[Frontiers in Bioscience 10, 2165-2176, September 1, 2005]

NA/K-ATPASE, ENDOGENOUS DIGITALIS-LIKE COMPOUNDS AND CANCER DEVELOPMENT - A HYPOTHESIS

Heidrun Weidemann

The Jewish Hospital Berlin, Department of Internal Medicine, Cardiology, Berlin, GFR

TABLE OF CONTENTS

1. ABSTRACT

The primary transport system of sodium and potassium across the plasma cell membrane, the Na/K-ATPase, is a vital enzyme involved in numerous cellular events. This enzyme is the receptor for plant and amphibian steroids such as ouabain, digoxin and bufalin. In the past decade several endogenous steroids, identical or similar to the plant and amphibian steroids, termed here collectively digitalis-like compounds (DLC), have been identified in human tissues. This paper raises the hypothesis that alterations in the metabolism of endogenous DLC and in their interactions with the Na/K-ATPase may be associated with the development of malignancies. This hypothesis is based on the review of the literature pointing to: 1. An abnormal activity of the Na/K-ATPase and its sensitivity to DLC in malignant cells; 2. Abnormal plasma DLC concentrations in cancer patients; 3. Abnormal synthesis and release of DLC in immune compromised mice; and 4. Beneficial effects of DLC in the treatment of cancer.

2. INTRODUCTION

2.1. The Na/K-ATPase

The sodium and potassium-adenosine triphosphatase (Na/K-ATPase, Na^+, K^+-pump, B.C.3.6.1.3) hydrolyses ATP and uses the free energy to transport potassium into the cell and sodium out of the cell, against their electrochemical gradients (1). The Na/K-ATPase is the major determinant of cytoplasmic sodium and potassium concentrations. Thus, it has an important role in regulating cell volume, cytoplasmic pH and Ca^{++} concentration through the Na^+/H^+ and Na^+/Ca^{++} exchangers, respectively, and in driving a variety of secondary transport processes such as Na^+-dependent glucose and amino acids transport. The Na/K-ATPase is an oligomer composed of stoichiometric amounts of two major polypeptides, the alpha- and the beta-subunits (1 - 2). The alpha-subunit is a multi-spanning membrane protein that is responsible for the catalytic and transport properties of the enzyme and contains the binding sites for the cations, ATP and digitalis (3 - 4).

The beta-subunit is a polypeptide that transverses the plasma cell membrane and appears to be involved in the modulation of the K^+ and Na^+ affinity of the enzyme. Moreover, the beta-subunit acts as a chaperone by stabilizing the folding of the alpha-polypeptide, facilitating hereby its delivery to the plasma cell membrane (4 - 5). A third group of proteins characterized by the FXYD motif has been identified and is considered to play an important regulatory role in Na/K-ATPase function (6). It is well established that four isoforms of the alpha polypeptides exist in vertebrates, designated alpha 1, alpha 2, alpha 3 and alpha 4 and three different Na/K-ATPase beta-isoforms (7, 8).

2.2. Digitalis and endogenous digitalis-like compounds (DLC)

Since the discovery of the Na/K-ATPase more than 45 years ago, it is known that plant steroids, collectively termed cardiac glycosides or digitalis-like compounds (DLC), bind to a specific site of the enzyme and that this binding results in the inhibition of ATP hydrolysis and ion transport. Twenty years ago several independent investigators found that mammalian tissues and body fluids (including brain, adrenal, heart, plasma, cerebrospinal fluid and urine) contain DLC. More than ten years elapsed before these compounds responsible for the mentioned effects were identified: Ouabain was identified in human plasma and the adrenal gland (9, 10), digoxin was shown to be present in human urine (11), an ouabain isomer was detected in bovine hypothalamus (12), 19-norbufalin and its peptide derivative were identified in cataractous human lenses (13) and dihydropyrone-substituted bufadienolide was discovered in human placenta (14). In addition, immunoreactivity of marinobufagenin-like (15) and proscillaridin A like compounds (16), which both also belong to the bufadienolide family, has been demonstrated in human plasma. All the identified digitalis-like compounds share a common general structure: a steroid nucleus with a lactone ring at C-17 and a hydroxyl group at C-14. The 5-member and 6-member-lactone rings, in cardenolides and bufadienolides, respectively, are considered the most essential functional group of these substances (17).

Strong evidences point to the fact that the adrenal gland is the main source of endogenous DLC in most species. The concentration of DLC in adrenal extracts is higher than in any other tissue analyzed (18, 19). In addition, minced adrenal tissue was found to release immunoreactive DLC into a serum-free incubation medium (20) as well as the reperfused organ itself (21). It was observed that ouabain is secreted by primary cultures of bovine adrenocortical cells (22) and that the secretion is augmented by ACTH and angiotensin II (23). Moreover, it was shown that the content of endogenous ouabain was 5.7-fold higher in the zona glomerulosa than in the zona fasiculata of the adrenal gland (24). Finally, the staining of the adrenal medulla by monoclonal anti-ouabain antibodies (25) also points to this tissue as the main source of DLC. With respect to DLC synthesis, it was demonstrated that cholesterol is a substrate for the biosynthesis of DLC in the adrenal gland and that 3 beta-hydroxysteroid dehydrogenase/isomerase, which respectively convert cholesterol to pregnenolone and progesterone, are required for the initial step, the P450-cholesterol side-chain cleavage, in the biosynthetic pathway of DLC (26). Recently, strong evidence has been presented for the endogenous synthesis of both ouabain and dihydroouabain also by human adrenocortical cells in culture (27).

2.3. Consequences of DLC and Na/K-ATPase interactions

As mentioned above, DLC bind to and inhibit the activity of the Na/K-ATPase. There are numerous established consequences of this inhibition such as the dissipation of sodium and potassium gradients, followed by increased depolarization of the plasma cell membrane, increased cytoplasmic Ca^{++} concentration, increased cell volume and intracellular acidification (2 - 3). Moreover, it recently was shown that, also by inhibition of Na/K-ATPase activity, DLC induce cell detachment via a signal transduction cascade (28). In addition, recent studies support the notion that DLC participate in physiological processes that are distinct from their action on the activity of the plasma membrane Na/K-ATPase (for review see 7, 29). It has been revealed that DLC can regulate growth of rat cardiac myocytes and influence growth-related gene expression (29, 30). Furthermore, these phenomena were associated with the activation of multiple signal transduction pathways (31) leading e.g. to apoptosis. The generation of radical oxygen species (ROS) is considered as one important step in the apoptosis-inducing cascade. There are different pathways known to produce ROS (32, 33) and recently the ouabain-induced activation of *Ras* was described as essential in the down-stream generation of ROS in the mitochondria (34). Based on these observations, Askari and his colleagues suggested that the Na/K-ATPase might act, through interactions with other proteins, as an intracellular signal transducer (29, 35). Similar conclusions stem from experiments demonstrating that ouabain at low concentrations induces intracellular Ca^{++} oscillations which lead to activation of the transcription factor NF-kappaB in rat kidney proximal cells (36). An additional example of this concept is the interaction between the Na/K-ATPase and parts of the cellular cytoskeleton, such as actin filaments, ankyrin and spectrin (37, 38). It is generally accepted that these interactions play a crucial role in sorting, targeting, and regulating the distribution of the Na/K-ATPase in various sub-cellular compartments (39, 40). Finally, it was recently demonstrated that DLC, by interacting with the Na/K-ATPase, elicit changes in endocytosed membrane traffic (41). This effect, too, is independent on the inhibition of Na/K-ATPase activity at the plasma cell membrane.

3. REVIEW of LITERATURE

3.1. Changes of the Na/K-ATPase in malignant cells

3.1.1. Hyperactivity of Na/K-ATPase in malignant cells – the "leakage theory"

Numerous studies already in the 70^{ties} dealt with changes in the trans-membranous transport of cations in the course of malignant cell transformation, due to a change of Na/K-ATPase activity (for review see 42). Widely accepted

seemed to be the "leakage" theory describing the increased Na/K-ATPase activity in neoplastic cells as secondary to an increased (passive) Na^+ load, as well as a loss of K^+ ions across the plasma membrane of tumor cells due to an increased membrane permeability (43). As a result of this increased Na/K-ATPase activity, the co-transport and intracellular accumulation of nutrients, which both depend on Na^+ concentration gradients and are necessary for malignant transformation, would be facilitated. Kaplan, supporting the leakage theory, stressed the importance of high intracellular K^+ concentrations and low cAMP concentrations for cell proliferation, both of which are "by-products" of an increased Na/K-ATPase activity (42). Additionally, he dealt with changes in Na/K-ATPase activity in transformed cell lines induced by a variety of oncogenic viruses. For example, mouse 3T3 fibroblasts, transformed by SV40, show a 4-5-fold increase in Na/K-ATPase activity (44). Recently, the oncogenic properties of the principal Ebstein-Barr virus oncoprotein, latent membrane protein (LMP-1), were analyzed (45). Besides a bundle of metastatic factors, also the expression of angiogenic fibroblast growth factor-2 (FGF-2) was induced by LMP-1. As shown previously, the secretion of FGF-2 is suppressed by ouabain, hence, linking EBV-triggered cell transformation to Na/K-ATPase pathways.

Some evidence points to the fact that kinetic changes in Na/K-ATPase activity already are present at very early stages of tumor genesis. For instance, properties of Na/K-ATPase have been characterized in thymocytes and thymoblasts from mice of two strains: AKR, in which thymoma developed spontaneously and C57Bl, in which the development was induced by X-irradiation. Remarkably, even before thymoma could be discerned morphologically, the activity of Na/K-ATPase has altered (46).

Furthermore, Na/K-ATPase activity may vary during the life span of malignant cells. For example, a depolarization of the plasma cell membrane in chick-embryo fibroblasts, transformed by Rous sarcoma virus, was described reflecting a reduced Na/K-ATPase activity, maybe shortly before apoptosis (47). More recent data, dealing with the impact of ischemia and oxidative stress (e.g. caused by reperfusion) on Na/K-ATPase activity in non-malignant cells, revealed a decrease in enzyme activity mainly due to radical oxygen species (48, 49). In malignant cells exposed to ROS you may expect similar changes in Na/K-ATPase activity.

A correlation between Na/K-ATPase activity, plasma cell membrane depolarization and apoptosis is also supported by studies on the *Bcl-2* gene. The 26kD proto-oncogene *Bcl-2* belongs to a family of intracellular anti-apoptotic proteins (e.g. *Bcl*-X_L, Mcl-1) and is located mainly at the membranes of the mitochondria, the endoplasmic reticulum and the nucleus (50). This anti-apoptotic gene was abundantly investigated especially in the development of B cell lymphomas (51) but little is known about the interactions with the Na/K-ATPase. Some evidence, however, points to a direct stimulating effect of *Bcl-2* on Na/K-ATPase activity with concomitant changes of the plasma cell membrane potential. Gilbert and Knox demonstrated, firstly, that *Bcl-2*-transfected human B cell lymphoma cells have an increased resting activity of the Na/K-ATPase which was not significantly altered after radiation (52). They also showed that *Bcl-2* over-expression was correlated with an increased resistance towards radiation-induced apoptosis and that after pre-incubation with ouabain, which did not yet induce apoptosis, but depolarized the plasma cell membrane, the *Bcl-2*-induced protection against radiation-induced apoptosis is abolished (52). These data imply that the depolarization of the plasma cell membrane resulting from Na/K-ATPase activity inhibition may be a crucial factor in the process of apoptosis induction, or, *vice versa*, that the *Bcl-2*-associated resistance towards radiation-induced apoptosis may stem from hyperpolarization of the plasma cell membrane. These results, moreover, reveal that *Bcl-2* apparently interacts with the Na/K-ATPase. There is no evidence for a *Bcl-2*-protein anchored in the plasma cell membrane, hence, one may assume a free cytosolic *Bcl-2* (isoform) which interacts with the Na/K-ATPase, e.g. by interfering with binding regions of ROS, and hereby increasing K^+ channel activity (53). In fact, *in-vitro* experiments have shown that a fragmented cytosolic *Bcl-2* (without C-terminal anchor) is able to render fully resistance towards apoptosis (54).

It is well established that Angiotensin II (Ang II) affects cell growth (mainly via the AT_2-receptor) or induces apoptosis (mainly via the AT_1 receptor) in cardiac cells by triggering signaling pathways (55 - 57). Additionally, it was demonstrated that, in the human breast cancer cell line MCF-7, Ang II increases Na/K-ATPase activity via the AT_1 receptor in a dose dependant manner (58). This Na/K-ATPase activation corresponded with stimulation of proliferation, apparently independently on Ca^{2+} signaling mechanisms. Finally, it was shown that Ang II (via AT_2 receptor) stimulates ouabain synthesis/secretion (59). Hence, it may be postulated that the proliferative effect of Ang II on tumor cells is linked to its stimulating effect on Na/K-ATPase activity and that DLC, the endogenous Na/K-ATPase inhibitors, are modulating (malignant) cell growth, in between others, via Angiotensin II feed-back systems.

3.1.2. Changes in sensitivity of the Na/K-ATPase to DLC in malignant cells

Not only the activity of Na/K-ATPase differs between normal and malignant cells, but also its sensitivity towards DLC. This change in sensitivity may be due to a reduced density of the Na/K-ATPase at the plasma cell membrane of tumor cells as well as a change of isoforms. The first option was supported by studies 20 years ago on resting sarcoma cells as compared to fibroblasts, demonstrating a reduced density of ouabain-receptors, i.e. Na/K-ATPase, on the malignant plasma cell membrane (60). These results recently were supported by showing a down-regulation of the Na/K-ATPase alpha 1-isoform in the process of malignant transformation of prostate cells which renders the malignant cell more resistant towards ouabain (61).

The second option was supported especially by the work of Repke who revealed in malignant transformed

cells a switch of Na/K-ATPase configuration to more fetal isozymes explaining hereby their resistance towards ouabain (62). For example, the IC50 value for suppression of growth of malignant MCA cells was found to be 4-fold higher than the K 0.5 value for inhibition of the cardiac muscle Na/K-ATPase. There is increasing evidence that Na/K-ATPase isoform expression may play a crucial role in organogenesis and cell function specialization with region- and time-specific isoform expression patterns, even within single cell types (63, 64). Considering that tumorigenesis implies a process of cell de-differentiation, you may expect a more immature isoform pattern.

In general, Na/K-ATPase beta-isoform expression in malignant cells often is down-regulated, as shown for human clear cell renal (65), gastric (66) and bladder cancer (67). This beta-down-regulation has been suggested to be associated with the loss of tight junctions and epithelial polarity in cancer cells (68). It also was demonstrated that decreased expression of the beta 1-subunit in poorly differentiated carcinoma cell lines correlated with increased expression of the transcription factor Snail, known to down-regulate E-cadherin, with consequently a transition from epithelial to a mesenchymal phenotype (69).

In contrast, the alpha-subunits of the Na/K-ATPase seem to be up-regulated in malignant cells (67, 70). It even was suggested that this pattern of Na/K-ATPase expression serves as a potentially useful predictor of prognosis in bladder cancer (67). A more detailed analysis of alpha-subunits expression, however, revealed a decreased alpha 1 expression and increased alpha 3 expression in prostate (61) as well as in colorectal cancers (70). Conversely, the induction of cell differentiation in human HL60 leukemia cells, e.g. by retinoic acid, was shown to be accompanied by down-regulation of the alpha 3 isoform (71). Considering the fact that in humans the alpha 3 isoform has the lowest affinity for ouabain (72), it can be concluded that the process of malignant cell transformation is characterized not only by down-regulation of high-affinity alpha 1/alpha 2 isoforms but also by up-regulation of low-affinity Na/K-ATPase isoforms. Noteworthy, low-dose ouabain increases the beta 1- and decreases the alpha 3 subunits of the Na/K-ATPase (73).

3.2. Plasma DLC concentrations in cancer patients

DLC plasma concentrations in humans were only scarcely examined. Most of the available data deal with DLC plasma concentrations in patients with chronic heart disease (74-76). To our knowledge, no data exist about DLC concentrations in cancer patients. In a preliminary study we found that a majority (73.6%) of breast cancer patients (n = 84) expressed lower DLC plasma concentrations (<50 pmol/L) than a control group (150 ± 30 pmol/L), but about 10.8% of the patients revealed extremely high (>2000 pmol/L) DLC plasma concentrations (Weidemann and Harwood, unpublished data). In this subgroup with 40-fold increased DLC concentrations no common feature, which might explain this phenomenon, was found; especially, there were no clinical or laboratory signs indicating an ectopic ACTH syndrome (Goto *et al.* 1996). In addition, patients with a negative lymph node status (n = 21) had higher, although statistically not yet significant, DLC plasma concentrations than the subgroup (n = 59) with a positive lymph node status (mean 95 pmol/L vs. mean 33 pmol/L, $P < 0.07$). Our finding that the concentration of DLC in the supernatants of seven tumor lines, in between others, MCF-7, T-47-7, K562 and BT474, is minimal (< 5 pmol/L) excludes a direct autocrine production of DLC in tumor cells.

DLC concentrations were also determined in the plasma of 10 colon cancer patients and compared to age- and sex-matched controls. DLC plasma concentrations were 4.5 folds higher ($P < 0.001$) in cancer patients than in healthy individuals. These results may represent an "up-regulation" before the (adrenal) exhaustion develops (see below).

3.3. Plasma and adrenal DLC concentrations in immune compromised individuals

The bi-directional relationship between the immune (thymus) and the neuroendocrine Hypothalamo-Pituitary-Adrenal gland (HPA) axis systems is well described (77-79). There is increasing evidence that the maturation and function of both systems is dependent on each other, e.g. thymulin increases the synthesis of pro-opiomelanocortin derivatives such as ACTH (78). Conversely, the thymus seems to be a target of the permissive function of glucocorticoids (80) and may undergo early apoptosis when exposed to high concentrations of glucocorticoids (81). One reason for a misbalance of this relationship is a phenomenon, which recently was abundantly explored, called "De-sensitization of the HPA axis" (82-87). This endocrine disorder is caused by certain conditions, such as aging (83, 84), chronic stress (85), chronic inflammation (86) and depression (87)**.** A desensitized HPA axis is characterized by elevated basic plasma cortisol concentrations, an increased cortisol and ACTH response towards stress stimulation and a delayed normalization (82, 83). Elevated plasma cortisol concentrations, as mentioned above, are harming the morphology and function of the thymus. Recent data point to a possible regulation of DLC metabolism by components of the HPA axis and anti-tumor properties of DLC. Hence, we suggested that the establishment of tumors in *athymic* nude mice is facilitated by an impaired DLC metabolism (at the expense of a normal or increased cortisol metabolism) with corresponding reduced plasma and tissue DLC concentrations. Athymic nude mice have been widely used as experimental animals for transplantation of tumors and tumor cell lines (88). The autosomal recessive nude gene in homozygous (*nu/nu*) mice causes the lack of fur and an abnormal thymus (89).

The aim of a recent *in-vivo* study was to elucidate this hypothetical impairment of DLC metabolism in immune-compromised animals. In short, the study showed that DLC response to acute stress is differently regulated in normal and nude mice (90). Acute stress did induce the production of DLC in the adrenal gland and the hypothalamus of both the normal and the nude mice. However, acute stress induced an increase in DLC plasma

concentration only in normal mice whereas DLC plasma concentrations in nude mice did not change. Interestingly, the basal adrenal DLC concentrations were already significantly lower in nude as compared to normal mice (11.4 ± 3 vs. 49.4 ± 9.2 pmol/g, $p<0.001$). This raises the possibility that a relative adrenal dysfunction with insufficient DLC supply may be at least one factor which facilitates the growth of tumors in nude mice (see below: hypothesis). The corticosterone response, on the other hand, was generally better maintained as compared to DLC: Whereas an additional ACTH stimulus provoked an increase in plasma corticosterone concentrations in both, normal and nude mice, the DLC response in normal mice did not change towards exogenous ACTH and even dropped in nude mice (90).

3.4. DLC in the treatment of cancer

The examination of DLC for the treatment of cancer (91-93) started already forty years ago, but was abandoned again because of the inherent high toxicity of these compounds. Recently, however, the use of DLC for cancer treatment is being reconsidered (94-96). Epidemiological data are indicating a lower mortality in breast cancer patients who were on digitalis at time of first diagnosis, as compared to patients without digitalis therapy (94, 97).

The potential use of DLC as anti-cancer drugs was examined at the cellular level in several cancer cell lines. It was shown that DLC induce differentiation and/or apoptosis in human myeloid leukaemia HL-60, U937 and K562 cells (98 -100). Moreover, it was demonstrated that over-expression of the anti-apoptotic *Bcl-2*-gene antagonizes specifically the DLC-induced apoptosis in human leukaemia cells (101, 102). Recently, for the prostate cell lines PC-3, LNCaP and DU145 a bundle of data has accumulated demonstrating the ability of digoxin, ouabain and bufalin to induce specifically apoptosis via activation of caspase-3, early cytochrome c release from mitochondria and ROS generation without damaging other cells (103 -106). Moreover, it was shown that, Cdk5 kinase and *p25* formation (via *p35* cleavage), two important players in the apoptosis cascade, are directly activated by digoxin (107). Most of these effects were shown to be associated with, or dependent on, sustained elevation of intracellular calcium concentrations (96, 98). But, there are also data pointing to an Ca^{++}-independent pathway which mainly is responsible for ROS generation.

Xie *et al.* (2000) proposed a *dual* activation modus of Na/K-ATPase at the plasma cell membrane (34). This may correspond to the observation, as pointed out above, that ouabain exerts a dual effect on proliferation and apoptosis in e.g. human prostate smooth muscle cells: while at nanomolar concentrations it caused significant cell proliferation, at higher concentrations it induced cell apoptosis (108).

4. HYPOTHESIS

In view of the above described literature we propose the following hypothesis (Figure 1):

- The normal cell in vertebrates is characterized by a Na/K-ATPase activity according to the physiologic needs to maintain normal cell growth and function due to a balanced intracellular homeostasis. This homeostasis is incessantly challenged by malignant transformation. Since decades evidence accumulated describing the process of malignant cell transformation as an event which can be observed constantly on the molecular level in every living mechanism. In the course of malignant transformation plasma cell membrane permeability, in between other things, may change ("leakage theory") with passive load of Na^+ and loss of K^+, resulting in a 'compensatory' hyperactivity of the Na/K-ATPase. At this point the elimination of the (pre)tumor cell still is possible, but may require already specific immune defense mechanisms, involving the HPA axis.

- In normal physiologic conditions the adrenal gland, the end organ of the HPA axis, responds to a given stress stimulus, including tumor manifestation, with an increase in stress hormones, such as cortisol. The adequate response is guaranteed by the complex feedback system of the HPA axis. Increasing evidence points to stress-induced synthesis and release of digitalis-like compounds (DLC), similarly to cortisol, in the adrenal gland. The balanced regulation and interplay of these two hormones is supposed to be crucial for a competent immune response, especially an effective tumor defense reaction. In short, whereas chronic elevated cortisol plasma concentrations are known to affect the immune system negatively and hence favor the development of malignant tumors, the DLC can be considered as tumor protective hormones due to their ability to induce apoptosis specifically in malignant cells via inhibition of Na/K-ATPase and/or induction of signaling pathways.

- In a still unknown way, e.g. by IL-6 release, the (pre)tumor cell could send a 'stress' signal to the HPA axis transmitting a higher demand of DLC for the 'purpose' of tumor defense. A number of studies have demonstrated that plasma IL-6 concentration correlates with tumor progression and is a predictor of survival in metastatic tumor disease. Hence, IL-6 may be considered as a hypothetical "signal" transmitted to the HPA axis, either directly from the pre-malignant cell, or indirectly via release from activated monocytic cells triggered by a tumor-related antigen on the cell membrane.

- In the case that the tumor cell survives and acquires higher malignancy, e.g. by over-expressing the *Bcl-2* gene, the need of increasing DLC synthesis becomes essential for the organism to resist tumor expansion. This step in malignant cell transformation is characterized by a down-regulation of Na/K-ATPase, especially beta- and alpha 1 subunits, with a concomitantly declining sensitivity of this enzyme towards DLC. Additionally, the *Bcl-2* protein may directly stimulate Na/K-ATPase activity.

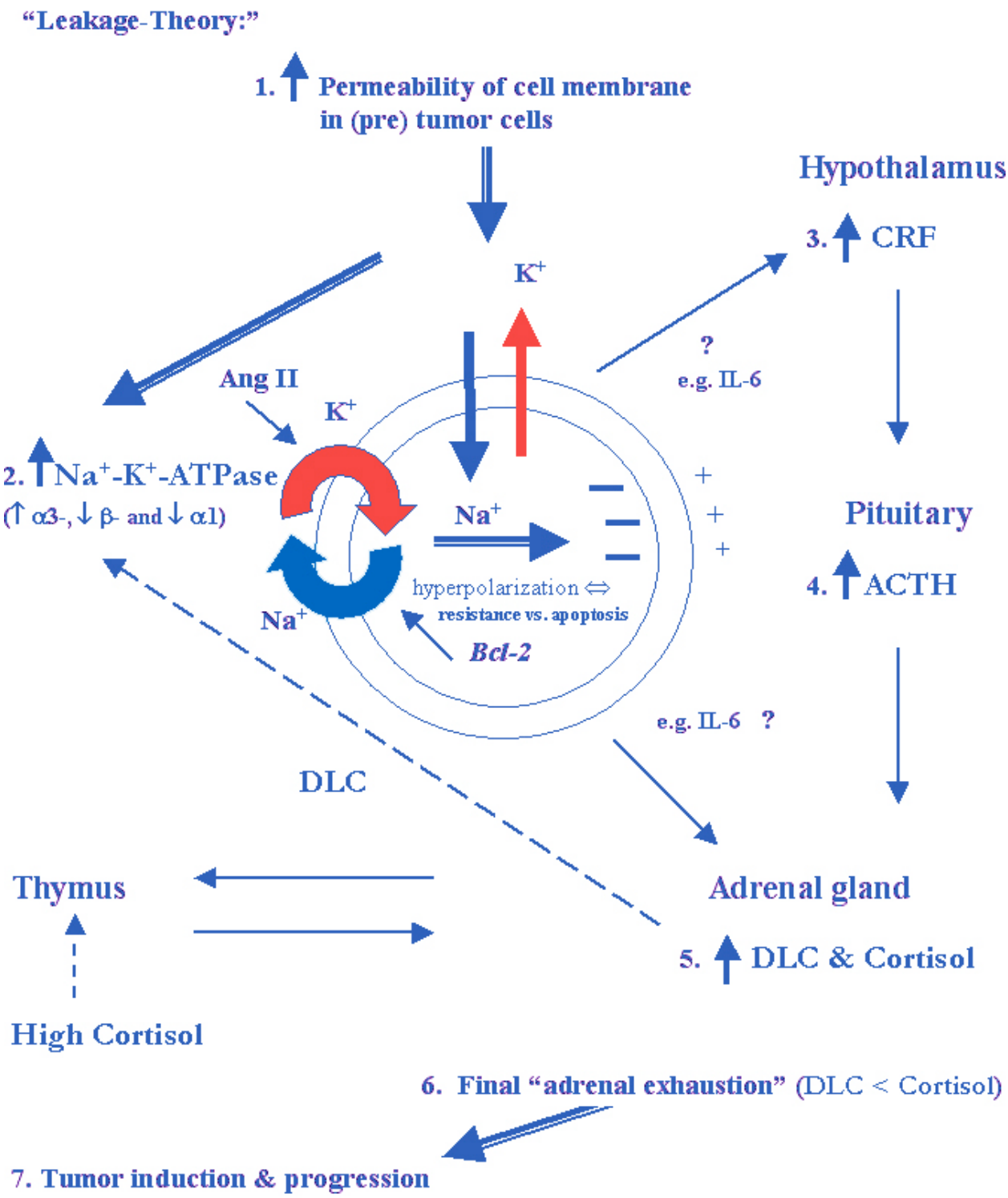

Figure 1. Hypothesis - linking Na/K-ATPase activity, endogenous digitalis-like compounds and tumor induction and progression. De-sensitization of the HPA axis and accelerated thymus involution, both due to chronic stress, result in "adrenal exhaustion" and facilitate tumor induction. See text for details. HPA- Hypothalamo-Pituitary-adrenal; CRF-Corticotropine-releasing factor; ACTH-Adrenocorticotropine hormone; DLC-Digitalis-like compounds; filled arrow = stimulation and/or supporting effects; broken arrow = inhibition and/or harming effects; shadowed arrow = result.

Similarly, Angiotensin II may contribute to accelerated Na/K-ATPase activity in tumor cells. Altogether, the cell membrane now is hyperpolarized and apoptosis fails to eliminate the tumor cell, in between others, due to increased resistance towards e.g. radiation induced apoptosis.

- In normal conditions, the HPA axis responses effectively to this increasing tumor challenge by increasing the output of stress hormones, especially DLC. However, under certain circumstances, the adrenal gland may fail to provide the demanded stress hormones. As shown in recent years, the physiologic stress response system can be disturbed by at least two mechanisms:

  Firstly, chronic elevated cortisol plasma concentrations (due to chronic stress exposure) cause a degeneration of neurons in the hippocampus and, consequently, a loss of cortisol receptors predominantly localized in this region. As a result, the sensitivity of the HPA axis towards negative feed back inhibition decreases with consequently a further increase in cortisol plasma concentrations. Moreover, the response of cortisol and ACTH towards acute stress stimuli becomes inadequately high and the normalization of cortisol plasma concentrations is delayed. Hence, a vicious circle is triggered, called a "De-sensitization" of the HPA axis.

  Secondly, the function of the HPA axis may be impaired directly by the thymus. As described above, the maturation and function of both endocrine systems are highly dependent on each other. Chronic elevated cortisol plasma concentrations may not only lead to an impairment of the function of HPA axis but also harm the thymus by inducing an accelerated apoptosis of thymocytes (praecox thymus involution). As a result, the stimulating effect of thymulin on the adrenal gland vanishes.

- This de-regulation of two important neuroendocrine systems may prepare the ground for tumor development because, in this situation, the fine-tuned balance between cortisol and DLC production begins to collapse: our recent observation, that plasma cortisol concentrations in immune compromised mice apparently can be maintained over a long period, while the DLC plasma concentrations seem to drop more quickly (90), is in accord with this proposal.

- Hence, in the presence of a new strong stress stimulus the adrenal gland becomes more and more 'exhausted' which is primarily seen in an inadequate synthesis/release of DLC. To close the loop, in this scenario the (pre)malignant cell itself may present a stimulus for the adrenal gland to release DLC. After a presumed short time of "last fight" with extreme up-regulation of DLC synthesis, supported by own data, in the end the adrenal gland is failing to provide the hormones necessary for tumor cell elimination, e.g. by apoptosis. The process of tumor induction hereby is facilitated, developing in the end its own aggressive dynamics.

This hypothesis raises many open questions, e.g. it is unclear whether the influence of DLC on tumor induction is exclusively positive or also negative in certain circumstances. Similarly, it is unclear whether and how much the tumor itself, e.g. by positive amplification, may influence the described endocrine processes. Nevertheless, this hypothesis may provide new venues for future studies aimed at early cancer detection, risk stratification and treatment.

5. ACKNOWLEDGEMENT

The author thanks with great appreciation and respect Prof. David Lichtstein, Prof. Haim Rosen and Prof. Joseph Weidenfeld at The Hadassah University Jerusalem, with whom this hypothesis was discussed during the period of a scholarship dedicated to DLC research in cancer (2000-2002). Furthermore the author thanks all friends in Israel as well as in Germany and England, who supported and encouraged me to pursue this hypothesis, especially Eudice Yudelevich, Steven Harwood and Ralf Fohs.

6. REFERENCES

1. Scheiner-Bobis G: The sodium pump, its molecular properties and mechanics of ion transport. *Eur J Biochem* 269, 2424-2433 (2002)

2. Blanco G & R. W. Mercer: Isozymes of the Na^+, K^+-ATPase: heterogeneity in structure, diversity in function. *Am J Physiol* 275, F633-F650 (1998)

3. Kaplan J.H: Biochemistry of Na^+, K^+-ATPase. *Annu Rev Biochem* 71, 511-535 (2002)

4. Lingrel J.B: Na^+, K^+-ATPase: isoform structure, function, and expression. *J Bioenerg Biomembr* 24, 263-270 (1992)

5. Arystarkhova E & K. J. Sweadner: Tissue-specific expression of the Na^+, K^+-ATPase beta-3 subunit. The presence of beta3 in lung and liver addresses the problem of the missing subunit. *J Biol Chem* 272, 22405-22408 (1997)

6. Garty H, M. Lindzen, M. Fuzesi, R. Aizman, R. Goldshleger, C. Asher & S.J. Karlish: A specific functional interaction between CHIF and Na/K-ATPase: role of FXYD proteins in the cellular regulation of the pump. *Ann N.Y. Acad Sci* 986, 395-400 (2003)

7. Mobasheri A, J. Avila, I. Cozar-Castellano, M.D. Brownleader, M. Trevan, M.J. Francis, J.F. Lamb & P. Martin-Vasallo: Na/K-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. *Biosci Rep* 20, 51-91 (2000)

8. Jorgensen P.L, K.O. Hakansson & S.J. Karlish: Structure and mechanism of Na/K-ATPase: functional sites and their interactions. *Annu Rev Physiol* 65, 817-849 (2003)

9. Hamlyn J.M, M.P. Blaustein, S. Bova, D.W. DuCharme, D.W. Harris, F. Mandel, M.R. Mathews & J.H. Ludens: Identification and characterization of ouabain-like compound from human plasma. *Proc Natl Acad Sci USA* 88, 6259-6263 (1991)

10. Doris P.A, A. Hayward-Lester, D. Bourne & D.M. Stocco: Ouabain production by cultured adrenal cells. *Endocrinology* 137, 533-39 (1996)

11. Goto A, T. Ishiguro, K. Yamada, M. Ishii, M. Yoshioka, C. Eguchi, M. Shimura & T. Sugimoto: Isolation of a urinary digitalis-like factor indistinguishable from digoxin. *Biochem Biophys Res Commun* 173, 1093-1101 (1990)

12. Tymiak A.A, J.A. Norman, M. Bolgar, G. DiDonato, H. Lee, W.L. Parker, L.C. Lo, N. Berova, K. Nakanishi, E. Haber & G.T. Haupert: Physico-chemical characterization of a ouabain isomer isolated from bovine hypothalamus. *Proc Natl Acad Sci USA* 90, 8189-8193 (1993)

13. Lichtstein D, I. Gati, S. Samuelov, D. Berson, Y. Rozenman, L. Landau & J. Deutsch: Identification of digitalis-like compounds in human cataractous lenses. *Eur J Biochem* 216, 261-268 (1993)

14. Hilton P.J, R.W. White, G. A. Lord, G.V. Garner, D.B. Gordon, M.J. Hilton & L.G. Forni: An inhibitor of the sodium pump obtained from human placenta. *Lancet* 348, 303-305 (1996)

15. Bagrov A.Y, O.V. Fedorova, J.L. Austin-Lane, R.I. Dmitrieva & D.E. Anderson: Endogenous marino-bufagenin-like immunoreactive factor and Na^+, K^+-ATPase inhibition during voluntary hypoventilation. *Hypertension* 26, 781-788 (1995)

16. Sich B, U. Kirch, M. Tepel, W. Zidek, W. Schoner: Pulse pressure correlates in humans with a Proscillaridin A immunoreactive compound. *Hypertension* 7,1073-1078 (1996)

17. Kelly R.A. & T.W. Smith: Pharmacological treatment of heart failure. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics. Eds: Herdam J.G. & Limbird L.E. McGraw-Hill comp. 809-838 (1996)

18. Rauch A.L. & V.M. Buckalew Jr: Tissue distribution of an endogenous ligand to the sodium, potassium ATPase molecule. *Biochem Biophys Res Commun* 152, 818-824 (1988)

19. Doris P.A: Immunological evidence that the adrenal gland is a source of an endogenous digitalis-like factor. *Endocrinology* 123, 2440-2444 (1988)

20. Doris P.A. & D.M. Stocco: An endogenous digitalis-like factor derived from the adrenal gland: studies of adrenal tissue from various sources. *Endocrinology* 125, 2573-79 (1989)

21. Dawnay A, S. Harwood & J.P. Hinson: Release of ouabain-like compound (OLC) from the intact perfused rat adrenal gland. *Endocrin Res* 24, 721-24 (1998)

22. Laredo J, B. B. Hamilton & J.M. Hamlyn: Ouabain is secreted by bovine adrenocortical cells. *Endocrinology* 135, 794-797 (1994)

23. Shah J.R, J. Laredo, B.P. Hamilton & J.M. Hamlyn: Different signaling pathways mediate stimulated secretions of endogenous ouabain and aldosterone from bovine adrenocortical cells. *Hypertension* 31, 463-468 (1998)

24. Laredo J, B.P. Hamilton & J.M. Hamlyn: Secretion of endogenous ouabain from bovine adrenocortical cells: Role of the zona glomerulosa and zona fasiculata. Biochim. Biophys. *Res Commun* 212, 487-493 (1995)

25. Takahashi H. & Y. Terano: Ouabain-like immunoreactivity in the brain and the adrenal medulla is increased in DOCA-salt hypertensive rats. In: The Sodium Pump. Eds: Bamberg E. & Schoner W. Springer, New York, 775-778 (1994)

26. Lichtstein D, M. Steinitz, I. Gati, S. Samuelov, J. Deutsch & J. Orly: Biosynthesis of digitalis-like compounds in rat adrenal cells: Hydroxycholesterol as possible precursor. *Life Sci* 62, 2109-2126 (1998)

27. el-Masri M.A., B.J. Clark, H. M. Qazzaz, R. Valdes Jr. Human adrenal cells in culture produce both ouabain-like and dihydroouabain-like factors. *Clin Chem* 48, 1720-1730 (2002)

28. Contreras R.G, C. Flores-Maldonado, A. Lázaro, L. Shoshani, D. Flores-Benitez, I. Larré & M. Cereijido: Ouabain binding to Na/K-ATPase relaxes cell attachment and sends a specific signal (NACos) to the nucleus. *J Membr Biol* 198, 147-158 (2004)

29. Askari A: Significance of protein-protein inter-actions to Na/K-ATPase functions. In: Na/K-ATPase and related ATPases. Eds: Taniguchi K. & Kaya S. Elsevier Science BV, 17-26 (2000)

30. Huang L, H. Li & Z. Xie: Ouabain-induced hypertrophy in cultured cardiac myocytes is accompanied by changes in expression of several late response genes. *J Mol Cell Cardiol* 29, 429-437 (1997)

31. Mohammadi K, P. Kometiani, Z. Xie & A. Askari: Role of protein kinase C in the signal pathways that link Na/K-ATPase to ERK1/2. *J Biol Chem* 276, 42050-42056 (2001)

32. Sauer H, M. Wartenberg & J. Hescheler: Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol Biochem* 4, 173-86 (2001)

33. Boonstra J. & J.A. Post: Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene* 337, 1-13 (2004)

34. Liu J, J. Tian, M. Haas, J.I. Shapiro, A. Askari & Z. Xie: Ouabain interaction with cardiac Na/K-ATPase initiates signal cascades independent of changes in intracellular Na^+ and Ca^{2+} concentrations. *J Biol Chem* 36, 27838-44 (2000)

35. Aizman O. & A. Aperia: Na/K-ATPase as a signal transducer. *Ann NY Acad Sci* 986, 489-496 (2003)

36. Miyakawa-Naito A, P. Uhlen, M. Lal, O. Aizman, K. Mikoshiba, H. Brismar, S. Zelenin & A. Aperia: Cell signaling microdomain with Na/K-ATPase and inositol 1,4,5-trisphosphate receptor generates calcium oscillations. *J Biol Chem* 278, 50355-61 (2003)

37. Cantiello H.F: Actin filaments stimulate the Cantiello H.F: Actin filaments stimulate the Na/K-ATPase. *Am J Physiol* 269, F637-F643 (1995)

38. Nelson W.J. & R.W. Hammerton: A membrane-cytoskeletal complex containing Na/K-ATPase, ankyrin, and fodrin in Madin-Darby canine kidney (MDCK) cells: implications for the biogenesis of epithelial cell polarity. *J Cell Biol* 108, 893-902 (1989)

39. Caplan M.J: Ion pumps in epithelial cells: sorting, stabilization, and polarity. *Am J Physiol* 272, G1304-G1213 (1997)

40. Muth T.R, C.J. Gottardi, D.L. Roush & M.J. Caplan: A basolateral sorting signal is encoded in the alpha-subunit of Na/ K-ATPase. *Am J Physiol* 274, C688-C696 (1998)

41. Rosen H, V. Glukhman, T. Feldmann, E. Fridman & D. Lichtstein: Cardiac steroids induce changes in recycling of the plasma membrane in human NT2 cells. *Mol Biol Cell* 15, 1044-1054 (2004)

42. Kaplan J.G: Membrane cation transport and the control of proliferation of mammalian cells. *Ann Rev Physiol* 40, 19-41 (1978)

43. Shen S.S, S.T. Hamamoto, H.A. Bern & R.A. Steinhardt: Alteration of sodium transport in mouse mammary epithelium associated with neoplastic transformation. *Cancer Res* 38, 1356-1361 (1978)

44. Kimelberg H. & E: Increased ouabain-sensitive $86Rb^+$ uptake and sodium and potassium ion-activated adenosine triphosphatase activity in transformed cell lines. *J Biol Chem* 250, 100-104 (1975)

45. Wakisaki N. & J.S. Pagano: Epstein-Barr virus induces invasion and metastatic factors. *Anticancer Res* 23, 2133-38 (2003)

46. Gonta-Gabriec K, W. Rossowski & I. Szumiel: Properties of Na/K-ATPase and alkaline phosphatase alter during spontaneous and radiation-induced leukemogenesis in mice. *Neoplasma* 33, 141-155 (1986)

47. Banerjee S.P, H.B. Bosmann & H.R. Morgan: Oncogenic transformation of chick-embryo fibroblasts by Rous sarcoma virus alters rubidium uptake and ouabain binding. *Exp Cell Res* 104, 111-117 (1977)

48. Franzon R, M.L. Lamers, F.M. Stefanello, C.M. Wannmacher, M. Wajne & A.T. Wyse: Evidence that oxidative stress is involved in the inhibitory effect of proline on Na/K-ATPase activity in synaptic plasma membrane of rat hippocampus. *Int J Dev Neurosci* 21, 303-307 (2003)

49. Streck E.L, A.I. Zugno, B. Tagliari, R. Franzon, C.M. Wannmacher, M. Wajne & A.T. Wyse: Inhibition of rat brain Na/ K-ATPase activity induced by homocysteine is probably mediated by oxidative stress. *Neurochem Res* 26, 1195-1200 (2001)

50. Zimmermann K.C, C. Bonzon & D.R. Green: The machinery of programmed cell death. *Pharmacol Ther* 92, 57-70 (2002)

51. Pileri S.A, P.L. Zinzani, G. Gaidano, B. Falini, P. Gaulard, E. Zucca, E. Sabattini, S. Ascani, M. Rossi, F. Cavalli: International Extranodal Lymphoma Study Group. Pathobiology of primary mediastinal B-cell lymphoma. *Leuk Lymphoma* 44, Suppl 3:S21-6 (2003)

52. Gilbert M. & S. Knox: Influence of *Bcl-2* overexpression on Na/K-ATPase pump activity: correlation with radiation-induced programmed cell death. *J Cell Physiol* 171, 299-304 (1997)

53. Wang L, P. Zhou, R.W. Craig & L. Lu: Protection from cell death by *mcl-1* is mediated by membrane hyperpolarization induced by K^+ channel activation. *J Membr Biol* 172, 113-20 (1999)

54. Borner Ch, I. Martinou, C. Mattmann, M. Irmler, E. Schaerer, J.C. Martinou & J. Tschopp: The protein *Bcl-2a* does not require membrane attachment, but two conserved domains to suppress apoptosis. *J Cell Biol* 126, 1059-1068 (1994)

55. Kajstura J, E. Cigola, A. Malhotra, P. Li, W. Cheng, L.G. Meggs & P. Anversa: Angiotensin II induces apoptosis of adult ventricular myoytes *in vitro*. *Mol Cell Cardiol* 29, 859-870 (1997)

56. Diep Q.N, J.S. Li, E.L. Schiffrin: In vivo study of AT_1 and AT_2 angiotensin receptors in apoptosis in rat blood vessels. *Hypertension* 34, 617-624 (1999)

57. Touyz R.M. & C. Berry: Recent advances in angiotensin II signaling. *Braz J Med Biol Res* 35, 1001-1015 (2002)

58. Muscella A, S. Greco, M.G. Elia, C. Storelli, S. Marsigliante: Angiotensin II stimulation of Na/K-ATPase

activity and cell growth by calcium independent pathway in MCF-7 breast cancer cells. *J Endocrinol* 173, 315-23 (2002)

59. Laredo J, J.R. Shah, Z. Lu, B.P. Hamilton, J.M. Hamlyn: Angiotensin II stimulates secretion of endogenous ouabain from bovine adrenocortical cells vi angiotensin type 2 receptors. *Hypertension* 29, (part 2) 401-407 (1997)

60. Latzkovits L, C. Torday, T. Janossy & E. Erdös: Manifestation of K^+ transport alterations in cultered tumour cells of mice. *Acta Chir Hung* 21, 287-294 (1983)

61. Mobasheri A, R. Fox, I. Evans, F. Cullinham, P. Martin-Vasallo & C.S. Foster: Epithelial Na/K-ATPase is down-regulated in canine prostate cancer; a possible consequence of metabolic transformation in the process of prostate malignancy. *Cancer Cell Int* 13, 8 (2003)

62. Repke K.R.H, R. Schön, R. Megges, J. Weiland, E. Nissen & E. Matthes: Potential suitability of Na-K-transporting ATPase in pre-screens for anti-cancer agents. *Anti-Cancer Drug Design* 10, 177-87 (1995)

63. Corthesy-Theulaz I, A.M. Merillat, P. Honegger & B.C. Rossier: Differential regulation of Na/K-ATPase isoform gene expression by T3 during rat brain development. *Am J Physiol* 261, C124-31 (1998)

64. Herrera V.L, T. Cova, D. Sassoon & N. Ruiz-Opazo: Developmental cell-specific regulation of Na/K-ATPase alpha 1-, alpha 2-, and alpha 3-isoform gene expression. *Am J Physiol* 266, C1301-12 (1994)

65. Rajasekaran S.A, W.J. Ball Jr, N.H. Bander, J.D. Pardee & A.K. Rajasekaran: Reduced expression of beta-subunit of Na/ K-ATPase in human clear cell renal cell carcinoma. *J Urol* 162, 574-80 (1999)

66. Avila J, E. Lecuona, M. Morales, A. Soriano, T. Alonso & P. Martin-Vasallo: Opposite expression pattern of the human Na/K-ATPase beta 1 isoform in stomach and colon adenocarcinomas. *Ann NY Acad Sci* 834, 633-635 (1997)

67. Espineda C, D.B. Seligson, J.W. Ball Jr, J. Rao, A. Palotie, S. Horva, Y. Huang, T. Shi & A.K. Rajasekaran: Analysis of the Na/K-ATPase alpha- and beta-subunit expression profiles of bladder cancer using tissue microarrays. *Cancer* 97, 1859-68 (2003)

68. Rajasekaran A.K. & S.A. Rajasekaran: Role of Na/K-ATPase in the assembly of tight junctions. *Am J Physiol Renal Physiol* 285, F388-F396 (2003)

69. Espineda C.E, J.H. Chang, J. Twiss, S.A. Rajasekaran & A.K. Rajasekaran: Repression of Na/K-ATPase beta-1-subunit by the transcription factor snail in carcinoma. *Mol Biol Cell* 15, 1364-73 (2004)

70. Sakai H, T. Suzuki, M. Maeda, Y. Takahashi, N. Horikawa, T. Minamim, K. Tsukada & N. Takeguchi: Up-regulation of Na/K-ATPase alpha 3-isoform and down-regulation of alpha 1-isoform in human colorectal cancer. *FEBS Lett* 563, 151-54 (2004)

71. Gilmore-Hebert M, J.W. Schneider, A.L. Greene, N. Berliner, C.A. Stolle, K. Lomax, R.W. Mercer & E.J. Benz Jr: Expression of multiple Na/K-adenosine triphosphatase isoform genes in human hematopoietic cells. Behavior of the novel alpha 3 isoform during induced maturation of HL60 cells. *J Clin Invest* 84, 347-51 (1989)

72. Crambert G, U. Hasler, A.T. Beggah, C. Yu, N.N. Modyanov, J.D. Horisberger, L. Lelievre & K. Geering: Transport and pharmacological properties of nine different human Na/K-ATPase isozymes. *J Biol Chem* 275, 1976-86 (2000)

73. Kometiani P, J. Tian, J. Li, Z. Nabih, G. Gick & Z. Xie: Regulation of Na/K-ATPase beta 1-subunit gene expression by ouabain and other hypertrophic stimuli in neonatal rat cardiac myocytes. *Mol Cell Biochem* 215, 65-72 (2000)

74. Haustein K.O: The relation of membrane-ATPase activity with congestive heart failure. *Int J Clin Pharmacol Ther Toxicol* 25, 651-55 (1987)

75. Liu Z.Q, A.Q. Ma & D.Y. Yang: Intra-cellular electrolytes changes and levels of endogenous digoxin-like substance within the plasma in patients with congestive heart failure. *Int J Cardiol* 27, 47-53 (1990)

76. Ko Y.S, S.Y. Yang & W.K. Yian: Clinical significance of changes in the serum level of endogenous digitalis-like factor in patients with chronic congestive heart failure. *Zhonghua Nei Ke Za Zhi* 29, 11-13 (1990)

77. Gaillard R.C: Interaction between the hypothalamo-pituitary-adrenal axis and the immunological system. *Ann Endocrinologie* 62, 155-163 (2001)

78. Savino W. & M. Dardenne: Neuroendocrine control of thymus physiology. *Endocrine Reviews* 21, 412-443 (2000)

79. Goya R.G, O.A. Brown & F. Bolognani: The Thymus-Pituitary axis and its changes during aging. *Neuroimmuno-modulation* 6, 137-142 (1999)

80. Sapolsky R.M, L.M. Romero & A.U. Munck: How do glucocorticoids influence stress responses ? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Reviews* 21, 55-89 (2000)

81. Tarcic N, H. Ovadia, D.W. Weiss & J. Weidenfeld: Restraint stress-induced thymic involution and cell apoptosis are dependent on endogenous glucocorticoids. *J Neuroimmunology* 82, 40-46 (1998)

82. Tizabi Y. & G. Aguilera: Desensitization of the hypothalamic-pituitary-adrenal axis following prolonged administration of corticotropin-releasing hormone or vasopressin. *Neuroendocrinology* 56, 611-8 (1992)

83. Wilkinson C.W, E.R. Peskind & M.A. Raskind: Decreased hypothalamic-pituitary-adrenal axis sensitivity

to cortisol feedback inhibition in human aging. *Neuroendocrinology* 65, 79-90 (1997)

84. Seeman T.E. & Robbins R.J: Aging and hypothalamic-pituitary-adrenal response to challenge in humans. *Endocrine Reviews* 15, 233-260 (1994)

85. Meaney M.J, S. Bhatnagar, S. Laroque, C. McCormick, N. Shanks, S. Sharma, J. Smythe, V. Viau & P.M. Plotsky: Individual differences in the hypothalamic-pituitary-adrenal stress response and the hypothalamic CRF system. *Ann NY Acad Sci* 19, 70-84 (2000)

86. Shanks N, S. Larocque & M.J. Meaney: Neonatal endotoxin exposure alters the development of the hypothalamic-pituitary-adrenal axis: early illness and later responsivity to stress. *J Neurosci* 15, 376-84 (1995)

87. Michelson D. & P.W. Gold: Pathophysiologic and somatic investigations of hypothalamic-pituitary-adrenal axis activation in patients with depression. *Ann NY. Acad Sci* 19, 717-722 (2000)

88. Isaacson J.H & B.M. Cattanach: Report. *Mouse News Lett* 27, 31 (1992)

89. In: Immunology. Eds: Goldsby R.A, T.J. Kindt, B.A. Osborne, J. Kuby. *W.H. Freeman Pub USA* 477-83 (2003)

90. Weidemann H, N. Salomon, T. Avnit-Sagi, J. Weidenfeld, H. Rosen & D. Lichtstein: Diverse effects of stress and additional adrenocorticotropic hormone on digitalis-like compounds in normal and nude mice. *J Neuroendocrinology* 16, 458-463 (2004)

91. Shiratori O: Growth inhibitory effect of cardiac glycosides and aglycones on neoplastic cells: in vitro and in vivo studies. *Gann* 58, 521-28 (1967)

92. Mayhew E. & C. Levinson: Reversibility of ouabain induced inhibition of cell division and cation transport in Ehrlich ascites cells. *J Cell Physiol* 72, 73-75 (1968)

93. Hartwell J.L. & B.J. Abbott: Antineoplastic principles in plants: recent developments in the field. *Adv Pharmacol* 7, 117-209 (1969)

94. Stenkvist B: Is digitalis a therapy for breast carcinoma ? *Oncol Rep* 6, 493-96 (1999)

95. Haux J: Digitalis: impinges on more than just the (ion-) pump. *Med Hypotheses* 59, 781-82 (2002)

96. Lopez-Lazaro M, N. Palma del la Pena, N. Pastor, C. Martin-Codero, E. Navarro, F. Cortes, M.J. Ayuso & M.V. Toro: Anti-tumor activity of Digitalis purpurea subsp. heywoodii. *Planta Med* 69, 701-04 (2003)

97. Haux J: Digitoxin is a potential anticancer agent for several types of cancer. *Med Hypotheses* 53, 543-48 (1999)

98. Numazawa S, M.A. Shinoki, H. Ito, T. Yoshida & Y. Kuroiwa: Involvement of Na^+, K^+-ATPase inhibition in K562 cell differentiation induced by bufalin. *J Cell Physiol* 160, 113-120 (1994)

99. Zhang L, K. Nakaya, T. Yoshida & Y. Kuroiwa: Induction by bufalin of differentiation of human leukemia cells HL60, U937, and ML1 toward macrophage/ monocyte-like cells and its potent synergistic effect on the differentiation of human leukemia cells in combination with other inducers. *Cancer Res* 52, 4634-4641 (1992)

100. Jing Y, H. Ohizumi, N. Kawazoe, S. Hashimoto, Y. Masuda, S. Nakajo, T. Yoshida**,** Y. Kuroiwa & K. Nakaya: Selective inhibitory effect of bufalin on growth of human tumor cells in vitro: association with the induction of apoptosis in leukemia HL-60 cells. *Jpn J Cancer Res* 85, 645-651 (1994)

101. Watabe M, N. Kawazoe, Y. Masuda, S. Nakajo & K. Nakaya: *Bcl-2* protein inhibits bufalin-induced apoptosis through inhibition of mitogen-activated protein kinase activation in human leukemia U937 cells. *Cancer Res* 57, 3097-3100 (1997)

102. Watabe M, Y. Masuda, S. Nakajo, T. Yoshida, Y. Kuroiwa & K. Nakaya: The cooperative interaction of two different signaling pathways in response to bufalin induces apoptosis in human leukemia U937 cells. *J Biol Chem* 271, 14067-14072 (1996)

103. McConkey D.J, Y. Lin, L.K. Nutt, H.Z. Ozel & R.A. Newman: Cardiac glycosides stimulate Ca^{2+} increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. *Cancer Res* 60, 3807-12 (2000)

104. Yeh J.H, W.J. Huang, S.F. Kann & P.S. Wang: Inhibitory effects of digitalis on the proliferation of androgen dependent and independent prostate cancer cells. *J Urol* 166, 1937-42 (2001)

105. Yeh J.H, W.J. Huang, S.F. Kann & P.S. Wang: Effects of bufalin and cinobufalgin on the proliferation of androgen dependent and independent prostate cancer cells. *Prostate* 54, 112-24 (2003)

106. Huang Y.T, S.C. Chueh, C.M. Teng & J.H. Guh: Investigation of ouabain-induced anticancer effect in human androgen-independent prostate cancer PC-3 cells. *Biochem Pharmacol* 67, 727-33 (2004)

107. Lin H, J.L. Juang & P.S. Wang: Involvement of Cdk5/p25 in digoxin-triggered prostate cancer cell apoptosis. *J Biol Chem* 30, (2004) (ahead of print)

108. Chueh S.C, J.H. Guh, J. Chen, M.K. Lai & C.M. Teng: Dual effects of ouabain on the regulation of proliferation and apoptosis in human prostatic smooth muscle cells. *J Urol* 166, 347-53 (2001)

Key Words: Digitalis-Like-Compounds, Na^+,K^+-ATPase, Apoptosis, Hypothalamo-Pituitary-Adrenal Axis, Stress, Thymus, Cancer , Review

Send correspondence to: Dr Heidrun Weidemann, The Jewish Hospital Berlin, Department of Internal Medicine/Cardiology, Berlin, GFR, Tel. +49-30-21472812, Fax: +49-30-4994 2514, E-mail: hweidemann@freenet.de

http://www.bioscience.org/current/vol10.htm