Endogenous Cardiac Glycosidelike Compounds

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SUMMARY The possibility that endogenous inhibitors of the sodium pump exist and bind to the cardiac glycoside binding site on Na\(^+\),K\(^+\)-adenosine triphosphatase (ATPase) has been a source of much controversy. Although numerous hormones and inorganic ions that modulate Na\(^+\),K\(^+\)-ATPase activity have been described, most of these affect the sodium pump indirectly by varying the intracellular sodium concentration or by increasing the number of enzyme units. None of these endogenous compounds has been shown conclusively to modulate sodium pump activity by binding to the cardiac glycoside binding site on Na\(^+\),K\(^+\)-ATPase. However, the near-universal presence of three high-affinity binding sites on the α-subunit of the enzyme has engendered much speculation that endogenous ligands for these receptors must exist. In addition, none of the hormones known to indirectly affect sodium pump activity in vivo has been shown to modulate Na\(^+\),K\(^+\)-ATPase activity in response to extracellular volume expansion or to play a role in the pathogenesis of hypertension or chronic renal failure, conditions in which a circulating inhibitor of Na\(^+\),K\(^+\)-ATPase has been implicated. This report presents a condensed history of the search for endogenous inhibitors of Na\(^+\),K\(^+\)-ATPase and describes recent advances in the field. Despite progress in identifying and characterizing compounds that could affect Na\(^+\),K\(^+\)-ATPase activity in vivo, definitive proof for the existence of endogenous ligands for the cardiac glycoside binding site remains elusive.

Key Words • cardiac glycoside • digoxinlike factors • natriuretic factors • Na\(^+\),K\(^+\)-ATPase • hypertension

The possibility that endogenous inhibitors of the sodium pump exist and bind to the cardiac glycoside binding site on Na\(^+\),K\(^+\)-adenosine triphosphatase (ATPase) has been a source of much interest. A number of endogenous compounds that modulate the activity of the sodium pump have been identified, including catecholamines, insulin, thyroxine, mineralocorticoids, and other hormones. Most of these compounds affect the sodium pump indirectly by varying the intracellular sodium concentration, the rate-limiting substrate for the pump in vivo, or by increasing the number of pump units in the membrane. Evidence exists, however, that sodium pump activity may be directly affected by several hormones, possibly by altering the affinity of the pump for sodium, although the mechanism by which this occurs is not yet understood. In addition, inorganic ions also may modulate pump function in vivo; for example, vanadate, acting as a pyrophosphate analogue within the cell, can inhibit phosphorylation of several cation-transferring enzymes, including Na\(^+\),K\(^+\)-ATPase. Although all of these compounds may have physiologically important roles in modulating sodium pump activity in vivo, none has been shown to modulate sodium pump function in response to extracellular volume expansion or to play a role in the pathogenesis of some forms of hypertension, two conditions in which a circulating inhibitor of Na\(^+\),K\(^+\)-ATPase has been implicated. In addition, none of the endogenous compounds has been shown conclusively to modulate sodium pump activity by binding to the cardiac glycoside binding site on Na\(^+\),K\(^+\)-ATPase. Nevertheless, the near-universal presence of high-affinity binding sites for these cardiac glycosides on the α-subunit of the enzyme has engendered intense speculation that native ligands for these receptors must exist.

Historically, evidence for the existence of circulating inhibitors of the pump first came from studies suggesting the presence of a natriuretic hormone in the plasma of volume-expanded animals. As about one half of renal sodium reabsorption depends on the sodium pumps in the basolateral membrane of renal tubular cells, it would not be surprising if the Na\(^+\),K\(^+\)-ATPase enzyme proved to be a target for a hormonal system that regulates sodium reabsorption. Although the recent discovery of atrial natriuretic peptides has put...
much of the evidence for the presence of a natriuretic hormone in a new perspective, these peptides do not inhibit \( \text{Na}^+\text{K}^-\text{ATPase} \), and cannot explain data supporting the existence of sodium pump inhibitors during volume expansion. Gionick et al. were the first to demonstrate that a factor was released into the plasma of volume-expanded rats that inhibited \( \text{Na}^+\text{K}^-\text{ATPase} \) in vitro. De Wardener and Clarkson recently reviewed the data supporting a role for circulatory inhibitors of \( \text{Na}^+\text{K}^-\text{ATPase} \) as natriuretic hormones.

Concurrent with the description of a potential role for endogenous sodium pump inhibitors in the response to extracellular volume expansion, evidence has been increasing for the presence of circulating factors that could contribute to the pathogenesis of hypertension. Dahl et al. were the first to suggest the existence of a hormone that might cause vasoconstriction and enhance sodium excretion at the same time. Evidence for the existence of such a vasopressor substance, distinct from known vasopressor hormones, was first supplied by Michelakis and colleagues, who demonstrated that small amounts of plasma from hypertensive, uninephrectomized dogs could increase vascular responsiveness to infused norepinephrine or angiotensin II. Subsequently, Haddy and Overbeck published a review in which they suggested that a sodium pump inhibitor might lead to vasoconstriction by inducing depolarization of the vascular cell membrane. Blaustein later codified the hypothesis linking the existence of a possible vasopressor natriuretic hormone with inhibition of the sodium pump in vascular smooth muscle cells. He postulated that \( \text{Na}^+\text{K}^-\text{ATPase} \) inhibition in vascular smooth muscle would increase the intracellular sodium concentration and that this might lead to an increase in intracellular calcium by sodium-calcium exchange. Evidence consistent with this hypothesis has come from several sources. Haddy and Pamnani showed that \( \text{Na}^+\text{K}^-\text{ATPase} \) activity in vascular smooth muscle is suppressed in rats with volume-expanded forms of hypertension. The \( \text{Na}^+ \) efflux from the leukocytes of patients with essential hypertension was reported to be reduced, and several groups reported a circulating inhibitor of \( \text{Na}^+\text{K}^-\text{ATPase} \) in the plasma of patients with essential hypertension. However, there is disagreement as to whether the sodium-calcium exchange mechanism is physiologically important in vascular smooth muscle of arteriolar resistance vessels.

Evidence also indicates that sodium pump inhibitors can potentiate the effect of other vasopressor agents. This would be expected if these inhibitors acted to increase intracellular calcium concentration in vascular smooth muscle. Plasma from patients with essential hypertension and from dogs and rats with experimental hypertension potentiates the constrictor effects of angiotensin II and norepinephrine on isolated rat arterioles. Moreover, vascular reactivity to angiotensin II and norepinephrine is enhanced in rats with low renin, volume-expanded forms of hypertension. Ultrafiltrates of plasma from volume-expanded dogs have been reported to potentiate the constrictor responses of isolated arterioles to angiotensin II and norepinephrine. Also, Guthrie showed that digoxin, given in therapeutic doses to normal human subjects, increases vascular responsiveness to exogenously administered angiotensin II and norepinephrine. However, infusions of norepinephrine or ouabain into the brachial artery of hypertensive subjects with or without a low plasma renin level resulted in identical increases in forearm vascular resistance, tending to discount the role of an endogenous inhibitor of \( \text{Na}^+\text{K}^-\text{ATPase} \) in the pathogenesis of low renin hypertension.

It is also possible that a digitalislike endogenous sodium pump inhibitor substance acts in the central nervous system to enhance sympathetic nerve activity and thereby indirectly affects peripheral vascular resistance. Infusions of small amounts of ouabain into the cerebral ventricles of rats induces an immediate rise in sympathetic nerve outflow and blood pressure. The sustained rise in blood pressure and arrhythmogenic effects of a toxic dose of intravenously administered digitalis glycoside can be abolished by selective brainstem lesions.

Evidence also exists that the central nervous system either regulates the release, or is the source, of an endogenous \( \text{Na}^+\text{K}^-\text{ATPase} \) inhibitor. Disruption of neuronal pathways in the preoptic, hypothalamic, periventricular area of the anteroverentral third ventricular region (AV3V) of the rat can attenuate or prevent many forms of experimental hypertension, suggesting a role for this anatomical region as a central processing site for sympathetic nervous system control of blood pressure. The AV3V lesions also interrupt pathways involved in salt and water balance, including central angiotensin II- and vasopressin-dependent mechanisms. Also, AV3V-lesioned rats have higher levels of vascular \( \text{Na}^+\text{K}^-\text{ATPase} \) activity after volume expansion than sham-operated rats, consistent with the hypothesis that the levels of circulating pump inhibitor are lower in lesioned animals. Halperin et al. also described the presence of an endogenous ouabainlike substance in human cerebral spinal fluid and demonstrated that acute infusions of saline, but not dextrose, resulted in a rapid rise in cerebrospinal fluid levels of this factor. The existence of this ouabainlike factor in cerebrospinal fluid was confirmed by Lichstein et al.

In addition to this evidence linking the brain with the origin of an endogenous inhibitor of \( \text{Na}^+\text{K}^-\text{ATPase} \), several groups isolated an inhibitor of active sodium transport from hypothalamic tissue or from cultured rat neonatal hypothalamic cells. The hypothalamic factor isolated by Haupert et al. acts from the cytoplasmic side of the membrane and is a reversible inhibitor of \( \text{Na}^+\text{K}^-\text{ATPase} \). It is not a specific inhibitor of this enzyme, however, and it does not support partial reactions of \( \text{Na}^+\text{K}^-\text{ATPase} \) in a manner characteristic of the cardiac glycosides. The active sodium transport inhibitor from cultured hypothalamic cells can enhance vascular reactivity to \( \alpha \)-adrenergic agonists. Neither of these factors has been shown to be released or to increase in concentration in response to any physiological stimulus.
In addition to the evidence that an endogenous inhibitor of Na\(^+\),K\(^+\)-ATPase is present in the plasma of patients with primary hypertension and animals with some forms of experimental hypertension, several groups reported the presence in vertebrate species of an endogenous substance with immunological similarities to the digitalis glycosides. Flier et al.\(^{49}\) were the first to identify an endogenous substance, probably a bufodienolide steroid, in the plasma of toad *Bufo marinus* that cross-reacted with digoxin-specific antibodies and inhibited Na\(^+\),K\(^+\)-ATPase. Gruber and colleagues\(^{50,51}\) demonstrated the presence of a low-molecular-weight substance in the plasma of volume-expanded dogs that reacted with antidigoxin antibodies and that also appeared to be elevated in the plasma of some subhuman primate species with certain forms of experimental hypertension. Kojima\(^{52,53}\) reported that rats with deoxycorticosterone acetate–salt hypertension have increased plasma levels of factors with digoxinlike immunoreactivity, and they demonstrated that the infusion of digoxin-specific antibodies into rats with this form of experimental hypertension produces an immediate decline in blood pressure. Nevertheless, despite a number of studies purporting to have documented immunological cross-reactivity between endogenous Na\(^+\),K\(^+\)-ATPase inhibitors and digitalis glycosides, this issue remains controversial.\(^{54-56}\) Few investigators have used more than one antibody population or rigorously defined the potential artifacts of any given radioimmunoassay technique. Several recent reports reviewed the problems associated with this approach.\(^{57-59}\)

In humans, several studies suggested that plasma levels of an inhibitor of Na\(^+\),K\(^+\)-ATPase are increased in primary hypertension.\(^{27,28}\) It also was reported that sodium pump–dependent cation flux is abnormal in the erythrocytes and leukocytes of hypertensive patients.\(^{56-62}\) A recent study correlated changes in sodium efflux rates in leukocytes with those from resistance vessels obtained from omental biopsies in humans, suggesting that changes in the electrolyte content of nucleated blood cells reflect similar changes in arteriolar resistance vessels.\(^{63}\) Also, the observations of Smith and Welt\(^{64}\) that ouabain-sensitive erythrocyte cation flux is abnormal in patients with chronic renal failure have been extended by two recent reports.\(^{65,66}\) One group\(^{64}\) confirmed that this abnormality could be transferred to normal erythrocytes by incubating them with plasma of uremic, but not normal, subjects. They concluded that uremic patients have abnormally large quantities of a circulating sodium pump inhibitor. This is consistent with reports of false positive results of digoxin radioimmunoassays in the plasma of some patients with chronic renal failure, and a recent finding that plasma levels of endogenous cardiac glycosidelike activity, as defined by digoxinlike immunoreactivity or Na\(^+\),K\(^+\)-ATPase inhibitory activity, are increased in chronic renal failure.\(^{70-71}\)

Unfortunately, these reports have been largely inconclusive because workers have had little success in establishing the detailed characterization of these plasma inhibitors. To date, two different approaches have been used to provide evidence that endogenous factors exist in vivo that are capable of inhibiting Na\(^+\),K\(^+\)-ATPase directly. Some investigators determined that unextracted plasma or urine from hypertensive animals or humans inhibits sodium pump function and cross-reacts with digoxin-specific antibodies to a greater extent than control samples.\(^{26,49-53,60,72-74}\) The identity of these inhibitors remains elusive, however, and plasma levels often do not correlate well with the degree of blood pressure elevation or the extent of disease. A second approach has been to isolate and characterize sodium pump inhibitors from the plasma and tissues of humans and animals\(^{51-54,53-57}\); however, the biological relevance of these inhibitors remains to be proved.

Nevertheless, several groups have made considerable progress in isolating and identifying inhibitors of Na\(^+\),K\(^+\)-ATPase from biological sources. English and Cantley\(^{68,69}\) identified the 68-kDa protein, δ-endotoxin, as a potent inhibitor of Na\(^+\),K\(^+\)-ATPase. These are among the first data suggesting that a protein can directly inhibit Na\(^+\),K\(^+\)-ATPase activity; however, this protein acts from the cytoplasmic side of the cell membrane to inhibit sodium pump activity. Morgan, Mir, and colleagues\(^{72,73}\) demonstrated that their active sodium transport inhibitor derived from hypothalamus is probably a low-molecular-weight peptide.

The steroid nature of the cardiac glycosides and the bufodienolides has led to a search for endogenous mammalian steroid derivatives that might be relatively specific and for high-affinity inhibitors of Na\(^+\),K\(^+\)-ATPase.\(^{90-93}\) No compounds have yet been identified with the capacity both to bind to the cardiac glycoside binding site with high affinity and to induce a positive inotropic effect in heart. Nevertheless, recent work on ouabainlike compounds present in amniotic fluid by Graves et al.\(^ {74,75}\) (S. W. Graves, personal communication, 1987) and on similar factors present in human urine by Cloix et al.\(^ {67,76}\) (J-F. Cloix, personal communication, 1987) suggests that certain ouabainlike compounds may be structurally similar to steroids.

Several groups identified a variety of lipid compounds in extracts of tissue or plasma as being cardiac glycosidelike in that they inhibit Na\(^+\),K\(^+\)-ATPase activity, displace [\(^3\)H]ouabain from its binding site on the enzyme, and even nonspecifically interfere with ligand antibody binding in a digoxin radioimmunoassay. Biddard et al.\(^ {94}\) initially identified free fatty acids as the ouabainlike substances in an extract of the electric organ of the cell. In our laboratory, we isolated and characterized cardiac glycosidelike activity in extracts of plasma from normal humans and determined that most, if not all, of this activity could be attributed to a variety of lysophospholipids and free fatty acids.\(^ {95,96}\) Concurrently, Tamura et al.\(^ {97}\) showed that the increase in ouabainlike activity in plasma of hogs acutely volume-expanded with saline could be attributed to oleic and linoleic acids. Recent work from their laboratory also confirmed that this rise in ouabainlike activity in plasma with volume expansion includes a variety of unusual lysophospholipids as well.\(^ {98}\) In addition, we
recently showed that plasma levels of nonesterified fatty acids increased in Dahl salt-resistant rats in response to dietary salt loading but did not increase in Dahl salt-sensitive rats.\textsuperscript{98} The increase was predominantly due to a change in plasma levels of arachidonate and arachidonate precursors.\textsuperscript{99}

The fact that long-chain fatty acids and other amphipathic compounds, such as acylcarnitine and phospholipids, can modulate sodium pump activity has been known for some time.\textsuperscript{100, 101} However, it is very unlikely that these compounds are binding to the ouabain binding site. The Na\(^+\),K\(^+\)-ATPase, like other membrane-bound cation-transporting enzymes, has an absolute requirement for a specific phospholipid composition of the membrane immediately surrounding the enzyme.\textsuperscript{102-104} Unsaturated long-chain fatty acids might interact with these membrane lipids both to influence the affinity of the enzyme for the substrates and to facilitate or restrict the conformational changes the enzyme must undergo during transport of cations from one side of the lipid bilayer to the other. These lipids may influence membrane fluidity as a whole or selectively affect the lipid domain immediately surrounding the enzyme. High concentrations of certain phospholipids and fatty acids may also result in loss of enzyme function due to detergent effects. This could explain why low concentrations of phosphatidylserine stimulate Na\(^+\),K\(^+\)-ATPase in phospholipase or detergent-treated membranes, while higher concentrations of the phospholipid inhibit the enzyme.\textsuperscript{104}

Regardless of the potential of these compounds to induce large changes in Na\(^+\),K\(^+\)-ATPase activity in vitro, little evidence exists that they play an important role in modulating enzyme activity in vivo, except perhaps in certain diseases characterized by abnormalities in lipid metabolism (e.g., hyperlipidemia, chronic renal failure). Several groups, however, recently implicated arachidonic acid metabolites derived from renal tissue and urine as specific high-affinity inhibitors of Na\(^+\),K\(^+\)-ATPase.\textsuperscript{105-107} Although promising, these results require further confirmation and amplification.

A major objection to the concept of a circulating endogenous cardiac glycoside-like compound has been the fact that such an inhibitor would be nonselective, potentially binding to and inhibiting sodium pumps in a number of tissues indiscriminately. However, the identification of several isoforms of the sodium pump, each with different affinities for ouabain and perhaps with distinct physiological roles in terms of hormonal responsiveness, indicates that there could be selectivity among enzyme units in different tissues for an endogenous ligand for the ouabain binding site.\textsuperscript{5} The recent observation that the affinity of Na\(^+\),K\(^+\)-ATPase enzyme units for ouabain changes along the nephron, with the highest affinity occurring in the medullary collecting duct, lends credence to this supposition.\textsuperscript{109} Regardless, the evidence presented at this symposium indicates that a spirited search continues for a physiologically important inhibitor of sodium pump activity in vivo, an inhibitor that may be the true endogenous ligand for the cardiac glycoside binding site on Na\(^+\),K\(^+\)-ATPase.

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