BJP British Journal of Pharmacology

DOI:10.1111/bph.12270
www.brjpharmacol.org

Themed Section: Biological Sex and Cardiovascular Pharmacology

REVIEW

Sex-based differences in cardiac ischaemic injury and protection: therapeutic implications

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Keywords
acute coronary syndrome; cardioprotection; oestrogen; heart; ischaemia/reperfusion injury; sex differences; therapeutic implications

Received
25 January 2013
Revised
21 May 2013
Accepted
30 May 2013

Ischaemic heart disease (IHD) is the most frequent cause of mortality among men and women. Many epidemiological studies have demonstrated that premenopausal women have a reduced risk for IHD compared with their male counterparts. The incidence of IHD in women increases after menopause, suggesting that IHD is related to declining oestrogen levels. Experimental observations have confirmed the results of epidemiological studies investigating sex-specific differences in cardiac tolerance to ischaemia. Female sex appears also to favourably influence cardiac remodelling after ischaemia/reperfusion injury. Furthermore, sex-related differences in ischaemic tolerance of the adult myocardium can be influenced by interventions during the early phases of ontogenetic development. Detailed mechanisms of these sex-related differences remain unknown; however, they involve the genomic and non-genomic effects of sex steroid hormones, particularly the oestrogens, which have been the most extensively studied. Although the protective effects of oestrogen have many potential therapeutic implications, clinical trials have shown that oestrogen replacement in postmenopausal women may actually increase the incidence of IHD. The results of these trials have illustrated the complexity underlying the mechanisms involved in sex-related differences in cardiac tolerance to ischaemia. Sex-related differences in cardiac sensitivity to ischaemia/reperfusion injury may also influence therapeutic strategies in women with acute coronary syndrome. Women undergo coronary intervention less frequently and a lower proportion of women receive evidence-based therapy compared with men. Although our understanding of this important topic has increased in recent years, there is an urgent need for intensive experimental and clinical research to develop female-specific therapeutic strategies. Only then we will be able to offer patients better evidence-based treatment, a better quality of life and lower mortality.

LINKED ARTICLES
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Abbreviations
ACS, acute coronary syndrome; CaMKII, Ca^{2+} calmodulin-dependent protein kinase II; ER, oestrogen receptor; GPER, G protein coupled oestrogen receptor; HRT, hormone replacement therapy; IHD, ischaemic heart disease; PCI, percutaneous coronary intervention; ROS, reactive oxygen species

Introduction

Among cardiovascular diseases, ischaemic heart disease (IHD) is the single most frequent cause of death among men and women, and is responsible for more than one-half of all cardiovascular events (Steg *et al.*, 2012). Epidemiological studies have clearly shown that in premenopausal women the onset of IHD occurs, on average, 10 years later than in men, with myocardial infarction occurring 20 years later (Duvall, 2003; Bassuk and Manson, 2010).

The incidence of IHD in women increases markedly after menopause, suggesting that IHD is related to declining oestrogen levels. For these reasons, hormone replacement therapy (HRT) and the possible benefits of administering supplemental oestrogen to postmenopausal women have become popular research topics. However, randomized controlled trials (Hulley *et al.*, 1998; Herrington *et al.*, 2000), especially the Women's Health Initiative (WHI) study (Rossouw *et al.*, 2002), reported that oestrogen replacement in postmenopausal women actually increased the incidence

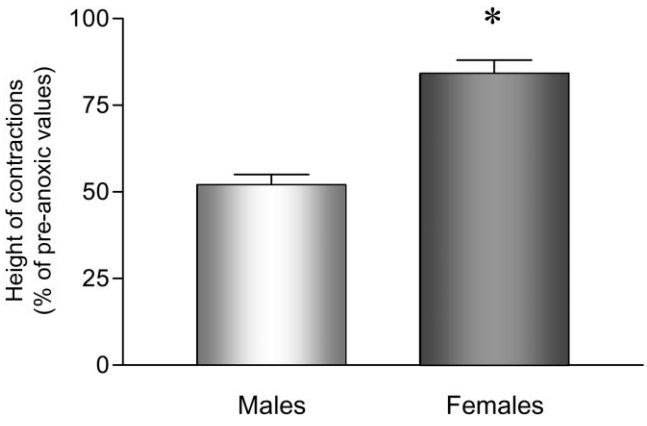

Figure 1

Ratio of the height of contractions of the isolated right ventricle before and after 20 min anoxic period in adult male and female Wistar rats, expressed as percent of value before the anoxic period. *$P < 0.01$, significantly different from males. Data from Ostadal *et al.* (1984).

of IHD. These results clearly illustrate the complexity of sex-related differences in cardiovascular disease.

Research focusing on IHD in women started in the late 1980s. The first experimental observation demonstrating that the hearts of female rats were more tolerant to oxygen deprivation than the hearts of male rats was published by our group in 1984 (Ostadal *et al.*, 1984) (Figure 1). In 1987, a stunning observation by Tobin *et al.* (1987) reported that women with symptoms suggestive of IHD were treated less aggressively than men. That article was followed by a second publication from the same investigators describing sex bias in the decision to recommend patients for coronary artery bypass surgery (Steingart *et al.*, 1991). The first book about women's unique experience with IHD was published by Legato and Colman (1991).

According to data from the Web of Science, the number of studies investigating sex-related differences in the cardiovascular system was negligible in 1989 (Ostadal *et al.*, 2009). However, the number of clinical and experimental studies has grown exponentially over the past 20 years. This increasing interest undoubtedly reflects the importance of this topic and the urgent need to explain underlying mechanisms, to better understand sex determinants of outcomes, and to minimize bias in the management and treatment of IHD in women. Accordingly, the present review attempts to summarize the available experimental data regarding sex-related differences in cardiac tolerance to ischaemia/reperfusion injury, the potential underlying mechanisms, the late effects of perinatal interventions and selected therapeutic implications.

Sex-related differences in normal myocardial function

Sex-related cardiac differences are apparent even in healthy individuals (reviewed in Legato and Leghe, 2010). For example, women have lower left ventricular mass (De Simone *et al.*, 1995), a stable number of myocytes during development compared with a decreasing number in men (Olivetti *et al.*, 1995) and a faster resting heart rate (Bazet, 1920; Czubryt *et al.*, 2006; Regitz-Zagrosek, 2006). Data on the myocardial contractile performance are controversial and not concise. Capasso *et al.* (1983) observed in rat papillary muscles that males had significantly lower rates of contraction and relaxation than females. Schaible and Scheuer (1984) using rat isolated working heart preparations observed increased cardiac output in males. And, finally, Leblanc *et al.* (1998) using papillary muscles found no difference between the sexes until rats were 6 months old. Another view arises from the study by Petre *et al.* (2007), despite virtually identical contractile performance of the feline heart under basal conditions, significant sex differences were found at higher stimulation frequencies. At the cellular and molecular levels, important differences have been observed in the mechanisms of cardiac excitation-contraction coupling (Farrell *et al.*, 2010), myocardial calcium metabolism, cardiac muscle responsiveness to Ca^{2+} and L-type Ca^{2+} channel modulation, mitochondrial calcium content, the production of reactive oxygen species (ROS) and the density of β-adrenoceptors (Chu *et al.*, 2005; Colom *et al.*, 2007; Curl *et al.*, 2008; Bell *et al.*, 2011; receptor nomenclature follows Alexander *et al.*, 2013). The presence of sex-related differences that are characteristic of the normal myocardium (the enumeration of which is far from complete) leads to the logical presumption that sex-related variations also exist in the reaction of cardiac muscle to diverse pathogenetic factors, including ischaemia/reperfusion injury, which, consequently, would lead to differences in therapeutic interventional strategies.

Sex-related differences in myocardial ischaemic injury

The decisive role in elucidating the mechanisms involved in sex-related differences in cardiac tolerance to ischaemia should be played by experimental studies involving laboratory animals. However, the major limiting factor in the vast majority of experiments that have been performed is the exclusive use of males (Leinwand, 2003). The primary reason is the fact that pathogenetic mechanisms in males – in contrast to females – are generally not influenced by fluctuations in hormonal activity. The often-repeated argument is the complicated comparison between age-matched males and females, with the body weight of females usually being lower. Most of the experimental observations have confirmed the results of epidemiological studies investigating sex-specific differences in the cardiac tolerance to ischaemia (see Booth and Lucchesi, 2008; Ostadal *et al.*, 2009; Deschamps *et al.*, 2010; Kolar and Ostadal, 2013). Although sex-related difference in cardiac tolerance to oxygen deficiency was first described in the 1980s (Ostadal *et al.*, 1984), detailed investigation of this issue started only recently. Increased resistance of the female myocardium to ischaemia/reperfusion injury was observed in mice, rats, rabbits and dogs (Johnson *et al.*, 2006; Murphy and Steenbergen, 2007, Ross and Howlet, 2012). Experiments using intact hearts from rats and mice

have shown that young adult females exhibit better recovery of contractile function and experience fewer arrhythmias during reperfusion than age-matched males (Bell *et al.*, 2008; Lujan and DiCarlo, 2008), although this has not been observed in all studies (Li and Kloner, 1995; Przyklenk *et al.*, 1995). Penna *et al.* (2009) stressed the importance of the duration of ischaemia: 30 min of ischaemia damages the female heart less than the male heart; the opposite, however, occurs in response to only 10 min of ischaemia. Improved functional recovery in females was accompanied by smaller infarct size, less lactate dehydrogenase release and decreased production of inflammatory cytokines (Lagranha *et al.*, 2010). Female hearts also exhibit less ischaemia and reperfusion injury than males under conditions that promote Ca^{2+} loading such as stimulation of β-adrenoceptors or increased external Ca^{2+} concentration (Cross *et al.*, 2002a). Similarly, female hearts exhibit less evidence of ischaemia/reperfusion injury, and enhanced contractility in transgenic models overexpressing the Na-Ca^{2+} exchanger and overexpressing the β_2-adrenoceptors, or ablation of phospholamban (Cross *et al.*, 1998; 1999; 2003). However, these studies used intact hearts, in which oestrogens in both cardiomyocytes and the vasculature potentially modify responses to ischaemia/reperfusion (Ross and Howlet, 2012). It is important to emphasize that even in spontaneously hypertensive rats, sex-related differences exist in the sensitivity to ischaemia/reperfusion: post-ischaemic recovery in female hearts was significantly better than the recovery in male hearts, in spite of comparable degrees of hypertension (Figure 2), (Besik *et al.*, 2007). Recently, it has been observed that obesity, another risk factor for IHD, increased myocardial infarct size in males but not in females (Clark *et al.*, 2011). Experimental and clinical studies have both indicated that female sex favourably influences the remodelling and adaptive response to myocardial infarction (Piro *et al.*, 2010; Regitz-Zagrosek *et al.*, 2010). Moreover, despite being older, women living with heart failure demonstrate a significantly better survival rate than men with this condition (Barker *et al.*, 2006). Thre are also sex-related differences in the extent of myocyte death following heart failure. Guerra *et al.* (1999) demonstrated that necrosis and apoptosis in the failing human heart were twice as high in men as in women, with the extent of necrosis being sevenfold greater than apoptosis in both sexes.

It follows that female sex favourably influences cardiac sensitivity to ischaemia/reperfusion injury. However, further analyses are required to clarify the pathogenetic mechanisms underlying the different processes. Better understanding of sex-related differences of the transition to heart failure following myocardial infarction will provide the necessary basis for the development of long-term therapeutic strategies.

Role of hormones in sex-related differences of cardiac sensitivity to ischaemia/reperfusion

The uncertainty of the effect of exogenous female hormones in postmenopausal women suggests that their protective effect alone does not fully explain sex-related differences in the susceptibility to IHD, which has stimulated intense experimental research of this complicated issue. Although the role of sex hormones is far from unambiguous, a large body of evidence suggests that oestrogen is involved in sex-related differences in cardiovascular diseases. The lack of protection conferred by HRT in women contrasts with observations in a number of animal studies in which oestrogen has been shown to be protective. Recently, Lagranha *et al.* (2010) clearly demonstrated that oestrogens contribute to the higher tolerance of the female heart; ovariectomy significantly increased infarct size in females, whereas the administration of oestrogen markedly reduced infarct size in males (Figure 3). Oestrogen has a number of effects on cardiovascular function and disease: it modulates vascular function, the inflammatory response, metabolism, insulin sensitivity, cardiac myo-

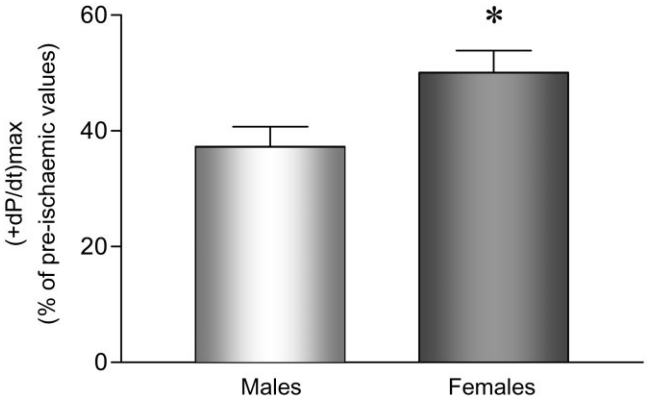

Figure 2
Recovery of peak rate of pressure development [$(+dP/dt)_{max}$] of the rat isolated heart (Langendorff preparation) during reperfusion after 20 min of global ischaemia, expressed as percentage of pre-ischaemic values. Adult SHR females versus SHR males. $^{**}P < 0.01$, significantly different from males. Data from Besik *et al.* (2007).

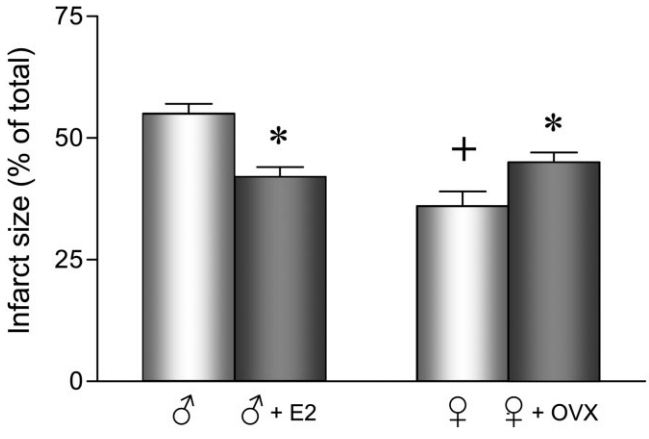

Figure 3
Infarct size in isolated perfused hearts from male and female rats after 30 min of ischaemia and 90 min of reperfusion. Sprague-Dawley rats. Male rats were treated with oestradiol (E2) for 2 weeks; female rats were ovariectomised (ovx). $^{*}P < 0.01$, significant effect of treatment. Data from Lagranha *et al.* (2010).

cytes, stem cell survival and the development of hypertrophy (Murphy, 2011). Based on many experimental studies, most of the action of this hormone has been attributed to oestrogen binding to either of the oestrogen receptors (ER), α or β, two nuclear hormone receptors. Apart from well-established cytosolic/nuclear localization, ERs have also been detected at the level of both the plasma membrane and the mitochondria (Chen *et al.*, 2005). It is important to note that both ER subtypes are widely expressed within cardiovascular tissues in a broad variety of species, ranging from mice to humans. In particular, cardiac myocytes, cardiac fibroblasts, vascular smooth muscle cells and endothelial cells express physiologically relevant levels of functional ER-α or ER-β proteins (Arias-Loza *et al.*, 2008). A number of studies have examined whether the protection afforded by oestrogen is mediated by ER-α or ER-β (Murphy and Steenbergen, 2007). These studies have been performed using either genetically altered mice that lack ER-α or ER-β, or by addition of a selective ER-α or ER-β agonist. Unfortunately, there is no consensus regarding which ER mediates the protection against ischaemia/reperfusion injury because there are data suggesting a role for both ER-α (Zhai *et al.*, 2000; Wang *et al.*, 2006b; Jeanes *et al.*, 2008) and ER-β (Gabel *et al.*, 2005; Nikolic *et al.*, 2007). To explain this discrepancy, different models of ischaemia/reperfusion, different endpoints, and the dose and timing of the addition of agonist should be taken into consideration (Murphy and Steenbergen, 2007). The activated ER complex triggers the synthesis of specific messenger RNAs and the production of several proteins that are responsible for the various effects elicited in different cell types.

In addition to these genomic effects of oestrogen, additional processes, termed 'non-genomic' (non-nuclear) processes, occur rapidly and are independent of protein synthesis (Di Lisa, 2006; Knowlton and Lee, 2012). Nitric oxide (NO) is one of the many agents that can modify the response of the myocardium in females. The expression of endothelial NO synthase (eNOS) is significantly higher in the female heart and inhibition of eNOS using L-NAME abolished the sex-related differences in cardiac sensitivity to ischaemia/reperfusion (Cross *et al.*, 2002b; Wang *et al.*, 2006b). Moreover, higher expression of eNOS leads to S-nitrosylation of L-type calcium channels, thereby reducing calcium overload, one of the main causes of ischaemia/reperfusion injury (Sun *et al.*, 2006). Also, NO relaxes smooth muscle in the arterial wall. The impact of oestrogen replacement therapy on serum lipid levels is well established; it is one of the few interventions that raise high-density lipoprotein cholesterol levels, and lower total cholesterol and low-density lipoprotein levels (Sack *et al.*, 1994). It is important to note, however, that oestrogen has no effect on established plaque (Mikkola and Clarkson, 2002). Another oestrogen signalling pathway involves the signalling kinase PI3K. It has been proposed that the ER can bind to PI3K in the membrane, and that oestrogen binding can activate PI3K signalling. Recent work has identified a third, membrane-associated ER, the G protein-coupled oestrogen receptor (GPER, Deschamps and Murphy, 2009), which may activate the non-nuclear protective response; for instance, GPER inhibited opening of the mitochondrial permeability transition pore (Bopassa *et al.*, 2010). Interestingly, an orphan GPER has also been suggested to bind oestrogen, resulting in activation of PI3K. Activation of the PI3K pathway may contribute to cardioprotection in females (Tong *et al.*, 2002). Thus, the cardioprotection observed in females may be mediated by altered protein expression or alterations in post-translational modifications mediated by signalling pathways.

Murphy and Steenbergen (2007) suggested that mitochondria are major targets of cardioprotective signalling. In fact, there are some studies suggesting that females exhibit altered cardiac mitochondrial function (Stirone *et al.*, 2005). Lagranha *et al.* (2010) observed that cardioprotection in females was mediated by altered mitochondrial proteins. They found that mitochondria isolated from females exhibited a number of post-translational modifications in mitochondrial enzymes involved in regulating the generation of ROS and oxidative metabolism. Therefore, females exhibit reduced ROS generation on reoxygenation. Recently, Morkuniene *et al.* (2010) and Pavon *et al.* (2012) described the relevance of oestrogens in maintaining proper mitochondrial function in response to the instability of mitochondrial membrane potential and mitochondrial permeability transition pore opening after ischaemia/reperfusion. They observed that the opening of this pore can be blocked by physiological concentrations of oestrogens, similar to blockade with the classic inhibitor cyclosporin.

These mechanisms are, however, not the only possible pathways responsible for the sex-related differences in the susceptibility of the heart to ischaemia. Thus, the cardioprotection associated with female sex was accompanied by greater expression of the sarcolemmal (Johnson *et al.*, 2006) and mitochondrial K_{ATP} (Lee *et al.*, 2000) channels, whose blockade increased the degree of injury in the female heart. Bae and Zhang (2005) hypothesized that the sex-related differences in heart susceptibility to ischaemia/reperfusion are reflected by increased protein kinase B (Akt) and PKCε levels. The proposed cardioprotective effect includes inhibition of apoptosis and, hence, reduction of myocardial infarction. The possible protective effect of Akt is supported by the observation that young women exhibit higher levels of nucleus-localized Akt compared with comparably aged men and postmenopausal women (Camper-Kirby *et al.*, 2001). In addition, Xu *et al.* (2006) suggested that the cardioprotective effects of oestrogens are mediated, in part, by inhibition of the expression of the pro-inflammatory cytokineTNF-α and modulation of TNF-α expression. TNF-α inhibition improved functional recovery and reduced apoptosis and myocardial necrosis. Ma *et al.* (2009) have studied the role of Ca^{2+} calmodulin dependent protein kinase II (CaMKII) in cardioprotection and found that both CaMKIIα and phosphoCaMKII were up-regulated in the hearts from oestrogen deficient rats, and these changes were reversed by oestrogen replacement. Furthermore, the deleterious effect of ischaemia/reperfusion on hearts from ovariectomized rats, poor contractile recovery and increased apoptosis were attenuated or abolished not only by oestrogen replacement but also by blockade of CaMKII. These results are evidence that oestrogen confers cardioprotection by suppressing the CaMKII pathway.

Possible role of androgens

Whether oestrogen alone is cardioprotective in humans needs to be clarified (Piro *et al.*, 2010). In fact, the observed

sex-related differences and the increased incidence in cardiac events among women after menopause may not entirely be due to the depletion of oestrogen, but may be related to levels of testosterone because postmenopausal ovaries produce significant amounts of androgens in the form of testosterone and androstendione. Similar to oestrogens, androgens are present in both sexes, albeit at different concentrations and ratios. Endogenous androgens (dehydroepiandrosterone, androstenedione and testosterone) are readily converted to oestradiol by the sequential actions of 17β-hydroxysteroid dehydrogenase and aromatase. Grohé *et al*. (1998) have shown that CYP450 aromatase is active in generating oestrogen and is expressed in cardiac myocytes. Incubation of cardiac myocytes with the oestrogen precursors androstenedione and testosterone stimulated expression of ER-α, ER-β and iNOS in a sex-specific fashion. These data suggest that local oestrogen biosynthesis contributes to the cardioprotective effects of oestrogens. Therefore, some of the beneficial effects observed in males may be due to the conversion of testosterone to oestradiol and oestradiol metabolites.

Testosterone activates androgen receptors that are expressed in cardiac myocytes; it increases endothelin-1 levels, circulating levels of homocysteine and, by stimulation of tyrosine hydroxylase, elevates the synthesis of catecholamines. In a mouse model of myocardial infarction, Cavasin *et al*. (2006) demonstrated for the first time that oestrogen and testosterone play different and opposing roles in the development of heart failure and long-term remodelling after myocardial infarction. In particular, oestrogens (either endogenous or supplemental) prevent maladaptive chronic remodelling and further deterioration of cardiac performance, whereas testosterone (either endogenous or supplemental) adversely affects myocardial healing (as indicated by higher rates of cardiac rupture), promotes cardiac dysfunction and remodelling, and exerts pronounced effects when oestrogen levels are reduced. However, it should be noted that plasma oestrogen and testosterone levels in males and females who received HRT were much higher than normal physiological levels (Piro *et al*., 2010). Furthermore, Tsang *et al*. (2008) observed that testosterone conferred cardioprotection by up-regulating the cardiac α_1-adrenoceptor and enhancing the effects of stimulation of this adrenoceptor. These effects of testosterone were abolished or attenuated by blockade of androgen receptors. It is necessary to stress that steroid hormone receptors do not act alone but interact with a broad array of co-regulatory proteins to alter transcription. Understanding co-regulator biology is important in the development of cardiovascular-specific ER modulators and modulators for other sex steroid hormones (Mendelsohn, 2005).

The number of hypotheses attempting to explain the mechanisms of sex-related differences in cardiac sensitivity to oxygen deprivation continues to increase. Nevertheless, a comprehensive and verified explanation of the molecular basis of this highly clinically relevant biological phenomenon remains elusive. Thus, a better understanding of the processes leading to differences in cardiac susceptibility to ischaemia/reperfusion injury in women is likely to open the way to new, better tailored HRT and selective modulators of steroid hormones for the prevention and treatment of cardiovascular diseases in women.

Sex-related differences in the effect of perinatal interventions on cardiac susceptibility to ischaemia/reperfusion in adults

Human epidemiological studies have shown a clear association between an adverse intrauterine environment and an increased risk of IHD in later adult life (Barker *et al*., 1989). This finding is in accordance with Barker's concept (Barker, 2000) of fetal and neonatal programming, which is based on epidemiological studies showing that perinatal pathogenetic factors are linked with the development of adult cardiovascular disease. Of all the stresses to which the foetus is subjected, perhaps the most important and clinically relevant is hypoxia (Xue and Zhang, 2009). The fetus may experience prolonged hypoxic stress under many different conditions, including pregnancy at high altitude, pregnancy with anaemia, placental insufficiency, cord compression, and heart, lung and kidney disease. One of the most common insults during the early stages of postnatal ontogenetic development is hypoxemia due to congenital cyanotic defects or pulmonary disease secondary to prematurity. Such hypoxemia may persist for several weeks or months until surgical repair of the structural defects, or improvement in pulmonary function returns the individual to a normoxemic state (Rohlicek *et al*., 2002). Moreover, these patients are growing older and are approaching the age characterized by significantly increased risk of IHD. Therefore, it can be expected that more of these patients will require diagnostic and therapeutic catheterization or cardiac surgery.

Experimental studies investigating the late effects of perinatal hypoxia on cardiac tolerance to ischaemia have started only recently and are, unfortunately, not concise; moreover, most have used male subjects, exclusively. Furthermore, they differ in the critical ontogenetic period studied (prenatal, perinatal, and postnatal), and the intensity and duration of hypoxia (Ostadal *et al*., 2012). Nevertheless, animal studies have repeatedly suggested a possible link between early hypoxia and increased risk for cardiovascular disease in offspring (Li *et al*., 2003; 2004). The question arises whether the effects of perinatal hypoxia on cardiac tolerance to ischaemia differ in adult males and females. In the rat model, we observed that the late myocardial effects of hypoxemia experienced in early life may be sex-dependent. Perinatal exposure to chronic hypoxia significantly increased cardiac tolerance – expressed as a lower incidence of ischaemic arrhythmias – to acute ischaemia/reperfusion injury in adult female rats, while the opposite effect on arrhythmias was observed in males (Figure 4), (Netuka *et al*., 2006). Similar sex-dependent effects of early hypoxia were later confirmed by Xue and Zhang (2009), who observed that prenatal hypoxia significantly decreased post-ischaemic recovery of left ventricular function, and increased cardiac enzyme release and infarct size in adult male, but not female, rats.

The mechanisms of these late effects of perinatal hypoxia on cardiac tolerance to ischaemia in adults are currently unknown. Prenatal chronic hypoxia sensitizes the apoptosis pathway in adult male hearts in response to ischaemia/reperfusion (Li *et al*., 2003). In addition, expression of cardiac

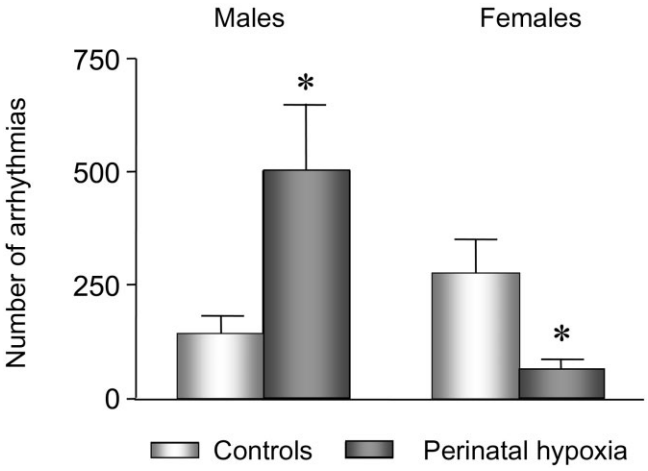

Figure 4
Total number of arrhythmias of the isolated heart (Langendorff preparation) over 30 min coronary artery occlusion in normoxic and perinatally hypoxic adult male and female Wistar rats. *$P < 0.01$, significantly different from controls Data from Netuka *et al.* (2006).

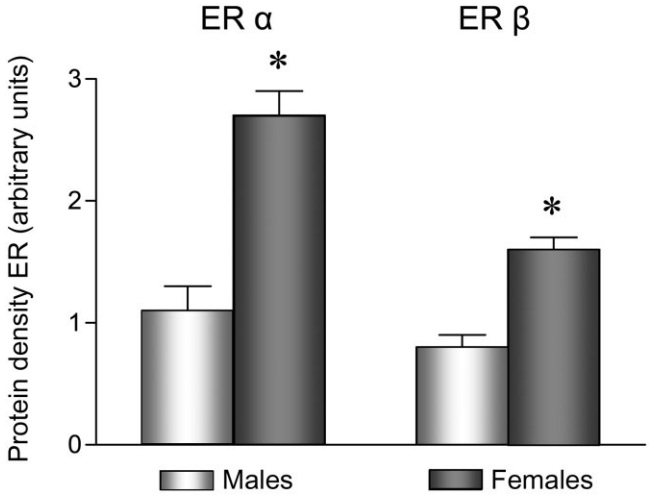

Figure 5
Oestrogen receptors (ER) α and β in the male and female fetal rat heart. Data shown represents the amount of ERs (expressed as optical density) *$P < 0.01$, significantly different from males. Data from Patterson *et al.* (2010).

heat shock protein 70 was significantly lower in prenatal hypoxic hearts than in controls, which may play a role in the increased susceptibility of the adult male heart to ischaemia/reperfusion injury (Li *et al.*, 2004). Decreased prenatal eNOS levels may also contribute to this increased susceptibility in adults. Recently, two studies analysed the possible mechanisms involved in prenatal hypoxia-induced sex-dependent changes in cardiac tolerance to ischaemia in adults. According to Xue and Zhang (2009), these changes are due to differences in fetal programming of the expression of the gene for PKCε, which plays a pivotal role in cardioprotection against ischaemia/reperfusion injury (Kolar *et al.*, 2009). Down-regulation of PKCε function was observed in the hearts of adult male offspring only. Patterson *et al.* (2010) found that chronic hypoxia during gestation down-regulated PKCε expression in the developing heart through an epigenetic modification. They found sex-related differences in the methylation of the binding sites for the transcription factor specificity protein (SP)1 and PKCε transcription, and hypoxia-induced methylation was significantly greater in the hearts of male fetuses. According to Patterson *et al.* (2010), this sex difference may be caused, in part, by the greater expression of ER-α and ER-β in the hearts of female fetuses (Figure 5). The finding that both ERs interacted with SP1 binding sites at the PKCε promoter in the fetal heart suggests a mechanism for the increased protection of SP1 binding sites and PKCε transcription in the female heart in response to hypoxic stress.

Although it may be difficult to translate the recent findings in animals directly to humans, the possibility that fetal interventions result in sex-dependent programming of specific genes in the offspring with the consequence of increased cardiac susceptibility to ischaemia/reperfusion injury warrants investigation in human subjects. This would have important clinical implications because cardiac sensitivity to oxygen deprivation in adult patients may be significantly influenced by perinatal disturbances in a sex-dependent manner.

Implications for female-specific therapeutic strategies

Significant sex-related differences in cardiac tolerance to ischaemia/reperfusion injury represent a vigorous argument supporting the development of sex-specific therapeutic strategies. This should be supported by sex-specific research on existing drugs and sex-specific strategies in the development of novel agents (Regitz-Zagrosek, 2006). The results available to date are, unfortunately, insufficient and inconclusive. This complex topic can be divided into three closely related areas: (i) therapeutic implications of the protective molecular, cellular and systemic effects of oestrogen; (ii) sex-related differences in cardiac protection strategies based on standard protective phenomena; and (iii) the management of women with acute coronary syndrome (ACS).

Therapeutic implications of the protective effects of oestrogen

The exact underlying molecular and cellular mechanisms by which oestrogen exerts its cardioprotective effects in myocytes are still largely unknown and require further research. Previous studies have shown numerous broad-range, interrelated genomic and non-genomic anti-apoptotic effects of oestrogen that may provide opportunities for alternative therapeutic interventions (Bouma *et al.*, 2010). These include the ER-α and ER-β, and PI3K-mediated Akt activation, PI3K-mediated PKCδ and PKCε activation, decreased NFκB activation, decreased p38 MAPK-mediated acute myocardial inflammation with decreased TNF α levels (Wang *et al.*, 2006a), free-radical scavenging and antioxidant effects, increased NOS and NO production, modulation of Ca^{2+} influx

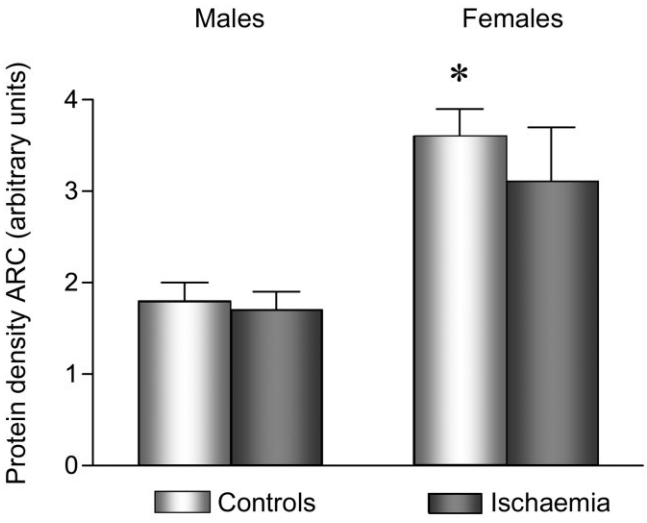

Figure 6

Apoptosis repressor with caspase recruitment domain (ARC) in the area at risk of the ischaemic group and in the corresponding segment of the left ventricle of the non-ischaemic (control) groups of hearts from male and female New Zealand White rabbits; *$P < 0.01$, significantly different from males. Data from Bouma *et al.* (2010).

and release, activation of mitochondrial ATP-sensitive K^+ channels (Murphy and Steenbergen, 2007), increased Bcl-2 levels and decreased caspase 3 and caspase 8 activity (Wang *et al.*, 2009). The concept of early therapeutic intervention with pro- and anti-apoptotic stimuli to limit infarct size appears to be very promising (Bouma *et al.*, 2010). However, a lack of understanding persists with regard to the importance of different apoptotic mechanisms in cardiomyocytes, their interactions and their organ specificity. Of particular promise, in this context, is the recent identification of a powerful cardiomyocyte-specific endogenous anti-apoptotic factor, the apoptosis repressor with caspase recruitment domain (ARC, Bouma *et al.*, 2010, Figure 6).

Similar problems are represented by the as yet unresolved systemic protective effects of oestrogen. A number of short- and long-term protective effects on the cardiovascular system have been described, including endothelium-dependent vasodilation, the beneficial influence on lipoprotein metabolism, acceleration of endothelial cell growth (Mendelsohn and Karas, 1999), inhibition of the renin-angiotensin system (Fischer *et al.*, 2002), protection against vascular injury by inhibition of vascular smooth muscle proliferation (Morey *et al.*, 1997), attenuation of myocardial inflammation (Smith *et al.*, 2000), increased cardiac output (Magness *et al.*, 1993), attenuation of the development of cardiac hypertrophy in response to pressure overload (van Eickels *et al.*, 2001) and likely anti-arrhythmic properties (McHugh *et al.*, 1995). On the other hand, potential harmful cardiovascular effects of oestrogen have also been described in mouse models such as ventricular remodelling after myocardial infarction, which is associated with increased mortality (Van Eickels *et al.*, 2001). Moreover, it has become clear that HRT has complex biological effects – anti-inflammatory and proinflammatory – both of which activate coagulation and improve fibrinolysis (Koh and Yoon, 2006). Nevertheless, a clinically relevant prothrombotic effect has been repeatedly demonstrated and adverse systemic effects of oestrogen may, therefore, mitigate beneficial effects at the cellular level.

Many potentially beneficial effects of oestrogens on cardiovascular physiology resulted in significant expectation of protective effects of postmenopausal HRT for IHD prevention in women (Vaccarino *et al.*, 2011). Initial observational studies showed a reduced incidence of IHD in postmenopausal women undergoing HRT compared with those who were not (Rossano *et al.*, 1993). As mentioned above, the Heart and Estrogen/Progestin Replacement Study and the WHI clinical trials did not support beneficial effects of HRT in postmenopausal women, either in secondary or in primary cardiovascular prevention (Hulley *et al.*, 1998; Rossouw *et al.*, 2002). In fact, the WHI study was terminated early due to a small but significant increase in cardiovascular events and other adverse outcomes in the HRT group. In trying to explain these unexpected results, it has been argued that the timing of initiation of HRT after the onset of menopause may influence the response to treatment for IHD prevention. *Post hoc* analyses of the WHI trial suggested that cardiovascular disease risk may be decreased when oestrogen therapy is initiated earlier (within 10 years of menopause) but the results were not statistically significant (Rossouw *et al.*, 2007). Thus, no trial of HRT has conclusively demonstrated a beneficial effect on cardiovascular disease; if anything, risk is slightly increased. The potential risks and benefits of postmenopausal hormone therapy were recently summarized by Rozenberg *et al.* (2013). It seems that an important distinction should be made between the treatment of climacteric symptoms in young, generally healthy, postmenopausal women and the prevention of chronic diseases in elderly women. Hormone therapy seems to be beneficial and safe for postmenopausal symptomatic women aged <60 years. Adding medroxy progesterone acetate to oestrogen replacement therapy might have an unfavourable effect on cardiovascular risk. Hormone therapy is generally contraindicated for women with a previous history of breast cancer, stroke and thromboembolic disease Therefore, HRT should not be used for the prevention of IHD in women (Vaccarino *et al.*, 2011).

It may be concluded that the protective effects of oestrogen on the cardiovascular system have many potential therapeutic implications; however, its effects are complex and need further intensive investigation. Eventually, a better understanding of these mechanisms may improve the clinical management of IHD in women because it may help devise and develop new strategies for the prevention, detection and treatment of IHD that are better tailored to women.

Sex-related differences in heart-protection strategies based on standard protective phenomena

Answering the question of whether it is possible to increase the already high tolerance of the female heart to ischaemia by the different types of known cardioprotective mechanisms is

not simple. Experimental studies investigating this problem are sporadic and inconclusive, and clinical observations are lacking (see Ostadal *et al*., 2009). We have observed that one of the protective phenomena, adaptation to chronic hypoxia, increases tolerance in both sexes, yet the sex difference was preserved: the female heart was significantly more tolerant (Ostadal *et al*., 1984). Two other well-known strategies for protecting the heart are ischaemic preconditioning and post-conditioning, which imply cardioprotection achieved by applying brief episodes of myocardial ischaemia and reperfusion either before or after the index ischaemia, respectively (see Hausenloy and Yellon, 2009). Data regarding the protective effect of ischaemic preconditioning and postconditioning are, however, inconsistent. Humphreys *et al*. (1999) observed a comparable degree of protection in both male and female rat hearts, while similar results were obtained by Talukder *et al*. (2010) in the mouse heart. On the other hand, Wang *et al*. (2006a) were unable to increase the tolerance of the female rabbit heart by preconditioning induced by isoflurane. Finally, Song *et al*. (2003) found that cardioprotection using endotoxin-induced preconditioning was attenuated in isolated hearts from female rats compared with those from male rats. Cao *et al*. (2008) found no sex-related difference in the degree of Met^5-enkephalin-induced protection, but there was a sex-related difference in the cardioprotective signalling pathway after the administration of this opioid peptide. Cardioprotection induced by Met^5-enkephalin in males primarily uses a PI3K/Akt1/2 pathway, whereas in females a PI3K/Akt/3 pathway was used. To us, it appears that the observation by Turcato *et al*. (2006), that preconditioning in females is age dependent, is particularly relevant. The protective effect was absent in young, highly tolerant hearts, but appeared with the decrease of natural tolerance during aging. This fact is most likely to be a general biological phenomenon and we have observed a similar effect in neonatal rats (Ostadalova *et al*., 1998; Ostadal *et al*., 1999). Their already high hypoxic tolerance could not increase further, either by adaptation to chronic hypoxia or by ischaemic preconditioning. The protective effect occurred only with the decrease of natural cardiac tolerance during the first postnatal week.

Very few studies have tested the difference between the sexes with regard to the effectiveness of postconditioning. Crisostomo *et al*. (2006) reported that this protective phenomenon is more pronounced in isolated hearts from male rats than in those from female rats. The protective effect of postconditioning in the female heart depends on the duration of ischaemic exposure; it was present in female hearts exposed to 20 min of ischaemia, but absent when exposed to 25 min of oxygen deprivation. Unfortunately, infarct size was not tested in this study (the endpoint was the post-ischaemic recovery of contractile function). Penna *et al*. (2009) observed that after 10 min, ischaemia postconditioning reduced infarct size and improved systolic function only in female hearts, in which a larger infarct size was observed; in male hearts, neither of these endpoints were affected. However, after 30 min of ischaemia, postconditioning reduced infarct size more in male than in female hearts, but improved systolic function in female hearts only. Similarly, Zheng *et al*. (2011) and Dow and Kloner (2007) also did not observe reduced infarct size in female hearts induced by 30 min to 45 min of ischaemia. It appears, therefore, that the protective effect of postconditioning is sex- and infarct size-dependent. Finally, for completeness, it is interesting to note the single finding of Lee *et al*. (2010a), that in rat isolated hearts (index ischaemia 30 min), none of the post-conditioning protocols improved myocardial tolerance to ischaemia/reperfusion injury nor decreased infarct size in males or females.

We agree with the opinion of Heusch (2004) that it is necessary to control for confounding variables, such as the size of the area at risk, the duration of preceding ischaemic insult and collateral status, in cardioprotection studies. We are convinced that in addition to the factors mentioned above, sex, age and the degree of cardiac tolerance to oxygen deprivation (Ostadal *et al*., 1999; Ostadal, 2009) are equally important. Neglect of these variables has probably contributed to the failure of translation of experimentally validated principles of cardioprotection to clinical practice (Bolli *et al*., 2004).

Management of women with ACS

Sex-related differences in cardiac sensitivity to ischaemia/reperfusion may also influence therapeutic strategies in women with acute ischaemia. Sex-related differences in the efficacy and safety of the use of individual groups of drugs are briefly discussed in the following section and, at greater length, by Ostadal and Ostadal, (2012).

ACS is an unstable form of IHD and comprises two diagnoses: acute myocardial infarction and unstable angina pectoris. It is generally accepted that women experience higher mortality after ACS compared with men. At the time of presentation, women with ACS are, however, typically older and more likely to have hypertension, diabetes and metabolic syndrome than men (Alfredsson *et al*., 2007). It must be emphasized that sex-related differences in mortality after ACS may disappear after adjustment for age and co-morbidities (Motovska *et al*., 2008; Akhter *et al*., 2009). At younger ages, women more often have ACS with angiographically 'normal' coronary arteries than men. The underlying mechanisms of this so called coronary microvascular dysfunction are diverse and may be related to endothelial reactivity, low endogenous oestrogen levels, coagulation disorders and abnormal inflammatory reactions. On the basis of experimental data and clinical observation, coronary microvascular dysfunction is put forth as a major etiological factor for IHD in women (Maas and Appelman, 2010; Vaccarino *et al*., 2011). Contemporary therapy of ACS includes coronary revascularization, anti-thrombotic therapy, antagonists of the β-adrenoceptors (beta-blockers), inhibitors of the renin-angiotensin-aldosterone system and statins.

Coronary revascularization. Women are less likely to undergo coronary revascularization for ACS than men (Lee *et al*., 2010b). In a study by Radovanovic *et al*. (2007), the in-hospital mortality rate of women who underwent percutaneous coronary intervention (PCI) was significantly higher than in men. Conversely, Lee *et al*. (2010b) reported that among individuals who underwent revascularization, there were no sex-related differences in survival or reinfarction rate

during hospitalization for ACS. Compared with medical therapy, PCI was associated with a significant reduction in coronary events in both women and men (Lee *et al.*, 2010b). However, women undergoing coronary angiography usually exhibit normal coronary arteries or only insignificant coronary atherosclerosis (Vikman *et al.*, 2007).

Anti-thrombotic therapy. After adjustment for baseline characteristics, data regarding mortality and morbidity in women compared with men in the setting of thrombolysis are contradictory (Nicolau *et al.*, 2004). In the International Tissue Plasminogen Activator/Streptokinase Mortality study, mortality was similar to that of men, but women experienced a higher rate of hemorrhagic stroke (White *et al.*, 1993). Female sex has been also associated with a higher risk of bleeding complications in other trials investigating fibrinolysis for acute myocardial infarction (Reynolds *et al.*, 2007).

As for anticoagulants, the effect varies with different drugs. Women are more likely to achieve a higher activated thromboplastin time after administration of unfractionated heparin (Granger *et al.*, 1996). Compared with men, women demonstrated a greater reduction in mortality rate and myocardial infarction after dalteparin administration (FRISC Study Group, 1996); however, dalteparin was responsible for more frequent minor bleeding (Toss *et al.*, 1999). Similarly, in the ESSENCE and TIMI trials, a significant benefit of enoxaparin over unfractionated heparin was observed in women but not in men (Cohen *et al.*, 2001). On the other hand, no sex-related differences have been observed in the efficacy of bivalirudin (Stone *et al.*, 2007) and fondaparinux (Yusuf *et al.*, 2006); however, the association between bivalirudin therapy and increased bleeding risk in women has been reported often (Yusuf *et al.*, 2006; Madsen *et al.*, 2008).

The glycoprotein IIb/IIIa receptor antagonists (abciximab, tirofiban, eptifibatid) block the final common pathway leading to platelet aggregation by inhibiting the binding of fibrinogen to the glycoprotein IIb/IIIa receptor on the surface of activated platelets. A meta-analysis of randomized trials showed considerable sex-related differences; whereas men benefited from the therapy (19% reduction in death rate and infarction), women showed a significantly increased risk (15%) (Boersma *et al.*, 2002). A possible explanation is the lower proportion of positive troponin patients among women than among men. Specifically, it has been shown that the beneficial effect of this drug is more pronounced in men and women with positive baseline troponin levels (Capodanno and Angiolillo, 2010).

The antiplatelet effects of aspirin (acetylsalicylic acid) are primarily related to its ability to block non-competitively the cyclooxygenase-1 in platelets, thereby blocking thromboxane synthesis. The magnitude of this effect in both sexes is comparable (Becker *et al.*, 2006). A meta-analysis of randomized trials showed that sex does not significantly affect the proportional reduction of serious cardiovascular events both in primary and secondary prevention (Baigent *et al.*, 2009). Similarly, no sex-related differences were observed after administration of the thienopyridine inhibitors of the platelet ADP $P2Y_{12}$ receptor (ticlodipin, clopidogrel, prasugrel), thereby blocking a key signalling pathway of platelet activation (Capodanno and Angiolillo, 2010).

Beta-blockers. A lower proportion of women with ACS are treated with beta-blockers, compared with men (Enriquez *et al.*, 2008). Women achieve significantly higher plasma levels of beta-blockers, such as metoprolol or propranolol, and consequently exhibit a more pronounced decrease in heart rate and systolic blood pressure during beta-blocker therapy than men (Jochmann *et al.*, 2005). However, a meta-analysis of randomized trials demonstrated a similar reduction in cardiovascular-related deaths in women and men (Olsson *et al.*, 1992).

Inhibitors of the renin-angiotensin system. Similarly, women are less likely to receive ACE inhibitors or angiotensin AT_1 receptor antagonists in therapy for ACS (Nguyen *et al.*, 2010). A meta-analysis of ACE inhibitor therapy early after myocardial infarction, complicated by left ventricular dysfunction, revealed comparable effects with respect to prognosis and hospitalization rate in both sexes (Flather *et al.*, 2000). Relevant sex-specific pharmacokinetic differences have not been observed for most of the AT_1 receptor antagonists and, similarly, no differences between women and men have been reported with respect to the efficacy and safety of these drugs (Jochmann *et al.*, 2005).

Statins. These drugs are also less frequently prescribed to women with ACS (Enriquez *et al.*, 2008; Nguyen *et al.*, 2010). Similar to men, women with ACS are likely to benefit more from intensive, than from standard, statin therapy (Murphy *et al.*, 2007; Wenger, 2008).

It is necessary to stress that, at the present time, only limited evidence is available with regard to female-specific aspects of management and outcomes in ACS. Women less frequently undergo coronary intervention and, compared with men, a lower proportion of women receive evidence-based pharmacotherapy. There is an urgent need for intensive experimental and clinical research for the development of female-specific therapeutic strategies in ACS. Future major trials should include a sizable number of women to enable valid and more pertinent conclusions regarding sex-specific differences.

Conclusions

It is important to stress that sex is a variable that should be considered in both experimental and clinical cardiology, and analysis of cardiac tolerance to ischaemia represents a typical example. Pooling data from males and females may obscure important cardiac differences in many parameters, under both physiological and pathological conditions. Although detailed mechanisms of these differences remain unknown, it is clear that they are sufficiently important to be considered in optimizing diagnostic and therapeutic procedures in clinical practice. Our understanding of this important topic has increased dramatically in recent years. With new basic and clinical information regarding sex-related differences in cardiac sensitivity to ischaemic injury, we will be able, in the future, to offer patients better evidence-based treatments, a better quality of life and lower mortality.

Acknowledgements

This study was supported by grants of the Czech Science Foundation 303/12/1162 and AVOZ 50110509.

Conflict of interest

There is no conflict of interest.

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