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The endogenous digitalis-like factor

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Abstract

Intensive search into the presence of endogenous digitalis-like factor (EDLF) started shortly after identification of the alpha subunit of the Na,K,-ATPase as being receptor for digitalis glycosides. After years of skepticism, present data testify EDLF really exists. Most probably, the EDLF has chemical structure of either ouabain or of one of its isomers. It is secreted by the adrenal cortex, and, under conditions of stress, it's secretion is regulated differently from the secretion of both gluco- and mineralocorticoids. The physiological role of the EDLF has not been fully understood yet. In the newborn's kidneys, the inhibition of the Na,K-ATPase may assist to increase elimination of surplus sodum from the organism. In individuals of any age, the inhibitory influence of EDLF upon Na,K-ATPase in the arterial wall smooth muscle cells increases peripheral vascular resistance and thus, blood pressure. In the tissue culture, direct positive inotropic influences of EDLF upon rat cardiomyocytes was observed. However, the importance of positive inotropic effect of the EDLF upon the heart in clinical medicine remains to be elucidated. (Mol Cell Biochem **160/161:** 111–115, 1996)

Key words: digitalis-like factor, ouabain, Na,K-ATPase, adrenal cortex

Introduction

Since the discovery of endogenous ligands for opioid receptors, the endorphins, the idea of endogenous ligands of humoral receptors became feasible. In this review, we present some of the fundamental facts regarding the discovery of endogenous digitalis-like factor (DLF), and review data on its possible source, chemical structure, as well as data on its potential role in the organism.

History of the DLF

Hypothesis on the potential existence of a DLF was most probably put forward by Ringer in 1885, and was reviewed again by Szent-Györgyi in 1953 (see [1]). First experimental data describing presence in blood serum of a substance with digoxin-like immunoreactivity appeared in 1979 and 1980 and originated from two independent laboratories: Gruber *et al.* [2, 3] described the presence of digoxin-like immunoreactivity in the serum of animals and men with hypertension and blood volume-expansion. Another observation of the presence in blood serum of a material capable to react upon enzymoimmunoanalysis like digoxin was published by Schreiber *et al.* [4, 5] in rats with pressure-induced cardiac hypertrophy and with hyperthyroidism. In both cases, the presence of the substance with digitalis-like reactivity was identified by means of commercially available kits designed for determination of serum digoxin levels. In parallel, also the ability of that material to inhibit the ^{86}Rb influx into human erythrocytes in a way similar to digitalis glycosides was proved. During the next years attempts started to answer three main questions: (1) identification of the chemical formula of DLF; (2) identification of its source in the organism; (3) identification of its physiological role.

Identification of the chemical formula of DLF

The chemical formula of aglycons, or genins of most substances inhibiting Na,K-ATPase in the way digoxin does is based on cyclopentanoperhydrophenanthrene nucleus with

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the lactone ring positioned on C 17 (Fig. 1). With respect to that, potential candidates for the endogenous DLF were searched mainly among lipids and among the steroid compounds. Of steroids, the candidates for the DLF were progesteron derivatives [6, 7] and dehydroepiandrosterone sulphate [8]. The possibility that dehydroepiandrosterone could be responsible for endogenous digitalis-like activity was critically evaluated later [9]. As digoxin-like immunoreactivity of 14-OH oestrone, and for 14-OH oestradiol were demonstrated, the number of steroid compounds candidating for the position of DLF was great. Besides that, also several nonsteroid compounds were listed as potential DLF, namely ascorbic acid, non-esterified fatty acids, bile acids, lysophospholipids and an unidentified amino-glyco-steroid (see [10]). It became evident later that in the human plasma was present a substance with properties very closely resembling ouabain and the precise identification of the structure of DLF was given [11]. The active fraction was isolated from 85 liters of human plasma of healthy individuals not receiving any digitalis compound. It was analysed by mass spectrometry, its ability to inhibit Na,K-ATPase was tested in two independent ways as well as the ability of this fraction to react with polyclonal antiserum against ouabain. All tests performed showed the fraction of human plasma was structurally, biologically, as well as immunologically indistinguishable from ouabain. The ability of that fraction to react with antibodies against ouabain leaves open the question whether this substance, endogeneous ouabain, is identical to substances detected in the past by means of their ability to cross-react with antidigoxin antibodies, and isolated from material other than blood serum. E.g., detailed analysis of a substance with DLF activity extracted from bovine hypothalamus disclosed this substance is an isomer of ouabain in which two hydroxyl groups are altered in comparison to ouabain [12]. This structural difference may explain differences of physiological effects of the hypothalmic DLF from those of ouabain. Mainly, this DLF had higher positive inotropic effect on cultured rat myocytes than ouabain without toxic component, and caused vasoconstriction of rat aorta and pulmonary artery at concentrations in which ouabain was ineffective [12].

Another question requiring further elucidation is, whether endogenous ouabain is the only one substance with digitalis-like properties that is synthesized in the organism. The analysis of DLF isolated from patients with chronic renal failure disclosed presence of two different substances. One of them had properties of ouabain, the other those of digoxin [13]. The synthesis of more than one substance with digitalis like properties would not be exceptional – e.g. the Bufo marinus toad, a producer of bufalin synthesizes, besides bufalin at least another one digitalis-like steroid with significant vasoconstrictory activity [14]. Nevertheless, it seems to be fairly clear the mammals including man are able to synthesize at least one substance identical with ouabain.

Identification of the source of the DLF

The DLF was isolated from a wide variety of tissues, e.g. bovine brain, porcine myocardium, liver, the adrenals, as well as biological fluids – human milk, colostrum, human plasma

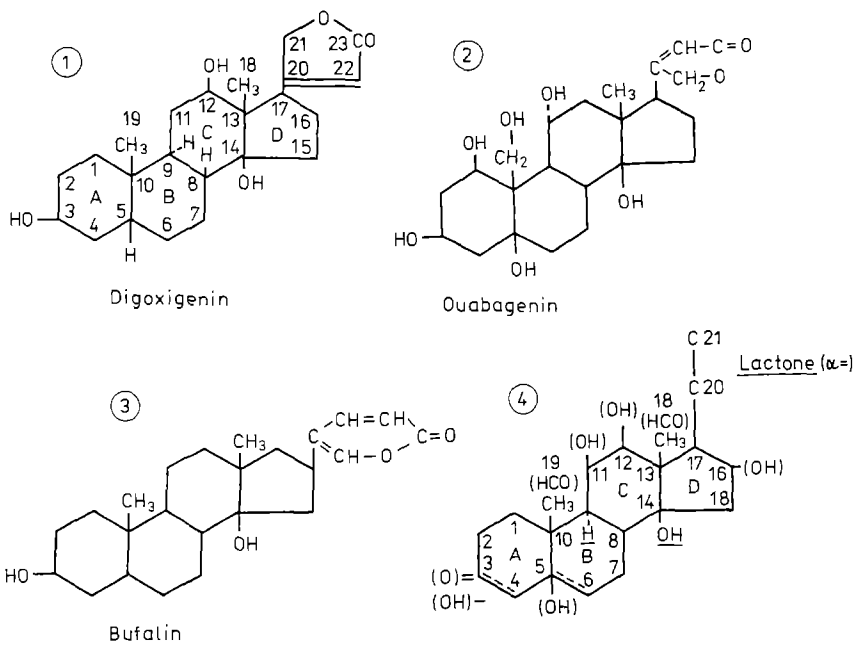

Fig. 1. Structure of digoxigenin (1), ouabagenin (2), of bufalin (3). General structure of genins in which formula is based on cyclopentanoperhydrophenanthrene nucleus (4). Underlined are substitutes essential for the cardiotonic action.

(Table 1) and, curiously, even from normal as well as from cataract-affected eye lenses [15]. From the very beginning of research of DLF, attempts were made to identify the site of its origin (see [10]). It was the opinion of first investigators that DLF originated in the brain [16], more precisely, in the hypothalamus [17–19] and adenohypophysis [20].

Potential interconnection between the development of cardiac hypertrophy and adrenal function was proposed in the past, at different times and at different levels by Liebegott – in humans [21], and by Hort [22], in experimental animals. Also the finding that in adrenalectomized rats, isoprenaline was not able to induce cardiac hypertrophy [23] was relevant and stressed the role of adrenal cortex in adaptive changes of the heart. When a good correlation between cardiac and adrenal weights was observed in animals with experimental cardiac hypertrophy of different origin it was hypothesized the DLF originated in adrenal cortex [5]. In rat adrenal extract the presence of a fraction was identified with chromatographic mobility close to aldosterone that exhibited strong digoxin-like immunoreactivity [24, 25]. Interesting in this respect was also the observation, in rats with cardiac pressure overload and hypertrophy, of decreased adrenal weight after long-term digitoxin treatment [26]. Pernollet and collaborators [27], Dorris [28], Dorris and Stocco [29] and Shaikh *et al.* [30] presented data indicating adrenal cortex was a source of a DLF. Presence of the endogenous ouabain-like substance was finally demonstrated in human, beef and rat adrenals in concentrations exceeding approximately 500 times that in the blood serum [11]. Likewise it was shown the adrenocortical cells in tissue culture secreted a compound possessing ouabain-like immunoreactivity. Their secretion was negatively influenced by raised concentration of potassium in the cultivation medium [11]. In humans, administration of dexamethasone suppressed secretion of both cortisol and of DLF, whereas administration of adrenocorticotrophic hormone (ACTH) increased plasma levels of both cortisol and of DLF [31]. Identical laboratory experiments has shown at the same time that both production and renal clearance play an important role in determination of the plasma level of DLF [31].

Doubts were thrown on these data by some other clinical observations. Although plasma level of immunoreactive ouabain in a patient with primary hyperaldosteronism decreased after unilateral adrenalectomy, in another two patients with bilateral adrenalectomy, the serum level of immunoreactive ouabain-like material remained normal [32]. This corresponds to older observation of digoxin-like immunoreactivity present in serum of adrenalectomized rats for which adrenocortical hormone was, most probably responsible [33]. Still another question is however, the specificity of the laboratory method or methods used to detect the presence of DLF. A logic requirement for future research in this field is standardisation of laboratory methods enabling us to compare data obtained at different laboratories. The key position of adrenal cortex in the secretion of DLF remains, in spite of all mentioned controversies, fairly clear.

Table 1. DLF content of different tissues and of biological fluids. Tissues were obtained from 300 g freshly killed male rat as well as from human cadaver

Tissue source	DLF (ng/g tissue)		Reference
	rat	human	
Adrenal	1.98	0.95	[30]
Liver	0.39	0.57	ibid.
Kidney	0.09	0.22	ibid.
Heart	< 0.04	ND	ibid .
Plasma (adult)	ND	0.1[1]	[50]
Plasma (neonatal)	ND	0.5[1]–1.0	ibid.
Milk	ND	35.6[1]	[41]
Colostrum	ND	61.3[1]	ibid.
Peritoneal dilysis fluid	ND	0.09[1]	[13]

[1]values in ng/ml; ND not determined

Another question is whether adrenal cortex is the only one source of DLF, at least in humans. The presence of DLF in human colostrum and human milk in concentrations exceeding those in blood make it probable the mammary gland either itself secretes the DLF or extracts it from blood, concentrates it and secretes it concentrated. Also the origin of DLF isolated from beef hypothalamus remains obscure as yet [12]. It is evident that further analysis of this problem is necessary.

Physiological role of DLF

Elevated levels of plasma DLF were found in different pathologic states, e.g. arterial hypertension in nondiabetic as well as in diabetic patients [34], primary aldosteronism, pheochromocytoma, acromegaly, and chronic renal failure (see [32]), in congestive heart failure [35], and in obese persons [36]. Further, elevated plasma levels of DLF were also found in patients with acute myocardial infarction [37], in pregnant women [38, 39], in pre-term and full-term neonates [40], in umbilical cord blood of newborns and in mothers colostrum [41]. Rare are observations of the coincidence of adrenal tumor, elevated plasma level of ouabain in the plasma of both peripheral and tumor corresponding adrenal veins, and with arterial hypertension ('ouabainoma'). From the broad spectrum of scattered clinical data, it is impossible to define the most probable physiological role of the DLF . Most probably, DLF is active primarily in inducing natriuresis. The presence in the urine of healthy salt loaded subjects of a natriuretic factor binding to specific digoxin antibodies was demonstrated during early stage of the investigation of the DLF [42]. Participation of the DLF in different experimental models of DOCA-high salt-intake hypertension without partial nephrectomy was demonstrated [43, 44] as well as in models of hypertension in which DOCA-high salt regimen was supple-

mented by partial nephrectomy [45–47]. Further, in a way not yet precisely identified, the DLF may rise blood pressure, most probably via its action on vascular smooth muscle Na^+-Ca^{2+} exchange [47]. Reports on the direct action of DLF upon the heart and heart muscle cells are scarce, but exist. In tissue culture, the DLF isolated from bovine hypothalamus exhibited positive inotropic effect at doses three orders of magnitude lower than ouabain [12]. Other data are less specific and, consequently less persuasive. Kaye *et al.* [48], and Bagrov *et al.* [37] both hypothesized on potential arrhythmogenic effects of DLF. As a curiosity, signs of digitalis intoxication with Mobitz type I atrioventricular block were described as a result of intoxication caused by endogenous DLF of the toad [49]. The physiological role of the appearance in the blood sera DLF both in experimental animals and in patients exposed to hemodynamic overload remains still to be elucidated. In connection with these attempts, we feel obliged to again underline the necessity of standardization of methods used for detection of the presence of DLF at different conditions, both in clinical research, and in experiments using laboratory animals.

Conclusions

Intensive research on the existence of DLF lasts approximately 15 years. In spite of initial general skepticism, relatively very small number of laboratories in several countries of the world collected data that are perceived critically [52], but cannot be overlooked. Mainly, it was demonstrated the organisms of a wide variety of species including humans are able to synthesize a substance meeting criteria of DLF. This substance is either ouabain or a compound very closely related to it. Further, it must be admitted that besides endogenous ouabain some other compound or compounds exist that belong into the larger family of endogenous digitalis-like compounds. The tissue most probably synthesizing DLF *de novo* (or, maybe, assembling it from precursors) is adrenal cortex. The physiological role of the DLF remains still to be mostly elucidated. Most probably, this substance (or, substances) participate in regulation of blood pressure and, perhaps also in regulation of the inotropic state of the heart. Standardization of laboratory methods used by different working groups for the detection of DLF in biological material under various experimental as well as clinical conditions seems to be one of important prerequisites of successfull research in this field in the future.

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