Endogenous Digitalislike Compounds
A Tentative Update of Chemical and Biological Studies

JEAN-FRANCOIS CLOIX

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.

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

SINCE the early work of de Wardener\(^1\) in 1961 on the third factor with a hormonal nature,\(^2\) which controls sodium excretion through inhibition of tubular Na\(^+\), K\(^+\)-adenosine triphosphatase (ATPase),\(^3\)\(^-4\) 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 eryth-
rocytes, 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 per-
formance liquid chromatography, or HPLC, on octa-
decyl 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 in a small disposable 
column filled with 40-μm octadecyl 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 normoten-
sive 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, vari-
dous HPLC on C18, diphenyl and phenyl columns.
The final active material was analyzed by nuclear mag-
netic resonance and mass spectrometry. It was hypothe-
sized that the urine-derived EDLC could be an amino 
glycosteroid 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. How-
ever, the recent results we obtained with a highly puri-
fied urine-derived EDLC confirmed that such a com-
 pound could be a glycosteroid with a molecular mass 
close to 500 daltons.

**Biological Properties**

Semipurified urine-derived EDLC has the following 
biological properties: 1) it specifically and reversibly 
hinders 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 E\(_2\)P form of 
Na\(^+\),K\(^+\)-ATPase; 5) it inhibits Na\(^+\),K\(^+\)-ATPase non-
competitively with adenosine 5'-triphosphate; 6) it in-
hibits 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 demon-
strates a dose-dependent increase of EDLC release; 
however, this increased release does not attain the bas-
al 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 elec-
tric 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 par-
ticipate 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-
HUMAN URINARY NA\(^{+}\), K\(^{+}\)-ATPase Inhibitor/Cloix

Hnatowich and LaBella\(^{22}\) 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 chlormanidone acetate (a progestosterone 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.

Chakravarty et al.\(^{35}\) reported that a natriuretic fraction (possibly EDLC) extracted from human urine produced dose-dependent contractions of the isolated anacoccygeus muscle of the rat. This fraction was shown to be different from catecholamines, acetylcholine, 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; 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 steroidial 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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J F Cloix

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