Endogenous Digitalislike Compounds
A Tentative Update of Chemical and Biological Studies

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SUMMARY  Endogenous digitalislike compound (or compounds) has been described as involved in some diseases. Questions remain concerning its chemical nature, origin, and biological properties. The methods of measuring the compound are based on biological properties of digitalis, mainly Na$^+$, K$^+$-adenosine triphosphatase (ATPase) inhibition and related properties. Chemically, digitalislike compound has been described as a peptide, as fatty acids, and as a steroid. Its origin could be the brain, particularly the hypothalamus and pituitary gland. The adrenal glands were also proposed as its origin. The reported biological properties of digitalislike compound are mainly dependent on Na$^+$, K$^+$-ATPase inhibition. No definitive conclusions can be drawn from the available data.

KEY WORDS  • endogenous Na$^+$, K$^+$-ATPase inhibitor • endogenous digitalislike compound • natriuretic hormone • natriuretic factor • sodium pump inhibitor

SINCE the early work of de Wardener in 1961 on the third factor with a hormonal nature, which controls sodium excretion through inhibition of tubular Na$^+$, K$^+$-adenosine triphosphatase (ATPase), numerous studies have been performed in an attempt to purify and identify chemically such a compound(s). As it is well established that Na$^+$, K$^+$-ATPase is specifically inhibited by digitalis compounds, it was tempting to speculate that endogenous digitalislike compound (EDLC) or compounds might be relevant and related to the third factor. The activity of EDLC was found to be increased in some diseases, including end-stage renal failure, active acromegaly, and experimental and essential hypertension, and controlled by sodium intake and extracellular volume.

The main questions to be addressed concern the chemical structure of EDLC, its origin, and its biological properties. We begin by reviewing our experience in this area, and then discuss the relevant findings of others. Initially, we summarize the various methods for EDLC measurement and purification.

Methods of EDLC Measurement

The first two bioassays used measured induction of natriuresis by intravenous injection in rats and inhibition of short-circuit current of anurian bladder (antinatriferic effect). The mechanism of the former was described as being inhibition of renal Na$^+$, K$^+$-ATPase. The discovery of atrial natriuretic factors complicated the understanding of the natriuretic hormone. Atrial natriuretic factors do not inhibit renal Na$^+$, K$^+$-ATPase; however, they may participate in the natriuretic action of plasma and tissue extracts containing EDLC. The antinatriferic effect of EDLC is mediated by its inhibitory action on Na$^+$, K$^+$-ATPase. These two bioassays are time-consuming and require a lot of material, and this explains the need for faster and more specific assays.

The measurement of EDLC is based on the similarities between the endogenous and exogenous digitalis compounds: 1) inhibition of Na$^+$, K$^+$-ATPase activity of renal or cerebral membrane preparations in particular; 2) inhibition of ouabain binding to whole cells, whole tissues, or purified Na$^+$, K$^+$-ATPase; 3) inhibition of sodium pump activity in different varieties of cells; 4) inhibition of $^{86}$Rb uptake (rubidium is an analogue of potassium for the sodium pump) by various types of cells or tissues; and 5) cross-reaction with antidigoxin antibodies. The data presented here were essentially based on assays of EDLC as an inhibitor of Na$^+$, K$^+$-ATPase.

Studies in Our Laboratory

EDLC from Human Plasma

We purified three different compounds from fresh human plasma obtained from a blood bank. The plas-
ma was first deproteinized by boiling and chromato-
graphed on AcA 54 and diethylaminoethyl (DEAE)-
cellulose. These compounds inhibited renal Na⁺,K⁺-ATPase activity, ouabain binding to human red blood cells, sodium transport out of human erythrocytes, and serotonin uptake by human platelets. When injected into the third ventricle of the rat, one of these compounds increased blood pressure. The chemical nature of these compounds was not studied due to the difficulties in obtaining an adequate amount of active material. Therefore, we decided to purify EDLC from human urine.

EDLC from Human Urine

Before purifying EDLC from human urine it was important to verify that our urine donors had the "same" EDLC in their plasma.

The same method of chromatography (high performance liquid chromatography, or HPLC, on octadeyl column with linear acetonitrile gradient) was used to analyze the plasma and urine of the same subjects. The plasma was resolved into three peaks with EDLC properties, and urine was resolved into two peaks. The latter had the same retention times as the first two of the three plasma EDLC peaks. In addition, when the same analyses were performed on plasma and urine of patients with essential hypertension, one EDLC peak was increased both in the plasma and urine, and these two compounds had the same retention time. These data suggested to us that in plasma and urine one EDLC peak is increased in essential hypertension, and that this plasma EDLC might be similar to that in urine.

Urine donors were screened by analysis of 24-hour urine by flash chromatography on a small disposable column filled with 40-μm octadeyl packing material with a stepwise acetonitrile gradient. The data, expressed in terms of ouabain-equivalent units excreted per 24 hours (nmol/24 hr), showed that the normotensive subjects with a family history of hypertension excreted more EDLC than normotensive controls (1.24 ± 0.11, n = 60 vs 0.48 ± 0.05, n = 58; p < 0.05). Using such a procedure we selected 35 urine donors producing the highest amounts of EDLC and collected 2000 liters of urine.

From 400 liters of urine we purified an apparent homogeneous compound having EDLC properties as follows: flash chromatography on C18 packing, anion exchange chromatography on DEAE-Trisacryl, various HPLC on C18, diphenyl and phenyl columns. The final active material was analyzed by nuclear magnetic resonance and mass spectrometry. It was hypothesized that the urine-derived EDLC could be an amino glycoesteroid with a molecular mass of 431 daltons. With increasing purity we did not confirm the presence of an amino group on the urine-derived EDLC. However, the recent results we obtained with a highly purified urine-derived EDLC confirmed that such a compound could be a glycoesteroid with a molecular mass close to 500 daltons.

Biological Properties

Semipurified urine-derived EDLC has the following biological properties: 1) it specifically and reversibly inhibits dog kidney Na⁺,K⁺-ATPase activity; 2) its inhibitory effect is reversed by potassium; 3) it is an apparent competitive inhibitor of ouabain binding to Na⁺,K⁺-ATPase; 4) it binds to the E₃P form of Na⁺,K⁺-ATPase; 5) it inhibits Na⁺,K⁺-ATPase non-competitively with adenosine 5'-triphosphate; 6) it inhibits sodium pump activity in human red blood cells; 7) it inhibits serotonin uptake by human platelets; and 8) it induces diuresis and natriuresis but not kaliuresis in a rat bioassay. It was suggested, therefore, that such an EDLC could be considered as one of the natriuretic hormones whose mechanism of action is similar to that of ouabain.

Origin of EDLC

The origin of EDLC is one of the most difficult questions to answer. A renal origin seems unlikely, as anephric patients have high plasma levels of EDLC. Measurements in rat tissue extracts suggest that the pituitary gland, hypothalamus, and adrenal glands contain the highest amounts of EDLC. Injection of adrenocorticotropic hormone (ACTH) into rat increases plasma EDLC levels. Removal of the adrenal glands induces a decrease in these levels. Incubation of rat tissue slices with Hanks' medium (J. F. Cloix, unpublished observations) suggests that the pituitary gland releases higher amounts of EDLC than the other organs tested thus far. This release is increased by long-term high sodium diet. Incubation of adrenal glands with various concentrations of ACTH demonstrates a dose-dependent increase of EDLC release; however, this increased release does not attain the basal release level of the pituitary gland.

Studies by Others

Chemical Features

Fatty Acids

The first to suggest that digitalislike compounds could be chemically similar to fatty acids were Bidard et al. They purified Na⁺,K⁺-ATPase from the electric organ of Electrophorus electricus, and from this enzyme preparation they extracted Na⁺,K⁺-ATPase inhibitors that were identified by mass spectrometry as arachidonic analogues and related unsaturated fatty acids, particularly linoleic, arachidonic, linolenic, and docosahexaenoic acids. These compounds could participate in the regulation of Na⁺,K⁺-ATPase activity, but they might not be endogenous compounds. Tamura et al. purified from the plasma of salt-loaded hogs two different compounds having EDLC properties and related them to oleic and linoleic acids. These two acids inhibit Na⁺,K⁺-ATPase with very low affinity, however. The very low specificity of these two fatty acids was reported by Lichtstein et al.

Kelly et al. described four different compounds in human plasma with digitalislike properties. They iden-
tified the active component of these fractions as long-chain nonesterified fatty acids and lysophospholipids. These lipids were present in quantities sufficient to account for all the Na\(^+\),K\(^+\)-ATPase inhibitory activity. They probably do not directly regulate Na\(^+\),K\(^+\)-ATPase in vivo under normal physiological conditions, but they may alter sodium pump activity in disease states characterized by abnormalities of lipid metabolism or plasma protein binding.

Hamlyn et al.\(^{18}\) purified from human plasma two compounds having digitalislike activity. Structural analysis by mass spectrometry suggested a major mass of 400 to 500 daltons for the first factor; the second factor was identified as lysophosphatidylcholine. They concluded that these plasma EDLCs are nonspecific inhibitors of Na\(^+\),K\(^+\)-ATPase that act by reversible perturbation of lipid-enzyme interactions required for normal catalytic activity.

**Peptides**

Some authors suggested that EDLCs extracted from various sources could be peptides. These studies were based on the sensitivity of EDLC to proteolytic enzymes or to the reaction with fluorescamine, which is a reagent specific for primary amines.

Gruber et al.\(^{19}\) purified from dog plasma two EDLCs reacting with fluorescamine. They therefore suggested that these compounds could be peptidic. Kramer et al.\(^{20}\) extracted natriuretic fractions with EDLC characteristics from the urine of salt-loaded normal subjects. Amino acid analysis revealed that both natriuretic factors directly purified and the natriuretic material bound to the digoxin antibody had four amino acids in similar ratios. The physicochemical properties, as evidenced by chromatographic and electrophoretic studies, and enzymatic inactivation suggest that the low-molecular-weight natriuretic factor(s) in human urine may be associated with a small peptide(s) of weak acidic nature.

Morgan et al.\(^{21}\) partially purified a Na\(^+\),K\(^+\)-ATPase inhibitor from the culture medium obtained from rat hypothalamic cells. Incubation of this material with dispase, carboxypeptidase A, chymotrypsin, and prolidase destroyed the inhibitory activity, whereas trypsin and leucine aminopeptidase were ineffective. They concluded that the hypothalamus releases a low-molecular-weight, heat-stable peptide with EDLC properties.

**Steroids**

Lichtstein et al.\(^{22}\) extracted a ouabainlike compound from toad skin. Using ultraviolet, nuclear magnetic resonance, and mass spectrometry, the structure of the purified compound was suggested to be resibufogenin (mono-hydroxy-14,15-epoxy-20,22-dienolide glycoside). They also presented evidence that this compound is present in toad plasma. This is the only structure of a digitalislike compound published so far. No data are available to suggest that resibufogenin is relevant in mammals.

Hnatowich and LaBella\(^{23}\) performed a screening of some steroids with respect to in vitro EDLC properties. They concluded that among the compounds reported to exhibit digitalislike activity and postulated to share structural features with an endogenous steroidal digitalislike factor, only chlormanidione acetate (a progesterone derivative), and its congeners appear to be possible candidates.

**Lignans**

Lignans are natural products, some of which were recently discovered in human urine, semen, and blood plasma. Fagoo et al.\(^{24}\) described that mammalian lignans showed EDLC activity. However, these compounds have a low affinity for Na\(^+\),K\(^+\)-ATPase.

**Origin of EDLC**

Based on tissue extract experiments, the origin of EDLC was suggested to be brain,\(^{21,25-30}\) cerebrospinal fluid,\(^{29,30}\) kidney,\(^{31,32}\) heart,\(^{33,34}\) and adrenals.\(^{13,33}\) Inside the brain, hypothalamus and pituitary glands seem to be the most likely sites of origin of the secretion of EDLC. Lesion of the anteroventral third ventricular (AV3V) area of the brain\(^{25}\) showed indeed, that the hypothalamus could either be the source of EDLC or be involved in the regulation of EDLC secretion. The data reported by Morgan et al.\(^{21}\) are the most relevant suggesting that the hypothalamus could secrete EDLC. At a satellite symposium of the 11th Congress of the International Society of Hypertension, Natriuretic Hormones in Hypertension, a great deal of information about the involvement of the brain in EDLC secretion and synthesis was presented (see Reference 16).

**Biological Properties**

Few data are available regarding the biological properties of EDLC, which should have natriuretic and inotropic effects. In general, EDLC should have an action on all processes depending on sodium pump activity.

Chakraverty et al.\(^{35}\) reported that a natriuretic fraction (possibly EDLC) extracted from human urine produced dose-dependent contractions of the isolated anococcygeus muscle of the rat. This fraction was shown to be different from catecholamines, acetycholine, serotonin, prostaglandin E, and angiotensin II. Martin and Favre\(^{36}\) extracted from the urine of salt-loaded men an EDLC that inhibited sodium transport in vitro preparations of rat colon. Shimoni et al.\(^{37,38}\) reported that EDLC, highly purified from toad skin and sheep brain, increases the force of contraction of frog and guinea pig atria\(^{37}\); and EDLCs obtained from toad skin and plasma have positive inotropic effects on frog cardiac muscle.\(^{38}\)

**Conclusion**

The chemical features of EDLC are now under investigation in various laboratories. Due to the heterogeneity of EDLC in the different sources used for its purification, it is possible that numerous EDLCs could
exist. This might explain the peptidic, lipidic, and steroidal features reported so far. Once the EDLC structures are determined and the synthetic products are available, it will be possible to perform pharmacological and physiological studies, which are now more or less impossible. The development of a drug is dependent upon studies of the mechanism of EDLC action in various tissues.

References

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Endogenous digitalislike compounds. A tentative update of chemical and biological studies.
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Hypertension. 1987;10:I67
doi: 10.1161/01.HYP.10.5_Pt_2.I67

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