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How Many Endobains Are There?*

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Oxidative metabolism is very active in brain, where large amounts of chemical energy as ATP molecules are consumed, mostly required to maintain cellular Na^+/K^+ gradients through the participation of the sodium pump (Na^+,K^+-ATPase), whose activity is selectively and potently inhibited by the alkaloid ouabain. Na^+/K^+ gradients are involved in nerve impulse propagation, in neurotransmitter release and cation homeostasis in the nervous system. Likewise, enzyme activity modulation is crucial for maintaining normal blood pressure and cardiovascular contractility as well as renal sodium excretion. The present article reviews the progress in disclosing putative ouabain-like substances, examines their denomination according to different research teams, tissue or biological fluid sources, extraction and purification, assays, biological properties and chemical and biophysical features. When data is available, comparison with ouabain itself is mentioned. Likewise, their potential action in normal physiology as well as in experimental and human pathology is summarized.

KEY WORDS: Endobains; ouabain-like factors; Na^+, K^+-ATPase inhibitors; sodium pump regulators; endogenous factors; brain factors.

INTRODUCTION

Oxidative metabolism is very active in brain, where large amounts of chemical energy as ATP molecules are consumed, mostly required to maintain cellular Na^+/K^+ gradients (1), which are involved in nerve impulse propagation, in neurotransmitter release and cation homeostasis in the nervous system. Na^+ extrusion from the cells requires its movement against a concentration gradient and electric potential, through an ATP-dependent mechanism, the sodium pump. Sodium pump and enzyme Na^+, K^+-ATPase (EC 3.6.1.3) (2) are chemical and mechanical manifestations of a single system (1), which is essential in normal cell cycle, in preventing cell membrane osmotic rupture as well as in nervous system differentiation (3). Likewise, modulation of such enzyme activity is crucial for maintaining normal blood pressure and cardiovascular contractility as well as renal sodium excretion.

Among Na^+, K^+-ATPase inhibitors, the alkaloid ouabain has proven both potent and selective, exerting pharmacological effects in several tissues, mainly heart and kidney (4).

The purpose of this overview was to summarize the progress performed by research teams engaged in the search for ouabain-like endogenous factors. Since previous reviews have dealt with the subject up to the beginning of the last decade (5,6), special emphasis was devoted to more recent findings. Research performed to date, particularly as regards to factor source, biological actions and chemical structure of endogenous substances that may act as ligands for the

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cardiac glycoside binding site of the sodium pump (Na^+, K^+-ATPase) is reviewed. Since information provided herein is far from exhaustive, the reader should resort to individual articles for further details.

DENOMINATION

Endogenous ouabain-like substances have been termed: endogenous digitalis-like factor (6–9) **EDLF;** digoxin- and ouabain-like immunoactivity, **DLIA** (10); endogenous digoxin-like immunoreactive factor, **DLIF** (11); digoxin-like immunoreactive substances, **DLIS** (12); endogenous digitalis-like factor, **DLF** (13); just **"ouabain"** between quotes (14,15); endogenous ouabain-like factor, **endobain E** (16,17); endogenous "ouabain", **EO** (18); uremic plasma factor, **F1** (19); hypothalamic and pituitary inhibitor factor, **HHIF** (20); hypothalamic inhibitory factor, **HIF** (21); ouabain-displacing compound, **ODC** (8); ouabain-like factors, **OLF** (22,23); ouabain-like substance, **OLS** (24); sodium-potassium pump inhibitor, **SPI** (25) and two inhibitors termed **A** and **B** (26). The suggested term **endobain** (27) is employed herein as a collective word to include the various ***endo***genous oua***bain***-like substances.

NA^+, K^+-ATPase MODIFIERS

Sodium pump or Na^+, K^+-ATPase activity requires ATP supply and activating cations, Na^+ and K^+, which are also considered as substrates. Among a wide spectrum of diverse substances, fatty acids (28,29), vanadate (30), as well as peptide molecules including insulin (31), hystidil-proline diketopiperazine (32), calcitonin (33), angiotensin 1—7 (34) and neurotensin (35) have proven enzyme modulators. As far as neurotransmitter substances are concerned, evidence indicates that some are able to modify Na^+, K^+-ATPase activity (see 3,36), particularly catecholamines norepinephrine and dopamine, which modify synaptosomal membrane Na^+, K^+-ATPase activity, behaving as enzyme inhibitors or stimulators according to the absence or presence of a brain soluble fraction during enzyme assay (37, see 38).

FACTOR SOURCES

Diverse studies have led to the isolation of sodium pump inhibitors from several tissues and biological fluids. From the central nervous system, studies were carried out in whole brain (14,39–42), cerebral cortex (16,37,43); hypothalamus (15,20,23,41,44–46), midbrain (21), medulla (15), and also from pituitary gland (20,23). Besides, factors were isolated from adrenal gland (14,26) and lens (47).

As regards biological fluids, active substances have been isolated from cerebrospinal fluid (48,49), plasma (11,18,19,23–25,46,50–55), urine (8,12,22,56) and peritoneal dialysate (57).

EXTRACTION AND PURIFICATION

As a first step, isolation procedures imply tissue homogenization in organic solvents or water. Some of the procedures start with acid acetone extraction (39–42,44) followed by gel filtration in Sephadex G-10 and desalting (39) or G-25 and ion-exchange chromatography (42,44). Another procedure starts with cerebral cortex homogenization in water followed successively by gel filtration in Sephadex G-10, Sephadex G-50 and anionic exchange HPLC (16,43).

From bovine adrenal gland, two sodium pump inhibitors were purified after extraction in methanol followed by acetone or chloroform; in either case, procedures imply ultrafiltration and several RP-HPLC runs (26).

Several authors have resorted to RP-HPLC for the purification of endogenous ouabain-like substances from serum (19) and urine (8,18), after TLC (56) or gel chromatography steps (22).

An approach employed for endobain purification from biological fluids implies affinity chromatography in which the ligand is an antibody (antiserum) to a cardiac glycoside, followed by one or more HPLC or RP-HPLC runs (12).

ASSAYS

In order to characterize endobain function, biological assays are performed at several levels, including whole tissue, and in cellular and subcellular preparations: in the whole animal to assess blood pressure (58) and diuresis (8,59); in chopped tissue for neurotransmitter release (60) and phosphoinosite hydrolysis (61); in whole cells (erythrocyte) for ^{86}Rb uptake (12,39,53, 62,63); and in cell-free systems for enzyme determination (16,23,37, 40–44,53,54) or ^3H-ouabain-binding (16, 39–42,44,64) in particulate membrane preparations. In the last case, results indicate direct action of enzyme modulators on respective receptors, regardless of ionic gradient participation.

Another approach implies immunoassays which may achieve high sensitivity and specificity, employing commercially available radioimmunoassay kits or self-produced anti-ouabain antisera. Substances cross-reacting with antibodies against ouabain (6,10,23,65), digoxin (10,11), digitoxin (see 6) and marinobufagenin (26) have been described.

Biophysical assays include: mass spectrometry, nuclear magnetic resonance spectroscopy, infrared spectroscopy, UV spectra, MW, as well as determination of HPLC retention time.

BIOLOGICAL PROPERTIES

Effect on Na^+, K^+-ATPase Activity. Enzyme determination is one of the most frequently used biological assays to study endogenous ouabain-like substances. Such assays are carried out in crude or purified membranes from central nervous system (16,37,40–43,53), kidney (23,42,44,53), or aortic sarcolemma (54). Determinations performed in the presence of Mg^{2+}, Na^+ and K^+, with and without ouabain, alow assessment of Na^+, K^+-dependent enzyme activity. As regards enzyme substrate, ATP or *p*-nitrophenylphosphate, a non-natural ATPase substrate, are currently employed. However, it should be taken into account that Na^+, K^+-ATPase or K^+-*p*-nitrophenylphosphatase activities (both ouabain-sensitive) may not necessarily provide the same information since differences in subcellular distribution (66), Km values and sensitivity to ouabain and F^- (67) have been reported.

In evaluating the effect of endobains on Na^+, K^+-ATPase inhibition, it is noteworthy that in general, though differing in binding properties, cardiac glycosides of chemical structure similar to ouabain behave similarly as enzyme inhibitors.

For different endobains, Na^+, K^+-ATPase inhibition curves are concentration-dependent (17,23,53); however, the slope of the curve may differ from (53) or strongly resemble (17,23) those for cardiac digitalis. Assays performed in the presence of endobain E and ouabain indicate that the effect is not truly additive, suggesting an interaction between endobain E and ouabain inhibitory mechanisms (17).

A comparative study of enzyme inhibition by ouabain *versus* a factor from peritoneal dialysate indicates a similar extent effect on fetal brain membranes in which $\alpha 3$ isoform is known to predominate; however, in other membrane preparations in which $\alpha 1$ (kidney) or $\alpha 2$ (skeletal muscle) isoforms are more abundant, the extent of enzyme inhibition is dissimilar (57).

Effect on Other Membrane-Bound Enzymes. To determine whether the effect is specific for Na^+, K^+-ATPase, an essential requirement for putative endobains is their failure to modify other membrane-bound enzymes.

One of the factors is known to entirely inhibit rat cardiac and renal Na^+, K^+-ATPase activities but has no detectable effects on cardiac Ca^{2+}-ATPases or ouabain-insensitive Mg^{2+}-ATPase assayed in the same membrane preparations (53).

The source fraction for endobain E isolation, peak II, besides inhibiting Na^+, K^+-ATPase activity, exerts other ouabain-like properties (5) and fails to affect other synaptosomal membrane enzymes such as Mg^{2+}-ATPase, acetylcholinesterase and 5′-nucleotidase activities (68).

Calcium pump, assayed as synaptosomal membrane Ca^{2+}-ATPase activity was inhibited by an hypothalamic factor HHIF (20) as well as by the cerebral cortex factor, endobain E (unpublished).

Na^+, K^+-ATPase Inhibition Versus ^{3}H-Ouabain Binding Blockade. The diverse endobains invariably inhibited ^{3}H-ouabain binding as detected in whole tissue (64), membrane fractions (17,39–42,44,64) or human erytrocytes (8).

For some Na^+, K^+-ATPase endogenous modulators, a close correlation between percentage enzyme inhibition and ouabain binding blockade has been found (7,40,51,69). At variance, ^{3}H-ouabain binding inhibition by endobain E was concentration-dependent over a 10-fold range, an effect similar to that found for Na^+, K^+-ATPase inhibition. However, a 50% blockade in the former corresponded with full enzyme inhibition, suggesting interaction between endobain E and ouabain inhibitory mechanisms; structural differences between endobain E and ouabain as disclosed by dissimilar UV spectra, chromatographic behaviour and alkali sensitivity (70), may well explain the differences between the extent of Na^+, K^+-ATPase inhibition and ^{3}H-ouabain binding blockade (17).

In this regard, K^+ proves to be eight-fold more effective to diminish phospho-enzyme levels than ^{3}H-ouabain binding (71) and no close correlation between the binding of cardiac glycoside ASI-222 (a digitoxigenine derivative) to Na^+, K^+-ATPase with its ability to inhibit the enzyme was found, suggesting drug binding not only to functional but also to non-functional sites when inhibiting Na^+, K^+-ATPase activity (72).

The slope of the concentration-response curve for the interaction between endobain E and Na^+, K^+-ATPase resembled that of ouabain, thus suggesting that both inhibitors may act by the same mechanism. Alternatively,

endobain E and ouabain may only partly share a common binding site, by binding to neighbouring sites rather than to the same site (17).

Interestingly, in the opioid system, in which exogenous ligands (opioid alkaloids) are chemically very different from endogenous ligands (opioid peptides), the same profile for binding competition with peptides and opioid alkaloids was recorded (73). Therefore, in this system, the binding of opioid peptides to their receptor occurs at the opioid alkaloid site.

K^+ Antagonism. The ability of K^+ to reduce phospho-enzyme levels and to stabilize a conformation with relatively low affinity for ouabain (74) as well as to antagonize cardiac glycosides binding to Na^+, K^+-ATPase (75) has been described; the magnitude of such K^+ antagonism on binding seems dependent on the lifespan of the cardiac glycoside-binding E_2P enzyme conformation (76). Antagonistic K^+ effect depends on the type of cardiac glycoside tested (77,78).

On increasing K^+ concentration to a physiological level, enzyme inhibition by ouabain was markedly diminished (9,72,79) whereas that induced by certain endobains remained unaltered (53,80), showing a dissimilar behaviour of both inhibitor types *versus* K^+.

Unlike ouabain, endobain E inhibits Na^+, K^+-ATPase activity over a wide concentration range of Na^+ (1.56–200 mM), K^+ (1.25–40 mM) and ATP (1–8 mM) (80), as well as that of K^+-*p*-nitrophenylphosphatase activity in the presence of several K^+ and substrate *p*-nitrophenylphosphate concentrations (81), when in no case inhibition proved competitive. Unexpectedly, endobain E has been found to stimulate Na^+, K^+-ATPase activity at low (0.5 mM) ATP concentration (80).

Effect on ^{86}Rb Uptake. Another way to characterize endobains is to measure ^{86}Rb uptake in intact erythrocytes (12,26,39). Inhibitory potencies of endogenous digitalis-like factors (EDLF) on Na^+, K^+-ATPase activity were only found in fractions capable of inhibiting ^{86}Rb uptake (53). Newborn plasma inhibits ^{86}Rb uptake in erythrocytes (62), an effect neutralized by anti-ouabain antibodies (63).

Effect on Neurotransmitter Release. A close relationship between Na^+, K^+-ATPase activity and neurotransmitter release has been disclosed and this enzyme seems to play a role in such mechanism (82–84). Ouabain increases the release of several neurotranmitters, including acetylcholine (82,84,85), 5-hydroxytryptamine (86) and catecholamines (87–89). Concomitantly, enzyme activating conditions decrease neurotransmitter release (84). Likewise, the ability of endogenous ouabain- like compounds to enhance neurotransmitter release has been reported (90).

A brain soluble fraction (peak II) is capable of releasing a neurotransmitter pool stored in pineal nerve synaptic vesicles, as demonstrated histochemically at electron microscope level (91). The more purified fraction endobain E increases norepinephrine release in a concentration-dependent fashion. Due to experimental conditions employed, such endobain E effect most likely occurs at presynaptic level and is independent of norepinephrine reuptake. On the whole, the effect resembles that of ouabain. It was postulated that endobain E enhances catecholamine availability in the synaptic gap, leading to an increase in noradrenergic activity (60).

Other Biological Properties. Ouabain stimulates phosphoinositide hydrolysis, an effect which is more marked in slices from neonatal than adult rats (92). Similarly, endobain E highly increases phosphoinositide hydrolysis in neonatal tissue but only slightly in adult tissue (61). This effect seems to involve not only Na^+, K^+-ATPase inhibition but also Na/Ca exchanger and a voltage-dependent Ca^{2+} channel (93).

Other properties shared by ouabain and endogenous ouabain-like substances are the increase in diuresis and natriuresis (8,59) and cardiac muscle contraction force (42) as well as enhancement of cardiovascular function (18).

Endobain E and commercial ouabain decreased specific binding of the muscarinic antagonist ^3H-quinuclidinyl benzilate to rat central nervous system membranes in a concentration-dependent manner; interestingly, such changes followed a pattern similar to the one disclosed for synaptosomal membrane Na^+, K^+-ATPase activity, suggesting that the sodium pump and cholinergic muscarinic receptor interrelate at functional level (94).

Although in general endobains share diverse biological properties with ouabain, a difference was recorded for the glutamatergic NMDA receptor on assaying ^3H-MK-801 binding to brain membranes. Whereas ouabain is able to stimulate such binding under certain experimental conditions, endobain E invariably reduces the binding (95), an effect which seems hardly attributable to enzyme inhibition (96).

CHEMICAL AND BIOPHYSICAL FEATURES

According to their chemical nature, endogenous substances may be grouped into either identical or closely resembling ouabain or else diverse non-steroidal compounds.

Ouabain and Ouabain Isomers. Endogenous digitalis-like immunoreactive factors (DLIF) with biologi-

cal and immunological properties similar to cardiotonic substances, such as digoxin, have been found in several tissues and body fluids of humans and animals (6).

An endogenous Na^+, K^+-ATPase inhibitor has been isolated from bovine hypothalamus and human plasma (HIF) and structurally characterized as an ouabain isomer (45,46). A factor which differs from ouabain in mass spectrum, in accurate mass, and in HPLC retention time has been isolated from bovine hypothalamus (20).

Sodium pump inhibitors of the bufodienolide type of cardiotonic steroids have been identified; from lens, a 19-norbufalin and a peptide derivative were isolated (47). A substance similar to amphibian marinobufogenin has been isolated from human urine (56) and another one which cross-reacts against bufodienolide proscillaridin A has been isolated from adrenal glands (26).

The presence of a factor in human plasma very similar to ouabain as determined by mass spectrometry has been reported (52,55).

One out of four plasma sodium pump inhibitors distinguishable biologically, on the basis of chromatographic, mass spectral, biochemical and physiological analyses has been shown to be a novel steroidal ouabain isomer; such substance is secreted by the adrenal cortex and enhances cardiovascular function. It has been advanced that it may belong to a broader family of novel mammalian steroids regulating the sodium pump and other processes (18).

An ouabain isomer rather than ouabain is present in bovine hypothalamus (45) and has been attributed to the same substance present in human plasma (46).

The presence of a factor identical or closely similar to ouabain in rat hypoyhalamus has been confirmed immunohistochemically (97) and biochemically in partially purified extracts (98).

In human urine, two ouabain-displacing compounds (ODC-1 and ODC-2) have been separated by RP-HPLC; the more polar ODC-1 is ubiquitously distributed in mammals, increases after salt loading in humans and behaves as a natriuretic factor with vasoactive properties, closely resembling ouabain in biological, physicochemical, and chromatographic properties. ODC-2 is indistinguishable from digoxin in proton nuclear magnetic resonance and fast atom bombardment mass spectrum (8).

Ouabain Detection. After detection of a factor indistinguishable from ouabain in human plasma (50), it has been contended that ouabain is an endogenous circulating agent (99).

Ouabain itself is present in hypothalamic and medullary neurons and mediates sympathoexcitatory and pressor responses not only to acute and chronic increases in sodium concentration in cerebrospinal fluid, but also to high sodium diet intake in SHR hypertensive patients (15). Likewise, HPLC retention time of an ouabain-like substance (OLS) purified from plasma and tissue is identical to that of standard ouabain (24).

Finally, an inhibitor termed B with molecular mass of 584 Da, which cross-reacts with antibodies against ouabain, behaves as ouabain in inhibiting erythrocyte sodium pump, in retention time in RP-C18 HPLC, molecular mass, UV and NMR spectroscopic data, jointly supporting that is actually the cardenolide ouabain (26).

Interestingly, a binding protein specific for cardiac glycosides has been detected in bovine serum (100).

Non-Steroidal Substances. Low MW factors, either non-peptidic (39,42) or peptidic in nature (41) have been described. Two inhibitors (termed A and B) of the sodium pump have been isolated from bovine adrenals. Inhibitor A has 600 Da molecular mass and maximal UV absorption at 250 nm; it cross-reacts against the bufodienolide proscillaridine A but not against the cardenolide ouabain, inhibits erythrocyte sodium pump and is slightly more hydrophilic than ouabain on RP-C18 HPLC (26).

Resembling ascorbic acid derivatives, a factor which inhibits Na^+, K^+-ATPase activity, raises intracellular free calcium and induces natriuresis has been identified in human urine; this ouabain-like factor (OLF) is chemically a vanadium diascorbate adduct, unrelated to ouabain (22).

Among non-steroid factors, endobain E is a low MW factor, highly hydrophilic, non-lipidic, non-peptidic anionic compound, acid stable but alkali labile (16), which differs from ouabain in HPLC retention time, chromatographic behavior and UV spectra (70), and most likely is an ascorbic acid derivative (81).

PHYSIOLOGICAL IMPLICATIONS

According to ^{3}H-ouabain binding affinity profile, three isoforms of the sodium pump have been identified, more extensively characterized in rat than in man (101, 102). In arterial smooth muscle, the high affinity ($\alpha 3$) isoform and the plasmalemmal Na / Ca exchanger are confined to plasmalemmal domains that overlie junctional sarcoplasmic reticulum; at variance, the low affinity ($\alpha 1$) isoform and the plasmalemmal Ca^{2+} pump are uniformly distributed in the plasma membrane (103).

In human arteries, marinobufagenin (a putative digitalis-like factor) and ouabain are able to modulate the sodium pump, exhibiting respectively greater affinity to

α1 and α3 isoforms, the latter proving more concentrated in nerve endings. These findings support the view that differential response to endogenous digitalis-like factors is attributable to specific Na^+, K^+-ATPase α-isoform behaviour (104).

Whereas several ouabain-like substances are effective as kidney enzyme inhibitors, peak II (the source fraction for endobain E), failed to inhibit kidney ATPase activity (105). This finding may receive a plausible explanation in that α1 is the main isoform in this tissue whereas all three isoforms are present in brain (106).

The distinct pattern of isoform sensitivity displayed by the various digitalis-like factors (DLFs) and ouabain further differentiates these substances and raises several physiological implications of these endogenous factors (57).

As regards brain development, it should be mentioned that Na^+, K^+-ATPase inhibition by endobain E obtained from brain of a four-day-old rat is higher than that from adult rat. Since cerebral Na^+, K^+-ATPase expression increases 10-fold during development, the effect of endobain E should be greater at early postnatal stages of development than during adult life and may play a role in neuronal development (107).

Marinobufagenin, an amphibian endogenous digitalis-like factor (EDLF), is a vasoconstrictor bufodienolide which inhibits aortic sarcolemmal Na^+, K^+-ATPase. Interestingly, marinobufagenin-like activity but not ouabain-like activity increases after treating rats for eight days with ACTH (54).

Hypoxia is a potent enhancer of endogenous substances release; this observation suggested that a Na^+, K^+-ATPase inhibitor may be involved in energy-conserving cellular adaptive responses to hypoxic or ischemic insult through ATP maintainance (21).

Since potential sodium pump regulators may be physiologically released, it is reasonable to posit their involvement in maintaining homeostasis. Some of these novel compounds may act as regulators not only of chemical neurotransmission but also natriuresis and blood pressure.

PATHOLOGICAL CONDITIONS

After acute myocardial infarction, a bufodienolide (MW ≅ 400D) has been isolated from urine and eluted from HPLC columns with the same retention time as amphibian marinobufagenin, from which it is indistinguishable. Since hypoxia stimulates endobain release, such factors may be involved in the pathogenesis of myocardial ischemia (56).

Although renal Na^+, K^+-ATPase activity remains unchanged in the low renin hypertension model, increased plasma ouabain and Na^+, K^+-ATPase inhibitory activity has been recorded, helping to explain the development of this hypertension type (25). Interestingly, sera from patients on chronic dialysis renders an endobain termed F1 which displays marked electrophysiological effects and modifies transepithelial ^{22}Na flux pattern, whereas control sera produce no change (19).

The concentration of an ouabain-like factor (OLF) has been determined in plasma and tissues from an hypertensive rat strain *versus* a normotensive one and from healthy human volunteers. A single HPLC fraction identical to ouabain was found in rat hypothalamus and hypophysis and in both rat and human plasma, whose dilution curve paralleled that of ouabain; inhibitory factor is present at larger concentrations in hypertensive rat strain, thus suggesting its pathogenetic role (98).

Studies performed both in hypertensive humans and animals have shown a correlation between plasma concentrations of endogenous digitalis-like factors and blood pressure (58,108). Furthermore, an ouabain-like compound extracted from toad skin which enhances basal and K^+-stimulated norepinephrine release from pulmonary artery has been associated to hypertension (109).

A circulating factor is increased in hypertension, which cross-reacts with antibodies against proscillaridin A; at variance with ouabain, its concentration fails to correlate with blood pressure. Although its retention time in HPLC is identical to that of ouabain, both substances differ in UV spectra (26). The ability of a factor which mediates sympathetic hyperactivity in congestive heart failure has been reported (15).

Raised digoxin-like immunoactivity (DLIA) has been documented in diabetic patients, which may be secondary to sodium retention and volume expansion. Whether DLIA increase occurs via their action on Na^+, K^+-ATPase and $[Ca^{2+}]i$ stores, actually leading to hypertension and/or modulate insulin sensitivity or secretion remains to be elucidated (10).

As regards central nervous system disorders, the suggestion that an endogenous digoxin-like immunoreactive factor (DLIF) is involved in the pathophysiology of mania has been advanced; although normal controls exhibit seasonal changes in serum DLIF concentration, in bipolar patients no such pattern is observed and low levels persist throughout the year (11).

Ouabain-like activity in the central nervous system correlates with diverse pathophysiological conditions. Ouabain enhance was observed in spontaneously hypertensive and in Dahl salt-sensitive rats, and appears to

be responsible for increased sympathoexcitation, decreased sympathoinhibition, desensitized arterial baroreflex function and the development of hypertension (110–112). Congestive heart failure has been associated to marked increases in both peripheral and brain ouabain-like activity (113), appearing to mediate the increase in resting sympathetic tone and to enhance sympathoexcitatory responses to stress.

Regarding neurotransmitter release, endobain E plus ouabain show no synergic or additive effects; therefore, both substances may act on distinct sites or by dissimilar mechanisms to enhance norepinephrine release. Since endobain E enhances norepinephrine release in hypothalamic neurons, this factor may be involved in the development and/or maintenance of cardiovascular disorders (60).

Na^+, K^+-ATPase from purified basolateral membranes is inhibited by incubation with uremic plasma factor F1, indicating the presence of an endogenous Na^+, K^+-ATPase inhibitor, which may participate in the development of unpredictable responses to digitalis therapy in pathophysiologic status (19).

CONCLUDING REMARKS

Sustained efforts have been devoted in several laboratories to disclose endogenous regulators for the sodium pump. The purpose of the present article, rather than an exhaustive summary of all available literature, was to provide a state-of-the-art overview of the subject.

It is worthwile mentioning that to explain the pharmacological action of plant opioid alkaloids, their receptors and later a wide spectrum of endogenous peptides related to their function have been disclosed (114). Plant cannabinoids present specific receptors (115) as well as endogenous ligands (116); likewise, the plant-ouabain has its receptor in human and animal tissues, for which the binding site is in the sodium pump (1,3). The presentation of a site capable of binding cardiac glucosides as a characteristic feature of the sodium pump provides a useful approach for the study of endogenous ouabain-like factors (endobains).

Progress on the knowledge of endobains is essential not only for nervous system but also for other tissues like kidney, heart or skin.

Na^+, K^+-ATPase, the enzymatic version of the sodium pump, participates in the regulation of several cell functions and is crucial for Na^+ extrusion and K^+ recovery from neurons following the passage of nerve impulse. Reasonably enough, tissues presenting the highest ionic exchange rates have the highest Na^+, K^+-ATPase activities (1,3). Since such enzyme concentrates at nerve ending membranes (66), its regulation is crucial for neurotransmission at central and peripheral system levels.

Why do we need so many endobains?

The wide spectrum of neuronal functions which depend on Na^+, K^+-ATPase activity may provide an answer. Furthermore, differential response to endogenous digitalis-like factors is attributable to the behaviour of specific Na^+, K^+-ATPase α-isoforms.

Since circulating Na^+, K^+-ATPase inhibitors are most likely generated by the central nervous system and/or adrenal glands, they may be considered hormonal factors. Accumulating evidence suggests central nervous system as a site of hypertensinogenic action of ouabain-like compounds. The putative role of brain "ouabain" in cardiovascular regulation affords a novel approach to the development of antihypertensive agents.

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REFERENCES

1. Albers, R. W. and Siegel, G. J. 1999. Membrane transport. Pages 95–118, *in* Siegel, G. J., Agranoff, B. W., Albers, R. W., Fisher, S. K., and Uhler, M. D., (eds.), Basic Neurochemistry, 6th edn., Lippincott-Raven, Philadelphia.
2. Skou, J. 1957. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochem. Biophys. Acta 23:394–401.
3. Stahl, W. L. 1986. The Na^+, K^+-ATPase of nervous tissue. Neurochem. Int. 8:449–476.
4. Kelly, R. A. and Smith, T. W. Pharmacological treatment of heart failure. 1996. Pages 809–838, *in* Hardman, J. G., and Limbird, L. E., (eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th edn., McGraw-Hill, New York.
5. Rodríguez de Lores Arnaiz, G. 1992. In search of synaptosomal Na^+, K^+-ATPase regulators. Mol. Neurobiol. 6:359–375.
6. Goto, A., Yamada, K., Yagi, N., Yoshioka, M., and Sugimoto, T. 1992. Physiology and pharmacology of endogenous digitalis-like factors. Pharmacol. Rev. 44:377–399.
7. Tamura, M., Lam, T. T., and Inagami, T. 1987. Specific endogenous Na^+, K^+-ATPase inhibitor purified from bovine adrenal. Biochem. Biophys. Res. Comm. 149:468–474.
8. Goto, A. and Yamada, K. 1998. Purification of endogenous digitalis-like factors from normal human urine. 1998. Clin Exp. Hypertens. 20:551–556.
9. Balzan, S., Ghione, S., Pieraccini, L., Biver, P., Di Bartolo, V., and Montali, U. 1994. Endogenous digitalis-like factor from umbilical cord and ouabain: comparison of biochemical properties. Pages 755–758, *in* Bamberg, E., and Schoner, W., (eds.), The Sodium Pump, Steinkopff, Darmstadt.

10. Martinka, E., Galajada, P., Ochodnicky, M., Lichardus, B., Straka, S., and Mokan, M. 1997. Endogenous digoxin-like immunoactivity and diabetes mellitus: facts and hypotheses. Med. Hypotheses 49:271–275.
11. Grider, G., El Mallakh, R. S., Huff, M. O., Buss, T. J., Miller, J., and Valdes, R. Jr. 1999. Endogenous digoxin-like immunoreactive factor (DLIF) serum concentrations are decreased in manic bipolar patients compared to normal controls. J. Affect. Disord. 54:261–267.
12. De Angelis, C., Riscazzi, M., Salvini, R., Piccoli, A., Ferri, C., and Santucci, A. 1997. Isolation and characterization of a digoxin-like immunoreactive substance from human urine by affinity chromatography. Clin. Chem. 43:1416–1420.
13. Bagrov, Y. Y., Dmitrieva, R. I., Manusova, N. B., Zvartau, E. E., Patkina, N. A., and Bagrov, A. Y. 1999. Involvement of endogenous digitalis-like factors in voluntary selection of alcohol by rats. Life Sci. 64:219–225.
14. Van Huysse, J. W. and Leenen, F. H. 1998. Role of endogenous brain "ouabain" in the sympathoexcitatory and pressor effects of sodium. Clin. Exp. Hypertens. 20:657–667.
15. Budzikowski, A. S., Huang, B. S., and Leenen, F. H. 1998. Brain "ouabain", a neurosteroid, mediates sympathetic hyperactivity in salt-sensitive hypertension. Clin. Exp. Hypertens. 20:119–140.
16. Rodríguez de Lores Arnaiz, G. and Peña, C. 1995. Characterization of synaptosomal membrane Na^+, K^+-ATPase inhibitors. Neurochem. Int. 27:319–327.
17. Rodríguez de Lores Arnaiz, G., Reinés, A., Herbin, T., and Peña, C. 1998. Na^+, K^+-ATPase interaction with a brain endogenous inhibitor (endobain E). Neurochem. Int. 33:425–433.
18. Hamlyn, J. M., Lu, Z. R., Manunta, P., Ludens, J. H., Kimura, K., Shah, J. R., Laredo, J., Hamilton, J. P., Hamilton, M. J., and Hamilton, B. P. 1998. Observations on the nature, biosynthesis, secretion and significance of endogenous ouabain. Clin. Exp. Hypertens. 20:523–533.
19. Calderaro, V., Steffanini, R., Matera, M. G., Vacca, C., Dini, I., and Rossi, F. 1997. Physiological and pharmacological properties of an endogenous sodium pump inhibitor. Life Sci. 61:1457–1468.
20. Sancho, J. M. 1998. A non-ouabain Na / K ATPase inhibitor isolated from bovine hypothalamus. Its relation to hypothalamic ouabain. Clin. Exp. Hypertens. 20:535–542.
21. De Angelis, C. and Haupert, G. T. Jr. 1998. Hypoxia triggers release of an endogenous inhibitor of Na^+, K^+-ATPase from midbrain and adrenal. Am. J. Physiol. 274:F182–188.
22. Kramer, H. J., Krampitz, G., Bäcker, A., and Meyer Lehnert, H. 1998. Ouabain-like factors in human urine: identification of a Na-K-ATPase inhibitor as vanadium-diascorbate adduct. Clin. Exp. Hypertens. 20:557–571.
23. Ferrandi, M., Manunta, P., Balzan, S., Hamlyn, J. M., Bianchi, G., and Ferrari, P. 1997. Ouabain-like factor quantification in mammalian tissues and plasma: comparison of two independent assays. Hypertension 30:886–896.
24. Butt, A. N., Semra, Y. K., Ho, C. S., and Swaminathan, R. 1997. Effect of high salt intake on plasma and tissue concentration of endogenous ouabain-like substance in the rat. Life Sci. 61:2367–2373.
25. Pamnani, M. B., Swindall, B. T., Schooley, J. F., Ghai, R., and Haddy, F. J. 1999. Sodium-potassium pump inhibitor in the mechanism of one-kidney, one wrap hypertension in dogs. Cell Mol. Biol. 45:115–121.
26. Schneider, R., Wray, V., Nimtz, M., Lehmann, W. D., Kirch, U., Antolovic, R., and Schoner, W. 1998. Bovine adrenals contain, in addition to ouabain, a second inhibitor of the sodium pump. J. Biol. Chem. 273:784–792.
27. Rodríguez de Lores Arnaiz, G. 1993. An endogenous factor which interacts with synaptosomal membrane Na^+, K^+-ATPase activation by K^+. Neurochem. Res. 18:655–661.
28. Vasdev, S. C., Longerich, L., Prabhakaran, V. M., Triggle, C. R., and Gault, M. H. 1989. Lipids as endogenous Na^+, K^+-ATPase inhibitors in plasma of healthy individuals and in dialysis dependent patients. Clin. Biochem. 22:313–319.
29. Tal, D. M., Yanuck, M. D., van Hall, G., and Karlish, S. J. D. 1989. Identification of Na^+, K^+-ATPase inhibitors in bovine plasma as fatty acids and hydrocarbons. Biochem. Biophys. Acta 985:55–59.
30. Cantley, L. C. Jr., Cantley, L. G., and Josephson, L. 1978. A characterization of vanadate interactions with the (Na,K)-ATPase. J. Biol. Chem. 253:7361–7368.
31. Bojorge, G. and Rodríguez de Lores Arnaiz, G. 1987. Insulin modifies Na^+, K^+-ATPase activity of synaptosomal membranes and whole homogenates prepared from rat cerebral cortex. Neurochem. Int. 11:11–16.
32. Battaini, F. and Peterkofsky, A. 1980. Histidyl-proline diketopiperazine, an endogenous brain peptide that inhibits (Na^+, K^+)-ATPase. Biochem. Biophys. Res. Comm. 94:240–247.
33. Rodríguez de Lores Arnaiz, G. and López Ordieres, M. G. 1997. A study of calcitonin effect on synaptosomal membrane enzymes. Peptides 18:613–615.
34. López Ordieres, M. G., Gironacci, M., Rodríguez de Lores Arnaiz, G., and Peña, C. 1998. Effect of angiotensin-(1—7) on ATPase activities in several tissues. Regulatory Peptides 77:135–139.
35. López Ordieres, M. G. and Rodríguez de Lores Arnaiz, G. 2000. Neurotensin inhibits neuronal Na^+, K^+-ATPase activity through high affinity peptide receptor. Peptides 21:571–576.
36. Wu, P. H. 1986. Na^+, K^+-ATPase in nervous tissue. Pages 451–502, *in* Boulton, A. A., Baker, G. B., and Wu, P. H., (eds.), Neuromethods, Enzymes, Humana Press, Clifton, NJ.
37. Rodríguez de Lores Arnaiz, G. and Mistrorigo de Pacheco, M. 1978. Regulation of (Na^+,K^+) adenosine triphosphatase of nerve ending membranes: action of norepinephrine and a soluble factor. Neurochem. Res. 3:733–744.
38. Rodríguez de Lores Arnaiz, G. 1983. Neuronal Na^+, K^+-ATPase and its regulation by catecholamines. Pages 147–158, *in* Caputto, R., and Ajmone Marsand, C., (eds.), Neural Transmission, Learning and Memory, Raven Press, New York.
39. Fishman, M. C. 1979. Endogenous digitalis-like activity in mammalian brain. Proc. Natl. Acad. Sci. USA 76:4661–4663.
40. Lichtstein, D. and Samuelov, S. 1980. Endogenous ouabain like activity in rat brain. Biochem. Biophys. Res. Comm. 96:1518–1523.
41. Akagawa, K., Hara, N., and Tsukada, Y. 1984. Partial purification and properties of the inhibitors of Na^+, K^+-ATPase and ouabain-binding in bovine central nervous system. J. Neurochem. 42:775–780.
42. Shimoni, Y., Gotsman, M., Deutsch, J., Kachalsky, S., and Lichtstein, D. 1984. Endogenous ouabain-like compound increases heart muscle contractility. Nature 307:369–371.
43. Rodríguez de Lores Arnaiz, G. and Antonelli de Gómez de Lima, M. 1986. Partial characterization of an endogenous factor which modulates the effect of catecholamines on synaptosomal Na^+, K^+-ATPase. Neurochem. Res. 11:933–947.
44. Haupert, G. T. and Sancho, J. M. 1979. Sodium transport inhibitor from bovine hypothalamus. Proc. Natl. Acad. Sci. USA 75:5735–5741.
45. Tymiak, A. A., Norman, J. A., Bolgar, M., Didonato, G. C., Lee, H., Parker, W. L., Lo, L. C., Berova, N., Nakanishi, K., Haber, E., and Haupert, G. T. Jr. 1993. Physicochemical characterization of a ouabain isomer isolated from bovine hypothalamus. Proc. Natl. Acad. Sci. USA 90:8189–8193.
46. Zhao, N., Lo, L., Berova, N., Nakanishi, K., Tymiak, A. A., Ludens, J., and Haupert, G. 1995. Na,K-ATPase inhibitors from bovine hypothalamus and human plasma are different from ouabain: Nanogram scale CD structural analysis. Biochemistry 34:9893–9896.

47. Lichtstein, D., Gati I., Samuelov S., Berson D., Rozenman Y., Landau L., and Deutsch, J. 1993. Identification of digitalis-like compounds in human cataractous lenses. Eur. J. Biochem. 216:261–268.
48. Halperin, J. A. 1989. Digitalis-like properties of an inhibitor of the Na^+, K^+ pump in human cerebrospinal fluid. J. Neurophysiol. Sci. 90:217–230.
49. Lichtstein, D., Minc, D., Bourrit, A., Deutsch, J., Karlish, S. J. D., Belmaker, H., Rimon, R., and Palo, J. 1985. Evidence for the presence of "ouabain like" compound in human cerebrospinal fluid. Brain Res. 325:13–19.
50. Ludens, J. H., Clark., M. A., Ducharme, D. W., Harris, D. W., Lutske, B. S., Mandel, F., Mathews, W. R., Sutter, D. M., and Hamlyn, J. M. 1991. Purification fron human plasma of an endogenous digitalis-like factor for structural analysis. Hypertension 17:923–929.
51. Hamlyn, J. M., Harris, D. W., Clark, M. A., Rogowski, A. C., White, R. J., and Ludens, J. H. 1989. Isolation and characterization of a sodium pump inhibitor from human plasma. Hypertension 13:681–689.
52. Hamlyn, J. M., Blaustein, M. P., Bova, S., DuCharme, D. W., Harris, D. W., Mandel, F., Mathews, W. R., and Ludens, J. H. 1991. Identification and characterization of a ouabain-like compound from human plasma. Proc. Natl. Acad. Sci. USA 88:6259–6263.
53. Crambert, G., Balzan, S., Paci, A., Decollogne, S., Montali, U., Ghione, S., and Lelièvre, L. G. 1997. Functional characterization of an endogenous digitalis-like factor in human newborn plasma. Ann. NY Acad. Sci. 834:621–625.
54. Fedorova, O. V., Anderson, D. E., and Bagrov, A. Y. 1998. Plasma marinobufagenin-like and ouabain-like immunoreactivity in adrenocorticotropin-treated rats. Am. J. Hypertens. 11:796–802.
55. Mathews, W. R., DuCharme, D. W., Hamlyn, J. M., Harris, D. W., Mandel, F., Clark, M. A., and Ludens, J. H. 1991. Mass spectral characterization of an endogenous digitalis-like factor from human plasma. Hypertension 17:930–935.
56. Bagrov, A. Y., Fedorova, O. V., Dmitrieva, R. I., Howald, W. N., Hunter, A. P., Kuznetsova, E. A., and Shpen, V. M. 1998. Characterization of a urinary bufodienolide Na^+, K^+-ATPase inhibitor in patients after acute myocardial infarction. Hypertension 31:1097–1103.
57. Tao, Q. F., Hollenberg, N. K., Price, D. A., and Graves, S. W. 1997. Sodium pump isoform specificity for the digitalis-like factor isolated from human peritoneal dialysate. Hypertension 29:815–821.
58. Hamlyn, J. M., Ringel, R., Schaeffer, J., Levinson, P. D., Hamilton, B. P., Kowarski, A. A., and Blaustein, M. P. 1982. A circulating inhibitor of (Na^+, K^+) ATPase associated with essential hypertension. Nature 300:650–652.
59. Nowicki, S., Enero, M. A., and Rodríguez de Lores Arnaiz, G. 1990. Diuretic and natriuretic effect of a brain soluble fraction that inhibits neuronal Na^+, K^+-ATPase. Life Sci. 47:1091–1098.
60. Vatta, M., Peña, C., Fernández, B., and Rodríguez de Lores Arnaiz, G. 1998. A brain Na^+, K^+-ATPase inhibitor (endobain E) enhances norepinephrine release in rat hypothalamus. Neuroscience 90:573–579.
61. Calviño, M. A., Peña, C., and Rodríguez de Lores Arnaiz, G. 1999. Differential effect of an endogenous Na^+, K^+-ATPase inhibitor on phosphoinositide hydrolysis in neonatal and adult rat brain cortex. J. Neurochem. 72 (Suppl.):S25B.
62. Di Bartolo, V., Balzan, S., Pieraccini, L., Ghione, S., Pegoraro, S., Biver, P., Revoltella, R., and Montali, U. 1995. Evidence for an endogenous ouabain-like immunoreactive factor in human plasma coeluted with ouabain on HPLC. Life Sci. 57:1417–1425.
63. Balzan, S., Montali, U., and Ghione, S. 1997. Evidence of an endogenous ouabain-like immunoreactive compound with digitalis-like properties in the human. Ann. NY Acad. Sci. 834:626–630.
64. Antonelli, M., Casillas, T., and Rodríguez de Lores Arnaiz, G. 1991. Effect of Na^+, K^+-ATPase modifiers on high-affinity ouabain binding determined by quantitative autoradiography. J. Neurosci. Res. 28:342–331.
65. Li, S., Eim, C., Kirch, U., Lang, R. E., and Schoner, W. 1998. Bovine adrenals and hypothalamus are a major source of proscillaridin A- and ouabain-immunoreactivities. Life Sci. 62:1023–1033.
66. Rodríguez de Lores Arnaiz, G., Alberici, M., and De Robertis, E. 1967. Ultrastructural and enzymic studies of cholinergic and non-cholinergic synaptic membranes isolated from brain cortex. J. Neurochem. 14:215–225.
67. Yoshida, H., Nagai, K., Ohashi, T., and Nakagawa, Y. 1969. K^+-dependent phosphatase activity observed in the presence of both adenosine triphosphate and Na^+. Biochem. Biophys. Acta 171:178–185.
68. Rodríguez de Lores Arnaiz, G., Antonelli de Gómez de Lima, M., and Girardi, E. 1988. Different properties of two brain fractions separated in Sephadex G-50 that modify synaptosomal ATPase activities. Neurochem. Res. 3:229–235.
69. Illescas, M., Ricote, M., Méndez, E., G.-Robles, R., and Sancho, J. 1990. Complete purification of two identical Na^+-pump inhibitors isolated from bovine hypothalamus and hypophysis. FEBS Lett. 261:436–440.
70. Peña, C. and Rodríguez de Lores Arnaiz, G. 1997. Differential properties between an endogenous brain Na^+, K^+-ATPase inhibitor and ouabain. Neurochem. Res. 22:379–383.
71. Han, C. S., Tobin, T., Akera, T., and Brody, T. M. 1976. Effects of alkali metal cations on phospho-enzyme levels and [^{3}H]ouabain binding to (Na^+ + K^+)-ATPase. Biochem. Biophys. Acta 429:993–1005.
72. Songu-Mize, E., Gunter, J. L., and Caldwell, R. W. 1989. Comparative ability of digoxin and an aminosugar cardiac glycoside to bind to and inhibit Na^+, K^+-adenosine triphosphatase. Effect of potassium. Biochem. Pharmacol. 38:3689–3695.
73. Goldstein, A., Goldstein, J. S., and Cox, B. M. 1975. A synthetic peptide with morphine-like pharmacologic action. Life Sci. 17:1643–1654.
74. Hansen, O. and Skou, J. C. 1973. A study on the influence of the concentration of Mg^{2+}, P_i, K^+, Na^+, and Tris on (Mg^{2+} + P_i)-supported g-strophanthin binding to Na^+ + K^+)-activated ATPase from ox brain. Biochem. Biophys. Acta 311:51–66.
75. Hansen, O. 1984. Interaction of cardiac glycosides with (Na^+ + K^+)-activated ATPase. A biochemical link to digitalis-induced inotropy. Pharmacol. Rev. 36:143–163.
76. Gleitz, J. and Peters, M. 1997. Influence of extracellular K^+ concentration on the time-course of Na^+, K^+-ATPase inhibition by cardiac glycosides with fast and low binding kinetics. Eur. J. Pharmacol. 335:89–97.
77. Akera, T., Temma, K., Wiest, S. A., and Brody, T. M. 1978. Reduction of the equilibrium binding of cardiac glycosides and related compounds to Na^+, K^+-ATPase as a possible mechanism for the potassium-induced reversal of their toxicity. Naunyn-Schmiedeberg' Arch. Pharmacol. 304:157–165.
78. Akera, T., Ng, Y.-C., Shieh, I. S., Bero, E., Brody, T. M., and Braselton, W. E. 1985. Effects of K^+ on the interaction between cardiac glycosides and Na^+, K^+-ATPase. Eur. J. Pharmacol. 111:147–157.
79. Tamura, M., Harris, T. M., Konishi, F., and Inagami, T. 1993. Isolation and characterization of an endogenous Na^+, K^+-ATPase-specific inhibitor from pig urine. Eur. J. Biochem. 211:317–327.
80. Herbin, T., Peña, C., and Rodríguez de Lores Arnaiz, G. 1998. Kinetics of Na^+, K^+-ATPase inhibition by a rat brain endogenous factor (endobain E). Neurochem. Res. 23:33–37.

81. Rodríguez de Lores Arnaiz, G., Herbin, T., and Peña, C. 1998. A comparative study between ascorbic acid and a brain Na^+, K^+-ATPase inhibitor (endobain E). J. Neurochem. 70 (Suppl. 1): S60B.
82. Paton, W. D. M., Vizi, E. S., and Zar, M. A. 1971. The mechanism of acetylcholine release from parasympathetic nerves. J. Physiol. Lond. 215:819–848.
83. Vizi, E. S. 1972. Stimulation, by inhibition of (Na^+, K^+, Mg^{2+})-activated ATPase, of acetylcholine release in cortical slices from rat brain. J. Physiol. Lond. 226:95–117.
84. Vizi, E. S. 1978. Na^+, K^+-activated adenosinetriphosphatase as a trigger in transmitter release. Neuroscience 3:367–384.
85. Birks, R. I. 1963. The role of sodium ions in the metabolism of acetylcholine. Can. J. Biochem. Physiol. 39:2573–2597.
86. Gaitonde, B. B. and Joglekar, S. N. 1977. Mechanism of neurotoxicity of cardiotonic glycosides. Br. J. Pharmac. Chemother. 59:223–229.
87. García, A. G., García-López, E., Horga, J. F., Kirpekar, S. M., Montiel, C., and Sánchez-García, P. 1981. Potentiation of K^+-evoked catecholamine release in the cat adrenal gland treated with ouabain. Br. J. Pharmacol. 74:673–680.
88. Wakade, A. R. 1981. Facilitation of secretion of catecholamines from rat and guinea-pig adrenal glands in potassium-free medium or after ouabain. J. Physiol. (London) 313:481–498.
89. Pocock, G. 1983. Ion movements in isolated bovine adrenal medullary cells treated with ouabain. Mol. Pharmacol. 23: 681–697.
90. Vizi, E. S. and Oberfrank, F. 1992. Na^+, K^+-ATPase, its endogenous ligands and neurotransmitter release. Neurochem. Int. 20:11–17.
91. Rodríguez de Lores Arnaiz, G. and Pellegrino de Iraldi, A. 1991. The release of catecholamines by an endogenous factor that inhibits neuronal Na^+, K^+-ATPase. Micr. Electr. Biol. Cell. 15:93–106.
92. Balduini, W. and Costa, L. G. 1990. Characterization of ouabain-induced phosphoinositide hydrolysis in brain slices of the neonatal rat. Neurochem. Res. 15:1023–1030.
93. Calviño, M. A., Peña, C., and Rodríguez de Lores Arnaiz, G. 2000. Na^+/Ca^{2+} exchanger and voltage-dependent Ca^{2+} channel participation in neonatal rat brain phosphoinositide hydrolysis stimulation by an endogenous Na^+, K^+-ATPase inhibitor. J. Neurochem. 74 (Suppl.):S19D.
94. Rodríguez de Lores Arnaiz, G., Schneider, P., and Peña, C. 1999. Brain soluble fractions which modulate Na^+, K^+-ATPase activity likewise modify muscarinic receptor. Neurochem. Res. 24:1417–1422.
95. Reinés, A., Peña, C., and Rodríguez de Lores Arnaiz, G. 1999. Ouabain and an endogenous Na^+, K^+-ATPase inhibitor modulate [^{3}H]MK-801 binding to NMDA receptor. J. Neurochem. 72 (Suppl.):S78D.
96. Reinés, A., Peña, C., and Rodríguez de Lores Arnaiz, G. 2000. Decreased [^{3}H]MK-801 binding to cerebral cortex nmda receptors by an endogenous Na^+, K^+-ATPase inhibitor. J. Neurochem. 74 (Suppl.):S65B.
97. Yamada, H. M., Naruse, M., Naruse, K., Demura, H., Takahashi, H., Yoshimura, M., and Ochi, J. 1992. Histological study on ouabain immunoreactivities in the mammalian hypothalamus. Neurosci. Lett. 141:143–146.
98. Ferrandi, M., Minotti, E., Salardi, S., Florio, M., Bianchi, G., and Ferrari, P. 1992. Ouabain-like factor in Milan hypertensive rats. Am. J. Physiol. 263:F739–F748.
99. Hamlyn, J. M. and Manunta, P. 1992. Ouabain, digitalis-like factors and hypertension. J. Hypertension 10(Suppl.):952–1178.
100. Antolovic, R., Kost, H., Mohadjerani, M., Linder, D., Linder, M., and Schoner, W. 1998. A specific binding protein for cardiac glycosides exists in bovine serum. J. Biol. Chem. 273: 16259–16264.
101. Sweadner, K. 1989. Isozymes of Na^+, K^+-ATPase. Biochem. Biophys. Acta 988:185–220.
102. Berrebi-Bertrand, I., Maixent, J. M., Christe, G., and Leleièvre, L. G. 1990. Two active Na/K-ATPases of high affinity for ouabain in adult rat brain membranes. Biochem. Biophys. Acta 1021:148–156.
103. Blaustein, M. P., Juhaszova, M., and Golovina, V. A. 1998. The cellular mechanism of action of cardiotonic steroids: a new hypothesis. Clin Exp. Hypertens. 20:691–703.
104. Bagrov, A. Y. and Fedorova, O. V. 1998. Effects of two putative endogenous digitalis-like factors, marinobufagenin and ouabain, on the Na^+, K^+-pump in human mesenteric arteries. J. Hypertens. 16:1953–1958.
105. Rodríguez de Lores Arnaiz, G. 1990. A study of tissue specificity of brain soluble fractions on Na^+, K^+-ATPase activity. Neurochem. Res. 15:289–294.
106. McGrail, K. M., Phillips, J. M., and Sweadner, K. J. 1991. Immunofluorescent localization of three Na^+, K^+-ATPase isozymes in the rat central nervous system: both neurons and glia express more than one Na^+, K^+-ATPase. J. Neurosci. 11: 381–391.
107. Calviño, M. A., Peña, C., and Rodríguez de Lores Arnaiz, G. 1998. Endogenous modulators of brain at early postnatal stages of rat development. Int. J. Devl. Neuroscience 16:97–102.
108. Kunes, J., Stolba, P., Pohlova, I., Jelinek, J., and Zicha, J. 1985. The importance of endogenous digoxin-like factors in rats with various forms of experimental hypertension. Clin. Exp. Hypert. A7:707.
109. Vizi, E. S., Oberfrank, F., Bernath, S., and Lichtstein, D. 1987. Noradrenaline releasing effect of an ouabain-like compound on pulmonary artery. Neuropharmacology 26:1541–1544.
110. Huang, B. S. and Leenen, F. H. H. 1995. Brain ouabain, sodium, and arterial baroreflex in spontaneous hypertensive rats. Hypertension 25 (part 2):814–817.
111. Leenen, F. H. H., Harmsen, E., Yu, H., and Ou, C. 1993. Effects of dietary sodium on central and peripheral ouabain-like activity in spontaneously hypertensive rats. Am. J. Physiol. 264:H2051–H2055.
112. Leenen, F. H. H., Harmsen, E., and Yu, H. 1994. Dietary sodium and central vs. peripheral ouabain-like activity in Dahl salt-sensitive vs. salt-resistant rats. Am. J. Physiol. 267:H1916–H1920.
113. Leenen, F. H. H., Huang, B. S., Yu, H., and Yuan, B. 1995. Brain "ouabain" mediates sympathetic hyperactivity in congestive heart failure. Circ. Res. 77:993–1000.
114. Reisine, T. and Pasternak, G. 1996. Opioid analgesics and antagonists. Pages 521–555, *in* Hardman, J. G., and Limbird, L. E., (eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th edn., McGraw-Hill, New York.
115. Pertwee, R. G. 1999. Pharmacology of cannabinoid receptor ligands. Curr. Med. Chem. 6:635–664.
116. Martin, B. R., Mechoulam, R., and Razdan, R. K. 1999. Discovery and characterization of endogenous cannabinoids. Life Sci. 65:573–595.