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New Members of the Insulin Family: Regulators of Metabolism, Growth and Now . . . Reproduction

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ABSTRACT

Insulin, IGF, and relaxin are established members of the insulin protein superfamily. The application of the techniques of cellular, molecular, and computational biology has permitted the identification of new insulin-like ligands and their cognate receptors. Information regarding the biologic role is available for some of these newly identified ligand-receptor systems and indicates novel roles in diverse processes such as testicular descent, germ cell function, and cell migration. (***Pediatr Res* 57: 70R–73R, 2005**)

Abbreviations
GPCR, G protein-coupled receptor
GREAT, G protein-coupled receptor affecting testis descent
INSL, insulin-like protein
LGR, leucine-rich repeat-containing G protein-coupled receptor

The insulin/IGF/relaxin superfamily is an ancient family of functionally diverse proteins. Insulin or insulin-like proteins have been described in unicellular eukaryotes as well as in such primitive species as insects, tunicates, annelids, and molluscans (1–6). Despite the broad magnitude of functional divergence present within the family, all proteins of the insulin family exhibit a high degree of structural conservation (Fig. 1). The primary peptide sequence of each member of the family is characterized by three domains comprised of an amino terminal B peptide (or chain) joined to a carboxyl A peptide by an intervening C peptide (B-C-A) (7). Between the different hormones within the family (paralog) and similar hormones in different species (ortholog), the B and A chain peptides are relatively invariant and exhibit a pattern of distinct and highly conserved cysteine motifs. These cysteine motifs characterize the family; specifically the motif (CC-3X-C-8X-C) present in the A peptide has been termed the insulin signature. Many members of the insulin family of hormones are synthesized as preprohormones, with the primary peptide undergoing post-translational modification to generate a cysteine-linked heterodimer of the B and A peptides that functions as the active hormone.

A decade ago the insulin family was comprised of four members in mammals: insulin, IGF-I, IGF-II, and relaxin. In recent years, additional members of the family, termed INSL3 (8,9), INSL4 (10,11), INSL5 (12,13), INSL6 (13–15), and INSL7 (16), have been identified (17) (Fig. 2). The discovery of these new ligands spurred efforts to identify their cognate receptors. These efforts resulted in the discovery that LGR8, a member of the LGR (*l*eucine-rich repeat-containing *G* protein-coupled *r*eceptors) subfamily of GPCR (*G p*rotein-*c*oupled *r*eceptors) receptors, was the cognate receptor for INSL3 (18). This review will describe new information regarding these new members of the insulin family and their cognate receptors with emphasis on the putative biologic role(s) of these proteins.

INSL3

Having been initially identified in boar testes (19), the product of the INSL3 gene, relaxin-like factor, or Ley-I-L (Leydig insulin-like peptide precursor), was subsequently identified in both the human and the mouse as a single copy gene containing two exons and a single intron (8,20). Human INSL3 is located on the short arm of chromosome 19 and is structurally similar to relaxin. The major site of expression of INSL3 is in Leydig cells of the testes, but it is also expressed in the theca cells of the corpus luteum, the trophoblast, breast, and a variety of other tissues (21–25). The first indication of an essential role for INSL3 in reproduction came from studies of knockout models in mice. Insl3 null male mice were cryptorchid suggesting that Insl3 gene plays a major role in the development of the gubernaculum and subsequent testicular

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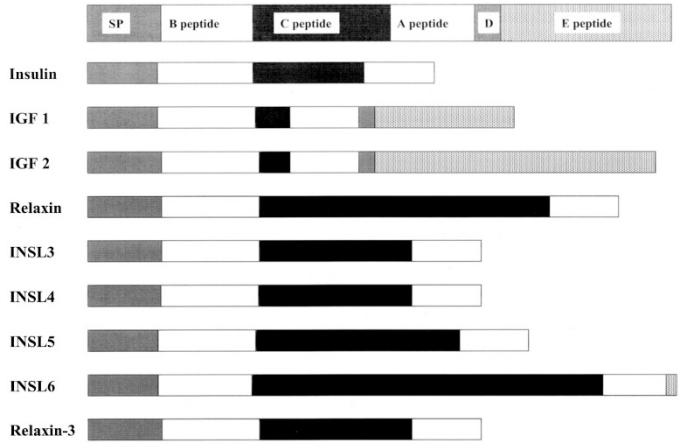

Figure 1. Schematic representation of the products of the genes of the insulin/IGF/relaxin family. The peptide domains are represented in relative scale. *Adapted from Kasik et al., Pediatr Diabetes 1:169–177. © Munksgard 2000 with permission.*

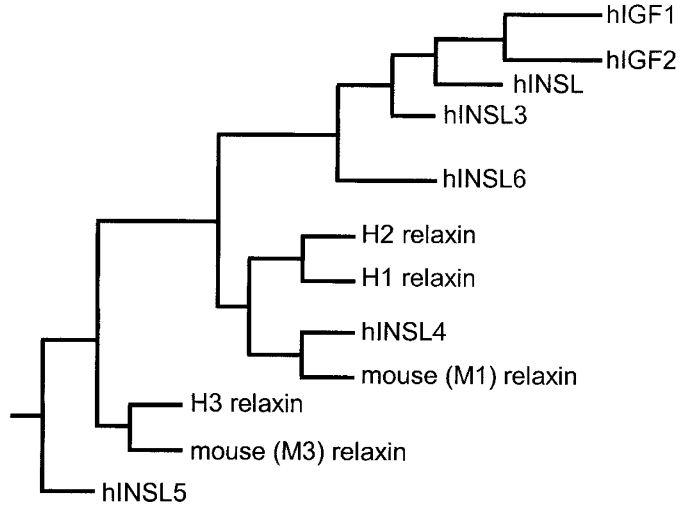

Figure 2. Phylogenetic tree of evolution of the insulin/relaxin superfamily. *Adapted from Bathgate et al., J Biol Chem 277:1148–1157. © 2002 by The American Society for Biochemistry and Molecular Biology, Inc. with permission.*

development (9,26). The phenotype of the Insl3 null mice was similar to that of the LGR8 (GREAT) null mice, leading to identification of LGR8 as the cognate receptor for INSL3 (18,27). Subsequent screening of boys with cryptorchidism, the most frequent congenital abnormality in humans, resulted in the identification of mutations within the INSL3 gene and its promoter sequence and the LGR8 gene (28–30), although the mutations in these two genes are responsible for only a small proportion (<10%) of familial cryptorchidism (31). The contribution of abnormalities in LGR expression, cognate signaling pathways, and target genes in the pathogenesis of cryptorchidism remains to be investigated.

In the testis, in addition to its actions on the gubernaculum, INSL3 suppresses germ cell apoptosis and acts in a paracrine manner as a survival factor for male germ cells (32). Whereas much of the interest regarding the biologic role of INSL3 has been focused on the testes, recent experimental observations provide clues to actions of INSL3 in the ovary. LH transiently

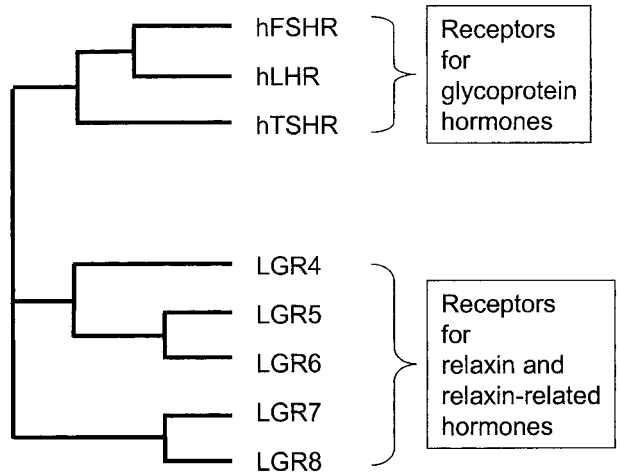

Figure 3. Phylogenetic tree of evolution of the LGR family of receptors. *Adapted from Hsu, Trend Endocrinol Metab 14:303–309. © 2003 Elsevier Ltd. with permission.*

increases INSL3 expression in theca cells of the ovary and INSL3 suppresses intra-oocyte cAMP levels with stimulation of induction of oocyte maturation, suggesting a paracrine role for INSL3 in mediating preovulatory LH actions on the ovary (32).

INSL4

Efforts directed at identifying differentially expressed genes within the human placenta led to the identification of the product of the INSL4 gene, early placenta insulin-like peptide (EPIL) or placentin (10,11). In contrast to other members of the insulin superfamily, INSL4 is primate-specific as INSL4 nucleic acid sequences are absent in rodent, horse, and lemur genomes (33). Structurally, the gene is similar to insulin, relaxin, and INSL3 and contains two exons and a single intron. It is located on 9p24, where it is clustered with the two human relaxin genes (34). The predominant site of expression of the INSL4 gene is in the placenta during early pregnancy, but it is also expressed to a lesser degree in interbone ligaments, perichondrium, and the uterus (35). Given that it is detectable in maternal serum during pregnancy, INSL4 presumably functions as a hormone. It is also likely that INSL4 has paracrine and autocrine actions. There is a paucity of information regarding the cognate receptor(s) for INSL4, although conditioned media from cells overexpressing INSL4 increases total cellular tyrosine phosphorylation *via* a pathway distinct from that of the insulin receptor (11). Recent reports of INSL4 being overexpressed and secreted in erb-3–positive breast cancer cells with high invasive potential and in hydatiform moles suggest a role for INSL4 in tissue invasiveness and cell migration (36).

INSL5 and INSL6

Three laboratories, working independently, identified through the application of the techniques of computational biology, two new genes: INSL5 (relaxin/insulin-like factor 2, RIF2) and INSL6 (relaxin/insulin-like factor 1, RIF1) (12–15). Human, mouse, and rat orthologs of these genes have also been

identified. Both genes encode proteins that are clearly members of the insulin superfamily; they contain signal peptides and exhibit the requisite cysteine motifs. The human INSL5 gene is located on chromosome 1 and the orthologous mouse Insl5 is located on mouse chromosome 4. Human INSL6 is located in the region 9p24 in proximity to the human relaxin genes as well as to INSL 4. The mouse ortholog, Insl6, is located on chromosome 19, the chromosome that also contains the single mouse relaxin gene. In humans, INSL5 is maximally expressed in the uterus and the digestive tract, with highest levels of expression in the rectum. In the mouse, expression of mouse Insl5 is described in thymus, kidney, heart, brain, and testis. INSL6 is maximally expressed in germ cells of the testis, with lower levels of expression detectable in a variety of other tissues including intestine, thymus, kidney, uterus, ovary, spleen, breast, lung, and liver (15,37). The INSL6 peptide undergoes posttranslational modifications including glycosylation and ubiquitination (37). A paracrine role for INSL6 in modulating Sertoli cell function is suggested by studies that demonstrate augmentation of FSH-stimulated cAMP in Sertoli cells (Lu C, Menon RK, unpublished data).

LGR FAMILY OF RECEPTORS

The discovery of new putative ligands in the insulin family spurred efforts to identify their cognate receptors. These efforts resulted in the deorphanizing of a group of GPCR receptors termed LGR (38), with the discovery that LGR8 was the cognate receptor for INSL3 (18). This discovery was soon followed by the identification of LGR7 and LGR8 as cognate receptors for relaxin (39), a discovery of some significance inasmuch as the identity of the relaxin receptor(s) had eluded identification since the time relaxin was the first discovered in 1926 (40). LGR are structurally similar to receptors for gonadotrophins (LH and FSH) and TSH. These receptors are characterized by a large N-terminal extracellular domain containing leucine-rich repeat preceding a GPCR seven transmembrane spanning domain. LGR can be divided into three subgroups, with the first group consisting of the gonadotropin and TSH receptors, the second subgroup consisting of LGR4, LGR5, and LGR6, and the third subgroup constituted by LGR7 and LGR8 (41) (Fig. 3). At present, LGR4, LGR5, and LGR6 remain as orphan receptors. LGR7 and LGR8 have been identified as receptors for relaxin (39). Relaxin-3 is an additional ligand for LGR7 (42) and INSL3 a ligand for LGR8 (18). The overlapping of ligand specificity between the LGR receptors suggest that tissue-specific expression profile of these receptors may enable these ligands to have differential biologic actions in various tissues. It is of interest that the LGR4 null mice exhibits intrauterine growth retardation and embryonic and perinatal lethality (43) and the LGR5 null mice displays a phenotype similar to ankyloglossia (44), suggesting that the LGR4 and LGR5 systems may have biologic roles distinct from LGR7 and 8 (Figs 1–3).

CONCLUSIONS

In relatively rapid succession, through the application of the techniques of molecular and computational biology, five new members of the insulin family and some of their cognate receptors have been identified. The two major themes that emerge from these discoveries are the expanding role of the insulin family of proteins in reproductive physiology and the identification of the GPCR receptors as cognate receptors for some of these new ligands. It is noteworthy that a recent study also implicated the "established" members of the insulin family in male sexual differentiation (45). Thus, male mice with triple knockout of the insulin, IGF-I, and insulin-related receptor displayed ovaries and a female phenotype. The identification of GPCR receptors as cognate receptors for the newer members of the insulin family is especially noteworthy because canonical insulin/IGF-I receptors are tyrosine kinase receptors and the identification of GPCR as cognate receptors for this family of ligands represents a paradigm shift. The challenge now lies in identifying the biologic function of these ligands and receptor

Table 1. *Mammalian insulin family of proteins*

Name	Alternate names	Gene symbol	Principal site of expression	Major biological function	Cognate receptor(s)
Insulin		INS	Pancreas	Metabolism, growth	InsulInsulin
Insulin-like growth factors	IGF-I and IGF-II	IGF1 IGF2	Widespread prenatal (IGF-II) and postnatal (IGF-II) expression	Growth and differentiation	IGF1R-I (IGF-I) and IGF2R/M6PR (IGF-II)
Relaxin H1		RLN1	Decidua, placenta, prostate	? Pseudogene	LGR7 and 8 (?)
Relaxin H2	Relaxin	RLN2	Corpus luteum	Parturition	LGR7 and 8
Insulin-like protein 3	Relaxin-like factor	INSL3	Testes (Leydig cell)	Testicular descent, paracrine regulation of oocyte maturation, and male gem cell survival	LGR 8
Insulin-like protein 4	Early placental insulin-like peptide (EPIL), placentin	INSL4	Placenta	Tissue invasiveness and cell migration (?)	?
Insulin-like protein 5	RIF2	INSL5	GI tract/kidney	?	GPCR142
Insulin-like protein 6	RIF1	INSL6	Testes (germ cells)	Paracrine modulation of Sertoli cell function (?)	?
Relaxin-3	Insulin-like protein 7	RLN3, H3, RXN	Brain	?	GPCR135, GPCR142, and LGR7

systems. A more complete understanding of the biologic roles of these new proteins may enable the design of novel therapeutic strategies in the treatment of conditions such as premature labor, infertility, and malignancy.

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