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(54) **LHRH antagonist peptides**

LHRH antagonistische Peptide

Peptides antagonistes de LHRH

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EP 1 188 768 B1

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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

Background of the Invention

[0001] The present invention relates to LHRH antagonist peptides and uses thereof.

[0002] Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are hormones released by the pituitary gland. These hormones regulate the functioning of the gonads and the production and maturation of gametes. LH and FSH are generally released by the pituitary gland upon prior release of triggering hormone from the hypothalamus. Luteinizing hormone-releasing hormone (LHRH; also known as gonadotropin-releasing hormone or GnRH) is one of the principal hypothalamic hormones which triggers the release of LH. Thus, release of LHRH represents a control point in the physiological regulation of gonadal function. The structure of mammalian LHRH has been determined, and has been found to be a decapeptide:

p-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (SEQ ID NO: 1)

1 2 3 4 5 6 7 8 9 10

[0003] LH release is necessary for ovulation; thus, compounds which inhibit LH release by blocking the action of LHRH are useful as contraceptive agents. LHRH antagonists are also useful for regulating secretion of gonadotropins in male mammals, and thus can be used as male contraceptives. In addition, LHRH antagonists can be used in the treatment of sex-hormone dependent cancers (for example, prostate cancer), where increased levels of gonadotropins increase the rate of tumor growth.

[0004] Many modified LHRH analog peptides have been synthesized in an attempt to increase the potency of the antagonists, preferably while also increasing the resistance of the antagonist to enzymatic degradation. For example, synthetic LHRH antagonist peptides which incorporate modified or unnatural amino acids have been tested. Common substitutions include, for example, substitution of 4-Cl-D-Phe for His at position 2, or substitution of D-Ala-NH_2 for Gly-NH_2 at position 10.

[0005] One problem frequently encountered in LHRH antagonist peptides is the occurrence of histamine-releasing activity. This histamine-releasing activity represents a serious obstacle to the clinical use of such antagonists because histamine release results in adverse side effects such as edema and itching. Thus, LHRH antagonist peptides which have low histamine releasing activity are particularly desirable. Although the LHRH antagonist and histamine-releasing properties are not necessarily related, very few prior art compounds combine low histamine-releasing activity with high LHRH antagonist activity. Many prior art LHRH antagonist peptides also suffer from poor water-solubility, which complicates formulation of the antagonist for administration.

[0006] Octa-and nonapeptides which are antagonistic analogs of LHR1-1 are disclosed in FR-A-2329294 and FR-A-2329293.

[0007] A series of des-His^2 octa- and nonapeptide analogs of LHRH with modifications in the 1 and 6 positions and, in some instances the 10 position are disclosed in Edgren et al J. Med. Chem. (1979) 22,935-943.

Summary of the Invention

[0008] An aspect of the invention provides an LHRH anatagonist as set out in claim 1. More preferably, the peptide compound comprises 10 residues.

[0009] A peptide compound may comprise a structure:

A-B-C-D-E-F-G-H-I-J

wherein

A is pyro-Glu, Ac-D-Nal, Ac-D-Qal, Ac-Sar, or Ac-D-Pal;
B is His or 4-Cl-D-Phe;
C is Trp. D-Pal, D-Nal, L-Nal, D-Pal(N-O), or D-Trp;
D is Ser;
E is N-Me-Ala, Tyr, N-Me-Tyr, Ser, Lys(iPr). 4-Cl-Phe, His, Asn, Met, Ala, Arg or Ile;
F is D/L-Asn or L-Gln;
G is Leu or Trp;

H is Lys(iPr), Gln, Met, or Arg;
I is Pro; and
J is D-Ala-NH_2;

or a pharmaceutically acceptable salt thereof.

[0010] The disclosure also provides pharmaceutical compositions of the LHRH antagonist peptides.

[0011] A method of inhibiting LHRH activity in a subject, may comprise administering to a subject an effective amount of an LHRH antagonist, such that LHRH activity is inhibited.

[0012] A method of inhibiting LHRH activity in a cell, may comprise contacting a cell with an LHRH antagonist, such that LHRH activity is inhibited.

[0013] A method of inhibiting growth of a hormone-dependent tumor in a subject, may comprise administering to a subject an effective amount of an LHRH antagonist, such that tumor growth is inhibited.

[0014] A method of inhibiting ovulation in a subject, may comprise administering to a subject an effective amount of an LHRH antagonist, such that ovulation is inhibited.

[0015] A packaged formulation for treating a subject for a disorder associated with LHRH activity may comprise an LHRH antagonist packaged with instructions for using the LHRH antagonist for treating a subject having a disorder associated with LHRH activity.

Brief Description of the Drawings

[0016]

Figure 1 is a graph depicting plasma testosterone levels (in ng/ml) in adult male rats administered eight subcutaneous injections of the LHRH antagonist #3827, one injection every three hours, at doses of 1, 10 or 100 μg/kg.

Figure 2 is a graph depicting plasma testosterone levels (in ng/ml) in adult male rats subcutaneously administered the LHRH antagonist #3827 via an osmotic pump. Doses were either 300 μg/kg/day for two weeks, followed by 15 μg/kg/day for an additional two weeks or 1000 μg/kg/day for two weeks, followed by 5, 15 or 50 μg/kg/day for an additional two weeks.

Figure 3 is a graph depicting the plasma testosterone levels (in ng/ml) in adult male rats subcutaneously administered the LHRH antagonist #3827 via an osmotic pump. Doses were either 30 μg/kg/day for two weeks, followed by 5 μg/kg/day for an additional two weeks, 100 μgkg/day for two weeks, followed by 5 μg/kg/day for an additional two weeks, or 300 μg/kg/day for two weeks, followed by 0 or 5 μg/kg/day for an additional two weeks.

Detailed Description of the Invention

[0017] In order that the present invention may be more readily understood, certain terms are first defined.

[0018] As used herein, "LHRH antagonist peptide" is intended to include peptides and peptide analogs which inhibit LHRH, activity (i.e., has "LHRH antagonist activity") *in vivo* or *in vitro*. Candidate LHRH antagonist peptides can be assayed, for example, in the animal model described in Corbin and Beattie, Endocrine Res. Commun. 2:1 (1975) (and see *infra*). In this assay, the LHRH antagonistic activity of a candidate compound is assayed by measuring the antiovulatory activity (AOA) of the compound in rats.

[0019] The term "histamine-releasing activity", as used herein, refers to the tendency of a compound to release histamine when administered to a subject. The histamine-releasing activity of a compound can be measured with an *in vitro* assay (described in more detail, *infra*). Preferred LHRH antagonist peptides have high activity in the rat antiovulatory activity assay, but low histamine releasing activity. Preferred LHRH antagonist peptides have an ED_{50} in the histamine release assay of at least 3 μg/ml, more preferably at least 5 μ g/ml, and still more preferably at least 10 μg/ml.

[0020] The term "alkyl", as used herein, refers to a straight or branched chain hydrocarbon group having from about 1 to about 10 carbon atoms. The term "lower alkyl" refers to an alkyl group having from about 1 to about 6 carbon atoms. Exemplary lower alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, and n-hexyl. An alkyl group may be unsubstituted, or may be substituted at one or more positions, with, e.g., halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, heterocycles, hydroxyls, aminos, nitros, thiols, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, $-CF_3$, -CN, or the like. Preferred alkyls are lower alkyls.

[0021] The term "cycloalkyl" refers to a cyclic saturated hydrocarbon group having from 3 to 8 carbon atoms. Exemplary cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Cycloalkyl groups may be unsubstituted or substituted at one or more ring positions as described for alkyls. Thus, a cycloalkyl may be substituted with, e.g., halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, heterocycles, hydroxyls, aminos, nitros, thiols amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones,

aldehydes, esters, - CF_3, -CN, or the like.

[0022] The terms "alkenyl" and "alkynyl", as used herein, refer to unsaturated groups analogous in length and possible substitution to the alkyls described above, but which contain at least one carbon-carbon double or triple bond respectively.

[0023] The term "aryl" as used herein includes 4-, 5-, 6- and 7-membered single-ring aromatic groups which may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycle" or "heteroaromatic". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, heterocycles, hydroxyls, aminos, nitros, thiols, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, -CF_3, -CN, or the like. An aromatic ring may also be substituted with another aromatic ring, as in, for example, a biphenyl. Aryl groups also include fused or polycyclic aromatic systems.

[0024] The terms "heterocycle" or "heterocyclic group" refer to 4- to 10-membered ring structures, more preferably 5 to 7 membered rings, which ring structures include one to four heteroatoms. Heterocyclic groups include pyrrolidine, oxolane, thiolane, imidazole, oxazole, piperidine, piperazine, morpholine. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, other heterocycles, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, -CF_3, -CN, or the like. Heterocycles may also be bridged or fused to other cyclic groups as described below.

[0025] The terms "polycycle" or "polycyclic group" refer to two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocycles) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, -CF_3, -CN, or the like.

[0026] The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur, phosphorus and selenium.

[0027] The term "arylalkyl", as used herein, refers to an aryl group appended to an alkyl group, including, but not limited to, benzyl, naphthylmethyl, pyridyl methyl, and the like.

[0028] The term "ylid" is known in the art and refers to a moiety in which a positively charged atom (especially from Groups V and VI of the periodic table) is bonded to a carbon atom which bears an unshared pair of electrons. Thus, an ylid has the resonance forms:

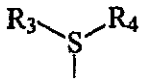


in which W is a heteroatom such as S, or P, and R_1 and R_2 are, independently, H, alkyl, cycloalkyls, alkenyl, alkynyl, aryl, alkoxy, thioalkoxy, and the like. The heteroatom is substituted with an appropriate number of substituents (i.e., two for N and S, and three for P); the substituents are independently alkyl, cycloalkyl, aryl, and the like. Nitrogen ylides do not have a significant contribution from the non-charge-separated resonance form (on the right, above).

[0029] The term "dipolar moiety", as used herein, refers to a covalently bonded moiety having both positive and negative charges (e.g., a zwitterionic moiety). Exemplary dipolar groups include ylids (e.g., of S, N, or P), tertiary amine oxides, nitrones, pyridine-N-oxides, nitrile oxides, quaternary amino acids (e.g., 2-(N,N,N-trialkylammonium) acetate), amino acids, sulfonium arene oxides (as described in, for example, U.S. Patent 4,111,914), betaines (e.g., trigonellin), and the like. In certain preferred embodiments, the dipolar moiety is a pyridine-N-oxide. In other preferred embodiments, the dipolar moiety is a zwitterionic pyridinium moiety. In certain other embodiments, the dipolar moiety is not a zwitterionic pyridinium moiety.

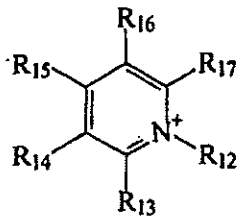
[0030] As used herein, a "cationic moiety" is a moiety in which at least one atom bears a positive charge, and the moiety has a net positive charge. Thus, for example, an N-alkyl (or N-alkenyl, -alkynyl, or -aryl, collectively referred to herein as "N-substituted pyridinium") pyridinium moiety can be a cationic moiety (and is referred to herein as a "cationic pyridinium moiety"), but a pyridine-N-oxide is not, unless it has a net positive charge. As described above, a pyridine-N-oxide can be a dipolar moiety. Other exemplary cationic moieties include quaternary amines, sulfonium salts, phosphonium salts, and the like. In certain preferred embodiments, the cationic moiety is a sulfonium moiety.

[0031] A sulfonium moiety has the following structure:



in which R_3 and R_4 are each, independently, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, and the like.

[0032] In other preferred embodiments, the cationic moiety is a cationic pyridinium moiety. A cationic pyridinium moiety has the following structure:

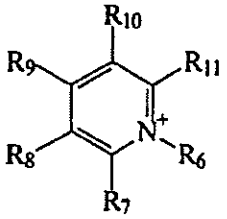


wherein R_{12} is alkyl or aryl, and R_{13}-R_{17} are each, independently, hydrogen, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, other heterocycles, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, $-CF_3$, -CN, or the like. Preferred cationic pyridinium moieties include Pal(iPr) and Pal(Bzl). N-methyl pyridinium moieties are not preferred.

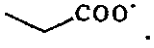
[0033] Although the above examples describe pyridine (or pyridinium) moieties, it will be apparent to the skilled artisan that other N-heteroaromatic moieties (that is, a moiety in which at least one nitrogen is present in an aromatic ring) may be substituted for the pyridine (or pyridinium) moieties described herein. Exemplary N-heteroaromatics include thiazole, triazole, tetrazole, pyrazole, pyrazine, pyridazine and pyrimidine, and the like. Thus, N-substituted pyrazines, pyridazines, and the like, are contemplated for use in the present invention.

[0034] As used herein, "tertiary amine" includes trialkyl amines, triaryl amines, and amines which have both alkyl and aryl substituents.

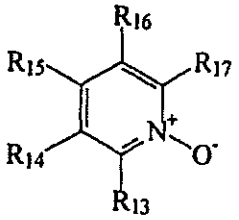
[0035] As used herein, "zwitterionic pyridinium moiety" refers to a moiety having the form:



in which R_6 comprises an alkyl, cycloalkyl, alkenyl, alkynyl, or aryl moiety , and R_7-R_{11} are each, independently, hydrogen, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, other heterocycles, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, $-CF_3$, -CN, or the like, with the proviso that at least one of R_6-R_{11} is substituted with an anionic moiety. An "anionic moiety", as used herein, is a moiety which has a net negative charge. The anionic moiety is chosen to be compatible with other moieties, and to form a stable compound. Illustrative anionic moieties include carboxylates, phosphates, phosphonates, sulfates, sulfonates, and the like. In certain preferred embodiments, the anionic moiety is a carboxylate. In other preferred embodiments, the anionic moiety is a sulfonate. In a preferred embodiment, R_6 comprises:

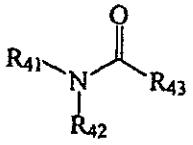


[0036] A pyridine N-oxide is a moiety which has the form:



where R_{13}-R_{17} have the meanings defined above.

[0037] The term "hydrophilic N-acyl moiety", as used herein, refers to a moiety which comprises a nitrogen atom acylated so as to form a hydrophilic moiety. Thus, a hydrophilic N-acyl moiety can have the form:



where R_{41} and R_{42}, are each, independently, H, alkyl, cycloalkyl, aryl and the like; and R_{43} is alkyl, cycloalkyl, alkenyl, alkynyl, aryl, and the like; and R_{41}-R_{43} are selected to form a hydrophilic moiety. In preferred embodiments, R_{41} and R_{42} are not both H.

[0038] Relative hydrophilicity can be determined by any of several methods known in the art (Hansch, ed., "Comprehensive Medicinal Chemistry", Vol. 4, Pergamon Press, Oxford, 1990), and can be used to guide the choice of potential hydrophilic moieties for use in the invention. The partition coefficient, P, between 1-octanol and water has been used as a reference for measuring the hydrophilicity of a compound. Hydrophilicity can be expressed as log P, the logarithm of the partition coefficient (Hansch et al., Nature 194:178 (1962); Fujita et al., J. Am. Chem. Soc. 86:5175 (1964)). Standard tables of hydrophilicity for many molecules, and lipophilicity (hydrophobicity) substituent constants (denoted π) for many functional groups, have been compiled (see, e.g., Hansch and Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology," Wiley, New York, New York, (1979)). The hydrophilicity of a vast range of candidate hydrophilicity moieties can be quite accurately predicted with the aid of these tables. For example, the measured log P (octanol/water) of naphthalene is 3.45. The substituent constant p for -OH is -0.67. Therefore, the predicted log P for β-naphthol is 3.45 + (-0.67) = 2.78. This value is in good agreement with the measured log P for β-naphthol, which is 2.84. In certain preferred embodiments, the hydrophilic N-acyl moiety has a value of log P between -1 and +2, more preferably between -0.5 and +1.5.

[0039] The term "small polar moiety" refers to a moiety which has small steric bulk and is relatively polar. In certain embodiments, a small polar moiety is not D-Cit, D-Hci or lower alkyl derivatives thereof. Polarity is measured as hydrophilicity by the P scale described above. In certain preferred embodiments, the small polar moieties have a log P between -1 and +2. In particularly preferred embodiments, the small polar moiety modifies residue 6. In preferred embodiments, the steric bulk of the small polar moiety is less than the steric bulk of Trp. Examples of residues incorporating preferred small polar moieties are D- or L-Asn, and L-Gln. In an especially preferred embodiment, the small polar moiety is D-Asn.

[0040] The term "leaving group" is known in the art and, as used herein, refers to a functionality which upon heterolytic bond cleavage departs with an electron pair. In general, good leaving groups are those moieties which are expelled from the substrate as weak bases, whether charged or uncharged. For example, sulfates, sulfonates, sulfides, chloride, bromide, iodide, phosphates, phosphinates, and the like are good leaving groups. In other words, when, for example, a C-S bond of a sulfonium moiety is cleaved, a sulfide departs (with an electron pair).

[0041] The term "receptor-modifying moiety", as used herein, refers to a moiety which can modify, covalently or non-covalently, a receptor for an LHRH antagonist peptide. For example, it has recently been shown (C.A. Flanagan et al., (1994) J. Biol. Chem. 269:22636) that Glu301 of the mouse LHRH receptor (which corresponds to Asp301 in the human LHRH receptor) interacts with Arg^8 in LHRH agonists. Thus, a carboxylate-modifying reagent (such as an alkylating agent) can modify Glu301 (Asp301) and thus modify the mouse (or human) LHRH receptor. A receptor-modifying moiety may act to bond the LHRH antagonist peptide to the receptor (e.g., esterifying the antagonist to the receptor by displacement of a leaving group), or it may modify the receptor without bonding the LRHR antagonist peptide to the receptor (e.g., by methylation with a methylsulfonium moiety). Other residues of an LHRH receptor can also be modified, and moieties which can modify such residues are also receptor-modifying moieties. Exemplary receptor-modifying reagents include alkyl and benzyl halides (e.g., methyl iodide or benzyl bromide), α-haloketones, α-haloesters and α-haloamides

(collectively referred to as "α-halocarbonyls"), sulfonium salts, sulfates, alkyl or aryl sulfonates, and other reagents which comprise a good leaving group as described above. Other receptor-modifying reagents are described in, for example, A.J. Barrett and G. Salvesen, eds. (1986) "Proteinase Inhibitors", Research Monographs in Cell and Tissue Physiology, Vol. 12, Elsevier Press, Amsterdam.

[0042] An LHRH antagonist may contain a receptor-modifying moiety, the disclosure is not intended to be limited to those antagonists that actually modify a receptor residue. An LHRH antagonist comprising a receptor-modifying moiety but which does not actually modify the receptor may nonetheless be an effective LHRH antagonist. However, for those antagonists that do modify a receptor residue, one advantage is that such moieties can be designed to selectively modify only the targeted receptor, thereby reducing non-specific reactions and decreasing the probability of toxic side effects.

[0043] The term "a peptide having a sidechain modified by" a moiety, as used herein, refers to a peptide (or peptide mimetic, see below) in which at least one residue has a sidechain comprising that moiety. Thus, for example, a "peptide having a sidechain modified by a dipolar moiety" means a peptide in which at least one side chain comprises a dipolar moiety.

[0044] The LHRH antagonist peptides also include peptide analogs and peptide mimetics. The term "peptide compound" as used herein is intended to encompass peptides, peptide analogs, peptide derivatives and peptide mimetics. The terms "peptide analog", "peptide derivative" and "peptide mimetic" as used herein are intended to include molecules which mimic the chemical structure of a peptide and retain the functional properties of the peptide. A "residue" refers to an amino acid or amino acid mimetic incorporated in the peptide compound by an amide bond or amide bond mimetic. Approaches to designing peptide analogs are known in the art. For example, see Farmer, P.S. in Drug Design (E.J. Ariens, ed.) Academic Press, New York, 1980, vol. 10, pp. 119-143; Ball. J.B. and Alewood, P.F. (1990) J. Mol. Recognition 3:55; Morgan, B.A. and Gainor, J.A. (1989) Ann. Rep. Med Chem. 24:243; and Freidinger, R.M. (1989) Trends Pharmacol. Sci. 10:270.

[0045] An "amino acid mimetic" refers to a moiety, other than a naturally occurring amino acid, that conformationally and functionally serves as a substitute for a particular amino acid in a peptide compound without adversely interfering to a significant extent with the function of the peptide (e.g., interaction of the peptide with an LHRH receptor). In some circumstances, substitution with an amino acid mimetic may actually enhance properties of the peptide (e.g., interaction of the peptide with an LHRH receptor). Examples of amino acid mimetics include D-amino acids. LHRH antagonist peptides substituted with one or more D-amino acids may be made using well known peptide synthesis procedures. The effect of amino acid substitutions with D-amino acids or other amino-acid mimetics can be tested using assays, e.g., the AOA and histamine-release assays as described below. Other methods of determining the effect of substitution with an amino acid mimetic will be apparent to the skilled artisan.

[0046] The peptide analogs or mimetics include isosteres. The term "isostere" as used herein refers to a sequence of two or more residues that can be substituted for a second sequence because the steric conformation of the first sequence fits a binding site specific for the second sequence. The term specifically includes peptide back-bone modifications (i.e., amide bond mimetics) well known to those skilled in the art. Such modifications include modifications of the amide nitrogen, the α-carbon, amide carbonyl, complete replacement of the amide bond, extensions, deletions or backbone crosslinks. Several peptide backbone modifications are known, including $\psi[CH_2S]$, $\psi[CH_2NH]$, $\psi[C(S)NH_2]$, ψ[NHCO], $\psi[C(O)CH_2]$, and ψ[(E) or (Z) CH=CH]. In the nomenclature used above, ψ indicates the absence of an amide bond. The structure that replaces the amide group is specified within the brackets. Other examples of isosteres include peptides substituted with one or more benzodiazepine molecules (see e.g., James, G.L. et al. (1993) Science 260: 1937-1942)

[0047] Other possible modifications include an N-alkyl (or aryl) substitution (ψ[CONR]), backbone crosslinking to construct lactams and other cyclic structures, or retro-inverso amino acid incorporation (ψ[NHCO]). By "inverso" is meant replacing L-amino acids of a sequence with D-amino acids, and by "retro-inverso" or "enantio-retro" is meant reversing the sequence of the amino acids ("retro") and replacing the L-amino acids with D-amino acids. For example, if the parent peptide is Thr-Ala-Tyr, the retro modified form is Tyr-Ala-Thr, the inverso form is thr-ala-tyr, and the retro-inverso form is tyr-ala-thr (lower case letters refer to D-amino acids). Compared to the parent peptide, a retro-inverso peptide has a reversed backbone while retaining substantially the original spatial conformation of the side chains, resulting in a retro-inverso isomer with a topology that closely resembles the parent peptide and is able to bind the selected LHRH receptor. See Goodman et al. "Perspectives in Peptide Chemistry" pp. 283-294 (1981). See also U.S. Patent No. 4,522,752 by Sisto for further description of "retro-inverso" peptides.

[0048] In addition to amino acid-substituted LHRH antagonist peptides, LHRH antagonist peptide compounds may have other modifications. For example, the amino-terminus or carboxy-terminus of the peptide can be modified. The term "amino-derivative group" is intended to include amino-terminal modifications of the peptide compounds of the invention. Examples of N-terminal modifications include alkyl, cycloalkyl, aryl, arylalkyl, and acyl groups. A preferred N-terminal modification is acetylation. The N-terminal residue may be linked to a variety of moieties other than amino acids such as polyethylene glycols (such as tetraethylene glycol carboxylic acid monomethyl ether), pyroglutamic acid, succinoyl, methoxy succinoyl, benzoyl, phenylacetyl, 2-, 3-, or 4-pyridylalkanoyl, aroyl, alkanoyl (including acetyl and cy-

cloalkanoyl e.g., cyclohexylpropanoyl), arylalkanoyl, arylaminocarbonyl, alkylaminocarbonyl, cycloalkylaminocarbonyl, alkyloxycarbonyl (carbamate caps), and cycloalkoxycarbonyl, among others.

[0049] The term "carboxy-derivative group" is intended to include carboxy-terminal modifications of the peptide compounds. Examples of modifications of the C-terminus include modification of the carbonyl carbon of the C-terminal residue to form a carboxyterminal amide or alcohol (i.e., a reduced form). In general, the amide nitrogen, covalently bound to the carbonyl carbon on the C-terminal residue, will have two substitution groups, each of which can be hydrogen, alkyl or an alkylaryl group (substituted or unsubstituted). Preferably the C-terminal is an amido group, such as $-CONH_2$, $-CONHCH_3$, $-CONHCH_2C_6H_5$ or $-CON(CH_3)_2$, most preferably $-CONH_2$, but may also be 2-, 3-, or 4-pyridylmethyl, 2-, 3-, or 4-pyridylethyl, carboxylic acid, ethers, carbonyl esters, alkyl, arylalkyl, aryl, cyclohexylamide, piperidineamide, other mono or disubstituted amides, ureas, or carbamates. Other moieties that can be linked to the C-terminal residue include piperidine-4-carboxylic acid or amide and cis- or trans- 4-amino-cyclohexanecarboxylic acid or amide.

[0050] The modified forms of LHRH antagonist peptides, including L- or D-amino acid substitutions, covalent modification of end termini or side chains, and peptide analogs and mimetics can be selected for desired alterations of the physical or chemical properties of the peptide, for example, increased stability, solubility, bioavailability, increased or decreased immunogenicity, etc. The peptides can be targeted to particular organs (e.g. the brain) by methods known in the art, for example, the dihydropyridine-pyridinium carrier of Bodor (see, e.g., U.S. patent 4,540,564). When a sidechain modified by a pyridinium moiety is desired, the corresponding N-alkylated dihydropyridine sidechain is incorporated into the peptide. When the peptide is administered to a subject, the N-alkylated dihydropyridine sidechain is oxidized *in vivo* to the desired pyridinium moiety.

[0051] Preferred LHRH antagonist peptides range in length from about 8 to about 12 residues, more preferably from 9 to 11 residues, and most preferably are 10 residues in length.

[0052] The LHRH antagonist peptides can be prepared by any suitable method for peptide synthesis, including solution-phase and solid-phase chemical synthesis. Preferably, the peptides are synthesized on a solid support. Methods for chemically synthesizing peptides are well known in the art (see, e.g., Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant, G.A (ed.). Synthetic Peptides: A User's Guide, W.H. Freeman and Company, New York (1992). Automated peptide synthesizers are commercially available.

[0053] The use of combinatorial libraries to identify ligands is now well established (see, e.g., M.A. Gallop et al., (1994) J Med. Chem. 37:1233; and E.M. Gordon et al., (1994) J Med. Chem. 37:1385; and references cited therein). Therefore, LHRH antagonist peptides can be identified by chemical (e.g., solution or solid-phase) synthesis of combinatorial libraries (e.g., of peptides or peptoids) and screening of the resulting libraries according to known techniques. Thus, many potential ligands can be synthesized and screened in a short period of time, and the most active ligands selected for further testing or use.

[0054] Standard abbreviations and conventions are used throughout this disclosure when describing the peptides. Peptides are written with the N-terminus on the left, the carboxyl terminus on the right. Amino acids are of the L-form unless stated otherwise, e.g., D-Lys means the D-form of lysine. Ac-Xaa means the N-terminal residue Xaa is N-acetylated; C-terminal amides are denoted Xaa-NH_2. In Table 1, only residues which differ from native mammalian LHRH are noted; thus, the notation Met(S^+Me)^8-LHRH.TFA means a peptide which differs from native mammalian LHRH only in the substitution of Met(S^+Me) for the native Arg at position 8 (TFA indicates the trifluoroacetate salt). Lys(iPr) denotes N-ε-2-propyl-lysinyl; other alkylating and acylating moieties are similar indicated. Thus, for example, Met ($S^+CH_2C_6H_5$) denotes S-benzyl methionine. Certain other non-standard residues and moieties are abbreviated as follows:

Abbreviation	Residue or moiety
pGlu	pyro-glutamyl
Nal	3-(2-naphthyl)alaninyl
Ada	3-(1-adamantanyl)alaninyl
4-Cl-Phe	(4'-chlorophenyl)alaninyl
Qal	3-(2'-quinolinyl)alaninyl
Pal	3-(3'-pyridyl)alaninyl
Pal(N-O)	3-(3'-pyridine-N-oxide)alaninyl
Pal(iPr)	3-(N-(2-propyl)-3'-pyridinium)alaninyl
Pal(Bzl)	3-(N-(benzyl)-3'-pyridinium)alaninyl
Pal(CH_2COO^-)	3-(3'-pyridinium-N-(2-acetate))alaninyl
Lys(iPr)	N-ε-2-propyl-lysinyl
Imdac	2-oxo-4-imidazolinyl
Otac	2-oxo-4-thiazolinyl

(continued)

Abbreviation	Residue or moiety
Ppic	3-(piperidin-1-yl)-propanoyl
Dodac	2,5-dioxo-4-imidazolinyl
Met(S^+Me)	S-methyl methioninyl
PEG	polyethylene glycol
Cit	citrullinyl
Hci	homocitrullinyl
Glu(Taurine)	5-(2-sulfoethylamido)glutamyl
Pyz	1,4-pyrazinecarbonyl
Pip	pipecolyl
CNa	(2-cyano)acetyl
Dea	diethylamide
Onic	3-nicotinyl-N-oxide
Glc	gluconate
Orotic	orotate
Orn	ornithine
Dap	2,4-diaminopropionyl

I. LHRH Antagonist Peptides

[0055] The disclosure pertains to LHRH antagonist peptides.

[0056] An LHRH antagonist may comprise a peptide compound, wherein a residue of the peptide compound corresponding to the amino acid at position 6 of natural mammalian LHRH comprises a small polar moiety such that the peptide compound has LHRH antagonist activity as defined in claim 1.

[0057] Preferably, the antagonist has an antiovulatory activity of less than about 1 μg per rat in a rat antiovulation assay and/or the antagonist has an ED_{50} in a histamine release assay of at least about 5 μl/ml. Preferably, the peptide compound comprises 10 residues.

[0058] A peptide compound may comprise a structure:

A-B-C-D-E-F-G-H-I-J

wherein

A is pyro-Glu, Ac-D-Nal , Ac-D-Qal, Ac-Sar, or Ac-D-Pal
B is His or 4-Cl-D-Phe
C is Trp, D-Pal, D-Nal, L-Nal, D-Pal(N-O), or D-Trp
D is Ser
E is N-Me-Ala, Tyr, N-Nlc-Tyr, Ser, Lys(iPr), 4-Cl-Phe, His, Asn, Met, Ala, Arg or Ile;
F is D/L-Asn or L-Gln;
G is Leu or Trp;
H is Lys(iPr), Gln, Met, or Arg
I is Pro; and
J is D-Ala-NH_2;

or a pharmaceutically acceptable salt thereof.

[0059] The disclosure provides peptide compounds of the following structures:

Ac-D-Nal-4-Cl-D-Phe-D-Pal-Ser-N-Me-Tyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH_2; and pharmaceutically acceptable salts thereof.

Ac-D-Nal-4-Cl-D-Phe-D-Pal-Ser-Tyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH_2; and pharmaceutically acceptable salts thereof.

II. Pharmaceutical Compositions

[0060] The LHRH antagonist peptides can be incorporated into pharmaceutical compositions suitable for administration to a subject. The pharmaceutical composition may comprise an LHRH antagonist peptide and a pharmaceutically acceptable carrier.

[0061] A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired result. A therapeutically effective amount of an LHRH antagonist peptide may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antagonist to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antagonist are outweighed by the therapeutically beneficial effects. A non-limiting range for a therapeutically effective amount of an LHRH antagonist is 0.01 μg/kg-10 mg/kg, preferably between about 0.01 and 5 mg/kg. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0062] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous or parenteral administration (e.g., by injection) Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids and other natural conditions which may inactivate the compound.

[0063] A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects. Examples of such salts are salts of acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosporic acid, nitric acid, and the like; acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, benzoic acid, pamoic acid, alginic acid, methanesulfonic acid, naphthalenesulfonic acid, and the like. Also included are salts of cations such as sodium, potassium, lithium, zinc, copper, barium, bismuth, calcium, and the like; or organic cations such as trialkylammonium. Combinations of the above salts are also useful.

[0064] An LHRH-R antagonist can be administered by a variety of methods known in the art. In a preferred embodiment, the LHRH-R antagonist is administered in a time release formulation, for example in a composition which includes a slow release polymer, or by depot injection. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Particularly preferred formulations include controlled-release compositions such as are known in the art for the administration of leuprolide (trade name: Lupron®), *e.g.*, microcapsules (U.S. Patents 4,652,441 and 4,917,893), injectable formulations (U.S. Patent 4,849,228), lactic acid-glycolic acid copolymers useful in making microcapsules or injectable formulations (U.S. Patents 4,677,191 and 4,728,721), and sustained-release compositions for water-soluble polypeptides (U.S. Patent 4,675,189).

[0065] When appropriately formulated, an LHRH-R antagonist may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The LHRH antagonists and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the LHRH antagonists may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the LHRH antagonists in the compositions and preparations may, of course, be varied. The amount of the LHRH antagonists in such therapeutically useful compositions is such that a suitable dosage will be obtained.

[0066] To administer the LHRH antagonists by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the LHRH antagonists may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, (1984) *J. Neuroimmunol.* 7:27). Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0067] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage.

The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin.

[0068] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., LHRH antagonist) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0069] Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

III. Methods of Using the LHRH Antagonists

[0070] LHRH antagonist peptides are useful for the treatment of such conditions as precocious puberty, prostate cancer, ovarian cancer, benign prostatic hypertrophy, endometriosis, uterine fibroids, breast cancer, premenstrual syndrome, polycystic ovary syndrome, and diseases which result from excesses of gonadal hormones in humans or animals of either sex. The LHRH antagonist peptides are also useful for behavior modification (e.g., "chemical castration"). The LHRH antagonist peptides are also useful for controlling reproduction in both males and females. Furthermore, the peptides may be used to treat immunosuppressed patients, as described in, for example, U.S. Patent 5,003,011.

[0071] A method of inhibiting LHRH activity in a subject may comprise administering to a subject an effective amount of an LHRH antagonist, such that LHRH activity is inhibited.

[0072] A method of inhibiting LHRH activity in a cell may comprise contacting a cell with an LHRH antagonist, such that LHRH activity is inhibited.

[0073] A method of inhibiting growth of a hormone-dependent tumor in a subject may comprise administering to a subject an effective amount of an LHRH antagonist, such that tumor growth is inhibited. A LHRH antagonist may be administered to a subject suffering from prostate cancer to inhibit the growth of the prostatic tumor.

[0074] A method of inhibiting ovulation in a subject may comprise administering to a subject an effective amount of an LHRH antagonist, such that ovulation is inhibited.

[0075] A packaged formulation for treating a subject for a disorder associated with LHRH activity may comprise an LHRH antagonist packaged with instructions for using the LHRH antagonist for treating a subject having a disorder associated with LHRH activity.

[0076] Peptide compounds may be used in the manufacture of a medicament for the treatment of a disorder in which inhibition of LHRH activity is beneficial. For example, the disorder in which inhibition of LHRH activity is beneficial can be selected from the group consisting of precocious puberty, prostate cancer, ovarian cancer, benign prostatic hypertrophy, endometriosis, uterine fibroids, breast cancer, premenstrual syndrome, polycystic ovary syndrome, and diseases which result from excesses of gonadal hormones.

[0077] The following illustrative examples should not be construed as limiting.

[0078] In the examples, the following abbreviations are used:

Boc: N-t-butoxycarbonyl
HOBt: 1-hydroxybenzotriazole
MCPBA: m-chloroperbenzoic acid
DCC: dicyclohexylcarbodiimide

EXAMPLE 1: Anti-Ovalulatory and Histamine Release Activity of LHRH Antagonists

[0079] Anti-ovulatory activity (AOA) was measured by an *in vivo* assay in rats, as described in Corbin and Beattie, Endocrine Res. Commun. 2:1 (1975). In brief, female rats are injected with a candidate LHRH antagonist on the day of proestrus; in general, the candidate LHRH antagonist was dissolved in 0.1% DMSO. The ability of the candidate peptide to inhibit ovulation is measured by determining the number of rats which ovulate. A candidate peptide is considered to have LHRH antagonist qualities if it inhibits ovulation in at least 50% of the treated rats at a dose of 5 μg per rat. Preferred LHRH antagonists inhibit ovulation in at least 50% of rats at a dose of 2 μg per rat, more preferably at a dose of 1 μg per rat, and still more preferably at a dose of 0.5 μg per rat.

[0080] Histamine-releasing activity was assayed by the method described in U.S. Patent 4,851,385 to Roeske. Briefly, a suspension of rat mast cells was added to increasing concentrations of an LHRH antagonist peptide and incubated for 15 minutes at 37°C. The buffer contained 25 mM PIPES, pH 7.4, NaCl (119 mM), KCl (5 mM), NaOH (40mM) glucose (5.6 mM), $CaCl_2$ (1 mM) and 0.1% bovine serum albumin. The reaction was stopped by centrifugation at 400 x g for 15 minutes at 4°C, and the supernatant assayed for histamine content by a published method (Siriganian (1974) Anal. Biochem. 57:383 and Siriganian and Hook (1986) in "Manual of Clinical Immunology", 3rd ed., N.R. Rose, H. Friedman, and J.L Fahey, eds., p. 808), or by a manual method which gave similar results. Maximal histamine release occurred rapidly, typically in less than one minute. No evidence of cell toxicity was seen for any of the peptides tested. The histamine-releasing activity of peptides is measured as the ED_{50}, in μg/ml; a higher ED_{50} represents lower histamine release.

[0081] The AOA and histamine-releasing activities of several peptides are summarized in Table 1. Compounds with an x are comparative compounds.

Table I. LHRH Antagonists

Compound	Sequence	AO activity (AOA)	Histamine release
3341 x	Ac-D-Nal[1],4-Cl-D-Phe[2], D-Pal[3], D-Gln[6]-Lys(iPr)[8], D-Ala[10]-LHRH.TFA	4/10@1 μg 2/10@2 μg	106
3342	Ac-D-Nal[1], 4-Cl-D-Phe[2], D-Pal[3], D-Asn[6]-Lys(iPr)[8], D-Ala[10]-LHRH.TFA	2/10@2 μg	126
3343 x	Ac-D-Nal[1], 4-Cl-D-Phe[2], D-Pal[3], D-Thr[6]-Lys(iPr)[8], D-Ala[10]-LHRH.TFA	5/10@1 μg	62
3344 x	Ac-D-Nal[1], 4-Cl-D-Phe[2], D-Pal[3], D-Cit[6]-Lys(iPr)[8], Pip[9]-D-Ala[10]-LHRH.TFA	9/10@1 μg	32
3361 x	Ac-D-Nal[1], 4-Cl-D-Phe[2], D-Pal[3], D-Glu(Taurine)[6]-Lys (iPr)[8], D-Ala[10]-LHRH.TFA	1/8@5 μg	131
3362 x	Ac-D-Nal[1], 4-Cl-D-Phe[2], D-Pal[3], D-Cit[6]-Lys (iPr)[8]-LHRH.TFA	4/8@1 μg	22
3363 x	Ac-D-Nal[1], 4-Cl-D-Phe[2], D-Pal[3], D-Cit[6]-Lys(iPr)[8], Pip[9]-LHRH.TFA	6/8@1 μg	25
3364 x	Ac-D-Nal[1],4-Cl-D-Phe[2], D-Pal[3], D-Phe(4-NO_2)[6]-Lys (iPr)[8], D-Ala[10]-LHRH.TFA	6/8@1 μg	14
3365 x	Ac-D-Nal[1], 4-Cl-D-Phe[2], D-Pal[3], D-Cit[6]-Lys(iPr)[8], ProNHEt[9]-des-Gly[10]-LHRH.TFA		24
3366 x	Ac-D-(or L)-9-anthryl-Ala[1], 4-Cl-D-Phe[2], D-Pal[3], D-Cit[6]-Lys(iPr)[8], D-Ala[10]-LHRH.TFA	6/8@5 μg	
3367 x	Ac-L-(or D)-9-anthryl-Ala[1], 4-Cl-D-Phe[2], D-Pal[3], D-Cit[6]-Lys(iPr)[8], D-Ala[10]-LHRH,TFA	7/8@5 μg	
3763 x	Ac-Sar[1],4-Cl-D-Phe[2],D-Nal[3],D-Pal(Bzl)[6], Lys(iPr)[8],D-Ala[10]-LHRH.2TFA	4/8@1.0	102[1],76[b]
3769 x	Ac-Sar[1],4-Cl-D-Phe[2],1-1-Nal[3],D-Pal(Bzl)[6], Lys(iPr)8,D-Ala[10]-LHRH.2TFA	6/8@1.0	

(continued)

Compound	Sequence	AO activity (AOA)	Histamine release
3770 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Pal(CH_2COOH)[6],Lys(iPr)[8],D-Ala[10]-LHRH.TFA	6/8@1.0 0.1DMSO	
3771 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys(Ala)[6], Lys(iPr)[8],D-Ala[10]-LHRH.TFA	7/8@1.0	
3772 x	Ac-Sar[1],4-Cl-D-Phe[2],D-1-Nal[3],D-Pal(iPr)[6], Lys(iPr)[8],D-Ala[10]-LHRH.2TFA	8/8@1.0	
3773 x	Ac-Sar[1],4-Cl-D-Phe[2],L-1-Nal[3],D-Pal(iPr)[6], Lys(iPr)[8],D-Ala[10]-LHRH.2TFA	7/8@1.0	
3785 x	Ac-D-Qal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys(Gulonyl)[6],Lys(iPr)[8],D-Ala[10]-LHRH.TFA	7/8@1.0 0.1DMSO	
3786 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Pal(N-O)[6], Lys(iPr)[8], D-Ala[10]-LHRH.TFA	4/8@1.0	
3787 x	Ac-D-Qal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys(Ppic)[6], Lys(iPr)[8], D-Ala[10]-LHRH.TFA	7/8@1.0	
3800 x	Ac-D-Qal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys(Imdac)[6], Lys(iPr)[8],D-Ala[10]-LHRH.TFA	7/8@1.0	
3801 x	Ac-D-Qal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys(Onic)[6], Lys(iPr)[8], D-Ala[10]-LHRH.TFA	8/8@1.0	
3802 x	Ac-D-Qal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys(Otac)[6], Lys(iPr)[8], D-Ala[10]-LHRH.TFA	5/8@1.0	
3803 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys(Dodac)[6], Lys(iPr)[8],D-Ala[10]-LHRH.TFA	2/8@1.0	
3804 x	Ac-D-Pal[1],4-Cl-D-Phe[2]-D-Pal[3],D-Pal(iPr)[6], Lys(iPr)[8],D-Ala[10]-LHRH.2TFA	8/8@1.0	
3827	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],N-Me-Tyr[5],D-Asn[6],Lys(iPr)[8],D-Ala[10]-LHRH.TFA	4/8@1.0	
3828 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],N-Me-Tyr[5],D-Lys(Onic)[8], Lys(iPr)[8],D-Ala10-LHRH.TFA	4/8@1.0	42[a],39[b]
3829 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],N-Me-Tyr[5],D-Lys(Ac)[6], Lys(iPr)[8],D-Ala[10]-LHRH.TFA	8/8@1.0	
3852 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],Lys(iPr)[5],D-His[6],Trp[7], Orn[8],D-Ala[10]-LHRH.TFA	8/8@5.0	
3853 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],His[5],D-Arg[6], Trp[7],Orn[8], D-Ala[10]-LHRH.TFA	0/8@5.0	
3854 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],Arg[5],D-His[6], Trp[7],Orn[8], D-Ala[10]-LHRH.TFA	6/8@5.0	
3855 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],Lys(iPr)[5],D-Trp[6],Trp[7], Orn[8],D-Ala[10]-LHRH.TFA	4/8@1.0	
3851 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],4-Cl-Phe[5],D-Pal(iPr)[6], Lys(iPr)[8],D-Ala[10]-LHRH.2TFA	0/8@1.0 6/8@0.5	4.1[a],5.4[b]
3882 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal(N-O)[3],D-Pal(iPr)[6],Lys(iPr)[8],D-Ala[10]-LHRH.2TFA	6/8@1.0	
4014 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Lys($COCH_2S^+Me_2$)[6], D-Ala[10]-LHRH.2TFA	0/8@1.00	

(continued)

Compound	Sequence	AO activity (AOA)	Histamine release
4015 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Arg[6], Lys ($COCH_2S^+Me_2$)[8],D-Ala[10]-LHRH.2TFA	1/8@1.00	
4016 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Met(S^+Me)[6], Met (S^+Me)[8],D-Ala[10]-LHRH.2TFA	6/8@1.00	
4013 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Orn($COCH_2S^+Me_2$)[6], D-Ala[10]-LHRH.2TFA	0/8@1.00	
4023 x	Ac-D-Nal[1],4-Cl-D-Phe[2],D-Pal[3],D-Arg[6], Dap ($COCH_2S^+Me_2$)[8],D-Ala[10]-LHRH.2TFA	0/8@1.00	
x = Mass spectral analysis did not confirm the structure, Nal arg (reference) histamine release values: a=0.11, b=0.14.			

[0082] Compounds having a small polar moiety at position 6 exhibited favorable combinations of AOA and histamine-releasing activity. For example, compound 3827, which has D-Asn (a small, hydrophilic moiety) at position 6 showed moderate AO activity. Compounds 3341, 3342, and 3343 having D-Gln, D-Asn, and D-Thr, respectively, combined moderate AOA with very low histamine release. Compound 3361, which had the taurine amide of Glu at position 6, also showed very low histamine release.

[0083] All of the analogs listed in Table 1 can by synthesized by the solid phase method using an automated synthesizer (e.g., Beckman Model 990). The amino acid residues used can be purchased from commercial sources (e.g. Aldrich Chemical Co., Milwaukee, WI), or can be produced from commercially available starting materials according to known methods. For example, pyridinium-N-oxides can be produced by oxidation of the corresponding pyridine with, e.g., peroxyacids such as mCPBA (see, e.g., Example 3, *infra*). Pyridinium moieties, for example, N-benzyl pyridinium compounds, can be produced by N-alkylation of the corresponding pyridine, e.g., by heating in an inert solvent with benzyl bromide (see Examples 1 and 2, *infra*). Similarly, sulfonium and phosphonium salts can be produced by S- or P- alkylation of a sulfide or phosphine, respectively, with an alkylating agent such as, for example, methyl iodide. Amino acids which are not obtained commercially can be synthesized in a protected form for coupling, or, if appropriate, can be coupled to form a peptide and subsequently modified to the desired form.

EXAMPLE 2: Synthesis of Boc-D-Pal(Bzl) hydrobromide salt:

[0084] Boc-D-Pal (1.36g, 6.0 mmol) was suspended in 60 ml of acetonitrile. Benzyl bromide (about 50 mmol) was added and the mixture was warmed to 50°C on a water bath. A cleasr solution resulted, and was stirred at room temperature for 16 hours. A white precipitate formed; TLC after 17 hours showed some starting remaining starting material; stirring continued for a total of 5 days, when the reaction was complete. The solvent was evaporated under reduced pressure, and the residue recrystallized from EtOH/ethyl acetate. Yield: 85%; m.p. 166-170°C.

EXAMPLE 3: Synthesis of Boc-D-Pal(iPr)

[0085] Boc-D-Pal (4.0 g, 17.7 mmol) and Ag_2O (8.0 g, 34.4 mmol) in 22 ml water was stirred at room temperature for 4 hours. The reaction vessel was cooled to 0°C, and 2-iodopropane (20.4 g, 120 mmol) in 40 ml 2-propanol was added. After addition was complete, the mixture was allowed to warm to room temperature and stirred for 4 days. Additional Ag_2O (2 g) and 2-iodopropane (2 g) were added after 24 hours and again after 48 hours. The mixture was filtered, and the precipitate was washed with ethanol (2 x 15 ml). The filtrate was evaporated to yield 4.3 g of a yellow oil. Crystallization from ethanol/ethyl acetate gave light yellow crystals (3.0 g); Yield: 63%; m.p. 182-185°C.

EXAMPLE 4: Synthesis of Boc-D-Pal(N-O)

[0086] Boc-D-Pal (2.0 g, 7.5 mmole) was dissolved in 40 ml acetone and 2.48 g (16.5 mmol) of MCPBA (57-86%; purchased from Aldrich and used as received) in 80 ml acetone was added in one portion. The mixture was stirred at room temperature for 40 hours; a small amount of white precipitate formed as the reaction proceeded. The precipitated was filtered and the mother liquor evaporated to yield a white precipitate. The combined solids were washed with ether (to remove chlorobenzoic acid) and recrystallized from ethyl acetate/hexane. Yield: 1.7 g (80%); m.p. 155-157°C.

EXAMPLE 5: Peptide Synthesis

[0087] A typical coupling cycle for peptide synthesis with Boc-amino acids on a peptide synthesizer (Beckman Model 990) was as follows:

[0088] Methylbenzyhydramine (MBHA) resin (1.18 g, 0.85 meq amino groups/g resin) was weighed into the reaction vessel and washed with two protions of chloroform (26 ml each). The resin was prewashed with 22% thioanisole (5 ml) /66% trifluoroacetic acid (TFA) in 14 ml dichloromethane (DCM) for 5 minutes, and then deprotected for 30 minutes with the same thioanisole/TFA mixture. The resin was washed with three portions of chloroform (20 ml each), two portions of 2-propanol (26 ml each) and two portions of DCM (26 ml each). The resin was neutralized with two portions of 12% diisopropylethylamine (DIPEA) (26 ml each), and then washed with four portions of DCM (26 ml each), followed by two portions of 1:1 DCM:dimethylformamide (DMF) (26 ml each). A solution of a Boc-protected amino acid (2.5 mole equivalents) and HOBt (2.5 mole equivalents) was introduced as a solution in 10 ml DMF, and DCC was added (256 mg in 6 DMF). Coupling was allowed to proceed for three hours, or overnight. Hindered residues (e.g., backbone N-methyl amino acids) required longer coupling times. The resin was washed with two 26 ml portions of DMF, followed by two 26 ml portions of 2-propanol and then two 26 ml portions of DCM. Completion of coupling was assessed by Kaiser's test (ninhydrin test). If coupling is not complete, a double coupling was performed (i.e., the resin was neutralized as above and the coupling step repeated). When complete coupling is achieved, the cycle was repeated with the next amino acid.

[0089] Upon completion of the synthesis, the peptide was cleaved from the resin by treatment with liquid hydrofluoric acid (HF) for 45 minutes at 0°C. The HF was evaporated and the the peptide treated with aqueous acetic acid and lyophilized. The crude peptide was then purified by high performance liquid chromatography (HPLC) on a C_{18} column, eluting with a mixture of acetonitrile and 0.1% TFA in water. Purified fractions (homogeneous by UV and TLC analysis) were combined and lyophilized. Analytical HPLC was used to determine the purity of the final product; all peptides synthesized were at least 98% pure.

EXAMPLE 6: Suppression of Plasma Testosterone Levels

[0090] The ability of an LHRH antagonist of the invention to suppress plasma testosterone levels was examined in adult male rats. The rats were administered the LHRH antagonist # 3827 (the structure of which is shown in Table 1). In one experiment, a single injection of LHRH antagonist was administered subcutaneously at doses of 300 or 1000 μg/kg. Animals showed a rapid, pronounced decrease in plasma testosterone to nearly undetectable levels by hours 6 hours post-administration. Testosterone levels returned to normal by 24 hours after the 300 μg/kg dose, but not until 72 hours after the 1000 μg/kg dose.

[0091] In another experiment, the LHRH antagonist # 3827 was administered in eight intravenous doses of 1, 10 or 100 μg/kg, each every three hours, corresponding respectively to 8, 80 or 800 μg/kg/day. The results are shown graphically in Figure 1. Castrate levels of testosterone were achieved at doses of 10 μg/kg every three hours. A dose as low as 1 μg/kg. equivalent to 8 μg/kg/day, induced a significant decrease in plasma testosterone levels throughout the experimental period.

[0092] In another experiment, continuous subcutaneous infusion of the LHRH antagonist # 3827 was achieved through use of an osmotic pump. Doses of 300 or 1000 μg/kg/day ("High Dose") were administered subcutaneously for two weeks via Alzet osmotic minipumps in each of five male Sprague-Dawley rats. The animals that received 1000 μg/kg/day were then maintained for an additional two weeks with 5, 15 or 50 μg/kg/day ("Low Dose") before the pumps were removed completely. The results are shown graphically in Figure 2. Chronic treatment of rats with the LHRH antagonist at initial doses of 300 or 1000 μg/kg/day resulted in castrate levels of testosterone within the first 7 days (and presumably within the first 24 hours) with either initial dose, and were maintained by all three subsequent doses for an additional 21 days. Castration was maintained throughout this period with no apparent "castration response" as has been described with other inhibitors. When the High Dose pumps were replaced with maintenance Low Dose pumps giving doses as low as 5 μg/kg/day, animals remained fully castrated throughout the experiment. Following completion of LHRH antagonist treatment, a dose dependent recovery in plasma testosterone was observed: animals that received the highest maintenance dose appeared to recover more slowly than animals treated with the lower maintenance doses. Recovery of testosterone levels following pump removal was complete within two weeks. In a follow up study, the results of which are shown graphically in Figure 3, complete suppression of testosterone to castrate levels was achieved by doses as low as 30 μg/kg/day. Castrate levels were again maintained with a dose of LHRH antagonist as low as 5 μg/kg/day

SEQUENCE LISTING

[0093]

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: LHRH Antagonist Peptides

(iii) NUMBER OF SEQUENCES: 1

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS.
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 000000
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: USSN 08/480,494
(B) FILING DATE: 07-JUN-1995

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(C) REFERENCE/DOCKET NUMBER: PPI-007CPPC

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Glu His Trp Ser Tyr Gly Leu Arg Pro Gly
1               5                  10
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Claims

1. An LHRH antagonist, wherein said LHRH antagonist is a polypeptide compound consisting of 8 to 12 amino acid residues and is designed based on the naturally occurring mammalian LHRH structure, wherein an amino acid residue corresponding to position 4 of the naturally occurring mammalian LHRH is serine, an amino acid residue corresponding to position 6 of the naturally occurring mammalian LHRH is D- asparagine, L-asparagine, or L-glutamine, an amino acid residue corresponding to position 7 of the naturally occurring mammalian LHRH is leucine or tryptophan, an amino acid residue corresponding to position 9 of the naturally occurring mammalian LHRH is proline; and an amino acid residue corresponding to position 10 of the naturally occurring mammalian LHRH is D-alanine, such that the polypeptide compound has LHRH antagonist activity, or a pharmaceutically acceptable salt thereof.

2. The LHRH antagonist of claim 1, wherein said LHRH antagonist is a decapeptide.

3. The LHRH antagonist of claim 1, wherein said LHRH antagonist is a nonapeptide.

4. The LHRH antagonist of claim 1, wherein said LHRH antagonist is a octapeptide.

5. The LHRH antagonist of claim 1, which inhibits ovulation in at least 50% of treated rats in a standard rat antiovulatory assay at a dose of 5 μg/rat and has an ED50 for histamine release of at least 3 μg/ml.

6. The LHRH antagonist of claim 5, which inhibits ovulation in at least 50% of treated rats in a standard rat antiovulatory assay at a dose of 2 μg/rat

7. The LHRH antagonist of claim 5, which inhibits ovulation in at least 50% of treated rats in a standard rat antiovulatory assay at a dose of l μg/rat

8. The LHRH antagonist of claim 5, which has an ED50 for histamine release of at least 5 μg/m).

9. The LHRH antagonist of claim 5, which has an ED50 for histamine release of at least 10 μg/ml.

10. The LHRH antagonist of claim 1, wherein the polypeptide compound comprises a structure: A-B-C-D-E-F-G-H-I-J wherein

    A is pyro-Glu, Ac-D-Nal , Ac-D-Qal, Ac-Sar, or Ac-D-Pal;
    B is His or 4-Cl-D-Phe;
    C is Trp, D-Pal, D-Nal, L-Nal, D-Pal(N-O), or D-Trp;
    D is Ser;
    E is N-Me-Ala, Tyr, N-Me-Tyr, Ser, Lys(iPr), 4-Cl-Phe, His, Asn, Met, Ala, Arg or Ile;
    F is D/L-Asn or L-Gln
    G is Leu or Trp;
    H is Lys(iPr), Gln, Met, or Arg;
    I is Pro; and
    J is D-Ala-NH2;

    or a pharmaceutically acceptable salt thereof.

11. An LHRH antagonist comprising a structure selected from the group consisting of:

Ac-D-Nal1, 4-Cl-D-Phe2, D-Pal3, D-Asn6-Lys(iPr)8, D-Ala10-LHRH

and pharmaceutically acceptable salts thereof.

12. A pharmaceutical composition comprising a LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

13. Use of an LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for inhibiting LHRH activity in a subject.

14. Use of an LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for inhibiting growth of a hormone-dependent tumor in a subject.

15. Use of claim 14 wherein said hormone-dependent tumor is prostate cancer.

16. Use of an LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for inhibiting ovulation in a subject.

17. A packaged formulation for treating a subject for a disorder associated with LHRH activity, comprising the LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof packaged with instructions for using the LHRH antagonist for treating a subject having a disorder associated with LHRH activity.

18. The packaged formulation of claim 17 wherein said disorder is endometriosis.

19. A pharmaceutical composition comprising the LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier; and

(a) which comprises a slow release polymer; or
(b) which is suitable for depot injection.

20. Use of an LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a disorder selected from the group consisting of precocious puberty, prostate cancer, ovarian cancer, benign prostatic hypertrophy, endometriosis, uterine fibroids, breast cancer, premenstrual syndrome, polycystic ovary syndrome, and diseases which result from excesses of gonadal hormones.

21. The LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof for use in therapy, optionally the treatment of a disorder selected from the group consisting of precocious puberty, prostate cancer, ovarian cancer, benign prostatic hypertrophy, endometriosis, uterine fibroids, breast cancer, premenstrual syndrome, polycystic ovary syndrome, and diseases which result from excesses of gonadal hormones.

22. Use of the LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for controlling reproduction.

23. Use of the LHRH antagonist of any one of claims 1 to 11 or a pharmaceutically acceptable salt thereof for controlling reproduction in males or females, excluding methods for treatment of the human or animal body by therapy.

Patentansprüche

1. LHRH-Antagonist, worin der LHRH-Antagonist eine Polypeptid-Verbindung ist, die aus 8 bis 12 Aminosäureresten besteht und basierend auf der natürlich vorkommenden Säugetier-LHRH-Struktur kreiert wurde, worin ein Aminosäurerest, der Position 4 des natürlich vorkommenden Säugetier-LHRH entspricht, Serin ist, ein Aminosäurerest, der Position 6 des natürlich vorkommenden Säugetier-LHRH entspricht, D-Asparagin, L-Asparagin oder L-Glutamin ist, ein Aminosäurerest, der Position 7 des natürlich vorkommenden Säugetier-LHRH entspricht, Leucin oder Tryptophan ist, ein Aminosäurerest, der Position 9 des natürlich vorkommenden Säugetier-LHRH entspricht, Prolin ist;

und ein Aminosäurerest, der Position 10 des natürlich vorkommenden Säugetier-LHRH entspricht, D-Alanin ist, so dass die Polypeptid-Verbindung LHRH-antagonistische Aktivität aufweist, oder ein pharmazeutisch annehmbares Salz davon.

2. LHRH-Antagonist nach Anspruch 1, worin der LHRH-Antagonist ein Decapeptid ist.

3. LHRH-Antagonist nach Anspruch 1, worin der LHRH-Antagonist ein Nonapeptid ist.

4. LHRH-Antagonist nach Anspruch 1, worin der LHRH-Antagonist ein Octapeptid ist.

5. LHRH-Antagonist nach Anspruch 1, der die Ovulation bei zumindest 50 % der behandelten Ratten in einem Standard-Ratten-Antiovulationstest bei einer Dosis von 5 µg/Ratte inhibiert und eine ED50 für die Histaminfreisetzung von zumindest 3 µg/ml aufweist.

6. LHRH-Antagonist nach Anspruch 5, der die Ovulation bei zumindest 50 % der behandelten Ratten in einem Standard-Ratten-Antiovulationstest bei einer Dosis von 2 µg/Ratte inhibiert.

7. LHRH-Antagonist nach Anspruch 5, der die Ovulation bei zumindest 50 % der behandelten Ratten in einem Standard-Ratten-Antiovulationstest bei einer Dosis von 1 µg/Ratte inhibiert.

8. LHRH-Antagonist nach Anspruch 5, der eine ED50 für die Histaminfreisetzung von zumindest 5 µg/ml aufweist.

9. LHRH-Antagonist nach Anspruch 5, der eine ED50 für die Histaminfreisetzung von zumindest 10 µg/ml aufweist.

10. LHRH-Antagonist nach Anspruch 1, worin die Polypeptid-Verbindung eine Struktur A-B-C-D-E-F-G-H-I-J aufweist, worin:

A Pyro-Glu, Ac-D-Nal, Ac-D-Qal, Ac-Sar oder Ac-D-Pal ist;
B His oder 4-Cl-D-Phe ist;
C Trp, D-Pal, D-Nal, L-Nal, D-Pal(N-O) oder D-Trp ist;
D Ser ist;
E N-Me-Ala, Tyr, N-Me-Tyr, Ser, Lys(iPr), 4-Cl-Phe, His, Asn, Met, Ala, Arg oder Ile ist;
F D/L-Asn oder L-Gln ist;
G Leu oder Trp ist;
H Lys(iPr), Gln, Met oder Arg ist;
I Pro ist; und
J D-Ala-NH2 ist;

oder ein pharmazeutisch annehmbares Salz davon.

11. LHRH-Antagonist, umfassend eine Struktur, die aus der aus Folgendem bestehenden Gruppe ausgewählt ist:

Ac-D-Nal1,4-Cl-D-Phe2,D-Pal3,D-Asn6-Lys(iPr)8,D-Ala10-LHRH
Ac-D-Nal1,4-Cl-D-Phe2,D-Pa13,N-Me-Tyrs,D-Asn6,Lys(iPr)8,D-Ala10-LHRH,

sowie pharmazeutisch annehmbare Salze davon.

12. Pharmazeutische Zusammensetzung, umfassend einen LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder ein pharmazeutisch annehmbares Salz davon und einen pharmazeutisch annehmbaren Träger.

13. Verwendung eines LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder eines pharmazeutisch annehmbaren Salzes davon zur Herstellung eines Medikaments zur Inhibierung der LHRH-Aktivität bei einem Individuum.

14. Verwendung eines LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder eines pharmazeutisch annehmbaren Salzes davon zur Herstellung eines Medikaments zur Inhibierung des Wachstums eines hormonabhängigen Tumors bei einem Individuum.

15. Verwendung nach Anspruch 14, worin es sich bei dem hormonabhängigen Tumor um Prostatakrebs handelt.

16. Verwendung eines LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder eines pharmazeutisch annehmbaren Salzes davon zur Herstellung eines Medikaments zur Inhibierung der Ovulation bei einem Individuum.

17. Verpackte Formulierung zur Behandlung eines Individuums wegen einer Erkrankung, die mit LHRH-Aktivität assoziiert ist, umfassend den LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder ein pharmazeutisch annehmbares Salz davon, verpackt mit Anweisungen zur Verwendung des LHRH-Antagonisten zur Behandlung eines Individuums mit einer Erkrankung, die mit LHRH-Aktivität assoziiert ist.

18. Verpackte Formulierung nach Anspruch 17, worin die Erkrankung Endometriose ist.

19. Pharmazeutische Zusammensetzung, umfassend einen LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder ein pharmazeutisch annehmbares Salz davon und einen pharmazeutisch annehmbaren Träger; sowie

    (a) die ein Polymer mit verzögerter Freisetzung umfasst; oder
    (b) die zur Depot-Injektion geeignet ist.

20. Verwendung eines LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder eines pharmazeutisch annehmbaren Salzes davon zur Herstellung eines Medikaments zur Behandlung einer Erkrankung, die aus der aus verfrühter Pubertät, Prostatakrebs, Ovarialkrebs, benignem Prostataadenom, Endometriose, Uterusfibrom, Brustkrebs, prämenstruellem Syndrom, polyzystischem Ovarialsyndrom sowie Erkrankungen, die aus Überschüssen von Gonadenhormonen resultieren, bestehenden Gruppe ausgewählt ist.

21. LHRH-Antagonist nach einem der Ansprüche 1 bis 11 oder ein pharmazeutisch annehmbares Salz davon zur Verwendung in der Therapie, gegebenenfalls zur Behandlung, einer Erkrankung, die aus der aus verfrühter Pubertät, Prostatakrebs, Ovarialkrebs, benignem Prostataadenom, Endometriose, Uterusfibrom, Brustkrebs, prämenstruellem Syndrom, polyzystischem Ovarialsyndrom sowie Erkrankungen, die aus Überschüssen von Gonadenhormonen resultieren, bestehenden Gruppe ausgewählt ist.

22. Verwendung eines LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder eines pharmazeutisch annehmbares Salzes davon zur Herstellung eines Medikaments zur Kontrolle der Reproduktion.

23. Verwendung eines LHRH-Antagonisten nach einem der Ansprüche 1 bis 11 oder eines pharmazeutisch annehmbares Salzes davon zur Kontrolle der Reproduktion bei Männern oder Frauen, unter Ausschluss von Verfahren zur Behandlung des menschlichen oder tierischen Körpers durch Therapie.

Revendications

1. Antagoniste de LHRH, où ledit antagoniste de LHRH est un composé de polypeptide consistant en 8 à 12 résidus d'acide aminé et est conçu sur la base de la structure de LHRH mammalienne se produisant naturellement, où un résidu d'acide aminé correspondant à la position 4 de la LHRH mammalienne se produisant naturellement est la sérine, un résidu d'acide aminé correspondant à la position 6 de la LHRH mammalienne se produisant naturellement est la D-asparagine, L-asparagine ou L-glutamine, un résidu d'acide aminé correspondant à la position 7 de la LHRH mammalienne se produisant naturellement est la leucine ou le tryptophane, un résidu d'acide aminé correspondant à la position 9 de la LHRH mammalienne se produisant normalement est la proline; et un résidu d'acide aminé correspondant à la position 10 de la LHRH mammalienne se produisant naturellement est la D-alanine, de sorte que le composé de polypeptide possède une activité antagoniste du LHRH, ou un sel pharmaceutiquement acceptable de celui-ci.

2. Antagoniste de LHRH selon la revendication 1, où ledit antagoniste de LHRH est un décapeptide.

3. Antagoniste de LHRH selon la revendication 1, où ledit antagoniste de LHRH est un nanopeptide.

4. Antagoniste de LHRH selon la revendication 1, où ledit antagoniste de LHRH est un octapeptide.

5. Antagoniste de LHRH selon la revendication 1, qui inhibe l'ovulation dans au moins 50% de rats traités dans un test anti-ovulatoire de rat standard à une dose de 5 µg/rat et possède une ED50 pour la libération de l'histamine d'au moins 3 µg/ml.

6. Antagoniste de LHRH selon la revendication 5, qui inhibe l'ovulation dans au moins 50% de rats traités dans un test anti-ovulatoire de rat standard à une dose de 2 µg/rats.

7. Antagoniste de LHRH selon la revendication 5, qui inhibe l'ovulation dans au moins 50% de rats traités dans un test anti-ovulatoire de rat standard à une dose de 1 µg/rat.

8. Antagoniste de LHRH selon la revendication 5, qui possède une ED50 pour la libération de l'histamine d'au moins 5 µg/ml.

9. Antagoniste de LHRH selon la revendication 5, qui possède une ED50 pour la libération de l'histamine d'au moins 10 µg/ml.

10. Antagoniste de LHRH selon la revendication 1, où le composé de polypeptide comprend une structure: A-B-C-D-E-F-G-H-I-J où

A est pyro-Glu, Ac-D-Nal, Ac-D-Qal, Ac-Sar ou Ac-D-Pal;
B est His ou 4-Cl-D-Phe;
C est Trp, D-Pal, D-Nal, L-Nal, D-Pal(N-O) ou D-Trp;
D est Ser;
E est N-Me-Ala, Tyr, N-Me-Tyr, Ser, Lys(iPr), 4-Cl-Phe, His, Asn, Met, Ala, Arg ouo Ile;
F est D/L-Asn ou L-Gln
G est Leu ou Trp;
H est Lys(iPr), Gln, Met ou Arg;
I est Pro; et
J est D-Ala-NH2;

ou un sel pharmaceutiquement acceptable de celui-ci.

11. Antagoniste de LHRH, comprenant une structure sélectionnée dans le groupe consistant en:

Ac-D-Nal1, 4-Cl-D-Phe2, D-Pal3, D-Asn6-Lys(iPr)8, D-Ala10-LHRH
Ac-D-Nal1, 4-Cl-D-Phe2, D-Pal3, _N-Me-Tyr5, _D-Asn6, _Lys(iPr)8, _D-Ala10-LHRH

et des sels pharmaceutiquement acceptables de celui-ci.

12. Composition pharmaceutique comprenant un antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou un sel pharmaceutiquement acceptable de celui-ci et un support pharmaceutiquement acceptable.

13. Utilisation d'un antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou d'un sel pharmaceutiquement acceptable de celui-ci pour la fabrication d'un médicament pour inhiber l'activité de la LHRH dans un sujet.

14. Utilisation d'un antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou d'un sel pharmaceutiquement acceptable de celui-ci pour la fabrication d'un médicament pour inhiber la croissance d'une tumeur hormonodépendante dans un sujet.

15. Utilisation selon la revendication 14, où ladite tumeur hormonodépendante est le cancer de la prostate.

16. Utilisation d'un antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou d'un sel pharmaceutiquement acceptable de celui-ci pour la fabrication d'un médicament pour inhiber l'ovulation dans un sujet.

17. Formulation emballée pour traiter un sujet pour un trouble associé à l'activité de la LHRH, comprenant l'antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou un sel pharmaceutiquement acceptable de celui-ci, emballé avec des instructions pour l'utilisation de l'antagoniste de la LHRH pour traiter un sujet ayant un trouble associé à l'activité de la LHRH.

18. Formulation emballée selon la revendication 17, où ledit trouble est l'endométriose.

19. Composition pharmaceutique comprenant l'antagoniste de LHRH selon l'une quelconque des revendications 1 à

11 ou un sel pharmaceutiquement acceptable de celui-ci et un support pharmaceutiquement acceptable; et

(a) qui comprend un polymère à libération lente; ou
(b) qui convient pour l'injection retard.

20. Utilisation d'un antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou d'un sel pharmaceutiquement acceptable de celui-ci pour la fabrication d'un médicament pour le traitement d'un trouble sélectionné dans le groupe consistant en puberté précoce, cancer de la prostate, cancer des ovaires, hypertrophie prostatique bénigne, endométriose, fibroïdes utérins, cancer du sein, syndrome prémenstruel, syndrome ovaire polycystique et de troubles qui résultent des excès d'hormones gonadiques.

21. Antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou un sel pharmaceutiquement acceptable de celui-ci pour utilisation en thérapie, en option pour le traitement d'un trouble sélectionné dans le groupe consistant en puberté précoce, cancer de la prostate, cancer des ovaires, hypertrophie prostatique bénigne, endométriose, fibroïdes utérins, cancer du sein, syndrome prémenstruel, syndrome ovaire polycystique et de troubles qui résultent des excès d'hormones gonadiques.

22. Utilisation de l'antagoniste de LHRH selon l'une quelconque des revendications 1 à 11 ou d'un sel pharmaceutiquement acceptable de celui-ci pour la fabrication d'un médicament pour contrôler la reproduction.

23. Utilisation de l'antagoniste de la LHRH selon l'une quelconque des revendications 1 à 11 ou d'un sel pharmaceutiquement acceptable de celui-ci pour contrôler la reproduction chez des mâles ou des femelles, excluant des méthodes de traitement du corps humain ou animal par thérapie.

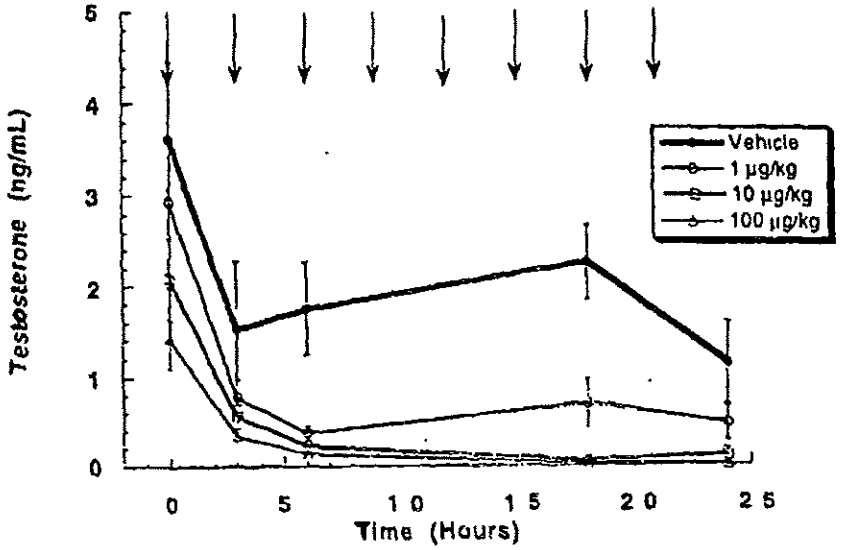

Testosterone (ng/mL)
5
4
3
2
1
0
0
5
10
15
20
25
Time (Hours)
Vehicle
1 µg/kg
10 µg/kg
100 µg/kg

FIGURE 1

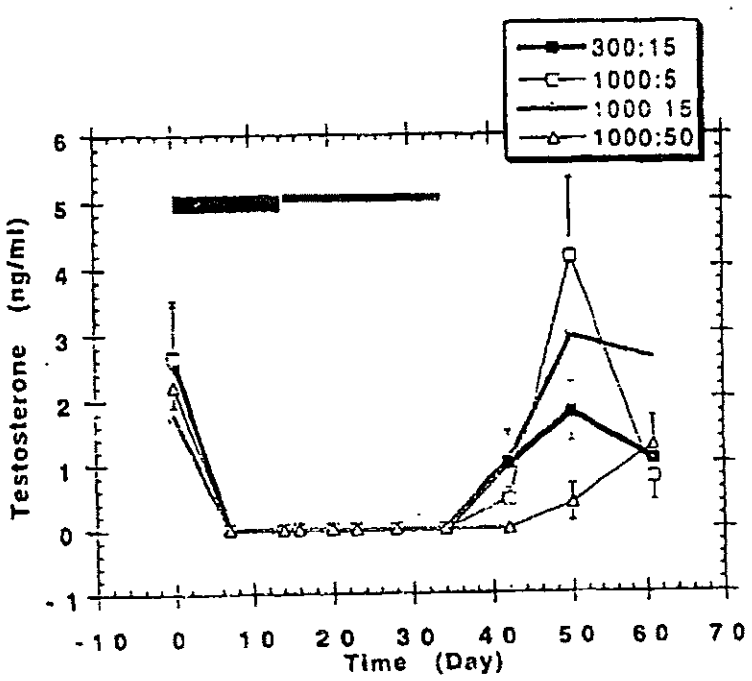

300:15
1000:5
1000 15
1000:50
Testosterone (ng/ml)
6
5
4
3
2
1
0
-1
-10
0
10
20
30
40
50
60
70
Time (Day)

FIGURE 2

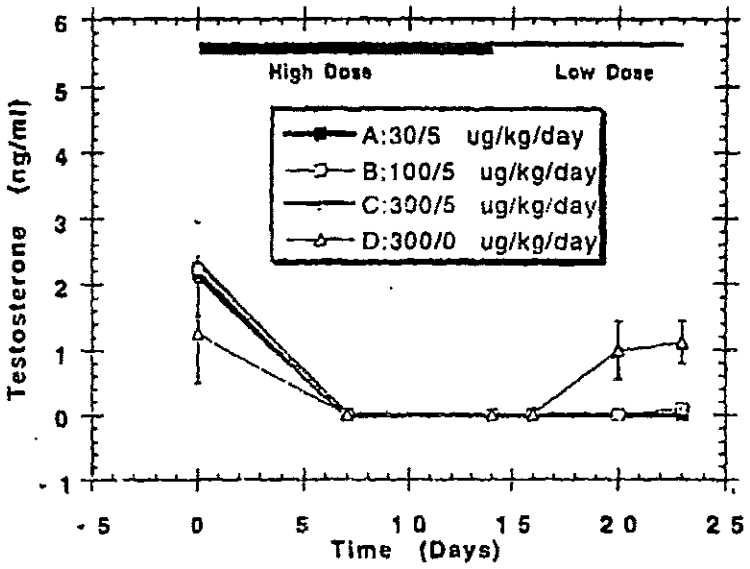

High Dose
Low Dose
A:30/5 ug/kg/day
B:100/5 ug/kg/day
C:300/5 ug/kg/day
D:300/0 ug/kg/day
Testosterone (ng/ml)
6
5
4
3
2
1
0
-1
-5
0
5
10
15
20
25
Time (Days)

FIGURE 3

REFERENCES CITED IN THE DESCRIPTION

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