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Review

Drugs of the future: the hormone relaxin

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Abstract. The peptide hormone relaxin is emerging as a multi-functional factor in a broad range of target tissues including several non-reproductive organs, in addition to its historical role as a hormone of pregnancy. This review discusses the evidence that collectively demonstrates the many diverse and vital roles of relaxin: the homeostatic role of endogenous relaxin in mammalian pregnancy and ageing; its gender-related effects; the therapeutic effects of relaxin in the treatment of fibrosis, inflammation, cardioprotection, vasodilation and wound healing (angiogenesis) amongst other pathophysiological conditions, and its potential mechanism of action. Furthermore, translational issues using experimental models (to humans) and its use in various clinical trials, are described, each with important lessons for the design of future trials involving relaxin. The diverse physiological and pathological roles for relaxin have led to the search for its significance in humans and highlight its potential as a drug of the future.

Keywords. Relaxin, RXFP1, therapeutic, fibrosis, inflammation, cardioprotection, vasodilation, wound healing.

Introduction

Relaxin was first identified in 1926 by Frederick Hisaw [1] as a substance that could noticeably relax the pelvic ligaments and influence the female reproductive tract. Subsequent studies in the 1970 s found relaxin to be a peptide hormone with a two-chain structure, similar to that of insulin [2] (Fig. 1), while the relaxin genes were eventually cloned in the 1980 s [3, 4] identifying a common B, C and A chain prohormone structure.

It is now well established that relaxin is a member of a family of peptide hormones that diverged from insulin early in vertebrate evolution to form the relaxin peptide family [5, 6]. This peptide family is encoded by seven genes in humans and includes the relaxin genes *RLN1*, *RLN2* and *RLN3* and the insulin-like peptide genes *INSL3*, *INSL4*, *INSL5* and *INSL6*. Most other species [5–7] contain only two relaxin genes, termed *RLN1* and *RLN3*. The product of the human *RLN2* gene, human gene-2 relaxin (H2 relaxin) is the functional orthologue of the *RLN1* gene product (relaxin) from non-primate species, and both these genes are the major stored and circulating forms of relaxin in their respective species. Although the peptides of these genes display relatively low primary amino acid sequence homology, phylogenetic analysis indicates that they evolved from an *RLN3* ancestral gene [5, 6]. Importantly, the peptides that make up this family have distinct expression profiles and biological func-

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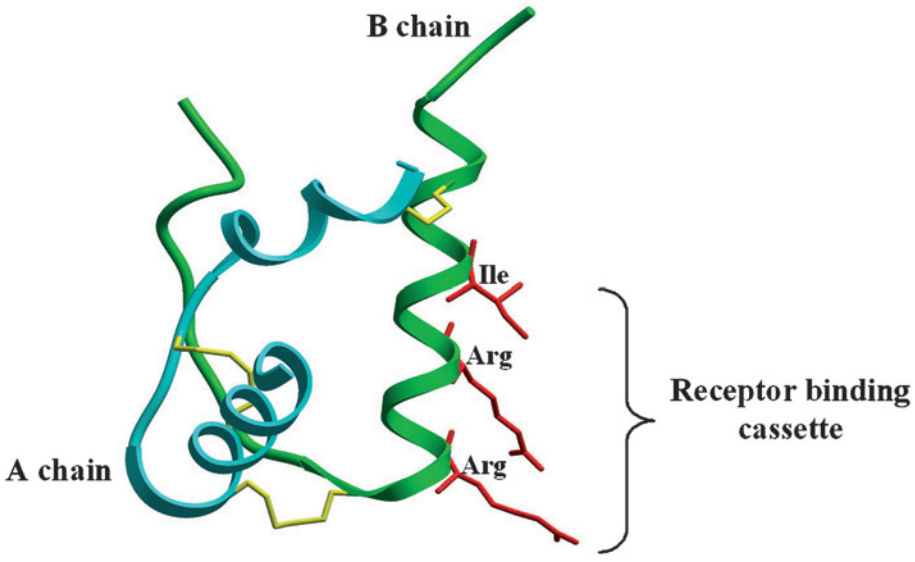

Figure 1. Representation of the structure of human gene-2 (H2) relaxin, with its constituent A (blue) and B (green) chains, linked by intra- and intermolecular disulphide (yellow) bonds. The conserved residues incorporating the relaxin receptor-binding motif (Arg-X-X-X-Arg-X-X-Ile/Val) are also shown. Kindly provided by Mr F. Shabanpoor and Assoc. Prof. J. Wade, Howard Florey Institute, University of Melbourne, Australia.

tions [reviewed in refs 8, 9]. For the purposes of this review we will specifically focus on the product of the human *RLN2* gene and its corresponding *RLN1* gene product from non-primate species, and, unless otherwise stated, will be specifically referring to these gene products when we use the term relaxin.

Relaxin is primarily produced in the ovary and/or placenta during pregnancy and was initially regarded as a hormone of pregnancy, based on its actions within the female reproductive tract to facilitate parturition ([reviewed in refs 8, 9]. Relaxin was found to promote elongation of the interpubic ligament in oestrogen-primed mammals [10, 11], inhibit spontaneous contractions of the uterine myometrium in estrogen-primed guinea pigs [12] and promote cervical softening in several species [13–16]. Many of these physiological actions of relaxin were thought to be associated with its ability to enhance collagen turnover in the pelvic girdle and female reproductive tract during pregnancy to facilitate successful parturition of many species. Relaxin is also produced in small quantities within the male reproductive tract, released into the seminal fluid and promotes several other actions. Both human gene-1 (H1) and H2 relaxin mRNA expression [17, 18] and immunostaining [19] have been detected in the human prostate and seminal vesicles, while relaxin expression has also been detected in the mouse [20] and rat [21] testis and prostate. Additionally, autoradiography has identified relaxin-binding sites in the male and female heart and brain [22], suggesting that relaxin may also target several non-reproductive organs.

It is therefore increasingly apparent that relaxin has a number of functions outside reproduction, with numerous reports suggesting potential therapeutic applications in non-reproductive processes such as fibrosis [8, 9, 23, 24], wound healing [8, 9], cardiac protection [24–27], allergic responses [28] and cancer [29, 30]. Not only are these actions surprisingly diverse, but unlike its actions in reproductive biology, occur in both males and females. This review therefore discusses (i) its homeostatic role in health and physiological processes, (ii) its therapeutic role in pathological disease states, (iii) its potential mechanism of action and (iv) its use in clinical trials, all of which are collectively enhancing its reputation as a potent but safe drug of the future.

Homeostatic role of endogenous relaxin

It has been increasingly recognised that there are a number of naturally occurring anti-fibrotic factors that are required to maintain homeostasis. Relaxin may be one such molecule. In the same way that hepatocyte growth factor and bone morphogenic protein-7 down-regulate transforming growth factor-beta1 (TGF-β1) signalling by interfering with Smad signal transduction, relaxin also moderates fibrogenesis at several levels, by inhibiting the influence of several profibrotic factors (such TGF-β, angiotensin II), inhibiting fibroblast proliferation and/or differentiation and stimulating matrix-metalloprotein (MMP) induced matrix degradation.

The ability of endogenous relaxin to protect the rodent female reproductive tract and mammary apparatus (which is required for successful parturition and lactation) was first identified in a series of studies that used neutralizing antibodies to rat relaxin [31–33]. Endogenous relaxin was confirmed to be required

for maintaining the length of gestation, litter delivery and survival, and these effects were attributed in part to its ability to promote cervical softening and extensibility in addition to vaginal growth [34]. These observed changes in the late-pregnant rat cervix and vagina were shown to be associated with a reduced density and organisation of collagen fibre bundles and altered length of elastin fibres [33, 35]. To further our understanding of the physiological role of endogenous relaxin, the relaxin gene knockout (*Rln1*−/−) mouse was later generated [36]. The initial phenotypic studies on late-pregnant *Rln1*−/− mice demonstrated that the development of the pubic symphysis, mammary gland, nipples and female reproductive organs during pregnancy was impaired [36], resulting in some level of reduced delivery and fetal survival and the inability of *Rln1*−/− mothers to lactate, consistent with the earlier studies which used neutralizing antibodies to rat relaxin [31–35]. The underlying pathology associated with these organs was attributed to a build up of collagen deposition and diminished collagen turnover, which would normally be mediated by relaxin in wild-type (*Rln1*+/+) mice [16]. Likewise, mice deficient in the relaxin family peptides receptor 1 (*Rxfp1*−/−) have impaired nipple development during late pregnancy and are unable to feed their young [37, 38], suggesting that the effects of relaxin associated with pregnancy are mediated via RXFP1 *in vivo*.

Male *Rln1*−/− mice also demonstrated underdeveloped reproductive tract development and inadequate growth of the prostate, testis and epididymis, leading to impaired male fertility [20]. Again the underlying pathology associated with these findings involved increased collagen deposition in all these organs, in addition to decreased sperm maturation and increased cell apoptosis in ageing male *Rln1*−/− mice [20]. While some first-generation *Rxfp1*−/− mice also underwent spermatogenesis leading to azoospermia and a reduction in male fertility [37], this spermatogenic defect was not observed in second- and third-generation mice, nor in separate *Rxfp1*−/− mice on a different genetic background [38], raising questions about the significance of the relaxin-RXFP1 interaction in the process of male reproductive tract development and function, and suggesting that other potential mechanisms are able to compensate for the loss of relaxin within the developing male reproductive tract.

The effects of long-term relaxin deficiency on ageing *Rln1*−/− mice have also been studied in several non-reproductive organs and have demonstrated an age-related progression of interstitial fibrosis in the heart [39], lung [40], kidney [41] and skin [42] of these animals, leading to organ damage and dysfunction [43]. While the effects of relaxin deficiency on these tissues are reportedly modest, they have been consistently found in most organs, particularly in male mice. Although long-term relaxin deficiency in mice did not lead to any apparent alterations in haemodynamics, it did lead to left ventricular diastolic dysfunction and increased atrial hypertrophy in male mice (by 9 months of age), which was most likely due to the increased left ventricle myocardial collagen concentration and procollagen type 1 mRNA measured in these animals [39]. Interestingly, this was not evident in the heart of female *Rln1*−/− mice. Similarly, the kidneys of male, but not female *Rln1*−/− mice were shown to develop an age-related accumulation of types I and III collagen (by 6 months of age), leading to renal interstitial fibrosis, increased glomerular matrix, cortical thickening and renal dysfunction [41]. In other organs, such as the lung [40] and skin [42], both male and female *Rln1*−/− mice underwent an age-dependent progression of fibrosis, resulting in increased bronchial epithelium thickening and altered airway/lung function in addition to increased dermal thickening. However, in each case, the onset and severity of fibrosis in these organs was more pronounced in male *Rln1*−/− mice compared to their age-matched female counterparts. Importantly, *Rxfp1*−/− mice also demonstrate pulmonary [38], renal and cardiac fibrosis [our unpublished data] at equivalent time points to these measured in *Rln1*−/− mice, suggesting that many of the protective actions of endogenous relaxin are mediated via RXFP1 in vivo. Furthermore, treatment of male *Rln1*−/− mice with exogenous H2 relaxin consistently led to the reversal of collagen accumulation (fibrosis) in the various organs studied [40–42, 44] (Table 1).

In more recent studies, the significance of endogenous relaxin in clinically relevant disease models has also been investigated using the *Rln1*−/− mouse. When subjected to a model of ovalbumin (OVA)-induced chronic allergic airways disease (AAD) over a 9-week period, which mimics several features of human asthma [45], both OVA-treated *Rln1*+/+ and *Rln1*−/− mice had increased airway inflammation, airway fibrosis and airway hyperresponsiveness (AHR) (lung function) compared to that measured in saline-treated control animals (Fig. 2). However, airway fibrosis was significantly elevated in OVA-treated *Rln1*−/− mice compared to that found in OVA-treated *Rln1*+/+ mice [46], leading to increased AHR, which correlated to either a reduction or failure to up-regulate MMP expression (in OVA-treated *Rln1*−/− animals) [46]. These findings confirmed that the specific role of relaxin in this model involved its ability to regulate extracellular matrix (ECM) and, in particular, collagen turnover within the airways/lung [46].

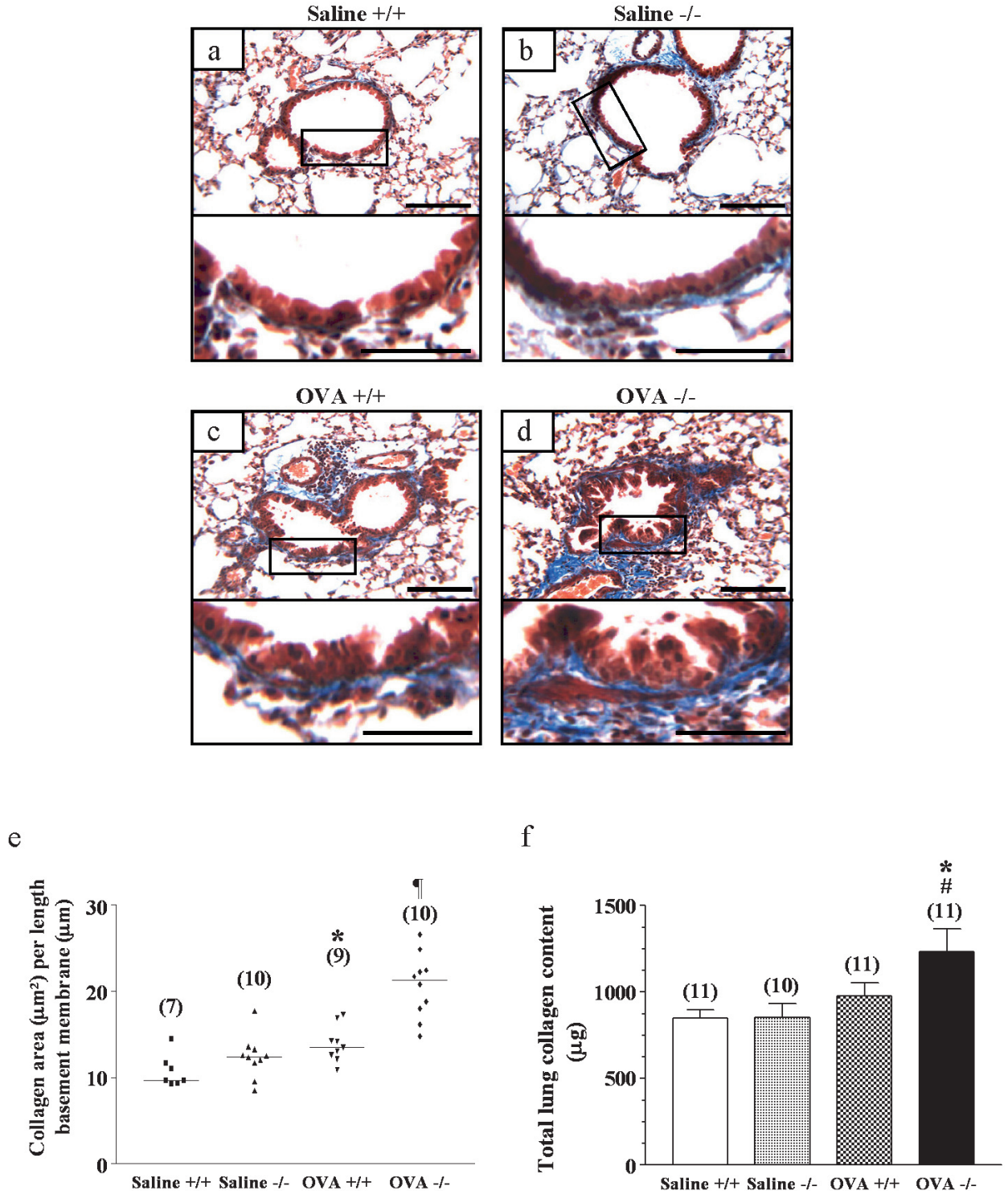

Figure 2. Lung collagen distribution and content in saline- and OVA-treated mice. Masson-trichrome-stained lung tissue sections were examined to assess collagen distribution in saline-treated RLX+/+ (*a*) and RLX−/− (*b*) mice and OVA-treated RLX+/+ (*c*) and RLX−/− mice (*d*). Bar, 100 μm in upper panels and 50 μm in lower panels. Morphometric analysis (*e*) was performed and results expressed as the area of collagen per basement membrane length (n=7–10 samples per group). *$p<0.02$ when compared to saline-treated RLX+/+ mice; ¶$p<0.001$ when compared to OVA-treated RLX+/+ mice. Hydroxyproline analysis (*f*) of collagen content was also performed on the four groups studied (n=10–11 samples per group) and expressed as the total collagen content per group. *$p<0.05$ when compared to saline-treated RLX+/+ mice and #$p<0.05$ when compared to saline-treated RLX−/− mice. Reproduced from Mookerjee et al. [46] with permission; copyright 2006, The Endocrine Society.

The relevance of endogenous relaxin has also been determined in a more rapidly progressive model of renal tubulointerstitial fibrosis, induced by unilateral ureteric obstruction (UUO) [47, 48]. At 3 days post-UUO, total collagen and myofibroblast accumulation were significantly greater in *Rln1*−/− mice than their *Rln1*+/+ counterparts [49]. This increase in collagen and myofibroblast accumulation was reversed by the administration of H2 relaxin to *Rln1*−/− mice 3 days post-UUO, again confirming that relaxin played a specific role in regulating collagen in this model. Furthermore, no significant differences in inflammation or MMP expression were observed between genotypes, suggesting that the ability of relaxin to inhibit renal fibrosis was mediated through inhibition of renal myofibroblast activity in this model [49] By 10 days post-UUO, the rapid progression of disease resulted in *Rln1*+/+ mice having equivalent levels of collagen and myofibroblast accumulation to that measured in *Rln1*−/− mice, suggesting that endogenous relaxin only delays but does not prevent the progression of fibrosis in such a rapid model of scarring. Nevertheless, the results indicate that even the very low levels of relaxin found endogenously are sufficient to modulate fibrosis.

These combined findings establish the important protective role that endogenous relaxin plays in several organs to inhibit the progression of collagen accumulation, associated with the progression of fibrosis. Furthermore, they demonstrate that the ability of relaxin to regulate collagen turnover has important implications during mammalian development and ageing, in addition to pregnancy.

Gender-specific effects of relaxin

Based on the more severe fibrotic phenotype of male relaxin-null mice, it was hypothesized that other female hormones and/or factors, such as oestrogen, may be compensating for the loss of the relaxin gene in female mice. In a recent study, the combined effects of oestrogen and/or relaxin deficiency, in addition to oestrogen replacement therapy (ERT) were analysed in ovariectomised female *Rln1*−/− and age-matched wild-type mice [50]. Ovariectomy of both *Rln1*−/− and wild-type mice had no noticeable effects on cardiac or renal fibrosis; however, cardiac hypertrophy was significantly elevated in mice lacking both relaxin and oestrogen [50]. In contrast, ovariectomy of *Rln1*−/− mice resulted in increased airway/pulmonary fibrosis, which resembled levels measured in age-matched male *Rln1*-/- by 12 months of age. ERT was able to significantly reverse the cardiac hypertrophy and airway/pulmonary fibrosis in these animals, suggesting that the protective effect of relaxin in inhibiting cardiac hypertrophy and airway/pulmonary fibrosis in female mice [50] could to a large degree be compensated for by ERT.

Therapeutic role of relaxin

In addition to its homeostatic and physiological roles, relaxin has also been demonstrated to play a number of significant roles in tissues associated with adverse pathology or disease, which collectively contribute to its therapeutic potential. Relaxin exhibits anti-fibrotic and anti-inflammatory actions, while promoting vasodilation, wound healing, and angiogenesis. These combined actions may even be linked at mulitiple levels (Fig. 3) and contribute to the ability of the hormone to protect several organs, as detailed below.

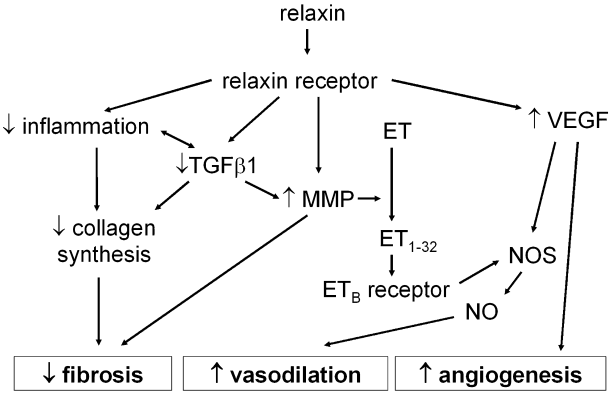

Figure 3. A schematic representation of the established effects of relaxin on fibrosis, vasodilation and angiogenesis. Upon binding to its primary receptor (RXFP1), relaxin inhibits the influx of inflammatory cells into injured organs, which may lead to the decreased recruitment of profibrotic cytokines by these cells. Relaxin also acts directly on profibrotic factors, primarily TGF-β1, to inhibit their ability to accelerate myofibroblast accumulation and collagen production, the net effect being a relaxin-induced reduction of fibrosis. Additionally, relaxin stimulates MMP-induced extracellular matrix breakdown, which contributes to its ability to decrease established fibrosis. The ability of relaxin to regulate MMP expression and activity also contributes to its vasodilatory effect, which is mainly facilitated via regulation of the ETB receptor and increased nitric oxide (NO) production. The ability of relaxin to promote vasodilation and angiogenesis can also be mediated through VEGF. These combined actions of relaxin lead to improved organ repair and function (see text for further details).

Anti-fibrotic effects of relaxin

Several investigators have demonstrated that recombinant H2 relaxin reduces the over expression of collagen, by inhibiting TGF-β1-stimulated collagen synthesis, while increasing MMP-induced collagen degradation and decreasing the actions of the tissue inhibitors of MMPs (TIMPs). Relaxin has been shown to inhibit collagen over expression *in vitro* when added to cultured TGF-β1-stimulated human dermal [51],

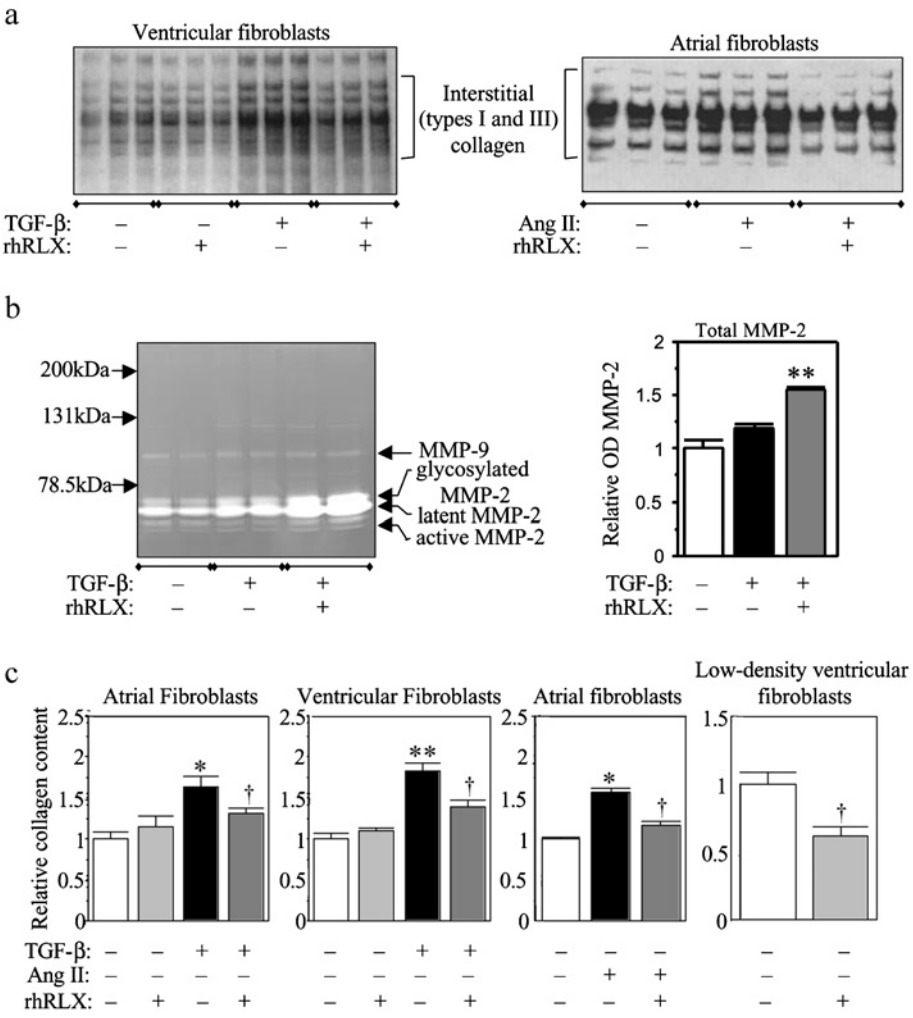

Figure 4. Modulation of collagen synthesis, degradation and deposition by recombinant H2 relaxin. Biosynthetically labelled interstitial collagens (*a*) from untreated cardiac fibroblasts (2×10^5/cm^2) and cells treated with either recombinant H2 relaxin (100°ng/ml) alone, TGF-β (1°ng/ml) alone or TGF-β (1°ng/ml) and recombinant H2 relaxin (100 ng/ml), or with Ang II ($5\times10-^7$ M) alone or Ang II ($5\times10-^7$ M) and recombinant H2 relaxin (100°ng/ml), were measured from the media samples after 72 h of culture. Shown are representative figures of triplicate samples from three separate experiments. MMP-2 and -9 expression and activity were determined by gelatin zymography (*b*) of media from untreated cultures and cells treated with either TGF-β (2°ng/ml) or TGF-β (2°ng/ml) and recombinant H2 relaxin (100°ng/ml) over 72 h. Shown is a representative zymograph of duplicate samples from each group, from four sets of samples/group. Also shown are the mean ± SE 'relative OD MMP-2' of the total MMP-2 (derived from the latent and active forms of MMP-2), as determined by densitometry scanning. Collagen content of cell layers (*c*) from untreated fibroblasts and cells treated with recombinant H2 relaxin (100°ng/ml) alone, TGF-β (2 ng/ml) alone or TGF-β (2 ng/ml) and recombinant H2 relaxin (100°ng/ml) or from untreated atrial fibroblasts and cells treated with Ang II ($10-^7$ M) alone or Ang II ($10-^7$ M) and recombinant H2 relaxin (100°ng/ml), after 72 h of culture were also measured. Results are presented as the mean ± SE 'relative collagen content' from three to four separate experiments. *$P<0.05$ and **$P<0.01$ compared with values from untreated cells. †$P<0.05$ compared with values from TGF-β or Ang-II-treated cells. Additionally, recombinant H2 relaxin (100°ng/ml) treatment of low-density cells (5/mm^2) over 7 days caused an inhibition of collagen deposition. Results are presented as the mean ± SE 'relative collagen content' from three separate experiments (six assays per group from each experiment). †$P<0.05$ compared with values from untreated cells. Reproduced from Samuel et al. [44] with permission; copyright 2004, The Endocrine Society.

pulmonary [52], uterine [53] and renal [54] fibroblasts, in addition to rat hepatic stellate cells [55, 56] and renal [57], atrial and ventricular fibroblasts [44] (Fig. 4). Relaxin was also shown to act in synergy with interferon-gamma (IFN-γ) to reduce collagen over expression by fibroblasts isolated from patients with scleroderma [58]. Furthermore, relaxin was shown to inhibit TGF-β, angiotensin-II or insulin-growth-factor-I-induced fibroblast proliferation [44], differentiation [44, 57] and collagen-I lattice contraction [57] as a means of down-regulating the stimulated collagen expression. In all these studies though (51–58), relaxin did not affect basal/unstimulated collagen expression, suggesting that relaxin does not directly inhibit the expression of collagen.

Human recombinant H2 relaxin has also been demonstrated to successfully reverse pathological collagen accumulation in every *in vivo* model of induced

Table 1. Rodent models of fibrosis, used to evaluate the anti-fibrotic effects of relaxin.

Organ affected	Species	Model of fibrosis used	Type of relaxin used	Treatment period	Reference
Skin	rat	fibrotic infiltration of polyvinyl alcohol sponge implants	recombinant H2	2 week	59
	mouse	fibrotic capsule formation around implanted osmotic mini-pumps	recombinant H2	2 weeks	59
		relaxin knockout: age-related progressive fibrosis	recombinant H2	2 weeks	42
Lung	mouse	bleomycin-induced model of pulmonary fibrosis	recombinant H2	2 weeks	52
		inflammation-induced model of airway fibrosis	recombinant H2	2 weeks	61
		relaxin knockout: age-related progressive fibrosis	recombinant H2	2 weeks	40
Liver	rat	carbon-tetrachloride-induced model of hepatic fibrosis	recombinant H2	4 weeks	55
Kidney	rat	bromoethylamine-induced model of chronic papillary necrosis	recombinant H2	4 weeks	63
		renal mass reduction by surgical excision or infarction-induced	recombinant H2	4 weeks	66
		hypertension and glomerulosclerosis	recombinant H2	4 weeks	64
		anti-glomerular basement membrane nephritis/fibrosis	recombinant H2	2 weeks	65
		spontaneous hypertension (and age)-induced fibrosis	recombinant H2	2 weeks	41
	mouse	relaxin knockout: age-related progressive fibrosis			
Heart	rat	spontaneous hypertension (and age)-induced fibrosis	recombinant H2	2-weeks	65
	mouse	isoproterenol-induced ischemic-injury-associated fibrosis	synthetic relaxin-3	10 days	69
		overexpression of β2-adrenergic-receptor-induced fibrosis	recombinant H2	2 weeks	44
		relaxin knockout: age-related progressive fibrosis	recombinant H2	2 weeks	44

fibrosis studied to date (by surgically, chemically or genetically modified means), involving the following organs.

Skin. Relaxin significantly inhibited fibrosis in a rat model of dermal scarring, induced by polyvinyl alcohol sponge implants, and in a mouse model of dermal fibrosis, induced by capsule formation around implanted osmotic mini-pumps [59] (Table 1). In both these models, relaxin significantly inhibited collagen deposition by 25–30% over a 2-week treatment period and altered the array of densely packed collagen fibres to those that were less abundant and randomly orientated. The ability of relaxin to reduce dermal scarring, while improving dermal extensibility, is thought to be mediated via the ability of relaxin to regulate fibrillin-2 expression [60].

Lung. In a bleomycin-induced model of pulmonary fibrosis in mice, the administration of recombinant H2 relaxin over a 2-week treatment period prevented lung-injury-induced collagen accumulation and restored lung function to that observed in normal mice [52] (Table 1). Furthermore, in a mouse model of allergic airways inflammation, relaxin was shown to inhibit collagen accumulation and reduce airway function [61]. In this model, exposure to ovalbunin (OVA) over time led to increased airway inflammation, mucus cell hypertrophy and hyperplasia in addition to airway remodelling and fibrosis [61] (Table 1). Relaxin administration over the second half of a 4 week exposure to OVA was able to markedly inhibit type I collagen accumulation and improved airway function, which led the authors to conclude that agents that can stimulate MMP-induced collagen breakdown could be used effectively as therapies for airway fibrosis [61].

Kidney. While skin wounds have a certain capacity to regenerate and heal from acute injury, chronic renal disease often results in irreversible scarring [62]. Continuous 2–4- week infusion of relaxin with osmotic mini-pumps has been shown to ameliorate renal fibrosis in several animal models of renal disease. Morphometric studies in a rat model of bromoethylamine-induced renal papillary necrosis demonstrated that 28 days of H2 relaxin infusion decreased the fractional area of interstitial collagen staining by 75% [63] (Table 1). This was associated with a parallel reduction in immunoreactive TGF-β1, macrophage infiltration and a preservation of glomerular filtration rate (GFR). Likewise, relaxin reduced glomerular and interstitial fibrosis in an anti-glomerular basement membrane nephritis model of disease [64] and normalised kidney collagen accumulation in spontaneously hypertensive rats [65] (Table 1).

In some cases, these benefits may be in part due to a reduction in blood pressure. Although similar effects were seen in both ablative and infarction models of 5/6 nephrectomy [66], relaxin was able to significantly decrease systolic blood pressure and glomerular lesions in an infarction model, but did not influence blood pressure in the normotensive ablation model, suggesting that the preservation of renal function by relaxin may be both blood pressure dependent and independent. Danielson et al. [67] have recently been able to demonstrate biphasic actions of relaxin: acute vasodilatory effects and a longer-term reduction in collagen deposition. Interestingly, serum concentra-

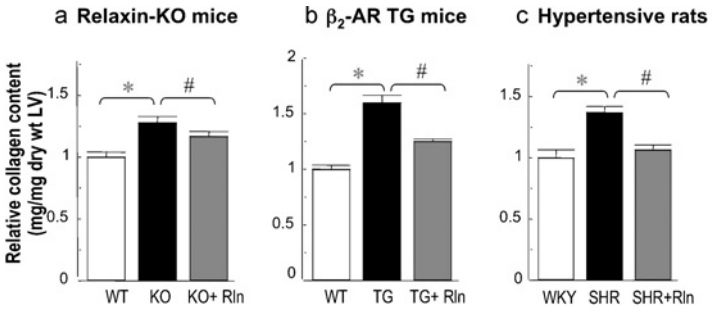

Figure 5. Effects of recombinant H2 relaxin treatment on collagen accumulation in three models of cardiac fibrosis *in vivo*. Collagen content/dry weight ventricular tissue was determined in 12-month-old relaxin wild-type (WT) mice (n=8), relaxin gene knockout (KO) [148] mice treated with vehicle alone (n=4) and relaxin KO mice treated with 500 μg/kg per day recombinant H2 relaxin (Rln) (n=4) for 14 days (*a*); from 5-month-old wild-type mice (n=8), β_2-adrenoceptor transgenic (TG) mice treated with vehicle alone (n=8) or with recombinant H2 relaxin (Rln) (n=8) for 14 days (*b*), and from 9-month-old (normotensive) Wistar Kyoto (WKY) rats (n=9) and spontaneously hypertensive rats (SHR) treated with vehicle alone (n=8) or with recombinant H2 relaxin (Rln) (n=8) for 14 days (*c*). *P<0.05 compared with values from WT mice/WKY rats; #P<0.05 compared with values from relaxin KO mice/β_2-adrenoceptor TG mice/SHRs treated with vehicle alone. Reproduced from Samuel et al. [44] with permission, copyright 2004, The Endocrine Society (*a, b*), and from Lekgabe et al. [65] with permission, copyright 2005, The American Heart Association (*c*).

tions of relaxin are increased in end-stage renal disease, with elevated serum concentrations predictive of death in male but not female patients on haemodialysis [68]. It is, however, unclear whether this association is causative, or merely a surrogate marker of chronic disease.

Heart. More recently, recombinant H2 relaxin was shown to reverse established cardiac fibrosis in relaxin null mice [44], mice with cardiac-restricted over expression of β2-adrenergic receptors (which undergo heart failure and premature death) [44] and in spontaneously hypertensive rats [65] (Table 1, Fig. 5) over a two-week treatment period. Importantly, relaxin only reduced cardiac fibrosis in diseased chambers of the heart, but did not influence basal collagen expression in unaffected regions of the myocardium.

While most of these studies have focused on the anti-fibrotic effects of H2 relaxin, limited studies have shown that relaxin-3 can also attenuate isoproterenol-induced myocardial fibrosis (in the rat) and subsequently improve left ventricular end-diastolic pressure following injury, while lowering plasma endothelin levels [69]. Additionally, H3 relaxin [70] and mouse relaxin-3 [71] have been shown to stimulate MMP expression upon administration to rat cardiac fibroblasts *in vitro* and the mouse lung *in vivo*, respectively, consistent with a matrix-remodelling effect of this peptide. Interestingly, human gene-3 relaxin (H3 relaxin) appeared to mediate its matrix remodelling effects via RXFP1, which is naturally expressed in cardiac fibroblasts [70], suggesting that both H2 and H3 relaxin act through RXFP1 to stimulate ECM remodeling. Furthermore, in all these studies, H2 and H3 relaxin demonstrated anti-fibrotic effects in all the diseased organs to which they were applied, but did not affect basal ECM and collagen remodelling in unaffected organs, demonstrating that relaxin is a potent, but safe anti-fibrotic hormone, with rapid efficacy in preclinical models of human disease. In most cases, the therapeutic effects of relaxin were shown to be rapid but safe when administration resulted in circulating levels of 20–50 ng/ml, which is within the physiological range of serum relaxin observed in pregnant rodents [8, 9]. While these concentrations of relaxin are much higher than those previously recorded in pregnant women undergoing single pregnancies (~1 ng/ml) [8, 9, 72], they are within the levels of circulating relaxin found in women undergoing multiple pregnancies.

Anti-inflammatory effects of relaxin

Relaxin has been shown to elicit anti-inflammatory effects in a variety of situations. Relaxin inhibits histamine release by mast cells in rat and guinea pig models of inflammation, which was shown to be mediated through nitric oxide production [73]. Relaxin also inhibits the influx of inflammatory cells such as neutrophils [74] and mast cells [75] into injured organs. Likewise, relaxin induced an increase in nitrite in coronary effluent while inhibiting the degranulation of mast cells, which would otherwise lead to an increase in histamine and subsequent myocardial cell injury [76]. In these circumstances, relaxin may indirectly reduce fibrosis through down-regulation of the acute inflammation/chronic inflammation-fibrosis continuum (Fig. 3).

Cardiovascular effects of relaxin

Chronotropic and inotropic effects of relaxin. Relaxin exerts a potent, positive, and dose-dependent chro-

notropic effect in the heart of rats and guinea pigs [77–81]. Relaxin increased heart rate in perfused intact heart, isolated right atria isolated left atria, and isolated ventricular tissue [77, 78, 81–85]. This chronotropic effect was also observed in conscious rats by us [65] and others [77, 79]. However, relaxin failed to induce a chronotropic effect on the human atria [86], which raises questions about the significance of this effect in non-rodent species. In some cases, relaxin was also observed to have a dose-dependent positive inotropic effect on the atria of rats and guinea pigs (isolated or whole heart) [77, 78, 81, 87], but this effect has not been demonstrated in all studies [83, 85]. In the isolated rat atria, relaxin was found to have greater potency in inducing chronotropy and ionotropy than endothelin-1, angiotensin II, isoprenaline, adrenaline, histamine or serotonin [78]. Additionally, relaxin has been reported to stimulate secretion of the atrial natriuretic peptide, a factor that is important in regulation of cardiovascular homeostasis, from the isolated heart of rats [85].

Vasodilatory effects of relaxin. Relaxin has been shown to cause dilation of vessels, such as arterioles, and also capillaries and postcapillary venules throughout the body.

The vasodilatation of coronary blood vessels in isolated and perfused hearts is more potent than other endothelium-dependent and -independent vasodilators (i.e. acetylcholine and sodium nitroprusside) [82, 88]. In support of this, relaxin decreases blood pressure and blunts the response of vasoconstrictors in mesenteric vessels of spontaneously hypertensive rats, bovine aorta smooth muscle cells and rat coronary endothelial cells [89–92]. Additionally, physiological vasodilatory effects of relaxin are supported by the observations that pregnant rats have a large decline in blood pressure during the 2–3 days preceding delivery [89, 93]. This decline in blood pressure was associated with a dose-dependent increase in coronary flow and a corresponding increase in nitric oxide (NO) concentration [82, 94, 95]. The increases in coronary flow and NO were inhibited by pretreatment with 0.1 mM N^g-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase II, suggesting that relaxin exerts its vasodilatory effects through an NO-driven mechanism [82]. However, relaxin does not exert vasodilatory effects in all blood vessels, as no vasodilatory effects of relaxin have been demonstrated in rat endothelium-intact aortic rings precontracted with noradrenaline [96].

Consistent with its vasodilatory effects in the heart, relaxin was able to promote dilation of alveolar blood capillaries in an experimental model of asthma [73]. Additionally, relaxin promotes renal vasodilation and hyperfiltration [97], while reducing the myogenic reactivity in small renal arteries of both pregnant [98] and conscious (relaxin-treated) non-pregnant rats [79, 99– 101]. Notably, these vasodilatory effects of relaxin have also been experimentally replicated in male animals [102]. It was found that relaxin could increase effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), attenuate the renal circulatory response to angiotensin II and reduce plasma osmolality regardless of gender [102, 103]. However, the significance of this vasodilation in the absence of pregnancy has yet to be elucidated.

From these combined findings, it has been hypothesised that relaxin may induce its haemodynamic effects in the cardiovascular and renal systems via a number of possible mechanisms. Firstly, relaxin has repeatedly been shown to stimulate coronary flow [82], renal vasodilation and hyperfiltration [97] by increasing NO production, via stimulation of NO synthase-II. This action was blocked by NO synthase inhibitors, suggesting an NO-dependent mechanism. Secondly, relaxin's actions on endothelin (ET) were thought to be mediated through the ETB receptor subtype, as it was this receptor subtype that was specifically involved in ET-induced stimulation of intracellular calcium in endothelial cells leading to the stimulation of prostacyclin and NO [97]. An essential role for the endothelial ETB receptor subtype in the renal vasodilation, hyperfiltration and reduced myogenic reactivity of small renal arteries was initially established in relaxin-treated rats [97]. However, controversy surrounds whether relaxin directly stimulates the ETB receptor. While Conrad and coworkers did not detect a relaxin-induced upregulation of ETB receptor protein expression [104], they were able to demonstrate increased processing of ET-1 to ET1–32 [100], thereby stimulating the ETB receptor and NO production in rat renal vessels/vasculature. Conversely, Dschietzig et al. [105] reported a relaxin-induced up-regulation of ETB receptor expression in human and bovine cell types *in vitro* and in rat thoracic aortas and renal and mesenteric arteries *ex vivo* [105]. Thus, from both studies [100, 105] it was concluded that relaxin was able to mediate its vasodilatory effects via regulation of the ETB receptor (either directly or indirectly) and increased NO production (Fig. 3).

Cardioprotective effects of relaxin. Several studies have implicated relaxin as a cardioprotective agent [reviewed in refs 24–27]. The expression of relaxin is low in normal cardiac tissues, but increases dramatically under pathological conditions, such as cardiomyopathy and heart failure [106]. Dschietzig and coworkers studied patients with congestive heart failure,

as a result of systolic dysfunction due to ischaemia or dilated cardiomyopathy, and identified relaxin as a novel compensatory hormone in humans, with the expression of both H1 and H2 relaxin being increased from patients with heart failure [106]. Additionally, plasma concentrations of H2 relaxin and myocardial expression of H1 and H2 relaxin mRNA in the atria and ventricles correlated with the severity of heart failure, while vasodilatory therapy with haemodynamic improvements resulted in a decline in circulating relaxin [106]. Interestingly, there was an inverse correlation between tissue levels of ET-1 (a powerful vasoconstrictor) and relaxin [106]. *In vitro* studies using isolated rat hearts confirmed that with elevation of ventricular filling pressure, which mimics the haemodynamic changes associated with heart failure, there was an up-regulation of ventricular relaxin expression and suppression of ET-1 secretion through induction of endothelin type-B receptors (which mediate ET-1 clearance and release of NO by endothelial cells) [106]. Others have shown that plasma H2 relaxin was increased in patients with chronic heart failure; however, the levels of H2 relaxin did not correlate with disease severity [107].

In separate studies, porcine relaxin was reported to be able to protect the heart of rats and guinea pigs following ischaemia-reperfusion-induced myocardial injury by improving cardiac contractility, maintaining coronary flow during ischaemia and increasing flow during reperfusion [76, 88]. In male rats, relaxin reduced the myocardial area damaged, ventricular arrhythmias and death, neutrophil invasion, platelet and mast cell activation, cellular calcium overload, lipid peroxidation of cell membranes and myocardial cell injury [88]. Additionally, relaxin pretreatment has been found to be protective against cardiac anaphylaxis induced by intraperitoneal injections of OVA in rats, which caused increased mast cell degranulation, histamine concentration, inotropy, chronotropy and decreased coronary flow [108].

H2 relaxin treatment was also found to be effective in reducing fibrosis in rodent models of heart disease, in mice with cardiac-restricted overexpression of β2-adrenergic receptors, and in spontaneously hypertensive rats. There is now good evidence to show that relaxin acts in the heart by down-regulating fibroblast proliferation and differentiation to inhibit collagen secretion, while stimulating an increase in MMP expression [44], both of which are associated with increased collagen degradation.

These combined actions do not of course occur in isolation. For example, vasodilation [76], increased coronary blood flow [82], increased production of endogenous NO production [76, 82], vascular endothelium growth factor and basic fibroblast growth factor (promoting vessel formation) [109], reduced mast cell degranulation and histamine release (mediators of allergic responses and inflammation) [110, 111], and reduction in neutrophil activation [74, 111] are just some of the mechanisms by which relaxin may protect the heart. Conversely, some of these actions may be contradictory. For example activation of the transcription factor nuclear factor-κB may simultaneously exacerbate inflammation, while reducing ET activity. Experimental *in vivo* models of human disease have therefore provided valuable and necessary insights into the interaction of these factors in disease.

Wound healing and proangiogenic effects of relaxin

Wound healing is a series of complex events that include inflammation, granulation, tissue formation and re-epithelialization, and eventually tissue remodelling. The effective healing of wounds is impaired by ischaemia or lack of regional blood perfusion and is dependent upon an increased local vascular supply of macrophages, platelets, neutrophils, smooth muscle cells and endothelial cells [9, 112]. Relaxin has also been shown to augment bronchial epithelial repair by increasing airway cell migration and ciliary beat frequency via a PKA-dependent mechanism [113].

Relaxin may also enhance wound healing through its proangiogenic effects. Relaxin was first thought to have angiogenic effects in the late 1950 s, when the clinical administration of impure porcine relaxin to patients with scleroderma and peripheral vascular disease resulted in healing of ischaemic ulcers on fingers and toes and improvement in peripheral vascular disease symptomology [114, 115]. Although some of these effects may be attributable to the increase in blood supply as a result of vasodilation, relaxin also has direct effects on the angiogenic process. Relaxin has also been found to stimulate blood vessel growth in the endometrial lining of the uterus and expression of the angiogenic cytokine, vascular endothelial growth factor (VEGF), in normal human endometrial stromal cells [116]. Consistent with its role in stimulating angiogenesis, when systemic relaxin was administered to women, they experienced a heavier than usual or irregular menstrual bleeding [116].

More recently, the potential angiogenic effects of relaxin were tested in rodent models of angiogenesis and wound healing. Continuous infusion of H2 relaxin stimulated new blood vessel formation, enhanced granulation tissue formation and induced both VEGF and basic fibroblast growth factor (bFGF) expression at the wound sites of both rat models and in cultures of human monocyte/macrophages (THP-1 cells) [116]. Furthermore, loss of capillaries parallels the patho-

genesis of progressive fibrosis in renal disease. Administration of exogenous VEGF improves experimental outcomes [117], suggesting that a relaxin-mediated increase in angiogenesis may be beneficial in scarring (Fig. 3).

However, enhancing angiogenesis is not always desirable. Maintenance of angiogensis is an enabling factor for tumour invasion, with relaxin having been implicated in increased tumour growth and angiogenesis of PC3 prostate xenografts [118].

Tumour-promoting role of relaxin

Relaxin has been implicated in having a role in breast cancer, as immunoreactive levels of H1 and H2 relaxin have been identified in normal and neoplastic breast tissue [119, 120]. While the specific role of these peptides in normal breast tissue has still to be determined, their expression was found to be up-regulated in human neoplastic tissue [119], and serum relaxin levels have been reported in breast cancer patients who developed metastases postsurgery, compared with healthy controls [121]. The administration of porcine relaxin to the human breast cancer cell line MCF7 [122, 123] was shown to promote cell growth, differentiation and invasiveness at low concentrations, but inhibited cell growth at high (nanomolar–micromolar) doses *in vitro*. These relaxin-induced effects on cell invasivion were later shown to be mediated via its ability to stimulate MMP expression [124]. An up-regulation of MMP activity has been implicated in tumorigenesis [125], while inhibition of MMPs is associated with reduced tumour growth and metastatic spread of breast tumour cells [126]. Similarly, adenoviral-mediated expression of human recombinant relaxin was shown to be biologically active by facilitating the invasive potential of canine mammary cancer cells [127], while *in vivo* studies showed that oestrogen-induced mammary tumours in rats underwent increased tumour growth with the addition of exogenous relaxin [29]. While these combined studies all point towards a tumour-promoting role of relaxin in breast cancer, it is still uncertain whether its effects are causative or the result of the metastases [121].

Relaxin has also been reported to stimulate the invasive potential of endometrial cancer cells and is highly expressed in endometrial metastatic tissues [30], suggesting that there are limitations to using relaxin under some pathological conditions and that in certain cases, inhibition of its activity is more desirable. In keeping with this view, a recent study demonstrated that an analogue of H2 relaxin was able to exhibit antagonistic properties and impaired prostate tumour growth progression *in vivo* [128], suggesting that mutating the H2 relaxin receptor-binding domain confers antagonistic properties on the hormone derivative. While the specific mechanism(s) of relaxin's actions in promoting mammary and endometrial tumours has still to be determined, these studies further suggest that regulation of relaxin's actions may offer a novel means of therapy in the treatment of various cancers.

Relaxin signaling

Relaxin receptor

While relaxin was one of the first peptides to be discovered, its receptor and that of the other relaxin family peptides were only recently discovered [reviewed in refs 7, 26] and found to interact with related G-protein-coupled receptors (GPCRs). The relaxin receptor is part of a subgroup (type C) of the family of leucine-rich-repeat-containing GPCRs (LGR) that include receptors for follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH). It has now been established that H2 relaxin (and relaxin in non-primate species) primarily binds with high affinity and activates the LGR7 receptor [129], which is now known as RXFP1 [7]. We recognize that a variety of tissues express mRNA for RXFP1 including, amongst others, the uterus, cervix/vagina, placenta, cerebral cortex, heart, lung and kidney.As seen in the relaxin null mouse, studies using RXFP1-deficient mice have demonstrated a quantitatively similar increase in interstitial collagen within the lung [38], confirming that relaxin's actions on collagen turnover *in vivo* are mediated through RXFP1. However, RXFP1 may bind multiple ligands, the significance of which is unknown.

Relaxin signal transduction

The ability of relaxin to down-regulate collagen production and increase collagen degradation is central to its physiological role. How it achieves this, however, is less clear. Several *in vitro* studies have provided the most promising insights into this process. As discussed above, H2 relaxin acts directly on TGF-β-stimulated fibroblasts from human [51–53, 58] and rodent [44, 55–57] origin to inhibit myofibroblast accumulation and collagen synthesis/secretion, while promoting MMP-induced collagen breakdown. Recent studies in human renal fibroblasts [54] suggested that these TGF-β-inhibitory effects of relaxin were mediated to a large extent by the Smad pathway. Phosphorylation of Smad2 and Smad3, formation of complexes with Smad4 and translocation of these complexes from the cytosol to the nucleus are key events in TGF-β-signalling [130]. Relaxin inhibited the phosphorylation and translocation of Smad2 to the

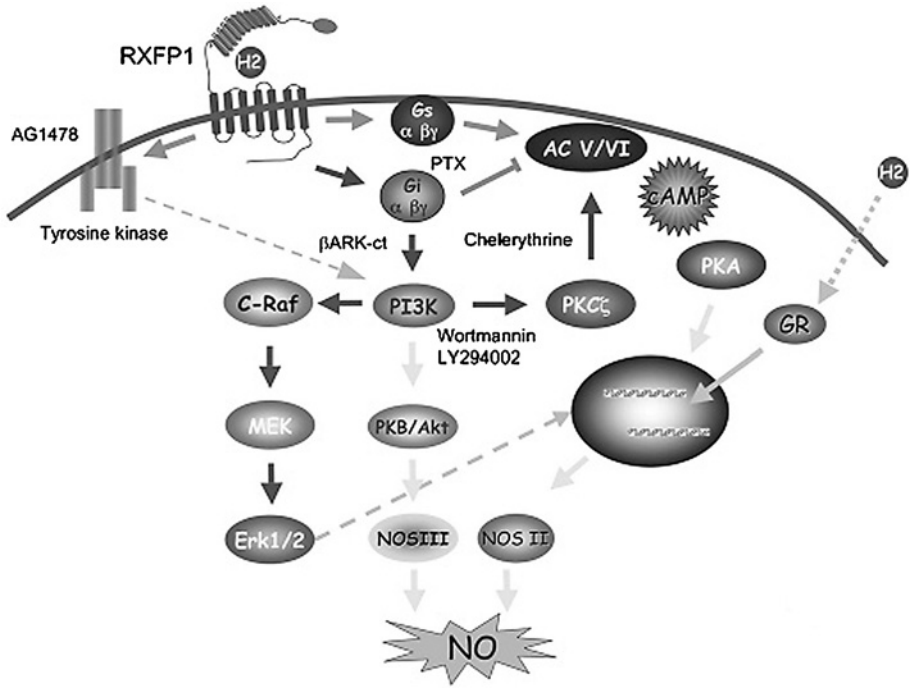

Figure 6. Potential signalling pathways utilised by the relaxin family peptide receptor 1 (RXFP1) receptor in a variety of cell types. The RXFP1 receptor can couple to adenylate cyclase (AC) via Gs and also couples to pertussis-toxin-sensitive G-proteins. G-protein βγ subunits then activate phosphatidylinositol-3-kinase (PI3-K) that in turn activates PKCζ, which can translocate to the cell membrane to both stimulate adenylate cyclase (AC V/VI) to enhance cyclic adenosine monophosphate (cAMP) production and also switch on the protein kinase B (PKB/Akt) pathway to increase activation of nitric oxide synthase (NOSIII). cAMP will activate PKA which can phosphorylate many signalling proteins but also enhance gene transcription of NOSII. Transactivation of tyrosine kinase receptors such as the epidermal growth factor receptor (EGFR) can also activate PI3-K that will switch on the P42/P44 (Erk1/2) MAP kinase pathway. There is also evidence that relaxin can directly activate nuclear glucocorticoid receptors (GR). Note that it is highly likely that the relative importance of the various mechanisms indicated will vary with the cell type in which the receptor is expressed. Reproduced from Samuel et al. [26] with permission, copyright 2006, Elsevier.

nucleus, in the absence of any effects on phosphorylation of Smad3, c-Jun NH_2-terminal kinase 1/2, extracellular signal-regulated kinase (ERK) or p3MAP kinase [54]. These findings are of significance, given that Smads play an important role in the regulation of collagen accumulation. However, recent findings demonstrate that cross-talk among a variety of pathways is necessary for the maximal stimulation of collagen expression [130], suggesting that relaxin, like many other ligands for GPCRs, is capable of activating multiple signal transduction pathways (Fig. 6).

This hypothesis has been confirmed by numerous other studies, which have demonstrated that relaxin acts on human endometrial cells to stimulate cAMP production and the activation of the p42/44 mitogen-activated protein kinase (MAPK) and ERK pathways [131]. A similar activation of ERK and antagonism of ET-1 (a downstream mediator of TGF-β) was observed when relaxin was administered to human umbilical vein endothelial cells and HeLa cells [105], while relaxin has also been shown to mediate some of its effects via the PI-3 kinase pathway [132] and protein kinase C zeta translocation [133]. Further work is now required to determine which of these pathways contributes to relaxin's matrix-remodelling actions on ECM-producing fibroblasts. On this issue, recent studies have demonstrated that relaxin administration to rat ventricular fibroblasts [44] is associated with a transient rise in cAMP, which is no longer detected in these cardiac fibroblasts [44] or human uterine fibroblasts [134] after 10 min, suggesting that the actions of relaxin on fibroblasts are not primarily mediated via a Gs-protein mediated pathway.

TGF-β1 activation

TGF-β1 is arguably the pre-eminent cytokine involved in the promotion of matrix synthesis and is consistently up-regulated in response to organ injury and/or disease. Many laboratories have repeatedly shown that relaxin abrogates TGF-β1 expression itself [63] or its ability to promote fibroblast differentiation (myofibroblast accumulation) and matrix synthesis [44, 51, 52, 54, 57]. These combined studies suggest that relaxin is able to consistently disrupt the fibrogenic influence of TGFβ1 in multiple organ systems.

Metalloproteinase activity

Relaxin's matrix-degrading properties are mediated by a net increase in the activity of various collagenases (MMP-1, MMP-13), gelatinases (MMP-2, MMP-9) and elastase (MMP-12), due to both increased expression of pro-collagenase and reduced expression of TIMPs [44, 51–53, 55–57, 135].

Furthermore, renal vasodilation and hyperfiltration were completely blocked in relaxin-treated rats by a specific peptide inhibitor of the gelatinases [100]. Conrad and colleagues concluded that a relaxin-induced increase in vascular gelatinase (MMP-2) activation led to the processing of ET-1 to ET1–32 [100], thereby stimulating the ETB receptor and NO production in rat renal vessels/vasculature.

Translational issues from experimental studies

While experimental animal studies have demonstrated the pleiotropic nature of relaxin as a potential therapeutic, a number of caveats to translating these findings to human therapeutics exist. Most of our understanding of relaxin biology comes from studies in rodents. Given that patterns of expression and putative roles for relaxin vary with species, it has been difficult to extrapolate a potential therapeutic use of relaxin in humans directly from preclinical, particularly rodent, studies. Questions regarding the significance of relaxin expression at non-ovarian sites and of receptor expression are the focus of intense study. As with other protein therapeutics, another challenge to developing relaxin as a therapy is the requirement for continuous administration due to its relatively short half-life. Nevertheless, relaxin is currently being tested in a number of clinical trials in which its pharmacological benefits may outweigh the inconvenience of continuous infusion. Studies examining alternative routes of administration have also been performed and can be advanced once a therapeutic benefit has been demonstrated.

Clinical trials with relaxin

Recombinant human relaxin (rhRlx) has been developed by three companies, Genentech, Connetics and BAS Medical, since the 1980 s. During that time, over 500 human subjects have been treated with rhRlx for indications that include cervical ripening, scleroderma, fibromyalgia and orthodontics. Although past trials have failed to show a definitive clinical use for relaxin, they are invaluable for having demonstrated specific biological activities in humans of this hormone, which holds promise of clinical utility in multiple indications.

Renal function

One of the most consistently observed findings in these trials was that rhRlx improved predicted creatinine clearance [136]. The rodent studies performed by the Conrad group [99, 102], together with observations that renal function increased during pregnancy in humans [137], suggested that rhRlx would improve GFR via increased renal blood flow (RBF). While clinical trials concluded to date have not directly measured GFR and RBF, they have shown that rhRlx caused statistically significant increases in predicted creatinine clearance across multiple trials. These increases were prolonged during chronic administration of rhRlx, lasting for 6 months during trials in which rhRlx was parenterally administered for this duration. Additionally, although studies involving bolus intravenous injection of rhRlx did not include a placebo arm, the onset of statistically significant increases in predicted creatinine clearance compared to baseline occurred within 24°h. This effect waned following cessation of both acute and chronic administration of rhRlx in these studies.

Other correlates of renal filtration, such as blood urea nitrogen and plasma uric acid, also changed in tandem with creatinine, suggesting improved GFR in subjects receiving rhRlx in these trials. The enhancement of renal function, as measured by these markers, occurred following both chronic and acute administration of rhRlx in subjects in whom creatinine clearance at baseline was normal, indicating the existence of a relaxin-sensitive mechanism for improvement in renal function in humans in whom there was no evidence of renal dysfunction. Furthermore, the renal responsiveness of male subjects to rhRlx in these trials indicated that this mechanism was present in the half of the population in whom the pregnancy imperative for improved renal function did not exist.

Why this would be the case evolutionarily is fascinating, but more pragmatically these findings strongly suggested that rhRlx could be useful in treating human disease impacted by impaired renal function. Congestive heart failure (CHF) is such a disease, affecting millions of ageing individuals, particularly men, with an economic impact of billions of dollars. Declining renal function in CHF patients is highly correlated with mortality [138], so a therapeutic agent that can improve or preserve renal function in this patient population would have a very large impact in this indication. A clinical trial studying the effect of short-term infusion of rhRlx in subjects with mild CHF has just concluded in Germany and data from this study

indicate that rhRlx can improve renal function. The results of this open-label trial, conducted by Dr. T. Dschietzig, are currently being analysed and will be presented in early 2007. A second study in CHF examining GFR and RBF using inulin and para-aminohippurate infusions is being initiated. A previously conducted small study examining GFR and RBF response to rhRlx in normal subjects showed that RBF, but not GFR, improved with short-term infusion of pregnancy levels (~1 ng/m) of rhRlx [138]. The study in CHF subjects will examine the same parameters in patients with mild-to-moderate renal insufficiency using pharmacological doses of rhRlx.

A multitude of other conditions, such as diabetic nephropathy and delayed graft function, would benefit from therapy with an agent that improves RBF and filtration capability and merit consideration for future trials using rhRlx.

Systemic vasodilation

Relaxin-mediated systemic vasodilation has been suggested by previous clinical trials, in which small but consistent decreases in blood pressure have been observed. Chronic administration of rhRlx in the scleroderma trials [136] was accompanied by decreases in blood pressure of a magnitude similar to that seen during pregnancy, and occurred over the 6 months of dosing, with blood pressure returning to baseline following cessation of dosing. Although the majority of the scleroderma patients had normal blood pressures at entry into the trials, a small subset was hypertensive. Interestingly, blood pressure decrements were greater in the hypertensive subset than in the normotensive patients. In the hypertensive subjects, statistically significant decreases from baseline in systolic blood pressure averaged 15–20 mm Hg, compared to the 5 -mm Hg drops that were observed in the normotensive subjects.

While vasodilation of renal vessels likely contributed to the decreased blood pressure observed in the subjects in the trials, systemic vasodilation is also known to accompany renal vasodilation during pregnancy. For example, systemic vascular resistance (SVR) decreased by approximately 30°% during the first trimester of pregnancy [139] when relaxin levels were peaking. Preclinical studies in rats have shown that rhRlx decreased SVR [79]. These observations also buttress the hypothesis that treatment of CHF patients with rhRlx could improve their symptoms by decreasing afterload. SVR, as well as other haemodynamic parameters, including pulmonary capillary wedge pressure and cardiac output, are being measured in the CHF trials described above.

Endometrial blood flow

Preclinical evidence that relaxin plays a role in the development and maintenance of the endometrial vasculature dates back to the 1950s when investigators observed remarkable histological changes in the endometrium following administration of a relatively crude preparation containing relaxin to monkeys [140]. More recently, Hayes at al. [141] observed that rhRlx treatment of monkeys during an in vitro fertilisation cycle increased subsequent 'implantation bleeding,' a phenomenon unique to non-human primates and believed to reflect embryonic implantation into the highly vascularized endometrium. The presence of circulating relaxin in humans during the mid-luteal phase of the menstrual cycle [142], its increase upon conception and the redundancy of its expression in the circulation and in the endometrium itself [143, 144] suggested that it played an important role in this tissue.

In clinical trials, reports of menorrhagia and metrorrhagia made by women during infusion of rhRlx have suggested that changes to the vasculature in the human endometrium may also occur following administration of pharmacologic doses of rhRlx [116]. In fact, this side effect has been reported more frequently than any other in the studies involving chronic (6-month) administration of rhRlx to human subjects. Because menstrual bleeding is a consequence of both blood vessel growth and vasodilation in the endometrium, it was not readily apparent which aspect rhRlx was influencing, but an rhRlx-mediated increase in blood flow can be concluded.

Increasing blood flow to endometrial tissue or to tissue derived from or associated with it, such as the placenta, could presumably be clinically useful in conditions in which there is a deficiency in this regard. Preeclampsia is such a condition. The deficiency is specific to women during pregnancy, can threaten the lives of both mother and baby, and is believed to be the result of suppressed bioactivity of growth factors, such as VEGF and placental growth factor (PlGF) [145]. The relative lack of growth factor activity is also believed to lead to ischaemia in multiple organs, including the kidney. Because rhRlx specifically upregulates VEGF in endometrial cells [116, 144], increases blood flow to the endometrium and improves renal function via increased blood flow, it is currently being tested in a small trial of women with severe preeclampsia. Renal function, uterine and umbilical blood flow, as well as clinical signs and symptoms of mother and baby, are being monitored in this study.

Connective tissue remodelling

Trials studying the effect of a therapeutic agent on diseases of fibrosis are difficult because fibrotic changes are usually gradual in the context of trial timelines and fibrotic endpoints can be difficult to quantify. These reasons may have contributed to the ultimate failure of the pivotal scleroderma trial in 2000 [136]. However, the preclinical evidence of rhRlx as a potent tissue remodelling hormone has spanned decades and is overwhelming. Relaxinologists who have studied its ability to remodel the cervix during parturition in rodents will attest to its remarkable and rapid activity on this tissue. Based on these findings, Genentech tested rhRlx for cervical ripening in its multinational clinical trials using topical application of rhRlx (intravaginal) in women with post-date pregnancies [146, 147]. Although these trials did not show efficacy, a reason for this failure may have been the lack of penetration of rhRlx into the cervical tissue, as suggested by the pharmacokinetic data from these trials. For these reasons, a trial testing the ability of pharmacological doses of intravenously administered rhRlx to ripen the cervix in women with post-date pregnancies is being conducted; its Results will be available early in 2007.

The anti-fibrotic aspect of rhRlx's pharmacology may also have an impact on the renal studies described above, but the acute vasodilatory effects induced by rhRlx facilitate rapid, quantitative and accurate measurements of endpoints related to renal function, so these will be selected as primary endpoints in clinical trials. Nevertheless, chronic administration of rhRlx may be necessary to sustain these vasodilatory effects and in this regard may also contribute to preserving renal function by inhibiting the fibrotic changes that occur in the kidney, as well as in the heart, in conditions such as CHF. Thus, both acute and chronic administration of rhRlx in the treatment of diseases of declining renal and heart function, such as CHF, may be useful.

Safety

In the over 500 human subjects dosed with rhRlx over the past 2 $\frac{1}{2}$ decades, rhRlx has shown an excellent safety profile, especially in healthy subjects. Chronic dosing for periods of up to 1 year in scleroderma patients was also very well tolerated and served as the foundation for future trials involving long-term dosing in other patient populations. The scleroderma patient population studied in the rhRlx trials was seriously ill and showed side effects that were expected in this patient population. Overall, the past trials provide valuable safety information and evidence of pharmacological activity, both of which support conducting future trials which aim to test the unique properties of rhRlx to treat human diseases.

Conclusion

Since the 1950s, research conducted on relaxin has progressively broadened its status from a reproductive hormone to a pleiotropic factor that plays several important roles in several non-reproductive organs, in addition to its well-known actions in tissues during pregnancy and lactation. Relaxin's most significant and consistent role in non-reproductive organs is its ability to stimulate connective tissue remodelling and induce a matrix-degrading phenotype in cells and tissues that are stimulated by chemical, surgical or genetic means to undergo diseased-related processes. Endogenous relaxin appears to be an important part of connective tissue homeostasis in a variety of organs, while exogenous (H2) relaxin treatment has been shown to effectively and rapidly inhibit the fibrotic process, at many levels. Importantly, relaxin dose not appear to influence the ECM under normal conditions, improving its potential as a safe therapeutic anti-fibrotic factor. Other consistent actions of relaxin in non-reproductive tissues include its ability to promote vasodilation, angiogenesis and wound healing, protect the cardiovascular system and influence fluid balance and body homeostasis, while inhibiting inflammation. It is highly conceivable that these various actions of the peptide hormone are interconnected.

Relaxin is a potentially important therapeutic agent of the future. Translating experimental findings to humans will however be essential in determining the clinical significance of relaxin. Several clinical trials have now demonstrated specific biological activities of the hormone, while current trials aim to validate its significance in human pathology.

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