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EDITORIAL

Biological sex themed section: Incorporating the female dimension into cardiovascular pharmacology

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LINKED ARTICLES

This article is part of a themed section on 'Biological Sex and Cardiovascular Pharmacology'.

To view the other articles in this section, see Fazal *et al.* (2013) DOI: 10.1111/bph.12279, Franconi and Campesi (2013) DOI: 10.1111/bph.12362, Mair *et al.* (2013) DOI: 10.1111/bph.12281, Ostadal and Ostadal (2013): DOI: 10.1111/bph.12270.

Previous linked articles are: Bubb *et al.* (2012) DOI: 10.1111/j.1476-5381.2012.02036.x, Chan *et al.* (2012) DOI: 10.1111/j.1476-5381.2012.02012.x, Fattore and Fratta (2010) DOI: 10.1111/j.1476-5381.2010.00776.x, Kittikulsuth *et al.* (2013) DOI: 10.1111/j.1476-5381.2012.01922.x, Nilsson *et al.* (2011) DOI: 10.1111/j.1476-5381.2011.01235.x, Thangavel *et al.* (2013) DOI: 10.1111/j.1476-5381.2012.02222.x, Varro and Baczko (2011) DOI: 10.1111/j.1476-5381.2011.01367.x.

Abbreviations

ACE, angiotensin converting enzyme; AT, angiotensin receptor; ET, endothelin; GPER1, G protein-coupled oestrogen receptor 1; HMG-CoA, hydroxymethylglutaryl co-enzyme A; NO, nitric oxide; ER, oestrogen receptor; PKC, protein kinase C; RAS, renin-angiotensin system

Gender medicine is a relatively new specialty, which takes into consideration the differential effects of sex or gender on health, applying across the spectrum of disease prevention, diagnosis, evaluation and treatment. It is generally considered that sex refers to the classification of organisms as male, female or intersex, using biological and physiological characteristics, whereas gender is defined within a social construct as masculine or feminine by a set of physical and behavioral features. Often the distinction is not strictly adhered to, such that sex is often integrated into the meaning of gender, although questions in medical research largely address the biological differences in males and females which impact on development of disease and its treatment.

Traditionally, research into disease processes and therapeutic approaches has been based on the premise that, apart from the reproductive system, differences between the sexes do not exist or are not pertinent. Notably, there has been under-representation of women in early clinical trials, preferential use of male animals in drug development programmes and lack of consideration of the existence of XX or XY cytology in studies using cultured cells. In recent years, however, an increasing amount of data revealing differences in disease prevalence between the sexes as well as variations in drug efficacy and side effect profiles has raised awareness of the importance of studying sex-based differences in disease and treatment. Indeed, much of the relevant literature has been brought together in an excellent Handbook of Pharmacology resource 'Sex and Gender Differences in Pharmacology' (Regitz-Zagrosek, 2012). In the more recent series of articles highlighted in this editorial, it has been our purpose to focus

particularly on the cardiovascular system. Here, what is currently known about the biological mechanisms responsible for sex-based differences in cardiovascular physiology and pathology is explored, and how some of this information is related to different outcomes after therapeutic intervention.

Unfortunately, few women identify cardiovascular disease as the greatest health problem facing them today, but it actually claims the lives of more women than all forms of cancer combined. Cardiovascular disease, including ischaemic heart disease and stroke, is the main cause of death for women in all 27 countries of the European Union (EU), accounting for 0.6 million deaths per year (Nichols *et al.*, 2012). In examination of the most recent data from the European Commission for standardised death rates per 100,000 inhabitants, diseases of the circulatory system, including those related to high blood pressure and cholesterol, accounted for 170 deaths in females across the EU-27 in 2010 and ischaemic heart disease for 53 deaths (European Commission – Eurostat). In contrast, development of the most common neoplasm in females, breast cancer, was responsible for 23 deaths per 100,000 inhabitants.

Although the incidence of death due to cardiovascular disease is lower in women than men, the gap has narrowed over the past 30 years in most EU countries, because of a larger reduction in men than in women. As an illustration, death rates decreased in the United Kingdom since 1980 by 75% and 66%, respectively. The disparity has been attributed to varying influences of major cardiovascular risk factors in males and females. In particular, cardiovascular disease has a greater impact on diabetic females than males, especially when diagnosed at a later stage (Roche and Wang, 2013). Also, women with diabetes more often have added risk factors, such as obesity, hypertension and elevated triglyceride levels. Furthermore, women who smoke have a 25% higher risk of ischaemic heart disease than do male smokers, and the longer women smoke, the bigger this risk becomes relative to men who smoke for the same number of years (Huxley and Woodward, 2011).

A significant body of epidemiological evidence supports the idea that once past the menopause, the female risk for cardiovascular disease increases more quickly than it does for same-aged men. Recent data from the World Health Organization highlights a progressive fold-increase in mortality from ischaemic heart disease with every decade of life, which is greater in men than in women across high income countries (Finegold *et al.*, 2013). An early menopause, either occurring spontaneously or after ovariectomy, has been associated with an age-adjusted hazard ratio of 2.2 for ischaemic heart disease and stroke, independent of traditional cardiovascular risk factors (Wellons *et al.*, 2012). So, the fundamental premise that has been addressed in both basic and clinical research is that oestrogens, oestradiol being the predominant oestrogen during reproductive years, have beneficial effects on the heart and vasculature. Increasing evidence suggests that the oestrogen/androgen balance is also likely to be key, as testosterone, long thought to have a detrimental influence on cardiovascular risk, has cardioprotective effects in men and women (Tirabassi *et al.*, 2013).

The generality of sex differences in cardiac physiology and pathology suggests that such distinctions must be fundamental, likely operating at a cellular level. In examination of molecular mechanisms determining cardiac structure and function that are affected by oestrogen, Fazal *et al.* (2013) have considered particular effects in the different cell types present in the heart. At cardiomyocyte level, oestrogenic signalling prevents loss of cardiac muscle mass through apoptotic and autophagic pathways, and contributes to antihypertrophic effects by increasing calcineurin degradation and release of natriuretic peptides. Oestrogen can modulate excitation-contraction coupling by regulating the level and activity of calcium and potassium channels and ion transporters, affecting contractile reserve of hypertrophied cells and susceptibility to arrhythmias. It is known that women are at a significantly greater risk for developing drug-induced ventricular arrhythmias. This has been related to them having a reduced repolarization reserve compared with men, and this is influenced by testosterone levels (Varro and Baczko, 2011). There are also male-female differences in mitochondrial electron transport in the myocyte that are sex hormone-dependent, such that female mitochondria generate less reactive oxygen species, which at high levels contribute to cardiac disease (Fazal *et al.*, 2013). Adverse remodelling processes involving cardiac fibroblasts and inflammatory cells also appear to have sex specificity, in that oestrogen can inhibit collagen I and II expression and turnover of the extracellular matrix. Oestrogen lowers blood levels of low density lipoprotein and, at least in animal studies, exogenous oestrogen is atheroprotective. In the coronary network, oestrogen is important for endothelial cell function, modulating nitric oxide (NO) release through NO synthase 3 to enhance vasorelaxation and re-endovascularization. Sexual dimorphism in vascular auto-regulation in mice has been described by Chan *et al.* (2012), who dissected out distinct endothelial pathways underlying NO-mediated activity, a soluble guanyl cyclase-dependent pathway being necessary in males, while endothelial derived hyperpolarising factor was the principal regulator in arteries of females.

Sex-related differences in cardiac tolerance to ischaemia are well documented in the findings of epidemiological studies, and are supported by experimental observations in models of ischaemia and reperfusion. Females are at reduced risk of injury and oestrogen administered acutely to males can reduce infarct size. The detailed mechanism of how oestrogen attenuates ischaemia-reperfusion injury is still under investigation. Indeed the ER_{α} versus ER_{β} receptor-mediated responses and the relative importance of genomic versus non-genomic effects are of considerable debate (Ostadal and Ostadal, 2013). In recent years, a third membrane-bound oestrogen receptor has been identified: G protein-coupled receptor 30 or G protein-coupled oestrogen receptor 1 (GPER1), which mediates oestrogenic responses in cardiovascular and metabolic regulation. GPER1 activation may have several beneficial effects in the cardiovascular system including protection of the myocardium against ischaemia-reperfusion injury. Ostadal and Ostadal (2013) have also addressed the probable role of endogenous androgens, when converted to oestradiol and its metabolites, in determining sex-related differences in cardiac sensitivity to oxygen deprivation. Another interesting facet of their work has concerned the condition of hypoxic stress experienced by the developing foetus or in the immediate post-natal period and how this may affect cardiac tolerance to ischaemia in later life. A clear

association of an adverse uterine environment and risk of adult ischaemic heart disease has been demonstrated in epidemiological studies, but the question of a male-female difference in risk has been addressed only in ischaemia and reperfusion models. In female rats, peri-natal exposure to hypoxia results in a lower incidence of arrythmias along with decreased infarct size after ischaemic injury than is observed in males. Mechanistically, it is suggested that sex-related differences may be due to increased ER_α and ER_β expression in female foetal hearts, resulting in greater protein kinase C (PKCε) transcription, which may protect against ischaemia and reperfusion induced injury. It might be expected that classical strategies for cardioprotection, such as chronic hypoxia, pre- or post-conditioning would produce different outcomes in males and females. Indeed, there is some evidence of lesser effects of ischaemic conditioning in females but this is age-dependent. In the clinical setting, there are implications for women with acute coronary syndrome, it being generally accepted that there is a higher incidence of mortality in women than in men. Generally women are less likely to undergo elective coronary revascularization and for those who do, they generally have worse outcomes than men, in part because they are usually older and have more co-morbidities. Current treatment of acute coronary syndrome includes also a range of drugs, particularly antithrombotic agents, inhibitors of the renin-angiotensin-aldosterone system and statins. Clear evidence for sex-specific differences in the pharmacokinetic and pharmacodynamic properties of drugs used to treat ischaemic and other cardiovascular conditions is accumulating.

In their review of drug handling and how biological differences between men and women interact with these processes, Franconi and Campesi (2013) have summarised data on the particular effects of sex-gender on pharmacokinetics, as well as the safety and efficacy of medications used in the treatment of hypertension, heart failure and arrhythmias. In general, pharmacokinetic differences are more numerous and consistent than disparities in pharmacodynamics, quite often being influenced by hormonal status. Variance in drug absorption from the gastrointestinal tract is based on a higher gastric pH, and lower gastric and bowel transit times in women than in men. Also, there is a large number of female-male differences in the expression and activity of drug transporters and metabolic enzymes of the cytochrome P450 system, the most important of which appears to be CYP3A4. Also, it has been reported recently that there is a dominant expression of human CYP3A5 in females which arises from an intrinsic, suboptimal activation of the transcription networks responsible for sex hormone-induced expression of the CYP isoform in men (Thangavel *et al*., 2013). The review has also highlighted that many genetic polymorphisms present sex-gender specificity. Angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor (AT_1) antagonists, aldosterone antagonists and renin inhibitors are integral components of pharmacotherapy for cardiovascular disease. Steroid sex hormones affect the renin angiotensin system (RAS), oestrogen decreasing renin and ACE activities and AT_1 expression, while the RAS is upregulated by androgens. Most definitive information concerns ACE inhibitors, which appear to be less effective in reducing mortality in women with symptomatic heart failure. However, a genetic variant of the ACE-1 enzyme (D/D genotype) affects the therapeutic response to ACE inhibition, conferring a more renoprotective effect. Another class of agents prescribed for hypertension and also angina, the calcium channel blocking agents, also present sexual dimorphism in pharmacokinetics. For example, the increased bioavailability of verapamil after controlled release dosage in women can be related to a higher activity of CYP3A4 and/or lower activity of P-glycoprotein than in men. Also, women treated with verapamil or amlodipine or the combination of olmesartan with amlopipine all experience greater blood pressure reduction than men do. The effects of β-adrenoceptor antagonists which are metabolised by CYP2D6, such as metoprolol, are particularly susceptible to the hormonal environment and plasma concentrations are increased in women taking oral contraceptive preparations. There is widespread use of hydroxymethylglutaryl co-enzyme A reductase inhibitors (statins) in lipid lowering treatment and it is known that oestrogens prevent the conversion of HMG-CoA to mevalonate, but use of statins for primary prevention of cardiovascular disease in women is still a matter of debate, uncertainty arising from under-representation in relevant clinical trials. Similarly, although there are a number of lines of evidence to indicate that platelet production and activation are influenced by action at oestrogen receptors, and platelet glycoproteins are subject to genetic variation and influence risk of atherothrombotic events in women, there is no clear view of a differential response to antiplatelet therapy. So overall, it must be stressed that the pharmacological status of women is less studied than that of men and ought to be considered more seriously. Investigations adopting a sex-gender approach are required in order to reach the most suitable and customized prescription for each patient independent of sex.

The endothelin (ET) system also plays an important part in regulation of cardiovascular function, as well as in the pathogenesis of hypertension and other cardiovascular diseases, the ET_A receptor primarily mediating vasoconstriction and anti-natriuresis, while stimulation of the ET_B receptor has opposite effects. Kittikulsuth *et al*. (2013) have reviewed the role of the ET system in animal models focusing on the inequality between the sexes associated with the susceptibility to and progression of hypertension and kidney diseases. Generally, females are relatively protected from elevation of blood pressure and kidney damage via increased ET_B versus ET_A receptor function. By comparison, males have higher circulating concentrations of ET-1 and renal expression of the peptide, along greater ET_A-mediated responses, including coronary and renal medullary vasoconstriction, seemingly influenced by testosterone. Almost all components of the endothelin system appear to be activated in the different types of pulmonary arterial hypertension. Evidence from recent epidemiological studies suggests the influence of gender on the development of pulmonary arterial hypertension with an approximate female to male ratio of 4:1, depending on the underlying disease pathology. Endogenous sex hormones, in particular 17β-oestradiol and its metabolites, have been implicated in the development of the disease, as reviewed by Mair *et al*. (2013). Knowledge of sex differences in the efficacy or adverse effects of ET_A receptor antagonists in the treatment of hypertension is poorly described, although in a recent combined analysis of six clinical trials including

1130 patients with pulmonary arterial hypertension, who were treated with ambrisentan, bosentan or sitaxentan, women had a superior therapeutic benefit.

Cannabinoids and their synthetic and endogenous analogs have a broad range of effects, including cardiovascular actions, the most important component of this being the profound hypotension associated with shock syndrome pathology. Furthermore, a protective role of endocannabinoids in myocardial ischemia has also been documented. Beside sex-based differences observed in the cannabinoid-induced effects related to cannabis abuse and dependence, cannabinoids have been shown to exert sex-dependent effects in other behavioural and physiological aspects. These have been discussed by Fattore and Fratta (2010), who have also considered male-female differences in cannabinoid pharmacokinetic and pharmacodynamic variables as potential factors underlying differences in cannabinoid effects.

While there has been progress in accounting for sex and gender in clinical studies, there has been relatively less application in addressing understanding of biological phenomena. For basic experimental biomedical research using cell cultures and laboratory animals, there are unique challenges to incorporation of the sex dimension in pursuit of fundamental scientific interests. In their article on sexual dimorphism in rodent models, Bubb *et al*. (2012) discuss the essential role that these animal models play in enabling research in hypertension and atherosclerosis, underlining strategies used to feasibility incorporate sex as a variable in fundamental investigations. There is currently huge impetus coming from the European Commission, which has called for systemic integration of sex-gender analysis into all funding proposals in the new European Union Research and Innovation Programme, Horizon 2020, starting in 2014, with the requirement to specify whether and in what sense this has relevance to the objectives and methodology of the particular research programme (Directorate General for Research and Innovation, European Commission, 2013). In support of this drive, an Expert Group 'Innovation through Gender' has produced a report to provide scientists with practical methods for sex and gender analysis, giving case studies as concrete illustrations of how sex and gender analysis leads to new ideas and excellence in research.

Sex-specific research has already uncovered some important differences in the aetiology and pharmacotherapy of cardiovascular diseases. With a more concentrated and concerted effort, many of the remaining issues could be solved, which will contribute to optimization of treatment outcomes for both men and women.

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