Pretreatment of dogs with the β -blocker atenolol as well as with the angiotensin-converting enzyme (ACE) inhibitor benazepril abolished the exercise-dependent increase in endogenous ouabain levels, indicating that the release of ouabain in dogs is under the control of epinephrine and angiotensin II.⁶³ Also, ACTH increases the plasma concentrations of endogenous ouabain in human beings and rats.^{65,66} Because plasma ouabain concentrations correlate with systolic and mean arterial blood pressure, endogenous ouabain behaves like a blood pressure–modulating factor (Fig. 3).^{62,67–69}

The interrelationship between endogenous ouabain and salt in the homeostatic regulation of blood pressure is rather complex.⁶² One may remember that the sodium pump maintains the cellular potassium concentrations and that K^+ on

the blood side of the plasma membrane counteract the action of ouabain.⁷⁰ In fish exposed to increasing salinity of their surrounding water, plasma ouabain and cortisol concentrations increase with the increase in plasma osmolality.⁷¹ Increased concentrations of endogenous ouabain have been reported under a number of conditions such as sodium imbalance, chronic renal failure, hyperaldosteronism, congestive heart failure, and pre-eclampsia.^{12,14,72} In hypertensive patients, however, endogenous ouabain increases with acute and chronic Na⁺ depletion. This argues against the hypothesis that ouabain acts as a natriuretic hormone.⁷³ In the general population, plasma ouabain increased with urinary K⁺ excretion but was dependent on neither urinary Na⁺ excretion nor serum concentrations of Na⁺ or K⁺.⁶⁷ It was suggested that ouabain is released by K⁺ to inhibit the pressure effect of excessive salt intake or to counteract the depressor action of sodium depletion.⁶⁷ Does the brain control ouabain's release by measuring the body's K⁺ content? We also may not exclude that the increase in plasma osmolality (as shown in fish⁷¹) and hence the increase in blood viscosity may affect ouabain secretion in mammals.

Similar to all rapidly acting hormones, ouabain shows long-term effects on protein synthesis (Fig. 2).^{6,7} Exposure of rats for a long period to small (nanomolar) doses of ouabain or other cardenolides leads to hypertension.⁷⁴⁻⁷⁷ The hypertensinogenic action of ouabain is unrelated to its inhibitory potency on Na⁺/K⁺-ATPase activity.⁷⁸ Approximately 50% of Caucasians with uncomplicated essential hypertension show increased concentrations of endogenous ouabain, reduced heart rate, and greater left ventricular mass and stroke volume of the heart.⁷⁹ This may be related to carriers of a mutated α -adducin Trp allele.⁶⁷ In these Caucasians, ouabain plasma concentrations correlate directly with the relative thickness of the left ventricular heart wall and the total peripheral resistance index.^{68,69} Immunization of rats against ouabain decreases arterial blood pressure,¹⁴ as does infusion of the commercially available Fab fragment of an antidigoxin antibody, Digibind (GlaxoSmithKline, Research Triangle Park, NC), that cross-reacts with ouabain in humans and rats.^{80,81}

Ouabain and digoxin affect cell proliferation and expression of isoforms of Na⁺/K⁺-ATPase in a different way in vitro⁶ and in vivo.⁸² Development of heart failure is associated with the down-regulation of the α_1 -isoform of Na⁺/K⁺-ATPase in the left ventricular myocardium,⁸³ and a reduced sensitivity to marinobufagenin, an up-regulation of the α_3 -isoform, and an enhanced sensitivity to ouabain.⁸⁴ Presumably, ouabain is involved in cardiac remodeling in the transition to heart failure.⁸⁴

Ouabain, a Neurosteroid, Mediates Sympathetic Hyperactivity in Salt-Sensitive Hypertension

Ouabain has been isolated from hypothalamus²¹ and it is present in the pituitary and in medullary neurons.¹⁵ In conscious rats, acute intracerebroventricular injection of ouabain increases sympathetic activity, blood pressure, and heart rate.

Such effects can be prevented by the simultaneous administration of Fab fragments of Digibind, which cross-react with ouabain.^{85,86} The effects of increased Na⁺ concentration in the cerebrospinal fluid and of ouabain are attenuated in transgenic rats that are deficient in brain angiotensinogen.⁸⁷ Apparently, even small increases of [Na⁺] in the cerebrospinal fluid are sensed by benzamil-sensitive Na⁺ channels,^{88,89} leading to the postulate that enhanced Na⁺ entry in relevant brain areas increase brain's ouabain release and subsequently sympathetic outflow and blood pressure.⁸⁸ In normal rats, sympathetic hyperactivity and hypertension induced by chronic ouabain and hypertonic saline treatment are prevented by angiotensin type 1-receptor blockade.⁸⁷ High salt intake also increases the expression and activity of ACE in hypothalamus and pons of Dahl salt-sensitive rats without a parallel increase in angiotensin II levels. Chronic blockade of brain ouabain by intraventricular infusion of a ouabain-binding antibody decreased the NaCl-dependent increase of ACE messenger RNA, which may indicate that the increase in ACE messenger RNA is secondary to the activation by brain ouabain.⁹⁰ Because ouabain may activate intracellular signaling cascades, resulting finally in an increase in [Ca²⁺]_i⁷ (Fig. 2), this also may lead to an increase in ACE expression and activity in neurons and finally to the amplification of the peripheral physiologic response. A tentative regulatory scheme based on the information available thus far is shown in Fig. 3.

Anti-Ouabain as an Antihypertensinogenic Agent

Can the hypertensinogenic action of ouabain be counteracted? In fact, PST 2238, a compound resembling CTS, acts as an antihypertensive when given orally at micromolar concentrations (Fig. 1).^{91,92} This new prototype of an antihypertensive drug also has effects in Milan hypertensive rats in which a genetic alteration of adducin genes is associated with hypertension and an up-regulation of renal Na⁺/K⁺-ATPase. Hence, PST 2238 might be useful for the treatment of human essential hypertension.⁹³

Endogenous Digoxin, a Factor Opposing Endogenous Ouabain

Although it is evident that digoxin is synthesized in the adrenal gland,⁴⁴ and despite the existence an impressive amount of literature on the action of this drug, not much data are available concerning why this CTS apparently differs from ouabain in its physiologic effects. The most striking difference is that digoxin acts as an antagonist of ouabain-induced hypertension.^{74,76,77,79,94} Additionally, the digoxin-induced arterial baroreflex opposes the sympathetic excitatory pressor responses to ouabain in the periphery and in the brain,^{79,94,95} and no longer activates the chemoreflex in patients with chronic heart failure (Fig. 3).⁹⁶ The plasma concentration of endogenous digoxin is increased in renal failure, hypertensive pregnancy, during prolonged strenuous exercise, and in newborn infants.^{13,15,97} Hence, it is an open

question whether the reported increase in plasma digoxin levels represents counteractive effects against the stress-induced increase in endogenous ouabain. One also may ask whether digoxin as a remedy, when used in therapy of heart failure, may act preferably via the suppression of the sympathetic excitatory pressor responses that had been exerted via ouabain's release from adrenals and brain^{94,95} as a result of an activation of the chemoreflex in patients with chronic heart failure,⁹⁶ rather than by direct inotropic response on heart muscle cells. It is unclear thus far how the 2 substances ouabain and digoxin, which are both specific inhibitors of the sodium pump, can produce opposite physiologic effects. One reason may lie in the higher hydrophobicity of digoxin as compared with ouabain, which would lead to a different tissue distribution. However, other reasons also may exist.

Endogenous Marinobufagenin, a Natriuretic Factor?

Endogenous marinobufagenin differs in its action from ouabain in 3 different aspects.⁶² First, it exhibits a greater affinity for the ouabain-resistant α_1 subunit of Na^+/K^+ -ATPase.^{98,99} Second, the acute NaCl load of rats and dogs is accompanied by a sustained increase in the level of endogenous marinobufagenin.^{99,100} Possibly the increased blood plasma marinobufagenin concentration promotes natriuresis and compensates for the genetically impaired pressure natriuretic mechanism.^{100,101} The bufadienolide acting like ouabain as a vasoconstrictor⁴⁶ is increased in volume expansion and pre-eclampsia and, similar to ouabain, is increased on voluntary hypoventilation of human volunteers.¹⁰² Finally, marinobufagenin levels increase in chronic heart failure progressively with the secretion of atrial natriuretic peptide, and this effect correlates with changes in left ventricular function.¹⁰³

Conclusions

Ouabain, digoxin, and marinobufagenin are steroid hormones using Na^+/K^+ -ATPase as a signal transducer. The steroids address different target isoforms and show differing physiologic responses: ouabain acts as a pressure-modulating factor, digoxin decreases blood pressure, and marinobufagenin seems to act as a natriuretic hormone.

References

1. Withering W: An account on the foxglove, and some of its medical uses with practical remark on dropsy and other diseases, in Robinson J (ed): An Account on the Foxglove, and Some of its Medical Uses With Practical Remark on Dropsy and Other Diseases. London, Pater-noster-Row p 1785
2. Schatzmann HJ: Herzglykoside als hemmstoffe für den aktiven kalium-und natriumtransport durch die erythrocytenmembran. *Helv Physiol Pharmacol Acta* 11:346-354, 1953
3. Eisner DA, Smith TW: The Na-K pump and its effectors in cardiac muscle, in Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE, (eds): The Na-K pump and its effectors in cardiac muscle (vol 1, ed 2). New York, Raven Press, 1992, pp 863-902
4. Blaustein MP, Juhaszova M, Golovina VA: The cellular mechanism of

- action of cardiotonic steroids: A new hypothesis. *Clin Exp Hypertens* 20:691-703, 1998
5. Juhaszova M, Blaustein MP: Na^+ pump low and high ouabain affinity α subunit isoforms are differently distributed in cells. *Proc Natl Acad Sci U S A* 94:1800-1805, 1997
 6. Xie Z, Askari A: Na^+/K^+ -ATPase as a signal inducer. *Eur J Biochem* 269:2434-2439, 2002
 7. Scheiner-Bobis G, Schoner W: A fresh facet for ouabain action. *Nat Med* 7:1288-1289, 2001
 8. Dahl LK, Knudsen KD, Heine M, et al: Effects of chronic excess salt ingestion. Genetic influence on the development of salt hypertension in parabiotic rats. Evidence for a circulating factor. *J Exp Med* 126: 687-699, 1967
 9. deWardener HE, Clarkson EM: Concept of natriuretic hormone. *Physiol Rev* 65:658-759, 1985
 10. Blaustein MP: Sodium ions, calcium ions, blood pressure regulation and hypertension. A reassessment and a hypothesis. *Am J Physiol* 232:C167-C173, 1977
 11. Hamlyn JM, Ringel R, Schaeffer J, et al: A circulating inhibitor of $(\text{Na}+\text{K})$ -ATPase associated with essential hypertension. *Nature* 300: 650-652, 1982
 12. Blaustein MP: Physiological effects of endogenous ouabain. Control of intracellular Ca^{2+} stores and cell responsiveness. *Am J Physiol* 264: C1367-C1387, 1993
 13. Goto A, Yamada K: Ouabain-like factor. *Curr Opin Nephrol Hyper-tens* 7:189-196, 1998
 14. Hamlyn JM, Lu Z, Manunta P, et al: Observations on the nature, biosynthesis, secretion and significance of endogenous ouabain. *Clin Exp Hypertens* 20:523-533, 1998
 15. Schoner W: Endogenous cardiac glycosides, a new class of steroid hormones. *Eur J Biochem* 269:2440-2448, 2002
 16. Reichstein T: Cardenolid- und pregnanglykoside. *Die Naturwissen-schaften* 3:53-76, 1967
 17. Höriger N, Zivanov D, Linde HH, et al: Cardenolide hydrogen suberates and other bufadienolide hydrogen suberates in Ch'an Su. *Helv Chim Acta* 53:1993-2002, 1970
 18. Hamlyn JM, Blaustein MP, Bova S, et al: Identification and character-ization of a ouabain-like compound from human plasma. *Proc Natl Acad Sci U S A* 88:6259-6263, 1991
 19. Mathews WR, DuCharme DW, Hamlyn JM, et al: Mass spectral char-acterization of an endogenous digitalis like factor from human plasma. *Hypertension* 17:930-935, 1991
 20. Schneider R, Wray V, Nimitz M, et al: Bovine adrenals contain, in addition to ouabain, a second inhibitor of the sodium pump. *J Biol Chem* 273:784-792, 1998
 21. Kawamura A, Guo J, Itagaki Y, et al: On the structure of endogenous ouabain. *Proc Natl Acad Sci U S A* 96:6654-6659, 1999
 22. Komiyama Y, Nishimura N, Munakata M, et al: Identification of endogenous ouabain in culture supernatant of PC12 cells. *J Hypertens* 19:229-236, 2001
 23. Hamlyn JM, Laredo J, Shah JR, et al: 11-Hydroxylation in the biosyn-thesis of endogenous ouabain: Multiple implications. *Ann N Y Acad Sci* 986:685-693, 2003
 24. Kitano S, Morimoto S, Nishibe A, et al: Exogenous ouabain is accu-mulated in the adrenals and mimics the kinetics of endogenous digi-talis-like factor in rats. *Hypertens Res* 21:47-56, 1998
 25. Greef K, Wirth KE: Pharmacokinetics of strophanthin glycosides. *Handbook Exp Pharmacol* 56/II:57-85, 1981
 26. Boulanger BR, Lilly MP, Hamlyn JM, et al: Ouabain is secreted by the adrenal gland of the awake dogs. *Am J Physiol* 264:E413-E419, 1993
 27. Masugi F, Ogihara T, Hasegawa T, et al: Normalization of high plasma level of ouabain-like immunoreactivity in primary aldosteronism after removal of adenoma. *J Hum Hypertens* 2:17-20, 1988
 28. Li S-Q, Eim C, Kirch U, et al: Bovine adrenals and hypothalamus are a major source of proscillaridin A- and ouabain-immunoreactivities. *Life Sci* 62:1023-1033, 1998
 29. Manunta P, Evans G, Hamilton BP, et al: A new syndrome with elevated plasma ouabain and hypertension secondary to an adrenocortical tumor. *J Hypertens* 10:S27, 1992

30. Komiya Y, Nishimura N, Munakata M, et al: Increases in plasma ouabain like immunoreactivity during surgical extirpation of pheochromocytoma. *Hypertens Res* 22:135-139, 1999
31. Qazzaz HM, El-Masri MA, Valdes RJ: Secretion of a lactone-hydrogenated ouabain-like effector of sodium, potassium-adenosine triphosphatase activity by adrenal cells. *Endocrinology* 141:3200-3209, 2000
32. Perrin A, Brasmes B, Chambaz EM, et al: Bovine adrenocortical cells in culture synthesize an ouabain-like compound. *Mol Cell Endocrinol* 126:7-15, 1997
33. Doris PA, Hayward-Lester A, Bourne D, et al: Ouabain production by cultured adrenal cells. *Endocrinology* 137:533-539, 1996
34. Laredo J, Hamilton BP, Hamlyn JM: Ouabain is secreted by bovine adrenocortical cells. *Endocrinology* 135:794-797, 1994
35. Lichtstein D, Steinitz M, Gati I, et al: Biosynthesis of digitalis-compound in rat adrenal cells: Hydroxycholesterol as a precursor. *Life Sci* 62:2109-2126, 1998
36. Laredo J, Hamilton JP, Hamlyn JM: Secretion of endogenous ouabain from bovine adrenal cells. Role of zona glomerulosa and zona fasciculata. *Biochem Biophys Res Commun* 212:487-493, 1995
37. Laredo J, Shah JR, Lu Z, et al: Angiotensin II stimulates secretion of endogenous ouabain from bovine adrenal cortical cells via angiotensin II receptors. *Hypertension* 29:401-107, 1997
38. Laredo J, Shah JR, Hamilton BP, et al: Alpha-1 adrenergic receptors stimulate secretion of endogenous ouabain from human and bovine adrenocortical cells, in Taniguchi K, Kaya S (eds): *Alpha-1 Adrenergic Receptors Stimulate Secretion of Endogenous Ouabain From Human and Bovine Adrenocortical Cells*. Amsterdam, Elsevier Science, 2000, pp 671-679
39. De Angelis C, Haupt GT Jr: Hypoxia triggers release of an endogenous inhibitor of Na⁺-K⁺-ATPase from midbrain and adrenal. *Am J Physiol* 274:F182-F188, 1998
40. Paci A, Marrone O, Lenzi S, et al: Endogenous digitalis like factors in obstructive sleep apnea. *Hypertens Res* 23:S87-S91, 2000 (suppl)
41. Goto A, Ishiguro T, Yamada K, et al: Isolation of an urinary digitalis-like factor indistinguishable from digoxin. *Biochem Biophys Res Commun* 173:1093-1101, 1990
42. Goto A, Yamada K: Purification of endogenous digitalis-like factors from normal human urine. *Clin Exp Hypertens* 20:551-556, 1998
43. Qazzaz HMAM, Goudy SL, Valdes RJ: Deglycosylated products of endogenous digoxin-like immunoreactive factor in mammalian tissue. *J Biol Chem* 271:8731-8737, 1996
44. Qazzaz HM, Cao Z, Bolanowski DD, et al: De novo biosynthesis and radiolabeling of mammalian digitalis-like factors. *Clin Chem* 50:612-620, 2004
45. Qazzaz HMA, Lane AN, Valdes RJ: Structural identification of digoxin-like factors (DLIFs) using NMR spectroscopy. *Clin Chem* 49:A130, 2003 (suppl 6)
46. Bagrov AY, Fedorova OV, Dmitrieva RI, et al: Characterization of a urinary bufodienolide Na⁺,K⁺-ATPase inhibitor in patients after acute myocardial infarction. *Hypertension* 31:1097-1103, 1998
47. Lichtstein D, Gati I, Samuelov S, et al: Identification of digitalis-like compounds in human cataractous lenses. *Eur J Biochem* 216:261-268, 1993
48. Huang L, Li H, Xie Z: Ouabain-induced hypertrophy in cultured cardiac myocytes is accompanied by changes in expression of several late response genes. *J Mol Cell Cardiol* 29:429-437, 1997
49. Kometiani P, Li J, Gnudi L, et al: Multiple signal transduction pathways link Na⁺/K⁺-ATPase to growth-related genes in cardiac myocytes. The roles of Ras and mitogen-activated protein kinases. *J Biol Chem* 273:15249-15256, 1998
50. Haas M, Askari A, Xie Z: Involvement of Src and epidermal growth factor receptor in the signal transducing function of Na⁺/K⁺-ATPase. *J Biol Chem* 275:27832-27837, 2000
51. Xie Z, Kometiani P, Liu J, et al: Intracellular reactive oxygen species mediate the linkage of Na⁺/K⁺-ATPase to hypertrophy and its marker genes in cardiac myocytes. *J Biol Chem* 274:19323-19328, 1999
52. Mohammadi K, Kometiani P, Xie Z, et al: Role of protein kinase C in the signal pathways that link Na⁺/K⁺-ATPase to ERK1/2. *J Biol Chem* 276:42050-42056, 2001
53. Miyakawa-Naito A, Uhlén P, Lal M, et al: Cell signaling microdomain with Na,K-ATPase and inositol 1,4,5-triphosphate receptor generates calcium oscillations. *J Biol Chem* 318:50355-50361, 2003
54. Aizman O, Uhlén P, Lal M, et al: Ouabain, a steroid hormone that signals with slow calcium oscillations. *Proc Natl Acad Sci U S A* 98:13420-13424, 2001
55. Saunders R, Scheiner-Bobis G: Ouabain stimulates endothelin release and expression in human endothelial cells without inhibiting the sodium pump. *Eur J Biochem* 271:1054-1062, 2004
56. Abramowitz J, Dai C, Hirschi KK, et al: Ouabain- and marinobufagein-induced proliferation of human umbilical vein smooth muscle cells and rat vascular smooth muscle cell line, A7r5. *Circulation* 108:3048-3053, 2003
57. Aydemir-Koksoy A, Allen JC: Low concentrations of ouabain induce vascular smooth muscle cell proliferation. *Cell Mol Biol (Noisy-le-grand)* 47:341-345, 2001
58. Golomb E, Hill MR, Brown RG, et al: Ouabain enhances the mitogenic effect of serum in vascular smooth muscle cells. *Am J Hypertens* 7:69-74, 1994
59. Arnon A, Hamlyn JM, Blaustein MP: Na⁺ entry via store-operated channels modulates Ca²⁺ signaling in arterial myocytes. *Am J Physiol* 278:C163-C173, 2000
60. Godfraind T, Ghysel-Burton J: Independence of the positive inotropic effect of ouabain from the inhibition of the heart Na⁺/K⁺ pump. *Proc Natl Acad Sci U S A* 77:3067-3069, 1980
61. Arnon A, Hamlyn JM, Blaustein MP: Ouabain augments Ca²⁺ transients in arterial smooth muscle without raising cytosolic Na⁺. *Am J Physiol* 279:H679-H691, 2000
62. Manunta P, Ferrandi M: Different effects of marinobufagein and endogenous ouabain. *J Hypertens* 25:257-259, 2004
63. Schoner W, Bauer N, Müller-Ehmsen J, et al: Ouabain as a mammalian hormone. *Ann N Y Acad Sci* 986:678-684, 2003
64. Goto A, Yamada K, Nagoshi H, et al: Stress-induced elevation of ouabain like compound in rat plasma and adrenal. *Hypertension* 26:1173-1176, 1995
65. Sopucleous A, Elmatzoglou I, Souvatzoglou A: Circulating endogenous digitalis-like factor(s) (EDLF) in man is derived from the adrenals and its secretion is ACTH-dependent. *J Endocrinol Invest* 26:668-674, 2003
66. Yamada K, Goto A, Omata M: Adrenocorticotropin-induced hypertension in rats: Role of ouabain-like compound. *Am J Hypertens* 10:403-408, 1997
67. Wang JG, Staessen JA, Messaggio E, et al: Salt, endogenous ouabain and blood pressure interactions in the general population. *J Hypertens* 2003:1475-1481, 2003
68. Manunta P, Stella P, Rivera R, et al: Left ventricular mass, stroke volume and ouabain-like factor in essential hypertension. *Hypertension* 34:450-456, 1999
69. Pierdomenico SD, Bucci A, Manunta P, et al: Endogenous ouabain and hemodynamic and left ventricular geometric patterns in essential hypertension. *Am J Hypertens* 14:44-50, 2001
70. Erdmann E, Schoner W: Ouabain-receptor interactions in Na⁺ + K⁺-ATPase preparations. II. Effect of cations and nucleotides on rate constants and dissociation constants. *Biochim Biophys Acta* 330:302-315, 1973
71. Kajimura S, Hirano T, Moriyama S, et al: Changes in plasma concentrations of immunoreactive ouabain in the talpia in response to changing salinity: Is ouabain a hormone in fish. *Gen Comp Endocrinol* 135:90-99, 2004
72. Gottlieb SS, Rogowski AC, Weinberg M, et al: Elevated concentrations of endogenous ouabain in patients with congestive heart failure. *Circulation* 86:420-425, 1992
73. Manunta P, Messaggio E, Ballabeni C, et al: Plasma ouabain-like factor during acute and chronic changes in sodium balance in essential hypertension. *Hypertension* 38:198-203, 2001
74. Manunta P, Rogowski AC, Hamilton BP, et al: Ouabain-induced hy-

- pertension in the rat: Relationships among plasma and tissue ouabain and blood pressure. *J Hypertens* 12:549-560, 1994
75. Pannani MB, Chen S, Yuan CM, et al: Chronic blood pressure effects of bufalin, a sodium-potassium ATPase inhibitor in rats. *Hypertension* 23:1106-1109, 1994
 76. Veerasingham SJ, Leenen FH: Ouabain- and central sodium-induced hypertension depend on the ventral anteroventral third ventricle region. *Am J Physiol* 276:H63-H70, 1999
 77. Yuan CM, Manunta P, Hamlyn JM, et al: Long-term ouabain administration produces hypertension in rats. *Hypertension* 22:178-187, 1993
 78. Manunta P, Hamilton BP, Hamlyn JM: Structure-activity relationship for hypertensinogenic activity of ouabain. Role of the sugar and lactone ring. *Hypertension* 37:472-477, 2001
 79. Manunta P, Hamilton J, Rogowski AC, et al: Chronic hypertension induced by ouabain but not digoxin in the rat: Antihypertensive effect of digoxin and digitoxin. *Hypertens Res* 23:S77-S85, 2000
 80. Adair CD, Buckalew V, Taylor K, et al: Elevated endoxin-like factor complicating a multifetal second trimester pregnancy: Treatment with digoxin-binding immunoglobulin. *Am J Nephrol* 16:529-531, 1996
 81. Goodlin RC: Antidigoxin antibodies in eclampsia. *N Engl J Med* 318:518-519, 1988
 82. Wang H, Yuan W-Q, Lu ZR: Differential regulation of the sodium pump alpha-subunit isoform gene by ouabain and digoxin in tissues of rats. *Hypertens Res* 23:S55-S60, 2000
 83. Schwinger RHG, Wang JG, Frank K, et al: Reduced sodium pump α_1 , α_3 and β_1 -isoform protein levels and Na/K-ATPase activity but unchanged Na^+ - Ca^{2+} exchanger protein levels in human heart failure. *Circulation* 99:2105-22237, 1999
 84. Shamraj OI, Grupp IL, Grupp G, et al: Characterization of Na/K-ATPase, its isoforms, and the inotropic response to ouabain in isolated failing hearts. *Cardiovasc Res* 72:2229-22237, 1993
 85. Huang BS, Harmsen E, Yu H, et al: Brain ouabain-like activity and the sympathexcitatory and pressor effects of central sodium in rats. *Circ Res* 71:1059-1066, 1992
 86. Takahashi H, Matsuzawa M, Okabayashi H, et al: Evidence for digitalis-like substance in the hypothalamopituitary axis in rats: Implications in the central cardiovascular regulation associated with excess intake of sodium. *Jpn Circ J* 51:1199-1207, 1987
 87. Huang BS, Ganten D, Leenen FHH: Responses to central Na^+ and ouabain are attenuated in transgenic rats deficient in brain angiotensin. *Hypertension* 37:683-686, 2001
 88. Wang H, Huang BS, Leenen FHH: Brain sodium channel and ouabain like compounds mediate central aldosterone-induced hypertension. *Am J Heart Circ Physiol* 285:H2516-H2523, 2003
 89. Wang H, Leenen FHH: Brain sodium channels mediate increases in brain "ouabain" and blood pressure in Dahl S rats. *Hypertension* 40:96-100, 2002
 90. Zhao X, White R, Huang BS, et al: High salt intake and the brain renin-angiotensin system in Dahl-sensitive rats. *J Hypertens* 19:89-98, 2001
 91. Ferrari P, Torielli L, Ferrandi M, et al: PST 2238: A new antihypertensive compound that antagonizes the long term pressor effect of ouabain. *J Pharmacol Exp Ther* 285:83-94, 1998
 92. Quadri L, Bianchi G, Cerri A, et al: 17 β -(3Furyl)-5 β -androstane-3 β -14 β -17a-triol (PST 2238). A very potent antihypertensive agent with a novel mechanism of action. *J Med Chem* 40:1561-1564, 1997
 93. Ferrari P, Ferrandi M, Tripoldi G, et al: PST 2238: A new antihypertensive compound that modulates Na,K-ATPase in genetic hypertension. *J Pharmacol Exp Ther* 288:1074-1083, 1999
 94. Huang BS, Kudlac M, Kumarathasan R, et al: Digoxin prevents ouabain and high salt intake-induced hypertension in rats with sino-aortic denervation. *Hypertension* 34:733-738, 1999
 95. Gheorghide M, Ferguson D: Digoxin: A neurohormone modulator in heart failure? *Circulation* 84:2181-2186, 1991
 96. Paganelli F, Maixent J-M, Gélisse R, et al: Effect of digoxin on chemoreflex in patients with chronic heart failure. *Cell Mol Biol* 47:335-340, 2001
 97. Valdes R Jr: Endogenous digoxin-immunoactive factor in human subjects. *Fed Proc* 44:2800-2805, 1985
 98. Fedorova OV, Bagrov AY: Inhibition of Na/K-ATPase from rat aorta by two endogenous Na/K pump inhibitors, ouabain and marinobufagenin. Evidence of interaction with different α -subunit isoforms. *Am J Hypertens* 10:929-935, 1997
 99. Fedorova OV, Lakatta EG, Bagrov AY: Endogenous Na,K pump ligands are differentially regulated during acute NaCl loading of DAHL rats. *Circulation* 102:3009-3014, 2000
 100. Bagrov AY, Feodorova OV, Dmitrieva RI, et al: Plasma marinobufagenin-like and ouabain-like immunoreactivity during saline volume expansion in anaesthetized dogs. *Cardiovasc Res* 31:296-305, 1996
 101. Fedorova OV, Kolodkin NI, Agalakova NI, et al: Marinobufagenin, an endogenous α -1 sodium pump ligand, in hypertensive Dahl salt-sensitive rats. *Hypertension* 37:462-466, 2001
 102. Bagrov AY, Roukayatkina NI, Fedorova OV, et al: Digitalis-like and vasoconstrictor effects of endogenous digoxin-like factor(s) from the venom of *Bufo marinus* toad. *Eur J Pharmacol* 234:165-172, 1993
 103. Fridman AI, Matveev SA, Agalakova NA, et al: Marinobufagenin, an endogenous ligand of alpha-1 sodium pump, is a marker of congestive heart failure severity. *J Hypertens* 20:1-6, 2002