

International Union of Basic and Clinical Pharmacology. LXXVII. Kisspeptin Receptor Nomenclature, Distribution, and Function

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Abstract—Kisspeptins are members of the Arg-Phe amide family of peptides, which have been identified as endogenous ligands for a G-protein-coupled receptor encoded by a gene originally called *GPR54*

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(also known as *AXOR12* or *hOT7T175*). After this pairing, the gene has been renamed *KISS1R*. The International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification recommends that the official name for the receptor is the kisspeptin receptor to follow the convention of naming the receptor protein after the endogenous ligand. The endogenous ligand was initially called metastatin, after its role as a metastasis suppressor, and is now referred to as kisspeptin-54 (KP-54), a C-terminally amidated 54-

amino acid peptide cleaved from the 145-amino acid gene product. Shorter C-terminal cleavage fragments [KP-14, KP-13 and KP-10 (the smallest active fragment)] are also biologically active. Both receptor and peptide are widely expressed in human, rat, and mouse; the receptor sequence shares more than 80% homology in these species. Activation of the kisspeptin receptor by kisspeptin is via coupling to $G_{q/11}$ and the phospholipase C pathway, causing

Ca^{2+} mobilization. Mutations in the *KISS1R* gene result in hypogonadotropic hypogonadism, and targeted disruption of *Kiss1r* in mice reproduces this phenotype, which led to the discovery of the remarkable ability of the kisspeptin receptor to act as a molecular switch for puberty. In addition to regulating the reproductive axis, the kisspeptin receptor is also implicated in cancer, placentation, diabetes, and the cardiovascular system.

I. Introduction

The endogenous ligand of the orphan G-protein-coupled receptor GPR54 was identified in 2001 (Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001) as the product of the *KISS1* gene, which was a known metastasis suppressor (Lee et al., 1996; Lee and Welch, 1997a,b). The ligand was initially christened metastin and is now referred to as kisspeptin. The *KISS1* gene product consists of 145 amino acids, which is cleaved to produce a C-terminally amidated 54 amino-acid peptide, KP-54. The C-terminal cleavage fragments KP-14, KP-13, and KP-10 also possess biological activity, although it is unclear to what extent these peptides are generated endogenously (Fig. 1). Cleavage of the three C-terminal amino acids of kisspeptin by the matrix-metalloproteases 2 and 9 (MMP-2/9) renders the peptide inactive (Fig. 1) (Takino et al., 2003). High expression levels of both receptor and peptide are present in the placenta, with lower levels in the brain, particularly the hypothalamus and the pituitary.

The original interest in the kisspeptin system focused on its effects in cancer. However, in 2003, several persons with hypogonadotropic hypogonadism were identified with mutations in the *KISS1R* gene (Seminara et al., 2003; de Roux et al., 2003). It has since been elucidated in an increasing number of species that kisspeptin, acting at the kisspeptin receptor, is responsible for the regulation of the reproductive axis by integrating internal and external cues. For more detailed information, the reviews listed in Table 1 should be consulted.

The standard NC-IUPHAR rules of nomenclature state that a receptor is named after its endogenous agonist (Vanhoutte et al., 1996), and therefore we support the suggestion of others that, at the protein level, GPR54 is referred to as the kisspeptin receptor (<http://www.iuphar-db.org/PRODDATABASE/ObjectDisplayForward?objectId=266>, Gottsch et al., 2009). They also suggest abbreviating the kisspeptin receptor to *KISS1R*; however, the use of the letter R in the abbreviation is not consistent with NC-IUPHAR nomenclature (Vanhoutte et al., 1996), although it helps to avoid confusion when refer-

ring to *KISS1* peptides. Kisspeptin can also be referred to as KP-54, KP-14, KP-13, or KP-10 depending on the length of the fragment. Gene names should be italicized and, consistent with the Human Genome Organization (HUGO), *KISS1R* refers to the receptor gene and *KISS1* to the peptide gene, with lower case letters used for nonhuman species (Table 2).

II. G-Protein-Coupled Receptor 54 Designated as the Kisspeptin Receptor

Lee et al. (1999) cloned a novel G-protein-coupled receptor from rat brain and identified this as a 396-amino acid polypeptide with the expected seven transmembrane domains. Metastin, the peptide product of the *KISS1* gene, was identified as the endogenous ligand for GPR54 by three independent groups (Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001). This peptide ligand was initially termed metastin after its ability to inhibit metastasis and was classified as a member of the Arg-Phe (RF)-amide family, a group that also includes PrRP, QRFP, and NPF (Kutzleb et al., 2005). The *KISS1* gene was named for its place of discovery, Hershey, Pennsylvania, home of the "Hershey's Kisses" sweets. The name incorporated "SS" for suppressor sequence, reflecting its metastatic effects. Following International Union of Pharmacology convention to name receptors after their endogenous agonists, GPR54 should be designated the kisspeptin receptor for the ligand kisspeptin (Table 3).

The kisspeptin receptor is a class A G-protein-coupled receptor coupled to $G_{q/11}$, causing activation of the phospholipase C (PLC) signaling pathway and resultant Ca^{2+} mobilization (see section VII). Rat and human kisspeptin receptors share 85% sequence identity, increasing to 98% in the transmembrane domains, whereas mouse and human share 82% (Fig. 2). The closest structural relative of the kisspeptin receptor is the galanin receptor GAL_1 , with 45% homology; however, galanin does not bind to it (Lee et al., 1999) (Fig. 3). KP-10, consisting of the 10 C-terminal amino acids of the full sequence, is the minimum fragment length needed to bind and activate the kisspeptin receptor, and it has a potency in vitro similar to that of the full-length 54-amino acid peptide (Kotani et al., 2001), although activities after peripheral delivery in vivo are slightly different (Thompson et al., 2006).

¹ Abbreviations: AVPV, anteroventral periventricular nucleus; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; KP, kisspeptin; LH, luteinizing hormone; MMP, matrix metalloprotease; NC-IUPHAR, Nomenclature Committee, International Union of Basic and Clinical Pharmacology; PeN, preoptic periventricular nucleus; PLC, phospholipase C.

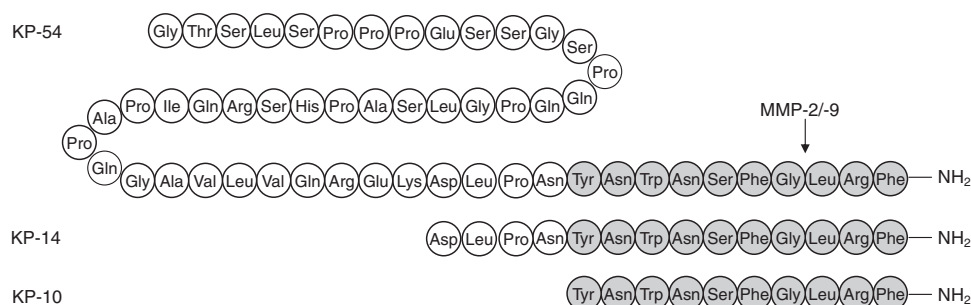


FIG. 1. Human kisspeptin (KP-54) and the cleavage fragments KP-14 and KP-10. The MMP-2/9 cleavage site is indicated.

TABLE 1
Detailed reviews

Focus of Review	Reference
Signalling	Castano et al., 2009
Distribution	Oakley et al., 2009
Function of kisspeptin	
General overview	Mead et al., 2007a
Cancer	Harms et al., 2003
Puberty	Messenger, 2005a; Kuohung and Kaiser, 2006; Seminara, 2006; Tena-Sempere, 2006b; Kauffman et al., 2007a; Navarro et al., 2007; Smith and Clarke, 2007; Roa et al., 2009
Seasonal regulation of reproduction	Revel et al., 2007; Clarke et al., 2009; Simonneaux et al., 2009
Metabolic regulation of reproduction	Castellano et al., 2009
Pregnancy	Reynolds et al., 2009
Transgenic mouse models	Colledge, 2009a,b

TABLE 2
Nomenclature for kisspeptin and its receptor

Kisspeptin	Species	Gene	Protein
Receptor	Human	<i>KISS1R</i>	Kisspeptin receptor
	Nonhuman	<i>Kiss1r</i>	Kisspeptin receptor
Peptide	Human	<i>KISS1</i>	Kisspeptin/KP-54/KP-14/ KP-13/KP-10
	Nonhuman	<i>Kiss1</i>	Kisspeptin/KP-54/KP-14/ KP-13/KP-10

III. Kisspeptin Receptor Distribution

A. Human

In humans, reverse transcriptase polymerase chain reaction has revealed high levels of *KISS1R* mRNA in placenta, pituitary, pancreas, and spinal cord (Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001) (Table 4). Northern blotting detected high *KISS1R* mRNA peripherally, in the heart, skeletal muscle, kidney, liver, and placenta, and also in regions of the brain (cerebral cortex, putamen, and medulla) and the spinal cord (Clements et al., 2001). Immunohistochemistry confirmed expression in the brain, detecting neuronal expression in the cerebral cortex, thalamus, pons-medulla, and cerebellum (Muir et al., 2001). In the periphery, specific ¹²⁵I-KP-14 binding was detected in aorta, coronary artery, and umbilical vein (Mead et al., 2007b), suggesting expression of the kisspeptin receptor in the cardiovascular system.

B. Mouse

In the mouse, *Kiss1r* mRNA is expressed in the central nervous system (Funes et al., 2003) and particularly

in gonadotropin-releasing hormone (GnRH) neurons (Han et al., 2005; Messenger et al., 2005b). With the use of X gal histochemistry, whereby β -galactosidase was knocked into the *Kiss1r* gene, expression has been detected in the dentate gyrus of the hippocampus and in both GnRH neurons and cells in the periventricular region of the posterior hypothalamus but not, interestingly, in the rostral part of the third ventricle or arcuate nucleus (Herbison et al., 2010).

C. Rat

In rat, *Kiss1r* mRNA has been detected in brain regions such as the pons, midbrain, thalamus, hypothalamus, hippocampus, amygdala, cortex, frontal cortex, and striatum in addition to peripheral regions such the liver and intestine (Lee et al., 1999). Another study also localized *Kiss1r* mRNA to areas of the brain, including diagonal band of Broca, medial septum, medial preoptic area, lateral preoptic area, median preoptic nucleus, and anterior and lateral hypothalamus (Irwig et al., 2004). *Kiss1r* mRNA has also been found in the pituitary (Richard et al., 2008).

D. Other Species

Kiss1r mRNA has been detected in the hypothalamus of pig (Li et al., 2008) and monkey (Shahab et al., 2005) and the pituitary of sheep (Smith et al., 2008). There has been recent interest in conservation of the kisspeptin regulation of reproduction during evolution and in keeping with this the kisspeptin receptor has been detected in several fish species, notably in the brain (Parhar et al., 2004; Mohamed et al., 2007; Nocillado et al., 2007;

TABLE 3
Classification of the kisspeptin receptor

Previous names	GPR54, AXOR12, hOT7T175	
Structural information	7TM	
Human	398 aa (SwissProt Q969F8) chr. 19p13.3 (Entrez 84634)	
Rat	396 aa (SwissProt Q924U1) chr. 7q11 (Entrez 78976)	
Mouse	396 aa (SwissProt Q91V45) chr. 10C1 (Entrez 114229)	
Functional assays	CHO cells transfected with the kisspeptin receptor	Hori et al., 2001
	In vitro pharmacology using human isolated vessels	Mead et al., 2007b
Endogenous agonists	KP-54 ($EC_{50} = 5.5$ nM)	Kotani et al., 2001
	KP-54 ($pEC_{50} = 8.9$ nM)	Mead et al., 2007b
Selective agonists	4-fluorobenzoyl-F-G-I-RW-NH ₂ ($EC_{50} = 0.7$ nM)	Tomita et al., 2007
	[dY] ¹ KP-10	Curtis et al., 2010
Antagonists	Peptide234 ac[(D)-A]NWNGFG[(D)-W]RF	Roseweir et al., 2009
Antagonist potencies	Peptide 234 ($IC_{50} = 7.0$ nM)	
Radioligands	¹²⁵ I-Tyr45-KP-54 ($K_D = 0.1$ nM)	Ohtaki et al., 2001
	¹²⁵ I-KP-14 ($K_D = 0.1$ nM)	Mead et al., 2007b
	¹²⁵ I-KP-10 ($K_D = 1.9$ nM)	Kotani et al., 2001
Transduction mechanisms	Coupled to G $\alpha_{q/11}$ proteins	Kotani et al., 2001; Muir et al., 2001; Becker et al., 2005
Receptor distribution		
Human	RT-PCR showed highest expression of KISS1R mRNA in placenta, pituitary, spinal cord and pancreas.	Kotani et al., 2001; Ohtaki et al., 2001.
	Kisspeptin receptor protein expression detected in the cerebral cortex, thalamus, pons-medulla and cerebellum by immunohistochemistry.	Muir et al., 2001
	Radioligand binding and immunohistochemistry found expression in aorta, coronary artery and umbilical vein.	Mead et al., 2007b
Rat	RT-PCR detected Kiss1r mRNA in cortex, frontal cortex and striatum and in ovary.	Lee et al., 1999; Castellano et al., 2006
	Kisspeptin-receptor immunoreactivity has been detected in the pituitary.	Richard et al., 2008
Mouse	RT-PCR showed <i>Kiss1r</i> expression in heart and in brain and reproductive tissues (uterus, oviduct, and testis).	Stafford et al., 2002; Funes et al., 2003
Tissue function	Metastasis inhibitor, stimulator of LH release, inhibitor of trophoblast migration, vasoconstrictor of coronary artery, and umbilical vein.	Ringel et al., 2002; Bilban et al., 2004; Irwig et al., 2004; Navarro et al., 2004a; Mead et al., 2007b

aa, amino acid(s); RT-PCR, reverse transcription-polymerase chain reaction.

Biran et al., 2008; Filby et al., 2008; van Aerle et al., 2008; Carrillo et al., 2009; Li et al., 2009; Mechaly et al., 2009; Yang et al., 2010) and in frog (Lee et al., 2009; Moon et al., 2009).

IV. Kisspeptin Distribution

A. Human

KISS1 mRNA showed a similar distribution to the receptor, with high levels in placenta, pancreas, testis, liver, and small intestine (Lee et al., 1996; Muir et al., 2001; Ohtaki et al., 2001). More recently, KISS1 mRNA-expressing neurons within the hypothalamus have been identified by in situ hybridization in the infundibular nucleus (Rometo et al., 2007), the human equivalent of the arcuate nucleus in animals.

B. Mouse

Kisspeptin has been detected at both the mRNA and protein level in several hypothalamic nuclei including the anteroventral periventricular nucleus (AVPV), the preoptic periventricular nucleus (PeN), and the arcuate nucleus (Gottsch et al., 2004; Clarkson and Herbison, 2006), areas implicated in the regulation of gonadotropin secretion. It is noteworthy that levels in the AVPV and PeN are 10-fold higher in female mice than in male mice (Clarkson and Herbison, 2006). The distribu-

tion of kisspeptin-immunoreactivity in the mouse brain has been mapped using a well characterized polyclonal rabbit anti-kisspeptin-10 antiserum (AC566) (Franceschini et al., 2006), identifying areas of expression in the arcuate nucleus, the rostral part of the third ventricle, including the AVPV region and in the dorsomedial nucleus and posterior hypothalamus (Clarkson et al., 2009).

C. Rat

Kiss1 mRNA has been detected in the central nervous system and in particular in specific hypothalamic regions, such as the arcuate nucleus, the AVPV, and the PeN (Irwig et al., 2004; Smith et al., 2006b; Adachi et al., 2007; Kauffman et al., 2007b; Kalamatianos et al., 2008). Kiss1 mRNA expression has also been found in the pituitary (Richard et al., 2008). At the protein level, kisspeptin-like immunoreactivity has been identified in the arcuate nucleus and also in the paraventricular and ventromedial nuclei of the hypothalamus (Brailoiu et al., 2005); however, there is some indication that antisera may cross-react with other RF-amide peptides (Colledge, 2009a,b).

D. Other Species

Kisspeptin has been identified in homologous regions of the brain in monkey (Shahab et al., 2005; Shibata et

Human	MHTVATSGPNASWGAPANASGCPGCGANASDGPVPSRAVDLWLVPLFFA	50
Rat	MAAEATLGNVSWWAPSNASGCPGCGVNASDGPVPSAPRPLDVLWLVPLFFA	50
Mouse	MATEATLAPNVTWAPSNASGCPGCGVNASDDPGSAPRPLDVLWLVPLFFA	50
	* : ** . ** . : * ** . ***** . **** . * . : ** . : *****	
Human	ALMLLGLVGNLSLVIYVICRHKPMRTVTNFYIANLAATDVTFLCCVPFTA	100
Rat	ALMLLGLVGNLSLVIYVICRHKHMQTVTNFYIANLAATDVTFLCCVPFTA	100
Mouse	TLMLLGLVGNLSLVIYVICRHKHMQTVTNFYIANLAATDVTFLCCVPFTA	100
	: ***** . ***** * : *****	
Human	LLYPLPGWVLGDFMCKFVNYIQQVSVQATCATLTAMSVDRWYVTFPLRA	150
Rat	LLYPLPTWVLGDFMCKFVNYIQQVSVQATCATLTAMSVDRWYVTFPLRA	150
Mouse	LLYPLPAWVLGDFMCKFVNYIQQVSVQATCATLTAMSVDRWYVTFPLRA	150
	***** *****	
Human	LHRRTPRLALAVSLSIWVGSAAVSAPVLALHRLSPGPRAYCSEAFPSRAL	200
Rat	LHRRTPRLALTVSLSIWVGSAAVSAPVLALHRLSPGPHTYCSEAFPSRAL	200
Mouse	LHRRTPRLALAVSLSIWVGSAAVSAPVLALHRLSPGPTYCSEAFPSRAL	200
	***** . *****	
Human	ERAFALYNLLALYLLPLLATCACYAAMLRHLGRVAVRPAPADSALQGGVL	250
Rat	ERAFALYNLLALYLLPLLATCACYGAMLRHLGRAAVRPAPTDGALQGGQLL	250
Mouse	ERAFALYNLLALYLLPLLATCACYGAMLRHLGRAAVRPAPTDGALQGGLL	250
	***** . ***** . ***** : * . ***** *	
Human	AERAGAVRAKVSRLVAAVLLFAACWGPIQLFLVLQALGPAGSWHPRSYA	300
Rat	AQRAGAVRTKVSRLVAAVLLFAACWGPIQLFLVLQALGPAGSWHPRSYA	300
Mouse	AQRAGAVRTKVSRLVAAVLLFAACWGPIQLFLVLQALGPAGSWHPRSYA	300
	* : ***** . *****	
Human	AYALKTWAHCMSYSNSALNPLLYAFLGSHFRQAFRRVPCAPRRRPRRR	350
Rat	AYALKIWAHCMSYSNSALNPLLYAFLGSHFRQAFRCVPCGPQRQRPHA	350
Mouse	AYAVKIWAHCMSYSNSALNPLLYAFLGSHFRQAFRCVPCCRQRQRPH	350
	** * . * ***** ***** * : * * * .	
Human	PGPSDPAAPHAELRLGSHPARAQAQKPGSSGLAARGLCVLGEDNAPL	398
Rat	SAHSDRAAPHVPHSRAAHPVVRTPEPGENP--VRRSPSVQDEHTAPL	396
Mouse	SAHSDRAATHVPHSRAAHPVIRSPPEGENP--VVRSPCAQSEHTASL	396
	. . ** ** . * : . : ** . * : * * . . . * . . * . * *	

FIG. 2. ClustalW (<http://www.ebi.ac.uk/clustalw/>) sequence alignment of the human, rat and mouse kisspeptin receptor. *, identical amino acid in both species; :, conserved amino acid substitution; ;, semiconserved amino acid substitution; -, break in the sequence.

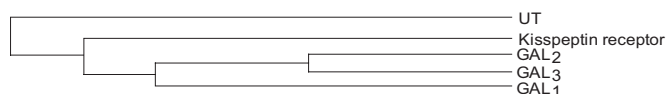


FIG. 3. Cladogram showing the relationship of the kisspeptin receptor with other receptors. UT, urotensin receptor; GAL₁₋₃, galanin receptors.

al., 2007; Ramaswamy et al., 2008, 2009), sheep (Estrada et al., 2006; Franceschini et al., 2006; Pompolo et al., 2006), horse (Decourt et al., 2008; Magee et al., 2009), hamster (Paul et al., 2009), goat (Ohkura et al., 2009), and pig (Tomikawa et al., 2010). Kisspeptin has also been found to be expressed in frog (Lee et al., 2009) and fish species (Biran et al., 2008; Felip et al., 2008; Kanda et al., 2008; van Aerle et al., 2008; Carrillo et al., 2009; Kitahashi et al., 2009; Li et al., 2009; Yang et al., 2010), suggesting that the kisspeptin system has been conserved during evolution.

V. Radiolabeled Ligands

Saturation binding analysis, in cells artificially expressing either human or rat *KISS1R*, has been used to determine dissociation constant, K_D , values for ¹²⁵I-Tyr45-KP-15 (95 pM, human) (Ohtaki et al., 2001) and ¹²⁵I-KP-10 (1.9 ± 0.4 nM, human; 1.0 ± 0.1 nM, rat) (Kotani et al., 2001). ¹²⁵I-KP-14 has been characterized

in human cardiovascular tissues, where it bound saturably, specifically, and reversibly in human aorta with a K_D of 0.20 ± 0.03 nM and a binding density, B_{max} , of 7.65 ± 0.95 fmol/mg protein. Binding had an association rate constant (k_{obs}) of 0.164 ± 0.054 min⁻¹, a dissociation rate constant of 0.0020 ± 0.0006 min⁻¹, a half-time ($t_{1/2}$) for association of 4 min, and $t_{1/2}$ for dissociation of 301 min (Mead et al., 2007b). Both KP-10 and KP-14 competed monophasically for ¹²⁵I-kisspeptin-14 binding with K_D values of 0.03 ± 0.007 and 0.26 ± 0.12 nM, respectively, suggesting that, together with the Hill slope of 1.12 ± 0.09 , there is only one receptor for kisspeptin despite the potential multiple endogenous peptides. Iodinated KP-54 has also been synthesized and used in radioimmunoassays (Dhillon et al., 2005).

VI. Agonists

A. Naturally Occurring Agonists

KP-54, the long isoform of kisspeptin in human, is predicted to be cleaved from the C-terminally amidated KP-145 precursor by furin or prohormone convertases, deduced by the presence of pairs of basic residues flanking this sequence (Kotani et al., 2001; Ohtaki et al., 2001; Harms et al., 2003). The original report describing the isolation of *KISS1* mRNA contained a sequencing

TABLE 4
Summary of the distribution of KISS1R mRNA in human compiled from various studies

Tissue	Ohtaki et al. (2001)	Muir et al. (2001)	Kotani et al. (2001)	Clements et al. (2001)
Adrenal gland	N.D.	N.D.	–	N.D.
Brain	++	N.D.	+	+++
Amygdala	N.D.	+++	+++++	N.D.
Caudate nucleus	N.D.	+++	+++++	N.D.
Cerebellum	N.D.	+	++	+++
Cerebral cortex	N.D.	N.D.	N.D.	+++
Cingulate gyrus	N.D.	++++	N.D.	N.D.
Corpus callosum	N.D.	N.D.	++	N.D.
Globus pallidus	N.D.	+++	N.D.	N.D.
Hippocampus	N.D.	+++	+++++	+
Hypothalamus	N.D.	+++	N.D.	N.D.
Locus ceruleus	N.D.	+++	N.D.	N.D.
Medial frontal gyrus	N.D.	++	N.D.	N.D.
Medulla oblongata	N.D.	+++	N.D.	+++
Nucleus accumbens	N.D.	+++++	N.D.	N.D.
Parahippocampal nucleus	N.D.	+++	N.D.	N.D.
Putamen	N.D.	+++	N.D.	+++
Striatum	N.D.	++	N.D.	N.D.
Substantia nigra	N.D.	++	+++++	N.D.
Superior frontal gyrus	N.D.	+++	N.D.	N.D.
Thalamus	N.D.	++	+++++	+
Breast	+	N.D.	N.D.	N.D.
Bone marrow	+	N.D.	N.D.	N.D.
Colon	+	N.D.	N.D.	–
Heart	+	N.D.	+	+++
Fetal brain	N.D.	N.D.	++	N.D.
Fetal liver	N.D.	N.D.	++	N.D.
Kidney	+	N.D.	+	+++
Liver	N.D.	N.D.	–	+++
Lung	+	N.D.	+	+
Lymph node	++	N.D.	N.D.	N.D.
Ovary	+	N.D.	N.D.	N.D.
Pancreas	+++++	N.D.	+++++	N.D.
Peripheral blood leukocyte	++	N.D.	N.D.	+
Pituitary	N.D.	N.D.	+++++	N.D.
Placenta	+++++	N.D.	+++++	+++
Prostate	+	N.D.	N.D.	N.D.
Spinal cord	N.D.	+	+++++	+++
Skeletal muscle	–	N.D.	–	+++
Small intestine	+	N.D.	N.D.	–
Spleen	++	N.D.	+	+
Testis	++	N.D.	+	N.D.
Thymus	+	N.D.	++	–
Tonsil	+	N.D.	N.D.	N.D.
Stomach	N.D.	N.D.	+	N.D.

N.D., tissue not included in the study; –, expression not detected as; + to +++++, levels of expression graded from low expression to high.

error (Lee et al., 1996) that introduced a frameshift mutation at amino acid 46 in the precursor peptide and led to a prediction that the resultant protein would consist of 164 amino acids. The corrected sequence, and the corresponding shorter open reading frame of 145 amino acids, was subsequently published (West et al., 1998). In rat and mouse, the long kisspeptin isoform is 52 amino acids long, with Arg-Try-NH₂ at the C terminus instead of Arg-Phe-NH₂ as in humans (Tena-Sempere 2006a).

Shorter isoforms of kisspeptin, consisting of the 10-, 13-, and 14-amino acid amidated C-terminal sequences, also possess biological activity and have been shown to have potency similar to that of the longer form in vitro (Mead et al., 2007b; Mikkelsen et al., 2009), although a discrepant report exists (Pheng et al., 2009). The shorter isoforms have been reported to have a lower potency in vivo than KP-54 (Thompson et al., 2009), possibly as a result of increased susceptibility to degradation in the

circulation. Is it currently not known which of the isoforms of kisspeptin are endogenous or whether the shorter forms are breakdown products, although KP-54, KP-14, and KP-13 have been detected in human placenta (Kotani et al., 2001) and KP-54 in plasma of pregnant women (Horikoshi et al., 2003; Dhillon et al., 2006). In cells artificially expressing the human kisspeptin receptor, KP-13, KP-14, and KP-54 bound with equal affinity (Kotani et al., 2001), and this is reflected in equipotent function of isoforms in native human tissue (Mead et al., 2007b).

B. Synthetic Analogs

A number of studies have attempted to identify the residues that are necessary and sufficient to activate the kisspeptin receptor. Alanine and D-amino acid scans revealed that the five C-terminal amino acids of KP-10 are crucial for agonist activity (Niida et al., 2006). The pharmacophore site has been identified as Phe9, Arg12,

and Phe13 of KP-13 (Orsini et al., 2007), with the peptide forming a helicoid conformation from Asn7 to Phe13. The extreme C terminus, the RF moiety, of the receptor seems to be evolutionarily conserved, suggesting that this motif is essential for receptor binding, in agreement with the role of these residues as a pharmacophore. In rat, alanine substitution indicated that residues 6 and 10 of KP-10 are essential for kisspeptin receptor activation, at least in this species (Gutiérrez-Pascual et al., 2009).

The synthesis of lower molecular weight derivatives of the KP-10 structure by 9-fluorenylmethoxycarbonyl-based solid-phase peptide synthesis produced molecules, such as the pentapeptide H-Amb-Nal(2)-Gly-Leu-Arg-Trp-NH₂, that were high-potency agonists comparable with kisspeptin (Tomita et al., 2006). Further investigations into the effect of terminal acyl groups on agonist potencies of pentapeptide analogs led to the synthesis of 4-fluorobenzoyl-Phe-Gly-Leu-Arg-Trp-NH₂, with an EC₅₀ of 0.69 nM (Tomita et al., 2007). Agonistic peptide analogs of this compound have been synthesized that were more stable against degradation by MMP-2 and -9 (Tomita et al., 2008), which should prove useful in increasing *in vivo* stability.

A recent structure-activity study revealed that the five N-terminal amino acids of KP-10 are required for receptor activation and also identified key residues, Ser5 and Leu8, that are obligatory for agonist activity. They also found that analogs comprising five amino acids possessed lower binding affinities than those containing the full 10 residues (Roseweir et al., 2009). In addition, a KP-10 analog, [dY]¹KP-10, has been reported to be a superagonist in mice *in vivo* (Curtis et al., 2010). However, previous work by Niida et al. (2006) found no stimulation of the kisspeptin receptor with this analog, which could be due to differences in reporter systems in the two studies.

VII. Antagonists

One peptide antagonist has been reported: ac[(D)-A]NWNGFG[(D)-W]RF (peptide 234) (Roseweir et al., 2009). It was discovered by systematically substituting amino acid residues in the KP-10 sequence and the resulting compounds were tested for the ability to inhibit kisspeptin-stimulated inositol phosphate release against chinese hamster ovary cells stably expressing the kisspeptin receptor. Peptide 234 contained seven residues conserved from KP-10, had an IC₅₀ of 7.0 nM, and competed for the binding of ¹²⁵I-KP-10 with an affinity of 2.7 nM. It was initially demonstrated to inhibit KP-10 actions on the hypothalamic-gonadotropic axis in female rhesus monkeys, male rats, and ovariectomized ewes upon acute central injections. However, recent studies have documented that both intracerebroventricular and systemic administration of a penetrating peptide 234 fusion molecule are capable of suppressing

gonadotropin secretion after central administration of KP-10 to male rats (Pineda et al., 2010).

VIII. Receptor Signaling

At present, knowledge of intracellular signaling pathways downstream of the kisspeptin receptor has mainly come from assays in cell lines. There is substantial evidence that the kisspeptin receptor couples to the G $\alpha_{q/11}$ signaling pathway, activating PLC, to result in phosphatidylinositol 4,5-bisphosphate hydrolysis followed by accumulation of inositol-(1,4,5)-triphosphate and diacylglycerol to cause subsequent Ca²⁺ mobilization (Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001). Other signaling pathways activated seem to be cell type-dependent, and proposed downstream mediators include protein kinase C, arachidonic acid, mitogen activated protein kinases (such as extracellular signal-regulated kinase 1/2 and p38), and phosphatidylinositol-3-kinase/Akt (Kotani et al., 2001; Muir et al., 2001; Ringel et al., 2002; Becker et al., 2005; Stathatos et al., 2005). Signaling through inositol-(1,4,5)-triphosphate is also consistent with the potential vasoconstrictor role of kisspeptin (see section VIII.C). It is noteworthy that confirmation of kisspeptin signaling via PLC and Ca²⁺-dependent pathways has been obtained from studies on GnRH neurons in brain slices (Castellano et al., 2006b; Liu et al., 2008), which have also shown involvement of transient receptor potential cation channels (Zhang et al., 2008).

Activation of the kisspeptin receptor by kisspeptins also causes phosphorylation of focal adhesion kinase and paxillin, leading to formation of focal adhesion and stress fibers (Kotani et al., 2001; Ohtaki et al., 2001), and this correlates with the role of kisspeptin in inhibiting chemotaxis. Kisspeptin receptor stimulation has been demonstrated to inhibit calcineurin activity (Stathatos et al., 2005), which could also contribute to metastasis suppression. The kisspeptin receptor pathway seems to have the ability to disrupt signaling via the chemokine receptor CXCR4, thus inhibiting the prometastatic activity arising from interaction with its ligand, stromal cell-derived factor 1 (Navenot et al., 2005). Kisspeptin has been reported to induce apoptosis in cells, although there are conflicting reports about the role apoptosis may play in the metastasis-suppressing actions of kisspeptin (Harms et al., 2003; Becker et al., 2005). However, recent evidence favors an involvement of apoptosis (Navenot et al., 2009b), possibly by activation of Rho and Rho-associated kinase (Navenot et al., 2009a).

Desensitization of the hypothalamic-pituitary-gonadal axis after continuous administration of kisspeptin has been demonstrated (Seminara et al., 2006; Thompson et al., 2006; Ramaswamy et al., 2007; Roa et al., 2008a). Further investigation revealed the kisspeptin receptor to be constitutively associated with the G-protein-coupled receptor serine/threonine kinases and β -arrestins-1

and -2, suggesting that they mediate kisspeptin receptor signaling and desensitization (Pampillo et al., 2009).

The precise mechanism responsible for the inhibitory action of kisspeptin on the expression of MMP-2/9 is not known. However, it is proposed that kisspeptin receptor activation decreases the binding of NF κ B binding to the promoter region of MMP-9, affecting its expression and resultant cell migration (Yan et al., 2001).

IX. Physiological Role

A. The Neuroendocrine Regulation of Reproduction

The crucial role that kisspeptin and its receptor play in the regulation of the reproductive axis was first indicated by observations of loss-of-function mutations in the kisspeptin receptor in some patients with idiopathic hypogonadotropic hypogonadism (Seminara et al., 2003; de Roux et al., 2003) and confirmed in transgenic mice models (Funes et al., 2003; Seminara et al., 2003; Kauffman et al., 2007c; Lapatto et al., 2007). Kisspeptin has since been identified as a major regulator of the hypothalamic-pituitary-gonadal axis, governing pubertal onset, in an increasing number of species. Kisspeptin is able to stimulate gonadotropin release in humans (Dhillon et al., 2005, 2007), mice (Gottsch et al., 2004; Messenger et al., 2005b), rats (Navarro et al., 2004a, 2005a,b; Thompson et al., 2004, 2006), monkeys (Plant et al., 2006; Seminara, 2006), and sheep (Messenger et al., 2005b; Caraty et al., 2007). GnRH is a direct mediator of this effect, as shown in monkeys (Keen et al., 2008), sheep (Messenger et al., 2005b), pigs (Lents et al., 2008), and goats (Hashizume et al., 2010). GnRH antagonists are able to block kisspeptin-induced release of gonadotropins (Gottsch et al., 2004; Matsui et al., 2004; Navarro et al., 2004a). Further investigations have shown GnRH neurons to express the kisspeptin receptor (Irwig et al., 2004; Parhar et al., 2004; Han et al., 2005; Messenger et al., 2005b) and key experiments involving *Kiss1r* and *Kiss1* knockout mice highlighted that a functional kisspeptin receptor is necessary for GnRH secretion and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Seminara et al., 2003; Dungan et al., 2007; Lapatto et al., 2007; d'Anglemont de Tassigny et al., 2007, 2008). Investigations into the mechanism of communication between kisspeptin and GnRH neurons have found that GnRH neuronal activity is increased by kisspeptin, as measured by c-Fos immunoreactivity (Irwig et al., 2004; Matsui et al., 2004), and that kisspeptin depolarizes GnRH neurons (Han et al., 2005; Zhang et al., 2008), increasing firing rates (Quaynor et al., 2007; Dumalska et al., 2008; Liu et al., 2008; Pielecka-Fortuna et al., 2008).

Within the hypothalamus, regulation of the expression of *Kiss1* and, to a lesser extent, *Kiss1r* mRNA by sex steroids has been documented in a range of species. Gonadectomized mice, sheep, and rhesus monkeys show increased *Kiss1* mRNA expression in the arcuate nu-

cleus or infundibular region (Irwig et al., 2004; Navarro et al., 2004a; Pompolo et al., 2006; Smith et al., 2005a,b, 2007; Rometo et al., 2007; Shibata et al., 2007). Sex steroid replacement decreases the elevated *Kiss1* mRNA expression to control levels (Navarro et al., 2004a; Smith et al., 2005b, 2007; Pompolo et al., 2006; Rometo et al., 2007; Shibata et al., 2007), suggesting that this circuitry represents negative feedback control of gonadotropin secretion. Conversely, the preovulatory LH surge involves positive feedback; in mice and rats, this seems to be regulated by neurons in the AVPV (Smith et al., 2005a, 2006b; Adachi et al., 2007). Ovariectomized female rats have decreased *Kiss1* mRNA expression in the AVPV, which is restored with estrogen replacement (Smith et al., 2005a). This seems to be mediated by the estrogen receptor ER α (Kinoshita et al., 2005; Adachi et al., 2007; Roa et al., 2008b,c). However, this mechanism of control varies between species; in sheep and primates, kisspeptin neurons are restricted to the arcuate nucleus and the preoptic area, and both positive and negative feedback regulation of gonadotropin secretion seems to occur in the arcuate nucleus (Estrada et al., 2006; Pompolo et al., 2006; Smith et al., 2007).

There is substantial evidence that kisspeptin receptor signaling is required for the initiation of puberty. Humans and mice with disruption of the *KISS1R* or *KISS1* gene fail to go through puberty (de Roux et al., 2003; Funes et al., 2003; Seminara et al., 2003; d'Anglemont de Tassigny et al., 2007; Lapatto et al., 2007). Kisspeptin administration induced precocious puberty (Navarro et al., 2004b), whereas central injection of a kisspeptin antagonist delayed puberty (Pineda et al., 2010), in prepubertal rats. During puberty, there seems to be increased communication between kisspeptin and GnRH neurons, demonstrated by increased hypothalamic *Kiss1* and *Kiss1r* mRNA expression in rats and monkeys (Navarro et al., 2004a; Shahab et al., 2005), increased appositions between kisspeptin fibers and GnRH neurons (Clarkson and Herbison, 2006), increased kisspeptin pulse frequency (Keen et al., 2008), and increased sensitivity of GnRH neurons to kisspeptin (Han et al., 2005). Kisspeptin may also act at the level of the pituitary, although this is controversial and warrants further investigation (Richard et al., 2009).

A growing body of evidence suggests that the kisspeptin/kisspeptin receptor system has the ability to integrate both metabolic cues, such as nutritional status and metabolism (Castellano et al., 2005; Smith et al., 2006a), and environmental cues such as photoperiod (Greives et al., 2007; Revel et al., 2007; Smith et al., 2007; Wagner et al., 2008) and act on these to affect the reproductive system.

B. Pregnancy

In addition to the well established role of kisspeptin and its receptor in the regulation of the reproductive axis, the kisspeptin system has been proposed to have effects on other physiological systems, such as gestation.

Initial reports of kisspeptin expression reported high levels in the human placenta (Lee et al., 1996; Muir et al., 2001; Ohtaki et al., 2001). More specifically, kisspeptin and kisspeptin receptor expression has now been demonstrated in human trophoblasts (Janneau et al., 2002; Bilban et al., 2004), with higher expression in the first trimester than the third, correlating with decreasing invasiveness. An in-house validated radioimmunoassay revealed that in male and nonpregnant female humans, plasma kisspeptin circulates at very low concentrations (Dhillon et al., 2005, 2006). However, during pregnancy, plasma kisspeptin concentrations increase dramatically, with a 1000-fold increase in the first trimester, rising to a 10,000-fold increase in the third trimester (Horikoshi et al., 2003). Kisspeptin inhibits the migration of trophoblasts, at least in vitro (Bilban et al., 2004), possibly via down-regulation of MMP-2. Kiss1 and Kiss1r mRNA expression has also been detected in the trophoblast giant cells of the rat placenta (Terao et al., 2004), although the relevance of kisspeptin in the physiology of gestation of nonhuman species has yet to be fully investigated.

C. Other Roles

In the human cardiovascular system, expression of both receptor and peptide has been identified in the coronary artery, aorta, and umbilical vein, and kisspeptin elicited potent vasoconstrictor effects in coronary artery and umbilical vein (Mead et al., 2007b). In addition, kisspeptin acts as a positive inotropic agent in human and mouse heart (Kirby et al., 2008), consistent with the ability of kisspeptin to increase intracellular Ca^{2+} (see section VI). This suggests it may function as a cardiovascular transmitter.

In the pancreas, high expression of peptide and receptor has been detected in human and mouse islet endocrine cells, where it can potentiate the secretion of insulin (Hauge-Evans et al., 2006; Bowe et al., 2009). Additional studies suggested that intravenous KP-10 increased insulin levels in rats (Bowe et al., 2009, although another group found that KP-13 reduced glucose-induced insulin secretion in a perfused-pancreas model (Silvestre et al., 2008), a discrepancy that may reflect the different peptides used or the importance of the in vivo environment.

Kiss1 and Kiss1r mRNA have been identified in the rat hippocampus (Arai et al., 2005) and have been shown to affect neuronal transmission there (Arai and Orwig, 2008), presenting the possibility that the kisspeptin system could be involved in neurogenesis, cognition, and the pathogenesis of epilepsy.

X. Pathophysiological Role

A. Metastasis

The initial discovery of kisspeptin was made from observations of an antimetastatic effect of chromosome 6

in human melanoma cell lines (Welch et al., 1994; Miele et al., 1996, 2000). Investigations by subtractive hybridization and differential display showed one gene in particular to be unregulated in those cells transfected with chromosome 6 and that this gene was a metastasis suppressor (Lee et al., 1996; Lee and Welch, 1997a). However, mapping showed that this gene was in fact located on chromosome 1q32, not 6 (Lee et al., 1996). Further studies have determined the presence of a trans-acting regulatory product on chromosome 6 (Lee et al., 1996; West et al., 1998; Goldberg et al., 2003; Mitchell et al., 2007).

The metastatic suppressor activity of the kisspeptin system was first identified in melanoma (Lee and Welch, 1997a; Ohtaki et al., 2001; Shirasaki et al., 2001). It was then shown that this effect was not solely limited to this cancer type by detection in breast cancer (Lee and Welch, 1997b; Martin et al., 2005; Stark et al., 2005; Kostadima et al., 2007; Marot et al., 2007; Mitchell et al., 2007). Most reports associated loss of *KISS1* with increasing cancer progression and metastases, although there are contradictions (Martin et al., 2005; Marot et al., 2007). To date, the metastasis suppressor activity of the kisspeptin system has been identified in thyroid (Ringel et al., 2002; Stathatos et al., 2005), ovarian (Jiang et al., 2005; Zhang et al., 2005; Gao et al., 2007; Hata et al., 2007), bladder (Sanchez-Carbayo et al., 2003), gastric (Dhar et al., 2004; Guan-Zhen et al., 2007; Yao et al., 2007), esophageal (Ikeguchi et al., 2004), hepatocellular (Ikeguchi et al., 2003; Hou et al., 2007; Schmid et al., 2007), pancreatic (Masui et al., 2004; Liang and Yang, 2007), and lung (Zohrabian et al., 2007) cancers.

B. Disorders of the Hypothalamic-Pituitary-Gonadal Axis

An understanding of the fundamental role played by the kisspeptin receptor in puberty was achieved from studies of patients with idiopathic hypogonadotropic hypogonadism. Linkage analysis of these patients, who displayed no sexual maturation and low levels of gonadotropins, revealed mutations in the *KISS1R* gene: a homozygous substitution of leucine to serine at nucleotide 148 (L148S) or heterozygous mutations at nucleotides 331 (R331X) and 399 (X399R) (Seminara et al., 2003). Since then, other loss-of-function mutations have been documented, and more detailed molecular analysis has shown the L148S mutation to inhibit the catalytic activation of G_q but not to affect the trafficking, plasma membrane expression, or ligand binding properties of the receptor (Wacker et al., 2008). A gain-of-function mutation (R386P) has been reported in a girl with central precocious puberty (Teles et al., 2008). The mutations in the kisspeptin receptor that are currently known are illustrated in Fig. 4. It is noteworthy that the phenotype observed in humans carrying loss-of-function mutations was mimicked in *Kiss1r*-null mice (Seminara

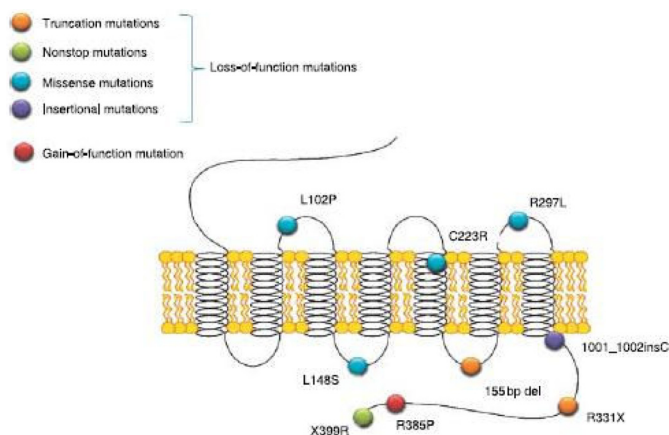


FIG. 4. Identified mutations in the human *KISS1R* gene. [Reprinted from Gianetti E and Seminara S (2008) Kisspeptin and KISS1R: a critical pathway in the reproductive system. *Reproduction* 136:295–301. Copyright © 2008 Society for Reproduction and Fertility. Used with permission.]

et al., 2003), confirming the essential role of the kisspeptin receptor in pubertal development.

C. Other Pathophysiologies

In concordance with the proposed role of the kisspeptin system in pregnancy, there are emerging, although contradictory, reports of changes in kisspeptin expression in preeclampsia (Qiao et al., 2005; Farina et al., 2006; Armstrong et al., 2009). In addition, Panidis et al. (2006) reported raised levels of KP-54 in normal-weight subjects with polycystic ovary syndrome, which correlates with its association with leptin and obesity. However, these results should be interpreted with caution owing to the different assays used. Kisspeptin and kisspeptin receptor expression has also been detected in human atherosclerotic plaques (Mead et al., 2007b).

In diabetes, where hypogonadism is common, disturbed kisspeptin function can be rescued by leptin (Castellano et al., 2006a). The link between leptin and kisspeptin was further strengthened by observations that leptin can influence the expression of kisspeptin in the arcuate nucleus of mice (Smith et al., 2006a).

XI. Genetically Modified Animals

A. Kisspeptin Receptor

Disruption of the *Kiss1r* gene in mice results in viable homozygous offspring with hypogonadotropic hypogonadism. Male mice show small testes, hypoplastic Leydig cells, spermatogenic arrest, and absence of development of secondary sex glands. Female mice have delayed vaginal opening, hypoplastic uterine horns, and small ovaries with no graafian follicles or corpora lutea. Both sexes display decreased levels of sex steroids (testosterone or β -estradiol) in addition to reduced concentrations of LH and FSH (Funes et al., 2003; Seminara et al., 2003; Kauffman et al., 2007c; Lapatto et al., 2007), and female mice lack the compensatory rise in LH that usu-

ally follows ovariectomy (Dungan et al., 2007). There is also a lack of stimulation of LH or FSH release upon injection of kisspeptin (Messenger et al., 2005b). This phenotype is consistent with that of humans with mutations in the *KISS1R* gene.

B. Kisspeptin

Targeted disruption of the *Kiss1* gene also results in viable offspring who have an inability to undergo sexual maturation. Mutant male mice have arrested spermatogenesis and small testes; female mice have small ovaries, threadlike uteri, and no progression through the estrous cycle; and both sexes have low levels of circulating LH, FSH, and sex steroids. The pituitary remains functional, however, as peripheral administration of kisspeptin results in LH secretion (d'Anglemon de Tasigny et al., 2007; Lapatto et al., 2007).

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