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Physiology and Pharmacology of Endogenous Digitalis-like Factors

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I. Introduction

In 1775, William Withering of England used extracts from the flowering plant "foxglove" to treat patients suffering from congestive heart failure, then known as dropsy (Skou, 1986). Since then, cardiac glycosides have been among the most frequently used classes of drugs for the treatment of heart failure and certain arrhythmias. However, the clinical use of cardiac glycosides and their cellular mechanisms of action continue to be controversial (Allen et al., 1985; Antman and Smith, 1989; Fozzard and Sheets, 1985; Smith, 1988; Panet et al., 1990; Thomas et al., 1990). There are at least three unresolved, interrelated issues of pharmacological interest: how cardiac glycosides produce cardiotoxicity, whether cardiac glycosides are effective for heart failure in patients with sinus rhythm, whether there are naturally occurring cardiac glycosides in the human body.

It is generally accepted that cardiac glycosides elicit their cardiotonic action through binding to the extracel-

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lularly exposed recognition site on Na^+,K^+-ATPase† (Akera, 1977; Anner, 1985). Cardiac glycosides are specific and unique inhibitors of Na^+,K^+-ATPase at normal concentration (10^{-8} to 10^{-9} M). The presence of a specific recognition site for cardiac glycosides on Na^+,K^+-ATPase raises the question as to whether certain endogenous compounds may interact physiologically with this structure and thus regulate the function of Na^+,K^+-ATPase in all tissues including the heart. This suggestion dates back many years, and as early as 1942 Rein indicated that the liver might elaborate a digitalis-like substance that improved the contraction of a failing heart (LaBella, 1985). Subsequently, Szent-Gyorgyi (1953) suggested that cardiac glycosides are not drugs but a substitute for an endogenous compound that may be a physiological regulator of heart muscle contraction. Since then, there has been speculation that endogenous regulators of Na^+,K^+-ATPase (sodium pump) with cardiac glycoside-like properties might exist in animals. This assumption is analogous to that which led to the identification of the opioid peptides (endorphins and enkephalins) as ligands of the opiate receptor (Hughes et al., 1975).

Much evidence has accumulated supporting the existence of a salt-excreting or natriuretic hormone since the early work by de Wardener et al. (1980, 1981, 1985). Dahl et al. (1969) proposed that salt-induced hypertension in the Dahl salt-sensitive rat must be caused by a low molecular weight humoral factor released in response to salt ingestion, possibly a salt-excreting hormone. Because this hypertensinogenic substance could be transferred via parabiosis, it was assumed to be stable and of long duration. This proposal received support from Overbeck and coworkers (Overbeck et al., 1976; Haddy and Overbeck, 1976; Overbeck, 1985). They found that vascular Na^+,K^+-ATPase was chronically inhibited in hypertensive animals and patients with low plasma renin activity and suggested that the vasoconstriction in these various types of volume-expanded hypertension was caused by a humoral inhibitor of vascular Na^+,K^+-ATPase. Subsequently, the idea was proposed that such an inhibitor of Na^+,K^+-ATPase acting as a natriuretic hormone by inhibiting the sodium pump also may produce human essential hypertension (Blaustein, 1977; de Wardener and MacGregor, 1980).

Recent studies have unified these two concepts and the research into the existence of an EDLF or inhibitor of Na^+,K^+-ATPase has been pursued by many researchers. Different investigators proposed different terms for EDLF, e.g., endigin, endoxin, endodigin, endocardin, endalin, or cardiodigin (Fishman, 1979; Gruber et al., 1980; Schwartz and Adams, 1980; Schreiber et al., 1981; Cloix et al., 1985; De Pover et al., 1982). However, despite a large body of evidence in support of the existence of an endogenous inhibitor of sodium transport, isolation of such an inhibitor has remained inconclusive. Actually, little advance has been made until recently except for the identification of nonspecific Na^+,K^+-ATPase inhibitors. Because atrial natriuretic peptide was isolated, identified, and sequenced so rapidly, several researchers viewed the delay in the characterization of EDLF with some suspicion, and even doubted the existence of EDLF (Wilkins, 1985; Tal et al., 1989). Moreover, Kelly and Smith (1989) proposed that the endogenous ligand for the digitalis receptor does not act as an extracellular hormonal inhibitor of Na^+,K^+-ATPase but may play a role in intracellular trafficking of nascent Na^+,K^+-ATPase to the appropriate plasma membrane domain.

We believe one of the reasons the isolation of true EDLF has been an extraordinarily difficult task is because of the screening system used for EDLF identification. The recognition that assay methodology is susceptible to many nonspecific interferences has accelerated the rate of recent progress. At last, similar substances, which appear to have some of the characteristics required for serious consideration as EDLF, have been independently purified to homogeneity from human plasma, urine, and bovine adrenal glands (Tamura et al., 1988; Goto et al., 1988c; Hamlyn et al., 1989b). Furthermore, Hamlyn et al. (1991) were able to identify EDLF from human plasma as either ouabain or a similar substance.

In the present review, we will focus on the purification of candidates for EDLF and the validity of those candidates. Please refer to other reviews for detailed discussions of the possible roles played by EDLF in the regulation of sodium homeostasis and blood pressure (Blaustein and Hamlyn, 1991; Buckalew and Gruber, 1983; de Wardener and Clarkson, 1985; Goto et al., 1991b; Graves, 1986; Haber and Haupert, 1987; Poston, 1987; Haddy, 1990; Wechter and Benaksas, 1990).

II. Search for an Endogenous Digitalis-like Factor

A. Definition of the Endogenous Digitalis-like Factor

Na^+,K^+-ATPase is the plasma membrane enzyme that catalyzes the active transport of Na^+ and K^+ across the cell membrane. It is found in virtually all cells of all members of the animal kingdom. Most animal cells have a high concentration of K^+ and a low concentration of Na^+ relative to the external medium. Na^+,K^+-ATPase generates and maintains these ionic gradients. Na^+,K^+-ATPase has two types of protein unit, α and β. The α subunit (112 kDa) is commonly referred to as a catalytic subunit and contains the binding sites for cardiac glycosides on its extracellular portion and the binding site for ATP on the intracellularly located portion of the

† Abbreviations: ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; EDLF, endogenous digitalis-like factor; 12(R)-HETE, 12(*R*)-hydroxy-5,8,10,14-icosatetraenoic acid; CMA, chlormadinone acetate; HPLC, high-performance liquid chromatography; ODC, ouabain-displacing compound; HIF, hypothalamic inhibitory factor; OLC, ouabain-like compound; DOCA, desoxycorticosterone acetate.

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protein (fig. 1). The β subunit (35 kDa) is a glycoprotein, but the role it plays is still unclear.

The search for a natural ligand for the cardiac glycoside receptor (EDLF) originated from the fact that a specific digitalis-binding site of high affinity and selectivity has been conserved on Na^+,K^+-ATPase in all animal species in the course of evolution. It is highly probable that the EDLF that binds to the receptor regulates in vivo Na^+,K^+-ATPase activity and plays an essential role in fundamental cellular functions associated with Na^+-K^+ gradients. Therefore, it is an absolute condition that EDLF binds to the same cardiac glycoside-binding site of Na^+,K^+-ATPase. There are no a priori reasons for assuming that the mammalian ligand would be restricted to being an inhibitor. It is possible that the endogenous ligand may act as a stimulator on Na^+,K^+-ATPase. However, this possibility may be remote because many substances increase Na^+ influx into cells and stimulate, secondarily, the sodium pump activity in vivo (Rossier et al., 1987).

The binding characteristics of a cardiac glycoside to its receptor have been extensively studied and were thought to be directly related to the properties of the sodium pump or Na^+,K^+-ATPase per se. Although we cannot exclude the possibility that EDLF may exert distinct pharmacological effects from those of a cardiac glycoside, EDLF likely shares those conditions or characteristics with cardiac glycosides if EDLF binds to the same receptor to which exogenous cardiac glycosides bind. The action of EDLF should be selective for Na^+,K^+-ATPase. Interactions with other membrane transport proteins or other ATPases must be excluded. The binding of EDLF should be enhanced by increased concentrations of intracellular Na^+ and inhibited by increased concentrations of extracellular K^+. In addition, EDLF should show a high affinity for Na^+,K^+-ATPase ($K_d < 10^{-8}$ M). The effect of EDLF should be reversible, and, hence, the dose-response curve for EDLF should be parallel to those for cardiac glycosides.

It is well known that the sensitivity to cardiac glycosides markedly differs among different animal species (Repke et al., 1965). Higher animals are, in general, more susceptible to the actions of cardiac glycosides. Species-dependent differences are observed with all cardiac glycosides and are related to the essential differences in the

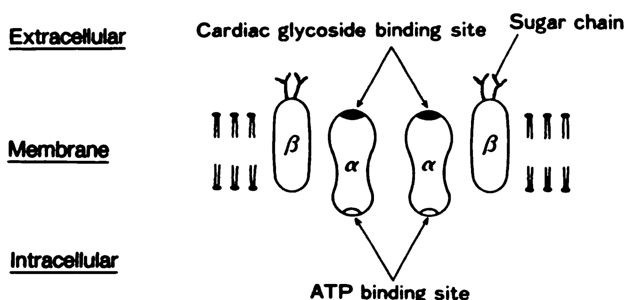

FIG. 1. Supposed structure of Na^+,K^+-ATPase.

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primary sequence of the cardiac glycoside receptor located on the α subunit of Na^+,K^+-ATPase (Akera and Ng, 1991). The changes in amino acids in the membrane-spanning regions H1 to H2 extracellular domain profoundly affect the affinity of the enzyme for cardiac glycosides (From et al., 1990). Similar species differences are also found when bufodienolides are used as ligands (Schoenfeld et al., 1987). Furthermore, Na^+,K^+-ATPase preparations from several species show different sensitivities to cassaine, a digitalis-like substance with a distinct chemical structure, which parallels their different sensitivities to the cardiac glycosides (Tobin et al., 1975). It appears that species differences are found in all digitalis-like compounds that act through a binding to the cardiac glycoside receptor. EDLF also should share similar differences in species sensitivity.

Thus, EDLF should fulfill most of the pharmacological criteria possessed by cardiac glycosides themselves (Godfraind and Fagoo, 1986): (*a*) EDLF should interact with Na^+,K^+-ATPase showing (1) inhibition with reversibility, high affinity, and selectivity; (2) competition with KCl and dependence of this on the Na^+/K^+ ratio; (3) competition with [^{3}H]ouabain binding; and (4) species sensitivity (namely a weak activity in rat heart as compared to guinea pig heart. (*b*) EDLF should inhibit the sodium pump and evoke a positive inotropic effect in isolated heart preparations. However, it is not a necessary condition that EDLF should interact with some of the specific cardiac glycoside antibodies, competing with radioligand in a way similar to the glycosides themselves.

B. Distinction between Endogenous Digitalis-like Factor and Na^+,K^+-ATPase Inhibitors

Because the sodium pump controls many essential cellular functions, such as cell volume, heat production, intracellular pH, free calcium concentration, secretion, neuronal transmission, muscle contractility, and membrane potential, the activity of the sodium pump needs to be finely controlled. Therefore, many regulatory mechanisms contribute to this process (Godfraind and Fagoo, 1986; Rossier et al., 1987). The regulators are made up of local factors and circulating hormones. The activity may be directly modulated by ligands, including monovalent cations, ATP and its analogs, or cardiac glycosides and their putative physiological counterparts (EDLF). In addition, the effects of some regulators of sodium pump activity may be mediated indirectly through cellular mechanisms other than the direct action on the Na^+,K^+-ATPase or the cardiac glycoside receptor of the enzyme. Many hormones and growth factors, such as aldosterone, cortisol, thyroid hormones, insulin, vasopressin, and epidermal growth factor, belong to this category (Bowen and McDonough, 1987). Their actions are mediated by two distinct mechanisms (*a*) induction of Na^+,K^+-ATPase gene expression and (*b*) posttranslational modifications of enzymes from a preexisting pool.

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It is reasonable that Na^+,K^+-ATPase is subject to regulatory influences of a wide variety. Hence, it is possible that putative circulating regulators of the sodium pump, which is thought to be involved in the regulation of sodium balance and blood pressure, might inhibit Na^+,K^+-ATPase, in part or wholely, by binding to another site than that to which cardiac glycosides bind. For example, dopamine inhibits Na^+,K^+-ATPase activity in permeabilized rat renal cells but has no direct effect either on the activity of the purified enzyme from rat kidney or on [^{3}H]ouabain binding to the enzyme (Bertorello and Aperia, 1989). Dopamine is a Na^+,K^+-ATPase inhibitor but does not satisfy the criteria for EDLF (fig. 2). Moreover, a number of agents that inhibit Na^+,K^+-ATPase in a manner different from cardiac glycosides are able to produce positive inotropic effects (Akera, 1977). Such agents include N-ethylmaleimide, para-chloromercuribenzoate, and thallium. This implies that inhibition of Na^+ transport per se, independent of the mechanism of inhibition, is needed for positive inotropy. Thus, Na^+,K^+-ATPase inhibitors that do not act through binding to the cardiac glycoside receptor and are distinct from EDLF, if present, are capable of regulating sodium pump activity in vivo and may play important physiological roles.

C. Detection of Endogenous Digitalis-like Factor

The factors that may influence the activity of the sodium pump can be detected by several different procedures. Mainly, three types of assay methods have been used: (*a*) Na^+-/K^+-dependent ATPase activity, (*b*) Na^+/K^+ transport in intact cells, and (*c*) receptor binding with radiolabeled cardiac glycosides (Sagnella and MacGregor, 1985). Differences in sensitivity to the actions of cardiac glycosides are found among these procedures. The radioreceptor procedure is generally more sensitive to the actions of cardiac glycosides than is the isolated enzyme assay procedure. Although the differences in sensitivity are considerably influenced by the assay conditions (e.g., K^+ concentration, the order of the ligand addition) or the species from which the Na^+,K^+-ATPase preparation is derived, cardiac glycosides inhibit Na^+,K^+-ATPase activity at concentrations higher than those needed for ouabain binding (Wallick et al., 1979). The inhibition of Na^+,K^+-ATPase activity or sodium pump activity does not provide evidence that the inhibition is mediated through the binding to the cardiac glycoside receptor. Thus, an inhibitory action on Na^+,K^+-ATPase or sodium pump is a necessary condition but not a sufficient condition to classify an endogenous substance as acting in a digitalis-like manner. In view of the definition of EDLF, competition for the specific cardiac glycoside-binding site would appear to be the *sine qua non* for EDLF.

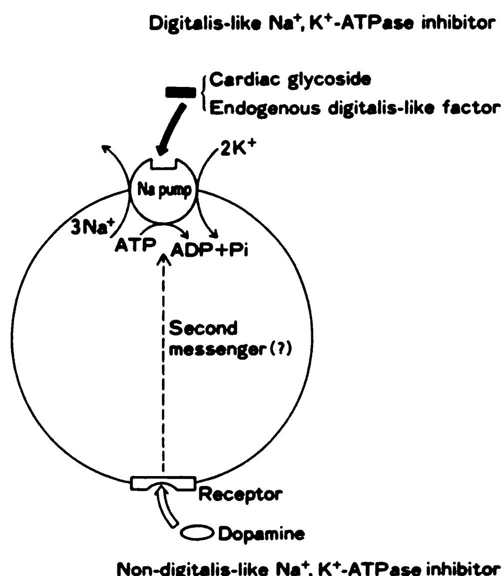

FIG. 2. Distinction between digitalis-like Na^+,K^+-ATPase inhibitor and non-digitalis-like Na^+,K^+-ATPase inhibitor.

Although each different procedure supposedly reflects, at least in part, the activity expected for EDLF, it is possible that each method may detect totally different substances. Clarkson and de Wardener (1985) reported that the crude extract isolated from human urine possessed all the appropriate digitalis-like activities, but with successive purifications, the activities separated from each other and were thus found to be due to different substances. Every assay has its limitations, and most researchers have tried to use multiple assays to eliminate as many false-positive results as possible in their search for EDLF.

Many researchers have chosen to use digoxin-like immunoreactivity to study EDLF on the premise that the digoxin antibody may be able to recognize the endogenous ligand of the cardiac glycoside receptor (Gruber et al., 1980). An antibody to digoxin is usually raised against a conjugate of bovine serum albumin and digoxin, which is prepared by periodate oxidation of the vicinal hydroxyl groups of the terminal sugar; the resulting aldehyde groups are then coupled to the amino groups of albumin (Soldin, 1986). The conjugate linkage is through the carbohydrate moiety of digoxin. The antibodies generated against this conjugate are directed, to a large extent, against the steroid moiety of digoxin. As a consequence, digoxin metabolites such as digoxingenin bis- and monodigitoxiside and digoxingenin itself all react with antibodies, but dihydrodigoxigenin and dihydrodigoxin metabolites, in which C22 is reduced, show little or no cross-reactivity. The specificity of commercially available anti-digoxin antibodies is very high, and other cardiac glycosides show only weak interaction with these antibodies. Most anti-digoxin antibodies do not recognize digitoxin, which differs from digoxin by the presence of a single hydroxyl group at position 12 on the steroid backbone. Hence, anti-digoxin antibodies recognize the adjacent portions of 12-OH in the steroid nucleus (fig. 3).

By contrast, digitalis receptors on the sodium pump

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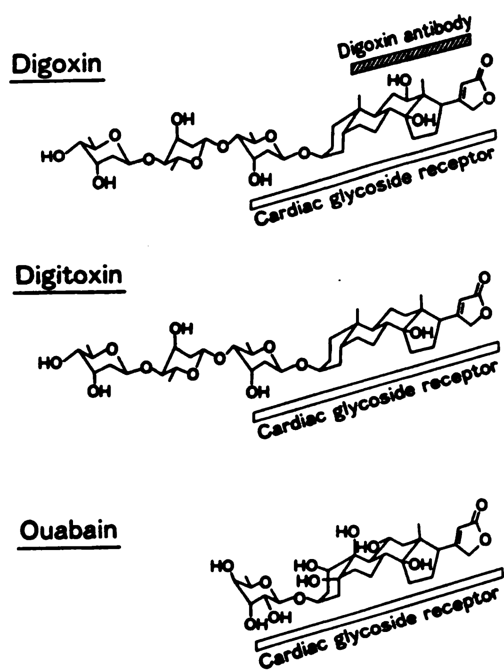

FIG. 3. Difference in the recognition sites of anti-digoxin antibody and cardiac glycoside receptor.

do not differentiate digoxin and digitoxin and bind other cardiac glycosides as well. There is evidence that the binding of cardiac glycosides to Na^+,K^+-ATPase involves at least three sites of attachment, one for the sugar moiety, a second for the steroid backbone of the genin, and a third for the C17 side chain (From et al., 1990). The recognition site for anti-digoxin antibodies is clearly distinct from that for the digitalis receptor on the sodium pump (fig. 3).

Radioimmunoassay of digoxin makes it possible to search for only those endogenous substances that are structurally related to digoxin. However, there is no a priori reason that EDLF in animals must resemble digoxin in structure. Rather, it would be a remarkable coincidence if the endogenous regulators of the sodium pump had a high affinity for digoxin antibodies. Immunoreactive digoxin-like factors have actually been found in body fluids taken from subjects with a variety of physiological and pathological conditions, including uremia, liver disease, and pregnancy, as well as during the neonatal period (Stone and Soldin, 1989). Many studies have also associated these digoxin-like immunoreactive factors with direct inhibition of semipurified Na^+,K^+-ATPase (Graves, 1987). It remains to be demonstrated whether these immunoreactive factors are really active in the regulation of sodium pump activity in cells in vivo.

It has been known for many years that the anti-digoxin antibody cross-reacts to some extent with most steroids. In fact, many steroids, including progesterone, cortisol, and dehydroepiandrosterone sulfate, give false-positive results for digoxin, as do many lipids and bile acids (Goto et al., 1991c). Furthermore, recent observations clearly point out that digoxin-like immunoreactivity may dissociate from digitalis-like biological activity (Clarkson and de Wardener, 1985; Illescas et al., 1990; Yamada et al., 1988, 1990). These findings indicate that digoxin antibodies do not recognize the functionally critical structural component intimately implicated in the binding to Na^+,K^+-ATPase (Hnatowich and LaBella, 1985). Thus, the radioimmunoassay for digoxin is, at most, an unreliable estimate of digitalis-like activity and has misled the search for EDLF. *Therefore, the results solely based on digoxin-like immunoreactivity are not included in this review.*

Estimates of the plasma concentration of EDLF based on digitalis-like biological activity vary greatly from laboratory to laboratory, ranging from 50 pM up to 80 nM (ouabain or digoxin equivalents) or greater (Hamlyn, 1988). Although tissue levels may be different from plasma levels, signs and symptoms suggesting digitalis toxicity should be manifest especially in such digitalis-sensitive species as humans if EDLF at the apparent concentration of >10 nM really acts on the sodium pump in vivo. However, those signs and symptoms have not been described in previous communications (Favre et al., 1987).

It is likely that the assays for EDLF falsely detected the apparent EDLF which does not act as an inhibitor of Na^+,K^+-ATPase in vivo. This fact clearly demonstrates that assay methods for EDLF are considerably susceptible to many types of interferences. In addition, several problems were encountered during various stages of extraction and purification of EDLF. These include a loss of EDLF related to its stability during isolation and an artifactual interference by the products of purification processes. Most investigators have stored starting materials (plasma, urine, hypothalamus, etc.) at −20 or −70°C for several months to accumulate sufficient quantities of them. However, the stability of EDLF has not been systematically examined. The storing or processing could have destroyed EDLF that was present in vivo. On the other hand, processing of source material could have generated several artifacts. Deproteinization of plasma by boiling may lead to the appearance of nonspecific inhibitors of several ATPases including Na^+,K^+-ATPase (Tal et al., 1986). The measurement of EDLF in plasma or tissue is complicated by the possible nonspecific interference resulting from the ionic composition such as K^+, vanadate, or divalent cations (Ca^{2+}, Hg^{2+}, Cu^{2+}, or Zn^{2+}) (Bidard et al., 1984). Ca^{2+} inhibits isolated Na^+,K^+-ATPase activity. K^+ decreases [^{3}H]ouabain binding to Na^+,K^+-ATPase and, similarly, its inotropic effects. Several researchers discussed the problems associated with the early publications concerning the isolation of EDLF from mammalian brain. Kracke (1983) pointed out that the results, in part, were due to ionic interference of the coeluting salt peak. Whitmer et al. (1982) demonstrated that the results were due to cations and phospholipids in the extract.

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These problems demanded serious consideration of the assays for EDLF by the investigators in this field. We (Goto et al., 1988a) found that assays of intact cells must be used to avoid the effects of substances acting as detergents or on the cytoplasmic side of the enzyme. An isolated enzyme preparation is devoid of the buffering action of the membrane associated with intact cells and, thus, is more susceptible to nonreceptor-mediated inhibition (Graves and Williams, 1987). Therefore, inhibition of the isolated enzyme may not provide evidence for inhibition of the sodium pump in intact cells. For example, the relaxant response to K^+ following exposure to zero K^+ buffer is considered a functional measure of the electrogenic pumping of Na^+ and K^+ and can be attenuated by ouabain (Webb and Bohr, 1978). An acid-acetone extract of guinea pig brain inhibited semipurified canine kidney Na^+,K^+-ATPase activity in vitro. Unlike ouabain, however, it did not prevent arterial muscles in K^+-free solutions from relaxing when K^+ was readded (James-Kracke et al., 1981).

It was further recognized that a bioassay for EDLF based on intact cells is superior to biochemical methods using isolated enzymes in terms of selectivity and specificity (Hamlyn et al., 1989b; Wenzel et al., 1990). However, even test methods using intact cells, which are still subject to multiple conditions, are not absolutely reliable and can be influenced by a host of compounds. Lipids, including nonesterified fatty acids, long-chain acylcarnitine, and phospholipids, which are unlikely to be the natural ligand of the digitalis receptor, are apparently capable of inhibiting leukocyte ouabain-sensitive $^{22}Na^+$ efflux rate constant, ^{86}Rb uptake in rat aorta, and ^{86}Rb uptake into human erythrocytes (Ng and Hockaday, 1986; Vasdev et al., 1989).

The sodium pump appears to be common to most mammalian cells, but the activity of the pump varies greatly from cell to cell, being greatest in the excitable cells of the nervous system and slowest in the erythrocytes. Furthermore, recent studies indicate the existence of Na^+,K^+-ATPase isoenzymes and the tissue-specific expression of those isoenzymes (Shull et al., 1986). In view of these findings, it is uncertain whether human blood cells can be used as a model for the sodium pump in cardiovascular cells. It has been shown that sodium pump regulation in human erythrocytes, in its response to hypokalemia, is different from that of heart muscle cells because of the lack of pump reserve in erythrocytes (Brown et al., 1986). Several researchers who sought to define the possible role of EDLF as a vasoactive and natriuretic substance have attempted to use aortic smooth muscle cells or renal tubular cells as the target cells for EDLF (Margargal and Overbeck, 1986; Goto et al., 1990a).

In many studies, putative EDLF was assayed under conditions in which the concentration of K^+ was higher than 10 mM. Cardiac glycosides apparently compete with K^+ at the extracellular binding site. Such a high K^+ concentration may mask the presence of EDLF. Masugi et al. (1987) attempted to discriminate ouabain-like Na^+,K^+-ATPase inhibitors from non-ouabain-like inhibitors by decreasing the K^+ concentration to 0.1 mM in the assay buffer. Furthermore, the competition of EDLF with K^+, i.e., a time-dependent binding in a manner sensitive to extracellular K^+, was used as evidence of a digitalis-like mechanism of action (Hamlyn et al., 1989b).

The growth of cultured cells partly depends on the Na^+-K^+ gradient generated by the sodium pump. Thus, cardiac glycosides inhibit the growth of cells derived from such digitalis-sensitive species as human. Gupta et al. (1982) developed mutants of HeLa cells that exhibited increased resistance to cardiac glycosides. These cells showed resistance to all of the compounds known to elicit digitalis-like properties. In contrast, they showed no cross-resistance to any of a large number of other compounds, including other types of Na^+,K^+-ATPase inhibitors such as ethacrynic acid, oligomycin, and sanguinarine, whose site of action is different from that of cardiac glycosides. Gupta et al. (1989) indicated that this highly specific cross-resistance pattern may be useful for the discrimination of EDLF from other types of Na^+,K^+-ATPase inhibitors.

The phosphorylation of the Na^+,K^+-ATPase from inorganic phosphorus is promoted by cardiac glycosides in the presence of Mg^{2+}. Repke et al. (1991) proposed that the promotion of ^{32}P incorporation from inorganic phosphorus may be the most suitable criterion for classifying a compound as digitalis like. Indeed, this effect is not produced by different types of Na^+,K^+-ATPase inhibitors such as Cibacron Blue F3GA. The compounds that inhibit Na^+,K^+-ATPase activity through a non-digitalis-like mechanism do not promote, or even suppress, enzyme phosphorylation. Those compounds that inhibit Na^+,K^+-ATPase through a digitalis-like mechanism promote enzyme phosphorylation. Thus, a positive or negative response in the phosphorylation promotion test may be able to strictly discriminate EDLF from other Na^+,K^+-ATPase inhibitors (Schoenfeld et al., 1987; Repke et al., 1991).

III. Candidates for Endogenous Digitalis-like Factor

A. Lipids

Bidard et al. (1984) identified a mixture of unsaturated fatty acids from a methanol extract of the electric organ of *Electrophrus electricus* as candidates for EDLF (Bidard et al., 1984). Linoleic, arachidonic, linolenic, and docosahexaenoic acids contributed to 60% of the total activity. It is noteworthy that saturated fatty acids were inactive. Tamura et al. (1985, 1987a) also identified linoleic acid, oleic acid, and γ-arachidoyl-, β-arachidoyl-, γ-linoleoyl-, and γ-oleoyl-lysophosphatidylcholines in the Na^+,K^+-ATPase inhibitors from the acetone/meth-

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anol (1:1) extract of acutely saline-infused hog plasma. These lipids increased 10-fold when pigs were infused with saline. Kelly et al. (1985, 1986) also showed that a large proportion of "digitalis-like activity" of boiled human plasma can be attributed to oleic and linoleic acids and phospholipids. Two Na^+,K^+-ATPase inhibitors were isolated from alkaline solvent extract of human plasma ultrafiltrate. Both substances with digoxin-like immunoreactivity showed ultraviolet absorption peaks at 190 and 220 nm (Dasgupta et al., 1987). One of these was found to be derived from a lysophosphatidyl serine containing a novel 19:4 fatty acid side chain (Dasgupta et al., 1988). In fact, a chemically synthesized lysophosphatidylserine with a 19:4 acyl group showed significant Na^+,K^+-ATPase inhibitory activity with an IC_{50} of 10^{-5} M (Inami et al., 1990).

More recently, Tal and associates (1989) identified Na^+,K^+-ATPase inhibitors in methanol extract of lyophilized bovine plasma as a mixture of three unesterified fatty acids, mainly oleic acid (72% of total) and three saturated hydrocarbons. Lichtstein et al. (1991) identified 11,13-dihydroxy-14-octadecanoic acid from a methanol extract of lyophilized bovine plasma as one of the EDLFs (Lichtstein et al., 1991). This substance, being a hydroxylic acid compound, was unstable and lost one or more water molecules and the large part of its biological activity. This compound manifested the two major effects of digitalis, e.g., inhibition of [^{3}H]ouabain binding and Na^+,K^+-ATPase activity, but it is uncertain whether the identified compound affects Na^+,K^+-ATPase by means of the same molecular mechanism as that of the cardiac glycosides. It also has been suggested that the Na^+,K^+-ATPase inhibitor in plasma from hypertensives may be unstable peroxides (Masugi et al., 1988). These studies, in which a semipurified enzyme preparation was used, were mainly based on the direct inhibition of Na^+,K^+-ATPase activity and ouabain binding. The inhibition of Na^+,K^+-ATPase activity produced by these lipids, unlike the cardiac glycosides, was always more sensitive than that of both ouabain binding and ^{86}Rb uptake activities.

In the study in which native erythrocytes were used, hemolysis was usually observed at higher concentrations of lipids. Unsaturated fatty acids have long been known to be inhibitors of Na^+,K^+-ATPase, at least in vitro (Swann, 1984), but the concentration-effect curves of the inhibition of Na^+,K^+-ATPase and in the displacement of [^{3}H]ouabain are steeper than those produced by the cardiac glycosides, indicating either membrane perturbation or a nonspecific effect on the incubation components (substrates or protein). Prolonged incubation with unsaturated fatty acids may lead to irreversible inactivation of the enzyme (Swarts et al., 1990). It is uncertain whether they inhibit the Na^+,K^+-ATPase in vivo, i.e., in the presence of plasma proteins. It has been shown that the presence of protein (albumin) virtually eliminated the inhibition of Na^+,K^+-ATPase by nonesterified fatty acids and lysophosphatidylcholines (Lau and Valdes, 1988). Linoleic, oleic, and arachidonic acids, at micromolar concentrations, nonselectively inhibited ouabain binding, opiate binding, and binding to the β-adrenergic receptor (Lichtstein et al., 1987). Taken together, these findings suggest that the effects of these lipids on Na^+,K^+-ATPase have limited affinity, are not specific for the enzyme, and are not reversible. It is unlikely that the nonesterified fatty acids and phospholipids are the long-sought physiological regulators of sodium transport.

There are, however, two indirect lines of evidence arguing for a physiological or pathophysiological role of the lipids in the regulation of Na^+,K^+-ATPase. First, Goodfriend et al. (1991) found that the levels of unesterified fatty acids in plasma increased considerably after a saline infusion as well as during a high salt diet in humans. Several reports indicate that the increase in levels of plasma fatty acids partially accounts for the increase in digitalis-like activity that follows saline challenge (Tamura et al., 1985; Buckalew et al., 1987; Lichtstein et al., 1987). It is possible that the increase in fatty acids would favor sodium excretion by inhibiting renal Na^+,K^+-ATPase. This would constitute one of the feedback loops contributing to salt balance. It has also been shown that lysophosphatidylcholine was increased by acute volume expansion and caused an increase in urinary sodium excretion (Buckalew et al., 1987; Rauch and Buckalew, 1988a).

Second, Vasdev et al. (1989) found a positive correlation between plasma Na^+,K^+-ATPase inhibitory activity and blood pressure in normotensive and hypertensive subjects. This is in accord with previous results from other laboratories (Hamlyn et al., 1982; Moreth et al., 1986). They also pointed out that the increased Na^+,K^+-ATPase inhibitory activity in plasma obtained from hypertensives was due to the lipids, including nonesterified fatty acids. It is noteworthy that the purification attempt of Hamlyn et al. (1987) based on the same biochemical method also led to the identification of lysophosphatidylcholine as an inhibitor of Na^+,K^+-ATPase from the plasma of volume-expanded individuals.

Schwarzman and coworkers (1985) isolated cells derived from the thick ascending limb of the loop of Henle of the outer medulla of rabbit kidney and identified a cytochrome P_{450}-dependent metabolite of arachidonic acid that potently inhibits cardiac Na^+,K^+-ATPase. The same authors incubated corneal microsomes with arachidonic acid and isolated the arachidonic acid derivative 12(R)-HETE (Schwartzman et al., 1987) as an inhibitor of the Na^+,K^+-ATPase having an IC_{50} of 50 nM. 12(R)-HETE also inhibited Na^+,K^+-ATPase from kidney and heart in a dose-dependent manner and at lower doses than 12(S)-HETE and arachidonic acid (Masferrer et al., 1990). Furthermore, the inhibition of Na^+,K^+-ATPase by 12(R)-HETE followed a mass action relationship, indicating a ligand receptor interaction. The relaxant

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response to K^+, following exposure to zero K^+ buffer, is caused by the stimulation of the sodium pump and can be attenuated by ouabain. 12(R)-HETE inhibited K^+-induced relaxations in a concentration-dependent manner (Masferrer and Mullane, 1988). It appears that 12(R)-HETE may act as a sodium pump inhibitor on intact vascular preparations. It remains to be determined whether the action of 12(R)-HETE mediates the binding to the cardiac glycoside receptor but not to another, distinct receptor.

B. Steroids

1. Modification of natural or synthetic steroids. In view of the steroid nature of the cardiac glycosides, it appears reasonable to expect that EDLF in animals may also be a steroid. The structural features that are generally considered to be important for the biological actions of the cardiac glycosides are a cyclopentaphenanthrene nucleus with AB *cis*, BC *trans*, and CD *cis*, a C-14 hydroxyl group, and an unsaturated lactone ring in the β configuration on C-17. However, this does not mean that EDLF has to share these structures. Although the erythrophleum alkaloids (cassaine) do not possess any of the above structural features, they exhibit biochemical and pharmacological effects similar to those of cardiac glycosides. The steroid moiety of the common hormonal steroids differ from that of the cardiac glycosides in the configuration of the AB and CD ring junctions; both junctions are *cis* in the cardiac glycosides and *trans* in common steroids. Testing various steroids may offer the potential structural clues to the chemical nature of EDLF. Along this line, LaBella et al. (1985, 1989) have been pursuing EDLF for many years. They screened a large number of steroids and found CMA, a derivative of progesterone, to be competent, in a ouabain-binding assay, of having about one-twentieth the potency of ouabain. CMA interacts at the digitalis receptor on Na^+,K^+-ATPase and inhibits the enzyme in the same rank order of species sensitivity as do cardiac glycosides. However, CMA exerts primarily cardiodepressant effects.

Incorporation of the 14-β-hydroxy into progesterone, which changes the CD junction to the *cis* configuration, enhances the receptor binding activity 10-fold and converts a cardiodepressive compound into a cardiostimulatory one (Bose et al., 1988). The cardiotoxicity produced by high concentrations of the 14-β-hydroxy-progesterone was indistinguishable from that evoked by the cardiac glycosides. Moreover, a glucoside of 14-β-hydroxy-progesterone was about 10 times more potent than the aglycone in a receptor-binding assay (Templeton et al., 1988). The glucoside enhanced the contractility of isolated cardiac muscle and, when infused into the renal artery of rats, markedly increased sodium excretion but not potassium excretion. On the other hand, ouabain induced kaliuresis but had no significant effect on sodium excretion at the same concentration.

Repke and co-workers (1991) reported from studies of structure-activity relationships that the animal structural requirement in cardiac glycosides for specific receptor recognition is the steroid nucleus present in cardiac glycosides, i.e., 5,14-androstane-3,14-diol. The prefix 5 β and 14 β indicates the AB *cis* and CD *cis* ring junctions, respectively. In other words, this structure means a cyclopentaphenanthrene nucleus with AB *cis*, BC *trans*, and CD *cis* as mentioned before. Of course, the individual activity of this lead structure is weak compared with that of cardiac glycosides proper. If any of these features are changed, most or all of the biological activity is lost. This lead (minimum) structure in cardiac glycosides is devoid of the unsaturated lactone ring, but it shows the same characteristics of interaction with Na^+,K^+-ATPase as do ordinary cardiac glycosides, including enhancement of phosphorylation from orthophosphate (Schoenfeld et al., 1987). Furthermore, the absence of the lactone function at C-17 β, the action of the sugar function at C-3 β, and the absence of the hydroxyl function at C-14 β do not eliminate the capacity to promote enzyme phosphorylation but only change the concentration required to reveal it.

All of the characteristic features of the steroid nucleus that have been thought to be important in the action of cardiac glycosides and are typically absent in the hormonal CD-*trans* steroids are not required for the competent occupancy of the digitalis recognition cleft. Moreover, the CD *cis* junction, occurring only in cardiotonic steroids of vegetable origin, is not an indispensable requirement for positive inotropic action. Glycosidation of the progesterone derivative, chlormadinol acetate, which contains a CD *trans* junction, evoked positive inotropic effects in vivo in cats (Weiland et al., 1987). Thus, animal hormone steroids with a CD *trans* junction may be able to elicit digitalis-like actions through conjugation with glucuronic or sulfuric acids. Indeed, other progesterone derivatives with a CD *trans* junction produced the inhibitory effects by the same molecular mechanism of interaction with Na^+,K^+-ATPase as is characteristic of cardiac glycosides (Weiland et al., 1991). It can be simply stated that the cyclopentanoperhydrophenanthrene nucleus is the common pharmacophoric lead structure (Repke et al., 1991). The erythrophlem alkaloid, cassaine, seems to satisfy none of the structural requirements usually associated with properties of cardiac glycosides. The perhydrophenanthrene nucleus may act as the pharmacophoric lead structure in this compound. Repke et al. (1991) suggested that the common CD *trans* steroid derivatives produced in animal metabolic pathways may provide the basic skeleton for an endogenous digitalis biosynthesis.

2. Bufodienolides. Cardiotonic steroids (bufodienolides) structurally related to the cardiac glycosides have long been known to exist in the poison glands of toads (Flier et al., 1980). High concentrations of digitalis-like

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activity have also been shown in the toad skin and plasma. Shimoni et al. (1984) purified a compound from methanol extract of toad skin that binds to the ouabain receptor, inhibits Na^+,K^+-ATPase activity, and increases the force of contraction of heart. The compound was identified as the steroidal dienolides derivative resibufogenin (Lichtstein et al., 1986). The substance was also present in toad plasma. Bufalin, a commercially available bufodienolide almost structurally identical with resibufogenin, blocked KCl vasodilation, potentiated norepinephrine vasoconstriction, and increased blood pressure in the dog, indicating that bufalin has some of the physiological properties required to be a candidate for the EDLF found in low renin hypertension (Eliades et al., 1989). The vasopressor effect of bufalin resulted from its direct vasoconstrictor effect as well as through an action on the heart.

Novel polyhydroxylated steroids, analogs of the known bufodienolides, were isolated from acetonitrile (0.5%) extract of the nuchodorsal glands of a snake. These steroids inhibited canine kidney Na^+,K^+-ATPase activity and produced positive inotropic actions in the guinea pig heart (Akizawa et al., 1985; Azuma et al., 1985).

3. Cardenolides. Tamura and coworkers (1987b, 1988) found an Na^+,K^+-ATPase inhibitor in the methanol extract of bovine adrenal glands, and activity was demonstrated mainly based on the inhibition of Rb uptake into human erythrocytes. This compound showed most properties required for EDLF: inhibitory activity of Na^+,K^+-ATPase, competitive displacing activity against [^{3}H]ouabain binding to the enzyme, and inhibitory activity for ^{86}Rb uptake into human erythrocytes. This water-soluble factor was purified to homogeneity by using several steps of HPLC. Molecular mass was estimated as 336 Da, and the activity was destroyed by both the acid treatment with 6 N HCl at 115°C for 21 hours and alkaline treatment with 0.2 N NaOH at 23°C for 2 hours. Digestion with various peptidases did not destroy the activity, and the substance had no effect on the Ca^{2+}-ATPase activity derived from skeletal muscle sarcoplasmic reticulum. The inhibitory potencies of the factor were diminished by increasing K^+ concentrations.

Goto et al. (1988c,d, 1989), using several steps of HPLC, isolated two ODCs from human urine on the basis of the inhibition of [^{3}H]ouabain binding to intact human erythrocytes. The first processing was adsorption onto an Amberlite XAD-2 resin. The polar ODC-1 and the less polar ODC-2 eluted from the C_{18} reverse phase HPLC column at 18 and 31% acetonitrile in water, respectively. The factor corresponding to ODC-1 also was consistently found in plasma from many mammals (Goto et al., 1988b). These ODCs also directly inhibited canine kidney Na^+,K^+-ATPase activity and ^{86}Rb uptake into human red blood cells. The dose-response curves were parallel to those of ouabain. These effects were dependent on extracellular K^+ concentration and were reversible. High-affinity anti-digoxin antibodies were capable of neutralizing the inhibitory activity of these ODCs on [^{3}H]ouabain binding to human erythrocytes, although the amount of antibodies required differed significantly between the two ODCs (Goto et al., 1991a). The actions of ODCs were sensitive to acid and base hydrolysis and were resistant to proteases. The effects were selective because they did not affect Ca^{2+}-ATPase activity derived from human erythrocytes. The molecular mass of ODC-1 appeared to be 342 Da.

Hamlyn et al. (1989a,b) purified an EDLF to homogeneity from plasma of mildly volume-expanded patients using a bioassay (Rb uptake into human red blood cells). The purification scheme involved large-scale dialysis, extraction of lyophilizate by methanol, followed by several steps of reverse phase HPLC. The purified factor inhibited the ouabain-sensitive ^{86}Rb uptake into human erythrocytes, the binding of [^{3}H]ouabain, and the activity of canine kidney Na^+,K^+-ATPase with high affinity (<0.3 nM). The molecular mass appeared to be between 300 and 900 Da. EDLF, like ouabain, supported phosphorylation of the Na^+,K^+-ATPase. EDLF was found to be a selective inhibitor of the sodium pump and did not inhibit sarcoplasmic reticulum Ca^{2+}-ATPase. EDLF was inactivated by acid or alkaline hydrolysis. It appeared to be insensitive to proteolytic enzymes. Human red blood cells were approximately 5000 times more sensitive to EDLF than were rat blood cells. Hamlyn et al. (1991) claimed that the adrenal is the probable source for plasma EDLF and the secretion of EDLF from adrenal cells is influenced by maneuvers thought to change intracellular calcium.

Thus, low molecular weight (<1000) nonpeptidic compounds have been identified from human plasma, human urine, and bovine adrenals based on digitalis-like biological activities in intact cells, as opposed to the biochemical techniques favored previously. These polar compounds, which share general chromatographic characteristics and are eluted at about 18 to 20% acetonitrile from a C_{18} reverse phase column, are sensitive to acid and base hydrolysis and apparently have no net charge. The sensitivity to acid and base hydrolysis is also observed in cardiac glycosides and is considered to be mainly caused by the opening of the lactone ring. It appears that these substances obtained from three sources fulfill the criteria expected for EDLF.

Schoner et al. (Schoner, 1991; Wenzel et al., 1990) found that patients with chronic renal failure had 12-fold higher serum concentrations of EDLF than did controls based on an inhibition of [^{3}H]ouabain binding to the isolated Na^+,K^+-ATPase. During hemodialysis, the serum EDLF decreased to normal values. Hemofiltrate from these patients was first passed through an Amberlite XAD-2 column, and EDLF was eluted with methanol. It was confirmed that 4000 liters of hemofiltrate contained partially purified EDLF which eluted at

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acetonitrile concentrations close to those mentioned above (Wenzel et al., 1990). An inhibitory activity of ^{86}Rb uptake into intact human erythrocytes with a similar chromatographic profile was partially purified also from the methanol extract of neonatal cord blood by reverse phase HPLC (Balzan et al., 1991). Fab fragments of anti-digoxin antibody neutralized the inhibitory activity of partially purified EDLF obtained from cord blood in the ^{86}Rb uptake assay (Montali et al., 1991)

4. Glycosteroids. Cloix and co-workers (Crabos et al., 1984; Cloix et al., 1985; Cloix, 1990) purified an apparently homogeneous compound from 3500 liters of urine obtained from 35 donors producing the highest amount of EDLF. They chose to purify the peak having all the digitalis-like properties, i.e., inhibition of Na^+,K^+-ATPase, inhibition of ouabain binding, inhibition of active sodium transport, and cross-reaction with anti-digoxin antibodies. Digoxin-like immunoreactivity, however, need not be the essential requirement for EDLF as discussed above. The first processing was low-pressure flash chromatography using octadecyl packing. The isolated digitalis-like compound, which was termed endalin, was less hydrophilic than those EDLFs from human plasma or urine or from bovine adrenals and was eluted from a C_{18} reverse phase HPLC column at 35% acetonitrile (Cloix et al., 1987). This compound was analyzed by nuclear magnetic resonance and mass spectrometry and was found to be a glucurosteroid with a molecular mass of approximately 500 Da. The biological properties of endalin were similar to those of ouabain. The main difference between ouabain and endalin was that endalin did not induce kaliuresis in a rat bioassay. A similar compound, which behaved like endalin, was partially purified from hemofiltrate in another laboratory (Kuske et al., 1987). It was reported (Wechter and Benaksas, 1990), however, that Cloix and co-workers were unable to reproduce this isolation in their subsequent attempts.

5. Adrenal or gonadal steroids. It was claimed that common steroids such as dehydroepiandrosterone sulfate, progesterone, and cortisol might account for apparent digitalis-like activity in plasma obtained from healthy adults or pregnant women (Vasdev et al., 1985; Longerich et al., 1988). These steroids inhibited ouabain binding to Na^+,K^+-ATPase and showed digoxin-like immunoreactivity, but these effects were found only at very high concentrations. Hence, they are unlikely to play roles as specific regulators of Na^+,K^+-ATPase (Lau and Valdes, 1988). This is also the case with bile salts (Vasdev et al., 1986).

6. Canrenone. Canrenone is the metabolic product of the synthetic steroids, spironolactone and K^+-canrenoate, and has been used as a diuretic or an antihypertensive agent based on its antimineralocorticoid action. Canrenone has been reported to act as a partial agonist at the cardiac glycoside-binding site (Finotti and Palatini, 1981). It was proposed that the antihypertensive and other pharmacological effects of canrenone might be related, in part, to its antagonistic effect on EDLF. Chronic canrenone administration in rats with reduced renal mass-saline hypertension resulted in a marked antihypertensive effect associated with a decrease in EDLF and a partial recovery of sodium pump activity (De Mendonca et al., 1988). Moreover, three derivatives of canrenone (6 α, 7 α; 6 β, 7 α; and 6 β, 7 β-dihydroxy-6,7-dihydrocanrenone) have been demonstrated to occur in human and animal urine, and these compounds caused changes in renal sodium excretion in rats (Genard et al., 1986). Canrenone and all of the 6,7-substituted derivatives partially inhibited pig kidney Na^+,K^+-ATPase activity. However, it appears that neither canrenone nor the 6,7 derivatives bind to the cardiac glycoside site but, rather, interact with it "allosterically" (Tal and Karlish, 1988).

C. Peptides

Gruber et al. (1980) reported the first isolation of two substances in diafiltrate of boiled dog plasma that compete with digoxin for specific digoxin antibodies and are inhibitors of Na^+,K^+-ATPase activity. Increased amounts of these factors were found in the plasma of volume-expanded dogs, suggesting that they may function as natriuretic factors. These authors suggested that the factors may be small peptides formed from precursor molecules as they eluted in fluorescencecamine-positive peaks and were sensitive to acid hydrolysis (Gruber and Buckalew, 1978).

Akagawa et al. (1984) partially purified an inhibitor of Na^+,K^+-ATPase and ouabain binding from acidified acetone extract of bovine hypothalamus. It was eluted in low molecular weight fractions by gel filtration and acted as both anion and cation at different pH values. The inhibitory activity was heat stable but was completely inactivated by charring. Thus, the inhibitory activity was not caused by inorganic metal ions. It was highly likely that the inhibitor was a small peptide because carboxypeptidase A considerably diminished the potency of the inhibitor. A seemingly similar peptidic inhibitor was found in rat cerebral cortex (Antonelli de Gomez de Lima and Rodriguez de Lores, 1988).

Kramer and coworkers (Kramer et al., 1985, 1986; Klingmuller et al., 1982) first presented evidence for an endogenous inhibitor of Na^+,K^+-ATPase which was natriuretic in a bioassay and inhibited sodium transport of isolated amphibian membrane. The activity eluted in post-salt fraction SIV when serum or urine was first subjected to gel chromatography on Sephadex G-25. The molecular weight of the inhibitor was <1000. The Na^+,K^+-ATPase inhibitory activity was found to approximately parallel the natriuretic activity at each stage of purification including anion exchange and reverse phase HPLC. This fraction also inhibited [^3H] ouabain binding to Na^+,K^+-ATPase. However, it is unlikely that the two

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biological properties, i.e., the ouabain-like and natriuretic activities, reside in a single compound (Kramer et al., 1991). The natriuretic activity was associated with a single fluorescence peak when treated with O-phthaldialdehyde and was completely destroyed by incubation with chymotrypsin. Thus, the natriuretic activity was considered to be due to the presence of small peptides (Klingmuller et al., 1982; Kramer et al., 1985, 1986). Favre et al. (Martin and Favre, 1984; Favre et al., 1987; Siegenthaler et al., 1987) confirmed, using a similar separation method, the presence of a natriuretic activity and a competitive inhibitor of ouabain binding to Na^+,K^+-ATPase in the post-salt fraction of salt-loaded human urine.

Halperin et al. (1988) showed that methanol (50%) extract of human cerebrospinal fluid contains a specific inhibitor of Na^+,K^+-ATPase with a molecular weight of approximately 600. This inhibitor increased during acute expansion of the extracellular fluid volume with a saline solution (Halperin et al., 1985). It is heat stable but sensitive to proteolytic digestion, suggesting that it is a small peptide, conceivably a neuropeptide.

A small peptide with a molecular weight of approximately 1000 has been partially purified as an active sodium transport inhibitor from cultured rat hypothalamic cells (Morgan et al., 1985). Gel filtration (Sephadex G-25) of heat-treated cells resulted in elution of inhibitor in the post-salt fraction. The factor increased tension, potentiated vasoconstrictor effects of noradrenaline, and caused calcium retention in rabbit aortic strips (Mir et al., 1988). Incubation of the factor with prolidase, chymotrypsin, or carboxypeptidase A destroyed its vasoconstrictor effects as well as its inhibitory effects on Na^+,K^+-ATPase.

It is uncertain whether these peptides exist in the circulation, but a recent report provides evidence that peptidic Na^+,K^+-ATPase inhibitors may be detected in the plasma of hypertensive patients, depending on the extraction methods (Giunta et al., 1990). These authors claim that they partially purified two peptidic Na^+,K^+-ATPase inhibitors in addition to a steroidal inhibitor from an aqueous, but not from a methanol, extract of plasma from patients with essential hypertension. However, their conclusion was mainly based on the molecular masses of those compounds (2000 and 4000 Da) and remains to be confirmed by more sophisticated analysis. Boschi et al. (1990) also partially purified a polar Na^+,K^+-ATPase inhibitor from plasma of borderline hypertensive patients. In preliminary experiments, the active fraction was eluted almost in the void volume from a C_{18} reverse phase HPLC column and showed fluorescent peaks typical for peptides.

Three unique peptidic inhibitors of Na^+,K^+-ATPase (SPAI-1, -2, and -3) have been isolated from porcine duodenum and consisted of 49 or 61 amino acids (Araki et al., 1989). The mechanism of inhibition was different from that produced by ouabain and was competitive with sodium but not with potassium. Other peptides or proteins, such as a thyrotropin-releasing hormone metabolite, mellitin, δ-endotoxin, theonellamine B, fragments of calmodulin, and opioid peptide, reportedly inhibit Na^+,K^+-ATPase activity, but it is unlikely that they are viable candidates because of their low affinity, nonselectivity, or irreversibility (Battaini and Peterkofsky, 1980; Chen and Lin-Shiau, 1985; English and Cantley, 1986; Nakamura et al., 1986; Teisinger et al., 1987; Ventra et al., 1987).

D. Unknown Substances

A low molecular (<1000) weight, nonpeptidic, nonlipidic compound has been purified from bovine hypothalamus and hypophysis (Haupert and Sancho, 1979). This material is very polar, eluted just after the void volume from a C_{18} reverse phase column, resistant to acid hydrolysis, and has both positive and negative charges. This substance is not selective for Na^+,K^+-ATPase and inhibits sarcoplasmic reticular, but not plasma membrane, Ca^{2+}-ATPase. This HIF binds to the cardiac glycoside receptor site located extracellularly on Na^+,K^+-ATPase (Carilli et al., 1985). HIF is not entirely ouabain like in the strict sense because it antagonizes phosphorylation of Na^+,K^+-ATPase by inorganic phosphorus, it has ligand requirements for maximal activity different from ouabain, and, finally, it is membrane permeable despite its water solubility (Haupert et al., 1984; Anner et al., 1990). EDLF, however, need not be identical with the cardiac glycosides in terms of the exact mechanism of binding to or inhibition of Na^+,K^+-ATPase. Furthermore, HIF inhibits low ouabain affinity kidney Na^+,K^+-ATPase with a 30-fold higher apparent affinity than ouabain. It increases the force of contraction in beating rat heart cells and leads to contractions of vascular rings of the rat aorta. A blockade of Ca^{2+} reuptake, through an inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase, may participate partly in these actions of HIF (Hallaq and Haupert, 1989).

The positive inotropic effect induced by HIF was more readily reversible than that produced by ouabain. Furthermore, this factor did not produce cardiotoxicity at a dose that produced similar pump inhibition and greater elevations in free calcium concentration than the toxic dose of ouabain. These findings are surely interesting, but it must be remembered that the isolated material was still not pure, and it may contain many uncharacterized substances. It is also unclear whether HIF is really found in the circulation. The attempt to completely purify and identify HIF has failed so far mainly because of the sparse amounts extractable from biological sources. Sancho et al. (Illescas et al., 1988, 1990) have extended their purification effect and purified an HIF from methanol extract of bovine hypothalamus and hypophysis. This compound showed a maximal absorbance

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at 247 nm, and the specific activity per weight of tissue was higher in hypophysis than in hypothalamus. No factor was found in cerebral cortex.

De Wardener et al. (1981) measured the ability of biological fluids to stimulate fresh guinea pig kidney glucose-6-phosphate dehydrogenase activity as an indication of their ability to inhibit Na^+,K^+-ATPase. This assay, however, has not been widely accepted and has been used only by this group of investigators. They demonstrated the presence of a nonpeptidic Na^+,K^+-ATPase inhibitor in the plasma of humans and the rat and in an acid acetone extract of rat hypothalamus (de Wardener et al., 1981, Alaghband-Zadeh et al., 1983). The glucose-6-phosphate dehydrogenase-stimulating activity survived boiling and acid hydrolysis, but its activity was destroyed by ashing and by base hydrolysis (Millett et al., 1987). Similar physicochemical properties suggested that the glucose-6-phosphate dehydrogenase-stimulating activity in the plasma and hypothalamus may be due to the same, heat-stable, nonpeptidic, organic compound.

Schwartz et al. (1982) obtained from acetone extracts of rat brain a low polar fraction that elutes at 75 to 80% acetonitrile containing 0.1% trifluoracetic acid from a C_{18} HPLC column based on the inhibition on purified lamb kidney Na^+,K^+-ATPase activity. The same concentration of extract inhibited lamb kidney Na^+,K^+-ATPase activity and [^{3}H]ouabain binding to the enzyme to exactly the same degree. The extract also inhibited rabbit skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase activity. Subsequently, these authors pointed out that peroxidized lipids and lysophospholipids, both of which inhibit Na^+,K^+-ATPase, were produced in acid-acetone extracts in the presence of air and that a specific inhibitor was found in aqueous acetone extracts under nitrogen (Whitmer et al., 1982).

Lichtstein and Samuelov (1980) found a nonpeptidic, low molecular weight (<500) compound with apparent digitalis-like properties in acidified acetone extract of rat brain. They also found a similar compound in sheep brain that inhibited the Na^+,K^+-ATPase activity in chicken embryo fibroblast and blocked the sodium ionophore (monensin)-induced hyperpolarization (Lichtstein and Samuelov, 1982). Furthermore, the compound obtained from sheep brain produced a positive inotropic effect on a trabeculum from sheep ventricle (Shimoni et al., 1984). A similar compound was demonstrated also in methanol extract of human cerebrospinal fluid (Lichtstein et al., 1985). It is unclear at present whether these compounds present in the nervous system belong to a hydroxylated fatty acid, a bufodienolide derivative, or another chemical entity.

A material coined "cardiodigin" has been isolated from water extracts of rat, guinea pig, cow, and dog hearts (de Pover et al., 1982; Fagoo and Godfraind, 1985; Castaneda-Hernandez, 1989). Cardiodigin inhibited human heart Na^+,K^+-ATPase and showed digoxin-like immunoreactivity. It was proposed that the EDLF may interact selectively with the high-affinity sites and produce a positive inotropic response without a reduction of whole cell ionic gradient (de Pover, 1984).

An endogenous inotropic substance was isolated from acetone-HCl extract of pig heart left ventricle based on an inotropic effect on guinea pig heart (Khatter et al., 1986, 1991). The partially purified substance was found to have most digitalis-like properties and produce a positive inotropic response by increasing sarcolemmal Ca^{2+} influx. Unlike cardiac glycosides, however, the substance facilitated the release of Ca^{2+} from sarcoplasmic reticulum and did not produce aftercontractions in larger doses. The chemical nature of this substance has yet to be described.

Substances able to block the binding of [^{3}H]ouabain to human red cell ghosts have been found in delipidated methanol extract of rat plasma (Rauch and Buckalew, 1988b). The circulating levels were increased by volume expansion. Although similar substances have also been reported in various rat tissues, their chemical identification remains to be determined.

E. Other Known Substances

1. Ascorbic acid. Ascorbic acid, which has been known as an inhibitor of Na^+,K^+-ATPase for decades, was isolated from $NaHCO_3$ extract of bovine brain or bovine adrenal as a potent inhibitor of isolated Na^+,K^+-ATPase (Ng et al., 1985; Kuske et al., 1987). Ascorbate inactivated the enzyme by a reduction of a group within the ATP-binding site and decreased the capacity and affinity of the ouabain-binding site. It also inhibited Ca^{2+}-ATPase derived from sarcoplasmic reticulum, but it did not affect sodium pump activity in intact cells even at high concentrations.

2. Lignans. Enterolactone, a lignan that has been identified in biological fluids from mammals, exhibited digitalis-like activities in the 10^{-4} M concentration range (Fagoo et al., 1986). In addition, a synthetic lignan produced a natriuresis in rats which may be related to an inhibition of Na^+,K^+-ATPase activity (Plante et al., 1987). However, their low affinity would appear to exclude lignans from serious consideration as EDLF.

3. Urodiolenone. The urodiolenone that appears as the glucuronide in the urine of hypertensive patients was found to be a potent inhibitor of Na^+,K^+-ATPase in guinea pig kidney as measured by a cytochemical assay (Neufeld et al., 1985). It is possible that urodiolenone has an exogenous origin (Chayen et al., 1988).

4. Hemin. Hemin (chloroprotohemin IX) was isolated from porcine blood cells and is a potent inhibitor of Na^+,K^+-ATPase activity and [^{3}H]ouabain binding to the enzyme (Yasuhara et al., 1991). Although hemin increased blood pressure in rats in a similar manner to ouabain, it remains to be determined whether hemin acts

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as an inhibitor of the sodium pump in intact cells through a binding to the cardiac glycoside receptor.

IV. Ouabain-like Compound

A. Identification of Ouabain-like Compound

As mentioned before, Hamlyn et al. (1989a,b) achieved a complete chemical identification of EDLF from human plasma. This EDLF was fully characterized and satisfied many of the criteria expected for EDLF (table 1). Hence, this EDLF appears to represent a naturally occurring ligand for the cardiac glycoside-binding site and, by virtue of its presence in plasma and its selectivity and high affinity, to play a significant role in the modulation of the sodium pump. Recently, this group of investigators modified the purification scheme and introduced Amberlite XAD-2 chromatography and the enzyme (Na^+,K^+-ATPase) affinity extraction. The XAD-2 chromatography and the enzyme affinity step improved the selectivity of purification and the scale of the process and enabled sufficient quantities of EDLF to be obtained for structural analysis. They processed 300 liters of human plasma and were able to isolate 31 μg of complete pure EDLF (Ludens et al., 1991). The purification factor was more than 0.6 billion-fold on a dry weight basis. The purified material was examined by a variety of mass spectrometric techniques, and the accurate mass was determined to be 585.295 Da. Based on this accurate mass, the EDLF was found to have the same chemical composition (the same carbon, oxygen, and hydrogen content) as ouabain. Direct comparison of ouabain and human plasma EDLF by mass spectrometry and analytical HPLC failed to reveal any differences (Mathews et al., 1991). Moreover, at each step in the purification of EDLF, inhibition of sodium pump (^{86}Rb uptake into human erythrocytes) and immunological cross-reactivity with anti-ouabain antibody were inseparable. Thus, the EDLF isolated from human plasma was immunologically as well as mass spectrometrically and biochemically identical with ouabain. These investigators concluded that human plasma EDLF is ouabain or is a closely related isomer of ouabain and proposed that more specific nomenclature such as OLC should be used. Mass spectrometry suggested that the sugar moiety of OLC also is a deoxyhexose, but it is still unknown whether or not it is rhamnose. It was really an unanticipated finding that EDLF from human plasma was identified as ouabain or a very similar substance following many years of efforts in various laboratories worldwide.

The bovine adrenal EDLF purified by Tamura et al. and the human urinary polar EDLF purified by us were actually very similar to, if not identical with, OLC purified by Hamlyn and coworkers as mentioned before. The bovine adrenal EDLF actually cross-reacted with highly specific anti-ouabain antibodies (Tamura, personal communications). The human urinary polar EDLF also cross-reacted with anti-ouabain antibodies, and the displacement of radiolabeled ouabain from the anti-ouabain antibody by serial dilutions of urinary EDLF was largely parallel to displacement by unlabeled ouabain. Furthermore, anti-ouabain IgG neutralized the inhibitory effect of urinary EDLF on [^{3}H]ouabain binding in a dose-related manner (Goto et al., 1992). These findings apparently support the idea that these EDLFs are at least recognized by highly specific anti-ouabain antibodies.

We (Goto et al., 1990b) recently reported that a different, less polar, urinary ODC-2 eluting at 31% acetonitrile from C_{18} reverse phase columns was indistinguishable from digoxin in three HPLC and three thin-layer chromatography systems. Because digoxin is one of the drugs most frequently used in clinical practice, we cannot completely discard the possibility of an eventual contamination with exogenous digoxin. Therefore, it is uncertain whether this digoxin-like compound is actually of an endogenous nature. Recently, a digoxin-like factor, which is similar to, but not identical with, digoxin in immunological, chromatographic, spectral properties, has been isolated to apparent homogeneity from mammalian adrenal cortex (Shaikh et al., 1991). Furthermore, a cross-reactive material with antibodies to digoxin was observed in a less polar fraction of human plasma and also was apparently associated with the inhibition of red blood cell sodium pump activity in the study by Hamlyn and coworkers (Harris et al., 1991). Taken together, different EDLFs belonging to cardenolides other than OLC may also exist in mammals.

B. Origin of Ouabain-like Compound

Ouabain is a cardiac glycoside that was previously thought to exist only in plants. If OLC is ouabain itself or a similar substance, it also belongs to a cardenolide with a *cis* junction in the steroidal ring CD, as opposed to the conventional CD-*trans* endogenous steroids. Biosynthetic pathways for such CD-*cis* steroids have never been described in humans. Moreover, rhamnose, the sugar component of ouabain, exists only in plants in the combined form of glycosides. Thus, the cardioactive CD-*cis* steroids do not appear to be produced in the human biosynthetic pathways, at least to date.

Since the report by Kelly (1986) emphasizing the importance of excluding exogenous sources of digitalis-like substances, the notion that genuine cardenolides are produced in the mammalian body should be regarded with caution (table 2). Exogenous sources of cardenolides, such as those derived from consumption of plants and vegetables, must be strictly excluded to prove an endogenous origin.

Hamlyn et al. (1991) indicated six lines of good evidence to support the endogenous nature of OLC: (*a*) high concentrations of OLC were found in the adrenals of species with different diets, (*b*) in rat plasma, levels of OLC decreased after bilateral adrenalectomy, (*c*) cultured adrenal cells release OLC, (*d*) plasma levels of

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TABLE 1
Proposed candidates for EDLF and criteria met by those substances

Candidates	Criteria*								
	1	2	3	4	5	6	7	8	9
Lipids									
Oleic acids	Yes	Yes	Yes	No	No	No	No	NT†	NT
Linoleic acids	Yes	Yes	Yes	No	No	No	No	NT	NT
Arachidonic acids	Yes	Yes	Yes	No	No	No	No	NT	NT
Lysophosphatidyl serine	Yes	Yes	Yes	No	No	No	No	NT	NT
Lysophosphatidyl choline	Yes	Yes	Yes	No	No	No	No	NT	NT
11,13-Dihydroxy-14-octadecanoic acid	Yes	Yes	NT	NT	NT	NT	NT	NT	NT
Peroxide	Yes	NT	NT	NT	NT	NT	NT	NT	NT
12(R)-HETE	Yes	NT	Yes	NT	Yes	NT	NT	NT	NT
Steroids									
CMA	Yes	Yes	Yes	NT	NT	NT	Yes	NT	No
14-β-Hydroxy-progesterone	Yes	Yes	Yes	NT	NT	NT	Yes	NT	Yes
14-β-Hydroxy-progesterone glucoside	Yes	Yes	Yes	NT	NT	NT	Yes	NT	Yes
Resibufogenin	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NT	Yes
Polyhydroxylated steroids	Yes	NT	NT	NT	Yes	NT	NT	NT	Yes
? (Tamura et al., 1987b, 1988)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NT	NT
? (ODC-1; Goto et al., 1988c,d, 1989)	Yes	Yes	Yes	Yes	NT	Yes	Yes	NT	NT
Digoxin-like compound (ODC-2; Goto et al., 1990a)	Yes	Yes	Yes	Yes	NT	Yes	Yes	NT	NT
OLC (Hamlyn et al., 1991)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
? (Schoner, 1991)	NT	Yes	Yes	NT	NT	NT	Yes	NT	NT
? (Balzan et al., 1991)	NT	Yes	Yes	NT	NT	NT	NT	NT	NT
Glycosteroid (Cloix et al., 1985, 1987)	Yes	Yes	NT	NT	NT	No	NT	NT	NT
Dehydroepiandrosterone sulfate	Yes	Yes	No	NT	No	NT	No	No	No
Progesterone	Yes	Yes	No	NT	No	NT	No	No	No
Cortisol	Yes	Yes	No	NT	No	NT	No	No	No
Bile salts	Yes	Yes	No	NT	No	NT	No	No	No
Canrenone and derivatives	Yes	Yes	NT	NT	No	NT	No	NT	NT
Peptides									
? (Gruber et al., 1980)	Yes	NT	NT	NT	NT	NT	NT	NT	NT
? (Akagawa et al., 1984)	Yes	Yes	NT	NT	NT	NT	Yes	NT	NT
? (Kramer et al., 1985, 1986)	Yes	Yes	NT	NT	NT	NT	No	NT	NT
? (Halperin et al., 1985, 1988)	Yes	Yes	Yes	NT	NT	Yes	Yes	NT	NT
? (Morgan et al., 1985)	Yes	Yes	Yes	NT	NT	NT	NT	NT	NT
? (Giunta et al., 1990)	Yes	NT	NT	NT	Yes	NT	NT	NT	NT
? (Boschi et al., 1990)	Yes	NT	Yes	NT	NT	NT	NT	NT	NT
SPAI (Araki et al., 1989)	Yes	No	NT	NT	NT	NT	No	NT	NT
Unknown substances									
? (Haupert and Sancho, 1979)	Yes	Yes	Yes	Yes	Yes	No	NT	NT	Yes
? (de Wardener et al., 1981)	Yes	NT	Yes	NT	NT	NT	NT	NT	NT
? (Schwartz et al., 1982)	Yes	Yes	NT	NT	NT	No	NT	NT	NT
? (Lichtstein and Samuelov, 1980, 1982)	Yes	Yes	Yes	NT	NT	NT	NT	NT	Yes
? (Godfraind and Fagoo, 1985)	Yes	Yes	NT	NT	NT	NT	NT	NT	NT
? (Khatter et al., 1986, 1991)	Yes	Yes	Yes	NT	NT	NT	Yes	NT	Yes
? (Rauch and Buckalew, 1988b)	Yes	Yes	NT	NT	NT	NT	Yes	NT	NT
Other known substances									
Ascorbic acid	Yes	Yes	No	No	No	No	No	NT	NT
Lignans	Yes	NT	NT	NT	No	NT	NT	NT	NT
Urodienolone	Yes	NT	NT	NT	NT	NT	NT	NT	NT
Hemin	Yes	Yes	NT	NT	NT	NT	NT	NT	NT

* Criteria for EDLF: 1, inhibition of Na^+, K^+-ATPase; 2, inhibition of [^{3}H]ouabain binding; 3, inhibition of sodium pump activity in cells; 4, reversibility; 5, high affinity; 6, selectivity; 7, competition with KCl; 8, species sensitivity; 9, positive inotropic effect.

† NT, not tested; ?, unidentified substance reported by the investigator shown in the parentheses.

OLC were elevated in hypertensive rats, (*e*) levels of OLC in plasma from humans receiving total parenteral nutrition were similar to those in matched controls, and (*f*) oral ouabain has minimal bioavailability. This evidence argues for the notion that mammals may have biosynthetic pathways for CD-*cis* steroids still to be discovered.

Recently, it was suggested that morphine, which has been believed to be exclusively of plant origin, is actually synthesized in the mammalian body (Donnerer et al., 1986; Weitz et al., 1987). In analogy, a genuine cardenolide, which was thought to be plant derived, may be produced in the mammalian body. The biosynthetic pathway of cardenolides has been studied in plants; cholesterol, pregnenolone, or progesterone is considered to be the precursor of cardenolides (Tschesche, 1972).

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TABLE 2
OLC as an EDLF

Strengths
OLC is consistently found in the plasma of all mammals with different diets. (We agree with this claim.) Similar levels of OLC is also present in plasma from humans receiving total parenteral nutrition.
Isolation and identification of OLC are based on established, sophisticated methods and are associated with no problems. In control studies, contamination from the components of the purification process were excluded.
Levels of OLC in rat plasma decrease after bilateral adrenalectomy and increase in DOCA-hypertension.
Cultured adrenal cells release OLC into serum-free medium.
Weaknesses
The biosynthetic pathway of cardenolides are not described in mammals.
It is uncertain whether OLC is solely responsible for the EDLFs (sodium transport inhibitors or Na^+,K^+-ATPase inhibitors) that have been detected during plasma volume expansion and in hypertension.
The presence of other EDLFs cannot be excluded yet, mainly because of the use of enzyme affinity extraction.

The presence of similar biosynthetic pathways should be documented in mammals to convincingly prove the endogenous origin of OLC.

C. Putative Physiological Roles of Ouabain-like Compound

1. Physiological implications of ouabain-like compound. The plasma levels of OLC, based on a specific enzyme-linked immunoassay, were similar in humans, dogs, and rats and had a concentration of 80 to 138 pM (Hamlyn et al., 1991). As mentioned repeatedly, sensitivity to cardiac glycosides varies among Na^+,K^+-ATPases isolated from different species. It has also been demonstrated that human red blood cells were >1000-fold more sensitive to inhibition by OLC from human plasma than were rat red blood cells.

It is known that the dissociation constant for the cardiac glycoside-receptor complex is in the nanomolar range in humans, comparable to the affinities of physiologically relevant binding sites for known hormones. For example, the K_d for ouabain and human heart muscle is 2.5 nM. Taken together, it is unclear whether the concentrations of OLC in the circulation are sufficient to induce physiological effects even in humans (Schoner, 1991). In DOCA-saline hypertensive rats, plasma OLC levels were 9-fold higher than in controls and were 975 pM (Hamlyn et al., 1991). It is still questionable whether circulating OLC at this concentration is physiologically meaningful in the rat, a species resistant to the actions of ouabain (Pamnani et al., 1991).

There are, however, precedents for endogenous steroids to be synthesized and/or concentrated in tissues. The enzymatic transformation of naturally secreted hormone to biologically active metabolites within its target tissues is a characteristic feature of the androgen (Sulcova et al., 1979). 19-Nor-corticosteroids, some of which are potent mineralocorticoids, are not circulating steroids and are synthesized by extraadrenal tissues in biologically active quantities (Melby et al., 1982). It is possible that OLC may be concentrated or modified to a more active form in target tissues.

The presence of OLC in the human body has an important clinical implication in the treatment of patients with cardiovascular diseases. If there is already OLC in the circulation that competes with the therapeutic cardiac glycoside (digoxin or digitoxin) for the inhibitory site on the sodium pump, it will be necessary to monitor the concentration of OLC to better understand the therapeutic role of cardiac glycoside. It should also be recognized that those patients with high levels of OLC may be at greater risk for the development of cardiotoxicity when an exogenous cardiac glycoside is given.

Although much attention has been paid to the possible roles of OLC in cardiovascular regulation, the biological purpose of OLC may be different from that which has been previously thought. It is generally accepted that the sodium pump activity is greatest in the excitable cells of the nervous system and the brain and is mainly composed of a Na^+,K^+-ATPase sensitive to cardiac glycosides even in digitalis-resistant rodents (Berrebi-Bertrand et al., 1990).

OLC likely plays an autocrine or paracrine role related to specific regulatory mechanisms, especially in the central nervous system. It is possible that OLC in the circulation may simply reflect that which has overflowed or spilled from the central nervous system. Most tissues, other than brain, also contain OLC (Hamlyn et al., 1991). Furthermore, recent preliminary findings suggest that various cultured cells or isolated vascular preparations release OLC (our unpublished observations). It remains to be explored whether or not OLC acts as a local factor and contributes to the electrolyte homeostasis through the regulation of the sodium pump.

The long-term effects of cardiac glycosides on the sodium pump have been examined in erythrocytes and myocardial cells. The sodium pump activity, as estimated from ^{86}Rb uptake, was significantly depressed during the first day after subjects had been given digoxin (Smith et al., 1987). The decreased pump activity could then bring about an increase in intracellular sodium concentration during several days of digoxin treatment. Adaptive changes, which may be completed in a period of 4 months, to treatment with cardiac glycosides appears to occur with a gradual increase in the number of Na^+,K^+-ATPase sites per cell, probably due to increased synthesis of Na^+,K^+-ATPase and a return toward predigoxin intracellular sodium concentrations (Malini et al., 1984).

A similar adaptation also has been found in myocardial

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cells (Bluschke et al., 1976). By contrast, in the same study, the number of sodium pumps did not increase in the brain during chronic treatment with cardiac glycosides. It is unclear whether chronically administered digoxin at this time of adaptation loses its main actions, such as positive inotropic activity. Does the phenomenon of up-regulation of sodium pump number also occur in cells chronically exposed to OLC? Do the effects of OLC disappear at this time? It was reported that chronic submaximal inhibition of Na^+,K^+-ATPase activity in cultured human cells or rat outer medullary tubular segments by incubation in medium containing ouabain resulted in an increased number of actively pumping Na^+,K^+-ATPase sites in the cell membrane (Lamb and McCall, 1972; Rayson 1989). The augmentation of the number of Na^+,K^+-ATPase sites after ouabain treatment was first induced by the increase in synthesis and, thereafter, was maintained by a reduction in the rate of Na^+,K^+-ATPase degradation. Intracellular Na^+ levels approximately doubled 2 to 4 hours after the addition of ouabain. After 18 hours, however, intracellular Na^+ levels were restored to levels not significantly different from controls. Thus, OLC may not be effective in eliciting chronic forms of hypertension unless such a homeostatic response of the target cell is impaired (Rayson, 1988).

2. Tissue selectivity of the action of ouabain-like compound. The site of action of OLC, if it functions as a circulating hormone, seems ubiquitous because the Na^+,K^+-ATPase is located in the covering plasma membranes of nearly all mammalian cells. However, it is a reasonable assumption that the effects of OLC as a hormone are exerted on more limited, selective target tissues. Two lines of reasoning may be plausible. First, the potential for organ-specific metabolism of circulating OLC imposes a means for restricting the scope of Na^+,K^+-ATPase inhibition. It has been demonstrated that sex steroids are converted in specific target tissues to derivatives with hormonal activities quantitatively and qualitatively distinct from those of the parent compound (Kim and LaBella, 1981).

Second, OLC may discriminate among tissues on the basis of difference in sensitivity. In addition to species-dependent differences in sensitivity, the tissue-specific difference in Na^+,K^+-ATPase sensitivity to cardiac glycosides has also been demonstrated. The tissue-specific difference in sensitivity is likely due to the expression of different Na^+,K^+-ATPase isoenzymes. It appears that the α subunit determines the sensitivity of the Na^+,K^+-ATPase to cardiac glycosides as mentioned before. Three forms of Na^+,K^+-ATPase, possessing distinct α-subunit isoforms, α 1, α 2, and α 3, have been isolated in humans and rats. It has been demonstrated that the three primary amino acid sequences exhibit differences between each isoform. These α isoforms are products of different genes, and expression of these genes is developmentally regulated and tissue specific (Shull et al., 1986; Herrera et al., 1987).

These Na^+,K^+-ATPase isozymes show different sensitivity to the actions of cardiac glycosides. Na^+,K^+-ATPases possessing the α-1 isoform are two orders of magnitude less sensitive to ouabain than those possessing the α-2 isoform. Therefore, it is tempting to speculate that the physiological effects of OLC are mediated by the cardiac glycoside-sensitive isoenzymes (α 2 and/or α 3). Thus, OLC may differentially regulate Na^+,K^+-ATPase isozymes that have different affinities for cardiac glycosides, and, hence, the actions of OLC may be tissue selective.

A systematic nomenclature by which Na^+,K^+-ATPase isozymes can be named and in which tissues they are found is still in its infancy (Sweadner, 1989). For example, the distribution and the function of the isozymes in the vascular tissue are not well established. Only recently, the presence of Na^+,K^+-ATPase isozymes has been confirmed in aortic smooth muscle cells. There is evidence for possible regulation of the messenger RNA for these isozymes in rat aorta during both DOCA-saline and angiotensin II-induced hypertension (Herrera et al., 1988). Two Na^+,K^+-ATPase isoforms also exist in canine aorta where the predominant expression is that of a high-affinity form (Maixent et al., 1991). It is likely that there exists a more complex molecular interaction of OLC with Na^+,K^+-ATPase than can be explained by the information obtained to date.

3. Positive inotropic effect. The notable action of cardiac glycosides is the enhancement of the force of contraction of cardiac muscle. If OLC is structurally and biologically identical with cardiac glycosides, it seems more logical to assume that the target tissue for OLC is the heart, at least in analogy with the effect of cardiac glycosides, and a promotion of positive inotropy is one of the properties of OLC.

Whether OLC exerts a significant effect on a specific tissue may depend on the influx rate of Na^+, the presence or absence of Na^+/Ca^{2+} exchange systems, and the importance of extracellular Ca^{2+} to the function of the particular tissue; Na^+,K^+-ATPase sensitivity to OLC is also of importance. The heart obviously meets most of these conditions. Indeed, OLC was increased in patients with congestive heart failure (Gottlieb et al., 1991). Human OLC had cardiotonic actions on guinea pig atria quantitatively similar to plant ouabain (Bova et al., 1991). Furthermore, increased plasma levels of OLC were considered to be a homeostatic response to the diminished cardiac output of patients with heart failure. Clearly, the physiological role of OLC is likely to be global rather than restricted to the myocardium.

4. Natriuretic effect. The view that OLC may act as a natriuretic hormone or a hypertensive, vasoactive substance was in large part responsible for the regeneration

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of interest in the subject of EDLF as mentioned in the "Introduction."

In most polarized cells, such as renal tubular epithelial cells, Na^+,K^+-ATPase is located primarily in the plasma membrane adjacent to the blood capillaries, i.e., on the basolateral surface. OLC could theoretically cause natriuresis by inhibiting Na^+,K^+-ATPase in the basolateral membrane and reducing Na^+ reabsorption. OLC potentially could inhibit sodium reabsorption along the full length of the renal tubule. The extent of inhibition in specific tubular segments would depend on the relative density of sodium pump units and their affinity for OLC. Sensitivity to ouabain has been reported to differ markedly in different segments of the nephron (Doucet and Barlet, 1986). The collecting tubular cells appear to be the most sensitive among various tubular cells. This observation may suggest the potential role of OLC as a determinant of the final urine sodium excretion.

Several researchers claim, however, that cardiac glycosides are at best poor models for the natriuretic factor (Wechter and Benaksas, 1990). Evidence proving a direct renal natriuretic effect of cardiac glycosides is limited and without clinical importance. Although there are several reports of the natriuretic action of cardiac glycosides, no cardiac glycoside shows any evidence of consistent natriuresis in vivo except when infused into the renal artery at toxic superphysiological doses (Hyman et al., 1956). Recent clinical studies also indicate that the sodium pump inhibition produced by digoxin did not facilitate natriuresis in normal subjects, despite the increases in intraerythrocyte sodium and decreases in the number of erythrocytic ouabain-binding sites (Luft et al., 1989). No change in plasma OLC levels was found in uninephrectomized rats drinking a 0.9% NaCl solution as compared with control rats (Hamlyn et al., 1991). The natriuretic activity of human OLC has not been described yet.

Gonick et al. (1977) first suggested that the natriuretic hormone may be an inhibitor of Na^+,K^+-ATPase. They measured the inhibitory effect of chromatographic fractions on semipurified Na^+,K^+-ATPase activity at a K^+ concentration of 20 mM. There is no evidence that this factor inhibited the enzyme activity by a mechanism related to the binding to the cardiac glycoside receptor. Furthermore, a small molecular weight sodium transport inhibitor isolated from the urine of normal subjects, who had undergone volume expansion by saline infusion, inhibited both the ouabain-sensitive and -insensitive components of the sodium efflux rate constants (Poston et al., 1982). This would suggest that the substance may not be a specific inhibitor of Na^+,K^+-ATPase. It has been claimed that a number of circulating ouabain-like factors may not be identical with a humoral natriuretic factor (Kramer et al., 1991).

5. Hypertensive effect. Much evidence indicates that the kidneys play a crucial role in the development and maintenance of high blood pressure in genetic hypertension, including human essential hypertension. Several investigators have presented similar hypotheses suggesting that a primary effect in renal sodium excretion exists that would evoke mechanisms within the organism to promote natriuresis. One such endogenous natriuretic material was proposed to be EDLF. EDLF would inhibit renal tubular sodium reabsorption, thereby restoring sodium balance to normal.

Inhibition of the sodium pump by EDLF would be expected to affect vascular smooth muscle and sympathetic nerve function. In these target tissues, EDLF would cause cell Na^+ to increase and membrane potential to decrease. Increased intracellular Na^+ or depolarization would act on plasma membrane Na^+-Ca^{2+} antiports or voltage-sensitive Ca^{2+} channels, respectively, and lead to increased intracellular Ca^{2+}. An increase in the cytosolic Ca^{2+} level would promote contraction of vascular smooth muscle and would also promote the release of norepinephrine from peripheral nerves and inhibit its reuptake. The resulting increase in blood pressure would induce a pressure natriuresis and would protect blood volume at the expense of an elevated blood pressure. In this context, hypertension is viewed as a homeostatic mechanism designed to normalize a primary alteration in renal sodium excretion (Haddy and Overbeck 1976; Blaustein, 1977; Haddy, 1987; Poston, 1987).

The acute vasoconstrictor action of cardiac glycosides is well established, i.e., injection of glycosides causes an acute increase in peripheral resistance, whereas exposure of isolated vascular preparations to ouabain potentiates the previously induced tone (Mulvany, 1985). The acute vasoconstrictor effect of ouabain in rat mesenteric small arteries was produced by the depolarizing action of ouabain, but the potentiating action of ouabain tended to be transient (Mulvany, 1985; Mulvany and Aalkjar, 1985). Prolonged incubation in ouabain (10^{-9} to 10^{-6} M) resulted in a concentration-dependent increase in the tone of resting human resistance arteries and a potentiation of tone (Woolfson et al., 1990). In human arteries, nanomolar levels of ouabain inhibited acetylcholine-induced endothelium-dependent vasodilation (Woolfson and Poston, 1991). Whether long-term inhibition of the sodium pump by cardiac glycosides would cause sustained vasoconstriction is still unclear. Chronic administration of digoxin to dogs and rats was accompanied by vascular wall waterlogging and, in rats, mild hypertension (Overbeck, 1985). Digoxin enhanced the increase in blood pressure during aldosterone infusion in conscious sheep (Spence et al., 1989). However, both ouabain and digitoxin failed to cause hypertension in one-kidney salt-loaded rats or one-kidney DOCA-treated rats (Nirasawa et al., 1985). Furthermore, ouabain produced a decrease in blood pressure in spontaneously hypertensive rats, probably due to sensitization of baroreceptors (Ayachi and Brown, 1980). In a clinical study, digoxin adminis-

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tration to normal subjects augmented pressor responsiveness to both norepinephrine and angiotensin. However, there were no concomitant alterations of blood pressure (Guthrie, 1984). Moreover, chronic treatment of humans with cardiac glycoside does not cause hypertension.

OLC purified from human plasma was capable of potentiating the histamine-induced contraction of a ring of guinea pig aorta (Bova et al., 1991). Human OLC had vasotonic actions qualitatively and quantitatively similar to those observed with plant ouabain. In hypertensive DOCA-treated uninephrectomized rats, plasma OLC levels were 9-fold higher than controls (Hamlyn et al., 1991). Masugi et al. (1986, 1987) measured ouabain-like immunoreactivity in human plasma and found that it increased in patients with essential hypertension or primary aldosteronism. However, the plasma immunoreactivity measured by this group was subsequently described as being due to an unstable lipid, and the assay appeared not to be specific for ouabain (Masugi et al., 1988). We need to accumulate data based on immunoassays of ouabain to define whether OLC is actually the EDLF implicated in the regulation of sodium excretion and blood pressure.

V. Conclusions

Through the search for EDLF, there has been agreement that EDLF need not be absolutely identical with the cardiac glycosides in terms of target organ specificity, mechanism of inhibition of the Na^+,K^+-ATPase, and chemical structure (Haupert, 1988). It is clear that such identity is not a requirement for relevance of EDLF to normal physiology or pathophysiology. Although we cannot completely discard the possibility that the approaches to date for searching and characterizing EDLF are missing the target, it turned out that at least one type of EDLF appears to have a similar chemical structure to that of plant-derived cardiotonic steroids. As summarized in table 2, we agree, from our own experience, that OLC has been found to exist consistently in the mammalian plasma and is the only compound that satisfies the narrow criteria required for EDLF. Furthermore, evidence suggests physiological and pathophysiological implications of OLC as an EDLF (table 2).

However, there are still some issues to be clarified with regard to OLC as an EDLF (table 2). Above all, production site and biosynthetic pathway of OLC should be determined. Furthermore, definitive evidence that the apparent EDLF activities reported in numerous previous studies, which were measured in whole or crude extracts and were based on other different assay methods than radioimmunoassay of ouabain, were mediated solely by OLC is still lacking and remains to be demonstrated. Even in studies carried out by Hamlyn and coworkers, it is possible that the EDLF they described may be different from OLC. Hamlyn and colleagues (1982) demonstrated a highly significant correlation between levels of a plasma inhibitor of canine kidney Na^+,K^+-ATPase activity and mean blood pressure in normotensive and hypertensive individuals using deproteinized plasma supernatants. In this study, however, they have showed a greater rate of association with Na^+,K^+-ATPase of the inhibitor in the human plasma in comparison with ouabain. This finding that the plasma inhibitor is distinct from ouabain in a kinetic analysis has been confirmed by other investigators (Toriyabe et al., 1988). Furthermore, Hamlyn et al. (1987) found inhibitors of Na^+,K^+-ATPase based on the same, direct inhibition of isolated Na^+,K^+-ATPase activity only in nonpolar fractions of plasma obtained from volume-expanded individuals and identified lysophosphatidylcholine as one of the inhibitors. In subsequent studies, a method to measure ^{86}Rb uptake was devised that allows incubation of human red blood cells with effectively undiluted intact plasma (Hamlyn et al., 1988). The basal levels of EDLF according to this method in human plasma were approximately 80 to 100 pM and the levels increased 8-to-10-fold by a massive acute saline infusion (Hamlyn, 1990). The temporal changes of plasma EDLF levels based on the same inhibition of ^{86}Rb uptake by human red blood cells supported the role of EDLF in DOCA-salt hypertension in pigs (Hamlyn, 1989). Although species differences were observed in the sensitivity of sodium pumps to EDLF based on this method, these differences were much smaller than the corresponding differences seen with ouabain (3-fold versus 10^4-fold; Hamlyn et al., 1988). Furthermore, inhibitory material of ^{86}Rb uptake was found in the nonpolar fractions of plasma analyzed by a reverse phase HPLC. This chromatographic behavior is totally different from that of ouabain. Taken together, it is uncertain whether OLC alone is able to completely explain the EDLF that has been reported in these various situations.

The enzyme affinity step used by Hamlyn et al. (1991) excluded all materials except those that bound specifically to Na^+,K^+-ATPase under the reaction conditions (in the presence of Mg^{2+} and inorganic phosphorus) known to support high-affinity binding of cardiac glycosides. In addition, only materials subsequently released from the enzyme under nonbinding conditions (in the presence of ethylene diaminetetraacetic acid) were used for further purification. Therefore, it is possible that other EDLFs with different mechanisms of action or with different binding characteristics were excluded from isolation. Many candidates, described in this paper, whose mechanism of action may not fit the strict criteria required for EDLF, may also be of importance in the regulation of the sodium pump in vivo (table 1). Particularly, the peptides from hypothalamic cells or cerebrospinal fluid (Morgan et al., 1985; Halperin et al., 1988) and the nonpeptidic hypothalamic or pituitary factor (Millet et al., 1987; Haupert and Sancho, 1979; Illescas et al., 1990) may also constitute the endogenous modu-

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lators of the sodium pump. It has been suggested that there may be more than one type of EDLF in the mammalian body in addition to a steroidal OLC (Schreiber, 1985). In view of the existence of at least three isoenzymes of the Na^+,K^+-ATPase with different sensitivities to cardiac glycosides, such a possibility may be highly tenable. Thus, many issues surrounding EDLF still remain to be resolved, in spite of the exciting discovery of OLC as an EDLF, and warrant further research.

REFERENCES

AKAGAWA, K., HARA, N., AND TSUKADA, Y.: Partial purification and properties of the inhibitors of Na,K,-ATPase and ouabain-binding in bovine central nervous system. J. Neurochem. **42:** 775–780, 1984.

AKERA, T.: Membrane adenosinetriphosphatase: a digitalis receptor? Science **198:** 569–574, 1977.

AKERA, T., AND NG, Y-C.: Digitalis sensitivity of Na^+,K^+-ATPase, myocytes and the heart. Life Sci. **48:** 97–106, 1991.

AKIZAWA, T., YASUHARA, T., KANO, R., AND NAKAJIMA, T.: Novel polyhydroxylated cardiac steroids in the nuchal glands of the snake, *Rhabdophis tigrinus*. Biomed. Res. **6:** 437–441, 1985.

ALAGHBAND-ZADEH, J., FENTON, S., HANCOCK, K., MILLETT, J., AND DE WARDENER, H. E.: Evidence that the hypothalamus may be a source of a circulating Na^+,K^+-ATPase inhibitor. J. Endocrinol. **98:** 221–226, 1983.

ALLEN, D. G., EISNER, D. A., AND WRAY, S. C.: Birthday present for digitalis. Nature **316:** 674–675, 1985.

ANNER, B. M.: The receptor function of the Na^+-K^+-activated adenosine triphosphatase system. Biochem. J. **227:** 1–11, 1985.

ANNER, B. M., REY, H. G., MOOSMAYER, M., MESZOELY, I., AND HAUPERT, G. T., JR.: Hypothalamic Na^+-K^+-ATPase inhibitor characterized in two-sided liposomes containing pure renal Na^+-K^+-ATPase. Am. J. Physiol. **258:** F144–F153, 1990.

ANTMAN, E. M., AND SMITH, T. W.: Current concepts in the use of digitalis. Adv. Intern. Med. **34:** 425–454, 1989.

ANTONELLI DE GOMEZ DE LIMA, M., AND RODRIGUEZ DE LORES, ARNAIZ, G.: The inhibitory activity of a brain extract on synaptosomal Na^+,K^+-ATPase is sensitive to carboxypeptidase A and to chelating agents. Neurochem. Res. **13:** 237–241, 1988.

ARAKI, K., KUROKI, J., ITO, O., KUWADA, M., AND TACHIBANA, S.: Novel peptide inhibitor of Na^+,K^+-ATPase from porcine intestine. Biochem. Biophys. Res. Commun. **164:** 496–502, 1989.

AYACHI, S., AND BROWN, A. M.: Hypotensive effects of cardiac glycosides in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. **213:** 520–524, 1980.

AZUMA, H., SEKIZAKI, S., AKIZAWA, T., YASUHARA, T., AND NAKAJIMA, T.: Activities of novel polyhydroxylated cardiotonic steroids purified from nuchal glands of the snake, *Rhabdophis tigrinus*. J. Pharm. Pharmacol. **38:** 388–390, 1985.

BALZAN, S., GHIONE, S., BIVER, P., GAZZETTI, P., AND MONTALI, U.: Partial purification of endogenous digitalis-like compounds in cord blood. Clin. Chem. **37:** 277–281, 1991.

BATTAINI, F., AND PETERKOFSKY, A.: Histidyl-proline diketopiperazine, an endogenous brain peptide that inhibits (Na^++K^+)-ATPase. Biochem. Biophys. Res. Commun. **94:** 240–247, 1980.

BERREBI-BERTRAND, I., MAIXENT, J-M., CHRISTE, G., AND LELIEVRE, L. G.: Two active Na^+/K^+-ATPases of high affinity for ouabain in adult rat brain membranes. Biochim. Biophys. Acta. **1021:** 148–156, 1990.

BERTORELLO, A., AND APERIA, A.: Regulation of Na^+-K^+-ATPase activity in kidney proximal tubules: involvement of GTP binding proteins. Am. J. Physiol. **256:** F57–F62, 1989.

BIDARD, J-N., ROSSI, B., RENAUD, J-F., AND LAZDUNSKI, M.: A search for an ouabain-like substance from the electric organ of *Electrophrus electricus* which led to arachidonic acid and related fatty acids. Biochim. Biophys. Acta. **769:** 245–252, 1984.

BLAUSTEIN, P.: Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. Am. J. Physiol. **232:** C165–C173, 1977.

BLAUSTEIN, M. P., AND HAMLYN, J. M.: Pathogenesis of essential hypertension: a link between dietary salt and high blood pressure. Hypertension **18** (Suppl. III): III-184–III-195, 1991.

BLUSCHKE, V., BONN, R., AND GREEFF, K.: Increase in the (Na^+,K^+-ATPase activity in heart muscle after chronic treatment with digoxin or potassium deficient diet. Eur. J. Pharmacol. **37:** 189–191, 1976.

BOSCHI, S., BORGHI, C., MUNARINI, A., COSTA, F. V., ZHUOREN, L., DEVRIES, D., AND AMBROSIONI, E.: Purification of a Na^+,K^+-ATPase inhibitor from borderline hypertensives' plasma. Biochem. Biophys. Res. Commun. **169:** 360–368, 1990.

BOSE, D., ELLIOTT, D., KOBAYASHI, T., TEMPLETON, J. F., KUMAR, V. P. S., AND LABELLA, F. S.: 14β-Hydroxyprogesterone binds to the digitalis receptor, inhibits the sodium pump and enhances cardiac contractility. Br. J. Pharmacol. **93:** 453–461, 1988.

BOVA, S., BLAUSTEIN, M. P., LUDENS, J. H., HARRIS, D. W., DUCHARME, D. W., AND HAMLYN, J. M.: Effects of an endogenous ouabainlike compound on heart and aorta. Hypertension **17:** 944–950, 1991.

BOWEN, J. W., AND MCDONOUGH, A.: Pretranslational regulation of Na-K-ATPase in cultured canine kidney cells by low K^+. Am. J. Physiol. **252:** C179–C189, 1987.

BROWN, L., WERDAN, K., AND ERDMANN, E.: The red blood cell: a model for ouabain receptor regulation in the heart? Klin. Wochenschr. **64:** 786–792, 1986.

BUCKALEW, V. M., JR., AND GRUBER, K. A.: Natriuretic hormone. *In* The Kidney in Liver Disease, ed. by M. Epstein, pp. 479–499, Elsevier, Amsterdam, The Netherlands, 1983.

BUCKALEW, V. M., JR., MORRIS, M., CAMPBELL, W. G., JR., AND RAUCH, A. L.: Plasma inhibitors of Na,K-ATPase: relation to salt balance and hypertension. Klin. Wochenschr. **65** (Suppl. 8): 133–138, 1987.

CARILLI, C., BERNE, M., CANTLEY, L. C., AND HAUPERT, G. T.: Hypothalamic factor inhibits the (Na,K)ATPase from the extracellular surface. J. Biol. Chem. **260:** 1027–1031, 1985.

CASTANEDA-HERNANDEZ, G.: Evidence for the existence of the same endogenous digitalis-like factor in several mammalian species. Comp. Biochem. Physiol. **94C:** 49–53, 1989.

CHAYEN, R., MEZUR, Y., WYLER, H., BERMAN, E., POTGIETER, E., AND GOLDBERG, S.: Urodiolenone from grapefruit juice, a urinary metabolite found in hypertensive subjects. Phytochemistry **27:** 369–372, 1988.

CHEN, C-C., AND LIN-SHIAU, S-Y.: Mode of inhibitory action of mellitin on Na^+-K^+-ATPase activity of the rat synaptic membrane. Biochem. Pharmacol. **34:** 2335–2341, 1985.

CLARKSON, E. M., AND DE WARDENER, H. E.: Observations on a low molecular weight natriuretic and Na-K-ATPase inhibitory material in urine. Clin. Exp. Hypertens. **A7:** 673–683, 1985.

CLOIX, J-F.: What is new in the chemistry of endogenous digitalis-like compounds? News Physiol. Sci. **5:** 204–207, 1990.

CLOIX, J. F., CRABOS, M., GRICHOIS, M. L., AND MEYER, P.: An endogenous digitalis-like compound extracted from human urine biochemical and chemical studies. Can. J. Physiol. Pharmacol. **65:** 1522–1527, 1987.

CLOIX, J-F., CRABOS, M., WAINER, I. W., RUEGG, U., SEILER, M., AND MEYER, P.: High yield-purification of a urinary Na^+-pump inhibitor. Biochem. Biophys. Res. Commun. **131:** 1234–1240, 1985.

CRABOS, M., WAINER, I. W., AND CLOIX, J. F.: Measurement of endogenous Na^+,K^+-ATPase inhibitors in human plasma and urine using high-performance liquid chromatography. FEBS Lett. **176:** 223–228, 1984.

DAHL, L. K., KNUDSEN, K. D., AND IWAI, J.: Humoral transmission of hypertension: evidence from parabiosis. Circ. Res. **14 and 15** (Suppl. I): I-21–I33, 1969.

DASGUPTA, A., MALIK, S., AHMAD, S., AND KENNY, M.: Mass spectrometry studies of a novel digoxin-like substance (DLIS-2) isolated from human plasma ultrafiltrate. Biochem. Biophys. Res. Commun. **152:** 1435–1440, 1988.

DASGUPTA, A., YEO, K-T., MALIK, S., SANDU, P., AHMAD, S., AND KENNY, M.: Two novel endogenous digoxin-like immunoreactive substances isolated from human plasma ultrafiltrate. Biochem. Biophys. Res. Commun. **148:** 623–628, 1987.

DE MENDONCA, M., GRICHIS, M-L., PERNOLLET, M. G., WAUQUIER, I., TROUILLET-THORMANN, B., MEYER, P., DEVYNCK, M-A., AND GARAY, R.: Antihypertensive effect of canrenone in a model where endogenous ouabain-like factors are present. J. Cardiovasc. Pharmacol. **11:** 75–83, 1988.

DE POVER, A.: Endogenous digitalis-like factor and inotropic receptor sites in rat heart. Eur. J. Pharmacol. **99:** 365–366, 1984.

DE POVER, A., CASTANEDA-HERNANDEZ, G., AND GODFRAIND, T.: Water versus acetone-HCl extraction of digitalis-like factor from guinea-pig heart. Biochem. Pharmacol. **31:** 267–271, 1982.

DE WARDENER, H. E., ALAGHBABD-ZADEH, J., BITENSKY, L., CHAYEN, J., CLARKSON, E. M., AND MACGREGOR, G. A.: Effect of sodium intake on ability of human plasma to inhibit renal Na-K-adenosine triphosphatase *in vitro*. Lancet **1:** 411–412, 1981.

DE WARDENER, H. E., AND CLARKSON, E. M.: Concepts of natriuretic hormones. Physiol. Rev. **65:** 658–759, 1985.

DE WARDENER, H. E., AND MACGREGOR, G. A.: Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. Kidney Int. **18:** 1–9, 1980.

DONNERER, J., OKA, K., BROSSI, A., RICE, K. C., AND SPECTOR, S.: Presence and formation of codeine and morphine in the rat. Proc. Natl. Acad. Sci. USA **83:** 4566–4567, 1986.

DOUCET, A., AND BARLET, C.: Evidence for differences in the sensitivity to

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ouabain of Na-K-ATPase along the nephrons of rabbit kidney. J. Biol. Chem. **261:** 993–995, 1986.

ELIADES, D., SWINDALL, B., JOHNSTON, J., PAMNANI, M., AND HADDY, F.: Hemodynamic effects of bufalin in the anesthetized dog. Hypertension **13:** 690–695, 1989.

ENGLISH, L. H., AND CANTLEY, L. C.: Delta endotoxin is a potent inhibitor of the (Na,K)-ATPase. J. Biol. Chem. **261:** 1170–1173, 1986.

FAGOO, M., BRAQUET, P., ROBIN, J. P., ESANU, A., AND GODFRAIND, T.: Evidence that mammalian lignans show endogenous digitalis-like activities. Biochem. Biophys. Res. Commun. **134:** 1064–1070, 1986.

FAGOO, M., AND GODFRAIND, T.: Further characterization of cardiodigin, Na^+,K^+-ATPase inhibitor extracted from mammalian tissues. FEBS Lett. **184:** 150–154, 1985.

FAVRE, H., SIEGENTHALER, G., MARTIN, B., ROTH, D., GRANGES, G., AND LOUIS, F.: Receptor-assay for endogenous inhibitors of Na-K ATPase. Klin. Wochenschr. **65** (Suppl. VIII): 49–52, 1987.

FINOTTI, P., AND PALATINI, P.: Canrenone as a partial agonist at the digitalis receptor site of sodium-potassium-activated adenosine triphosphatase. J. Pharmacol. Exp. Ther. **217:** 784–790, 1981.

FISHMAN, M. C.: Endogenous digitalis-like activity in mammalian brain. Proc. Natl. Acad. Sci. USA **76:** 4661–4663, 1979.

FLIER, J., EDWARDS, M. W., DALY, J. W., AND MYERS, C. W.: Widespread occurrence in frogs and toads of skin compounds interacting with the ouabain site of Na^+,K^+-ATPase. Science **208:** 503–505, 1980.

FOZZARD, H. A., AND SHEETS, M. F.: Cellular mechanism of action of cardiac glycosides. J. Am. Coll. Cardiol. **5:** 10A–15A, 1985.

FROM, A. H. L., FULLERTON, D. S., AND AHMED, K.: Digitalis receptor sugar binding site characteristics: a model based upon studies of Na^+,K^+-ATPase preparations with differing digitalis sensitivities. Mol. Cell. Biochem. **94:** 157–165, 1990.

GENARD, P., PALEM-VLIERS, M., LOMBA-PIGNON, M-R., CHRISTAENS, AND DE GRAEVE, J.: Identification of biologically active natural spirolactone derivatives in man and animal. Arch. Int. Physiol. Biochim. **94:** 45–55, 1986.

GIUNTA, C., FERRERO, P., PIZZUTO, M., CAVALETTO, M., PESSIONE, E., AND BRACCHINO, P.: Modulatory effect of some steroid hormones, their glucuronides and ouabain-like compounds on *Cavia cobaya* kidney Na^+,K^+-ATPase activity. Gen. Pharmacol. **21:** 435–442, 1990.

GODFRAIND, T., AND FAGOO, M.: Endogenous digitalis, cardiodigin and other regulators of the sodium pump. *In* Cardiac Glycosides 1785–1985, ed. by E. Erdmann, K. Greeff, and J. C. Skou, pp. 143–155, Steinkopff, Darmstadt, Germany, 1986.

GONICK, H. C., KRAMER, H. J., PAUL, W., AND LU, E.: Circulating inhibitor of sodium-potassium-activated adenosine triphosphatase after expansion of extracellular fluid volume in rats. Clin. Sci. Mol. Med. **53:** 329–334, 1977.

GOODFRIEND, T. L., BALL, D. B., WEINBERGER, M. H., MOORE, T. J., WEDER, A. B., AND EGAN, B. E.: Salt loads raise plasma fatty acids and lower insulin. Hypertension **17:** 958–964, 1991.

GOTO, A., ISHIGURO, T., YAMADA, K., ISHII, M., YOSHIOKA, M., EGUCHI, C., SHIMORA, M., AND SUGIMOTO, T.: Isolation of a urinary digitalis-like factor indistinguishable from digoxin. Biochem. Biophys. Res. Commun. **173:** 1093–1101, 1990a.

GOTO, A., YAMADA, K., ISHII, M., AND SUGIMOTO, T.: How best to detect endogenous digitalis-like factor? Clin. Chem. **34:** 2392–2393, 1988a.

GOTO, A., YAMADA, K., ISHII, M., AND SUGIMOTO, T.: Digitalis-like activity in human plasma: relation to blood pressure and sodium balance. Am. J. Med. **89:** 420–426, 1990b.

GOTO, A., YAMADA, K., ISHII, M., SUGIMOTO, T., AND YOSHIOKA, M.: Immunoreactivity of endogenous digitalis-like factors. Biochem. Pharmacol. **41:** 1261–1263, 1991a.

GOTO, A., YAMADA, K., ISHII, M., YOSHIOKA, M., ISHIGURO, T., EGUCHI, C., AND SUGIMOTO, T.: Presence of digitalis-like factor in mammalian plasma. Biochem. Biophys. Res. Commun. **152:** 322–327, 1988b.

GOTO, A., YAMADA, K., ISHII, M., YOSHIOKA, M., ISHIGURO, T., EGUCHI, C., AND SUGIMOTO, T.: Purification and characterization of human urine-derived digitalis-like factor. Biochem. Biophys. Res. Commun. **154:** 847–853, 1988c.

GOTO, A., YAMADA, K., ISHII, M., YOSHIOKA, M., ISHIGURO, T., EGUCHI, C., AND SUGIMOTO, T.: Urinary sodium pump inhibitor raises cytosolic free calcium concentration in rat aorta. Hypertension **13:** 916–921, 1989.

GOTO, A., YAMADA, K., ISHII, M., YOSHIOKA, M., ISHIGURO, T., AND SUGIMOTO, T.: The effects of urinary digitalis-like factor on cultured vascular smooth muscle cells. Hypertension **11:** 645–650, 1988d.

GOTO, A., YAMADA, K., AND SUGIMOTO, T.: Endogenous digitalis: reality or myth? Life Sci. **48:** 2109–2118, 1991b.

GOTO, A., YAMADA, K., YAGI, N., CHEN, H., AND SUGIMOTO, T.: Digoxin-like immunoreactivity: is it still worth measuring? Life Sci. **49:** 1667–1678, 1991c.

GOTO, A., YAMADA, K., YAGI, N., CHEN, H., TERANO, Y., AND SUGIMOTO, T.: Ouabain as the endogenous digitalis-like factor in animals? Clin. Chem. **38:** 161–162, 1992.

GOTTLIEB, S. S., ROGOWSKI, A. C., WEINBERG, M., KRICHTEN, C. M., HAMILTON, B. P., AND HAMLYN, J. M.: Elevated concentrations of a ouabain-like compound in congestive heart failure. Circulation **84:** II-54, 1991.

GRAVES, S. W.: Endogenous digitalis-like factors. Crit. Rev. Clin. Lab. Sci. **23:** 177–200, 1986.

GRAVES, S. W.: The possible role of digitalislike factors in pregnancy-induced hypertension. Hypertension **10** (Suppl. I): I-84–I-86, 1987.

GRAVES, S. W., AND WILLIAMS, G. H.: Endogenous digitalis-like natriuretic factors. Annu. Rev. Med. **38:** 433–444, 1987.

GRUBER, K. A., AND BUCKALEW, V. M., JR.: Further characterization and evidence for a precursor in the formation of plasma antinatriuretic factor. Proc. Soc. Exp. Biol. Med. **159:** 463–467, 1978.

GRUBER, K. A., WHITAKER, J. M., AND BUCKALEW, V. M., JR.: Endogenous digitalis-like substance in plasma of volume-expanded dogs. Nature **287:** 743–745, 1980.

GUPTA, R. S.: Mutants of HeLa cells resistant to ouabain and cassaine: genetic evidence for the common site of action of cardiac glycosides and erythrophleum alkaloids. Biochem. Pharmacol. **30:** 3039–3044, 1981.

GUPTA, R. S., CHOPRA, A., AND TROTT, A. B.: Development of a specific assay for cardiac glycoside-like compounds based on cross-resistance of human cell mutants. Biochem. Pharmacol. **38:** 2159–2168, 1989.

GUTHRIE, G. P.: Effects of digoxin on responsiveness to the pressor actions of angiotensin and norepinephrine in man. J. Clin. Endocrinol. Metab. **58:** 76–80, 1984.

HABER, E., AND HAUPERT, G. T.: The search for a hypothalamic Na,K-ATPase inhibitor. Hypertension **9:** 315–324, 1987.

HADDY, F. J.: Endogenous digoxin-like factors. ISI Atlas Sci. Pharmacol. **1:** 119–123, 1987.

HADDY, F. J.: Digitalislike circulating factor in hypertension: potential messenger between salt balance and intracellular sodium. Cardiovasc. Drugs Ther. **4:** 343–349, 1990.

HADDY, F. J., AND OVERBECK, H. W.: The role of humoral agents in volume expanded hypertension. Life Sci. **19:** 935–948, 1976.

HALLAQ, H. A., AND HAUPERT, G. T.: Positive inotropic effects of the endogenous Na^+/K^+-transporting ATPase inhibitor from the hypothalamus. Proc. Natl. Acad. Sci. USA **86:** 10080–10084, 1989.

HALPERIN, J. A., BRUGNARA, C., KOPIN, A. S., INGWALL, J., AND TOSTESON, D. C.: Increased digitalis-like activity in human cerebrospinal fluid after expansion of the extracellular fluid volume. Life Sci. **37:** 561–566, 1985.

HALPERIN, J. A., RIORDAN, J. F., AND TOSTESON, D. C.: Characteristics of an inhibitor of the Na^+,K^+-ATPase pump in human cerebrospinal fluid. J. Biol. Chem. **263:** 636–651, 1988.

HAMLYN, J. M.: Endogenous digitalis: where are we? ISI Atlas Sci. Pharmacology **2:** 339–344, 1988.

HAMLYN, J. M.: Increased levels of a humoral digitalis-like factor in deoxycorticosterone acetate-induced hypertension in the pig. J. Endocrinol. **122:** 409–420, 1989.

HAMLYN, J. M.: A humoral digitalis-like factor in man: kinetic inferences for its possible role in natriuresis. *In* Diuretics. III. Chemistry, Pharmacology, and Clinical Applications, ed. by J. B. Puschett, pp. 648–656, Elsevier, Amsterdam, The Netherlands, 1990.

HAMLYN, J. M., ASHEN, M. D., FORREST, B., ROGOWSKI, A. C., AND WHITE, R. J.: Species sensitivity of the sodium pump to a circulating ouabain-like inhibitor in acute hypervolemia and DOCA hypertension: Comparison with ouabain. Prog. Biochem. Pharmacol. **23:** 22–34, 1988.

HAMLYN, J. M., BLAUSTEIN, M. P., BOVA, S., DUCHARME, D. W., HARRIS, D. W., MANDEL, F., MATHEWS, W. R., AND LUDENS, J. H.: Identification and characterization of a ouabain-like compound from human plasma. Proc. Natl. Acad. Sci. USA **88:** 6259–6263, 1991.

HAMLYN, J. M., HARRIS, D. W., CLARK, M. A., ROGOWSKI, A. C., WHITE, R. J., AND LUDENS, J. H.: Isolation and characterization of a sodium pump inhibitor from human plasma. Hypertension **13:** 681–689, 1989a.

HAMLYN, J. M., HARRIS, D. W., AND LUDENS, J. H.: Digitalis-like activity in human plasma. J. Biol. Chem. **264:** 7395–7404, 1989b.

HAMLYN, J. M., RINGEL, R., SCHAEFFER, J., LEVINSON, P. D., HAMILTON, B. P., KOWARSKI, A. A., AND BLAUSTEIN, M. P.: A circulating inhibitor of (Na^++K^+)ATPase associated with essential hypertension. Nature **300:** 650–652, 1982.

HAMLYN, J. M., SCHENDEN, J. A., ZYREN, J., AND BACZYNSKJ, L.: Purification and characterization of digitalislike factors from human plasma. Hypertension **10** (Suppl. I): I-71–I-77, 1987.

HARRIS, D. W., CLARK, M. A., FISHER, J. F., HAMLYN, J. M., KOLBASA, K. P., LUDENS, J. H., AND DUCHARME, D. W.: Development of an immunoassay for endogenous digitalislike factor. Hypertension **17:** 936–943, 1991.

HAUPERT, G. T.: Physiological inhibitors of Na,K-ATPase: concept and status. In The Na^+,K^+-Pump, Part B: Cellular Aspects, pp. 297–320, Alan R. Liss, Inc., New York, 1988.

HAUPERT, G. T., JR., CARILLI, C. T., AND CANTLEY, L. C.: Hypothalamic sodium-transport inhibitor is a high affinity reversible inhibitor of Na^+,K^+-ATPase. Am. J. Physiol. **247:** F919–F924, 1984.

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HAUPERT, G. T., AND SANCHO, J. M.: Sodium transport inhibitor from bovine hypothalamus. Proc. Natl. Acad. Sci. USA **76**: 4658–4660, 1979.

HERRERA, V. L. M., CHOBANIAN, A. V., AND RUIZ-OPAZO, N.: Isoform-specific modulation of Na^+,K^+-ATPase alpha-subunit gene expression in hypertension. Science **241**: 221–223, 1988.

HERRERA, V. L. M., EMANUEL, J. R., RUIZ-OPAZO, N., LEVENSON, R., AND NADAL-GINARD, B.: Three differentially expressed Na,K-ATPase alpha subunit isoforms: structural and functional implications. J. Cell. Biol. **105**: 1855–1865, 1987.

HNATOWICH, M., AND LABELLA, F.: Endogenous digitalis-like factors: in vitro comparison of biological and immunological activities of peptides and steroid candidates. Eur. J. Pharmacol. **106**: 567–575, 1985.

HUGHES, J., SMITH, T., KOSTERLITZ, H., FOTHERGILL, L., MORGAN, B., AND MORRIS, H.: Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature **258**: 577–579, 1975.

HYMAN, A. L., JAQUES, W. E., AND HYMAN, E. S.: Observation on the direct effect of digoxin on renal excretion of sodium and water. Am. Heart J. **52**: 592–608, 1956.

ILLESCAS, M., RICOTE, M., MENDEZ, E., ROBLES, R. G., AND SANCHO, J. M.: Partial purification of a sodium inhibitor from bovine adenohypophysis. Its comparison with the natriuretic factor isolated from hypothalamus. Clin. Exp. Hypertens. **A10** (Suppl. 1): 301–307, 1988.

ILLESCAS, M., RICOTE, M., MENDEZ, E., ROBLES, R. G., AND SANCHO, J. M.: Complete purification of two identical Na^+-pump inhibitors isolated from bovine hypothalamus and hypophysis. FEBS Lett. **261**: 436–440, 1990.

INAMI, K., TESHIMA, T., EMURA, J., AND SHIBA, T.: Synthesis of lysophosphatidylserine with 19:4 acyl group, as a novel sodium-potassium ATPase inhibitor, in relation to DLIS-2, an endogenous digoxin-like substance. Tetrahedron Lett. **31**: 4033–4036, 1990.

JAMES-KRACKE, M. R., KRACKE, G. R., AND LANG, S.: Identification of a vasoactive substance (vasopressin) in a brain extract containing an unknown inhibitor of Na^+,K^+-ATPase. Clin. Exp. Hypertens. **3**: 523–538, 1981.

KELLY, R. A.: Excretion of artifactual endogenous digitalis-like factors. Am. J. Physiol. **251**: H205–H209, 1986.

KELLY, R. A., O'HARA, D. S., CANESSA, M. L., MITCH, W. E., AND SMITH, T. W.: Characterization of digitalis-like factors in human plasma. J. Biol. Chem. **260**: 11396–11405, 1985.

KELLY, R. A., O'HARA, D. S., MITCH, W. E., AND SMITH, T. W.: Identification of NaK-ATPase inhibitors in human plasma as nonesterified fatty acids and lysophospholipids. J. Biol. Chem. **261**: 11704–11711, 1986.

KELLY, R. A., AND SMITH, T. W.: The search for the endogenous digitalis: an alternative hypothesis. Am. J. Physiol. **256**: C937–C950, 1989.

KHATTER, J. C., AGBANYO, M., AND HOESCHEN, R. J.: Endogenous digitalis-like substance in pig left ventricle. Life Sci. **39**: 2483–2492, 1986.

KHATTER, J. C., AGBANYO, M., AND NAVARATNAM, S.: Endogenous inotropic substance from heart tissue has digitalis-like properties. Life Sci. **48**: 387–396, 1991.

KIM, R-S., S., AND LABELLA, F. S.: Endogenous ligands and modulators of the digitalis receptor: some candidates. Pharmacol. Ther. **14**: 391–409, 1981.

KLINGMULLER, D., WEILER, E., AND KRAMER, H. J.: Digoxin-like natriuretic activity in the urine of salt loaded healthy subjects. Klin. Wochenschr. **60**: 1249–1253, 1982.

KRACKE, G. R.: Na,K ATPase inhibitor from guinea pig brain is not ouabain-like. J. Lab. Clin. Med. **101**: 105–113, 1983.

KRAMER, H. J., BAECKER, A., SCHUERMANN, J., WEILER, E., AND KLINGMULLER, D.: Further characterization of the endogenous natriuretic and digoxin-like immunoreacting activities in human urine. Regul. Pept. 4 (Suppl. 4): 124–127, 1985.

KRAMER, H. J., BAECKER, A., WEILER, E., AND LIDDIARD, C.: Studies on ouabain-like endogenous natriuretic factors in human urine. Inhibition of Na-K-ATPase and ^3H-ouabain binding. Klin. Wochenschr. **64**: 760–766, 1986.

KRAMER, H. J., MEYER-LEHNERT, H., MICHEL, H., AND PREDEL, H-G.: Endogenous natriuretic and ouabain-like factors. Their role in body fluid volume and blood pressure regulation. Am. J. Hypertens. **4**: 81–89, 1991.

KUSKE, R., MORETH, K., RENNER, D., WIZEMANN, V., AND SCHONER, W.: Sodium pump inhibitor in the serum of patients with essential hypertension and its partial purification from hemofiltrate. Klin Wochencahr. **65** (Suppl. VIII): 53–59, 1987.

LABELLA, F. S.: Endogenous digitalis-like factors. Introductory remarks. Fed. Proc. **44**: 2780–2781, 1985.

LABELLA, F. S., BIHLER, I., TEMPLETON, J., KIM, F S., HNATOWICH, M., AND ROHRER, D.: Progesterone derivatives that bind to the digitalis receptor: effects on Na^+,K^+-ATPase and isolated tissues. Fed. Proc. **44**: 2806–2811, 1985.

LABELLA, F. S., TEMPLETON, J. F., KUMAR, V. P. S., AND BOSE, D.: Progesterone derivatives that bind to the digitalis receptor. Trends Pharmacol. Sci. **10**: 11–14, 1989.

LAMB, J. F., AND MCCALL, D.: Effect of prolonged ouabain treatment on Na, K, Cl and Ca concentration and fluxes in cultured human cells. J. Physiol. **225**: 599–617, 1972.

LAU, B. W. C., AND VALDES, R., JR.: Criteria for identifying endogenous compounds as digoxin-like immunoreactive factors in humans. Clin. Chim. Acta **175**: 67–78, 1988.

LICHTSTEIN, D., KACHALSKY, S., AND DEUTSCH, J.: Identification of a ouabain-like compound in toad skin and plasma as a bufodienolide derivative. Life Sci. **38**: 1261–1270, 1986.

LICHTSTEIN, D., MINC, D., BOURRIT, A., DEUTSCH, J., KARLISH, S. J. D., BELMAKER, H., RIMON, R., AND PALO, J.: Evidence for the presence of 'ouabain like' compound in human cerebrospinal fluid. Brain Res. **325**: 13–19, 1985.

LICHTSTEIN, D., AND SAMUELOV, S.: Endogenous 'ouabain like' activity in rat brain. Biochem. Biophys. Res. Commun. **96**: 1518–1523, 1980.

LICHTSTEIN, D., AND SAMUELOV, S.: Membrane potential changes induced by the ouabain-like compound extracted from mammalian brain. Proc. Natl. Acad. Sci. USA **79**: 1453–1456, 1982.

LICHTSTEIN, D., SAMUELOV, S., DEUTSCH, J., XU, H., LUTZ, R. A., CHERNICK, S. S., AND CHERNICK, S. S.: The ouabain receptor in animal tissues and its endogenous ligand. Klin. Wochenschr. **65** (Suppl. VIII): 40–48, 1987.

LICHTSTEIN, D., SAMUELOV, S., GATI, I., FELIX, A. M., GABRIEL, T. F., AND DEUTSCH, J.: Identification of 11,13-dihydroxy-14-octadecaenoic acid as a circulating Na^+/K^+-ATPase inhibitor. J. Endocrinol. **128**: 71–78, 1991.

LONGERICH, L., BRENT, D. A., JOHNSON, R. L., VASDEV, S., AND GAULT, M. H.: Identification of progesterone and cortisol as immunoreactive plasma digitalis like factors in pregnancy. Res. Commun. Chem. Pathol. Pharmacol. **59**: 383–393, 1988.

LUDENS, J. H., CLARK, M. A., DUCHARME, D. W., HARRIS, D. W., LUTZKE, B. S., MANDEL, F., MATHEWS, W. R., SUTTER, D. M., AND HAMLYN, J. M.: Purification of an endogenous digitalislike factor from human plasma for structural analysis. Hypertension **17**: 923–929, 1991.

LUFT, F. C., ARONOFF, G. R., FINEBERG, N. S., AND WEINBERGER, M. H.: Effects of oral calcium, potassium, digoxin, and nifedipine on natriuresis in normal humans. Am. J. Hypertens. **2**: 14–19, 1989.

MAIXENT, J. M., FENARD, S., AND KAWAMOTO, R. M.: Tissue localization of active Na, K-ATPase isoenzymes by determination of their profile of inhibition with ouabain, digoxin, digitoxigenin and LND 796, a new aminosteroid cardiotonic. J. Recept. Res. **11**: 687–698, 1991.

MALINI, P. L., STROCCHI, E., MARATA, A. M., AND AMBROSIONI, E.: Digitalis 'receptors' during chronic digoxin treatment. Clin. Exp. Pharmacol. Physiol. **11**: 285–290, 1984.

MARGARGAL, W. W., AND OVERBECK, H. W.: Effect of hypertensive rat plasma on ion transport of cultured vascular smooth muscle. Am. J. Physiol. **251**: H984–H990, 1986.

MARTIN, B., AND FAVRE, H.: Natriuretic factor exerts a ouabain-like activity in the rat colon in vitro. Pflugers Arch. **400**: 300–305, 1984.

MASFERRER, J., AND MULLANE, K. M.: Modulation of vascular tone by 12(R)-, but not 12(S)-hydroxyeicosatetraenoic acid. Eur. J. Pharmacol. **151**: 487–490, 1988.

MASFERRER, J., RIOS, A. P., AND SCHWARTZMAN, M. L.: Inhibition of renal, cardiac and corneal (Na^+-K^+)ATPase by 12(R)-hydroxyeicosatetraenoic acid. Biochem. Pharmacol. **39**: 1971–1974, 1990.

MASUGI, F., OGIHARA, T., HASEGAWA, T., AND KUMAHARA, Y.: Ouabain-like and non-ouabain-like factors in plasma of patients with essential hypertension. Clin. Exp. Hypertens. **A9**: 1233–1242, 1987.

MASUGI, F., OGIHARA, T., HASEGAWA, T., TOMII, A., NAGANO, M., HIGASHIMORI, K., KUMAHARA, Y., AND TERANO, Y.: Circulating factor with ouabain-like immunoreactivity in patients with primary aldosteronism. Biochem. Biophys. Res. Commun. **135**: 41–45, 1986.

MASUGI, F., OGIHARA, T., SAKAGUCHI, K., AND KUMAHARA, Y.: Partial purification and properties of a plasma ouabain-like inhibitor of Na^+,K^+-ATPase in patients with essential hypertension. J. Hypertens. **6** (Suppl. 4): S351–S353, 1988.

MATHEWS, W. R., DUCHARME, D. W., HAMLYN, J. M., HARRIS, D. W., MANDEL, F., CLARK, M. A., AND LUDENS, J. H.: Mass spectral characterization of an endogenous digitalislike factor from human plasma. Hypertension **17**: 930–935, 1991.

MELBY, L. C., DALE, S. L., HOLDBROOK, M., AND GRIFFING, G. T.: 19-Nor-corticosteroids in experimental and human hypertension. Clin. Exp. Hypertens. **A4**: 1851–1867, 1982.

MILLETT, J. A., HOLLAND, S. M., ALAGHBAND-ZADEH, J., AND DE WARDENER, H. E.: Extraction and characterization of a cytochemically assayable Na^+/K^+-ATPase inhibitor/glucose-6-phosphate dehydrogenase stimulator in the hypothalamus and plasma of man and the rat. J. Endocrinol. **112**: 299–303, 1987.

MIR, M. A., MORGAN, K., CHAPPEL, S., LEWIS, M., SPURLOCK, G., COLLINS, P., COLLINS, P., LEWIS, M., AND SCANLON, M.: Calcium retention and increased vascular reactivity caused by a hypothalamic sodium transport inhibitor. Clin. Sci. **75**: 197–202, 1988.

MONTALI, U., BALZAN, S., AND GHIONE, S.: Purification of endogenous digitalis-like factor(s) from cord blood of neonate by immunoaffinity chromatography. Biochem. Int. **25**: 853–859, 1991.

MORETH, K., RENNER, D., AND SCHONER, W.: A quantitative receptor assay for

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"digitalis-like" compounds in serum. Demonstration of raised concentrations in essential hypertension and correlation with arterial blood pressure. Klin. Wochenschr. 65: 179–184, 1986.

Morgan, K., Lewis, M. D., Spurlock, G., Collins, P. A., Foord, S. M., Southgate, K., Scanlon, M. F., and Mir, M. A.: Characterization and partial purification of the sodium-potassium-ATPase inhibitor released from cultured rat hypothalamic cells. J. Biol. Chem. 260: 13595–13600, 1985.

Mulvany, M. J.: Changes in sodium pump activity and vascular contraction. J. Hypertens. 3: 429–436, 1985.

Mulvany, M. J., and Aalkjar, C.: Vasoconstrictor effects of ouabain: mechanism of inhibition. Klin. Wochenschr. 63 (Suppl. III): 143–146, 1985.

Nakamura, H., Kobayashi, J., Nakamura, Y., and Ohizumi, Y.: Theonellamine B, a novel peptidal Na,K-ATPase inhibitor, from an Okinawan marine sponge of the genus *Theonella*. Tetrahedon Lett. 27: 4319–4322, 1986.

Neufeld, E., Sklarz, B., Goldberg, S., Gilad, S., Goldfarb, H., Laurian, L., Silverberg, D. S., and Chayen, R.: Observations on the chemical and physiological properties of urodiolenone, an urinary compound found in hypertension. Nephron 39: 146–148, 1985.

Ng, L. L., and Hockaday, T. D. R.: Non-esterified fatty acids may regulate human leucocyte sodium pump activity. Clin. Sci. 71: 737–742, 1986.

Ng, Y-C., Akera, T., Han, C-S., Braselton, W. E., Kennedy, R. H., Temma, K., Brody, T. M., and Sato, P. H.: Ascorbic acid: an endogenous inhibitor of isolated Na^+,K^+-ATPase. Biochem. Pharmacol. 34: 2525–2530, 1985.

Nirasawa, Y., Temma, K., Fink, G. D., and Akera, T.: Inability of Na^+,K^+-ATPase inhibitor to cause hypertension in sodium-loaded or deoxycorticosterone-treated one kidney rats. Life Sci. 37: 767–774, 1985.

Overbeck, H. W.: Salt and essential hypertension. *In* Essential Hypertension as an Endocrine Disease, ed. by C. R. W. Edwards and R. M. Carey, pp. 97–131, Butterworths, London, United Kingdom, 1985.

Overbeck, H. W., Pamnani, M. B., Akera, T., Brody, T. M., and Haddy, F. J.: Depressed function of a ouabain-sensitive sodium-potassium pump activity in blood vessels from renal hypertensive dogs. Circ.Res. 38 (Suppl. II): 48–52, 1976.

Pamnani, M., Haddy, F., Eliades, D., and Swindall, B.: A comparison of the acute effects of two putative endogenous Na^+,K^+-ATPase inhibitors on cardiorenal function in the dog and rat. Hypertension 17: 438–439, 1991.

Panet, R. M., Fixler, R., Snyder, D., Raz, S., Atlan, H., Eilam, Y., and Hasin, Y.: Role of the $Na^+/K^+/Cl^-$ transporter in the positive inotropic effect of ouabain in cardiac myocytes. J. Cell. Physiol. 145: 24–29, 1990.

Plante, G. E., Prevost, C., Chainey, A., Braquet, P., and Sirois, P.: Diuretic and natriuretic properties of prestegane B, a mammalian lignan. Am. J. Physiol. 253: R375–R378, 1987.

Poston, L.: Endogenous sodium pump inhibitors: a role in essential hypertension? Clin. Sci. 72: 647–655, 1987.

Poston, L., Wilkinson, S., Sewell, R. B., and Williams, R.: Sodium transport during the natriuresis of volume expansion: a study using peripheral blood leucocytes. Clin. Sci. 63: 243–249, 1982.

Rauch, A., and Buckalew, V. M.,Jr.: Lysophospholipids are natriuretic in rats. Am. J. Physiol. 254: F824–F827, 1988a.

Rauch, A. L., and Buckalew, V. M., Jr.: Tissue distribution of an endogenous ligand to the Na,K-ATPase molecule. Biochem. Biophys. Res. Commun. 152: 818–824, 1988b.

Rayson, B. M.: Na^+/K^+-ATPase regulation in Dahl salt-sensitive and salt-resistant rats. J. Biol. Chem. 263: 11056–11058, 1988.

Rayson, B. M.: Rates of synthesis and degradation of Na^+-KL^+-ATPase during chronic ouabain treatment. Am. J. Physiol. 256: C75–C80, 1989.

Repke, K. R. H., Est, M., and Portius, H. J.: Uber die Ursache der Speciesunterschiede in der Digitalisempfindlichkeit. Biochem. Pharmacol. 14: 1785–1802, 1965.

Repke, K. R. H., Weiland, J., and Menke, K-H.: Chemical models for the chemical nature of endogenous digitalis. J. Enzyme Inhibition 5: 25–32, 1991.

Rossier, B. C., Geering, K. G., and Kraehenbuhl, J. P.: Regulation of the sodium pump: how and why? Trends Biochem. Sci. 12: 483–487, 1987.

Sagnella, G. A., and MacGregor, G. A.: Problems in the identification and measurement of a sodium transport inhibitor in human plasma. Klin. Wochenschr. 63 (Suppl. III): 150–153, 1985.

Schoenfeld, W., Menke, K-H., Schoenfeld, R., and Repke, K. R. H.: 5 Beta, 14 beta-androstane-3 beta, 14-diol binds to the digitalis receptor site on Na/K-ATPase. J. Enzyme Inhibition 2: 37–45, 1987.

Schoner, W.: Endogenous digitalis-like factors. Trends Pharmacol. Sci. 12: 209–211, 1991.

Schreiber, V.: Endogenous digitalis-like factors revisited 1981–1985. Cor Vasa 27: 92–100, 1985.

Schreiber, V., Gregorova, I., Pribyl, T., and Stephan, T.: Digitalis-like biological activity and immunoreactivity in chromatographic fractions of rabbit adrenal extract. Endocrinol. Exp. 15: 229–230, 1981.

Schwartz, A., and Adams, R. J.: Studies on the digitalis receptor. Circ. Res. 46 (Suppl. I): I-154–I-160, 1980.

Schwartz, A., Whitmer, K., Grupp, G., Grupp, I., Adams, R. J., and Lee, S-W.: Mechanism of action of digitalis: Is the Na,K-ATPase the pharmacological receptor? Ann. NY Acad. Sci. 402: 253–271, 1982.

Schwartzman, M., Balazy, M., Masferrer, J., Abraham, N. G., McGiff, J. C., and Murphy, R. C.: 12(R)-Hydroxyicosatetraenoic acid: a cytochrome P450-dependent arachidonate metabolite that inhibits Na^+,K^+-ATPase in the cornea. Proc. Natl. Acad. Sci. USA 84: 8125–8129, 1987.

Schwartzman, M., Ferreri, N. R., Carrol, M. A., Song-Mize, E., and McGiff, J. C.: Renal cytochrome P450-related arachidonate metabolite inhibits (Na^++K^+)ATPase. Nature 314: 620–622, 1985.

Shaikh, I. M., Lau, B. W. C., Siegfried, B. A., and Valdes, R.: Isolation of digoxin-like immunoreactive factors from mammalian adrenal cortex. J. Biol. Chem. 266: 13672–13678, 1991.

Shimoni, Y., Gotsman, M., Deutsch, J., Kachalsky, S., and Lichtstein, D.: Endogenous ouabain-like compound increases heart muscle contractility. Nature 307: 369–371, 1984.

Shull, G. E., Greeb, J., and Lingrel, J. B.: Molecular cloning of three distinct forms of Na+,K+-ATPase alpha-subunit from rat brain. Biochemistry 25: 8125–8132, 1986.

Siegenthaler, G., Favre, H., Martin, B., and Roth, D.: A receptor binding assay for endoxin in human urine. Biochem. Biophys. Res. Commun. 142: 1013–1018, 1987.

Skou, J. C.: William Withering—the man and his work. *In* Cardiac glycosides 1785–1985, ed. by E. Erdmann, K. Greeff, and J. C. Skou, pp. 1–10, Steinkopff, Darmstadt, Germany, 1986.

Smith, J. B., Luft, F. C., Wade, M. B., Fineberg, N. S., and Weinberger, M. H.: Effect of short-term digoxin treatment on ouabain-sensitive sodium transport of erythrocytes. Clin. Chim. Acta 170: 89–96, 1987.

Smith, T. W.: Digitalis: mechanisms of action and clinical use. N. Engl. J. Med. 318: 358–365, 1988.

Soldin, S. J.: Digoxin-issues and controversies. Clin. Chem. 32: 5–12, 1986.

Spence, C. D., Coghlan, J. P., Whitworth, J. A., and Scoggins, B. A.: Digoxin enhances the pressor response to aldosterone administration in conscious sheep. Clin. Exp. Pharmacol. Physiol. 16: 211–222, 1989.

Stone, J. A., and Soldin, S. J.: An update on digoxin. Clin. Chem. 35: 1326–1331, 1991.

Sulcova, J., Rafter, J., and Starka, L.: 19-Nortestosterone in mouse kidney. Endocrinol. Exp. 13: 225–235, 1979.

Swann, A. C.: Free fatty acids and (Na^+,K^+)-ATPase: effects on cation regulation, enzyme conformation, and interactions with ethanol. Arch. Biochem. Biophys. 233: 354–361, 1984.

Swarts, H. G. P., Schuurmans Stekhoven, F. M. A. H., and de Pont, J. J. H. H. M.: Binding of unsaturated fatty acids to Na^+,K^+-ATPase leading to inhibition and inactivation. Biochim. Biophys. Acta. 1024: 32–40, 1990.

Sweadner, K. J.: Isoenzymes of the Na^+/K^+-ATPase. Biochim. Biophys. Acta. 988: 185–220, 1989.

Szent-Gyorgyi, A.: Chemical Physiology of Contraction in Body and Heart Muscle, Academic Press, New York, 1953.

Tal, D. M., and Karlish, S. J. D.: Do canrenone and 6,7-dihydroxylated derivatives compete with ouabain at the same site on Na,K-ATPase? Mol. Pharmacol. 34: 245–249, 1988.

Tal, D. M., Katchalsky, S., Lichtstein, D., and Karlish, S. J. D.: Endogenous "ouabain-like" activity in bovine plasma. Biochem. Biophys. Res. Commun. 135: 1–8, 1986.

Tal, D. M., Yanuck, M. D., Hall, G. V., and Karlish, S. J. D.: Identification of Na^+/K^+-ATPase inhibitors in bovine plasma as fatty acids and hydrocarbons. Biochim. Biophys. Acta. 985: 55–59, 1989.

Tamura, M., Harris, T. M., Higashimori, K., Sweetman, B. J., Blair, I. A., and Inagami, T.: Lysophosphatidylcholines containing polyunsaturated fatty acids were found as Na^+,K^+-ATPase inhibitors in acutely volume-expanded hog. Biochemistry 26: 2797–2806, 1987a.

Tamura, M., Kuwano, H., Kinoshita, T., and Inagami, T.: Identification of linoleic and oleic acids as endogenous Na^+,K^+-ATPase inhibitors from acute volume-expanded hog plasma. J. Biol. Chem. 260: 9672–9677, 1985.

Tamura, M., Lam, T-T., and Inagami, T.: Specific endogenous Na,K-ATPase inhibitor purified from bovine adrenal. Biochem. Biophys. Res. Commun. 149: 468–474, 1987b.

Tamura, M., Lam, T-T., and Inagami, T.: Isolation and characterization of a specific endogenous Na^+,K^+-ATPase inhibitor from bovine adrenal. Biochemistry 27: 4244–4253, 1988.

Teisinger, J., Janecek, J., Zemkova, H., Amler, E., and Vyskocil, F.: Inhibition of [^{3}H]ouabain binding and the electrogenic sodium pump by two fragments of calmodulin. Med. Sci. Res. 15: 189–190, 1987.

Templeton, J. F., Kumar, V. P. S., Bose, D., Smyth, D. D., Kim, R. S., and LaBella, F. S.: Digitalis-like pregnanes: cardiac and renal effects of a glycoside of 14β-hydroxyprogesterone. Can. J. Physiol. Pharmacol. 66: 1420–1424, 1988.

Thomas, R., Gray, P., and Andrews, J.: Digitalis: its mode of action, receptor, and structure-activity relationship. Adv. Drug Res. 19: 311–562, 1990.

Tobin, T., Akera, T., Brody, S. L., Ku, D., and Brody, T. M.: Cassaine: mechanism of inhibition of Na^+,K^+-ATPase and relationship of this inhibition to cardiotonic actions. Eur. J. Pharmacol. 32: 133–145, 1975.

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TORIYABE, S., MIURA, Y., KIMURA, S., MEGURO, Y., SUGAWARA, T., NOSHIRO, T., TAKAHASHI, M., OHASHI, H., SANO, E., WATANABE, H., AND YOSHINAGA, K.: A plasma inhibitor of sodium and potassium activated adenosine triphosphatase in patients with essential hypertension. Tohoku J. Exp. Med. **155:** 129–137, 1988.

TSCHESCHE, R.: Biosynthesis of cardenolides, bufadienolides and steroid sagopenins. Proc. R. Soc. Lond. B **180:** 187–202, 1972.

VASDEV, S. C., FERNANDEZ, P. G., LONGERICH, L., AND GAULT, M. H.: Higher plasma Na^+,K^+-ATPase inhibitory activity in essential hypertensive patients. Can. Cardiol. **5:** 249–254, 1989.

VASDEV, S. C., LONGERICH, L. L., ITTEL, T. H., JOHNSON, E., BARROWMAN, J. A., AND GAULT, M. H.: Bile salts as endogenous digitalis like factors. Clin. Invest. Med. **9:** 201–208, 1986.

VASDEV, S., LONGERICH, L., JOHNSON, E., BRENT, D., AND GAULT, M. H.: Dehydroepiandrosterone sulfate as a digitalis-like factor in plasma of healthy human adults. Res. Commun. Chem. Pathol. Pharmacol. **49:** 387–399, 1985.

VENTRA, C., MUSCARI, C., SPAMPINATO, S., BENARDI, P., AND CALDARERA, C. M.: Inhibitory action of opioid peptides on ouabain-sensitive Na^+-K^+ and Ca^{2+}-dependent ATPase activities in bovine cardiac sarcolemma. Peptides **8:** 709–713, 1987.

WALLICK, E. T., LANE, K. L., AND SCHWARTZ, A.: Biochemical mechanism of the sodium pump. Annu. Rev. Physiol. **41:** 397–411, 1979.

WEBB, R. C., AND BOHR, D. F.: Potassium-induced relaxation as an indicator of Na^+,K^+-ATPase activity in vascular smooth muscle. Blood Vessels **15:** 198–207, 1978.

WECHTER, W. J., AND BENAKSAS, E. J.: Natriuretic hormones. Prog. Drug Res. **34:** 231–260, 1990.

WEILAND, J., SCHOENFELD, W., MENKE, K-H., AND REPKE, K. R. H.: Interaction of progesterone derivatives with the digitalis target enzyme: impact of glycosidation of inhibitory action. Pharmacol. Res. **23:** 27–32, 1991.

WEILAND, J., SCHWABE, K., HUEBLER, D., SCOENFELD, W., AND REPKE, K. R. H.: Glycosidation of chlormadinol acetate alters its actions on Na^+,K^+-transporting ATPase and cardiac contractility: a contribution to the endogenous digitalis problem. J. Enzyme Inhibition **2:** 31–36, 1987.

WEITZ, C. L., FAULL, K. F., AND GOLDSTEIN, A.: Synthesis of the skeleton of the morphine molecule by mammalian liver. Nature **330:** 674–677, 1987.

WENZEL, M., HEIDRICH, E., KIRCH, U., MORETH-WOLFRAT, K., WIZEMANN, V., AND SCHONER, W.: Increased concentrations of a circulating sodium pump inhibitor in essential hypertension and uremia and its partial purification from hemofiltrate. Physiol. Bochemoslov. **39:** 79–85, 1990.

WHITMER, K. R., WALLICK, E. T., EPPS, D. E., LANE, L. K., COLLINS, J. H., AND SCHWARTZ, A.: Effects of extracts of rat brain on the digitalis receptor. Life Sci. **30:** 2261–2275, 1982.

WILKINS, M. R.: Endogenous digitalis: a review of the evidence. Trends Pharmacol. Sci. **6:** 286–288, 1985.

WOOLFSON, R. G., HILTON, P. J., AND POSTON, L.: Effects of ouabain and low sodium on contractility of human resistance arteries. Hypertension **15:** 583–590, 1990.

WOOLFSON, R. G., AND POSTON, L.: Effect of ouabain on endothelium-dependent relaxation of human resistance arteries. Hypertension **17:** 619–625, 1991.

YAMADA, K., GOTO, A., ISHII, M., YOSHIOKA, M., AND SUGIMOTO, T.: Dissociation of digoxin-like immunoreactivity and Na^+,K^+-ATPase inhibitory activity in rat plasma. Experientia **44:** 992–993, 1988.

YAMADA, K., GOTO, A., ISHII, M., YOSHIOKA, M., AND SUGIMOTO, T.: Further evidence for the dissociation of digoxin-like immunoreactivity from Na^+,K^+-ATPase inhibitory activity. Experientia **46:** 1041–1043, 1990.

YASUHARA, T., MORI, M., WAKAMATSU, K., AND KUBO, K.: Isolation and identification of hemin as an endogenous Na^+,K^+-ATPase inhibitor from porcine blood cells. Biochem. Biophys. Res. Commun. **178:** 95–103, 1991.

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