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Review

Relaxin: a pregnancy hormone as central player of body fluid and circulation homeostasis

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Abstract. The peptide relaxin has long been regarded as an important hormone of pregnancy, contributing to changes in connective tissue composition as well as to regulation of implantation, myometrial activity and labor. On the other hand, the astonishing pleiotropy of this hormone escaped scientific awareness. This review focuses on new facets of relaxin, including its antifibrotic effects, its role in the control of pituitary hormone release, its vasodilator and pro-angiogenic properties and its versatile myocardial actions. Recent progress in understanding relaxin's receptor and signaling mechanisms is also highlighted. The peptide will be characterized as potential regulator of body fluid and circulation homeostasis.

Key words. cAMP; G-protein receptor; MAP kinase; myocardium; nitric oxide; peptide; tyrosine kinase.

Introduction

The peptide hormone relaxin was discovered in 1926, when Frederick Hisaw performed experiments in female nonpregnant guinea pigs [1]. Following injections of serum from pregnant guinea pigs or rabbits, the interpubic ligament of the nonpregnant animals elongated. In 1930, Hisaw and co-workers succeeded in preparing a crude extract from sow corpora lutea which behaved like a peptide and retained the particular property of relaxing the interpubic ligament [2]. The new substance was given the name relaxin. Another 15–20 years later, relaxin was found to promote growth of the mammary gland [3], to inhibit uterine contractile activity [4], and to dilate and soften the cervix uteri [5]. In 1960, the first quantitative bioassay for relaxin – the pubic symphisis elongation assay in mice – was developed by Steinetz and co-workers [6].

Once assigned to the field of reproduction and fertility during the early years of its discovery, the other side of relaxin long escaped scientific awareness. Not until the 1980s did researchers begin to recognize the astonishing pleiotropy of this hormone. Among the first to reveal new facets of relaxin were Bani and Bigazzi in 1984 [7] and St. Louis and Massicotte in 1985 [8], whose results pointed to vasodilator effects of relaxin, Osheroff and coworkers in 1990 [9] who autoradiographically visualized relaxin binding sites in rat brain, and Dayanithi and coworkers in 1987 [10], who demonstrated the relaxin-induced pituitary secretion of vasopressin.

In very recent years, insight into the molecular mechanisms of relaxin actions has deepened substantially. Two G-protein-coupled receptors were finally identified as the elusive relaxin receptors [11], the gripping mode of which is now also known in detail [12]. Investigation of the signaling pathways of relaxin gained great momentum [13, 14], and human relaxin H3 – a new member of the relaxin familiy – was discovered [15].

This review will focus on the recent progress, with particular attention being paid to the versatile effects that relaxin exerts apart from being a hormone of reproduction.

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Synthesis and secretion of relaxin

Structure of human relaxins

The peptide belongs to the insulin familiy of structurally related molecules, which includes insulin, insulin-like growth factors, relaxin, the relaxin-like factor [16], placentin [17] and the relaxin/insulin-like factor(s) [18]. Mature relaxin, like insulin, has a molecular weight of approximately 6000 Da and consists of two chains, termed A and B, which are covalently linked by two interchain disulfide bonds with an intradisulfide bond in the A chain [19]. In the late 1970s, the growing availability of purified pig relaxin enabled the first determinations of relaxin's amino acid sequence [20, 21]. Whereas in most mammalian species only a single relaxin gene has been found [22], the great apes (chimpanzee, gorilla and orang-utan) [23] as well as rats and mice [15] possess two relaxin genes, and three different relaxin peptides originating from three different genes are currently known in humans: H1 and H2, the amino acid sequence of which was deduced from the nucleotide sequences by Hudson and co-workers [24, 25], and H3, recently identified by Bathgate and co-workers [15]. Among species, the sequence homology of relaxin is remarkably low, with differences of 30–60%, but the localization of the disulfide bonds and the cysteines is very similar, thus suggesting similar tertiary structures of the different forms of relaxin [26].

Gene expression and synthesis of human relaxins

The genes encoding H1 and H2 relaxin are localized in close proximity on chromosome 9 at 9p24 [24, 25], whereas the H3 gene is on chromosome 19 at 19p13.3 [15]. The coding region of all human genes starts with the signal peptide followed by the B chain, C peptide and A chain; the corresponding peptide is termed preprorelaxin. Cleavage of the signal peptide generates prorelaxin. As with the insulin genes, a single intron is localized within the C peptide region of all human relaxin genes. Alternative splicing leading to incorporation of an additional 101-bp exon in the C peptide region was reported for H1 and H2 relaxin by Gunnersen and co-workers [27] who detected these novel messenger RNA (mRNA) species, among the well-known transcripts, in human placenta and prostate gland as well as in chimpanzee placenta. The peptides deduced from this alternate message would be composed of a B chain identical to common (pro)relaxin, but lack the A chain owing to the creation of a stop codon within the C peptide region. The physiological relevance of this particular mRNA form is still uncertain.

Inferring from the physiology of insulin, two members of the subtilisin-like family of prohormon convertases that both convert proinsulin to insulin [28] are candidate proteases for generating mature relaxin from prorelaxin by endoproteolytic removal of the C peptide: prohormone convertase-1 (PC-1, often referred to as PC-3) and PC-2. This item, however, remains to be investigated in detail. Until now, only one report by Marriott and co-workers [29] pointed to PC-1 rather than PC-2 being (one of) the relaxin-processing protease(s). These experiments were carried out in cotransfected human kidney 293 cells; thus, it remains to be confirmed whether the results obtained truly reflect the in vivo situation.

Gene expression of human relaxin in different tissues

In the past, the H2 gene was considered the only functioning human relaxin gene because Northern analysis repeatedly failed to detect any H1 transcripts in various tissues [25, 30]. This supported the thesis that H1 may represent a pseudogene [24]. With the emergence of the sensitive reverse transcription polymerase chain reaction (RT-PCR) method, however, H1 mRNA transcripts became more easily detectable, and it is at present commonly accepted that the H1 gene is expressed in the prostate gland [27, 31]. The existence of H1 transcripts was furthermore reported for breast [32] and for decidua and trophoblast [31], although the latter finding was not confirmed in a work of Gunnersen and co-workers [27]. We have recently shown that H1 transcripts are present in human atrial and ventricular myocardium, arteries and veins, which represents the first report of H1 gene expression in nonreproductive organs [33]. Altogether, H1 appears to be transcribed in some tissues, but the decisive proof of H1 peptide existing in different tissues is still lacking owing to the lack of H1-specific peptide assays. This point remains to be addressed in future research.

In contrast to the controversy over H1 relaxin, there is general agreement on the main sites of H2 expression and secretion: it is the female reproductive organs – corpus luteum, endometrium, decidua, placenta, trophoblast and mammary gland [25, 27, 32, 34–36] – and prostate [27, 37, 38]. With regard to nonreproductive tissues, we recently found H2 mRNA as well as relaxin-like immunoreactivity in human atrial and ventricular myocardium and in vessels [33]. This finding was in part anticipated by Taylor and Clark in 1994 [39] who demonstrated relaxin secretion by cultured rat neonatal atrial myocytes.

From studies conducted in rats [40, 41] and mice [15], we have learned that the brain poses another relevant site of relaxin expression. The second relaxin gene in mice, referred to as M3, is particularly enriched in mouse brain [15]. Of note, relaxin peptide is expressed in the rat arcuate nucleus [40], which is known to regulate hypothalamo-pituitary secretory function. This corresponds well with neuroendocrine effects of relaxin that will be discussed later in this review.

As to the recently discovered H3 relaxin, in a human multitissue mRNA expression array, Bathgate and co-workers [15] observed weak hybridizing signals for the spleen, thymus, leucocytes, lymph node and testis. These findings, however, must be confirmed and will certainly be supplemented by protein data in the near future.

Regulation of relaxin gene expression

Considering the large body of data on the biological effects of relaxin, comparably little is known about regulation of the peptide itself. To date, some insight has been gained into the regulation of luteal relaxin, whereas relaxin-controlling pathways in distinct tissues are poorly defined. Regarding relaxin in the rat corpus luteum, it is well known that its expression is upregulated by a placental factor and that estrogen acts as enhancer of this regulation [42–44]. Recently, Peters and co-workers [45], in a model of rat cultured granulosa cells, elucidated the ensuing signaling cascade as being composed of prolactin receptor-activating agents, that is prolactin itself or placental lactogen(s), the protein kinase C isoform δ and the transcription factor Stat-3. Estrogen displays no relaxin-inducing effect on its own, but significantly enhances the prolactin receptor-mediated stimulation of relaxin.

Luteinizing hormone stimulated relaxin secretion in cultured granulosa cells from preovulatory porcine follicles [46], which corresponds well with the clinical observation that during the menstrual cycle, the relaxin rise (see below) follows undulation of plasma luteinizing hormone. As revealed by studies in humans [47], human luteinized granulosa cells [48], pigs [49] and monkeys [50], chorionic gonadotrophin represents another stimulus for relaxin expression in the corpus luteum. Finally, basic fibroblast growth factor, which is locally expressed in luteal tissue, was also shown to regulate luteal relaxin: it inhibited basal and stimulated relaxin secretion by cultured porcine luteal cells [51].

Circulating relaxin

In the 1970s, the first radioimmunoassays sufficiently sensitive to detect plasma relaxin became available [52–54]. Until now, H2 has been considered the only circulating form of relaxin [55], but this hypothesis must be confirmed using monoclonal antibodies specific for H1, H2 or H3 relaxin. The highest plasma levels of human relaxin can be measured in the first trimester of pregnancy (~900 pg/ml at week 10); afterwards, relaxin levels are lower, but stable until term [56]. In nonpregnant women, a smaller relaxin rise (~100 pg/ml) occurs ~ 10 days after the plasma surge of the luteinizing hormone during the menstrual cycle [57]. In our recent studies, we used a homologous human relaxin enzyme-linked immunosorbant assay (ELISA) (Immundiagnostik, Bensheim, Germany), which allows for detection of the peptide even in postmenopausal women, and men in whom plasma relaxin amounts to <10 pg/ml [33]. This commercially available kit and its validation have been described in detail in [58]. The ELISA has a detection limit of <1 pg/ml. This until now unprecedented sensitivity was achieved by using the sandwich immunoassay technique with two high-affinity antibodies.

Signal transduction of relaxin

Relaxin receptors

The development of radioactively labeled relaxin preparations was the prerequisite to examine characteristics and distribution of relaxin-binding sites, which were first analysed in reproductive tissues and in the fibroblast model [59–62]. Encouraged by early observations that the uterus becomes refractory to relaxin following prolonged exposure to the peptide [63, 64], Mercado-Simmen and co-workers [62] were the first to demonstrate, in the rat myometrium, downregulation of relaxin-binding sites by relaxin itself, whereas estrogen upregulated relaxin receptors. Neither peptide affected the binding constant of relaxin, which was in the high picomolar range.

In 1990, Osheroff and co-workers [9] published their first report on specific ^{32}P-relaxin binding sites in the rat brain. Further characterization revealed a single class of high-affinity (K_D ~1 nM) sites in different regions of the brain, including the circumventricular organs and the neurosecretory hypothalamic nuclei [65]. In 1992, these researchers also demonstrated relaxin binding to the rat heart atrium, which occurred with comparable affinity [66]. These and later works undertaken in the rat [67–69] unequivocally showed the inability of relaxin-related peptides, that is insulin and insulin-like growth factors, to displace relaxin from its binding sites – a fact that clearly implicated the existence of a specific relaxin receptor distinct from insulin or insulin-like growth factor sites. This receptor exhibits a novel and unusual gripping mode which was elucidated in detail mainly by Buellesbach and Schwabe [12, 70–72]: two charged arginine residues in positions 13 and 17 of the B chain project like fingers from the helix opposed by the hydrophobic isoleucine in position B20, thus generating a trivalent interaction mechanism. Despite many efforts to identify the receptor, including cross-linking studies [67] and approaches using biotinylated relaxin [73, 74], it remained elusive until 2002 when Hsu and co-workers [11] eventually described two orphan receptors, LGR 7 and LGR 8, that fulfilled the requirements of a relaxin receptor. Amazingly, LGR 7 and LGR 8 turned out to be G-protein-coupled seven-transmembrane domain receptors – a fact that clearly distinguished them from the tyrosine kinase receptors for in-

sulin and insulin-like factors. In other words, judging from the nature of its receptor, relaxin closely resembles other reproductive hormones such as luteinizing hormone and follicle-stimulating hormone, rather than the peptides of its own superfamily [75]. This may account for the immense difficulties in isolating the relaxin receptor.

Relaxin signaling

In accordance with the features of its receptor, relaxin is known to act through stimulation of adenylate cyclase and the resulting increase in cyclic AMP (cAMP), with the first reports originating from the field of reproduction research: Braddon demonstrated relaxin-induced cAMP changes in the mouse symphysis in 1978 [76]; Sanborn and co-workers established the causal relation between relaxin exposure, cAMP rise and inhibition of spontaneous contractile activity in the rat uterus in 1980 [77]; and Hsu and co-workers confirmed the latter findings in cultured rat myometrial cells [78]. These observations were confirmed for endometrial glandular cells [79, 80] and breast cells [81]. Proof of cAMP as a pivotal and abundant messenger of relaxin actions also emerged from the field of brain research by Cronin and co-workers [82] who conducted experiments in anterior pituitary cells and from cardiovascular reports by Toth and co-workers [83] describing relaxin-mediated release of atrial natriuretic peptide in rat hearts, as well as by Piedras-Renteria and co-workers [84] who elucidated the positive inotropic effect of relaxin on rat atrial myocytes.

Bartsch and co-workers [85] demonstrated in human endometrial stromal cells and in the human monocytic cell line THP-1 that the G-protein-coupled relaxin receptor also initiates tyrosine kinase activation, which has long been advocated [13, 75]: the results of these authors imply that activation of the relaxin receptor leads to tyrosine phosphorylation, which, in turn, inhibits phosphodiesterase activity and further upregulates cAMP levels.

In 1994, Masini and co-workers [86] were the first to discover a substantially novel mechanism of action of relaxin. They were able to show that relaxin attenuated calcium ionophore-induced granule exocytosis by isolated rat serosal mast cells and that this effect was mediated via nitric oxide. Further evidence for this new concept was soon provided by scientists from the same group who proved the existence of a relaxin-nitric oxide pathway leading to increased levels of cyclic GMP in rat and guinea-pig hearts [87], in human and rabbit platelets [88], in human breast cancer cells [89] and in mouse small bowel [90]. In this context, relaxin influence on the different nitric oxide synthases appears to depend on the cell type under investigation. Whereas in rat coronary endothelial cells [14] and in bovine aortic vascular smooth muscle cells [91] relaxin promoted expression and activity of the inducible nitric oxide synthase (NOS II) with negligible effects on constitutive endothelial-type nitric oxide synthase (NOS III), the peptide had the opposite effect in the mouse uterus, that is, upregulation of NOS III expression in epithelium, glands, endometrial stromal cells and myometrium while leaving inducible NOS (NOS II) expression unaffected [92].

Compared with the above-mentioned experimental work on cyclid nucleotides and tyrosine phosphorylation, research on downstream signaling cascades of relaxin is still in its infancy. However, evidence is accumulating that the extracellular signal-regulated kinase-1/2 cascade (p42/44 MAP kinase) represents a key mediator of relaxin action. This was demonstrated in human endometrial stromal cells and in the human monocytic cell line THP-1 [93], as well as in human uterine fibroblasts [13]. Undoubtedly, much will emerge from this rapidly evolving field of research in the next few years.

Biological effects of relaxin

First, one should mention that prorelaxin, in contrast to proinsulin, possesses a bioactivity that is at least comparable to that of the mature peptide – which implies that circulating prorelaxin is physiologically relevant and that the precursor may exert cellular effects prior to being processed to mature relaxin [94].

Reproductive effects of relaxin

Because this topic has already been reviewed very extensively [95–98] we will confine ourselves here to briefly summarizing the main facts.

First, the peptide received its name owing to its outstanding property of lengthening the interpubic ligament and softening the tissues of the birth canal, thereby facilitating passage of the fetus at birth [1, 2, 5]. The ensuing mechanisms of collagen remodeling will be delineated later in this review.

In the luteal phase of nonpregnant cycles, relaxin – stimulated itself by the gonadotropin surge – appears to be one of the hormones that promote decidualization of endometrial stromal cells: prolactin – the marker gene of decidualization – is induced by relaxin in a cAMP-dependent manner [99, 100]. If then the luteal phase of the cycle proceeds into pregnancy, relaxin may favor implantation of the embryo by different mechanisms. Bani and co-workers [101] demonstrated in mice that relaxin is capable of inducing laminin production in endometrial stromal cells. Laminin, in turn, is needed for trophoblast adherence and its invasion into the endometrial stroma. Relaxin furthermore upregulates the expression of the glycoprotein glycodelin during the last week of the luteal phase and during the periimplantation phase [102]. Endometrium-derived glycodelin-A – also referred to as

placental protein 14 (PP 14) – has contraceptive and immunosuppressive properties [103].

Next, inhibition of uterine contractility is one of the classic actions of relaxin [4], which was shown to be mediated via cAMP [77, 78]. Protein kinase A-stimulated calcium-activated potassium channels may contribute to this effect [104], although involvement of potassium channels is not undisputed [105]. Surprisingly, the human myometrium proved to be relatively insensitive to relaxin, in contrast to a variety of other species. Whereas relaxin decreased both the frequency and amplitude of spontaneous or electrically driven myometrial contractions in the rat, pig and guinea-pig, it showed no or only negligible effects in the human myometrium [26, 106–109]. Thus, relaxin-induced uterine quiescence seems of little importance in humans.

Another of the early discoveries in the field of relaxin is the mammotrophic property of the peptide, first described by Hamolsky and Sparrow in the rat [3]. During the early 1980s, studies conducted mainly by Bani and co-workers in rodents confirmed that relaxin promotes growth and differentiation of the mammary parenchyma (epithelial and myoepithelial cells) and the mammary stroma (fibroblasts, adipocytes and collagen) [110–112]. Moreover, relaxin was demonstrated to be essential for development of normal mammary nipples and nipple function in pregnant rats [113–115]. Finally, because relaxin immunoreactivity was detected in normal as well as in neoplastic human mammary tissue [116], the question arose whether relaxin influences the growth of breast neoplasms. In the MCF-7 cell line (human breast adenocarcinoma cells), relaxin exerted differentiation-promoting and growth-retarding effects which proved dependent on the nitric oxide pathway [89, 117, 118].

Effects of relaxin on connective tissue

Incentivized by early descriptions of relaxin-mediated lengthening of the interpubic ligament [1, 2, 5], researchers began to focus on relaxin's differential effects on the various components of connective tissue in the 1980s. Too and co-workers revealed stimulation by relaxin of the release of plasminogen activator, collagenase and proteoglycanase in rat granulosa cells in vitro [119]. Downing and Sherwood [120] concluded from their experiments in nonpregnant, intact pregnant and ovariectomized hormone-treated pregnant rats that relaxin caused a decrease in cervical collagen concentration along with an increase in collagen solubility during pregnancy. Later on, these effects were found to be enhanced by estrogen and antagonized by progesteron [121]. In 1990, Unemori and Amento characterized, in human dermal fibroblasts, modulation by relaxin of collagenase, tissue inhibitor of metalloproteinases (TIMP) and collagen secretion [122]. Under basal conditions, they observed stimulation of procollagenase mRNA and protein expression, modest downregulation of TIMP expression, as well as downregulation of collagen secretion by relaxin. Moreover, relaxin was remarkably capable of blunting the cytokine-stimulated increase in collagen secretion in these cells. These observations were extended by studies in human lung fibroblasts [123], where Unemori and co-workers showed a relaxin-dependent inhibition of the transforming growth factor-β-mediated overexpression of interstitial collagen types I and III together with increased expression of procollagenase (MMP-1). In 2001, Palejwala and co-workers provided a more subtle analysis of relaxin-related modulation of matrix metalloproteinases (MMPs) in human lower uterine segment fibroblasts [13]: relaxin stimulated mRNA and protein levels of MMP-1 (procollagenase), MMP-2 (gelatinase) and MMP-3 (prostromelysin), whereas the peptide decreased protein levels of TIMP-1. In this model, relaxin signaling depended on tyrosine phosphorylation and on the c-Raf kinase, the latter corresponding well with the above-mentioned involvement of the ERK-1/2 pathway in the action of relaxin.

Altogether, relaxin appears to induce a collagen-degrading phenotype in a variety of experimental settings. This property is reflected by the fact that the petide effectively inhibited organ fibrosis in numerous experimental models. Unemori and co-workers reported on relaxin-mediated inhibition of fibrosis around different implants in rats and mice [124] and on inhibition of bleomycin-induced murine lung fibrosis [123]. Similar results using relaxin were obtained by Garber and co-workers in a rat model of bromoethylamine-induced severe renal interstitial fibrosis [125] as well as by Williams and co-workers who proved weakening of liver fibrosis in rats as well as decreased collagen synthesis and increased expression of TIMP-1 and TIMP-2 in activated cultured hepatic stellate cells [126].

Until now, two randomized, double-blind, placebo-controlled trials using recombinant human relaxin in the treatment of scleroderma can be considered the most advanced clinical application of this peptide [127, 128]. In the first clinical study – a phase II trial enrolling 20 patients per arm [127] – administration of recombinant human relaxin over 24 weeks was associated with reduced skin thickening, improved mobility and improved function in patients with moderate to severe diffuse scleroderma. However, the subsequent phase II/III trial [128] failed to reveal any significant improvement with regard to primary and secondary outcomes (skin thickening, pulmonary function, Raynaud's activity, functional and quality of life questionnaires).

Effects of relaxin on the brain

Before Osheroff and co-workers performed, in 1990, their pioneering work on relaxin binding sites in the

brain, some evidence had already accumulated that relaxin may act on the brain and particularly on the pituitary gland. In 1984, Summerlee and co-workers were the first to detect such actions [129]. In an attempt to determine whether relaxin exerted a central action on the release of oxytocin, they studied the effect of intravenous injections of porcine relaxin on milk ejection in the anaesthetized lactating rat. They observed that reflex milk ejection was suppressed by relaxin in a dose-dependent manner, and that injection of relaxin into the cerebral ventricles profoundly disturbed the pattern of reflex milk ejection without affecting the response of the mammary gland to oxytocin. In 1986, these researchers proved inhibition by relaxin of the release of oxytocin from terminals in the neurohypophysis [130]. This relaxin-dependent regulation of pituitary oxytocin release was confirmed by different other reports, but with conflicting in part results: Dayanithi and co-workers [10] reported on a dual effect of relaxin on oxytocin release from isolated neural lobes of the pituitary and isolated neurosecretory nerve endings of the rat neurohypophysis. Under basal conditions, oxytocin release was inhibited by relaxin, but when the nerve endings were depolarized, oxytocin secretion was potentiated. Others found relaxin-mediated increases in the firing rate of supraoptic oxytocin neurones and, consequently, a plasma rise of oxytocin in the rat [131]. Different models leading to distinct states of pituitary activation may account for these controversial findings.

It soon became evident that relaxin also affected pituitary release of vasopressin [10]. This vasopressin release was partly mediated by the forebrain angiotensin II system and resulted in a vasopressor effect via vasopressin V1 receptors [132–135]. Central angiotensin II was also found to contribute to relaxin-evoked oxytocin regulation [133]. In addition, central administration of relaxin proved to be dipsogenic [136–138]. This effect depended on the photoperiod and led to pronounced nighttime drinking behavior in pregnant rats [136]. The physiological consequence of relaxin-induced vasopressin secretion is the well-known reduction in plasma osmolality during pregnancy [139, 140].

In the meantime, evidence suggests that the anatomic site of central relaxin action is the so-called lamina terminalis in the anterior wall of the third ventricle [141–143]. Consisting of two organs that lack a blood-brain barrier – the subfornical organ and the organum vasculosum – the lamina terminalis distributes neural output to nuclei adjacent to the pituitary gland and influences vasopressin and oxytocin release, central cardiovascular regulation, as well as drinking. This regulatory circuit involves the forebrain angiotensin II system acting via angiotensin II type-1 receptors [137].

In addition to affecting secretion of the posterior pituitary, relaxin may also affect hormones of the anterior pituitary gland. Relaxin promoted prolactin secretion in rats and monkeys [144, 145], and there are single reports on relaxin-stimulated secretion of growth hormone in monkeys [145] and on inhibited release of luteinizing hormone in rats [146].

Circulatory and renal effects of relaxin

The first descriptions of relaxin's vasodilatory potential date back to the early 1980s when Bani and Bigazzi observed relaxin-induced vasodilation in the mouse mammary gland [7] and St. Louis and Massicotte showed that chronic infusion of relaxin caused a substantial decline of blood pressure in spontaneously hypertensive rats [8]. Thereafter, several reports described relaxin-related vasodilation in different vascular beds, including the rat uterine endometrium [147], rat mesocoecum [148], guinea pig and rat coronary arteries [87] and rat liver sinusoids [149]. In the latter two studies, relaxin was shown to act via the nitric oxide pathway, which was also confirmed in a study with cultured bovine aortic smooth muscle cells [91].

With regard to the renal effects of relaxin, recent work in rats supports the view that the peptide is the elusive renal vasodilator of pregnancy. Conrad's group was able to demonstrate renal vasodilation, glomerular hyperfiltration and mitigation of angiotensin II-induced reduction in effective renal plasma flow prompted by chronic infusion of relaxin into conscious rats [140]. These effects – as well as the recently reported reduction by relaxin of myogenic reactivity in small renal arteries [150] – were sensitive to endothelin type-B receptor antagonism and to inhibition of nitric oxide, suggesting mediation via endothelial endothelin type-B receptors that activate release of nitric oxide [151]. Upon elimination of relaxin or its biological activity from circulation, by ovariectomy or administration of neutralizing antibodies, the gestational elevation in renal perfusion and glomerular filtration in pregnant rats was completely abrogated [152].

Our own [unpublished] data corroborate the hypothesis that stimulated expression of endothelin type-B receptors plays a central role in mediating chronic vasodilatory effects of relaxin: in our experiments, relaxin selectively stimulated the expression of endothelial and epithelial, but not of vascular smooth muscle endothelin type-B receptors. This is of outstanding importance because the endothelial receptors mediate release of vasodilatory nitric oxide and prostacyclin, whereas the endothelin type-B receptors located on vascular smooth muscle cells would contribute to endothelin-1-mediated vasoconstriction [153].

Finally, accumulating evidence indicates that relaxin may also promote angiogenesis. Thus, Unemori and co-workers revealed relaxin-mediated stimulation of vascular endothelial growth factor and basic fibroblast growth factor in human endometrial cells [154] and, even more impor-

tant, in macrophages from wound sites in rodent models of angiogenesis and wound healing [155]. Palejwala and co-workers also reported on stimulation by relaxin of vascular endothelial growth factor, at the protein level, in human endometrial cells [156]. The fact that in the above-mentioned clinical scleroderma trial, the most frequent adverse effect of chronic relaxin application was hypermenorrhagia [127] might indicate the in vivo relevance of relaxin-related angiogenesis.

Myocardial effects of relaxin

We have already mentioned that myocardial relaxin receptors were detected in rat atria [66–69] and that coronary arteries were shown to represent target vessels for relaxin-mediated nitric oxide-dependent vasodilation [87].

Incentivized by a report on the relaxin-dependent increase in heart rate in anesthetized rats by Parry and co-workers [157], Kakouris and co-workers identified, in 1992, relaxin's direct positive chronotropic and inotropic effects on atrial myocardium [158]. These scientists demonstrated that human relaxin produced concentration-dependent positive chronotropic effects in spontaneously beating right atria of the rat [EC_{50} (effective concentration 50%) = 0.09 ± 0.03 nmol/l] and concentration-dependent positive inotropic effects in electrically driven left atria (EC_{50} = 0.31 ± 0.02 nmol/l). The potency of relaxin in this model was greater than that of endothelin-1, angiotensin II, and isoprenaline. This positive chronotropy was confirmed in vivo in rats [159] and in the isolated rat heart model [160]. Until now, inotropic effects on ventricular myocardium could not be evidenced. Our own unpublished observations in the rat clearly indicate positive atrial inotropy of relaxin, but we have never noticed inotropic effects of the peptide in ventricular preparations. At present, there are no data indicating whether relaxin receptors reside on human atrial or ventricular myocardium.

With regard to the mechanism of the relaxin-mediated positive chronotropic and inotropic effects, Han and co-workers demonstrated, in rabbit single sinoatrial pacemaker cells, a cAMP-dependent increase in the currrent gated by L-type calcium channels leading to dose-dependent chronotropic effects of relaxin in the high picomolar to low nanomolar concentration range [161]. In the rat, the following mechanism appears to underlie the inotropic atrial action of relaxin: relaxin was found to inhibit the transient potassium outward current, which resulted in prolongation of the action potential and increased calcium influx in single quiescent atrial cells [84, 162].

Relaxin also affects secretory functions of the myocardium, as evidenced by its ability to stimulate the release of atrial natriuretic peptide in rat isolated hearts [83].

Based on its stimulatory effect on nitric oxide release and on its angiogenic properties, relaxin may furthermore offer great potential to prevent ischemic myocardial injury: both in isolated guinea-pig and rat hearts and in vivo, relaxin counteracted myocardial damage induced by ischemia and reperfusion as indicated by its ability to reduce (i) the extension of damaged myocardial areas; (ii) the occurrence of ventricular arrhythmias and death; (iii) the extent of myocardial neutrophil invasion; (iv) lipid peroxidation and mast cell granule release and (v) cellular calcium overload [163–165]. These data are substantiated by an early report on relaxin-related stimulation of neoangiogenesis in a rat in vivo model of myocardial infarction [166].

Coronary artery disease is the leading cause of congestive heart failure, which, in turn, is one of the high-ranking factors of morbidity and mortality in the industrialized world. Heart failure is characterized by complex neurohumoral activation associated with the upregulation of vasoconstricting and salt-retaining mediators – such as catecholamines, angiotensin II, vasopressin and endothelin-1 – and the compensatory rise of counterregulatory hormones, including atrial and brain natriuretic peptides as well as adrenomedullin [167]. We identified relaxin as a novel compensatory mediator in human congestive heart failure [33]: plasma concentrations of relaxin and myocardial expression of the relaxin H1 and H2 genes in atria and ventricles correlated with the severity of heart failure. In patients with decompensated heart failure, circulating relaxin declined in response to hemodynamic improvement by vasodilator therapy. We established that the failing human heart is a relevant source of circulating relaxin peptides, and that myocytes as well as interstitial cells produce relaxin. Functional experiments using isolated rat hearts demonstrated upregulation of ventricular relaxin expression by elevation of ventricular filling pressure, a hallmark of heart failure.

Our previous work showed that pulmonary circulation represents the major source of elevated circulating endothelin-1 in severe heart failure [168], and that this most potent vasoconstrictor in the pathophysiology of heart failure is presumably upregulated in pulmonary endothelium by the increase in pulmonary microvascular pressure [169]. Relaxin was capable of suppressing stimulated endothelin-1 release by pulmonary endothelial cells [33]. It blunted the angiotensin II-mediated rise of endothelin-1 by upregulating endothelin-1 type-B receptors, which function as endothelin-1 clearance receptors. Relaxin furthermore inhibited the pressure-induced endothelin-1 surge in pulmonary endothelial cells. Correspondingly, in patients demonstrating severe heart failure, we found an inverse correlation between circulating endothelin-1 and relaxin, which indicated the potential relevance of our in vitro results.

Effects of relaxin on hemostasis

Single studies in the rat indicated that relaxin inhibits platelet aggregation via stimulation of nitric oxide [170] and that the peptide may decrease the number of circulating platelets by suppressing their release from megakaryocytes [171]. If this could be substantiated in further studies, it would implicate an antithrombotic profile of relaxin.

Concerning the fibrinolytic system, promotion by relaxin of the release of tissue plasminogen activator was described in the reproductive tract [119, 172, 173]. However, whether this local effect of relaxin affects systemic hemostatic balance is unknown at present.

Effects of relaxin on the respiratory system

An antiasthmatic profile of relaxin was suggested by Bani and co-workers based on a study which revealed significant suppression of the asthma-like reaction to inhaled antigen in ovalbumin-sensitized guinea pigs [174]. The results of this study correspond well with the already mentioned mast cell-stabilizing and antiplatelet features of relaxin [86, 170].

Immunomodulatory effects of relaxin

In accordance with its proposed antiasthmatic effects, relaxin was found to favor the in vitro development of human antigen-specific T cells into type-1 helper T cells and to enhance interferon-γ production by human T cell clones [175]. In parallel, it inhibited the development of allergen-reactive type-2 helper T cells, which play a triggering role in the activation and recruitment of immunoglobulin E antibody-producing B cells, mast cells and eosinophils, that is in the cellular triad involved in the allergic inflammation [176].

Conclusions and future perspectives

The numerous facts we have itemized here regarding the biological effects of relaxin are best summarized by data obtained from the first placebo-controlled clinical trials using recombinant human relaxin in the treatment of scleroderma [127, 128, 177]. Continuous subcutaneous infusion of relaxin over 24 weeks caused sustained physiological changes which closely mimicked those seen in human pregnancy, that is increased blood volume, lower plasma osmolality, increased cardiac output owing to decreased cardiac afterload, decreased systolic and diastolic blood pressure, increased renal and endometrial blood flow, and increased glomerular filtration rate. The most frequent adverse effects were mild anemia and hypermenorrhagia. These clinical observations in humans confirmed that relaxin may play an outstanding role in regulating body fluid and circulation homeostasis. In concert with its antifibrotic, pro-angiogenic, antiischemic and immunomodulatory profile, these properties render relaxin an important regulator of human reproductive and nonreproductive physiology.

It is our opinion that future research will have to deepen our understanding of the following aspects of relaxin. From a physiologist's view, sites and regulation of gene expression of the different human relaxins – H1, H2 and H3 – will have to be better defined, as well as their distinct physiological roles in humans and their signaling. It appears furthermore crucial to compare the different contributions to body homeostasis made by endogenous relaxin in menstruating women, postmenopausal women and men. In spite of the disappointing results regarding estrogen replacement therapy to blunt the increase in postmenopausal women's cardiovascular risk [178, 179], Bani and co-workers have speculated that relaxin could represent the elusive 'cardiovascular protection shield' in menstruating women [180]. This tempting hypothesis, which still lacks clinical evidence, is based mainly on the fact that menstruating women show higher circulating relaxin levels than men [57] and on experimental data demonstrating mitigation of ischemia and reperfusion injury [163–165], platelet inhibition [170–171] and pro-angiogenic properties [154–156]. Moreover, our own work regarding human heart failure [33] has shown a protective, that is compensatory, role in the pathophysiology of the disease regardless of the gender of patients. From a pharmacologist's view, the recent identification of two human relaxin receptors should greatly expedite the development of relaxin agonists. As clinically physicians, we expect relaxin to be more widely investigated as a potential therapeutic tool in the near future. Until now, the above-mentioned scleroderma trials [127, 128] represent the only clinical investigations using relaxin. Apart from potential applications in the field of human reproduction, for example the use for fertilization, and as an antifibrotic agent, many intriguing results can be awaited from cardiovascular medicine. Relaxin's antiischemic and angiogenic properties strongly favor its use in coronary artery disease, be it in the case of stable angina or in the postinfarction period when the antifibrotic profile of the peptide may also come into play. Finally, it is also tempting to speculate that relaxin could prove useful in the chronic treatment of congestive heart failure, as elaborated earlier. Certainly, much work, especially in animal models, has to be done until these ideas can become clinical reality

Relaxin, the pregnancy hormone, has reentered the scientific scene as a 'global player', and much is to be expected from its new facets.

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