

International Union of Pharmacology LVII: Recommendations for the Nomenclature of Receptors for Relaxin Family Peptides

ROSS A. BATHGATE, RICHARD IVELL, BARBARA M. SANBORN, O. DAVID SHERWOOD, AND ROGER J. SUMMERS

Howard Florey Institute, University of Melbourne, Melbourne, Victoria, Australia (R.A.B.); School of Molecular and Biomedical Science, The University of Adelaide, South Australia, Australia (R.I.); Department of Biological Sciences, Colorado State University, Fort Collins, Colorado (B.M.S.); Department of Molecular and Integrative Physiology and College of Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois (O.D.S.); Department of Pharmacology, Monash University, Clayton, Victoria, Australia (R.J.S.)

Abstract	8
I. Brief historical background of relaxin family peptides and their receptors	8
II. Receptor distribution and function	11
A. Reproductive tissues	11
B. Brain	13
C. Cardiovascular and renal systems	14
D. Other sites of action of relaxin family peptides	15
E. Roles of relaxin family peptides determined from studies in receptor knockout mice	16
III. Structure of relaxin family peptides	17
A. Structural features of relaxin	17
B. Structural features of other relaxin family peptides	17
IV. Structure-activity relationships	17
A. Relaxin	17
B. INSL3	18
V. Binding of relaxin and relaxin family peptides	18
A. Relaxin binding	18
B. Binding of other relaxin family peptides	19
VI. Relaxin family peptide receptors	19
A. RXFP1 and RXFP2	19
B. RXFP3 and RXFP4	21
VII. Functional domains of receptors for relaxin family peptides	22
A. General features of leucine-rich repeat-containing receptors	22
B. Functional domains of RXFP1 and RXFP2 (LGR7 and LGR8)	23
C. Functional domains of RXFP3 and RXFP4 (GPCR135 and GPCR142)	24
VIII. Signaling pathways activated by relaxin family peptides	24
A. Signaling in response to relaxin	24
B. Signaling in response to other relaxin family peptides	27
IX. Regulation of relaxin family peptide receptors	27
X. Nomenclature issues for relaxin family peptides and their receptors	27
Acknowledgments	28
References	28

Address correspondence to: Professor R. J. Summers, P.O. Box 13E, Department of Pharmacology, Monash University, Victoria 3800, Australia. E-mail: roger.summers@med.monash.edu.au

This work was supported in part by a National Health and Medical Research Council (NH&MRC) block grant to the Howard Florey Institute (Reg Key 983001), NH&MRC project grants to R.A.D.B. and R.J.S. (300012) and R.I. (349502), Australian Research Council Linkage Grant LP0211545 to R.J.S., and Grant Iv7/11 from the Deutsche Forschungsgemeinschaft to R.I.

Article, publication date, and citation information can be found at <http://pharmrev.aspetjournals.org>.

doi:10.1124/pr.58.1.9.

Abstract—Although the hormone relaxin was discovered 80 years ago, only in the past 5 years have the receptors for relaxin and three other receptors that respond to related peptides been identified with all four receptors being G-protein-coupled receptors. In this review it is suggested that the receptors for relaxin (LGR7) and those for the related peptides insulin-like peptide 3 (LGR8), relaxin-3 (GPCR135), and insulin-like peptide 5 (LGR142) be named the relaxin family peptide receptors 1 through 4 (RXFP1–4). RXFP1 and RXFP2 are leucine-rich repeat-containing G-protein-coupled receptors with complex binding characteristics involving both the large ectodomain and the transmembrane loops. RXFP1 activates adenylate cyclase, protein kinase A, protein kinase C, phosphatidylinositol 3-kinase, and extracellular signaling regulated kinase (Erk1/2) and also interacts with nitric oxide signaling. RXFP2 activates adenylate cyclase in recombinant systems, but physiological responses are sensitive to pertussis toxin. RXFP3 and

RXFP4 resemble more conventional peptide liganded receptors and both inhibit adenylate cyclase, and in addition RXFP3 activates Erk1/2 signaling. Physiological studies and examination of the phenotypes of transgenic mice have established that relaxin has roles as a reproductive hormone involved in uterine relaxation (some species), reproductive tissue growth, and collagen remodeling but also in the cardiovascular and renal systems and in the brain. The connective tissue remodeling properties of relaxin acting at RXFP1 receptors have potential for the development of agents effective for the treatment of cardiac and renal fibrosis, asthma, and scleroderma and for orthodontic remodelling. Agents acting at RXFP2 receptors may be useful for the treatment of cryptorchidism and infertility, whereas antagonists may be used as contraceptives. The brain distribution of RXFP3 receptors suggests that actions at these receptors have the potential for the development of antianxiety and antiobesity drugs.

I. Brief Historical Background of Relaxin Family Peptides and Their Receptors

The circulating hormone relaxin was discovered when Hisaw (1926) observed that the injection of serum from pregnant guinea pigs or rabbits into virgin guinea pigs shortly after estrus induced a relaxation of the pubic ligament. Between the mid 1970s and 1990s the primary sequence of relaxin was determined for the pig (Schwabe et al., 1976; Schwabe and McDonald, 1977a), rat (John et al., 1981), mouse (Evans et al., 1993), human (Hudson et al., 1983, 1984), and >20 other species (Bryant-Greenwood and Schwabe, 1994; Sherwood, 1994; Bathgate et al., 2002b). In the human and four great ape species, there are two forms of relaxin that are encoded by separate genes *RLN1*¹ and *RLN2* (Hudson et al.,

1983, 1984) and are thought to be the consequence of a gene duplication during primate evolution. In the human, these two forms of relaxin are designated H1 relaxin and H2 relaxin. Only H2 relaxin is known to be secreted into the blood. In this review, circulating relaxin is simply designated relaxin. Relaxin is produced and secreted by the ovary during pregnancy in pigs, rats, mice, and humans (Sherwood, 1994). The structure of relaxin is superficially similar to that of insulin, and they are thought to have evolved from a common ancestral gene. Whereas the overall sequence identity of relaxin and insulin is only about 25%, both hormones are first synthesized as a prohormone that is composed of a signal sequence and a B-C-A domain configuration. Within the B and A domains, there are highly conserved cysteine residues that link the A and B domains by two interdomain disulfide bonds and form an A chain intradomain disulfide bond (Fig. 1). There has been limited evolutionary conservation of the primary structure of relaxin among species. For example, the extent of amino acid sequence identity between pig, rat, and human relaxin ranges from 42 to 49%. Nevertheless, several of the amino acids in relaxin are invariant or highly conserved among species. In addition to the six cysteine residues involved in disulfide bond formation, the amino acid motif Arg-X-X-Arg-X-X-Ile/Val-X located in the middle of the B chain is retained and is required for relaxin-receptor interaction (Büllesbach and Schwabe, 2000) (Fig. 2).

Recently, another human insulin/relaxin-like molecule and its nearly identical orthologs in the mouse, rat,

cytic cell line; LRR, leucine-rich repeat; LDLa, low-density lipoprotein class A; TM, transmembrane; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PDE, phosphodiesterase; HEK293, human embryonic kidney (cells); LY294002, 2-(4-morpholinyl)-8-phenyl-1(4H)-benzopyran-4-one hydrochloride; PI3-K, phosphatidylinositol 3 kinase; Erk1/2, extracellular signalling regulated kinase; GR, glucocorticoid receptor; RU486, 17 β -hydroxy-11 β -[4-dimethylamino phenyl]-17 α -[1-propynyl]estra-4,9-dien-3-one.

¹ Abbreviations: *RLN1*, gene encoding H1 relaxin; *RLN2*, gene encoding H2 relaxin; H1 relaxin, human gene 1 relaxin; H2 relaxin, human gene 2 relaxin; H3 relaxin, human gene 3 relaxin; (species) relaxin-3, peptide corresponding to human gene 3 (H3) relaxin in other species; Ley-I-L, Leydig insulin-like peptide; *INSL3*, gene encoding insulin-like peptide 3; *INSL4*, gene encoding insulin-like peptide 4; *INSL5*, gene encoding insulin-like peptide 5; *INSL6*, gene encoding insulin-like peptide 6; RLF, relaxin-like factor; *INSL3*, insulin-like peptide 3; *INSL4*, insulin-like peptide 4; *INSL5*, insulin-like peptide 5; *INSL6*, insulin-like peptide 6; LGR, leucine-rich repeat-containing guanine nucleotide binding (G-protein)-coupled receptors; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; Great, G-protein coupled receptor affecting testis descent; LGR7, leucine-rich repeat-containing guanine nucleotide binding (G-protein)-coupled receptor 7; LGR8, leucine-rich repeat-containing guanine nucleotide binding (G-protein)-coupled receptor 8; GPCR, G-protein coupled receptor; GPCR135, G-protein coupled receptor 135 (orphan receptor nomenclature); GPCR142, G-protein coupled receptor 142 (orphan receptor nomenclature); RXFP1, relaxin family peptide receptor 1; RXFP2, relaxin family peptide receptor 2; RXFP3, relaxin family peptide receptor 3; RXFP4, relaxin family peptide receptor 4; RT-PCR, reverse transcription-polymerase chain reaction; PKA, protein kinase A; SFO, subfornical organ; OVLT, organum vasculosum of the lamina terminalis; ET₁₋₃₂, first 32 amino acids of endothelin peptide; NO, nitric oxide; NOS, nitric-oxide synthase; THP-1, human mono-

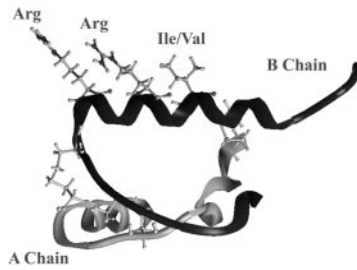


FIG. 1. Representation of the structure of H2 relaxin based on the crystal structure (Eigenbrot et al., 1991). The conserved residues incorporating the relaxin receptor binding motif (Arg-X-X-X-Arg-X-X-Ile/Val) are indicated as are the separate A and B chains. Reproduced from Bathgate et al. (2006), with permission; copyright © 2006 Elsevier, Inc.

and pig were discovered (Bathgate et al., 2002a; Burazin et al., 2002; Kizawa et al., 2003). Because this peptide has the amino acid motif Arg-X-X-X-Arg-X-X-Ile required for relaxin bioactivity in the middle of the B chain and it activates the relaxin receptor (Sudo et al., 2003), it was designated relaxin-3 or in the case of the human relaxin-3 peptide, H3 relaxin (Fig. 2). Relaxin-3 is expressed in greatest levels in the ventromedial dorsal tegmental nucleus [also called nucleus incertus (Goto et al., 2001)] in the rat and mouse brain, where it has been postulated to act locally as a neuropeptide (Burazin et al., 2002; Liu et al., 2003b).

Another insulin-like protein was discovered by differential screening of a boar testis cDNA library (Adham et al., 1993). Because the mRNA for this molecule was found in Leydig cells of the testis, it was originally designated Leydig insulin-like peptide (Ley-I-L). The gene for Ley-I-L was designated *INSL3* (Burkhardt et al., 1994). Ley-I-L contains a portion (Arg-X-X-X-Arg) of the putative relaxin receptor-binding region Arg-X-X-X-Arg-X-X-Ile, which is offset by four amino acid residues toward the C terminus relative to its position in relaxin. Subsequent reports (Büllesbach and Schwabe, 1995) revealed that synthetic human Ley-I-L augmented relaxin activity in a mouse interpubic ligament bioassay, leading to the suggestion that the term relaxin-like-factor (RLF) was the more appropriate term for this hormone. Although both *INSL3* and RLF are used in the literature, for the purposes of this review the hormone will be designated insulin-like peptide 3 (*INSL3*). *INSL3* expression is not confined to the male; it is also expressed in the ovary within both the follicular theca interna cells and the corpora lutea (Bathgate et al., 1996; Roche et al., 1996; Kawamura et al., 2004), as well as in the placenta (Hombach-Klonisch et al., 2001), breast (Hombach-Klonisch et al., 2000), and thyroid gland (Hombach-Klonisch et al., 2003).

Shortly after the discovery of *INSL3*, a cDNA clone for another member of the relaxin peptide family was identified independently by two groups during screening of cDNA libraries of first-trimester human placenta (Chassin et al., 1995; Koman et al., 1996). The protein product of this gene was named early placenta insulin-like pep-

ptide (Chassin et al., 1995) and placentin (Koman et al., 1996), respectively. The gene has been named insulin-like 4 (*INSL4*) based on the nomenclature used for the *INSL3* gene. The *INSL4* gene emerged before the divergence of New and Old World monkeys (Bieche et al., 2003), predating the emergence of the *RLN1* gene. The *INSL4* gene is highly expressed in the human placenta but its function is unknown.

More recently, two novel genes were independently discovered by three groups via searching of the Expressed Sequence Tags database using the conserved cysteine pattern of this peptide family. Hence, insulin-like peptide-5 (*INSL5*) (Conklin et al., 1999; Hsu, 1999) and insulin-like peptide-6 (*INSL6*) (Hsu, 1999; Lok et al., 2000) were added to the relaxin peptide family (Fig. 2). Both genes are present in rodent genomes and evolved from the ancestral *RLN3* gene (Wilkinson et al., 2005). Their functions are currently unknown, although *INSL5* has been identified as the cognate ligand for GPCR142 (RXFP4) (see below). The occurrence of the relaxin family peptides in human, rat and mouse is summarized in Table 1.

The receptors for relaxin, relaxin-3, *INSL3*, and *INSL5* were identified recently (Table 2). Relaxin and *INSL3* receptors are a subgroup (type C) of the family of leucine-rich repeat-containing guanine nucleotide binding (G-protein)-coupled receptors designated LGRs, that include the receptors for FSH, LH, and TSH. By using inferences from similar phenotypic expression following mutation and inactivation of *INSL3* (Nef and Parada, 1999; Zimmermann et al., 1999) and a transgenic insertional mutation in mouse chromosome 5, an orphan LGR designated either G-protein-coupled receptor affecting testis descent (Great) (Overbeek et al., 2001) or LGR8 (Hsu et al., 2002; Kumagai et al., 2002) was identified as the *INSL3* receptor. The discovery that the orphan receptor LGR7 is the relaxin receptor was largely attributable to the pursuit of a hunch raised by the combination of the similarity of the structure of LGR7 to LGR8 and the similarity of the structure of relaxin to *INSL3* (Hsu et al., 2002). LGR7 and LGR8, which are 757 (Hsu et al., 2002) and 737 (Overbeek et al., 2001) amino acids in length, respectively, share about 60% amino acid sequence identity and contain 10 leucine-rich repeats in their large N-terminal extracellular domain. Two orphan G-protein-coupled receptors designated GPCR135 and GPCR142 were recently proposed as putative receptors for relaxin-3 (Liu et al., 2003a,b). Both receptors belong to the type I family of GPCRs. Unlike LGR7 and LGR8, GPCR135 and GPCR142 have short N-terminal extracellular domains, and they contain only 469 and 374 amino acid residues, respectively. Chinese hamster ovary-K1 cells transfected with orphan receptor GPCR135, which is also known as somatostatin and angiotensin-like peptide receptor (Matsumoto et al., 2000) were used to identify relaxin-3 as a ligand in porcine brain extracts (Liu et al., 2003a). GPCR142, the

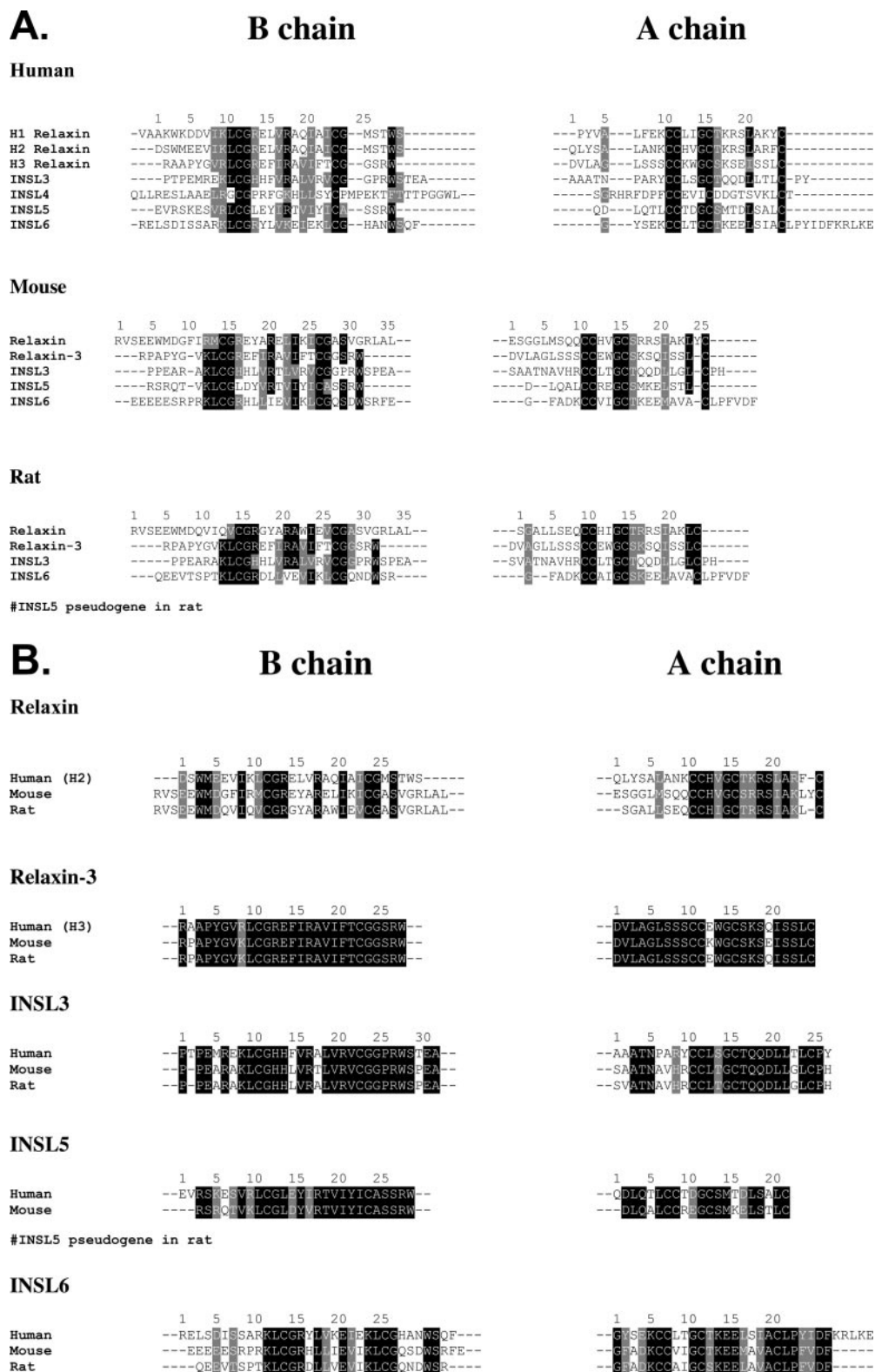


FIG. 2. Alignment of A and B chain sequences of all human relaxin-like peptides. Conserved amino acid residues are boxed in black, and conservative amino acid substitutions are boxed and shaded. Gaps have been introduced to aid the alignment where necessary. The chains are numbered according to the H2 relaxin sequence. A, alignment showing the relationship between relaxin family peptides in human, rat, and mouse. B, alignment showing the relationship between each peptide across species.

remaining orphan GPCR receptor that shares the closest amino acid sequence identity to GPC135 (43%), was also shown to be a receptor for relaxin-3 (Liu et al., 2003a).

More recent experiments have demonstrated that GPCR142 is probably the receptor for INSL5 (Liu et al., 2005). Thus, most of the receptors and cognate ligands

TABLE 1
The occurrence of relaxin and related peptides in human, rat, and mouse

Gene identification (ID) numbers are from Entrez Gene (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>).

Gene	Protein	Human Gene ID	Mouse Gene ID	Rat Gene ID
<i>RLN1</i>	H1 relaxin	6013	Not present	Not present
<i>RLN2</i>	H2 relaxin	6019	19773 ^a	25616 ^a
<i>RLN3</i>	H3 relaxin	117579	212108	266997
<i>INSL3</i>	INSL3	3640	16336	114215
<i>INSL4</i>	INSL4	3641	Not present	Not present
<i>INSL5</i>	INSL5	10022	23919	Pseudogene ^b
<i>INSL6</i>	INSL6	11172	27356	50546

^a Genes for rat and mouse relaxin are named *RLN1* in the databases but are the rodent equivalents of *RLN2* in the human.

^b *INSL5* is a pseudogene in the rat (Liu et al., 2005).

TABLE 2
Expression of receptors for relaxin and relaxin-related peptides in vertebrates

Gene identification (ID) numbers are from Entrez Gene (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>).

Family	IUPHAR Receptor Code	Ligand	Official IUPHAR Nomenclature	Human Gene Symbol	Human Gene ID	Mouse Gene ID	Rat Gene ID
Relaxin family peptide (RXFP)	2.1:RXFP:1:RXFP1:	H2 relaxin	RXFP1	LGR7	59350	381489	295144
Relaxin family peptide (RXFP)	2.1:RXFP:2:RXFP2:	INSL3	RXFP2	LGR8	122042	140498	363866
Relaxin family peptide (RXFP)	2.1:RXFP:3:RXFP3:	H3 relaxin	RXFP3	RLN3R1	51289	239336	294807
Relaxin family peptide (RXFP)	2.1:RXFP:4:RXFP4:	INSL5	RXFP4	RLN3R2	339403	242093	Pseudogene ^a

IUPHAR, International Union of Pharmacology.

^a *RLN3R2* is a pseudogene in the rat (Liu et al., 2005).

TABLE 3
Agonists and antagonists at receptors for relaxin and relaxin-related peptides

LDLa is the unique N-terminal region found in type C-glycoprotein receptors; the RXFP1-truncate is a naturally occurring splice variant of the receptor that includes the LDLa region and acts as a functional antagonist at the RXFP1 receptor (Scott et al., 2005c).

Receptor	Previous Name	Agonist	Antagonist	Radioligand
RXFP1	LGR7	H2 relaxin > H1 relaxin > H3 relaxin >> INSL3	RXFP1-truncate	³³ P-H2 relaxin, ¹²⁵ I-H2 relaxin
RXFP2	LGR8	INSL3 > H2 relaxin = H1 relaxin >> H3 relaxin	INSL3 B chain analog ^a ; (des 1–8) A chain INSL3 analog ^b	³³ P-H2 relaxin, ¹²⁵ I-H2 relaxin, ¹²⁵ I-INSL3
RXFP3	GPCR135, SALPR	H3 relaxin > H3 relaxin B chain	INSL5	¹²⁵ I-H3 relaxin, ¹²⁵ I-H3-B/INSL5 A chimera
RXFP4	GPCR142, GPR100	INSL5 = H3 relaxin > H3 relaxin B chain		¹²⁵ I-H3 relaxin, ¹²⁵ I-H3-B/INSL5 A chimera

^a Del Borgo et al. (2004); ^b Büllesbach and Schwabe (2005a).

for the relaxin family peptides have been identified. No specific receptors have yet been identified for *INSL4* and *INSL6*. It is recommended that *LGR7*, *LGR8*, *GPCR135*, and *GPCR142* now be known as relaxin family peptide receptors 1 through 4 (RXFP1–4), respectively (Table 3).

II. Receptor Distribution and Function

Receptors for relaxin family peptides have been localized by their mRNA expression patterns using RT-PCR, Northern blotting, or in situ hybridization or as protein by immunohistochemistry or receptor autoradiography (Table 4). Several studies have been conducted to identify the tissues that contain receptors for relaxin (RXFP1, *LGR7*), *INSL3* (RXFP2, *LGR8*), relaxin-3 (RXFP3, *GPCR135*), and *INSL5* (RXFP4, *GPCR142*). As Table 4 indicates, the receptor for relaxin has received the most experimental attention. *LGR7* (RXFP1) has been identified in female and male reproductive tissues, brain, and numerous other nonreproductive tissues such as kidney, heart, and lung. Neither the cell types within

tissues that express *LGR7* (RXFP1) nor the factors that regulate the relaxin receptor are well characterized.

A. Reproductive Tissues

A few investigators have used immunohistochemical analysis to locate RXFP1 (*LGR7*) protein in female reproductive tissues. In the rat uterus, RXFP1 was reported in both myometrial cells and epithelial cells (Hsu et al., 2002). RXFP1 has been reported in human endometrial stroma, as well as in endometrial epithelial cells (Luna et al., 2004), albeit in the latter cell type to differing extents. Another study demonstrated specific relaxin binding localized to the epithelium of the endometrial glands and uterine lumen and that both binding and RXFP1 mRNA levels increased markedly in the early secretory phase of the menstrual cycle compared with the proliferative phases (Bond et al., 2004). Weaker binding was observed in the endometrial stromal tissue and little in the myometrium (Bond et al., 2004). In two other primates, the marmoset and macaque monkeys,

TABLE 4
Distribution of receptors for relaxin and relaxin-related peptides

Tissue/Species	RXFP1			RXFP2			RXFP3 Human	RXFP4 Human
	Rat	Mouse	Human	Rat	Mouse	Human		
Ovary	mRNA ^a	Protein ^w	mRNA ^b					mRNA ^{ab}
Oviduct	mRNA ^a	mRNA ^c						
Uterus	mRNA ^a ; protein ^{b,t,u}	mRNA ^{c,n,ad}	mRNA ^{b,h,j} ; protein ^{h,y}			mRNA ^{b,j}		mRNA ^{ab}
Cervix, vagina	Protein ^{b,t,v}	mRNA ^{c,ad}	Protein ^y					
Placenta		mRNA ^{a,d}	mRNA ^b					mRNA ^{s,ab}
Nipple	Protein ^v	mRNA ^{c,ad}	Protein ^y					
Breast	Protein ^v	Protein ^d	Protein ^y					
Testis	mRNA ^{a,f}	mRNA ^{c,o,ad}	mRNA ^b	mRNA ^{l,q}	mRNA ^k	mRNA ^b	mRNA ^r	mRNA ^{s,ab}
Prostate		mRNA ^o	mRNA ^b					mRNA ^{s,ab}
Gubernaculum				mRNA ^l	mRNA ^k			
Brain	mRNA ^{a,e,f,i} ; protein ^u	mRNA ^{c,ad}	mRNA ^b	mRNA ^{ae}	mRNA ^k	mRNA ^b	mRNA ^{r,ac}	mRNA ^{s,ab}
Pituitary		mRNA ^c					mRNA ^{ac}	
Kidney	mRNA ^a		mRNA ^b	mRNA ^{af}		mRNA ^b		mRNA ^s
Heart	mRNA ^{a,e,i} ; protein ^{u,x}	mRNA ^{c,ad}	mRNA ^b					mRNA ^{ab}
Lung		mRNA ^{g,ad}	mRNA ^b					
Liver			mRNA ^b					
Intestine	mRNA ^a	mRNA ^{ad}						mRNA ^{ab}
Colon	mRNA ^a							mRNA ^{s,ab}
Adrenal	mRNA ^a		mRNA ^b				mRNA ^{r,ac}	mRNA ^{ab}
Thyroid			mRNA ^b			mRNA ^{b,p}		mRNA ^{s,ab}
Thymus							mRNA ^r	mRNA ^{s,ab}
Salivary glands			mRNA ^b					mRNA ^{s,ab}
Muscle			mRNA ^b			mRNA ^b		mRNA ^{ab}
Peripheral blood cells			mRNA ^b			mRNA ^b		mRNA ^{ab}
Monocyte cell line THP-1			mRNA ^m ; protein ^z			mRNA ^m		mRNA ^{ab}
Bone marrow			mRNA ^b			mRNA ^b		
Skin		mRNA ^{a,d}	mRNA ^b					mRNA ^{ab}

^a mRNA by Northern blot analysis (Hsu et al., 2000); ^b mRNA by RT-PCR, protein by immunohistochemical analysis (Hsu et al., 2002); ^c mRNA by lacZ reporter expression (Krajnc-Franken et al., 2004); ^d protein by immunohistochemical analysis (Ivell et al., 2003); ^e mRNA by RT-PCR (Kompa et al., 2002); ^f mRNA by RT-PCR (Kubota et al., 2002); ^g mRNA by RT-PCR (Samuel et al., 2003b); ^h mRNA by RT-PCR, protein by immunohistochemical analysis (Luna et al., 2004); ⁱ mRNA by RT-PCR (Samuel et al., 2004a); ^j mRNA by RT-PCR (Mazella et al., 2004); ^k mRNA by RT-PCR (Overbeek et al., 2001); ^l mRNA by Northern blot analysis (Kumagai et al., 2002); ^m mRNA by RT-PCR (Nguyen et al., 2003); ⁿ mRNA by RT-PCR (Siebel et al., 2003); ^o mRNA by RT-PCR (Samuel et al., 2003b); ^p mRNA by RT-PCR (Hombach-Klonisch et al., 2003); ^q mRNA by Northern blot analysis and in situ hybridization (Kawamura et al., 2004); ^r mRNA by RT-PCR (Liu et al., 2003b); ^s mRNA by RT-PCR (Liu et al., 2003a); ^t protein by receptor autoradiography (Osheroff et al., 1990); ^u protein by receptor autoradiography (Tan et al., 1999); ^v protein by immunohistochemistry (Kuenzi and Sherwood, 1995); ^w protein by receptor autoradiography (Yang et al., 1992); ^x protein by receptor autoradiography (Osheroff et al., 1992); ^y protein by immunohistochemistry (Kohsaka et al., 1998); ^z protein receptor binding (Parsell et al., 1996); ^{ab} mRNA by multitissue expression array or Northern blot analysis (Boels and Schaller, 2003); ^{ac} mRNA by RT-PCR (Matsumoto et al., 2000); ^{ad} mRNA by RT-PCR or Northern blot analysis (Scott et al., 2004); ^{ae} mRNA by in situ hybridization (Shen et al., 2005); ^{af} mRNA by RT-PCR and in situ hybridization (Fu et al., 2005).

strong RXFP1 immunoreactivity was found in the endometrial stroma and weaker staining was found in the epithelium (Ivell et al., 2003). RXFP1 immunostaining has also been reported in rat vagina and cervix smooth muscle (Hsu et al., 2002) and human breast subepithelial stroma (Ivell et al., 2003).

Histological studies confirm the many detailed functional reports establishing that relaxin has important roles in pregnancy and parturition. Established target tissues include pubic symphysis, cervix, uterus, nipples, and mammary glands although the relative importance of the functions varies with species. In many species including humans there is growth and an increase in elasticity of pubic joint cartilage during pregnancy (for reviews, see Sherwood, 1994, 2004; Bathgate et al., 2005). There is strong evidence from animal studies that these effects are caused by relaxin (Hisaw, 1926; Zhao et al., 1999; Sherwood, 1994). The softening and increase in size of the cervix during the second half of pregnancy is a general phenomenon in mammals and is thought to involve effects of relaxin on the collagen, elastin, proteoglycans, and glycosaminoglycans that are responsible for the tensile properties of the cervix. The mechanisms involved are complex and involve interactions with steroid hormones and prostaglandins. In humans, although

it has been demonstrated that relaxins can promote ripening of the cervix, clinical trials of topically applied relaxin failed, probably due to poor pharmacokinetic properties of the preparation (see Bathgate et al., 2005). The uterus is another target tissue for relaxin, although the effects on myometrial contractile activity are highly species-dependent. Relaxin clearly inhibits uterine contractility in rats, mice, and pigs but not in sheep, cows, or humans (Sherwood, 1994, 2004; Bathgate et al., 2005). In rats, the relaxation is associated with increases in intracellular levels of cAMP, PKA activity and stimulation of Ca²⁺-activated K⁺ channels (Sanborn, 2001). Relaxin also has a uterotrophic effect in some species that is associated with increased vascularization. In pigs but not rats this may have a role in the development of the uterus in pregnancy. The vagina, rather like the cervix, increases in size and softens during pregnancy, and there is good evidence from experiments in rats and mice that these effects involve relaxin (Bathgate et al., 2005).

Relaxin also has an important role in the development of the mammary nipple in rats and mice and in the development of the mammary gland in pigs. Rats treated with monoclonal relaxin antibody during the second half of pregnancy display a lack of nipple devel-

opment and cannot suckle their young (Hwang et al., 1991). An identical phenotype was observed in relaxin knockout mice (Zhao et al., 1999). In relaxin-deficient pigs, however, nipple development is relatively normal. The situation is reversed with respect to the mammary gland. In mice and rats, relaxin is not essential for mammary gland development although it may affect the differentiation of the tissue, whereas in pigs relaxin is important for the development of the mammary gland parenchyma. Relaxin-binding sites are present in the mammary glands of pigs but also rats and humans. In humans and other primates, there is increasing evidence for a role for relaxin in preparing the endometrium for implantation. A number of studies indicated that relaxin is associated with increased endometrial angiogenesis, thickening, and bleeding (Einspanier et al., 2001; Goldsmith et al., 2004). A link is also suggested by the plasma levels of relaxin that are highest in the first trimester, the time corresponding to embryo implantation. However the role of relaxin is probably facilitatory rather than mandatory for implantation because this still occurs in humans and other primates that lack ovaries (Johnson et al., 1991). Relaxin is also produced in the male reproductive tract, is present in semen, and increases sperm motility and penetration into oocytes (Weiss, 1989). As well as the potential function in males, it is also tempting to speculate that relaxin in semen targets the female reproductive tract to prepare the endometrium for implantation.

Studies of the distribution of RXFP2 are less extensive, but RXFP2 mRNA has been demonstrated in rat ovary, testis, and gubernaculum by Northern analysis, RT-PCR, and in situ hybridization (Kubota et al., 2002; Kumagai et al., 2002; Kawamura et al., 2004; Scott et al., 2005a). In mouse, RXFP2 mRNA is present in testis and gubernaculum (Overbeek et al., 2001). In human, RXFP2 mRNA is found in uterus and testis (Hsu et al., 2002; Mazella et al., 2004). By immunohistochemistry, RXFP2 epitopes have been identified in spermatocytes, spermatids, and Leydig cells within the human testis, and in the epididymal epithelium (R. Ivell, unpublished data). The pattern of localization clearly indicates a role for INSL3/RXFP2 in reproductive physiology. This role has clearly been identified as testis descent because both *INSL3* and *LGR8* male knockout mice display bilateral cryptorchidism (Nef and Parada, 1999; Zimmermann et al., 1999; Overbeek et al., 2001). The action of INSL3 is on RXFP2 receptors located on the gubernaculum (Kumagai et al., 2002).

Both RXFP3 and RXFP4 mRNA are found in human testis (Liu et al., 2003a,b). RXFP4 is also found in placenta (Boels and Schaller, 2003) and prostate (Liu et al., 2003a,b).

B. Brain

Relaxin receptors determined by receptor autoradiography are widely distributed in the brain (Fig. 3). Re-

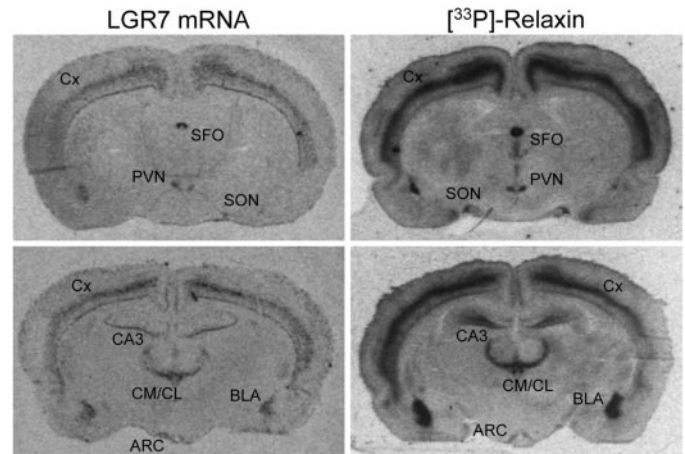


FIG. 3. Distribution of RXFP1 (*LGR7*) mRNA and ^{33}P -relaxin-binding sites in rat brain. Strong RXFP1 mRNA hybridization was associated with magnocellular neurons in the supraoptic nucleus (SON) and paraventricular nucleus (PVN), layers V and VI of neocortex (Cx), the subfornical organ (SFO), the basolateral amygdala (BLA), centromedial and controlateral thalamic nuclei (CM/CL), interneurons in the hippocampus (CA3), and arcuate nucleus (ARC). Most of these brain regions also contain high densities of ^{33}P -relaxin-binding sites. Levels of nonspecific hybridization and nonspecific binding were both equivalent to film background (data not shown). From Burazin et al. (2005), with permission; copyright © 2005 HighWire Press.

laxin receptors are localized to discrete regions of the olfactory system, neocortex, hypothalamus, hippocampus, thalamus, amygdala, midbrain, and medulla of the male and female rat brain (Osheroff and Phillips, 1991; Osheroff, 1995; Tan et al., 1999) (Fig. 3). Binding of relaxin to two of the circumventricular organs [subfornical organ (SFO); and organum vasculosum of the lamina terminalis (OVLT)] and the neurosecretory magnocellular hypothalamic nuclei (i.e., paraventricular and supraoptic nuclei) (Osheroff and Phillips, 1991; Osheroff, 1995; Tan et al., 1999) provides the anatomical and biochemical basis for the central role for relaxin in the control of plasma osmolality (McKinley et al., 1997, 1998; Sinnayah et al., 1999; Sunn et al., 2001, 2002). During the second half of pregnancy in rats there is a decline in plasma osmolality that is associated with increased serum relaxin levels (Sherwood et al., 1980; Lindheimer et al., 1989) that does not occur in pregnant relaxin-deficient rats (Novak et al., 2001). Likewise, decreases in plasma osmolality in wild-type mice in late pregnancy are not observed in relaxin knockout mice (Zhao et al., 1999). However in humans, although there is a decrease in plasma osmolality with pregnancy, this may not be an effect of relaxin, because women that become pregnant after ovum donation have undetectable relaxin levels yet still show the decrease in plasma osmolality (Johnson et al., 1991, 1996). In rats, water consumption is both increased in the second half of pregnancy and strongly stimulated by relaxin (Omi et al., 1997; Summerlee et al., 1998). Intracerebroventricular or intravenous relaxin promotes drinking in nonpregnant rats (Summerlee et al., 1998; Bathgate et al., 2005). Administration of monoclonal antibodies to relaxin to

rats in the second half of pregnancy reduces water consumption. These actions of relaxin are mediated by RXFP1 receptors located in the SFO and OVLT. The intravenous administration of porcine or H2 relaxin causes increased expression of *c-fos*, the gene encoding proto-oncogene protein, in neurons located in the peripheral and dorsal segments of the SFO and in the dorsal cap region of the OVLT, as well as the supraoptic and paraventricular nuclei of the hypothalamus (McKinley et al., 1997, 1998; Sunn et al., 2001, 2002), all sites of localization of relaxin receptors. These regions of the brain are accessible to circulating relaxin and may be responsible for the plasma osmolality changes that occur during pregnancy (Weisinger et al., 1993).

RXFP1 mRNA has also been demonstrated in rat brain by Northern blotting (Hsu et al., 2000; Scott et al., 2004) and RT-PCR (Kompa et al., 2002; Kubota et al., 2002; Samuel et al., 2004a), in mouse brain using a reporter gene approach (Krajnc-Franken et al., 2004) and by Northern blotting (Scott et al., 2004), and in human brain using RT-PCR (Hsu et al., 2002). RXFP2 mRNA is also expressed in the rat brain (Shen et al., 2005). RXFP3 is predominantly expressed in the brain (Table 4) whereas RXFP4 is expressed in various tissues including the brain (Liu et al., 2003a). RXFP3 mRNA has been demonstrated in human brain by RT-PCR and in rat brain by in situ hybridization where it is localized to the supraoptic and paraventricular nucleus (Liu et al., 2003a,b). Recently, a chimeric peptide, relaxin-3 B chain/INSL5 A chain, has been developed, radioiodinated, and shown to be a selective, high-affinity ligand for RXFP3 and RXFP4. In the rat, in which RXFP4 is a pseudogene, ¹²⁵I-relaxin-3/INSL5 labels RXFP3 receptors in the cerebral cortex, olfactory bulb, and superior colliculus (Fig. 4) (Sutton et al., 2004; Liu et al., 2005).

C. Cardiovascular and Renal Systems

There is increasing evidence that relaxin has important roles in the cardiovascular adaptive changes that are associated with pregnancy. These changes include not only increases in plasma volume, cardiac output, and heart rate but also decreased blood pressure and vascular resistance. In the kidney there is evidence for the presence of RXFP1 mRNA (Hsu et al., 2000, 2002) and in pregnant rats glomerular filtration rate and effective renal plasma flow increase whereas vascular resistance decreases in parallel with increases in plasma levels of relaxin (Novak et al., 2001). In both female and male rats administration of relaxin increases renal plasma flow and glomerular filtration rate and also causes the classic reduction in plasma osmolality associated with pregnancy (Danielson et al., 1999). Ovariectomy or passive immunization with monoclonal antibodies for rat relaxin prevents these adaptive changes to the kidney during pregnancy (Novak et al., 2001). The mechanism suggested to explain the actions of relaxin on the kidney

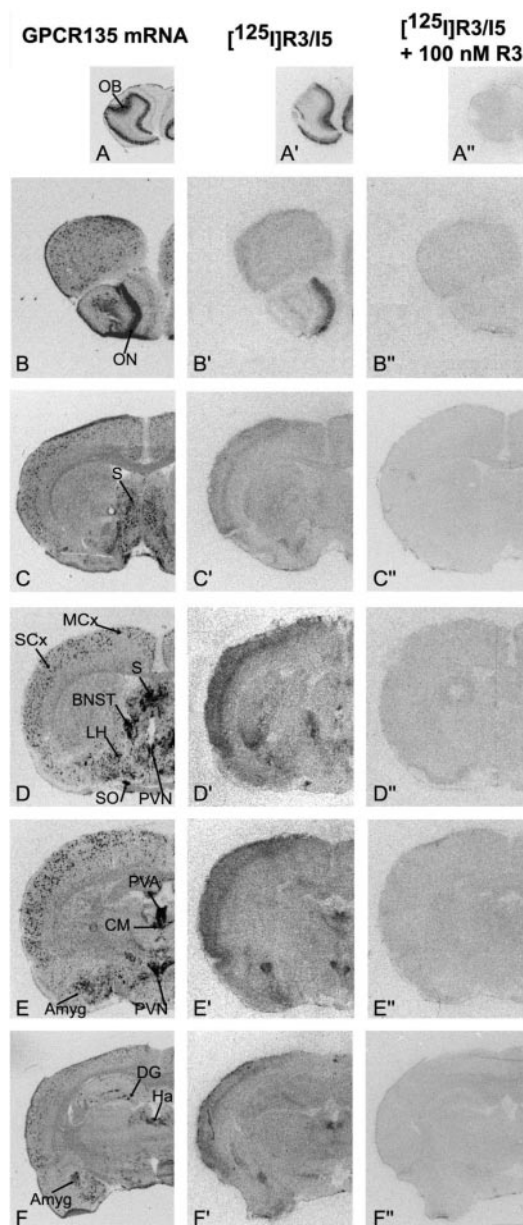


FIG. 4. Distribution of RXFP3 (GPCR135) mRNA (A–F) and ¹²⁵I-R3/I5 (relaxin-3 B chain/INSL5 A chain chimeric peptide) binding sites (A'–F') in rat brain. Autoradiograms from a series of coronal sections through the rat front and mid-brain (left hemisphere). Nonspecific binding was determined in the presence of 100 nM relaxin-3 (A''–F''). OB, olfactory bulb; ON, olfactory nucleus; S, septum; MCx, motor cortex; SCx, somatosensory cortex; BNST, bed nucleus of stria terminalis; LH, lateral hypothalamus; SO, supraoptic nucleus; PVN, paraventricular hypothalamic nucleus; PVA, paraventricular thalamic nucleus; CM, central thalamic nucleus; Amyg, amygdala; DG, dentate gyrus; Ha, habenula. Scale bar = 0.2 cm. From Sutton et al. (2004), with permission; copyright © 2004 S. Karger AG, Basel.

are the induction of vascular gelatinase that cleaves big endothelin to yield ET₁₋₃₂ which acts on the ET_B receptor to increase the production of the vasodilator NO (Conrad and Novak, 2004).

Vasodilation in response to relaxin is a common action in arterioles, capillaries, and venules in reproductive tissues (Bani et al., 1988; Lee et al., 1992; Vasilenko et al., 1986), heart (Bani et al., 1998b; Bani-Sacchi et al.,

1995; Masini et al., 1997), liver (Bani et al., 2001) and cecum (Bigazzi et al., 1986). Relaxin acts as a physiological antagonist of vasoconstrictors in mesenteric arteries (St-Louis and Massicotte, 1985; Massicotte et al., 1989), primary bovine aortic smooth muscle cells (Bani et al., 1998a), and uterine artery (Longo et al., 2003). The hormone also reduces the rise in intracellular Ca^{2+} produced by α -thrombin or angiotensin II (Bani et al., 1998a; Failli et al., 2002). The mechanisms suggested for the vasodilator actions of relaxin are activation of nitric-oxide synthase (NOS) III via cAMP, induction of NOSII (Nistri and Bani, 2003) and modification of the extracellular matrix of the vessel walls (Conrad and Novak, 2004).

The heart is clearly a target organ for relaxin in rodents. Atria of male and female rats possess high-affinity binding sites for relaxin (Osheroff et al., 1992; Osheroff and Ho, 1993; Tan et al., 1999), and RXFP1 mRNA has been detected in the rat (Hsu et al., 2000; Kompa et al., 2002), mouse (Krajnc-Franken et al., 2004), and human (Hsu et al., 2002) heart. Numerous *in vitro* and *in vivo* studies demonstrate that relaxin has potent, direct, and concentration-dependent chronotropic and inotropic effects on the rat heart. The positive chronotropic effects of relaxin have been reported in perfused intact hearts (Thomas and Vandlen, 1993; Bani-Sacchi et al., 1995; Coulson et al., 1996; Toth et al., 1996) and isolated right atria (Kakouris et al., 1992; Ward et al., 1992; Wade et al., 1994; Tan et al., 1998; Mathieu et al., 2001) and inotropic effects in left atria (Kakouris et al., 1992); (Ward et al., 1992; Wade et al., 1994; Tan et al., 1998; Mathieu et al., 2001). Little is known about the mechanism or site of action of relaxin in the heart. The chronotropic effects are accompanied by the secretion of atrial natriuretic peptide in isolated perfused rat hearts (Toth et al., 1996). In rat atrial myocytes, relaxin inhibits outward potassium currents, increases action potential duration, and enhances Ca^{2+} entry (Piedras-Renteria et al., 1997a,b). In rabbit sinoatrial node cells, relaxin increased the rate of action potentials and L-type Ca^{2+} currents (Han et al., 1994) by a PKA-dependent mechanism. These actions of relaxin on the heart are largely confined to rodents, and the hormone has no positive inotropic effect in the atria of sheep (Bathgate et al., 2001) or humans (R. J. Summers and L. J. Castro, unpublished data). Radiolabeled relaxin does not bind specifically to sheep or human atria (Y. Y. Tan and R. J. Summers, unpublished data). mRNA for RXFP2 has been demonstrated in human (Hsu et al., 2002) and rat (Fu et al., 2005) kidney as has RXFP4 (Liu et al., 2003a), but there are no known functional correlates for these expression patterns to date.

D. Other Sites of Action of Relaxin Family Peptides

In addition to the specific roles of relaxin already described, it is becoming increasingly clear that it has more general physiological roles. The role of relaxin in

inhibiting collagen biosynthesis and promoting collagen breakdown in reproductive tissues is well established, but it is also clear that it has similar effects in nonreproductive tissues (Gavino and Furst, 2001; Bathgate et al., 2003), which have led to the suggestion that relaxin would be an effective treatment for fibrotic diseases. Relaxin acts directly on transforming growth factor- β -stimulated human dermal fibroblasts (Unemori and Amento, 1990), lung fibroblasts (Unemori et al., 1996), and cardiac fibroblasts (Samuel et al., 2004a) to promote a decrease in type I and type III collagen synthesis and an increase in matrix metalloproteinase expression and activation. It has been used successfully to modify the extracellular matrix in the dermis (Kibblewhite et al., 1992; Unemori et al., 1993), lung (Unemori et al., 1996), liver (Williams et al., 2001), and kidney (Garber et al., 2001). In all of these experimental paradigms, relaxin is effective only during artificially induced collagen deposition, induced by profibrotic stimuli, surgery, or chemical means.

Relaxin has been used in humans to treat scleroderma (systemic sclerosis), a connective tissue disease of unknown etiology in which tissue fibrosis is the predominant clinical feature (Casten and Boucek, 1958). Clinical trials using recombinant H2 relaxin to treat scleroderma have had mixed success. A phase II trial infusing recombinant H2 relaxin subcutaneously for a 24-week period (Seibold et al., 2000) were encouraging and the peptide was found to be safe and well tolerated. Twenty-four weeks of treatment with a low but not a high dose of relaxin had significant beneficial effects on skin thickness and mobility. However, a larger study showed that skin elasticity, hand extension, oral aperture, cutaneous ulcers, and pulmonary function in recombinant H2-treated patients did not differ significantly from those in the placebo control subjects (Erikson and Unemori, 2001) even though relaxin did have beneficial actions in some patients. The positive results obtained in numerous *in vivo* and *in vitro* studies, in which relaxin has been able to reverse fibrosis, suggest that it still has enormous potential as an antifibrotic agent and that more research needs to be done to better understand its mechanism of action and to identify those patients who are likely to derive benefit from treatment.

Studies with the relaxin knockout mouse demonstrate that relaxin is an endogenous mediator of collagen turnover in nonreproductive tissues. In these animals, increased interstitial collagen was detected in heart (Du et al., 2003), lung (Samuel et al., 2003b), kidney (Samuel et al., 2004a), and skin (Amento et al., 2001). The effect was particularly prevalent in male mice and associated with abnormal function of these organs. In the heart, there is increased atrial hypertrophy and impaired left ventricular diastolic filling and venous return in older mice, associated with increased left ventricular collagen content and ventricular chamber stiffness (Du et al., 2003). In the lung, there was increased weight, collagen

content, and collagen concentration with age, distortion of alveolar structure, and thickening of the bronchioles. The structural changes were associated with a significantly altered peak expiratory flow and lung recoil (Samuel et al., 2003b). Increased kidney collagen in male relaxin knockout mice was associated with increased cortical thickening, focal increases in interstitial fibrosis, and a general increase in glomerulosclerosis, from 6 months of age and onward (Samuel et al., 2004b). This was associated with impaired renal function, demonstrated by increased serum creatinine and urinary protein, compared with wild-type mice (Samuel et al., 2004b). Collagen levels also increased in the subcutaneous layer of the aging dermis (Amento et al., 2001). Treatment of relaxin knockout mice with recombinant H2 relaxin in the early and developed stages of fibrosis resulted in the reversal of collagen deposition in the lung (Samuel et al., 2003b), heart (Samuel et al., 2004a), and kidney (Samuel et al., 2004b) consistent with an antifibrotic action.

E. Roles of Relaxin Family Peptides Determined from Studies in Receptor Knockout Mice

The recent identification of RXFP1 and RXFP2 as receptors for relaxin and INSL3, respectively, has enabled the creation of mice that are devoid of functional receptors. The consequences of the relaxin receptor RXFP1 knockout on reproduction have been reported for both females and males. Whereas studies with RXFP1 knockout female mice showed normal fertility and litter size (Krajnc-Franken et al., 2004), a small portion of the females were incapable of delivering their litters and approximately 15% of the pups were found dead soon after birth. Moreover, all offspring died within 24 to 48 h. These reproductive findings are consistent with those observed with relaxin knockout female mice (Zhao et al., 1999, 2000). The available evidence indicates that relaxin facilitates rapid and safe birth in mice, as it does in rats and pigs, by promoting growth and softening of the cervix (Sherwood, 1994, 2004; Zhao et al., 2000). Those pups that survived delivery died within 2 days of birth because relaxin is also required for nipple development (Hwang et al., 1991; Kuenzi et al., 1995), and pups are unable to grasp the small nipples in mice that result from lack of relaxin or its receptor RXFP1 (Krajnc-Franken et al., 2004; Zhao et al., 1999, 2000). Phenotypic consequences also occur in RXFP1 knockout males (Krajnc-Franken et al., 2004). Fertility was diminished to a marked degree. Sexually mature male RXFP1 knockout mice demonstrated disrupted spermatogenesis that was associated with an increased rate of apoptosis of meiotic spermatocytes (Krajnc-Franken et al., 2004). The testis and epididymidis weights in RXFP1 knockout mice were lower than those in wild-type controls. These reproductive findings are consistent with those observed with relaxin knockout male mice (Samuel et al., 2003a). Importantly, the reproductive pheno-

type of the RXFP1 knockout mouse was lost in later generations of animals (Krajnc-Franken et al., 2004), and in another strain of RXFP1 knockout mice developed independently (Kamat et al., 2004), there is no deleterious male reproductive tract phenotype. It is possible that the actions of relaxin on the male reproductive tract may be redundant and/or dependent on the genetic background of the animals. In tissues other than reproductive tissues, recent studies in the RXFP1 knockout mouse have demonstrated an increase in interstitial collagen in the lung, whereas other organs have not yet been examined (Kamat et al., 2004).

The consequences of RXFP2 receptor (LGR8/GREAT) knockout on reproduction are profound in the male mouse. The testes fail to descend into the scrotum and the mouse is sterile (Overbeek et al., 2001; Gorlov et al., 2002; Tomiyama et al., 2003). The testes in adult RXFP2 knockout mice are much smaller than those in wild-type controls, and spermatogenesis is arrested (Overbeek et al., 2001; Gorlov et al., 2002). These findings are in close agreement with those obtained with INSL3 knockout mice (Nef and Parada, 1999; Zimmermann et al., 1999). Testicular descent, which occurs during the latter stages of embryonic development in mice, is dependent upon modifications of two ligaments that attach the testis to the abdominal wall. The cephalic cranial suspensory ligament, which attaches the upper gonadal tip of the testis to the posterior abdominal wall, undergoes androgen-dependent regression. In contrast, the caudal ridge-shaped condensation of mesenchymal cells, which projects from the caudal pole of the testis to the future intra-abdominal ring of the inguinal canal, is retained and undergoes development (Ivell and Hartung, 2003; Klonisch et al., 2004). During development, the mesenchymal cells within the caudal ligament, the gubernaculum, proliferate. The ligament thickens, and the testes are retained close to the region where the inguinal canal will develop. Cryptorchidism in male RXFP2 knockout mice and INSL3 mice is attributable to failure of the gubernacular ligament to develop (Nef and Parada, 1999; Zimmermann et al., 1999; Overbeek et al., 2001; Gorlov et al., 2002). Available evidence indicates that INSL3 acts directly on the gubernaculum because this tissue contains RXFP2 receptors (Kumagai et al., 2002) and in transgenic female mice overexpressing INSL3 during embryonic development, the ovaries descend (Adham et al., 2002). The lack of fertility in male mice associated with knockout of RXFP2 or INSL3, appears to be attributable, at least in part, to the failure of the testes to descend into the scrotum. Histological examination of the seminiferous tubules in testes of RXFP2 knockout mice that were surgically descended (orchiopexy) appeared to demonstrate normal spermatogenesis (Overbeek et al., 2001), and a small proportion of RXFP2 knockout mice demonstrated fertility following this procedure (Nguyen et al., 2002). INSL3 may also play a direct role in promoting spermatogenesis. A recent study

in rats provides limited evidence that INSL3 produced by Leydig cells may act directly on RXFP2 receptors in germ cells to suppress apoptosis (Kawamura et al., 2004). Leydig cell function does not appear to be influenced in RXFP2 knockout mice. These animals display normal testosterone levels, reproductive behavior, seminal vesicle weight, and prostate weight (Overbeek et al., 2001; Gorlov et al., 2002)

The influence of RXFP2 knockout in female mice has received limited experimental attention. It is known that these animals are fertile (Overbeek et al., 2001). However, in the absence of INSL3 activity, female fertility may be impaired. Approximately 20% of INSL3-deficient female mice were reported to be infertile (Nef and Parada, 1999). In addition, there is evidence that follicular atresia and luteolysis are accelerated in INSL3 knockout mice, probably because of increased apoptosis (Spanel-Borowski et al., 2001). Consistent with the findings in INSL3 knockout mice, a recent study with cultured rat cumulus-enclosed oocytes or preovulatory follicles provided evidence that INSL3 produced by thecal cells may act directly on oocytes to enhance meiotic progression of arrested oocytes in preovulatory follicles (Kawamura et al., 2004), and in cow ovaries INSL3 expression correlates with protection of neighboring follicular cells from undergoing apoptosis (Irving-Rodgers et al., 2002).

The phenotypes of neither RXFP3 nor RXFP4 knockout mice have been reported. It remains to be determined if relaxin-3 acts through RXFP3 and/or RXFP4 to bring about a detectable physiological response.

III. Structure of Relaxin Family Peptides

A. Structural Features of Relaxin

The first information on the structure of the mature relaxin peptide came from studies that determined the amino acid sequence of the A and B chains of various native relaxins by peptide sequence analysis (Sherwood, 1994, 2004). Nucleotide sequence analysis not only confirmed the amino acid sequences but also predicted the amino acid sequences of H1 relaxin and H2 relaxin. The conformation of the disulfide linkages has been determined for porcine relaxin (Schwabe and McDonald, 1977b) and H2 relaxin (Canova et al., 1991), with an intrachain bond in the A chain between A10 and A15 and two interchain bonds between A11 and B11 and A24 and B23 (Fig. 2). In all species for which the amino acid sequences of relaxin are known, with the exception of mouse relaxin (Evans et al., 1993), the half-cysteine residues are found in positions comparable to those in porcine and human relaxin (Fig. 2). Although the structure of relaxin has diverged considerably among species during evolution and only 30 to 60% amino acid sequence identity generally exists among species, there are invariant positions in relaxin from all species that are largely confined to the cysteine residues and adja-

cent glycine residues as well as the relaxin-binding motif (Arg-X-X-X-Arg-X-X-Ile/Val) (Fig. 2). Although the presence of this relaxin receptor binding cassette generally means that relaxin peptides from different species are bioactive, there are marked differences in biological activities among species. Importantly, relaxin does not interact with insulin receptors, show any insulin-like activity, or cross-react with any insulin immunoassays (Sherwood, 1994). This is consistent with the relaxin receptor being a G-protein coupled receptor, completely distinct from the insulin and insulin-like growth factor receptors that are tyrosine kinases. A further difference is that, unlike insulin, relaxin is able to interact with and activate its cognate receptor as the uncleaved pro-form retaining the long C domain (Zarreh-Hoshyari-Khah et al., 2001).

Direct analysis of the tertiary structure of H2 relaxin confirmed the predicted structural homology of relaxin to insulin first deduced from molecular modeling techniques (Eigenbrot et al., 1991). H2 relaxin, like insulin, forms an asymmetric peptide dimer, although the orientation of the A and B chains in the relaxin dimer is completely different from those in the insulin dimer. Principal features of the relaxin molecule are a 24-residue A chain with two α -helices extending from A3 to A9 and from A13 to A20 and a 29-residue B chain containing one α -helix extending from B7 to B22. The relaxin model does not include the C terminus of the B chain, which appears to be disordered.

B. Structural Features of Other Relaxin Family Peptides

Unlike relaxin, the structure of all other relaxin family peptides was first determined from their cDNA sequences. However, the derived amino acid sequences all show the characteristic relaxin/insulin structure. The native peptide has only been isolated for bovine INSL3 (Büllesbach and Schwabe, 2002) and porcine relaxin-3 (Liu et al., 2003b). The disulfide linkages characteristic of relaxin and insulin were confirmed for the bovine INSL3 peptide (Fig. 2). The native peptides of INSL4 through 6 have not been isolated. There is currently no other structural information on any other relaxin peptide, although circular dichroism analysis of INSL3 generally indicates that it has a tertiary structure similar to that of relaxin (Büllesbach and Schwabe, 1995; Dawson et al., 1999; Smith et al., 2001).

IV. Structure-Activity Relationships

A. Relaxin

Comparison of relaxin sequences from many species as well as the testing of synthetic peptide analogs has defined many of the key structural features of relaxin. Relaxin A and B chains on their own have no biological activity (Tan et al., 1998). In addition, the intrachain molecular bond in the A chain is necessary for activity.

The amino acids in the N-terminal regions of the A and B chain are not directly involved in activity; however, retaining the secondary structure in this region of the chains is essential (Tregear et al., 1981; Büllesbach and Schwabe, 1986, 1987). The carboxyl-terminal region of the B chain does not appear to be important and up to eight amino acids can be deleted from this region of pig relaxin (Tregear et al., 1981) or H1 relaxin (Tan et al., 1999) without loss of activity. One of the invariant amino acids across all relaxin peptides, the glycine residue at A14 is absolutely essential for maintaining chain flexibility (Büllesbach and Schwabe, 1994). Hence the overall structure of the A chain seems to be more important than the individual amino acids and the main role of the A chain is to act as a scaffold to hold the B chain in the correct formation. In contrast, individual amino acids in the B chain are vital for receptor binding. The absolutely conserved arginine B13 and B17 residues (Fig. 2) are essential for interaction with the relaxin receptor (Büllesbach et al., 1992) and together with the B20 isoleucine residue are essential for relaxin binding (Büllesbach and Schwabe, 2000). The B20 isoleucine can be substituted with valine without loss of activity, and virtually all of the native relaxins have these amino acids in this position. Hence these amino acid residues, Arg-X-X-X-Arg-X-X-Ile/Val (Fig. 2), constitute what is now regarded as the “relaxin-binding cassette.” These residues are also conserved in relaxin-3 (Fig. 2) and are the basis for its interaction with relaxin receptors (Bathgate et al., 2002a).

Although these residues are undoubtedly the key to relaxin-specific actions, other structural determinants are essential for full relaxin activity. Substitution of the relaxin-binding cassette into a sheep INSL3 peptide produced only weak relaxin-like activity (Claasz et al., 2002; Tan et al., 2002), and insertion of a partial cassette into a porcine insulin analog failed to produce full relaxin activity (Büllesbach and Schwabe, 1996). Based on the marked differences in relaxin structure between species it is unlikely that there are other key residues for receptor interaction. As mentioned above, the structure of the B chain is especially important, with a full α -helical structure necessary for the correct positioning of the relaxin-binding cassette (Büllesbach and Schwabe, 1991; Büllesbach et al., 1992). Importantly, the INSL3 analogs with a full relaxin-binding cassette do not have the full α -helix structure of H2 relaxin (Claasz et al., 2002). Interestingly, surface regions on the relaxin structure involved in receptor binding that comprise the relaxin-binding cassette were accurately predicted from molecular modeling studies more than 20 years ago (Dodson et al., 1982).

B. INSL3

Although all INSL3 peptides contain a partial “relaxin-like” binding motif with two arginine residues in a pattern resembling relaxin (Fig. 2), these are displaced

toward the C terminus by four residues and show only minimal relaxin activity (Table 3) (Büllesbach and Schwabe, 1995; Dawson et al., 1999; Claasz et al., 2002). Furthermore, INSL3 peptides show only limited ability to interact with the relaxin receptor (RXFP1) (Sudo et al., 2003) and are the cognate ligands for the related RXFP2 receptor (Table 3) (Kumagai et al., 2002). The determinants for INSL3 binding to its receptor include a tryptophan residue at B27 located in the N terminus of the B chain (Büllesbach and Schwabe, 1999a) (Fig. 2). More recently it has been shown that the N terminus of the A chain of INSL3 can be truncated by up to nine residues and retain full binding activity (Büllesbach and Schwabe, 2005a). Truncation of up to six residues can be performed with the peptides still able to induce cAMP signaling although the loss of six residues is associated with lower potency and efficacy. Interestingly, further truncation produces peptides that bind with high affinity but are unable to induce cAMP signaling in RXFP2 transfected cells and act as competitive antagonists (Büllesbach and Schwabe, 2005a). Mutation to Arg8Ala or Tyr9Ala in the A chain produces peptides that retain agonist properties indicating that the arginine and tyrosine side chains are not essential for signal transduction. Hence, regions of the N terminus of the A chain of INSL3 appear to be essential for RXFP2 activation and peptides lacking this region of the peptide are the first high-affinity RXFP2 antagonists (Büllesbach and Schwabe, 2005a).

V. Binding of Relaxin and Relaxin Family Peptides

A. Relaxin Binding

Relaxin-binding sites have been identified in numerous reproductive tissues as well as in some nonreproductive tissues. Early studies used purified porcine relaxin (Sherwood et al., 1975; McMurtry et al., 1978) that retained its bioactivity after iodination although the final product was often a mix of multiply iodinated forms. These ligands were subsequently used to perform the first localization of relaxin receptors in multiple tissues from various species. Specific binding was not displaced by insulin or insulin-like growth factor I or II. Relaxin-binding sites were identified in the uterus of rat (McMurtry et al., 1978; Cheah and Sherwood, 1980; Mercado-Simmen et al., 1980), mouse (McMurtry et al., 1978; Yang et al., 1992) and pig (Mercado et al., 1982); the pubic symphysis of mouse (McMurtry et al., 1978; Yang et al., 1992) and guinea pig (McMurtry et al., 1978); the cervix of pig (Mercado et al., 1982), rat (Weiss and Bryant, 1982) and guinea pig (McMurtry et al., 1978); the rat mammary gland (McMurtry et al., 1978); human fetal membranes (Koay et al., 1986); and fibroblasts from the mouse pubic symphysis and human skin (McMurtry et al., 1980).

H2 relaxin contains a tyrosine residue that can be labeled by conventional chloramine-T iodination (Tang and Chegini, 1995). ^{125}I -H2 relaxin binds to epithelial, stromal, and smooth muscle cells in human fallopian tube, smooth muscle cells in the arterioles (Tang and Chegini, 1995), and fibroblasts isolated from human lower uterine segments (Palejwala et al., 1998). ^{125}I -H2 relaxin labeled by an alternate strategy has also been used to characterize the binding activity of relaxin analogs (Büllesbach and Schwabe, 2000). H2 relaxin (B-33) can also be phosphorylated using ^{32}P (Osheroff et al., 1990) to produce a specific high-activity radioligand monolabeled at the B-32 serine residue (Osheroff et al., 1990; Tan et al., 1999). ^{32}P -H2 relaxin has been shown to bind to rat uterus, cervix, and brain (Osheroff et al., 1990), rat heart atrium (Osheroff et al., 1992), rat atrial cardiomyocytes (Osheroff, 1995), and a number of regions of the rat brain (Osheroff and Ho, 1993; Osheroff and Phillips, 1991). In addition, binding sites have been identified in human uterine cells (Osheroff and King, 1995), in human fetal membranes (Garibay-Tupas et al., 1995), and in the human monocytic cell line THP-1 (Parsell et al., 1996). More recently ^{33}P -H2 relaxin has been used to localize relaxin binding in the rat uterus, atria, and brain (Tan et al., 1999) (Fig. 3) and for numerous structure function studies (Bathgate et al., 2002a,b; Sudo et al., 2003).

Cross-linking of ^{32}P -H2 relaxin to human uterine cells and rat atrial cardiomyocytes demonstrated binding to a >200-kDa protein that was postulated (Osheroff and King, 1995) to be a tyrosine kinase. Later, a 220-kDa protein was shown to be tyrosine phosphorylated following relaxin stimulation in human lower uterine segment fibroblasts (Palejwala et al., 1998). These data were interpreted as evidence for the relaxin receptor being a tyrosine kinase.

Biotinylated relaxin has been used to localize relaxin binding in the cervix, mammary glands, and nipples of rats (Kuenzi and Sherwood, 1995); the cervix, mammary gland, nipples, small intestine, skin (Min and Sherwood, 1996), ovary, and testis (Min and Sherwood, 1998) of pigs; the uterus, vagina, cervix, mammary gland, nipples, and placenta of pregnant women (Kohsaka et al., 1998), and the uterine endometrium and myometrium of the marmoset monkey (Einspanier et al., 2001). A monobiotinylated rat relaxin peptide was produced by peptide synthesis and shown to be biologically active (Mathieu et al., 2001).

B. Binding of Other Relaxin Family Peptides

Very little is known of specific binding sites for other relaxin family peptides. Binding studies with labeled INSL3 have not been carried out in the gubernaculum and ovary, the likely target tissues for the peptide. However, ^{125}I -INSL3 has been used to demonstrate specific binding sites in mouse uterus and brain (Büllesbach and Schwabe, 1995), although the significance of these sites

has yet to be established. These binding sites were specific for INSL3 and had a 1000-fold lower affinity for H2 relaxin. ^{125}I -INSL3 has been chemically cross-linked to mouse uterus and demonstrated a ~200-kDa band (Büllesbach and Schwabe, 1999b) similar to that shown for the relaxin receptor (Osheroff and King, 1995).

^{125}I -H3 relaxin (Liu et al., 2003b) binds specifically to RXFP3 and RXFP4 receptors expressed in mammalian cells. As H3 relaxin also binds to the relaxin receptor (RXFP1) with high affinity (Bathgate et al., 2002a; Sudo et al., 2003), determination of specific receptor binding sites for H3 relaxin distinct from relaxin-binding sites was a challenge. The recent development of a chimeric relaxin-3/INSL5 peptide has provided a ligand that is selective for RXFP3 and RXFP4 and because the rat does not express RXFP4, the ligand can be used to map RXFP3 in rat brain (Fig. 4) (Liu et al., 2005).

VI. Relaxin Family Peptide Receptors

A. RXFP1 and RXFP2

Because of their two-chain structure, the relaxin and INSL3 genes have traditionally been thought to belong to the insulin ligand family, and several of the relaxin paralogs have been named INSL3 to 7 based on their order of discovery. Based on the hypothesized coevolution of peptide ligands and their receptors, it was originally believed that the receptors for relaxin and INSL3 were related to the known insulin receptors and likely to be tyrosine kinases.

Recent advances in genome sequencing have facilitated the identification of novel genes based on their sequence relatedness to known genes in the hormonal signaling pathway (Hsu et al., 2000). Searches for paralogs of the known gonadotropin and thyrotropin receptors led to the identification of a group of GPCRs called LGRs. LGRs, similar to glycoprotein hormone receptors (Hsu et al., 1998, 2000), are mosaic proteins that contain an extracellular domain with multiple leucine-rich repeats (LRRs) that are important in ligand binding, and a typical GPCR seven-transmembrane (TM) domain. Studies of LGRs from different species suggest that three LGR subtypes (A, B, and C) evolved during the early evolution of metazoans and that each subtype of LGR shares a similar LRR domain and a unique hinge region between the LRR and the transmembrane region (Hsu, 2003). The type A LGRs include the follicle-stimulating hormone receptor, the luteinizing hormone receptor, and the thyroid-stimulating hormone receptor, important for signaling of the heterodimeric glycoprotein hormones FSH, LH, and TSH, respectively. In mammals, the type B LGR comprises three members, LGR4 through 6, which remain orphan GPCRs at the present time. By contrast, type C LGRs have only two members, RXFP1 and RXFP2 (Fig. 5). Because type A LGRs and the coevolved genes encoding glycoprotein hormone subunits could be traced to both nematodes

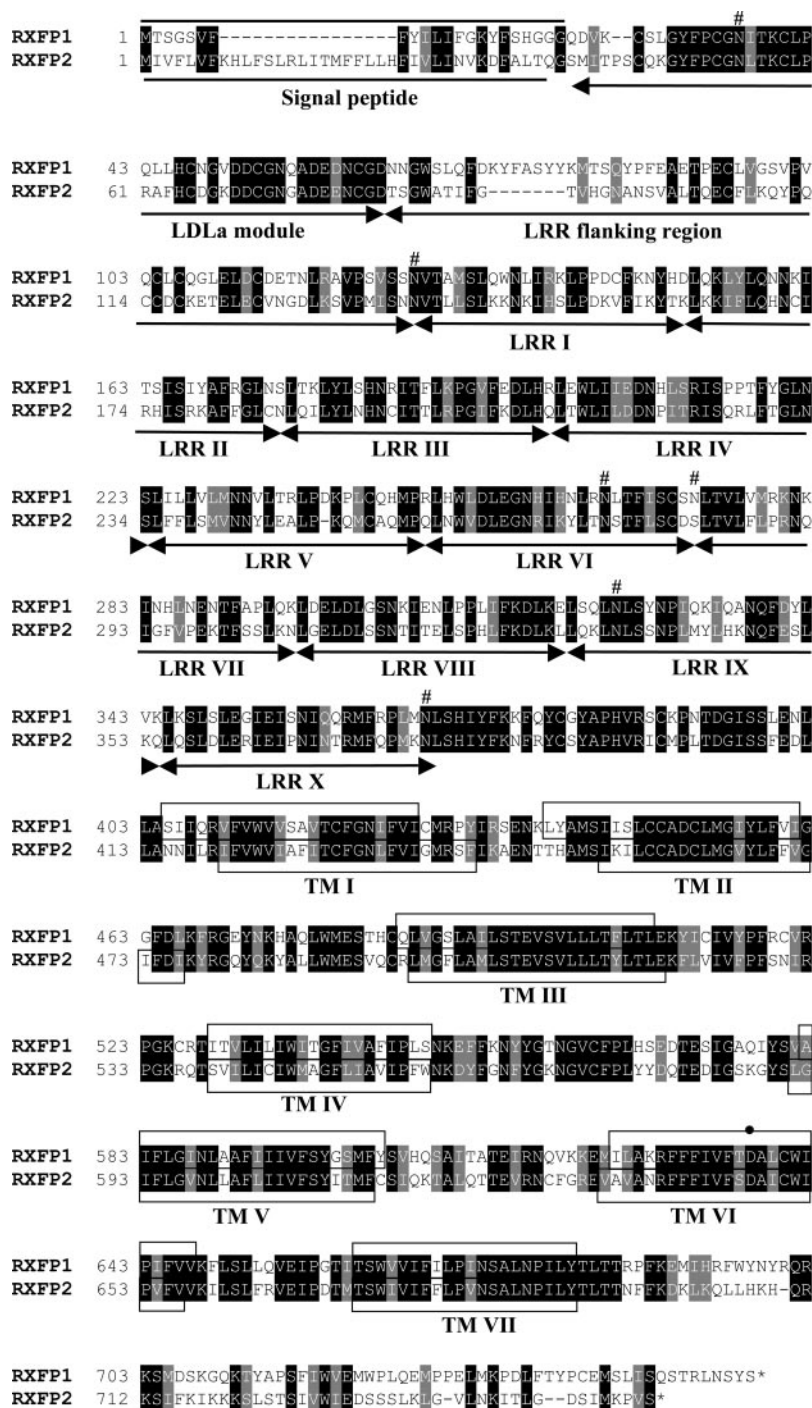


FIG. 5. Boxshade alignment of the human RXFP1 and RXFP2 receptors. Conserved amino acid residues are boxed in black, and conservative amino acid substitutions are boxed and shaded. Gaps have been introduced to aid the alignment where necessary. The signal peptides for both receptors are indicated by lines, the LRRs are marked by arrows, and the putative transmembrane domains (derived from ENSEMBL) are boxed. Putative *N*-glycosylation sites are indicated (#) above the RXFP1 sequence; four of the sites are common to both receptors. An aspartic acid residue (RXFP1—D637; RXFP2—D647), which when mutated to a tyrosine results in constitutive cAMP activity for both receptors, is marked (●) (Hsu et al., 2002).

and insects, it was concluded that the three LGR subtypes evolved before the emergence of vertebrates and nematodes (Hsu et al., 2000). Therefore, the type C LGR signaling pathway represents one of the earliest forms of GPCR signaling. The type C LGR ectodomain consists of a low-density lipoprotein class A (LDLa) module, followed by an alternatively spliced flanking region, and

the LRRs. Cysteine-rich regions form “caps” at each end of the LRRs, and these caps have been demonstrated to be an integral part of the LRR structure in many proteins (Kobe and Kajava, 2001). The ectodomain is connected to the seven-transmembrane-spanning regions followed by the C-terminal tail (Fig. 5). Based on the differential splicing in the ectodomain, many different

isoforms of the receptors can be generated (Muda et al., 2005; Scott et al., 2005c). An exon 4-deleted RXFP1 splice variant has been discovered in mouse, rat, and pig (Scott et al., 2005c). Removal of exon 4 causes a frameshift, resulting in a premature stop codon and production of a truncated protein (RXFP1-truncate) that encodes primarily the LDLa module without the LRRs or transmembrane region. When cells were cotransfected with plasmids containing RXFP1 and the secreted RXFP1-truncate, relaxin-induced RXFP1 signaling was significantly reduced, suggesting that this protein acts as a functional antagonist *in vitro* and may be an endogenous regulator of RXFP1 function.

A comparison of phenotypes of mice deficient in INSL3 (Nef and Parada, 1999; Zimmermann et al., 1999) and mice lacking a 550-kilobase region of chromosome 3, which contained the RXFP2 gene (Overbeek et al., 2001), led to the hypothesis that relaxin family peptides were cognate ligands for type C LGRs (Hsu et al., 2002). Functional studies established the fact that porcine relaxin (Hsu et al., 2002) and H2 relaxin (Sudo et al., 2003) activate both RXFP1 and RXFP2 to increase cAMP. RXFP1 and RXFP2 have more than 700 residues, share approximately 60% amino acid sequence identity, and contain 10 leucine-rich repeats in their extracellular domain. RXFP1 transcripts are found in reproductive tissues, as well as in brain, kidney, heart, and lung, in which actions of relaxin have been reported. The rat and mouse orthologs of RXFP1 that have recently been cloned (Scott et al., 2004) bind H2 relaxin with high affinity and when activated increase cAMP accumulation. Both receptors had a higher affinity for rat relaxin than the human receptor and also bind H3 relaxin with high affinity (Scott et al., 2005b). Examination of the closely related relaxin-3 and INSL3 has demonstrated that the peptides act as selective agonists for RXFP1 and RXFP2, respectively (Kumagai et al., 2002; Sudo et al., 2003). The ectodomains of both receptors are important for ligand binding, as in the case of type A LGRs (Osuga et al., 1997). When the soluble ligand-binding region of RXFP1 (7BP) was administered subcutaneously to antagonize endogenous circulating relaxin during the last 4 days of pregnancy, delivery was delayed by 27 h, and nipple development was retarded (Hsu et al., 2002). It is likely that RXFP1-BP blocks the action of relaxin in late pregnancy by acting as a relaxin-binding protein and sequestering circulating relaxin. Although the phenotype is consistent with earlier work on relaxin (Zhao et al., 1999) and RXFP1 (Krajnc-Franken et al., 2004) null mice, the delay in parturition seems inconsistent. However, delivery of pups is prolonged in rats treated with a monoclonal antibody against relaxin (Lao-Guico et al., 1988), and some relaxin knockout mice were observed to have difficulties in giving birth (Zhao et al., 1999).

Relaxin is therefore the cognate ligand for RXFP1 and, although relaxin peptides from some species will activate RXFP2 at high (supraphysiological) concentrations, rat relaxin does not activate human, rat, or mouse RXFP2, indicating that in rodents, relaxin is not an RXFP2 ligand (Scott et al., 2005a). INSL3 is the cognate ligand for RXFP2, and the RXFP2 knockout mouse (Gorlov et al., 2002) shows an identical phenotype to the INSL3 knockout (Nef and Parada, 1999; Zimmermann et al., 1999). Studies crossing INSL3 overexpressing mice and RXFP2 and RXFP1 knockout mice establish that RXFP2 is the only receptor for INSL3, and there is no interaction between the INSL3/RXFP2 and relaxin/RXFP1 signaling systems *in vivo* (Bogatcheva et al., 2003; Kamat et al., 2004). However, as rodent relaxin will not interact with RXFP2, it is still possible that in other species relaxin peptides will interact with RXFP2 *in vivo*.

Thus, despite their structural similarity, relaxin and insulin family peptides act through independent signaling pathways: the relaxin group activates the GPCRs whereas the insulin group activates tyrosine kinases. Phylogenetic analysis of LGRs and coevolved relaxin family peptides from different metazoans suggests that, whereas the number of relaxin receptors remained constant during vertebrate evolution, the ancestral gene for relaxin duplicated multiple times in a vertebrate branch-specific manner (Hsu, 2003). Therefore, relaxin family peptides from different branches of vertebrates may have adapted to distinct physiological roles via a limited number of receptor genes. Preliminary studies with synthetic INSL4 (Lin et al., 2004) and INSL6 (Bogatcheva et al., 2003) indicate that they do not interact with RXFP1 or RXFP2.

B. RXFP3 and RXFP4

In contrast to the high-affinity interactions between relaxin and RXFP1 and INSL3 and RXFP2, relaxin-3 has a lower affinity for RXFP1 than relaxin (Bathgate et al., 2002a; Sudo et al., 2003). A recent study indicates that relaxin-3 is a ligand for two orphan receptors, RXFP3 (GPCR135; somatostatin and angiotensin-like peptide receptor) and RXFP4 (GPCR142; GPR100) (Liu et al., 2003a,b). These receptors (Fig. 6) differ structurally and functionally from RXFP1 and RXFP2 (Fig. 5). They have relatively short N-terminal extracellular domains and apparently couple exclusively to G_i. Studies using both native relaxin-3 purified from brain extracts and recombinant human relaxin-3 indicated that this hormone potently stimulated guanosine 5'-[thio]triphosphate binding and inhibited cAMP accumulation in cells overexpressing RXFP3 and RXFP4. Endogenous expression of RXFP3 in the rat brain is found in the paraventricular and supraoptic nuclei of the hypothalamus (Liu et al., 2003b) and regions with connections to the nucleus incertus, where relaxin-3 is also expressed (Bathgate

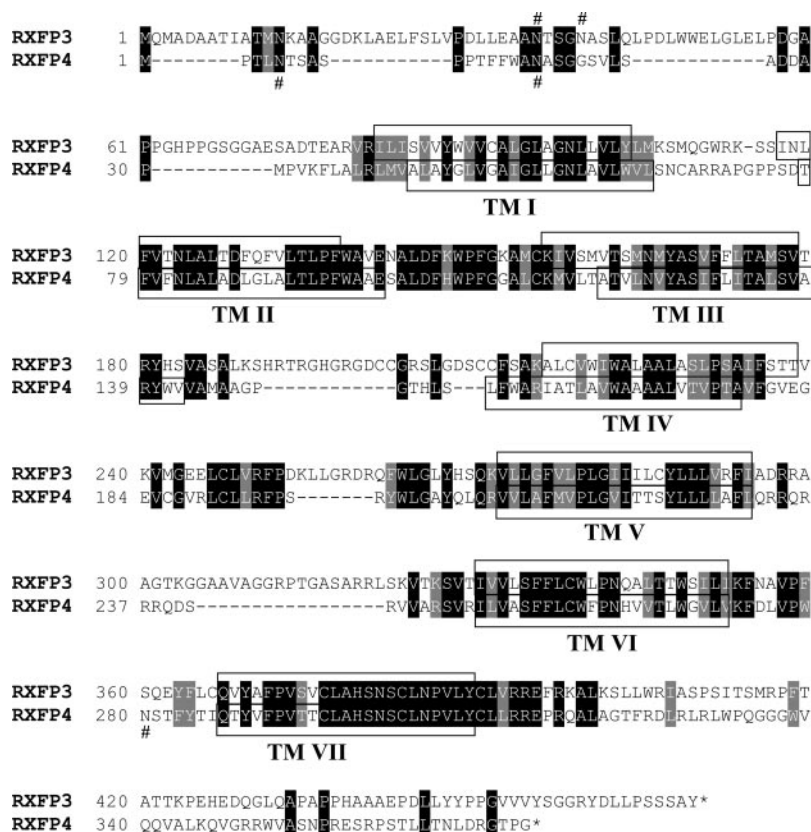


FIG. 6. Boxshade alignment of the human RXFP3 and RXFP4 receptors. Conserved amino acid residues are boxed in black, and conservative amino acid substitutions are boxed and shaded. Gaps have been introduced to aid the alignment where necessary. The putative transmembrane domains (derived from ENSEMBL) are boxed, and putative *N*-glycosylation sites are indicated (#)

et al., 2002a; Burazin et al., 2002). Interestingly, these are also regions where relaxin-binding sites were found (Osheroff and Phillips, 1991). RXFP4 binds to relaxin-3 with slightly lower affinity than RXFP3 and is less highly localized, with mRNA detected in the colon, thyroid, salivary gland, prostate, placenta, thymus, testis, kidney, and brain (Liu et al., 2003a). Although relaxin, INSL3, and INSL6 were shown not to interact with RXFP3 and RXFP4, a recent study demonstrated that INSL5 is a specific ligand for RXFP4 (Liu et al., 2005), and expressions of INSL5 and RXFP4 overlap extensively, indicating that RXFP4 is probably the endogenous receptor for INSL5.

VII. Functional Domains of Receptors for Relaxin Family Peptides

A. General Features of Leucine-Rich Repeat-Containing Receptors

There are three LGR subgroups that all have multiple LRRs but have differences in their ectodomain features including the unique “hinge” region linking the LRRs to the transmembrane domains (Fig. 7). Type A ectodomains contain 9 LRRs and include the receptors for FSH, LH, and TSH. Type B ectodomains contain 17 LRR and are currently orphan receptors. Type C ectodomains

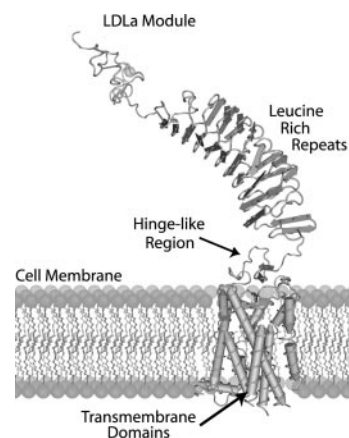


FIG. 7. Schematic representation of the putative structure of the RXFP1 and RXFP2 receptors. The major structural features include seven-transmembrane-spanning domains, hinge-like region, extracellular LRRs, and LDLa module. The model was constructed based on the structures of bovine rhodopsin (Okada and Palczewski, 2001) for the transmembrane domains, FSH receptor (Fan and Hendrickson, 2005) for the LRRs, and complement-like repeat CR8 from the low-density lipoprotein receptor-related protein (Huang et al., 1999) for the LDLa module. The LDLa module structure contains a Ca^{2+} molecule, which is necessary for the structure (Hopkins et al., 2005).

contain 10 LRRs and an N-terminal LDLa module and are the RXFP1 (LGR7) and RXFP2 (LGR8) receptors (Hsu, 2003). RXFP1 and RXFP2 receptors are highly conserved across species, with more than 90% sequence

similarity observed between rodent and primate receptors. Both receptors have N-linked glycosylation sites and activate adenylate cyclase to cause cAMP accumulation and stimulation of PKA pathways (Hsu, 2003). The LRR region of these receptors is made up of the individual repeats consisting of a β -strand and an α -helix connected by a turn (Fig. 7). The crystal structure of the ectodomain of the related FSH receptor has been solved (Fan and Hendrickson, 2005). The LRRs of the FSH receptor form an elongated tube-like structure, which is slightly curved, and FSH binds in a hand clasp fashion to the β -strands, which make up the inner concave surface of the LRRs. There is a differential distribution of electrostatic charge across the surfaces of the LRR region that is important for ligand recognition (Vassart et al., 2004). The crystallized FSH-FSH receptor complex demonstrates that the ligand receptor interface contains an exceptionally high buried-charge density. There are many direct interactions between acidic residues in the LRRs and basic residues in FSH.

It has been proposed that LGRs have an inherently noisy transmembrane domain, which is inhibited by the ectodomain that acts as a tethered inverse agonist. Once the hormone is bound to the LGR the ectodomain switches from a tethered inverse agonist to a full agonist interacting with the transmembrane region of the receptor (Vassart et al., 2004).

The interaction of the glycoprotein hormone receptors with their cognate ligands does not seem to require the seven-transmembrane region because there is high-affinity binding to the soluble ectodomain (Vassart et al., 2004; Fan and Hendrickson, 2005). Mutation of the exoloops of the seven TM region indicate that exoloop 2 and the hinge region have an important role in ligand recognition but appear to be important in binding and positioning of the ligand for interaction with the LRR rather than directly causing activation of the receptor (Vassart et al., 2004). The role of the seven TM region is also indicated by naturally occurring polymorphisms of the FSH receptor involving the seven TM region that show increased sensitivity to human chorionic gonadotropin and constitutive activity (Vassart et al., 2004).

The ectodomains of RXFP1 and RXFP2 are also the site of primary ligand binding. Ectodomain-only LGR7 (7BP; RXFP1-BP) and RXFP2 (8BP; RXFP2-BP) membrane anchored receptors demonstrate high-affinity binding of relaxin or INSL3 (Halls et al., 2005c). RXFP1-BP when cleaved from the cell surface will also bind relaxin and blocks its actions at the RXFP1 receptor presumably by acting as a relaxin-binding protein (Hsu et al., 2002). Indeed, RXFP1-BP acted in the same manner as antirelaxin antibodies (Hsu et al., 2002), and the effects were not observed if RXFP1-BP was boiled before addition to the system (Hsu et al., 2002).

B. Functional Domains of RXFP1 and RXFP2 (LGR7 and LGR8)

Both receptors are leucine-rich repeat-containing G-protein-coupled receptors with complex ectodomains (Fig. 7). For both the RXFP1 and RXFP2 a number of regions have been identified that are critical for ligand binding and signal transduction. These are the LDLa module, the LRRs and exoloop 2 of the TM domain (Fig. 7). Studies carried out using the wild-type receptors, receptor chimeras, and membrane-anchored ectodomains show that H2 relaxin activates both RXFP1 and RXFP2, whereas H3 relaxin only activates RXFP1 but not RXFP2 (Sudo et al., 2003). For both RXFP1 and RXFP2 the primary binding site is located in the LRR region because the RXFP1-BP and RXFP2-BP membrane anchored ectodomains, display high-affinity binding for ^{33}P -H2 relaxin (Halls et al., 2005c; Yan et al., 2005). Loss of the LDLa module does not affect binding, but signaling is abolished (R. A. D. Bathgate and A. J. W. Hsueh, unpublished data). Furthermore, optimal binding and signal transduction is only observed in receptors having an intact TM domain. The chimeric receptor LGR7/8 (RXFP1/2) containing the ectodomain of LGR7 and the TM domain of LGR8 displays decreased cAMP production in response to H3 relaxin and a reduced ability of H3 relaxin to compete for ^{33}P -H2 relaxin binding (Sudo et al., 2003). Replacement of exoloop 2 in the chimera with that of LGR7 restored both H3 relaxin binding and cAMP production (Sudo et al., 2003). This suggests that the TM2 loop has a role to play in binding and signal transduction. This is supported by recent studies (Halls et al., 2005c) demonstrating in the LGR7/8 chimera that there is reduced affinity and efficacy of H2 relaxin and increased affinity and efficacy of INSL3. In the RXFP2 (LGR8) receptor, the evidence for the presence of two binding sites is much stronger because ^{33}P -H2 relaxin identifies two binding sites with differing affinities (Halls et al., 2005c). The corresponding RXFP2-BP (LGR8-BP) displays only a single high-affinity site (Halls et al., 2005c).

Thus, in both the RXFP1 and RXFP2 receptors the LRRs form the primary binding site for the cognate ligands relaxin and INSL3. For RXFP1 it has been suggested that the contact motif is RXXXXXX(I/V) (Sudo et al., 2003). It has recently been demonstrated that this motif interacts with specific residues on the inner β -sheets of the LRRs of RXFP1 (Büllesbach and Schwabe, 2005b). Arg B-13 interacts with Glu²⁷⁷ and Asp²⁷⁹ of human RXFP1, whereas Arg B-16 interacts with Glu²³³ and Asp²³¹. Hydrophobic interactions between Ile B-20 and various amino acids on the inner β -sheets of the LRRs probably stabilize the binding interaction. It is likely that interaction of this motif with the LRRs would then allow the ligand to make contact with exoloop 2 of the TM domain that causes the conformation of the ectodomain to change, allowing the LDLa

module to direct G-protein coupling and subsequently adenylate cyclase activation.

Examination of the amino acid sequence of both the RXFP1 and RXFP2 receptors reveals *N*-glycosylation sites in the ectodomains and the sizes of RXFP1, RXFP2, RXFP1-BP, and RXFP2-BP on Western blots are consistent with the ectodomain being highly glycosylated (Hsu et al., 2002; Kumagai et al., 2002; Yan et al., 2005). The C-terminal regions of RXFP1 and RXFP2 have been examined for Ser, Thr, and Tyr residues that could represent sites for protein kinase phosphorylation. The following criteria were applied to determine the likely phosphorylation sites: 1) a score >0.5 derived from the program NetPhos (<http://www.cbs.dtu.dk/services/NetPhos>), 2) the vicinity of basic or acidic residues, proline residues, or other phosphorylated residues that are generally required for protein kinase binding, and 3) the conservation of target sequences across the species orthologs of each receptor. The RXFP1 receptor has two conserved motifs in the C-terminal tail that fulfill these criteria, an RQRKSMDSK motif immediately distal to helix 8 and a SQSTRLNYSYS motif at the C terminus (Fig. 5). These are similar to predicted and/or consensus sequences for protein kinase C (PKC) isozymes, including PKC ζ . In particular, the C-terminal motif has the required Arg at the -3 position and a hydrophobic residue at +1 (Nishikawa et al., 1997).

The RXFP2 receptor has a motif KHQRKSIF distal to helix 8 and similar to that in RXFP1 and an adjacent motif KIKKKSLS (Fig. 5). Again these motifs may represent target sites for particular PKC isozyme(s). The second motif is unlikely to represent a PKA phosphorylation site, as this kinase has high selectivity for Arg over Lys residues at the -3 and -2 positions (Pinna and Ruzzene, 1996). There is a third conserved motif (D/E)(D/E)SSSLK in the RXFP2 C-terminal tail (Fig. 5) that complies with the G-protein receptor kinase consensus sequence (Pinna and Ruzzene, 1996). G-protein receptor kinase phosphorylation of activated RXFP2 would represent an interesting point of divergence between this receptor and RXFP1 and may contribute to differences in activation of secondary signaling pathways.

C. Functional Domains of RXFP3 and RXFP4 (GPCR135 and GPCR142)

Much less is known about the functional domains of the RXFP3 and RXFP4 receptors. In contrast to RXFP1 and RXFP2 that have large (>300 amino acid), complex N-terminal ectodomains, the RXFP3 and RXFP4 receptors are typical neuropeptide-type receptors with shorter (<100 amino acid) N-terminal domains (Liu et al., 2003a,b) (Fig. 6). In contrast to RXFP1 and RXFP2 that signal through G α s to activate adenylate cyclase and cause increases in intracellular levels of cAMP, RXFP3, and RXFP4 signal through G α i to inhibit cAMP (Liu et al., 2003a,b). There are also differences in the

way in which the ligands interact with the receptors. RXFP1 and RXFP2 are activated only by relaxin family peptides that have A and B chains linked by the characteristic disulfide bonds and the separated A or B chains are totally inactive (Tan et al., 1998). However current evidence suggests that RXFP3 and RXFP4 do not interact with porcine relaxin, insulin, INSL3, INSL4, INSL6, or the relaxin-3 A chain but do interact with relaxin-3 and the relaxin-3 B chain (Liu et al., 2003a,b).

Examination of the C-terminal region of RXFP3 and RXFP4 for putative protein kinase phosphorylation sites shows that RXFP3 has conserved RKSSI and RR(L/R)SKV motifs at the distal ends of intracellular loops i1 and i3, respectively. Although these motifs are very similar to those for PKA and PKC isozymes, they do not comply completely with the consensus sequences. The i1 motif may be a PKA phosphorylation site, although the Lys at -2 is less favored than Arg or may be a substrate for PKC isozymes including PKC δ , PKC ϵ , and PKC ζ (Nishikawa et al., 1997). The i3 motif is less convincing, as the lack of a hydrophobic residue at +1 would be highly unusual for a PKA or PKC target site. Like RXFP3, RXFP4 has an R(Q/R)(Q/R)(Q/R)(Q/R/W)QD-SRV motif at the distal end of intracellular loop i3, but again this lacks the hydrophobic residue at the +1 position.

The suggestions regarding phosphorylation of the RXFP receptors have one caveat, namely that there are known kinase substrates that lack the typical consensus sequences. As seen for other G-protein-coupled receptors, hypotheses about receptor phosphorylation will have to be tested by experimental approaches including use of inhibitors, direct measurements of phosphorylation in the presence or absence of dominant-negative kinase mutants, and study of receptor mutants lacking the putative target residues.

VIII. Signaling Pathways Activated by Relaxin Family Peptides

A. Signaling in Response to Relaxin

Early studies of the biochemical effects of relaxin demonstrated an association with increases in cAMP in target tissues (Braddon, 1978; Cheah and Sherwood, 1980; Sanborn et al., 1980). Increased intracellular cAMP was observed in mouse pubic symphysis (Braddon, 1978), in rat uterus (Cheah and Sherwood, 1980; Judson et al., 1980; Sanborn et al., 1980), and in cultures of rat (Hsu et al., 1985) and rhesus monkey myometrium (Kramer et al., 1990), human endometrium (Chen et al., 1988; Fei et al., 1990), rat anterior pituitary cell (Cronin et al., 1987), and human THP-1 monocytes (Parsell et al., 1996). The increases in cAMP are important components in relaxin-induced myometrial inhibition in the rat (Dodge et al., 1999) and decidualization of human endometrial stromal cells (Huang et al., 1987; Tabanelli et al., 1992).

Although relaxin stimulation is clearly linked to increased cAMP in many tissues, it is not clear exactly how relaxin activates adenylate cyclase (Fig. 8). There seems to be at least some G-protein component of this increase in cAMP, consistent with the relaxin receptor RXFP1 being a G-protein coupled receptor (Hsu et al., 2002). The cAMP elevation in THP-1 and human endo-

metrial stromal cells can be blocked by inhibitors of tyrosine kinase or mitogen-activated protein kinase (MAPK) (Bartsch et al., 2001) and relaxin appears to activate MAPK in these cells (Zhang et al., 2002), although the activation is sporadic and can be inhibited by blocking PKA suggesting that it is a downstream event that follows elevation of intracellular cAMP (R. J. K.

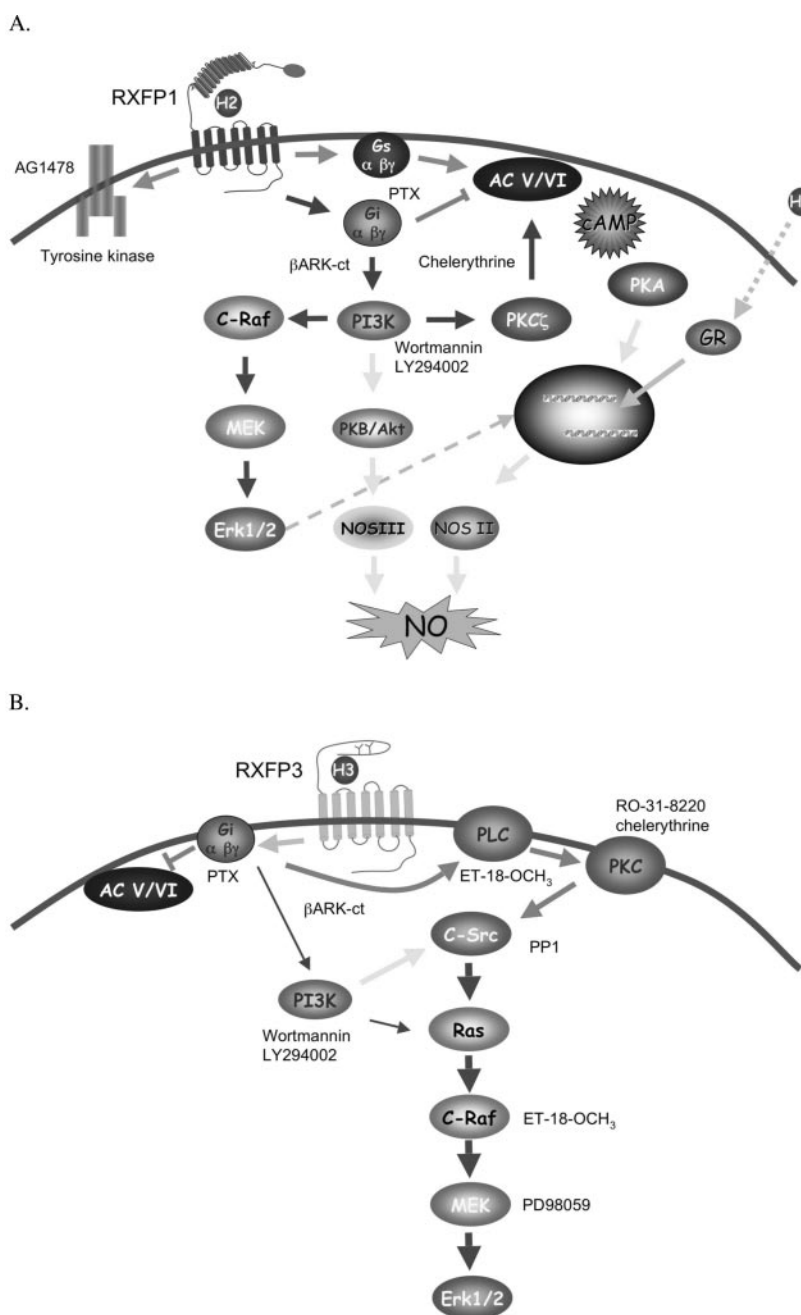


FIG. 8. Potential signaling pathways used by (A) the relaxin family peptide receptor 1 (RXFP1 or LGR7) or (B) the RXFP3 receptor in a variety of cell types. In A, the RXFP1 receptor can couple to adenylate cyclase (AC) via G_s and also couples to G_i . G-protein $\beta\gamma$ subunits then activate PI3-K that in turn activates PKC ζ , which can translocate to the cell membrane to both stimulate AC V/VI to enhance cAMP production and also switch on the protein kinase B (PKB/Akt) pathway to increase activation of NOSIII. cAMP will activate PKA, which can phosphorylate many signaling 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) MAPK pathway. There is also evidence that relaxin can enter the cell and directly activate nuclear GRs. 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. In B, the RXFP3 receptor couples to G_i to inhibit adenylate cyclase and accumulation of cAMP in response to forskolin. G_i $\beta\gamma$ subunits activate PKC (major pathway) and PI-3-kinase (minor pathway) that in turn activates the P42/P44 (Erk1/2) MAPK pathway. ET, endothelin; MEK, mitogen-activated protein kinase; PTX, pertussis toxin.

Anand-Ivell and O. Bartsch, personal communication). One suggestion from experiments in human endothelial stromal cells is that relaxin stimulation leads to the inhibition of a cell-specific phosphodiesterase (PDE) to induce sustained increases in cAMP (Bartsch et al., 2004). In THP-1 cells, the findings are equivocal with some studies indicating that the increase in cAMP is associated with PDE inhibition (Ivell et al., 2005) and others showing that blockade of PDEs by the general inhibitor isobutylmethylxanthine or the PDE8 inhibitor dipyridamole fails to alter the cAMP response to relaxin (Nguyen et al., 2003). In HEK293 cells expressing RXFP1 and a secreted alkaline phosphatase reporter gene containing a cAMP-response element, relaxin caused a marked response commensurate with an increase in intracellular cAMP (Halls et al., 2005a). In THP-1 cells or HEK293T cells expressing RXFP1 receptors cAMP responses to relaxin during the first 5 min of stimulation were not sensitive to PI3-K inhibition whereas responses to longer periods of stimulation were inhibited by wortmannin or LY294002 (Nguyen et al., 2003; Halls et al., 2005b). In HEK293 cells it was of interest that the PI3-K inhibitor-sensitive component of the response was abolished by pretreatment of cells with pertussis toxin, suggesting that PI3-K activation in HEK293 cells expressing RXFP1 may be via $\beta\gamma$ subunits derived from G_i (Halls et al., 2005b). Pertussis toxin sensitivity was not a feature of the responses in THP-1 cells, suggesting that if activation of PI3-K is by $\beta\gamma$ subunits, then they may be derived from G-proteins other than G_i in THP-1 cells (Nguyen et al., 2003). There is now (Nguyen and Dessauer, 2004) evidence that PKC ζ is the next step in PI3-K-mediated increases in cAMP production because inhibition of PKC with chelerythrine reduced and activation with sphingomyelinase increased the cAMP response to relaxin (Fig. 8). Relaxin-stimulated translocation of PKC ζ to the cell membrane in a number of cell lines, an effect that was inhibited by PKC ζ antisense oligonucleotide treatment that reduced PKC ζ mRNA and protein levels. The antisense oligonucleotide also reduced the relaxin-induced increase in cAMP levels, and the remaining response was insensitive to blockade by PI3-K inhibitors (Nguyen and Dessauer, 2004). The cAMP response to activation of RXFP1 receptors is clearly complex and can depend to some extent on the cell type and conditions of study.

This view is emphasized by the action of relaxin in other cell types where it is not associated with increases in cAMP but may still involve some component of G-protein-coupled signaling. In rat ventricular fibroblasts relaxin causes only a weak transient increase in cAMP (Samuel et al., 2004a) and studies in human lower uterine segment fibroblasts showed no increase in cAMP but did show MAPK activation (Palejwala et al., 1998, 2001). Thus, in fibroblasts, cAMP does not appear to be the predominant signaling mechanism but may link to MAPK signaling, which exerts the biological response.

Relaxin also activates Erk1/2 in a variety of cells known to express RXFP1 receptors (Zhang et al., 2002). In human endothelial stromal cells, THP-1 cells and in primary cultures of human coronary artery and pulmonary artery smooth muscle cells addition of H2 relaxin produced rapid (<5 min) phosphorylation of Erk1/2 with no change in levels of total Erk and the effect was blocked by inhibition of mitogen-activated protein kinase (Zhang et al., 2002) (Fig. 8). No evidence was obtained for activation of Akt or c-Jun N-terminal kinase in these cells by relaxin. The transcription factor cAMP-response element-binding protein was also activated by relaxin in endothelial stromal cells, but whether this results from the Erk1/2 cascade or the cAMP/PKA pathway is not clear at present (Zhang et al., 2002). Erk1/2 activation by relaxin has also been demonstrated in HeLa cells and human umbilical vein endothelial cells although the much longer time course of the effect (45–90 min) suggests that this may not be the result of a direct interaction with relaxin receptors (Dschiezig et al., 2003).

The positive inotropic and chronotropic effects of relaxin are also equivocal. Rabbit sinoatrial node cells respond to relaxin, and the effects appear to be mediated by increases in intracellular cAMP and activation of cAMP-dependent protein kinase (Han et al., 1994). Relaxin also stimulates the secretion of atrial natriuretic peptide from isolated perfused rat hearts (Toth et al., 1996). These effects were mediated through protein kinase C, although treatment with a cAMP-dependent protein kinase inhibitor also blocked atrial natriuretic peptide secretion as well as the chronotropic response, suggesting an involvement of cAMP. Measurement of cAMP levels in atria, which are likely to reflect the inotropic response, demonstrates only weak coupling to cAMP (Kompa et al., 2002). It is feasible that the chronotropic response of the few pacemaker cells in the sinoatrial node are mediated by cAMP, whereas the increased force of contraction of atrial myocytes uses another mechanism.

There is also considerable evidence that relaxin can act on cells both acutely and chronically by increasing the expression and or activity of NOS (Nistri and Bani, 2003). Two mechanisms have been suggested, activation of NOSIII by a G-protein-coupled receptor mechanisms and induction of NOSII mediated by cAMP (Fig. 8). Activation of NOSIII by relaxin would involve stimulation of RXFP1, interaction of G-protein $\beta\gamma$ subunits with PI3-K followed by activation of Akt and subsequently endothelial NOS by phosphorylation at Ser¹¹⁷⁹. Increased expression of NOSII would probably involve the increase in cAMP levels, activation of PKA, phosphorylation, and inactivation of I κ B thus promoting increased NOSII expression via nuclear factor κ B (Nistri and Bani, 2003). Recent studies also suggest that renal vasodilation in response to relaxin involves endothelin receptors. This mechanism involves up-regulation of matrix met-

aloproteinase activity by relaxin, followed by increased processing of big endothelin to yield bioactive ET₁₋₃₂, leading via ET_B receptors to activation of NOSIII and increased synthesis of NO (Conrad and Novak, 2004).

Relaxin has also been reported to interact with the glucocorticoid receptor (GR). Treatment with relaxin reduces the production of inflammatory cytokines by human macrophages in response to endotoxin (Dschietzig et al., 2004), and the effect is blocked by the GR antagonist RU486. Relaxin enters intact cells and is concentrated in the nucleus where it induces GR activation, nuclear translocation, and DNA binding (Fig. 8). It appears to act as a GR agonist and competes with GR agonists for binding to the GR. Because relaxins modified to have no action at the RXFP1 receptor retain activity at the GR, the effects appear to be mediated independently of an action on RXFP1.

B. Signaling in Response to Other Relaxin Family Peptides

Little is currently known of the mechanisms involved in signaling of other relaxin family peptides. The most data have been gleaned from studies on cloned receptors in transfected cell systems. When expressed in mammalian cells, LGR8 (RXFP2) responds to relaxin (Hsu et al., 2002) and INSL3 (Kumagai et al., 2002) with an increase in cAMP measured either directly (Hsu et al., 2002) or by an increase in secreted alkaline phosphatase in cells expressing RXFP2 and a cAMP-response element-responsive reporter gene (Halls et al., 2005a). Rat primary gubernacular cells in culture respond to both INSL3 and relaxin with an increase in cAMP (Kumagai et al., 2002). It is likely that RXFP2 shares signaling pathways with RXFP1, with cAMP being an important mediator of its cellular actions. However, recent studies indicate that INSL3 produces effects in testicular germ cells and oocytes by decreases in cAMP (Kawamura et al., 2004). These effects of INSL3 are blocked by pertussis toxin.

H3 relaxin will also increase cAMP in mammalian cells transfected with RXFP1 (Sudo et al., 2003) and THP-1 cells (Bathgate et al., 2002a), suggesting the same signaling pathway as relaxin. However, H3 relaxin acts at RXFP3 and RXFP4 receptors to inhibit cAMP accumulation, and the effect is blocked by pertussis toxin, indicating that the receptor is coupled to G_i, G_o, or G_z (Liu et al., 2003a,b) (Fig. 8). Recent studies also suggest that stimulation of RXFP3 expressed in Chinese hamster ovary-K1 cells activates Erk1/2 signaling (Van der Westhuizen et al., 2005). The signaling mechanisms for relaxin-3 via RXFP3/RXFP4 in vivo are currently unknown.

IX. Regulation of Relaxin Family Peptide Receptors

Very few studies have been conducted to examine the regulation of RXFP receptors. The most information is

available for RXFP1, and this information has primarily been obtained in studies not specifically designed to examine receptor regulation. Thus, in a study of relaxin receptor signaling in a rat model of cardiac failure, it was shown that RXFP1 mRNA was decreased, and this decrease corresponded with a reduced response to relaxin in animals in failure (Kompa et al., 2002). In male rats, treatment with 17 β -estradiol decreases the number of ³³P-H2 relaxin binding sites in atria and cerebral cortex whereas the same treatment in female rats had no effect in these tissues but increased the number of binding sites in uterine myometrium (Osheroff et al., 1992; Tan et al., 1999). More recently studies in human endometrium have shown marked increases in ³³P-H2 relaxin binding and RXFP1 mRNA expression in the secretory phase of the menstrual cycle although the agent responsible for the changes was not identified (Bond et al., 2004).

X. Nomenclature Issues for Relaxin Family Peptides and Their Receptors

A brief survey of the literature over the last 3 years provided a dozen or more synonyms for relaxin including human relaxin (Rln) H-2, human relaxin-2, relaxin-2, H2 relaxin, human gene 2 relaxin (hRLX2), human RLX, relaxin (RLX), rln-1, rhRLX (recombinant human relaxin), human relaxin 2, and H2-relaxin. To prevent further possible confusion among newcomers to the field (and even some of us in the field), we recommend that the human gene 1, gene 2, and gene 3 relaxin peptides be known as H1, H2, and H3 relaxin, respectively. Because most other animals possess the equivalent of H3 relaxin, it is suggested that these be known as "species" relaxin-3 (e.g., mouse relaxin-3). Only primates have the equivalent of H1 and H2 relaxins with most other species expressing only a single relaxin equivalent to H2 relaxin. It is suggested that these be known as "species" relaxin (e.g., mouse relaxin) to avoid the potential confusion caused by the attachment of numbers and comparison with the human forms.

Fortunately, INSL3 has a shorter history than relaxin, and there are fewer synonyms in the literature for this peptide. However, INSL3 was formerly known as Ley-I-L (Leydig insulin-like peptide) and RLF (relaxin-like factor) and has also been referred to as Leydig cell relaxin. It is suggested that "species" INSL3 is the appropriate nomenclature.

With regard to the receptors for relaxin family it is possible that RXFP1, RXFP2, RXFP3, and RXFP4 were all originally receptors for relaxin-3, which is the most ancient of the relaxin peptides. However, to avoid the potential confusion inherent in suggesting that all four receptors are relaxin receptors because the receptors have now evolved to recognize a wider range of more specialized ligands, it is suggested that the receptors be known as relaxin family peptide receptors (RXFP recep-

tors). The four receptors for relaxin family peptides, LGR7, LGR8, GPCR135, and GPCR142, would thus become "species" RXFP1, RXFP2, RXFP3, and RXFP4 receptors.

In conclusion, although the hormone relaxin was discovered almost 80 years ago, the isolation of the receptor that it activates and the elucidation of the signaling pathways involved in the cellular responses to the peptide have proved elusive. The past 5 years has seen the identification, not only of the receptor for relaxin but also three other receptors responding to relaxin family peptides with all four receptors being G-protein-coupled receptors. These discoveries have led very rapidly to the determination of the cognate ligands for the receptors, the tracing of their evolutionary history, and the determination of their signaling pathways. Although much still needs to be learned about the way in which relaxin family peptides alter the activity of cells, the promising progress in the area of development of antagonists for the receptors will provide valuable new tools to assist in this process.

Acknowledgments. We thank Professor Geoffrey Tregear for advice and encouragement, Dr. Bronwyn Evans, who analyzed RXFP1–4 receptors for putative protein kinase phosphorylation sites, and Daniel Scott and Tracey Wilkinson for assistance with figures.

REFERENCES

- Adham IM, Burkhardt E, Benahmed M, and Engel W (1993) Cloning of a cDNA for a novel insulin-like peptide of the testicular Leydig cells. *J Biol Chem* **268**:26668–26672.
- Adham IM, Steding G, Thamm T, Büllesbach EE, Schwabe C, Paprotta I, and Engel W (2002) The overexpression of the insl3 in female mice causes descent of the ovaries. *Mol Endocrinol* **16**:244–252.
- Amento EP, Samuel CS, Hu M, and Tian H (2001) Deletion of the relaxin gene causes age-related progressive dermal fibrosis. *Arthritis Rheum* **44**:A847.
- Bani D, Failli P, Bello MG, Thiemermann C, Bani Sacchi T, Bigazzi M, and Masini E (1998a) Relaxin activates the L-arginine-nitric oxide pathway in vascular smooth muscle cells in culture. *Hypertension* **31**:1240–1247.
- Bani D, Masini E, Bello MG, Bigazzi M, and Sacchi TB (1998b) Relaxin protects against myocardial injury caused by ischemia and reperfusion in rat heart. *Am J Pathol* **152**:1367–1376.
- Bani D, Nistri S, Quattrone S, Bigazzi M, and Sacchi TB (2001) Relaxin causes changes of the liver: in vivo studies in rats. *Horm Metab Res* **33**:175–180.
- Bani G, Bani ST, Bigazzi M, and Bianchi S (1988) Effects of relaxin on the microvasculature of mouse mammary gland. *Histol Histopathol* **3**:337–343.
- Bani-Sacchi T, Bigazzi M, Bani D, Mannaioni PF and Masini E (1995) Relaxin-induced increased coronary flow through stimulation of nitric oxide production. *Br J Pharmacol* **116**:1589–1594.
- Bartsch O, Bartlick B, and Ivell R (2001) Relaxin signalling links tyrosine phosphorylation to phosphodiesterase and adenylyl cyclase activity. *Mol Hum Reprod* **7**:799–809.
- Bartsch O, Bartlick B, and Ivell R (2004) Phosphodiesterase 4 inhibition synergizes with relaxin signaling to promote decidualization of human endometrial stromal cells. *J Clin Endocrinol Metab* **89**:324–334.
- Bathgate RAD, Balvers M, Hunt N, and Ivell R (1996) Relaxin-like factor gene is highly expressed in the bovine ovary of the cycle and pregnancy: sequence and messenger ribonucleic acid analysis. *Biol Reprod* **55**:1452–1457.
- Bathgate RAD, Hsueh AJW, and Sherwood OD (2006) Physiology and molecular biology of the relaxin peptide family, in *Knobil and Neill's Physiology of Reproduction* (Neill JD ed) pp 679–768, Academic Press, San Diego.
- Bathgate RAD, Moniac N, Bartlick B, Balvers M, Domagalski R, Dawson N, Wade JD, Tregear GW, and Ivell R (2001) The relaxin-like factor (RLF Insl3) is highly expressed in the ruminant ovary, in *Relaxin 2000: Proceedings of the Third International Conference on Relaxin and Related Peptides* (Tregear GW, Ivell R, Bathgate RA, and Wade JD eds) pp 349–356, Kluwer, Dordrecht, The Netherlands.
- Bathgate RAD, Samuel CS, Burazin TC, Gundlach AL, and Tregear GW (2003) Relaxin: new peptides, receptors and novel actions. *Trends Endocrinol Metab* **14**:207–213, 2003.
- Bathgate RAD, Samuel CS, Burazin TC, Layfield S, Claasz AA, Reytomas IG, Dawson NF, Zhao C, Bond CP, Summers RJ, et al. (2002a) Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene: novel members of the relaxin peptide family. *J Biol Chem* **277**:1148–1157.
- Bathgate RAD, Siebel AL, Tovote P, Claasz A, Macris M, Tregear GW, and Parry LJ (2002b) Purification and characterization of relaxin from the tammar wallaby (*Macropus eugenii*): bioactivity and expression in the corpus luteum. *Biol Reprod* **67**:293–300.
- Bieche I, Laurent A, Laurendeau I, Duret L, Giovangrandi Y, Frendo JL, Olivi M, Fausser JL, Evain-Brion D, and Vidaud M (2003) Placenta-specific INSL4 expression is mediated by a human endogenous retrovirus element. *Biol Reprod* **68**:1422–1429.
- Bigazzi M, Del MA, Petrucci F, Casali R, and Novelli GP (1986) The local administration of relaxin induces changes in the microcirculation of the rat mesocaecum. *Acta Endocrinol Copenh* **112**:296–299.
- Boels K and Schaller HC (2003) Identification and characterisation of GPR100 as a novel human G-protein-coupled bradykinin receptor. *Br J Pharmacol* **140**:932–938.
- Bogatcheva NV, Truong A, Feng S, Engel W, Adham IM, and Agoulnik AI (2003) GREAT/LGR8 is the only receptor for insulin-like 3 peptide. *Mol Endocrinol* **17**:2639–2646.
- Bond CP, Parry LJ, Samuel CS, Gehring HM, Lederman FL, Rogers PA, and Summers RJ (2004) Increased expression of the relaxin receptor (LGR7) in human endometrium during the secretory phase of the menstrual cycle. *J Clin Endocrinol Metab* **89**:3477–3485.
- Braddon SA (1978) Relaxin-dependent adenosine 6'5'-monophosphate concentration changes in the mouse pubic symphysis. *Endocrinology* **102**:1292–1299.
- Bryant-Greenwood G and Schwabe C (1994) Human relaxins: chemistry and biology. *Endocr Rev* **15**:5–26.
- Büllesbach EE and Schwabe C (1986) Preparation and properties of porcine relaxin derivatives shortened at the amino terminus of the A chain. *Biochemistry* **25**:5998–6004.
- Büllesbach EE and Schwabe C (1987) Relaxin structure: quasi allosteric effect of the NH₂-terminal A-chain helix. *J Biol Chem* **262**:12496–12501.
- Büllesbach EE and Schwabe C (1991) Total synthesis of human relaxin and human relaxin derivatives by solid-phase peptide synthesis and site-directed chain combination. *J Biol Chem* **266**:10754–10861.
- Büllesbach EE and Schwabe C (1994) Functional importance of the A chain loop in relaxin and insulin. *J Biol Chem* **269**:13124–13128.
- Büllesbach EE and Schwabe C (1995) A novel Leydig cell cDNA-derived protein is a relaxin-like factor. *J Biol Chem* **270**:16011–16015.
- Büllesbach EE and Schwabe C (1996) Introduction of relaxin properties into other hormones of insulin-like structure. *SAAS Bull Biochem Biotechnol* **9**:63–68.
- Büllesbach EE and Schwabe C (1999a) Tryptophan B27 in the relaxin-like factor (RLF) is crucial for RLF receptor-binding. *Biochemistry* **38**:3073–3078.
- Büllesbach EE and Schwabe C (1999b) Specific high affinity relaxin-like factor receptors. *J Biol Chem* **274**:22354–22358.
- Büllesbach EE and Schwabe C (2000) The relaxin receptor-binding site geometry suggests a novel gripping mode of interaction. *J Biol Chem* **275**:35276–35280.
- Büllesbach EE and Schwabe C (2002) The primary structure and the disulfide links of the bovine relaxin-like factor (RLF). *Biochemistry* **41**:274–281.
- Büllesbach EE and Schwabe C (2005a) LGR8 signal activation by the relaxin-like factor. *J Biol Chem* **280**:14586–14590.
- Büllesbach EE and Schwabe C (2005b) The trap-like relaxin-binding site of LGR7. *J Biol Chem* **280**:14051–14056.
- Büllesbach EE, Yang S, and Schwabe C (1992) The receptor-binding site of human relaxin II A dual prong-binding mechanism. *J Biol Chem* **267**:22957–22960.
- Burazin TC, Bathgate RAD, Macris M, Layfield S, Gundlach AL, and Tregear GW (2002) Restricted but abundant expression of the novel rat gene-3 (R3) relaxin in the dorsal tegmental region of brain. *J Neurochem* **82**:1553–1557.
- Burazin TCD, Johnson KJ, Ma S, Bathgate RAD, Tregear GW, and Gundlach AL (2005) Localization of LGR7 (relaxin receptor) mRNA and protein in rat forebrain: correlation with relaxin binding site distribution. *Ann NY Acad Sci* **1041**:205–210.
- Burkhardt E, Adham IM, Brosig B, Gastmann A, Mattei MG and Engel W (1994) Structural organization of the porcine and human genes coding for a Leydig cell-specific insulin-like peptide (LEY I-L) and chromosomal localization of the human gene (INSL3). *Genomics* **20**:13–19.
- Canova DE, Kessler TJ, Lee PJ, Fei DT, Griffin P, Stults JT, Wade JD, and Rinderknecht E (1991) Use of recombinant DNA derived human relaxin to probe the structure of the native protein. *Biochemistry* **30**:6006–6013.
- Casten GG and Boucek RJ (1958) Use of relaxin in the treatment of scleroderma. *J Am Med Assoc* **166**:319–324.
- Chassin D, Laurent A, Janneau JL, Berger R, and Bellet D (1995) Cloning of a new member of the insulin gene superfamily (INSL4) expressed in human placenta. *Genomics* **29**:465–470.
- Cheah SH and Sherwood OD (1980) Target tissues for relaxin in the rat: tissue distribution of injected ¹²⁵I-labeled relaxin and tissue changes in adenosine 3'5'-monophosphate levels after in vitro relaxin incubation. *Endocrinology* **106**:1203–1209.
- Chen GA, Huang JR, and Tseng L (1988) The effect of relaxin on cyclic adenosine 3'5'-monophosphate concentrations in human endometrial glandular epithelial cells. *Biol Reprod* **39**:519–525.
- Claasz AA, Bond CP, Bathgate RA, Otvos L, Dawson NF, Summers RJ, Tregear GW, and Wade JD (2002) Relaxin-like bioactivity of ovine insulin 3 (INSL3) analogues. *Eur J Biochem* **269**:6287–6293.
- Conklin D, Lofton-Day CE, Haldeman BA, Ching A, Whitmore TE, Lok S, and Jaspers S (1999) Identification of INSL5: a new member of the insulin superfamily. *Genomics* **60**:50–56.
- Conrad KP and Novak J (2004) Emerging role of relaxin in renal and cardiovascular function. *Am J Physiol* **287**:R250–R261.
- Coulson CC, Thorp JM Jr, Mayer DC and Cefalo RC (1996) Central hemodynamic effects of recombinant human relaxin in the isolated perfused rat heart model. *Obstet Gynecol* **87**:610–612.
- Cronin MJ, Malaska T and Bakht C (1987) Human relaxin increases cyclic AMP

- levels in cultured anterior pituitary cells. *Biochem Biophys Res Commun* **148**: 1246–1251.
- Danielson LA, Sherwood OD and Conrad KP (1999) Relaxin is a potent renal vasodilator in conscious rats. *J Clin Invest* **103**:525–533.
- Dawson NF, Tan YY, Macris M, Otvos L Jr, Summers RJ, Tregear GW, and Wade JD (1999) Solid-phase synthesis of ovine Leydig cell insulin-like peptide—a putative ovine relaxin? *J Pept Res* **53**:542–547.
- Del Borgo MP, Hughes RA, and Wade JD (2004) Design synthesis and analysis of single chain mimetics of relaxin and relaxin-like factor (INSL3), in *Peptides – Peptide Revolution: Genomics, Proteomics and Therapeutics* (Chorev M and Sawyer TK eds) pp 727–728, Springer, New York. Proceedings of the 18th American Peptide Symposium; 2003 Jul 19–23; Boston, MA.
- Dodge KL, Carr DW, and Sanborn BM (1999) Protein kinase A anchoring to the myometrial plasma membrane is required for cyclic adenosine 3'5'-monophosphate regulation of phosphatidylinositol turnover. *Endocrinology* **140**:5165–5170.
- Dodson GG, Eliopoulos EE, Isaacs NW, McCall MJ, Niall HD, and North ACT (1982) Rat relaxin: insulin-like fold predicts a likely receptor binding region. *Int J Biol Macromol* **4**:399–405.
- Dschietzig T, Bartsch C, Richter C, Laule M, Baumann G, and Stangl K (2003) Relaxin, a pregnancy hormone, is a functional endothelin-1 antagonist: attenuation of endothelin-1-mediated vasoconstriction by stimulation of endothelin type-B receptor expression via ERK-1/2 and nuclear factor- κ B. *Circ Res* **92**:32–40.
- Dschietzig T, Bartsch C, Stangl V, Baumann G, and Stangl K (2004) Identification of the pregnancy hormone relaxin as glucocorticoid receptor agonist. *FASEB J* **18**: 1536–1538.
- Du X-J, Samuel CS, Gao X-M, Zhao L, Parry LJ, and Tregear GW (2003) Increased myocardial collagen and ventricular diastolic dysfunction in relaxin deficient mice: a gender-specific phenotype. *Cardiovasc Res* **57**:395–404.
- Eigenbrot C, Randal M, Quan C, Burnier J, O'Connell L, Rinderknecht E, and Kossiakoff AA (1991) X-ray structure of human relaxin at 1.5 Å. Comparison to insulin and implications for receptor binding determinants. *J Mol Biol* **221**:15–21.
- Einspanier A, Muller D, Lubberstedt J, Bartsch O, Jurdzinski A, Fuhrmann K, and Ivell R (2001) Characterization of relaxin binding in the uterus of the marmoset monkey. *Mol Hum Reprod* **7**:963–970.
- Erikson MS and Unemori EN (2001) Relaxin clinical trials in systemic sclerosis, in *Proceedings of the Third International Conference on Relaxin and Related Peptides, 22–27 October 2000, Broome, Australia* (Tregear GW, Ivell R, Bathgate RA, and Wade JD eds) pp 373–381, Kluwer Publishers, Amsterdam, The Netherlands.
- Evans BA, John M, Fowler KJ, Summers RJ, Cronk M, Shine J, and Tregear GW (1993) The mouse relaxin gene: nucleotide sequence and expression. *J Mol Endocrinol* **10**:15–23.
- Failli P, Nistri S, Quattrone S, Mazzetti L, Bigazzi M, Sacchi TB, and Bani D (2002) Relaxin up-regulates inducible nitric oxide synthase expression and nitric oxide generation in rat coronary endothelial cells. *FASEB J* **16**:252–254.
- Fan QR and Hendrickson WA (2005) Structure of human follicle-stimulating hormone in complex with its receptor. *Nature (Lond)* **433**:269–277.
- Fei DT, Gross MC, Lofgren JL, Mora-Worms M, and Chen AB (1990) Cyclic AMP response to recombinant human relaxin by cultured human endometrial cells—a specific and high throughput in vitro bioassay. *Biochem Biophys Res Commun* **170**:214–222.
- Fu P, Shen P-J, Zhao C-X, Scott DJ, Samuel CS, Wade JD, Tregear GW, Bathgate RAD, and Gundlach AL (2005) Detection localization and action of LGR8 receptors in adult and developing rat kidney. *Ann NY Acad Sci* **1041**:516–519.
- Garber SL, Mirochnik Y, Brecklin CS, Unemori EN, Singh AK, Slobodskoy L, Grove BH, Arruda JA, and Dunea G (2001) Relaxin decreases renal interstitial fibrosis and slows progression of renal disease. *Kidney Int* **59**:876–882.
- Garibay-Tupas JL, Maaskant RA, Greenwood FC, and Bryant-Greenwood GD (1995) Characteristics of the binding of ³²P-labelled human relaxins to the human fetal membranes. *J Endocrinol* **145**:441–448.
- Gavino ES and Furst DE (2001) Recombinant relaxin: a review of pharmacology and potential therapeutic use. *BioDrugs* **15**:609–614.
- Goldsmith LT, Weiss G, Palejwala S, Plant TM, Wojtczuk A, Lambert WC, Ammur N, Heller D, Skurnick JH, Edwards D, et al. (2004) Relaxin regulation of endometrial structure and function in the rhesus monkey *Proc Natl Acad Sci USA* **101**:4685–4689.
- Gorlov IP, Kamat A, Bogatcheva NV, Jones E, Lamb DJ, Truong A, Bishop CE, McElreavey K, and Agoulnik AI (2002) Mutations of the GREAT gene cause cryptorchidism. *Hum Mol Genet* **11**:2309–2318.
- Goto M, Swanson LW, and Canteras NS (2001) Connections of the nucleus incertus. *J Comp Neurol* **438**:86–122.
- Halls ML, Bathgate RAD, Roche PJ, and Summers RJ (2005a) Signaling pathways of the LGR7 and LGR8 receptors determined by reporter genes. *Ann NY Acad Sci* **1041**:292–295.
- Halls ML, Bathgate RAD, and Summers RJ (2005b) Signal switching following stimulation of LGR7 receptors by human relaxin 2. *Ann NY Acad Sci* **1041**:288–291.
- Halls ML, Bond CP, Sudo S, Kumagai J, Ferraro T, Layfield S, Bathgate RAD, and Summers RJ (2005c) Multiple binding sites revealed by interaction of relaxin family peptides with native and chimeric relaxin family peptide receptors 1 and 2 (LGR7 & LGR8). *J Pharmacol Exp Ther* **313**:677–687.
- Han X, Habuchi Y, and Giles WR (1994) Relaxin increases heart rate by modulating calcium current in cardiac pacemaker cells. *Circ Res* **74**:537–541.
- Hisaw FL (1926) Experimental relaxation of the pubic ligament of the guinea pig. *Proc Soc Exp Biol Med* **23**:661–663.
- Hombach-Klonisch S, Buchmann J, Sarun S, Fischer B, and Klonisch T (2000) Relaxin-like factor (RLF) is differentially expressed in the normal and neoplastic human mammary gland. *Cancer* **89**:2161–2168.
- Hombach-Klonisch S, Hoang-Vu C, Kehlens A, Hinze R, Holzhausen HJ, Weber E, Fischer B, Dralle H, and Klonisch T (2003) INSL-3 is expressed in human hyperplastic and neoplastic thyrocytes. *Int J Oncol* **22**:993–1001.
- Hombach-Klonisch S, Seeger S, Tschedschilsuren G, Buchmann J, Huppertz B, Seliger G, Fischer B, and Klonisch T (2001) Cellular localization of human relaxin-like factor in the cyclic endometrium and placenta. *Mol Hum Reprod* **7**:349–356.
- Hopkins EJ, Bathgate RAD, and Gooley PR (2005) The human LGR7 low-density lipoprotein class A module requires calcium for structure. *Ann NY Acad Sci* **1040**:27–34.
- Hsu CJ, McCormack SM, and Sanborn BM (1985) The effect of relaxin on cyclic adenosine 3'5'-monophosphate concentrations in rat myometrial cells in culture. *Endocrinology* **116**:2029–2035.
- Hsu S (1999) Cloning of two novel mammalian paralogs of relaxin/insulin family proteins and their expression in testis and kidney *Mol Endocrinol* **13**:2163–2174.
- Hsu SY (2003) New insights into the evolution of the relaxin-LGR signaling system. *Trends Endocrinol Metab* **14**:303–309.
- Hsu SY, Kudo M, Chen T, Nakabayashi K, Bhalla A, van der Spek PJ, van Duin M, and Hsueh AJ (2000) The three subfamilies of leucine-rich repeat-containing G protein-coupled receptors (LGR): identification of LGR6 and LGR7 and the signaling mechanism for LGR7. *Mol Endocrinol* **14**:1257–1271.
- Hsu SY, Liang SG, and Hsueh AJ (1998) Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled seven-transmembrane region. *Mol Endocrinol* **12**:1830–1845.
- Hsu SY, Nakabayashi K, Nishi S, Kumagai J, Kudo M, Sherwood OD, and Hsueh AJ (2002) Activation of orphan receptor by the hormone relaxin *Science (Wash DC)* **295**:637–638.
- Huang JR, Tseng L, Bischof P, and Janne OA (1987) Regulation of prolactin production by progestin estrogen and relaxin in human endometrial stromal cells. *Endocrinology* **121**:2011–2017.
- Huang W, Dolmer K, and Gettins PG (1999) NMR solution structure of complement-like repeat CR8 from the low density lipoprotein receptor-related protein. *J Biol Chem* **274**:14130–14136.
- Hudson P, Haley J, John M, Cronk M, Crawford R, Haralambidis J, Tregear G, Shine J, and Niall H (1983) Structure of a genomic clone encoding biologically active human relaxin. *Nature (Lond)* **301**:628–631.
- Hudson P, John M, Crawford R, Haralambidis J, Scanlon D, Gorman J, Tregear G, Shine J, and Niall H (1984) Relaxin gene expression in human ovaries and the predicted structure of a human preprorelaxin by analysis of cDNA clones. *EMBO (Eur Mol Biol Organ) J* **3**:2333–2339.
- Hwang JJ, Lee AB, Fields PA, Haab LM, Mojonnier LE, and Sherwood OD (1991) Monoclonal antibodies specific for rat relaxin V: passive immunization with monoclonal antibodies throughout the second half of pregnancy disrupts development of the mammary apparatus and hence lactational performance in rats. *Endocrinology* **129**:3034–3042.
- Irving-Rodgers HF, Bathgate RAD, Ivell R, Domagalski R, and Rodgers RJ (2002) Dynamic changes in the expression of relaxin-like factor (INSL3), cholesterol side-chain cleavage cytochrome p450, and β -hydroxysteroid dehydrogenase in bovine ovarian follicles during growth and atresia. *Biol Reprod* **66**:934–943.
- Ivell R, Anand-Ivell R, and Bartsch O (2005) Relaxin signaling from natural receptors. *Ann NY Acad Sci* **1041**:280–287.
- Ivell R, Balvers M, Pohnke Y, Telgmann R, Bartsch O, Milde-Langosch K, Bamberger A-M, and Einspanier A (2003) Immunoeexpression of the relaxin receptor LGR7 in breast and uterine tissues of humans and primates. *Reprod Biol Endocrinol* **1**:114.
- Ivell R and Hartung S (2003) The molecular basis of cryptorchidism. *Mol Hum Reprod* **9**:175–181.
- John MJ, Borjesson BW, Walsh JR, and Niall HD (1981) Limited sequence homology between porcine and rat relaxins: implications for physiological studies. *Endocrinology* **108**:726–729.
- Johnson MR, Abdalla H, Allman AC, Wren ME, Kirkland A, and Lightman SL (1991) Relaxin levels in ovum donation pregnancies. *Fertil Steril* **56**:59–61.
- Johnson MR, Brooks AA, and Steer PJ (1996) The role of relaxin in the pregnancy associated reduction in plasma osmolality. *Hum Reprod* **11**:1105–1108.
- Judson DG, Pay S, and Bhoola KD (1980) Modulation of cyclic AMP in isolated rat uterine tissue slices by porcine relaxin. *J Endocrinol* **87**:153–159.
- Kakouris H, Eddie LW, and Summers RJ (1992) Cardiac effects of relaxin in rats. *Lancet* **339**:1076–1078.
- Kamat AA, Feng S, Bogatcheva NV, Truong A, Bishop CE, and Agoulnik AI (2004) Genetic targeting of relaxin and insulin-like factor 3 receptors in mice. *Endocrinology* **145**:4712–4720.
- Kawamura K, Kumagai J, Sudo S, Chun SY, Pisarska M, Morita H, Toppari J, Fu P, Wade JD, Bathgate RAD, et al. (2004) Paracrine regulation of mammalian oocyte maturation and male germ cell survival. *Proc Natl Acad Sci USA* **101**:7323–7328.
- Kibblewhite D, Larrabee WJ, and Sutton D (1992) The effect of relaxin on tissue expansion. *Arch Otolaryngol Head Neck Surg* **118**:153–156.
- Kizawa H, Nishi K, Ishibashi Y, Harada M, Asano T, Ito Y, Suzuki N, Hinuma S, Fujisawa Y, Onda H, et al. (2003) Production of recombinant human relaxin 3 in AtT20 cells. *Regul Pept* **113**:79–84.
- Klonisch T, Fowler PA, and Hombach-Klonisch S (2004) Molecular and genetic regulation of testis descent and external genitalia development. *Dev Biol* **270**:1–18.
- Koay ES, Bryant GG, Yamamoto SY, and Greenwood FC (1986) The human fetal membranes: a target tissue for relaxin. *J Clin Endocrinol Metab* **62**:513–521.
- Kobe B and Kajava AV (2001) The leucine-rich repeat as a protein recognition motif. *Curr Opin Struct Biol* **11**:725–732.
- Kohsaka T, Min G, Lukas G, Trupin S, Campbell ET, and Sherwood OD (1998) Identification of specific relaxin-binding cells in the human female. *Biol Reprod* **59**:991–999.
- Koman A, Cazaubon S, Couraud PO, Ullrich A, and Strosberg AD (1996) Molecular characterization and in vitro biological activity of placentin, a new member of the insulin gene family. *J Biol Chem* **271**:20238–20241.
- Kompa AR, Samuel CS, and Summers RJ (2002) Inotropic responses to human gene

- 2 (B29) relaxin in a rat model of myocardial infarction (MI): effect of pertussis toxin. *Br J Pharmacol* **137**:710–718.
- Krajnc-Franken MA, van Disseldorp AJ, Koenders JE, Mosselman S, van Duin M, and Gossen JA (2004) Impaired nipple development and parturition in LGR7 knockout mice. *Mol Cell Biol* **24**:687–696.
- Kramer SM, Gibson UE, Fendly BM, Mohler MA, Drolet DW, and Johnston PD (1990) Increase in cyclic AMP levels by relaxin in newborn rhesus monkey uterus cell culture. *In Vitro Cell Dev Biol* **26**:647–656.
- Kubota Y, Temelcos C, Bathgate RAD, Smith KJ, Scott D, Zhao C, and Hutson JM (2002) The role of insulin 3 testosterone Müllerian inhibiting substance and relaxin in rat gubernacular growth. *Mol Hum Reprod* **8**:900–905.
- Kuenzi MJ, Connolly BA, and Sherwood OD (1995) Relaxin acts directly on rat mammary nipples to stimulate their growth. *Endocrinology* **136**:2943–2947.
- Kuenzi MJ and Sherwood OD (1995) Immunohistochemical localization of specific relaxin-binding cells in the cervix, mammary glands, and nipples of pregnant rats. *Endocrinology* **136**:1367–1373.
- Kumagai J, Hsu SY, Matsumi H, Roh JS, Fu P, Wade JD, Bathgate RAD, and Hsueh AJ (2002) INSL3/Leydig insulin-like peptide activates the LGR8 receptor important in testis descent. *J Biol Chem* **277**:31283–31286.
- Lao-Guico LM, Voss EWJ, and Sherwood OD (1988) Monoclonal antibodies specific for rat relaxin. I. Production and characterization of monoclonal antibodies that neutralize rat relaxin's bioactivity in vivo. *Endocrinology* **123**:2472–2478.
- Lee AB, Hwang JJ, Haab LM, Fields PA, and Sherwood OD (1992) Monoclonal antibodies specific for rat relaxin. VI. Passive immunization with monoclonal antibodies throughout the second half of pregnancy disrupts histological changes associated with cervical softening at parturition in rats. *Endocrinology* **130**:2386–2391.
- Lin F, Otvos L Jr, Kumagai J, Tregear GW, Bathgate RAD, and Wade JD (2004) Synthetic human insulin 4 does not activate the G-protein-coupled receptors LGR7 or LGR8. *J Pept Sci* **10**:257–264.
- Lindheimer MD, Barron WM, and Davison JM (1989) Osmoregulation of thirst and vasopressin release in pregnancy. *Am J Physiol* **257** (Pt 2):F159–F169.
- Liu C, Chen J, Sutton S, Roland B, Kuei C, Farmer N, Sillard R, and Lovenberg TW (2003a) Identification of relaxin-3/INSL7 as a ligand for GPCR142. *J Biol Chem* **278**:50765–50770.
- Liu C, Eriste E, Sutton S, Chen J, Roland B, Kuei C, Farmer N, Jörnvall H, Sillard R, and Lovenberg TW (2003b) Identification of relaxin-3/INSL7 as an endogenous ligand for the orphan G-protein-coupled receptor GPCR135. *J Biol Chem* **278**:50754–50764.
- Liu C, Kuei C, Sutton S, Chen J, Bonaventure P, Wu J, Nepomuceno D, Kamme F, Tran DT, Zhu J, et al. (2005) INSL5 is a high affinity specific agonist for GPCR142 (GPR100). *J Biol Chem* **280**:292–300.
- Lok S, Johnston DS, Conklin D, Lofton-Day CE, Adams RL, Jelmberg AC, Whitmore TE, Schrader S, Griswold MD, and Jaspers RP (2000) Identification of INSL6, a new member of the insulin family that is expressed in the testis of the human and rat. *Biol Reprod* **62**:1593–1599.
- Longo M, Jain V, Vedernikov YP, Garfield RE, and Saade GR (2003) Effects of recombinant human relaxin on pregnant rat uterine artery and myometrium in vitro. *Am J Obstet Gynecol* **188**:1468–1474.
- Luna JJ, Riesewijk A, Horcajadas JA, de van Os R, Dominguez F, Mosselman S, Pellicer A, and Simon C (2004) Gene expression pattern and immunoreactive protein localisation of LGR7 receptor in human endometrium throughout the menstrual cycle. *Mol Hum Reprod* **10**:85–90.
- Masini E, Bani D, Bello MG, Bigazzi M, Mannaioni PF, and Sacchi TB (1997) Relaxin counteracts myocardial damage induced by ischemia-reperfusion in isolated guinea pig hearts: evidence for an involvement of nitric oxide. *Endocrinology* **138**:4713–4720.
- Massicotte G, Parent A, and St-Louis J (1989) Blunted responses to vasoconstrictors in mesenteric vasculature but not in portal vein of spontaneously hypertensive rats treated with relaxin. *Proc Soc Exp Biol Med* **190**:254–259.
- Mathieu MN, Wade JD, Tregear GW, Bond CP, Summers RJ, Catimel B, Nice EC, and Otvos L (2001) Synthesis conformational studies and biological activity of α -mono-biotinylated rat relaxin. *J Pept Res* **57**:374–382.
- Matsumoto M, Kamohara M, Sugimoto T, Hidaka K, Takasaki J, Saito T, Okada M, Yamaguchi T, and Furuichi K (2000) The novel G-protein coupled receptor SALPR shares sequence similarity with somatostatin and angiotensin receptors. *Gene* **248**:183–189.
- Mazella J, Tang M, and Tseng L (2004) Disparate effects of relaxin and TGF β 1: relaxin increases but TGF β 1 inhibits the relaxin receptor and the production of IGFBP-1 in human endometrial stromal/decidual cells. *Hum Reprod* **19**:1513–1518.
- McKinley MJ, Allen AM, Burns P, Colvill LM, and Oldfield BJ (1998) Interaction of circulating hormones with the brain: the roles of the subfornical organ and the organum vasculosum of the lamina terminalis. *Clin Exp Pharm Physiol* **25** (Suppl):S61–S67.
- McKinley MJ, Burns P, Colvill LM, Oldfield BJ, Wade JD, Weisinger RS, and Tregear GW (1997) Distribution of Fos immunoreactivity in the lamina terminalis and hypothalamus induced by centrally administered relaxin in conscious rats. *J Neuroendocrinol* **9**:431–437.
- McMurtry JP, Floersheim GL, and Bryant GG (1980) Characterization of the binding of 125 I-labelled succinylated porcine relaxin to human and mouse fibroblasts. *J Reprod Fertil* **58**:43–49.
- McMurtry JP, Kwok SC, and Bryant-Greenwood GD (1978) Target tissues for relaxin identified in vitro with 125 I-labelled porcine relaxin. *J Reprod Fertil* **53**:209–216.
- Mercado SR, Goodwin B, Ueno MS, Yamamoto SY, and Bryant GG (1982) Relaxin receptors in the myometrium and cervix of the pig. *Biol Reprod* **26**:120–128.
- Mercado-Simmen RC, Bryant-Greenwood GD, and Greenwood FC (1980) Characterization of the binding of 125 I-relaxin to rat uterus. *J Biol Chem* **255**:3617–3623.
- Min G and Sherwood OD (1996) Identification of specific relaxin-binding cells in the cervix, mammary glands, nipples, small intestine and skin of pregnant pigs. *Biol Reprod* **55**:1243–1252.
- Min G and Sherwood OD (1998) Localization of specific relaxin-binding cells in the ovary and testis of pigs. *Biol Reprod* **59**:401–408.
- Muda M, He C, Martini PG, Ferraro T, Layfield S, Taylor D, Chevrier C, Schweickhardt R, Kelton C, Ryan PL, et al. (2005) Splice variants of the relaxin and INSL3 receptors reveal unanticipated molecular complexity. *Mol Hum Reprod* **11**:591–600.
- Nef S and Parada LF (1999) Cryptorchidism in mice mutant for Insl3. *Nat Genet* **22**:295–299.
- Nguyen BT and Dessauer CW (2004) Relaxin stimulates protein kinase C ζ translocation: requirement for cyclic adenosine 3',5'-monophosphate production. *Mol Endocrinol* **19**:1012–1023.
- Nguyen BT, Yang L, Sanborn BM, and Dessauer CW (2003) Phosphoinositide 3-kinase activity is required for biphasic stimulation of cyclic adenosine 3',5'-monophosphate by relaxin. *Mol Endocrinol* **17**:1075–1084.
- Nguyen MT, Showalter PR, Timmons CF, Nef S, Parada LF, and Baker LA (2002) Effects of orchiectomy on congenitally cryptorchid insulin-3 knockout mice. *J Urol* **168**:1779–1783.
- Nishikawa K, Tokar A, Johannes FJ, Songyang Z, and Cantley LC (1997) Determination of the specific substrate sequence motifs of protein kinase C isozymes. *J Biol Chem* **272**:952–960.
- Nistri S and Bani D (2003) Relaxin receptors and nitric oxide synthases: search for the missing link. *Reprod Biol Endocrinol* **1**:5.
- Novak J, Danielson LA, Kerchner LJ, Sherwood OD, Ramirez RJ, Moalli PA, and Conrad KP (2001) Relaxin is essential for renal vasodilation during pregnancy in conscious rats. *J Clin Invest* **107**:1469–1475.
- Okada T and Palczewski K (2001) Crystal structure of rhodopsin: implications for vision and beyond. *Curr Opin Struct Biol* **11**:420–426.
- Omi EC, Zhao S, Shanks RD, and Sherwood OD (1997) Evidence that systemic relaxin promotes moderate water consumption during late pregnancy in rats. *J Endocrinol* **153**:33–40.
- Osheroff PL (1995) Relaxin receptors in the rat uterus brain and heart, in *Progress in Relaxin Research: The Proceedings of the Second International Congress on the Hormone Relaxin* (MacLennan AH, Tregear GW, and Bryant-Greenwood GD eds) pp 159–176, Global Publications, Adelaide, Australia.
- Osheroff PL, Cronin MJ, and Lofgren JA (1992) Relaxin binding in the rat heart atrium. *Proc Natl Acad Sci USA* **89**:2384–2388.
- Osheroff PL and Ho WH (1993) Expression of relaxin mRNA and relaxin receptors in postnatal and adult rat brains and hearts: localization and developmental patterns. *J Biol Chem* **268**:15193–15199.
- Osheroff PL and King KL (1995) Binding and cross-linking of 32 P-labeled human relaxin to human uterine cells and primary rat atrial cardiomyocytes. *Endocrinology* **136**:4377–4381.
- Osheroff PL, Ling VT, Vandlen RL, Cronin MJ, and Lofgren JA (1990) Preparation of biologically active 32 P-labeled human relaxin: displaceable binding to rat uterus cervix and brain. *J Biol Chem* **265**:9396–9401.
- Osheroff PL and Phillips HS (1991) Autoradiographic localization of relaxin binding sites in rat brain. *Proc Natl Acad Sci USA* **88**:6413–6417.
- Osuga Y, Hayashi M, Kudo M, Conti M, Kobilka B, and Hsueh AJ (1997) Co-expression of defective luteinizing hormone receptor fragments partially reconstitutes ligand-induced signal generation. *J Biol Chem* **272**:25006–25012.
- Overbeek PA, Gorlov IP, Sutherland RW, Houston JB, Harrison WR, Boettger-Tong HL, Bishop CE, and Agoulnik AI (2001) A transgenic insertion causing cryptorchidism in mice. *Genesis* **30**:26–35.
- Palejwala S, Stein D, Wojtczuk A, Weiss G, and Goldsmith LT (1998) Demonstration of a relaxin receptor and relaxin-stimulated tyrosine phosphorylation in human lower uterine segment fibroblasts. *Endocrinology* **139**:1208–1212.
- Palejwala S, Stein DE, Weiss G, Monia BP, Tortoriello D, and Goldsmith LT (2001) Relaxin positively regulates matrix metalloproteinase expression in human lower uterine segment fibroblasts using a tyrosine kinase signaling pathway. *Endocrinology* **142**:3405–3413.
- Parsell DA, Mak JY, Amento EP, and Unemori EN (1996) Relaxin binds to and elicits a response from cells of the human monocytic cell line THP-1. *J Biol Chem* **271**:27936–27941.
- Piedras-Renteria ES, Sherwood OD, and Best PM (1997a) Effects of relaxin on rat atrial myocytes. I. Inhibition of I_{to} via PKA-dependent phosphorylation. *Am J Physiol* **272** (Pt 2):H1791–H1797.
- Piedras-Renteria ES, Sherwood OD, and Best PM (1997b) Effects of relaxin on rat atrial myocytes. II. Increased calcium influx derived from action potential prolongation. *Am J Physiol* **272** (Pt 2):H1798–H1803.
- Pinna LA and Ruzzene M (1996) How do protein kinases recognize their substrates? *Biochim Biophys Acta* **1314**:191–225.
- Roche PJ, Butkus A, Wintour EM, and Tregear GW (1996) Structure and expression of Leydig insulin-like peptide mRNA in the sheep. *Mol Cell Endocrinol* **121**:171–177.
- Samuel CS, Tian H, Zhao L, and Amento EP (2003a) Relaxin is a key mediator of prostate growth and male reproductive tract development. *Lab Invest* **83**:1055–1067.
- Samuel CS, Unemori EN, Mookerjee I, Bathgate RAD, Layfield SL, Mak J, Tregear GW, and Du X (2004a) Relaxin modulates cardiac fibroblast proliferation differentiation and collagen production and reverses cardiac fibrosis in vivo. *Endocrinology* **145**:4125–4133.
- Samuel CS, Zhao C, Bathgate RAD, Bond CP, Burton MD, Parry LJ, Summers RJ, Tang ML, Amento EP, and Tregear GW (2003b) Relaxin deficiency in mice is associated with an age-related progression of pulmonary fibrosis. *FASEB J* **17**:121–123.
- Samuel CS, Zhao C, Bond CP, Hewitson TD, Amento EP, and Summers RJ (2004b) Relaxin-1-deficient mice develop an age-related progression of renal fibrosis. *Kidney Int* **65**:2054–2064.
- Sanborn BM (2001) Hormones and calcium: mechanisms controlling uterine smooth muscle contractile activity. The Litchfield Lecture. *Exp Physiol* **86**:223–237.
- Sanborn BM, Kuo HS, Weisbrodt NW, and Sherwood OD (1980) The interaction of

- relaxin with the rat uterus. I. Effect on cyclic nucleotide levels and spontaneous contractile activity. *Endocrinology* **106**:1210–1215.
- Schwabe C and McDonald JK (1977a) Primary structure of the B-chain of porcine relaxin. *Biochem Biophys Res Commun* **75**:503–510.
- Schwabe C and McDonald JK (1977b) Relaxin: a disulfide homolog of insulin. *Science (Wash DC)* **197**:914–915.
- Schwabe C, McDonald JK, and Steinetz BG (1976) Primary structure of the A chain of porcine relaxin. *Biochem Biophys Res Commun* **70**:397–405.
- Scott DJ, Fu P, Shen P-J, Gundlach A, Layfield S, Riesewijk A, Tregear GW, and Bathgate RAD (2005a) Characterisation of the rat INSL3 receptor. *Ann NY Acad Sci* **1041**:13–16.
- Scott DJ, Layfield S, Riesewijk A, Morita H, Tregear GW, and Bathgate RAD (2004) Identification and characterization of the mouse and rat relaxin receptors as the novel orthologues of human leucine-rich repeat-containing G-protein-coupled receptor 7. *Clin Exp Pharmacol Physiol* **31**:828–832.
- Scott DJ, Layfield S, Riesewijk A, Morita H, Tregear GW, and Bathgate RAD (2005b) Characterisation of the mouse and rat relaxin receptors. *Ann NY Acad Sci* **1041**: 8–12.
- Scott DJ, Tregear GW, and Bathgate RAD (2005c) LGR7-truncate is a splice variant of the relaxin receptor LGR7 and is a relaxin antagonist in vitro. *Ann NY Acad Sci* **1041**:22–26.
- Seibold JR, Korn JH, Simms R, Clements PJ, Moreland LW, Mayes MD, Furst DE, Rothfield N, Steen V, Weisman M, et al. (2000) Recombinant human relaxin in the treatment of scleroderma A randomized double-blind placebo-controlled trial. *Ann Intern Med* **132**:871–879.
- Shen P-J, Fu P, Phelan KD, Scott DJ, Layfield S, Tregear GW, Bathgate RAD, and Gundlach AL (2005) Restricted expression of LGR8 in intralaminar thalamic nuclei of rat brain suggests a role in sensorimotor systems. *Ann NY Acad Sci* **1041**:510–515.
- Sherwood OD (1994) Relaxin. in *The Physiology of Reproduction* (Knobil E and Neill JC eds) pp 861–1009, Raven Press, New York.
- Sherwood OD (2004) Relaxin's physiological roles and other diverse actions. *Endocr Rev* **25**:205–234.
- Sherwood OD, Crnekovic VE, Gordon WL, and Rutherford JE (1980) Radioimmunoassay of relaxin throughout pregnancy and during parturition in the rat. *Endocrinology* **107**:691–698.
- Sherwood OD, Rosentreter KR, and Birkhimer ML (1975) Development of a radioimmunoassay for porcine relaxin using ¹²⁵I-labeled polytyrosyl-relaxin. *Endocrinology* **96**:1106–1113.
- Siebel AL, Gehring HM, Reytomas IG, and Parry LJ (2003) Inhibition of oxytocin receptor and estrogen receptor- α expression but not relaxin receptors (LGR7) in the myometrium of late pregnant relaxin gene knockout mice. *Endocrinology* **144**:4272–4275.
- Sinnayah P, Burns P, Wade JD, Weisinger RS, and McKinley MJ (1999) Water drinking in rats resulting from intravenous relaxin and its modification by other dipsogenic factors. *Endocrinology* **140**:5082–5086.
- Smith KJ, Wade JD, Claasz AA, Otvos L Jr, Temelcos C, Kubota Y, Hutson JM, Tregear GW, and Bathgate RAD (2001) Chemical synthesis and biological activity of rat INSL3. *J Pept Sci* **7**:495–501.
- Spanel-Borowski K, Schafer I, Zimmermann S, Engel W, and Adham IM (2001) Increase in final stages of follicular atresia and premature decay of corpora lutea in Insl3-deficient mice. *Mol Reprod Dev* **58**:281–286.
- St-Louis J and Massicotte G (1985) Chronic decrease of blood pressure by rat relaxin in spontaneously hypertensive rats. *Life Sci* **37**:1351–1357.
- Sudo S, Kumagai J, Nishi S, Layfield S, Ferraro T, Bathgate RA, and Hsueh AJ (2003) H3 relaxin is a specific ligand for LGR7 and activates the receptor by interacting with both the ectodomain and the exoloop 2. *J Biol Chem* **278**:7855–7862.
- Summerlee AJ, Hornsby DJ, and Ramsey DG (1998) The dipsogenic effects of rat relaxin: the effect of photoperiod and the potential role of relaxin on drinking in pregnancy. *Endocrinology* **139**:2322–2328.
- Sunn N, Egli M, Burazin TC, Burns P, Colvill L, Davern P, Denton DA, Oldfield BJ, Weisinger RS, Rauch M, et al. (2002) Circulating relaxin acts on subfornical organ neurons to stimulate water drinking in the rat. *Proc Natl Acad Sci USA* **99**:1701–1706.
- Sunn N, McKinley MJ, and Oldfield BJ (2001) Identification of efferent neural pathways from the lamina terminalis activated by blood-borne relaxin. *J Neuroendocrinol* **13**:432–437.
- Tabanelli S, Tang B, and Gurdipe E (1992) In vitro decidualization of human endometrial stromal cells. *J Steroid Biochem Mol Biol* **42**:337–344.
- Tan YY, Dawson NF, Komp AR, Bond CP, Claasz A, Wade JD, Tregear GW, and Summers RJ (2002) Structural requirements for the interaction of sheep insulin-like factor 3 with relaxin receptors in rat atria. *Eur J Pharmacol* **457**:153–160.
- Tan YY, Wade JD, Tregear GW, and Summers RJ (1998) Comparison of relaxin receptors in rat isolated atria and uterus by use of synthetic and native relaxin analogues. *Br J Pharmacol* **123**:762–770.
- Tan YY, Wade JD, Tregear GW, and Summers RJ (1999) Quantitative autoradiographic studies of relaxin binding in rat atria uterus and cerebral cortex: characterization and effects of oestrogen treatment. *Br J Pharmacol* **127**:91–98.
- Tang X-M and Chegini N (1995) Human fallopian tube as an extraovarian source of relaxin: messenger ribonucleic acid expression and cellular localization of immunoreactive protein and ¹²⁵I-relaxin binding sites. *Biol Reprod* **52**:1343–1349.
- Thomas GR and Vandlen R (1993) The purely chronotropic effects of relaxin in the rat isolated heart. *J Pharm Pharmacol* **45**:927–928.
- Tomiyama H, Hutson JM, Truong A, and Agoulnik AI (2003) Transabdominal testicular descent is disrupted in mice with deletion of insulinlike factor 3 receptor. *J Pediatr Surg* **38**:1793–1798.
- Toth M, Taskinen P, and Ruskoaho H (1996) Relaxin stimulates atrial natriuretic peptide secretion in perfused rat heart. *J Endocrinol* **150**:487–495.
- Tregear GW, Fagan C, Reynolds H, Scanlon D, Jones P, Kemp B, and Niall HD (1981) Porcine relaxin: synthesis and structure activity relationships, in *Peptides: Synthesis, Structure, and Function* (Rich DH and Gross E eds) pp 249–252, Pierce Publishing, Rockford, IL.
- Unemori EN and Amento EP (1990) Relaxin modulates synthesis and secretion of procollagenase and collagen by human dermal fibroblasts. *J Biol Chem* **265**: 10681–11065.
- Unemori EN, Beck LS, Lee WP, Xu Y, Siegel M, Keller G, Liggitt HD, Bauer EA, and Amento EP (1993) Human relaxin decreases collagen accumulation in vivo in two rodent models of fibrosis. *J Invest Dermatol* **101**:280–285.
- Unemori EN, Pickford LB, Salles AL, Piercy CE, Grove BH, Erikson ME, and Amento EP (1996) Relaxin induces an extracellular matrix-degrading phenotype in human lung fibroblasts in vitro and inhibits lung fibrosis in a murine model in vivo. *J Clin Invest* **98**:2739–2745.
- Van der Westhuizen E, Sexton PM, Bathgate RAD, and Summers RJ (2005) Responses of GPCR135 to human gene 3 (H3) relaxin in CHO-K1 cells determined by microphysiometry. *Ann NY Acad Sci* **1041**:332–337.
- Vasilenko P, Mead JP, and Weidmann JE (1986) Uterine growth-promoting effects of relaxin: a morphometric and histological analysis. *Biol Reprod* **35**:987–995.
- Vassart G, Pardo L, and Costagliola S (2004) A molecular dissection of the glycoprotein hormone receptors. *Trends Biochem Sci* **29**:119–126.
- Wade JD, Layden SS, Lambert PF, Kakouris H, and Tregear GW (1994) Primate relaxin: synthesis of gorilla and rhesus monkey relaxins. *J Protein Chem* **13**:315–321.
- Ward DG, Thomas GR, and Cronin MJ (1992) Relaxin increases rat heart rate by a direct action on the cardiac atrium. *Biochem Biophys Res Commun* **186**:999–1005.
- Weisinger RS, Burns P, Eddie LW, and Wintour EM (1993) Relaxin alters the plasma osmolality-arginine vasopressin relationship in the rat. *J Endocrinol* **137**:505–510.
- Weiss G (1989) Relaxin in the male. *Biol Reprod* **40**:197–200.
- Weiss TJ and Bryant GG (1982) Localization of relaxin binding sites in the rat uterus and cervix by autoradiography. *Biol Reprod* **27**:673–679.
- Wilkinson TN, Speed TP, Tregear GW, and Bathgate RAD (2005) Evolution of the relaxin-like peptide family. *BMC Evol Biol* **5**:14.
- Williams EJ, Benyon RC, Trim N, Hadwin R, Grove BH, Arthur MJ, Unemori EN, and Iredale JP (2001) Relaxin inhibits effective collagen deposition by cultured hepatic stellate cells and decreases rat liver fibrosis in vivo. *Gut* **49**:577–583.
- Yan Y, Cai J, Fu P, Layfield S, Ferraro T, Kumagai J, Sudo S, Giannakisa E, Tregear G, Wade J, et al. (2005) Studies on soluble ectodomain proteins of relaxin (LGR7) and insulin 3 (LGR8) receptors. *Ann NY Acad Sci* **1041**:35–39.
- Yang S, Rembisa B, Bullesbach EE, and Schwabe C (1992) Relaxin receptors in mice: demonstration of ligand binding in symphyseal tissues and uterine membrane fragments. *Endocrinology* **130**:179–185.
- Zarreh-Hoshyari-Khah R, Bartsch O, Einspanier A, Pohnke Y, and Ivell R (2001) Bioactivity of recombinant prorelaxin from the marmoset monkey. *Regul Pept* **97**:139–146.
- Zhang Q, Liu SH, Erikson M, Lewis M, and Unemori E (2002) Relaxin activates the MAP kinase pathway in human endometrial stromal cells. *J Cell Biochem* **85**:536–544.
- Zhao L, Roche PJ, Gunnarsen JM, Hammond VE, Tregear GW, Wintour EM, and Beck F (1999) Mice without a functional relaxin gene are unable to deliver milk to their pups. *Endocrinology* **140**:445–453.
- Zhao L, Samuel CS, Tregear GW, Beck F, and Wintour EM (2000) Collagen studies in late pregnant relaxin null mice. *Biol Reprod* **63**:697–703.
- Zimmermann S, Steding G, Emmen JM, Brinkmann AO, Nayernia K, Holstein AF, Engel W, and Adham IM (1999) Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. *Mol Endocrinol* **13**:681–691.