Effects of obesity and estradiol on Na⁺/K⁺-ATPase and their relevance to cardiovascular diseases

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Abstract

Obesity is associated with aberrant sodium/potassium-ATPase (Na⁺/K⁺-ATPase) activity, apparently linked to hyperglycemic hyperinsulinemia, which may repress or inactivate the enzyme. The reduction of Na⁺/K⁺-ATPase activity in cardiac tissue induces myocyte death and cardiac dysfunction, leading to the development of myocardial dilation in animal models; this has also been documented in patients with heart failure (HF). During several pathological situations (cardiac insufficiency and HF) and in experimental models (obesity), the heart becomes more sensitive to the effect of cardiac glycosides, due to a decrease in Na⁺/K⁺-ATPase levels. The primary female sex steroid estradiol has long been recognized to be important in a wide variety of physiological processes. Numerous studies, including ours, have shown that estradiol is one of the major factors controlling the activity and expression of Na⁺/K⁺-ATPase in the cardiovascular (CV) system. However, the effects of estradiol on Na⁺/K⁺-ATPase in both normal and pathological conditions, such as obesity, remain unclear. Increasing our understanding of the molecular mechanisms by which estradiol mediates its effects on Na⁺/K⁺-ATPase function may help to develop new strategies for the treatment of CV diseases. Herein, we discuss the latest data from animal and clinical studies that have examined how pathophysiological conditions such as obesity and the action of estradiol regulate Na⁺/K⁺-ATPase activity.

Key Words

- ▶ estradiol
- ▶ sodium/potassium adenosine triphosphatase
- ▶ obesity
- ▶ cardiovascular diseases

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Introduction

Sodium/potassium-ATPase (Na⁺/K⁺-ATPase) is a membrane protein responsible for the active transport of Na⁺ and K⁺ ions across the plasma membranes of most higher eukaryotes (Therien & Blostein 2000, Kaplan 2002). The energy for this transport is derived from the hydrolysis of

the terminal phosphate bond of ATP, during which the acyl phosphate intermediate is formed, a hallmark of the diverse membership of the P-type ATPase family (Kaplan 2002). A reduction in Na⁺/K⁺-ATPase levels is associated with obesity, apparently linked to hyperglycemic

hyperinsulinemia, which may repress or inactivate the enzyme (Fig. 1; Iannello *et al.* 2007a,b). A decrease in cardiac Na⁺/K⁺-ATPase activity or protein concentration contributes to the deficiencies in cardiac contractility in animal models and has been documented in patients with heart failure (HF; Schwinger *et al.* 2003, Liu *et al.* 2012). The majority of hormones (e.g. insulin, estradiol, aldosterone, thyroid hormone, catecholamines, and glucocorticoids) exert a positive effect on Na⁺/K⁺-ATPase by increasing its activity or synthesis of new alpha (α)- and beta (β)-subunits as well as by activating specific signaling cascades (Ewart & Klip 1995, Dzurba *et al.* 1997, Devarajan & Benz 2000, Al-Khalili *et al.* 2004, Liu *et al.* 2007).

The primary female sex steroid estradiol has long been recognized as an important hormone in a wide variety of physiological processes (Knowlton & Lee 2012). Epidemiological and retrospective studies have provided important evidence for the diverse roles of estradiol in human physiology and disease (Barros & Gustafsson 2011, Burns & Korach 2012). Premenopausal women are protected against cardiovascular (CV) diseases, while postmenopausal women have the same risk for this disease as men do (Rosano & Fini 2002, Katsiki *et al.* 2011c). Obviously, estradiol deficiency plays a key role. Also, after menopause when estradiol levels are decreased, lipid accumulation and visceral fat mass are increased, which all lead to an increased risk for the development of CV diseases (Geer & Shen 2009). One of the cardioprotective mechanisms proposed for estradiol involves its ability to increase the activity and expression of Na⁺/K⁺-ATPase *in vitro* (Dzurba *et al.* 1997, Isenovic *et al.* 2002, Sudar *et al.* 2008). Numerous studies, including ours, have shown that estradiol is one of the major regulators of Na⁺/K⁺-ATPase in the CV system (Dzurba *et al.* 1997, Isenovic *et al.* 2002,

Palacios *et al.* 2004). This explanation is also supported by the observation that estradiol-induced increase in Na⁺/K⁺-ATPase α2 expression leads to a significantly higher activity of Na⁺/K⁺-ATPase (Palacios *et al.* 2004). However, the mechanisms by which estradiol affects Na⁺/K⁺-ATPase remain unclear, in both normal and pathological conditions (such as obesity). Increasing our understanding of the molecular mechanisms determining the action of estradiol on Na⁺/K⁺-ATPase in humans may help to develop new strategies for the treatment of CV diseases, particularly in women. Therefore, in the present review, we discuss the latest data from animal and clinical studies focusing on the regulation of Na⁺/K⁺-ATPase in pathophysiological conditions such as obesity and also the effects of estradiol on the regulation of Na⁺/K⁺-ATPase.

Na⁺/K⁺-ATPase: structure and function

The primary function of Na⁺/K⁺-ATPase is the maintenance of low intracellular Na⁺ and high intracellular K⁺ concentrations required for a multitude of cellular functions. This occurs in several steps (Therien & Blostein 2000, Kaplan 2002). Following binding of ATP to Na⁺/K⁺-ATPase, three Na⁺ ions from the cytoplasm associate with the molecule. The transfer of a phosphate group (via the hydrolysis of ATP) to Na⁺/K⁺-ATPase results in a conformational change that creates an opening outside the cell that allows the three bound Na⁺ ions to be released. Two extracellular K⁺ ions then bind, which following cleavage of the phosphate group are released inside the cell (Therien & Blostein 2000, Kaplan 2002, Shinoda *et al.* 2009).

The Na⁺/K⁺-ATPase molecule is a hetero-oligomer composed of α- and β-subunits, in a 1:1 ratio (Therien & Blostein 2000, Kaplan 2002). In addition, other proteins such as a family of seven homologous single transmembrane segment proteins (FXD), which are often referred to as γ-subunits, contribute to the stabilization and attenuation of Na⁺/K⁺-ATPase function (Fig. 2; Garty & Karlish 2006). The large catalytic α-subunit (molecular weight 110 kDa) contains binding sites for Na⁺ and K⁺ ions and cardiac glycosides (CGs; Therien & Blostein 2000, Kaplan 2002). It also possesses the transient phosphorylation site, where the terminal phosphate of ATP is attached to the protein via an aspartate³⁶⁹ residue (Pedersen *et al.* 1996, Ziegelhoffer *et al.* 2000). The α-subunit has ten transmembrane domains and two large intracellular loops, oriented such that the amino and carboxyl ends are located intracellularly (Fig. 2; Ziegelhoffer *et al.* 2000, Morth *et al.* 2007). The ATP

Figure 1

Na⁺/K⁺-ATPase regulation in normal condition and obesity. PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PKG, protein kinase G; [Na⁺]_i, intracellular Na⁺; [K⁺]_i, intracellular K⁺; CGs, cardiac glycosides; IR, insulin resistance; FFAs, free fatty acids; upward arrow, increased level; downward arrow, decreased level; upper and lower right quadrants, inhibitory/reduction effect.

Figure 2

Diagram showing the topological arrangement of α - (yellow), β - (orange), and γ - (green; FXYD) subunits in the P-type Na⁺/K⁺-ATPase. Drawn using PDB:2ZXE (Shinoda *et al.* 2009). Full colour version of this figure available via <http://dx.doi.org/10.1530/JOE-13-0144>.

binding sites are located in the larger cytoplasmic loop (Kaplan 2002). The α -subunit has four isoforms (α_1 , α_2 , α_3 , and α_4) (Juhaszova & Blaustein 1997, Therien & Blostein 2000, Segall *et al.* 2001). While the α_1 -isoform is expressed ubiquitously, the α_2 -isoform is expressed largely in the brain, heart, and muscle, as well as in a number of other tissues (Kaplan 2002, Dostanic-Larson *et al.* 2006). The α_3 -isoform is found in neurons and ovaries, but it is also present in white blood cells and heart of some species, such as humans (Jewell & Lingrel 1991, Therien & Blostein 2000, Dostanic-Larson *et al.* 2006). The α_4 -isoform is localized to the testis. It is specifically synthesized at the spermatogenic stage and is required for sperm motility (Fig. 3; Woo *et al.* 2000).

Despite the fact that various α -isoforms share a high degree of sequence identity (~85% identity) (Jewell & Lingrel 1991, Blanco & Mercer 1998), it is still necessary to distinguish between tissue-specific and isoform-specific differences in relation to their functional properties (Therien & Blostein 2000, Kaplan 2002). It has also been shown in cardiac cells that different isoforms (α_1 and α_2) can localize in different regions of the same cell (James *et al.* 1999). This suggests that the different isoforms are capable of carrying out specific functions.

The smaller and highly glycosylated β -subunit (molecular weight 35–55 kDa) is required for the stabilization of the α -subunit and, it is thought, may modulate cation affinity (Therien & Blostein 2000, Kaplan 2002). *In vitro* studies using purified proteins indicate that the separation of α - and β -subunits results in a lack of measurable enzyme activity (Xie *et al.* 1996). The β -subunit acts as a chaperone to stabilize the correct folding and delivery of the α -subunit to the membrane (Kawamura & Noguchi 1991, Koksoy 2002). The β -subunit has a short cytoplasmic tail, one transmembrane segment, and a large, glycosylated extracellular segment (Fig. 2; Beggah *et al.* 1997, Morth *et al.* 2007). Three isoforms of the β -subunit exist (β_1 , β_2 , and β_3) (Therien & Blostein 2000, Kaplan 2002). The β_1 -isoform is, similar to the α_1 -isoform, ubiquitously expressed, suggesting a house-keeping role for the $\alpha_1\beta_1$ Na⁺/K⁺-ATPase in most cells (Tokhtaeva *et al.* 2012). The β_2 -isoform is expressed predominantly in the brain and muscle (Avila *et al.* 1998), while the β_3 -isoform is mainly expressed in the lungs, testis, skeletal muscle, brain, and liver (Appel *et al.* 1996, Tokhtaeva *et al.* 2012). In human heart, α_1 -, α_2 -, and

Figure 3

Distribution and function of Na⁺/K⁺-ATPase subunits.

$\alpha 3$ -isoforms are expressed together with the $\beta 1$ -isoform and very low levels of $\beta 2$ -isoform in a region-specific manner (Fig. 3; Schwinger *et al.* 1999, 2003).

The FXYD proteins are a family of seven homologous single transmembrane segment proteins (FXYD1–7), termed after the invariant FXYD amino acid motif in their extracellular domain (Fig. 2; Sweadner & Rael 2000, Garty & Karlish 2006). They act as tissue-specific regulatory subunits, which adjust the kinetic properties of Na⁺/K⁺-ATPase to the needs of the particular cell type or physiological state (Garty & Karlish 2006). FXYD1 (or phospholemman) is mainly expressed in the heart and skeletal muscle (Chen *et al.* 1997, Lifshitz *et al.* 2006, Geering 2008); its regulation is important for Na⁺/K⁺-ATPase function, especially in CV diseases. In addition, FXYD2, initially referred to as the γ -subunit, has been shown to be predominantly expressed in the kidneys (Fig. 3; Forbush *et al.* 1978, Kim *et al.* 1997, Garty & Karlish 2006). Later studies have described other γ -subunits that belong to the same family of proteins but expressed in other tissues (Garty & Karlish 2006, Geering 2008). However, to our knowledge, these are not known to be important for the regulation of Na⁺/K⁺-ATPase in the CV system.

It has been reported that the four α -subunit and three β -subunit isoforms of Na⁺/K⁺-ATPase are encoded by different genes and are synthesized independently of each other (Lingrel & Kuntzweiler 1994, Jorgensen *et al.* 2003). The isoforms combine to form a number of Na⁺/K⁺-ATPase isozymes expressed in a tissue-specific and cell-specific manner (Jorgensen *et al.* 2003). The heterodimeric protein subunits are synthesized independently in the endoplasmic reticulum and assembly during or very soon after synthesis in this organelle (Geering *et al.* 1996, Therien & Blostein 2000, Efendiev *et al.* 2007).

The concentration of Na⁺/K⁺-ATPase in tissues varies greatly; a large difference exists between the lowest (i.e. 250–500 sites/cell in erythrocytes) and the highest (i.e. 11 000–12 000 pmol/g wet weight in the brain cortex) measured concentrations (Lauf & Joiner 1976, Wiley & Shaller 1977, Schmidt *et al.* 1992, 1996, Koksoy 2002). A number of studies comparing different heterologously expressed human Na⁺/K⁺-ATPase isoforms have been conducted. Two studies revealed that ouabain had a twofold lower affinity for the $\alpha 2\beta 1$ -heteromer than for the $\alpha 1\beta 1$ - or $\alpha 3\beta 1$ -heteromer (Crambert *et al.* 2000, Muller-Ehmsen *et al.* 2001, Schwinger *et al.* 2003). In these studies, examining the apparent affinity for Na⁺ and K⁺ yielded conflicting results. The effects of Na⁺ and K⁺ on equilibrium ouabain binding were measured to estimate the affinity of Na⁺/K⁺-ATPase for these cations.

The apparent affinities of Na⁺ and K⁺ were measured as the half-activation constant for Na⁺ and K⁺ ($K_{0.5}$), which is different from the intrinsic affinity (K_m) (Jaisser *et al.* 1994). $K_{0.5}$ values (concentration of ligand that yields the half-maximal response) for Na⁺ and K⁺ were obtained from the Na⁺ and K⁺ antagonism of equilibrium ouabain binding. In one study, affinities for Na⁺ were found to be similar for all the cardiac heteromers (Muller-Ehmsen *et al.* 2001), while in another study $\alpha 1\beta 1$ -heteromer had the highest affinity for Na⁺ and the $\alpha 3\beta 1$ -heteromer had the lowest affinity (Crambert *et al.* 2000). Affinities toward K⁺ were similar for the $\alpha 1\beta 1$ - and $\alpha 3\beta 1$ -heteromers, but the affinity of the $\alpha 2\beta 1$ -heteromer was significantly lower in one study (Muller-Ehmsen *et al.* 2001), while in another study affinities toward K⁺ were found to be similar for these heteromers (Crambert *et al.* 2000).

Regulation of Na⁺/K⁺-ATPase in physiological and pathophysiological conditions

Hormones and environmental factors can regulate Na⁺/K⁺-ATPase activity through i) gene expression, ii) trafficking, and iii) phosphorylation (Ewart & Klip 1995, Isenovic *et al.* 2004a,b, Efendiev *et al.* 2007, Li *et al.* 2011). The first mechanism is through the regulation of gene transcription, generally occurring over days (Bonvalet 1998, Therien & Blostein 2000). The second mechanism of regulation is that new Na⁺/K⁺-ATPase subunits are delivered to the plasma membrane from intracellular stores when needed (Hundal *et al.* 1992, Al-Khalili *et al.* 2003). It has been shown that Na⁺/K⁺-ATPase-containing compartments are located just underneath the plasma membrane (Efendiev *et al.* 2007). Regulation performed through direct effects on the kinetic behavior of the enzyme occurs within minutes to hours and is accomplished through changes in the turnover rate of the existing pumps via the phosphorylation of protein kinase A (PKA), PKC, PKB, or PKG (Fig. 1; Bertorello & Katz 1993, Li *et al.* 1999, Therien & Blostein 2000, Sudar *et al.* 2008). The third proposed mechanism includes regulation through direct effects on the kinetic behavior of the pumps that are already present in the membrane, which occurs within minutes to hours (Therien & Blostein 2000). Despite this, it is important to point out that this mechanism is controversial (Chibalin *et al.* 1998, 1999, Fuller *et al.* 2004, 2013, Despa *et al.* 2005, Silverman *et al.* 2005).

Na⁺/K⁺-ATPase is the specific target for the action of digitalis and other CGs. These are produced in mammals in a manner similar to the production of steroid hormones

from cholesterol and act as indirect regulators of cardiac contractility (positive inotropy) (Bagrov *et al.* 2009, Lingrel 2010). CGs bind to the extracellular part of Na⁺/K⁺-ATPase to inhibit its activity (Kaplan 2002, Lingrel 2010); it is widely accepted that one ouabain binds to one αβ heteromer (Kaplan 2002). Thus, the inhibition of Na⁺/K⁺-ATPase in myocytes by endogenous CGs leads, at least locally, to an increased Na⁺ concentration, followed by increased intracellular Ca²⁺ levels (via Na⁺,Ca²⁺-exchangers) (Bagrov *et al.* 2009, Lingrel 2010). This rise in intracellular Ca²⁺ content triggers the release of Ca²⁺ from the sarcoplasmic reticulum, resulting in increased heart contraction (Grupp *et al.* 1985, Bagrov *et al.* 2009). Besides this, there is growing evidence that non-inhibitory doses of ouabain can modulate cell proliferation, apoptotic threshold, cell-to-cell contact, and cell migration (Aperia 2007). These effects generally require the binding of ouabain to Na⁺/K⁺-ATPase and then Na⁺/K⁺-ATPase can function as a signal transducer (Aperia 2007). Several years ago, Aperia's group made the observation that ouabain triggered Na⁺/K⁺-ATPase-dependent activation of the inositol 1,4,5-trisphosphate receptor (IP3R) via a direct interaction (Aizman *et al.* 2001, Khodus *et al.* 2011). The activation of IP3R results in oscillatory increases in intracellular Ca²⁺ content (Aizman *et al.* 2001, Aperia 2007). Ca²⁺ oscillations have emerged as the most versatile of all cell signals, as the cell can decode the frequency of the oscillations (Berridge 2007). Ca²⁺ oscillations generated by the Na⁺/K⁺-ATPase-IP3R complex have a low frequency and activate the pleiotropic transcriptional factor nuclear factor kappa B (NFκB), which protects from apoptosis (Li *et al.* 2006, Khodus *et al.* 2011). During several pathological situations (cardiac insufficiency and HF) and in obesity experimental models, the heart becomes more sensitive to the effect of CGs due to a decrease in the number of Na⁺/K⁺-ATPase molecules (Shamraj *et al.* 1993, Koksoy 2002, Liu *et al.* 2012). Therefore, the regulation of Na⁺/K⁺-ATPase activity and expression may be important for the treatment and possible prevention of these diseases (Fig. 1; Koksoy 2002).

The expression of Na⁺/K⁺-ATPase isoforms is regulated with high specificity in different regions of the heart during both physiological and pathophysiological states. In normal human left ventricular (LV) myocardium, a Na⁺/K⁺-ATPase concentration of ~700 pmol/g wet weight can be found (Schmidt *et al.* 1993, Schwinger *et al.* 2003). Also, in normal human right atrium, Na⁺/K⁺-ATPase activity is 40% lower vs that in LV myocardium (Schwinger *et al.* 2003, Wencker *et al.* 2003). Several studies have reported a consistent and

significant decrease of 26–32% in Na⁺/K⁺-ATPase protein content in human HF (Norgaard *et al.* 1988, Schwinger *et al.* 1990, 2003). Data show that the inhibition of cardiac myocyte death largely prevents the development of cardiac dilation and contractile dysfunction (Wencker *et al.* 2003), i.e. the hallmarks of HF. Recently, it has been found that a reduction in Na⁺/K⁺-ATPase levels in cardiac tissue induces myocyte death and cardiac dysfunction (Liu *et al.* 2012), thus possibly leading to the development of myocardial dilation and HF. Norgaard *et al.* (1988) also reported that decreased Na⁺/K⁺-ATPase concentration may be of importance for myocardial dysfunction and dilated cardiomyopathy. They enrolled 24 patients with suspected idiopathic dilated cardiomyopathy in their study (Norgaard *et al.* 1988). Nineteen patients had impaired LV function and a Na⁺/K⁺-ATPase concentration of 331 ± 19 pmol/g wet weight, whereas five patients had normal LV function and a Na⁺/K⁺-ATPase concentration of 559 ± 62 pmol/g wet weight (*P* < 0.001) (Norgaard *et al.* 1988).

In heart tissue biopsies from patients with dilated cardiomyopathy, total Na⁺/K⁺-ATPase concentration is decreased by up to 40% (Norgaard *et al.* 1988). Semb *et al.* (1998) examined changes in cardiac Na⁺/K⁺-ATPase expression and function in a post-infarction rat model of hypertrophy and congestive HF (CHF). They found that in the CHF group the ratio of heart weight to body weight was 70% greater than that in the control group (*P* < 0.05). Also, the expression of the α1- and β1-subunits (mRNA and protein) of the Na⁺/K⁺-ATPase was not significantly different in the CHF and control groups, but mRNA and protein levels of the α2-isoform were lower in the hearts of the CHF group by 25 and 55% respectively; mRNA levels of the α3-isoform were higher by 120% and cell volume of the isolated cardiomyocytes was 30% larger in the CHF group than in the control group (Semb *et al.* 1998). Allen *et al.* (1992) found no significant alteration in mRNA expressions of the isoforms, but total Na⁺/K⁺-ATPase concentration was non-significantly reduced by ~10% in patients with end-stage HF due to either ischemic or dilated cardiomyopathy, compared with the normal controls. Shamraj *et al.* (1993) reported for the first time that failing human hearts are more sensitive to ouabain, due to a mean reduction of 42% in Na⁺/K⁺-ATPase concentration. Schwinger *et al.* (1999) showed that the reduction of the expression and activity of Na⁺/K⁺-ATPase protein enhanced the sensitivity of failing human myocardium toward CGs; at the protein level, α1- and α3-isoform levels were lower (by –38 and –30% respectively) in failing human myocardium than

in the non-failing one. Similarly, the abundance of $\beta 1$ -isoform, maximal ouabain binding, and Na⁺/K⁺-ATPase activity were lower (by -39 , -39 , and -42% respectively), while the expression of the $\alpha 2$ -isoform showed only a small tendency toward reduction (Schwinger *et al.* 1999, 2003).

Regulation of Na⁺/K⁺-ATPase by estradiol

Several studies have shown that estradiol is one of the primary Na⁺/K⁺-ATPase regulators in the CV system (Dzurba *et al.* 1997, Isenovic *et al.* 2002, Palacios *et al.* 2004, Li *et al.* 2011). This finding is also supported by the observation that estradiol-induced increase in Na⁺/K⁺-ATPase $\alpha 2$ expression leads to a significantly higher Na⁺/K⁺-ATPase activity (Palacios *et al.* 2004). Estradiol has also been reported to enhance Na⁺/K⁺-ATPase activity in H9C2 cardiac myocytes and rat hearts (Liu *et al.* 2007, 2012). By contrast, ovariectomized rats exhibited a decreased Na⁺/K⁺-ATPase activity (Kaur *et al.* 1997, Li *et al.* 2011). In women, Na⁺/K⁺-ATPase activity in erythrocytes increased when estradiol levels reached their peak during the menstrual cycle (Melis *et al.* 1990).

Estradiol exerts these effects through the activation of multiple signaling cascades. One studied mechanism of the activation of Na⁺/K⁺-ATPase includes phosphatidylinositol 3-kinase (PI3K) and PKB (Akt) (Isenovic *et al.* 2002, 2004b, Sudar *et al.* 2008). The activation of PI3K and Akt also plays an important role in the heart through the regulation of the survival and function of cardiomyocytes (Huang & Kaley 2004, Matsui & Rosenzweig 2005). It has also been reported that premenopausal women display a significantly greater staining of Akt in the nuclei of cardiac myocytes than men or postmenopausal women (Camper-Kirby *et al.* 2001, Sugden & Clerk 2001). Also, elevated nuclear phospho-Akt⁴⁷³ localization in cultured cardiomyocytes after exposure to estradiol or the phytoestrogen genistein has also been shown (Camper-Kirby *et al.* 2001, Huang & Kaley 2004). The activation of Akt in a gender-dependent manner may help explain functional benefits for the heart provided by estrogenic stimulation and also differences in CV disease risks between the sexes (Camper-Kirby *et al.* 2001). In the CV system, the activation of estrogen receptor α (ER α) by estradiol has been shown to activate PI3K through the binding of phosphotyrosine-containing proteins such as insulin receptor substrate-1 (IRS1) and IRS2 and the association of p85 with IRS1 in different types of cells (Mauro *et al.* 2001, Isenovic *et al.* 2003, Sudar *et al.* 2008, Koricanac *et al.* 2009). Some authors, including our team, have already

described an inducing effect of estradiol on Akt serine (Ser⁴⁷³) phosphorylation, but data concerning threonine (Thr³⁰⁸) are rare (Ren *et al.* 2003, Patten *et al.* 2004, Koricanac *et al.* 2011).

Our previously published results together with others indicated that ERK1/2 are involved in the regulation of Na⁺/K⁺-ATPase (Al-Khalili *et al.* 2004, Isenovic *et al.* 2004a). We have shown that the ERK1/2 inhibitor PD98059 abrogates the stimulation of Na⁺/K⁺-ATPase activity, suggesting the involvement of ERK1/2 signaling as well (Isenovic *et al.* 2004a). Estradiol has been reported to rapidly activate ERK1/2, resulting from more proximal kinase activation, including Ras, Src, raf, and MEK stimulation (Migliaccio *et al.* 1996, Levin 2001, Koricanac *et al.* 2011).

Some of the effects of estradiol have been linked to the activity of high-molecular-weight (85 kDa) cytosolic phospholipase A₂ (cPLA₂; Burlando *et al.* 2002, Sudar *et al.* 2008), which is also abundantly expressed in the heart and vasculature (LaPointe & Isenovic 1999, Murakami & Kudo 2002, Sudar *et al.* 2008). Evidence that cPLA₂ plays a role in the regulation of Na⁺/K⁺-ATPase activity includes a report that the inhibition of Na⁺/K⁺-ATPase activity is mediated by the activation of cPLA₂ (Xia *et al.* 1995). However, to our knowledge, a direct correlation between estradiol-induced activation of cPLA₂ and Na⁺/K⁺-ATPase regulation has not been established yet. Maximal activation of cPLA₂ requires sustained dual phosphorylation of Ser⁵⁰⁵ and Ser⁷²⁷ by MAPKs and MAPK-activated protein kinases respectively (Lin *et al.* 1993, Murakami & Kudo 2002, Sudar *et al.* 2008). Other kinases have been shown to phosphorylate cPLA₂, including the stimulation of ERK1/2 (van Rossum *et al.* 2001, Sudar *et al.* 2008).

The results presented by Li *et al.* (2011) indicated that estradiol may influence both the specific activity and physiological function of the cardiac Na⁺/K⁺-ATPase and these effects may participate in the reported protective influence of the hormone against myocardial ischemia. Using a yeast two-hybrid assay, Na⁺/K⁺-ATPase $\beta 1$ -subunits have been identified as potential binding partners for N-myc downstream-regulated gene 2 (NDRG2). NDRG2 is a cytoplasmic protein and member of the NDRG family (Deng *et al.* 2003, Hu *et al.* 2006, Li *et al.* 2011). It has been reported that human NDRG2 is expressed in many tissues, especially in the brain, heart, skeletal muscle, and kidneys, where Na⁺/K⁺-ATPase is enriched (Hu *et al.* 2006, Li *et al.* 2011). Analysis of the promoter region flanking 5' of the NDRG2 gene revealed a putative estrogen-response element in the region (5'-nnAGTCAnnnTGACCnn-3'), which suggests

that estradiol may also play a regulative role in the expression of *NDRG2* (Li *et al.* 2011).

Estradiol has been reported earlier to improve the excitation contraction coupling and maintenance of cation homeostasis in cardiac myocytes via the stimulation of the Na⁺/K⁺-ATPase activity of cardiac sarcolemmal membranes (Dzurba *et al.* 1997, Barta *et al.* 1989). In addition, it was speculated by Dzurba *et al.* (1997) that estradiol may interact with the enzyme molecule at least in three different ways:

- (i) Directly – whereby a chemical modification of the enzyme molecule may be responsible for the observed effects (Kamernitskii *et al.* 1982).
- (ii) Through the digitalis receptor – in this case, the action may be based on some structural similarities between the molecules of digitalis and that of estradiol (Franck *et al.* 1984).
- (iii) Through a Ca²⁺-dependent protein kinase-mediated phosphorylation mechanism – where the enzyme molecule may be activated by phosphorylation to bind to estradiol (Migliaccio *et al.* 1982, Dzurba *et al.* 1997, Sudar *et al.* 2008).

Na⁺/K⁺-ATPase and obesity

In obese patients, Na⁺/K⁺-ATPase activity is reduced in adipose tissue and negatively correlated with BMI, oral glucose tolerance test, and blood pressure (Iannello *et al.* 2007a,b). Obesity is associated with tissue Na⁺/K⁺-ATPase reduction, apparently linked to hyperglycemic hyperinsulinemia, which may repress or inactivate the enzyme (Iannello *et al.* 2007a,b). Also, obesity is associated with a reduction of Na⁺/K⁺-ATPase activity in both humans and rodents (Iannello *et al.* 2007a,b). Bray and Yukimura reported decreased Na⁺/K⁺-ATPase activity in the liver of animals with experimental obesity (Bray & Yukimura 1978). Furthermore, Lin *et al.* (1978, 1981) have shown that the protein levels of Na⁺/K⁺-ATPase subunits are lower in the skeletal muscle and liver of adult obese (*ob/ob*) mice than in those of their lean counterparts, probably due to the decrease in the number of enzyme units. This, of course, cannot be due to an inhibitory effect, but could be the result of a repressing effect (Iannello *et al.* 2007a). In this context, Guernsey & Morishige (1979) have also shown the reduction of Na⁺/K⁺-ATPase activity in *ob/ob* mice (Fig. 1).

As with laboratory rodent models, human obesity is associated with reduced Na⁺/K⁺-ATPase activity in some tissues (Iannello *et al.* 2007a,b). Insulin resistance,

a phenomenon common in obesity, may repress Na⁺/K⁺-ATPase enzyme activity, probably through the mediation of free fatty acids (FFAs), which are elevated in such cases (Iannello *et al.* 2007a,b). FFAs, present in the membrane or as the products of phospholipase A₂ (PLA₂)-dependent regulatory pathway, tend to inhibit Na⁺/K⁺-ATPase (Oishi *et al.* 1990, Therien & Blostein 2000). Interestingly, Iannello *et al.* (1994) reported that Na⁺/K⁺-ATPase activity is reduced in the adipose tissue of obese hyperinsulinemic subjects (Fig. 1).

Obesity as well as diabetes is associated with hyperleptinemia in the presence of leptin resistance; various drugs frequently prescribed to such patients may also affect leptin levels (Katsiki *et al.* 2011a,b, Paspala *et al.* 2012). Leptin has been shown to decrease Na⁺/K⁺-ATPase activity in rat kidney via the PI3K pathway (Fig. 1; Beltowski *et al.* 2004). Impaired Na⁺/K⁺-ATPase function has also been observed in diabetes, hypertension, dyslipidemia, and metabolic syndrome (Koter *et al.* 2004, Chibalin 2007, Rodrigo *et al.* 2007, Javorkova *et al.* 2010). Interestingly, both metformin and statins have been reported to enhance the Na⁺/K⁺-ATPase activity of erythrocytes in diabetic and dyslipidemic patients respectively (Chakraborty *et al.* 2011, Uydu *et al.* 2012). Similarly, antihypertensive agents (i.e. losartan and enalapril) attenuated the decreased activity of sarcolemmal Na⁺/K⁺-ATPase in the failing heart of rats after myocardial infarction (Guo *et al.* 2008). Further studies are needed to investigate the impact of such CV risk-reducing drugs on Na⁺/K⁺-ATPase function.

Conclusions

Reduced Na⁺/K⁺-ATPase function seems to play a causal role in the development of CV diseases, probably due to the association of decreased Na⁺/K⁺-ATPase activity with other risk factors (e.g. obesity or impaired estradiol signaling). Thus, the regulation of Na⁺/K⁺-ATPase activity and expression as well as the regulation of different Na⁺/K⁺-ATPase isoforms may be important for the treatment and possible prevention of CV diseases. Despite many available data, the effects of estradiol on Na⁺/K⁺-ATPase still remain unclear, in both normal and pathological conditions, such as obesity. Increasing our understanding of the molecular mechanisms determining the action of estradiol on Na⁺/K⁺-ATPase in humans may help to develop new strategies for the treatment of CV diseases in women and possibly also in men.

Declaration of interest

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