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Themed Section: Recent Progress in the Understanding of Relaxin Family Peptides and their Receptors

REVIEW ARTICLE

Vascular actions of relaxin: nitric oxide and beyond

Correspondence Dr Chen Huei Leo, School of BioSciences, The University of Melbourne, Parkville, VIC, 3010, Australia. E-mail: chen.leo@unimelb.edu.au

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C H Leo[1], M Jelinic[1], H H Ng[1], S A Marshall[1], J Novak[2], M Tare[3,4], K P Conrad[5] and L J Parry[1]

[1]School of BioSciences, The University of Melbourne, Parkville, VIC, Australia, [2]Division of Mathematics and Science, Walsh University, North Canton, OH, USA, [3]Department of Physiology, Monash University, Clayton, VIC, Australia, [4]School of Rural Health, Monash University, Clayton, VIC, Australia, and [5]Department of Physiology and Functional Genomics Department of Obstetrics and Gynaecology, College of Medicine, University of Florida, Gainesville, FL, USA

The peptide hormone relaxin regulates the essential maternal haemodynamic adaptations in early pregnancy through direct actions on the renal and systemic vasculature. These vascular actions of relaxin occur mainly through endothelium-derived NO-mediated vasodilator pathways and improvements in arterial compliance in small resistance-size arteries. This work catalysed a plethora of studies which revealed quite heterogeneous responses across the different regions of the vasculature, and also uncovered NO-independent mechanisms of relaxin action. In this review, we first describe the role of endogenous relaxin in maintaining normal vascular function, largely referring to work in pregnant and male relaxin-deficient animals. We then discuss the diversity of mechanisms mediating relaxin action in different vascular beds, including the involvement of prostanoids, VEGF, endothelium-derived hyperpolarisation and antioxidant activity in addition to the classic NO-mediated vasodilatory pathway. We conclude the review with current perspectives on the vascular remodelling capabilities of relaxin.

LINKED ARTICLES

This article is part of a themed section on Recent Progress in the Understanding of Relaxin Family Peptides and their Receptors. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.10/issuetoc

Abbreviations

Ang II, angiotensin II; BK, bradykinin; EDH, endothelium-derived hyperpolarisation; eNOS, endothelial NOS; ET1, endothelin-1; ECs, endothelial cells; IK_{Ca}, intermediate-conductance calcium-activated K^+ channel; PAs, parenchymal arterioles; PlGF, placental growth factor; *Rln*$^{-/-}$, relaxin-deficient mice; RXFP1, relaxin/insulin-like family peptide receptor 1; SHRs, spontaneously hypertensive rats; VSMC, vascular smooth muscle cell

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Tables of Links

TARGETS	
GPCRs[a]	**Enzymes**[c]
AT_2 receptors	Adenylate cyclase
B_2 receptors	Akt (PKB)
ET_B receptors	COX
RXFP1 receptors	eNOS
Voltage-gated ion channels[b]	iNOS
IKCa (KCa3.1)	PI3K
SK_{Ca}	

LIGANDS	
ACh	MMP2
Ang II	NO
BK, bradykinin	PGI_2
cGMP	PlGF, placental growth factor
cAMP	Relaxin
ET-1	TNF-α
Indomethacin	VEGF
L-NAME	

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c]Alexander *et al.*, 2015a,b,c).

Introduction

The 6 kDa peptide relaxin is considered a hormone of pregnancy mainly because the highest circulating relaxin concentrations are measured during pregnancy in animals and women. It is now known to contribute to important maternal cardiovascular adaptations of pregnancy (Conrad, 2011a,b). These beneficial effects of relaxin are associated with haemodynamic changes in the maternal systemic and renal vasculature (Conrad, 2010, 2011a,b). They include increased GFR, effective renal plasma flow and global arterial compliance, and reduced systemic vascular resistance, and are explained by the vasodilatory actions of relaxin on small arteries. The negative consequences on the vasculature of a lack of circulating endogenous relaxin are best illustrated in relaxin-deficient ($Rln^{-/-}$) rodents during pregnancy (Novak *et al.*, 2001; Vodstrcil *et al.*, 2012; Gooi *et al.*, 2013; Marshall *et al.*, 2016). However, relaxin is also produced in arteries of non-pregnant female and male rodents (Novak *et al.*, 2006), and $Rln^{-/-}$ male mice have vascular phenotypes that affect normal blood vessel function (Leo *et al.*, 2014a; Ng *et al.*, 2015). This review considers the current literature and provides some new perspectives on the role of endogenous relaxin in maintaining normal vascular function.

The concept that relaxin is a vasodilator is strongly supported by immunohistochemical data that localized the relaxin receptor, RXFP1, in both endothelial and smooth muscle cells in a broad range of arteries and veins (Novak *et al.*, 2006; Jelinic *et al.*, 2014; Ng *et al.*, 2015). The unexpected finding was the differential expression of RXFP1 receptors across the vasculature that now seems to contribute to the diversity of vascular actions of the ligand. In small resistance-size arteries, vascular tone is mediated by endothelium-derived factors, including NO, prostanoids and endothelium-derived hyperpolarisation (EDH). The pioneering studies of Novak and Conrad (Novak *et al.*, 2002) on small renal arteries in rodents demonstrated that recombinant human relaxin (rhRLX) acts predominantly through endothelium-dependent NO-mediated vasodilator pathways involving MMPs, endothelial NOS (eNOS), PI3K activation and Akt phosphorylation (Conrad, 2010). More recent work has identified several alternative vasodilatory pathways that are activated by rhRLX and do not entirely involve NO (Leo *et al.*, 2014b; Leo *et al.*, 2016a,b). This review describes the heterogeneous actions of relaxin in different vascular beds and highlights the diversity of mechanisms involved, in addition to the classic NO-mediated vasodilatory pathway. Although much of the research has focused on modulation of vascular tone, relaxin is also capable of remodelling the extracellular matrix within the vessel wall, thus influencing distensibility and/or stiffness of blood vessels (Conrad and Shroff, 2011). Therefore, we conclude the review with current perspectives on the vascular remodelling capabilities of relaxin.

Relaxin regulates vascular homeostasis in pregnant females

The vascular actions of relaxin were first reported almost 30 years ago when Massicotte and colleagues demonstrated reduced vasoconstrictor responses to noradrenaline and arginine vasopressin in perfused rat mesenteric arteries after *in vivo* infusion with rat relaxin for 2 days (Massicotte *et al.*, 1989). Evidence that porcine relaxin is a vasodilator and alters coronary blood flow through a NO-dependent pathway in rat and guinea pig hearts originated from the work of Bani and co-workers (Bani-Sacchi *et al.*, 1995). These studies were pivotal in catalysing research on relaxin's role as a regulator of vascular function in pregnancy. Such research is epitomized by numerous studies from the Conrad group and others in which non-pregnant rats were treated exogenously with purified porcine relaxin or rhRLX to mimic circulating concentrations of relaxin in mid-pregnancy (20–40 $ng \cdot mL^{-1}$). Vascular responses, for example reduced myogenic reactivity, were demonstrated in rat small renal and mesenteric arteries after *in vivo* rhRLX treatment (Novak *et al.*, 2002; van Drongelen *et al.*, 2011; van Drongelen *et al.*, 2012), whereas *ex vivo* rhRLX application induced vasodilation in isolated rodent small renal and mesenteric arteries (Li *et al.*, 2005; McGuane *et al.*, 2011b) and human small gluteal and subcutaneous arteries but not in pulmonary resistance arteries (Fisher *et al.*, 2002; McGuane *et al.*, 2011b). Although the direct vasodilator

effects of rhRLX in isolated blood vessels have not been observed by all investigators (Leo *et al.*, 2016a), these studies established important vascular functions and underlying mechanisms of relaxin action in renal and mesenteric arteries (discussed in more detail later).

Early maternal vascular adaptations to pregnancy are essential to maternal health and the survival and normal development of the fetus. During pregnancy, the maternal vasculature becomes refractory to the actions of vasoconstrictors such as the thromboxane A_2 mimetic U46619 (Kim *et al.*, 1994) and angiotensin II (Ang II) (Novak *et al.*, 1997; Hermsteiner *et al.*, 2001). There is also enhanced endothelium-dependent vasodilation (Kim *et al.*, 1994; Cooke and Davidge, 2003) involving NO, prostacyclin (PGI_2) and EDH (Luksha *et al.*, 2010; Majed and Khalil, 2012; Johal *et al.*, 2014). The maternal uteroplacental vasculature also undergoes modifications to increase uterine blood flow and delivery of nutrients to the growing fetus. Specifically, there is dramatic remodelling of the uterine artery wall (Osol and Mandala, 2009). Initially, there is outward hypertrophic remodelling (increase in lumen diameter and wall cross-sectional area) caused by vascular smooth muscle cell (VSMC) de-differentiation. The lumen diameter then widens, but without a large expansion of vessel wall thickness. Uterine and radial arteries also become longer and more distensible in both axial and radial directions (Cipolla and Osol, 1994). Maximum remodelling of the main uterine artery occurs during late pregnancy and is associated with VSMC hypertrophy and hyperplasia (Cipolla and Osol, 1994; van der Heijden *et al.*, 2005; Osol and Mandala, 2009).

Endogenous relaxin is now a leading candidate molecule for regulating these essential vascular adaptations in early pregnancy. In a small pilot study of women with ovarian failure who become pregnant through egg donation, *in vitro* fertilization and embryo transfer (with no detectable circulating relaxin), the first trimester increase in GFR is blunted significantly. They also have increased plasma osmolality relative to women with normal ovarian function (Smith *et al.*, 2006). Serum relaxin concentrations are first detected 6–7 days after the lutenizing hormone peak in non-conceptive and conceptive cycles and reach highest circulating concentrations (1–2 $ng \cdot mL^{-1}$) in the first trimester (Stewart *et al.*, 1990). Correlated with this first trimester increase in relaxin are changes in the uterine artery (Anumba *et al.*, 2009), suggesting that relaxin mediates modifications to the uteroplacental vasculature. Analysis of serum relaxin levels in pregnancies complicated by preeclampsia or chronic kidney disease revealed no differences between normal pregnancies and those complicated by disease (Lafayette *et al.*, 2011; Bramham *et al.*, 2016). Nor was there a correlation between relaxin levels and GFR, mean arterial pressure, renal blood flow or renal vascular resistance (Lafayette *et al.*, 2011). However, these studies measured relaxin late in pregnancy, prior to delivery. Sampling of serum in the first trimester revealed that women were at increased risk of developing late-onset preeclampsia in combination with a small for gestational age newborn if they had lower than normal relaxin concentrations (Uiterweer *et al.*, 2014). Thus, a deficiency or complete lack of circulating relaxin, specifically in early pregnancy, may compromise the maternal vascular adaptations to pregnancy and affect both renal and placental function (Conrad, 2016).

Animal models of relaxin deficiency confirm the hypothesis that a lack of relaxin during pregnancy can have significant consequences on the vasculature (Table 1). Depletion of circulating relaxin with a neutralizing monoclonal

Table 1

Vascular phenotypes in relaxin-deficient rodents

Animal model	Artery	Vascular phenotype
MCA1 treated Long-Evans rats – mid pregnant (Novak *et al.*, 2001)	small renal artery	↑ myogenic constriction
Rln$^{-/-}$ mice – male and female (Novak *et al.*, 2006; Debrah *et al.*, 2011)	small renal artery	↑ myogenic constriction inward geometric remodelling, ↓ passive compliance
Rln$^{-/-}$ mice – late pregnant, 5 months old (Gooi *et al.*, 2013)	uterine artery	↑ passive circumferential wall stiffness
Rln$^{-/-}$ mice – late pregnant (Marshall *et al.*, 2016)	mesenteric artery	↑ sensitivity to Ang II (endothelium-independent)
Rln$^{-/-}$ mice – male (Leo *et al.*, 2014a)	mesenteric artery	↑ sensitivity to the α_1-adrenoceptor agonist phenylephrine and the thromboxane A_2 mimetic U46619
	–	↓ endothelium-dependent relaxation to ACh ↓ volume compliance but no change in circumferential wall stiffness
Rln$^{-/-}$ mice – male (Ng *et al.*, 2015)	aorta	↑ superoxide production ↓ total eNOS protein and basal NOS activity ↑ eNOS phosphorylation at Ser^{1177} ↓ sensitivity to the PGI_2 analogue, iloprost no reduction in endothelium-dependent relaxation to ACh
Rln$^{-/-}$ mice – male (Debrah *et al.*, 2011; Jelinic *et al.*, 2015)	femoral artery; external iliac artery	no effect

MCA1 = monoclonal antibody to rat relaxin.

antibody in midterm pregnant rats prevented the normal pregnancy-induced increase in GFR and effective renal plasma flow, and the normal reduction in renal vascular resistance, plasma osmolality and myogenic reactivity of small renal arteries (Novak *et al.*, 2001). These relaxin-neutralizing antibodies also blocked the gestational elevation in cardiac output and global arterial compliance, and decrease in systemic vascular resistance (Debrah *et al.*, 2006). In late pregnant rats, relaxin-neutralizing antibody treatment reduced uterine artery inner and outer diameters, and increased circumferential passive wall stiffness (Vodstrcil *et al.*, 2012), but there was no significant change in wall thickness or wall-to-lumen ratio. There was also no measureable change in collagen expression and composition, or expression of MMP2 and MMP9 (Vodstrcil *et al.*, 2012), so as yet, the precise mechanistic pathways underlying these structural modifications are unclear. Pregnant $Rln^{-/-}$ mice also have stiffer uterine arteries, with reduced elastin, MMP2, MMP10 and MMP14 gene expression in the vessel wall (Gooi *et al.*, 2013). The less profound changes in the arteries of the pregnant $Rln^{-/-}$ rats are explained by the relatively short antibody treatment time (they received the relaxin-neutralizing antibodies for 3 days), whereas the $Rln^{-/-}$ mice lacked relaxin for the duration of their pregnancy (19 days). This longer period of relaxin deficiency might be necessary to cause changes in the structural composition of the vascular wall in the absence of relaxin.

A novel vascular smooth muscle phenotype was recently identified in the mesenteric arteries of late pregnant female $Rln^{-/-}$ mice. Unlike their wild-type counterparts, responses to Ang II were not reduced, indicating that normal vascular adaptation to pregnancy is compromised in $Rln^{-/-}$ mice (Marshall *et al.*, 2016). Surprisingly, the hyper-responsiveness to Ang II was endothelium-independent and appeared to be associated with a reduction in vasodilator prostanoids derived from the vascular smooth muscle (Marshall *et al.*, 2016). Despite these vascular phenotypes, $Rln^{-/-}$ mice are not hypertensive during pregnancy (O'Sullivan *et al.*, 2016), although pups born to $Rln^{-/-}$ dams are growth restricted (Gooi *et al.*, 2013). It is tempting to speculate that a deficiency or complete lack of relaxin in the first trimester of pregnancy may predispose women to hypertensive disorders, including preeclampsia, because the renal and mesenteric vasculature fail to adapt sufficiently to pregnancy. However, there is no direct evidence to support this hypothesis in pregnant women.

Vascular actions of endogenous relaxin in males?

Despite being primarily classified as a pregnancy hormone, relaxin and RXFP1 receptors are detected in the small renal arteries, mesenteric arteries and aorta of male rodents (Novak *et al.*, 2006; Jelinic *et al.*, 2014; Ng *et al.*, 2015). This suggests that relaxin may act as an autocrine or paracrine factor to mediate vascular function. Studies in $Rln^{-/-}$ male mice demonstrated that both vascular function and structure were impaired in small renal and mesenteric arteries, and the aorta (Novak *et al.*, 2006; Leo *et al.*, 2014a; Ng *et al.*, 2015). Interestingly, the degree of vascular impairment is vessel-specific, highlighting the heterogeneity of vascular dysfunction associated with a deficiency of relaxin (Table 1).

Relaxin phenotypes in small renal and mesenteric arteries

In small renal arteries of $Rln^{-/-}$ mice, myogenic constriction was increased relative to wild-type mice (Novak *et al.*, 2006), a finding uncovered by incubating arteries in $_{L}$-arginine or $_{D}$-arginine. Although the exact mechanism of action for this increased myogenic reactivity in small renal arteries is not yet known (Novak *et al.*, 2006), it is likely to involve impaired NO signal transduction pathways. In addition, when compared with wild-type mice, the $Rln^{-/-}$ mice demonstrated relatively hypotrophic geometric remodelling associated with reduced VSMC density and unstressed wall area, as well as increased collagen-to-total protein ratio, leading to reduced passive compliance (Novak *et al.*, 2006; Debrah *et al.*, 2011). Therefore, it is possible that in $Rln^{-/-}$ mice, there is impaired vascular remodelling and compromised endothelium-derived NO function, resulting in a more 'constricted' and 'stiffer' small renal artery phenotype compared with that in wild-type mice.

Small mesenteric arteries (150–200 μm) of $Rln^{-/-}$ mice exhibit super-sensitivity to the α_1-adrenoceptor agonist, phenylephrine and the thromboxane A_2 mimetic, U46619 (Leo *et al.*, 2014a). The hyper-sensitivity to these vasoconstrictors is endothelium-dependent because these changes were absent in endothelium-denuded mesenteric arteries. Further analysis using pharmacological blockers revealed that the enhanced responses to these vasoconstrictors were underpinned by an impairment of NO and vasodilator prostanoid pathways (Leo *et al.*, 2014a). In further support of these findings, mesenteric arteries from $Rln^{-/-}$ mice were less responsive to the endothelium-dependent agonist, ACh, indicating endothelial dysfunction (Leo *et al.*, 2014a). In contrast to the small renal arteries (Novak *et al.*, 2006), endothelial dysfunction of the mesenteric arteries was not due to impaired NO or EDH. Instead, it was associated with an increased contribution of vasoconstrictor prostanoids, independent of changes in thromboxane receptors or COX enzymes (Leo *et al.*, 2014a).

Mesenteric arteries from $Rln^{-/-}$ mice also have reduced volume compliance but no changes in passive circumferential wall stiffness (Leo *et al.*, 2014a). A recent study that assessed the impact of ageing on mesenteric arteries demonstrated age-dependent increases in circumferential wall stiffness, but not volume compliance in wild-type mice with no additional detrimental effects of relaxin deficiency (Jelinic *et al.*, 2015). However, vascular stiffness was exacerbated in mesenteric arteries of younger $Rln^{-/-}$ mice compared with $Rln^{+/+}$ of equivalent age, highlighting the importance of endogenous relaxin in regulating vascular remodelling of these arteries in younger animals. However, the negative consequences of relaxin deficiency are surpassed by other influences during the ageing process.

Relaxin phenotypes in large arteries (>500 μm)

In contrast to small renal and mesenteric arteries, the vascular phenotypes in the aorta of $Rln^{-/-}$ mice are less pronounced. Superoxide production was increased but was

independent of changes in SOD or NADPH oxidase protein expression (Ng *et al.*, 2015). Furthermore, eNOS protein expression and basal NOS activity were reduced in $Rln^{-/-}$ mice. Despite reduced NO bioavailability and increased basal superoxide levels in the aorta, endothelium-dependent and -independent vasorelaxation were unaffected. The NO contribution to endothelium-dependent relaxation was impaired, but this was only revealed following blockade of COX activity (Ng *et al.*, 2015). There was also a compensatory increase in eNOS phosphorylation at Ser^{1177} to maintain endothelial function under conditions of increased oxidative stress (Ng *et al.*, 2015). In addition to NO, the aortae of $Rln^{-/-}$ mice also had modifications in the prostanoid pathway. Specifically, there was reduced smooth muscle sensitivity to the PGI_2 analogue, iloprost, which was partly explained by decreased expression of PGI_2 receptors. Expression of the PG synthase enzymes *Cox1*, *Cox2* and *Ptgis* were not affected (Ng *et al.*, 2015). In contrast, in the aorta of aged $Rln^{-/-}$ mice (16 months), *Cox1* was increased whereas *Cox2* was decreased (Ng *et al.*, 2015). Despite these changes in COX gene expression in the older mice, there was no overt evidence of vascular dysfunction.

There has been no analysis of the passive mechanical wall properties in the aorta, and there were no effects of relaxin deficiency on other larger arteries, for example femoral and external iliac (Debrah *et al.*, 2011; Jelinic *et al.*, 2015). To summarize, endogenous relaxin plays an important role in the maintenance of endothelial function and vascular remodelling in both males and females, particularly in small resistance-size arteries. Impaired vascular function in $Rln^{-/-}$ mice is artery-specific and is mediated by compromised NO and prostanoid production. The consequences of relaxin deficiency on cardiovascular function are unclear because mean arterial pressure, heart rate and left ventricular dimensions are not compromised in $Rln^{-/-}$ mice (Du *et al.*, 2003). Moreover, the lifespan of $Rln^{-/-}$ mice is not different from $Rln^{+/+}$ mice, which age up to 24 months (Jelinic *et al.*, 2015). However, old male $Rln^{-/-}$ mice have impeded left ventricular diastolic filling and increased atrial weights, most likely due to increased ventricular collagen and chamber stiffness (Du *et al.*, 2003). Although $Rln^{-/-}$ mice display no overt cardiovascular phenotype, the underlying impairments in vascular function may worsen the progression of cardiovascular disease when animals are exposed to a secondary 'insult'.

Heterogeneous vascular actions of relaxin

The vascular endothelium regulates the tone of the underlying smooth muscle via the production of various endothelium-derived relaxing and contracting factors. Different vascular beds exhibit a marked heterogeneity in the relative contribution of these factors to the regulation of tone (Edwards *et al.*, 2010; Zhao *et al.*, 2015). In the aorta, endothelium-dependent relaxation is entirely mediated by NO whereas in smaller resistance-size vessels, there is a pronounced contribution of EDH (Luksha *et al.*, 2009; Sandow *et al.*, 2012). We now know that RXFP1 receptors are present in a variety of blood vessels, with quite different localization patterns in the endothelium and underlying VSMCs (Vodstrcil *et al.*, 2012; Jelinic *et al.*, 2014). The differential localization pattern of RXFP1 receptors in various vessel and cell types also supports the hypothesis that relaxin treatment will produce differential responses between the different vessel beds. To date, expression of vascular RXFP1 receptors has not been compared between males and females, and the possible involvement of sex steroids in regulating vascular RFXP1 receptors is unknown. Neither age, obesity nor hypertension altered *Rxfp1* expression in rat mesenteric arteries (van Drongelen *et al.*, 2011; van Drongelen *et al.*, 2012; van Drongelen *et al.*, 2013). Similarly, the increase in mesenteric artery vascular stiffness associated with ageing in mice was not correlated with decreased *Rxfp1* expression in the vessel wall (Jelinic *et al.*, 2015). To conclude, factors that regulate vascular RXFP1 receptors in both healthy animals (male and female), and in the context of disease, are yet to be established.

Cultured endothelial and vascular smooth muscle cells

The signalling mechanisms underlying rhRLX-RXFP1 interactions are well-characterized using native cells that overexpress RXFP1 receptors (Bathgate *et al.*, 2013; Halls *et al.*, 2015). Although these cells are important in understanding agonist-receptor interactions, they have limited physiological relevance to cellular function in endothelial cells (ECs) and VSMCs. Recent work investigated rhRLX -RXFP1 receptor interactions using human umbilical vein and artery ECs and VSMCs, which highlighted distinct signalling mechanisms between different cell types (Sarwar *et al.*, 2015). Specifically, rhRLX increased cAMP and cGMP accumulation in a bell-shaped concentration-dependent manner in human umbilical vein ECs and VSMCs. Conversely, rhRLX concentration dependently increased cAMP and cGMP levels in human umbilical artery VSMCs, but in a sigmoidal-shaped manner. These heterogeneous patterns of activation were attributed to involvement of different G-proteins in the signalling pathways. Poor expression of RXFP1 receptors with no binding to relaxin in human umbilical artery ECs explains why relaxin did not evoke detectable cAMP and cGMP accumulation in these cells (Sarwar *et al.*, 2015).

Earlier work in human coronary artery and aortic ECs demonstrated that rhRLX treatment causes concentration-dependent phosphorylation of eNOS, leading to increased NO production (McGuane *et al.*, 2011b). However, relaxin had no effect on NO generation in human coronary VSMCs, indicating that it selectively increased NO production in ECs. Furthermore, rhRLX treatment did not affect calcium influx in human coronary and aortic ECs (McGuane *et al.*, 2011b), suggesting that increased NO production by relaxin was not due to increased calcium signalling in ECs. NO production in these cells was attenuated by inhibiting NOS, PI3K, Akt and a heterodynamic G-protein ($G\alpha_{i/o}$), suggesting that relaxin activates NO production via a relaxin-RXFP1-PI3K-Akt-NOS pathway (McGuane *et al.*, 2011b). Activation of Akt in response to rhRLX treatment was also observed in rat aortic ECs (Dschietzig *et al.*, 2012). In addition to its effects on ECs, porcine relaxin stimulated NO production and cGMP accumulation in bovine aortic VSMCs by increasing iNOS expression and activity (Bani *et al.*, 1998).

An EC-VSMC co-culture approach was recently used to assess the effects of relaxin on crosstalk between these vascular cells. In human coronary artery, ECs co-cultured with

VSMCs, rhRLX stimulated cGMP and cAMP accumulation; this was partially blocked by the COX inhibitor, indomethacin, implying the likely involvement of the vasodilator prostanoid, PGI_2. However, this only occurred in human coronary artery ECs, and not umbilical vein ECs (Sarwar *et al.*, 2016). These data show heterogeneous cellular signalling responses to relaxin in cells from different origins, and strengthen our idea that *in vivo* relaxin treatment will produce region-dependent vascular responses depending on the blood vessel type (arteries vs. veins) or vessel size (large vs. small).

Aorta

In normal healthy rats, rhRLX treatment for 2 but not 3 days augmented ACh-evoked relaxation, which was accompanied by increased phosphorylation of eNOS and enhanced NO-mediated relaxation in the aorta (Leo *et al.*, 2016b). rhRLX also inhibited agonist-evoked contraction. Specifically, *ex vivo* rhRLX treatment for 6 h suppressed endothelin-1 (ET1)-induced contraction in segments of rat isolated aortas. This effect was endothelium-dependent and involved activation of endothelin ET_B receptors. rhRLX treatment also enhanced vascular relaxation mediated by endothelin-3 (a selective agonist for ET_B receptors), an effect that was abolished by the ET_B-selective antagonist A-192621 (Dschietzig *et al.*, 2003). This work in the rat aorta, along with that which reported rhRLX-induced ET_B-dependent renal vasodilation in pregnant rats (Novak *et al.*, 2001), is strong evidence that an important mechanism of relaxin action in the aorta is to enhance ET_B-mediated vasodilation.

Other studies have shown that *ex vivo* treatment with rhRLX for 48 h prevented aortic vascular dysfunction induced by the proinflammatory cytokine TNF-α or high glucose. The first study involved incubation of rat aortic rings with TNF-α, which increases oxidative stress and causes endothelial dysfunction. This was accompanied by a reduction in expression of eNOS and increased eNOS phosphorylation at Thr^{495}, leading to impaired eNOS activity (Dschietzig *et al.*, 2012). Co-incubation of these aortic rings with rhRLX caused PI3K-dependent eNOS dephosphorylation at Thr^{495} and eNOS phosphorylation at Ser^{1177} and Ser^{633}, and attenuated arginase II expression. This results in increased eNOS activity, leading to improved ACh-mediated endothelium-dependent relaxation. These effects of rhRLX involved signalling via glucocorticoid receptors because incubation with the antagonist RU486 negated the restoration of vascular function (Dschietzig *et al.*, 2012). rhRLX treatment also attenuated TNF-α-induced increases in superoxide and nitrotyrosine formation, as well as restoring SOD expression (Dschietzig *et al.*, 2012).

The second *ex vivo* model involved incubation of mouse aortic rings with high glucose (30 mm) for 3 days (Ng *et al.*, 2016). This caused endothelial dysfunction (reduced sensitivity to the endothelium-dependent agonist, ACh) by increasing the contribution of reactive oxygen species and vasoconstrictor prostanoids. Co-incubation of aortae with rhRLX for 3 days prevented endothelial dysfunction independent of NO availability. The presence of the COX inhibitor indomethacin and the antioxidant, tempol improved endothelium-dependent relaxation in high glucose-treated aortae, but not in rhRLX-treated aortae. This suggests that rhRLX suppressed the vasoconstrictor prostanoid pathway, and increased endothelium-derived PGI_2 production (Ng *et al.*, 2016). However, rhRLX also has vasoprotective effects possibly by reducing free radicals or enhancing the antioxidant capacity in the aorta during acute hyperglycaemia. Importantly, *in vivo* rhRLX treatment for 4 weeks effectively reversed endothelial dysfunction and reduced atherosclerotic plaque size in the aorta of apolipoprotein-deficient mice, which was attributed to a reduction in vascular superoxide production (Tiyerili *et al.*, 2016). A recent study extended these *ex vivo* findings in an *in vivo* model of endothelial dysfunction. Specifically, rhRLX infusion for 8 weeks prevented endothelial dysfunction in the guinea pig aorta caused by cigarette smoke. There was also a reduction in reactive oxygen species and increased NO production (Pini *et al.*, 2016). Overall, these findings demonstrate that rhRLX is a vasoprotective molecule under conditions of acute inflammation or hyperglycaemia, as well as in chronic settings such as smoking and atherosclerosis by reducing vascular oxidative stress in the aorta.

Femoral arteries and veins

To date, only one study has investigated the effects of rhRLX treatment on endothelial function in femoral arteries and veins (Jelinic *et al.*, 2014). Despite the presence of RXFP1 receptors in these vessels, subcutaneous infusion of rhRLX for 5 days did not enhance endothelial vasodilator function. One explanation is that the RXFP1 receptors are predominantly localized to the VSMCs and to a lesser extent within ECs (Jelinic *et al.*, 2014); thus, rhRLX is not capable of enhancing endothelium-dependent relaxation. It is also possible that rhRLX modifies vascular function through an action on the VSMC, but this is yet to be explored in these blood vessels.

Small renal arteries

The effects of *in vivo* rhRLX treatment on small resistance-size arteries were first demonstrated in small renal arteries (Novak *et al.*, 2002), as continuous relaxin infusion for 5 days reduced myogenic contraction. These effects of rhRLX were obliterated by the NOS inhibitor $_L$-NAME or in endothelium-denuded arteries. This was compelling evidence that the vascular effects of rhRLX were mediated by endothelium-derived NO (Novak *et al.*, 2002). Subsequent studies showed that rhRLX increased arterial gelatinase activity and indirectly activated ET_B receptors (Jeyabalan *et al.*, 2003; Jeyabalan *et al.*, 2007) (Figure 1). Consistent with these *in vivo* studies, *ex vivo* incubation of vessels with rhRLX for 3 h also inhibited myogenic reactivity in mouse and rat small renal arteries (and in human subcutaneous arteries), effects that were similarly mediated by gelatinases, ET_B receptors and NO (McGuane *et al.*, 2011a). Interestingly, the mechanism by which rhRLX inhibits myogenic constriction is time-dependent and involves differential up-regulation of gelatinase activity. Chronic infusion of rhRLX for 5 days in rats increased MMP2 activity and inhibited myogenic reactivity (Jeyabalan *et al.*, 2003). In contrast, a shorter duration of rhRLX treatment (4 to 6 h) also inhibited myogenic reactivity but involved a selective increase MMP9 activity (Jeyabalan *et al.*, 2007). Regardless of the activation of either MMP2 or MMP9 by rhRLX, both MMPs can convert big ET

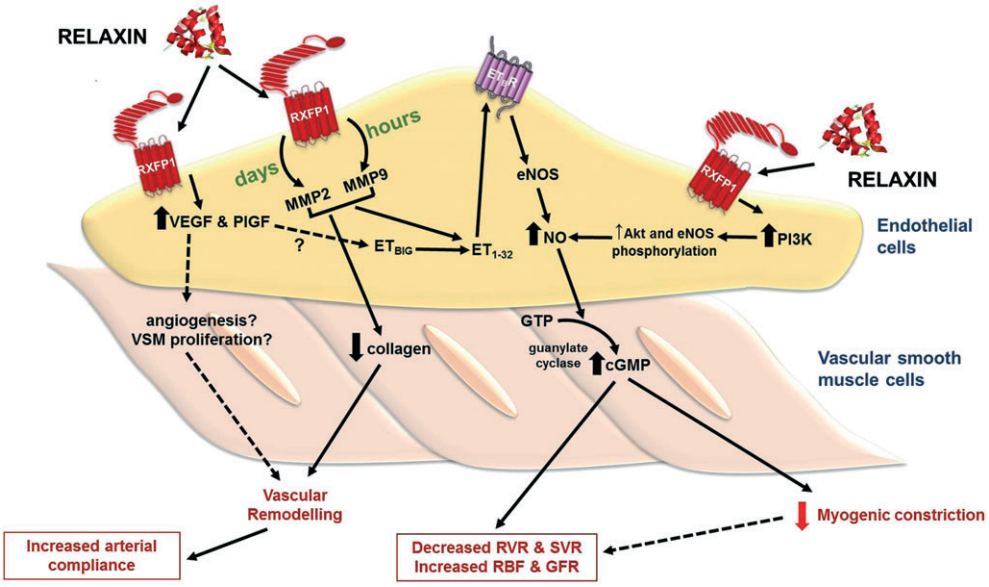

Figure 1

Effects of relaxin treatment in small renal arteries. Relaxin administration for hours and days increases MMP activities in ECs, leading to the conversion of big ET to $ET_{1–32}$, which activates endothelial ET_B receptors. Stimulation of endothelial ET_B receptors causes NO production and activates smooth muscle soluble guanylate cyclase, leading to cGMP accumulation and vasodilation. Vasodilation of small renal arteries reduces myogenic reactivity and renal vascular resistance (RVR) and increases renal blood flow (RBF) and GFR. Relaxin also directly acts on endothelial RXFP1 receptors to increase PI3 kinase-dependent Akt-eNOS phosphorylation, resulting in NO production. In addition to vasodilation, relaxin treatment causes vascular remodelling in the small renal arteries. Relaxin treatment increases angiogenic factors such as VEGF and PlGF, and reduces collagen content, causing vascular remodelling and increases arterial compliance. SVR, systemic vascular resistance.

into $ET_{1–32}$ which in turn activates ET_B receptors leading to NO production (Figure 1). The involvement of ET_B receptors was demonstrated in experiments using ET_B receptor antagonists that abolished the ability of rhRLX to reduce myogenic contraction in small renal arteries (Jeyabalan *et al.*, 2003; Jeyabalan *et al.*, 2007). The earlier work of Dschietzig *et al.* (2003) implied that rhRLX directly increases ET_B receptor expression in human umbilical vein ECs, but not human VSMCs. Moreover, ET1 binding to EC membranes was markedly increased after a 6 h exposure of the cells to rhRLX. However, *in vivo* rhRLX treatment had no effect on ET_B receptor expression and/or activity in rat small renal arteries (Kerchner *et al.*, 2005). Although there is strong agreement that rhRLX-induced vasodilation is mediated by ET_B receptors (most likely in endothelium), the ability of rhRLX to alter arterial ET_B receptor expression remains to be confirmed.

Angiogenic growth factors such as VEGF and the placental growth factor (PlGF) are also reported to mediate rhRLX's renal vascular actions (McGuane *et al.*, 2011a). In the presence of VEGF- or PlGF-neutralizing antibodies, rhRLX's ability to reduce myogenic reactivity *ex vivo* and increase endothelium-dependent relaxation of rodent renal arteries was abolished. Moreover, the renal vasodilatory effects of *in vivo* rhRLX were eliminated in conscious female rats co-treated with the VEGF receptor tyrosine kinase inhibitor, SU5416 (McGuane *et al.*, 2011a). One important point to note is that most of these studies involve rhRLX treatment in either male or non-pregnant female rats, so it remains a possibility that relaxin may mediate vasodilation through different pathways in the two sexes. There is no question that the most consistent component of rhRLX-mediated vasodilation in all studies is NO. The involvement of VEGF, PlGF, MMP2/9 and ET_B receptors in mediating the vasodilatory effects of rhRLX on small renal arteries is compelling (Figure 1), but it has yet to be shown if they are also involved in mediating the maternal renal vascular adaptations to pregnancy attributed to endogenous relaxin.

Cerebral parenchymal arterioles

The cerebral circulation is unique because the middle cerebral artery (MCA) has significant myogenic tone and contributes ~50% to cerebrovascular resistance (Faraci *et al.*, 1987). This allows local changes in cerebral blood flow while maintaining cerebrovascular resistance. Pial arteries eventually become brain parenchymal arterioles (PAs) after penetrating into the brain tissue (Hamel, 2006). Cipolla and colleagues investigated the role of rhRLX in both MCAs and PAs by infusing relaxin continuously for 10 days in male rats. rhRLX had no effect on the MCAs, but pressure-induced myogenic constriction slightly decreased in endothelium-intact cerebral PAs from rhRLX-treated rats. Furthermore, the contractile response to the IK_{Ca} channel inhibitor, TRAM34, was increased after rhRLX treatment (Chan and Cipolla, 2011). Although these effects were not statistically significant, they indicated that rhRLX may reduce myogenic tone and up-regulate IK_{Ca}-dependent EDH responses in PAs at a basal level. A subsequent study showed that 14 days of rhRLX infusion in spontaneously hypertensive rats (SHRs) resulted in decreases in cerebral PA myogenic tone. Similarly, rhRLX treatment improved EDH-mediated relaxation in cerebral PAs of SHRs

(Chan *et al.*, 2013). Interestingly, RXFP1 receptors were not detected in the PAs, but rhRLX treatment increased VEGF and MMP2 expression in the brain cortex, which expresses these receptors. Combined treatment of rhRLX, and a VEGF neutralizing antibody abolished the vascular effects of rhRLX in the cerebral PAs. Taken together, these findings suggest that rhRLX exerts its effects on the cerebral PAs through a RXFP1-VEGF signalling pathway in the adjacent brain tissue (Chan *et al.*, 2013).

Mesenteric arteries and veins

A large body of work has investigated the effects of relaxin treatment in the mesenteric vasculature of healthy rats and to a lesser extent under diseased conditions. Earlier work demonstrated that rhRLX reduced myogenic reactivity in the mesenteric arteries as observed in the small renal arteries (Novak *et al.*, 2002; Jeyabalan *et al.*, 2007). However, more recent studies revealed that exogenous rhRLX augments endothelium-dependent relaxation that was agonist-specific and time-dependent. Specifically, rhRLX administration in rats for 2 to 5 days enhanced bradykinin (BK)-dependent, but not ACh-induced relaxation (Jelinic *et al.*, 2014; Leo *et al.*, 2016b). Similarly, acute i.v. injection of rhRLX also enhanced BK-mediated relaxation (Leo *et al.*, 2014b). Enhancement of BK-evoked relaxation was not a result of increased expression of BK B_2 receptors (Leo *et al.*, 2014b). RXFP1 receptors are able to heterodimerise with angiotensin AT_2 receptors in the kidneys, forming RXFP1-AT_2 receptor heterodimer complexes that rhRLX could act on to reduce renal interstitial fibrosis (Chow *et al.*, 2014). As there are no changes to B_2 receptor expression, one possibility is that relaxin selectively signals through RXFP1-B_2 receptor heterodimer complexes to enhance endothelial function.

The ability of relaxin to enhance endothelial vasodilator function is abolished by $_L$-NAME, suggesting that NO is responsible for the vascular effects of rhRLX (Novak *et al.*, 2002; van Drongelen *et al.*, 2011; van Drongelen *et al.*, 2012; van Drongelen *et al.*, 2013; Jelinic *et al.*, 2014). However, 5 days of rhRLX treatment had no effect in mesenteric arteries from aged (40–46 weeks old) (van Drongelen *et al.*, 2011) or obese rats (van Drongelen *et al.*, 2012) but improved flow-mediated NO-dependent vasodilation in arteries of SHRs (aged 10–12 weeks) (van Drongelen *et al.*, 2013). It was important to show that the lack of a response to relaxin was not due to a ligand-mediated reduction in RXFP1 expression nor did age or disease compromise receptor expression in this artery.

A single i.v. injection of rhRLX enhanced basal NOS activity, with increased phosphorylation of Akt at Ser^{473} and iNOS expression after 3 h (Leo *et al.*, 2014b). In addition, this acute response to rhRLX injection enhanced BK-mediated relaxation, which was underpinned by IK_{Ca}-dependent EDH, but not NO or PGI_2 (Figure 2). Enhancement of EDH-type relaxation involved a signalling pathway through the BK receptor because opening of IK_{Ca} and small-conductance calcium-activated K^+ channel channels by NS309 was not directly affected by i.v. relaxin injection (Leo *et al.*, 2014b). Interestingly, despite the absence of detectable rhRLX in the circulation 24 h after a single injection of rhRLX, BK-mediated relaxation remained enhanced. The main mediator of the prolonged vascular response is likely to be PGI_2. Vascular *Ptgir* expression and responses to the PGI_2 analogue, iloprost, did not change after i.v. rhRLX injection, suggesting that this prolonged effect of i.v. relaxin relied on the endothelium (Leo *et al.*, 2014b). These acute i.v. experiments provided the first evidence that rhRLX-mediated endothelium-dependent relaxation involves the contribution of novel vasodilators, EDH and PGI_2 (Figure 2). Subsequent studies continuously infused rhRLX i.v. for 2–3 days; this also enhanced endothelial vasodilator function but activated

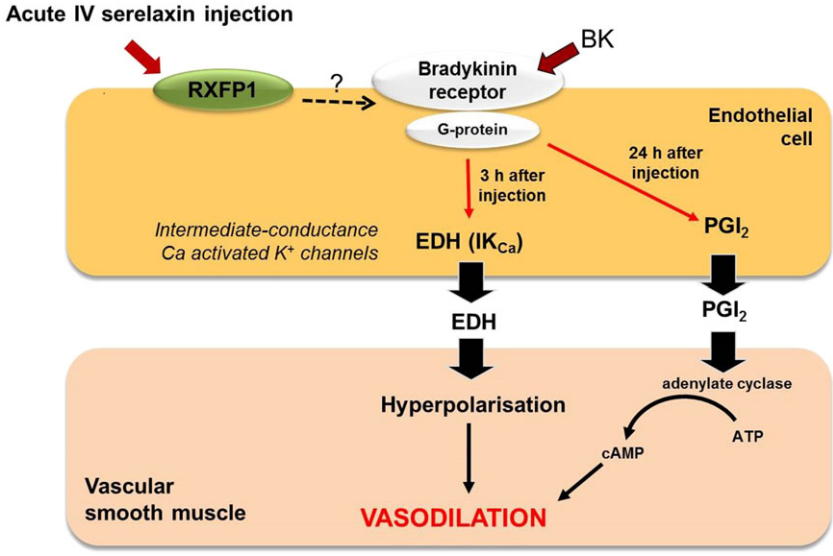

Figure 2

Proposed mechanism(s) of acute i.v. injection of relaxin in rat mesenteric arteries. Relaxin injection causes rapid (3 h) and sustained (24 h) augmentation of BK-evoked relaxation. The enhanced BK-mediated relaxation is dependent on the IK_{Ca} channels 3 h after acute i.v. relaxin injection. In contrast, the sustained (24 h) relaxation to BK is dependent on PGI_2.

different mechanisms (Leo *et al.*, 2016b). Specifically, rhRLX infusion for 2 days increased basal NOS activity and potentiated NO-mediated relaxation. This was attributed to increased eNOS expression (Leo *et al.*, 2016b). However, this enhanced basal NOS activity was not sustained after 3 days of rhRLX infusion because there was a compensatory down-regulation of eNOS phosphorylation and protein expression (Leo *et al.*, 2016b). Although the BK-mediated relaxation was sustained, it was dependent on COX2-derived PGI_2. Furthermore, rhRLX infusion for 3 days but not 2 days increased *Cox2* expression and BK-induced production of PGI_2, assayed as its metabolite, 6-keto $PGF_{1\alpha}$. This is compelling evidence that exogenous rhRLX stimulates PGI_2 production in mesenteric arteries (Leo *et al.*, 2016b) and it strongly suggests that the identity of the endothelial-derived vasorelaxing factors activated after rhRLX treatment is time-dependent.

The effect of rhRLX treatment in the mesenteric vasculature is limited to arteries as no studies to date have shown changes in mesenteric venous function. Neither myogenic reactivity nor endothelium-dependent relaxation of mesenteric vein were altered after 2, 3 or 5 days of rhRLX treatment despite the presence of RXFP1 receptors in these blood vessels (Li *et al.*, 2005; Jelinic *et al.*, 2014; Leo *et al.*, 2016b). Therefore, the vascular effects of relaxin on veins cannot be excluded and more studies are required to determine the optimum dose and duration of relaxin treatment needed to elicit changes in venous function.

Remodelling actions of relaxin in the vasculature

The vascular actions of exogenous relaxin extend to modifications of passive mechanical wall properties. Subcutaneous rhRLX infusion in rodents increased circumferential arterial compliance in small renal arteries (Conrad *et al.*, 2004; Debrah *et al.*, 2011). In mice treated with rhRLX for 5 days, the relative increase in small renal artery compliance was mediated by both geometric (outward) and compositional (decreased collagen) remodelling. Outward remodelling was characterized by an increase in unpressurised wall area and wall-thickness-to-lumen area ratio. rhRLX treatment also increased VSMC density and decreased total collagen content in the artery wall, without altering pro-MMP2 and MMP9 activities (Debrah *et al.*, 2011).

Although there is consensus that relaxin treatment enhances endothelium-dependent relaxation to vasodilators and reduces myogenic tone in mesenteric arteries, there are substantial differences in the data on passive compliance. Subcutaneous rhRLX treatment in young male Wistar rats for 3–5 days increased arterial compliance (circumferential and longitudinal) and passive volume compliance, and reduced passive circumferential stiffness (Li *et al.*, 2005; Jelinic *et al.*, 2014). The latter was associated with outward remodelling (increased cross-sectional area and inner diameter) but not changes to the total soluble collagen or elastin content (Jelinic *et al.*, 2014). However, 5 days of rhRLX treatment failed to alter mesenteric artery passive mechanical wall properties in young (10–12 weeks old) or old (40–46 weeks old) non-pregnant female Wistar Hannover rats (van Drongelen *et al.*, 2011). Recently unpublished data from our laboratory using a different sub-strain of Wistar rat also demonstrated that neither 3, 5 nor 10 days of relaxin treatment altered passive mechanical properties of mesenteric arteries in young animals (8 weeks old). In this study, plasma rhRLX concentrations were detectable in the range of 57–89 $ng{\cdot}mL^{-1}$ and resulted in significant decreases in plasma osmolality, demonstrating that the relaxin was biologically active. One explanation of these data is that the potential beneficial effects of rhRLX on vascular remodelling are hard to distinguish from normal physiological remodelling because these studies used healthy young adult rats with arteries that were in a growth and development phase. Another is that there may be strain differences in the expression of receptors.

Consistent with the data in mesenteric arteries, relaxin treatment has subtle or no remodelling effects in other vascular beds in healthy animals. For example, rhRLX increased wall thickness and lumen diameter of brain PAs, indicative of outward remodelling, without affecting passive compliance in non-pregnant female rats (Chan and Cipolla, 2011). These effects on remodelling were mediated by PPAR-γ. Conversely, rhRLX treatment (for 5 or more days) had no effect on passive mechanical wall properties in the external iliac arteries and veins (Debrah *et al.*, 2011; Jelinic *et al.*, 2014), middle cerebral arteries (Chan and Cipolla, 2011) or mesenteric veins (Li *et al.*, 2005; Jelinic *et al.*, 2014). Thus, the effects of relaxin on passive compliance appear to be highly region-specific. Table 2 highlights the differences between studies in rats including sex and age of the animals, and duration of relaxin treatment. These are all factors that could contribute to the regional effects of relaxin on different vascular beds and need to be considered when undertaking work on vascular remodelling. As mentioned previously, strain differences in responsiveness to relaxin may also contribute to differences between studies.

The potential effects of relaxin on vascular remodelling are more pronounced in animal studies where vascular structure and distensibility have been compromised by disease. Treatment of aged (17 months old) male SHRs for 14 days with rhRLX, followed by a 7 day washout period, increased vessel diameter and elastin content and reduced collagen content of the aorta. It also enhanced passive compliance (circumferential) in the carotid artery (Xu *et al.*, 2010). Similarly, rhRLX treatment for 14 days in young (14–16 weeks old) SHRs reversed the hypertension – induced inward remodelling to increase passive distensibility in brain PAs but had no effect on middle cerebral arteries (Chan *et al.*, 2013). This remodelling was associated with increased MMP2 and VEGF expression in the brain cortex. These factors are hypothesized to interact with brain PAs to enhance distensibility (Chan *et al.*, 2013). Conversely, a shorter duration of rhRLX infusion for 5 days appeared to have no effect on the circumferential remodelling of mesenteric arteries from old, obese and hypertensive (SHR) male rats (van Drongelen *et al.*, 2011; van Drongelen *et al.*, 2012; van Drongelen *et al.*, 2013). This suggests that a longer duration of relaxin may be necessary to affect passive mechanical properties of mesenteric arteries in disease models. Therefore, we conclude that vascular remodelling is one of the beneficial effects of

Table 2

Summary of the effects of exogenous *in vivo* relaxin treatment on passive mechanical wall properties in rats

Sex	Age (weeks)	Treatment duration	Vessel	Effect of serelaxin	Reference
F	12–14	5 days	small renal artery	↑ passive compliance, outward hypertrophic remodelling	Conrad *et al.* (2004)
M	12	5 days	mesenteric artery	↓ circumferential stiffness & ↑ passive compliance	Jelinic *et al.* (2014)
M	8–12	3, 5 and 10 days		no effect	Jelinic *et al.*, *unpublished*
F	20–24	3 days		↑ passive compliance	Li *et al.* (2005)
F	10–12; 40–46				–
F	10–12 (control) (obese)	5 days		no effect	Van Drongelen *et al.* (2011, 2012, 2013)
F	10–12 (control) (SHR)				–
M	12	5 days	FA, FV, MV	no effect	Li *et al.* (2005); Jelinic *et al.* (2014)
F	14–16	10 days	brain parenchymal artery middle cerebral artery	outward remodelling no effect	Chan and Cipolla (2011)
		14 days (WKY and SHR)	brain parenchymal artery middle cerebral artery	↑ distensibility, outward remodelling no effect	Chan *et al.* (2013)
M	68 (SHR)	14 days +7 days washout	carotid artery	↑ distensibility, outward remodelling	Xu *et al.* (2010)

FA, femoral artery; FV, femoral vein; MV, mesenteric vein; WKY, Wistar Kyoto rats; M, male; F, female.

relaxin but this may limited to specific vessel beds and under disease conditions, and of course, in response to pregnancy.

Conclusion and future directions

During the last decade, a large body of research has not only identified an important vasodilatory role for endogenous relaxin, but has also uncovered novel mechanisms of relaxin action in the vasculature that involve prostanoids, EDH and antioxidant activity. Of importance, the detrimental effects of a lack of endogenous relaxin on the maternal renal, mesenteric and uterine vasculature during pregnancy are now established. Pregnancy-associated hypertensive disease and placental insufficiency (leading to fetal growth restriction) stem from inadequate maternal vascular adaptations, thus relaxin deficiency should be considered when assessing potential causes of these disease entities. The use of $Rln^{-/-}$ animal models (rats and mice) has enabled the discovery of novel vascular phenotypes in males and non-pregnant females and propose a new role for endogenous relaxin as mediator of vascular homeostasis. This work also emphasized the heterogeneous actions of relaxin between vascular beds. The major development in this area of research has been the breadth of *in vivo* relaxin treatment studies in conscious animals to assess relaxin actions in different vascular beds. Clearly, relaxin acts through endothelium-dependent NO-mediated vasodilator pathways involving MMPs, eNOS and NO to modulate myogenic reactivity in small renal arteries. But analysis of agonist-evoked endothelium-dependent relaxation that is enhanced by relaxin has also uncovered the involvement of EDH (IK_{Ca} channel activity) and vasodilator prostanoids (synthesis of PGI_2) pathways.

Much of this review has focused on the endothelium, but RXFP1 receptors are also located in VSMCs so future research needs to investigate the direct effects of relaxin on these cells. The rationale for this is best illustrated in recent work in late pregnant $Rln^{-/-}$ mice in which the normal pregnancy-associated attenuation of Ang II-mediated vasoconstriction in mesenteric arteries did not occur (Marshall *et al.*, 2016). This adaptive failure was endothelium-independent and was likely due to reduced smooth muscle-derived vasodilator prostanoids. It will also be important to revisit the work of Bani *et al.* (1998), which showed porcine relaxin stimulates NO production and cGMP accumulation in bovine aortic VSMCs by increasing iNOS expression and activity. Decreases in intracellular Ca^{2+} concentrations, increases in myosin light chain phosphatase activity and calcium-activated K^+ channel activity all contribute to smooth muscle cell relaxation. Previous work on uterine smooth muscle has shown that porcine relaxin targets each of these pathways to cause relaxation (Nishikori *et al.*, 1983; Rao and Sanborn, 1986; Meera *et al.*, 1995). Based on this evidence, future research needs to investigate if relaxin acts directly on RXFP1 receptors in VSMCs and stimulates similar intracellular mechanisms to cause relaxation.

The evidence that relaxin can remodel the vasculature and improve arterial compliance is less compelling outside the setting of pregnancy. The inconsistency between studies is hampered by different experimental approaches (duration

of relaxin treatment, age and sex of animals). Most studies in young rats report that rhRLX treatment fails to alter passive mechanical wall properties or circumferential wall stiffness, suggesting that rhRLX's beneficial effects are not evident in healthy animals. However, work in SHRs treated with rhRLX for 14 days revealed reduced collagen content and changes in aorta vessel diameter, improvements in carotid artery distensibility (Xu *et al.*, 2010) and increased passive distensibility in brain PAs (Chan *et al.*, 2013). Thus, we conclude that relaxin is capable of vascular remodelling, but further work will be needed to address the mechanisms of action as it is not clear that relaxin acts directly on the extracellular matrix to reduce arterial stiffness. Moreover, it needs to be established if the actions of relaxin on vascular remodelling are limited to disease conditions and specific vessel beds.

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Conflict of interest

The authors disclose that this project was partially funded by Novartis Pharma AG, who also provided the serelaxin as a condition of an Australian Research Council Linkage Grant. L.J.P. was also a paid consultant for Novartis Pharma AG. K.P.C. is an inventor or co-inventor of use patents for relaxin, and has served as a paid or unpaid consultant to Connetics, BAS Medical, Corthera, Novartis and other companies concerning the use of relaxin.

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