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[54] LHRH ANTAGONISTS

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[58] Field of Search 530/313; 525/54.11; 514/15, 800

[56] References Cited

PUBLICATIONS

Chem. Abstr. vol. 82 (1975) 73465h.
Chem. Abstr. vol. 86 (1977) 5813c.

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[57] ABSTRACT

The present invention deals with LHRH antagonists which possess improved water solubility and while having the high antagonist potency of the basic peptides, are free of the edematogenic effects. These compounds are highly potent in inhibiting the release of gonadotropins from the pituitary gland in mammals, including humans.

The compounds of this invention are represented by the formula

$$X\text{-}R^1\text{-}R^2\text{-}R^3\text{-Ser-Tyr-}R^6\text{-Leu-Arg-Pro-}R^{10}\text{-}NH_2$$

wherein

X is an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms,

R^1 is D- or L-Pro, D- or L- Δ^3-Pro, D-Phe, D-Phe(4-Hl), D-Ser, D-Thr, D-Ala, D-Nal (1) or D-Nal (2),

R^2 is D-Phe or D-Phe(4-Hl)

R^3 is D-Trp, D-Phe, D-Pal, D-Nal(1) or D-Nal (2),

R^6 is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) and

R^{10} is Gly or D-Ala

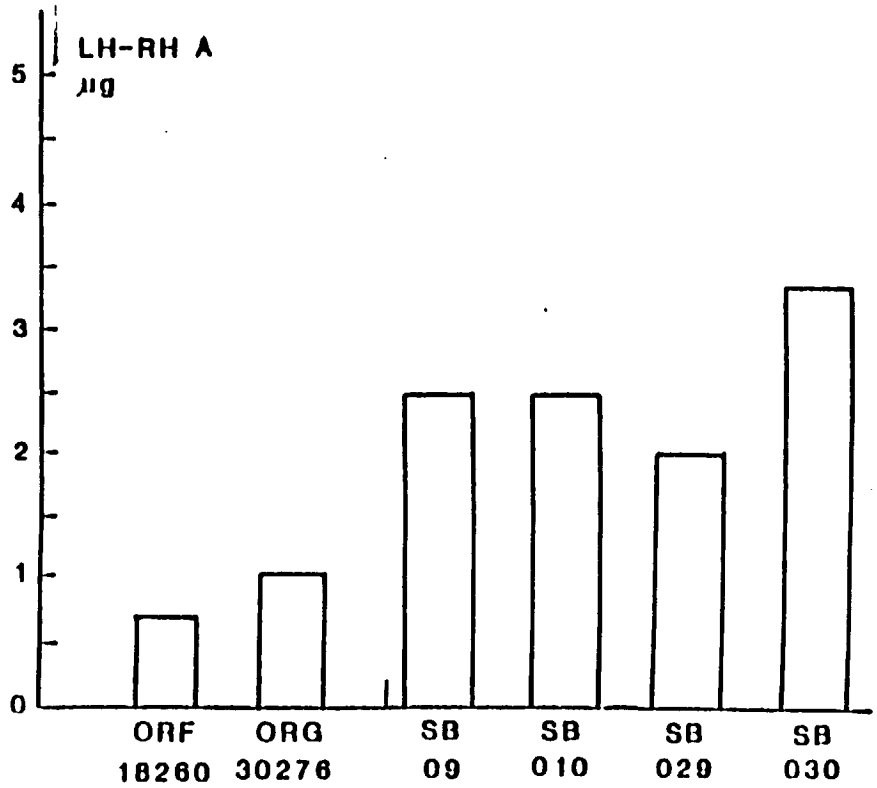
where Q is lower alkyl of 1–3 carbon atoms and Hl is fluoro, chloro or bromo,

and the pharmaceutically acceptable acid addition salts thereof and methods of use pertaining to these compounds.

19 Claims, 1 Drawing Sheet

APPEARS THIS WAY
ON ORIGINAL

HISTAMINE RELEASE IN VITRO FROM PERITONEAL MAST CELLS



CONCENTRATION OF LH-RH ANTAGONISTS REQUIRED TO INDUCE RELEASE OF 50% OF PERITONEAL MAST CELL HISTAMINE (HRD_{50})

LHRH ANTAGONISTS

This invention was made with Government support under Grant Nos. CA40003 and 40004, awarded by the N.C.I. (NIH). The U.S. Government has certain rights in this application.

BACKGROUND OF THE INVENTION

The present invention relates to novel peptides which inhibit the release of gonadotropins by the pituitary gland in mammals without inducing edematous reactions. More specifically, the present invention relates to analogs of the luteinizing hormone releasing hormone (LHRH), which has the structure:

p-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2,

salts thereof, and to pharmaceutical compositions and methods of use pertaining to these analogs.

DISCUSSION OF THE PRIOR ART

For more than 15 years, investigators have been searching for selective, potent antagonists of the LHRH decapeptide (M. Karten and J. E. Rivier, Endocrine Reviews, 7, 44–66 (1986)). The high degree of interest in such antagonists is due to their usefulness in the fields of endocrinology, gynecology, contraception and cancer. A large number of compounds have been prepared as potential LHRH antagonists. The most interesting antagonists to date have been compounds whose structure is a modification of the structure of LHRH.

The first series of potent antagonists was obtained by introduction of aromatic acid residues into positions 1, 2, 3 and 6, or, 2, 3, and 6. The compounds are expressed as LHRH modified by replacement of the original amino acid residues by others at the position indicated by the superscript numbers. The known antagonists include:

[Ac-D-Phe(4-Cl)^{1,2}, D-Trp^{3,6}] LHRH (D. H. Coy, et al., In: Gross, E. and Meienhofer, J. (eds) Peptides, Proceedings of the 6th. American Peptide Symposium, pp. 775–779, Pierce Chem. Co., Rockville, IL, 1979);

[Ac-Pro, D-Phe(4-Cl)^2 D-Nal(2)^{1,6}]LHRH (U.S. Pat. No. 4,419,347); and

[Ac-ΔPro, D-Phe(4-Cl)^2, D-Trp^{3,6}]LHRH (J. L. Pineda, et al., J. Clin. Endocrinol. Metab. 56, 420, 1983).

Later, in order to increase the water solubility of antagonists, basic amino acids, such as D-Arg, were introduced into position 6. For instance,

[Ac-D-Phe(4-Cl)^{1,2}, D-Trp^3, D-Arg^6, D-Ala^{10}]LHRH (ORG-30276) (D. H. Coy, et al., Endocrinology, 100, 1445, 1982); and

[Ac-D-Nal(2)^1, D-Phe(4-F)^2, D-Trp^3, D-Arg^6]LHRH (ORF-18260) (J. E. Rivier, et al., In: Vickery B. H., Nestor, Jr. J. J., Hafez, E. S. E. (eds), LHRH and Its Analogs, pp. 11–22, MTP Press, Lancaster, UK, 1984).

These analogs not only possessed the expected improved water solubility but also showed increased antagonistic activity. However, these highly potent, hydrophilic analogs containing D-Arg and other basic side chains at position 6 proved to produce transient edema of the face and extremities when administered subcutaneously in rats at 1.25 or 1.5 mg/kg (F. Schmidt, et al., Contraception, 29, 283, 1984; J. E. Morgan, et al., Int. Archs. Allergy Appl. Immun. 80, 70, (1986). Since the occurrence of edematogenic effects after administration of these antagonists to rats cast doubts on their safety

for the use in humans and delayed the introduction of these drugs for clinical use, it is desirable to provide antagonistic peptides which are free of these side effects.

SUMMARY OF THE INVENTION

The present invention deals with LHRH antagonists which possess an improved water solubility and high antagonist potency of the basic peptides, and are free of the edematogenic effects. These compounds are highly potent in inhibiting the release of gonadotropins from the pituitary gland in mammals, including humans.

The compounds of this invention are represented by formula I

$$X\text{-}R^1\text{-}R^2\text{-}R^3\text{-Ser-Tyr-}R^6\text{-Leu-Arg-Pro-}R^{10}\text{-}NH_2 \quad I$$

wherein

X is an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms,

R^1 is D- or L-Pro, D- or L-Δ^1-Pro, D-Phe, D-Phe(4-H1), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal (2),

R_3 is D-Phe or D-Phe(4-H1)

R_6 is D-Trp, D-Phe, D-Pal, D-Nal(1) or D-Nal (2),

R_{10} is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) and

R^{10} is Gly or D-Ala

where Q is lower alkyl of 1–3 carbon atoms and H1 is fluoro, chloro or bromo,

and the pharmaceutically acceptable acid addition salts thereof.

The compounds of Formula I can be prepared by several known techniques of the classical (solution) or solid phase peptide synthesis. Preferably, the compounds of Formula I are prepared from the analogous peptides of Formula II.

$$X_1\text{-}R^1\text{-}R^2\text{-}R^3\text{-Ser}(X^4)\text{-Tyr}(X^5)\text{-}R^{*6}(X^6)\text{-Leu-Arg}(X^6)\text{-Pro-}R^{10}\text{-NH-}X^{10} \quad II$$

wherein

X_1 is an acyl group derived from straight and branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms, t-Boc or hydrogen,

X^4 is hydrogen or a protecting group for the Ser hydroxyl group,

X^5 is hydrogen or a protecting group for the Tyr phenolic hydroxyl group,

X^6 is hydrogen or a protecting group for the Arg guanidino group,

X^{10} is hydrogen or a resin support containing benzhydryl or methylbenzyhydryl groups

R^1 is D- or L-Pro, D- or L-Δ^1-Pro, D-Phe, D-Phe(4-H1), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal(2),

R^2 is D-Phe or D-Phe(4-H1),

^{3} is D-Trp, D-Phe, D-Pal, D-Nal(1) or D-Nal (2),

R^{*6} is D-Lys or D-Orn

and R^{10} is Gly or D-Ala

where H1 is fluoro, chloro or bromo, provided that where X_1 is hydrogen or t-Boc, X^4, X^5, X^6, and X^8 must all be other than hydrogen.

The process comprises reacting a peptide of Formula II wherein X^6 is hydrogen, with a source of cyanate to yield a peptide of Formula III:

$$X_1\text{-}R^1\text{-}R^2\text{-}R^3\text{-Ser}(X^4)\text{-Tyr}(X^5)\text{-}R^{*6}\text{-Leu-Arg}(X^6)\text{-Pro-}R^{10}\text{-NH-}X^{10} \quad III$$

wherein
X_1, R^1, R^2, R^3, X^4, X^5, X^6, R^{10} and X^{10} are as defined above, and
R^{**6} is Cit or Hci. Suitably, the reaction is carried out when X is acyl and all other X moieties are hydrogen. Suitable cyanate sources are alkali metal cyanates, e.g., potassium cyanate, or an N-alkyl isocyanate, e.g., N-ethyl-isocyanate.

The peptide of Formula II are preferably synthesized by a known solid phase technique.

A gonadotropin antagonizing pharmaceutical composition is provided by admixing the compound of Formula I with a pharmaceutically acceptable carrier including microcapsules (microspheres) for delayed delivery.

There is also provided a method for relieving complications resulting from the physiological availability of amounts of pituitary gonadotropins in a mammal, in excess of the desired amount, which involves administering to the mammal a gonadotropin antagonizing dose of the compound of Formula I.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar graph of certain compounds of this invention and prior art compounds showing concentration required to induce release of 50% of rat peritoneal mast cell histamine.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The nomenclature used to define the peptides is that specified by the IUPAC-IUB Commission on Biochemical Nomenclature (*European J. Biochem.*, 1984, 138, 9–37), wherein in accordance with conventional representation the amino groups at the N-terminus appears to the left and the carboxyl group at the C-terminus to the right. By natural amino acid is meant one of the common, naturally occurring amino acids found in proteins comprising Gly, Ala, Val, Leu, Ile, Ser, Thr, Lys, Arg, Asp, Asn, Glu, Gln, Cys, Met, Phe, Tyr, Pro, Trp and His. The abbreviations for the individual amino acid residues are based on the trivial name of the amino acid and are Ala, alanine; Arg, arginine, Cit, citrulline, Gly, glycine; Hci, homocitrulline; Leu, leucine; Lys, lysine; Pal, 3-(3-pyridyl) alanine; Nal(2), 3-(2-naphthyl)alanine; Orn, ornithine; Phe, phenylalanine; Phe(4-Cl), 4-chlorophenylalanine; Phe (4-F), 4-fluorophenylalanine; Pro, proline; Ser, serine; Trp, tryptophan and Tyr, tyrosine. All amino acids described herein are of the L-series unless stated otherwise, e.g., D-Trp represents D-tryptophan and D-Nal (2) represents 3-(2-naphthyl)-D-alanine.

Other abbreviations used are:
AcOH: acetic acid
AcOEt: ethyl acetate
Ac_2O: acetic anhydride
Boc-: tert.butyloxycarbonyl-
DIC: diisopropylcarbodiimide
DIEA: diisopropylethylamine
DMF: dimethylformamide
HOBt: 1-hydroxybenzenetriazole hydrate
HPLC: high performance liquid chromatography
MeOH: methyl alcohol
TEA: triethylamine
DCC: dicyclohexylcarbodiimide
MeCN: acetonitrile
IpOH: isopropanol
Z(2-Cl): 2-chloro-benzyloxycarbonyl
DCB: 2,6-dichlorobenzyl
Tos: p-toluenesulfonyl
TFA: trifluoroacetic acid
Z: benzyloxycarbonyl

Especially preferred are LHRH analogs of Formula I wherein:
X is acetyl
R_2 is Pro, D-Phe, D-Phe(4-Cl) or D-Nal(2),
R_3 is D-Phe(4-Cl) or D-Phe(4-F),
R_6 is D-Trp.
R_{10} is D-Cit, D-Hci, D-Cit(Et) or D-Hci(Et) and
R^{10} is D-Ala.

The peptides are synthesized by a suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution phase synthesis. (See M. Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984).

For example, the techniques of exclusively solid-phase synthesis are set forth in the textbook "Solid Phase Peptide Synthesis", J. M. Stewart and J. D. Young, Pierce Chem. Company, Rockford, Ill., 1984 (2nd. ed.), G. Barany and R. B. Merrifield, "The Peptides", Ch. 1, 1–285, pp. 1979, Academic Press, Inc.

Classical solution synthesis is described in detail in the treatise "Methoden der Organischen Chemie (Houben-Weyl): Synthese von Peptiden", E. Wunsch (editor) (1974) Georg Thieme Verlag, Stuttgart, W. Germany.

Common to such synthesis is the protection of the reactive side chain functional groups of the various amino acid moieties with suitable protecting groups which will prevent a chemical reaction from occurring at that site until the group is ultimately removed. Usually also common is the protection of an alpha-amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha-amino protecting groups to allow subsequent reaction to take place at that location. Accordingly, it is common that, as a step in the synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with side-chain protecting groups linked to the appropriate residues.

The procedures of the present invention may be carried out using a variety of support phases. These support phases may be for example, resins such as benzhydrylamine resins (suitably 2% cross linked), p-methylbenzhydrylamine resins (suitably 2% cross linked) and the like.

In Formula II:
R^1, R^2, and R^3 are as defined hereinabove,
X_1 is hydrogen or an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms, or an alpha-amino protecting group. The alpha-amino protecting groups contemplated by X^1 are those well known to be useful in the art of step-wise synthesis of polypeptides. Among the classes of alpha-amino protecting groups which may be employed as X^1 may be mentioned fluoroenylmethyloxycarbonyl (Fmoc) or t-butyloxycarbonyl (Boc).
X^4 may be a suitable protecting group for the hydroxyl group of Ser such as benzyl (Bzl), and 2,6-dichlorobenzyl (DCB). The preferred protecting group is Bzl.
X^5 may be a suitable protecting group for the phenolic hydroxyl group of Tyr, such as Bzl, 2-Br-Z and 2,6-

dichloro-benzyl (DCB). The preferred protecting group is DCB.

X^6 is a suitable protecting group for the side chain amino group of Lys or Orn. Illustrative of suitable side chain amino protecting groups are benzyloxycarbonyl (Z), and 2-chloro-benzyloxycarbonyl (Z-(2-Cl).

X^8 is a suitable protecting group for the guanidino group of Arg, such as nitro, Tos, methyl-(t-butyl benzene)-sulfonyl, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; Tos is the preferred group.

Provided that where X is hydrogen or t-Boc, X^4, X^5, X^6 and X^8 are other than hydrogen.

The selection of a side chain amino protecting group is not critical except that generally one is chosen which is not removed during deprotection of the alpha-amino groups during the synthesis.

For intermediates A, the values for X^4, X^5, X^6, X^8 and X^{10} are hydrogen, for intermediates B, the values for X^4, X^5, X^6, X^8, and X^{10} are protecting groups.

The peptides of Formula I are preferably prepared from intermediates A which are obtained from intermediates B by procedures known in the art.

Intermediates B are preferably prepared by a solid-phase synthesis, such as described by Merrifield, J. Am. Chem. Soc., 85, p. 2149 (1963). Solid phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected α-amino acid to a suitable resin. Such a starting material can be prepared by attaching α-amino protected Gly or D-Ala by an amide bond to a benzyhydrylamine resin. Such resin supports are commercially available and generally used when the desired polypeptide being synthesized has an α-carboxamide at the C-terminal.

In one embodiment of the synthesis, the primary amino group of Gly or D-Ala is protected with a t-butoxy carbonylating agent and the coupling carried out using any of the known dialkyl carbodiimide coupling procedures.

The selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as a coupling reagent is N,N'-diisopropyl carbodiimide (DIC).

Activating reagents and their use in peptide coupling are described by M. Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984.

Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in about a two-fold excess, and the coupling may be carried out in a medium of DMF:CH_2Cl_2 (1:1) or in CH_2Cl_2 alone. In cases where incomplete coupling occurs, the coupling procedure is repeated before removal of the alpha-amino protecting group prior to the coupling of the next amino acid. The success of the coupling reaction at each stage of the synthesis, is preferably monitored by the ninhydrin reaction, as described by E. Kaiser, et al., *Anal. Biochem.*, 34, 595 (1970).

After the desired amino acid sequence of intermediates B has been completed. The terminal Boc group is removed and N-terminal acylation carried out using the appropriate acyl anhydride or acid chloride in 50-fold excess in a halogenated hydrocarbon solvent; suitably, acetic anhydride in methylene chloride for 30 minutes. The intermediate peptide can be removed from the resin support by treatment with a reagent such as liquid hydrogen fluoride, which not only cleaves the peptide from the resin but also cleaves all remaining side chain protecting groups X^4, X^5, X^6, X^8, and X^{10}.

When using hydrogen fluoride for cleaving, anisole and, if desired, methylethyl sulfide are included as scavengers in the reaction vessel, to yield Intermediates A.

Intermediates A are converted into peptides of Formula I by treatment with cyanate, suitably an alkali metal cyanate, preferably potassium cyanate, or an N-alkylisocyanate, for instance, N-ethylisocyanate, in DMF or aqueous DMF. The latter reaction, i.e., transformation of Orn/Lys-peptides into the corresponding Cit/Hci-peptides can be readily followed by HPLC using MeCN-aqueous TFA systems because of a characteristic 2.6±0.3 minutes increase of the retention times of Cit/Hci—and, for example, Cit (Et)/Hci(Et)-peptides relative to the corresponding Orn/Lys-peptides respectively.

Although a partial solid-phase synthesis of compounds of Formula I is disclosed herein, the preparation of the compounds also can be realized by exclusively solid-phase synthesis or by classical solution-phase methods.

The synthetic peptides prepared as described in the Examples are compared with two of the most potent LHRH antagonists reported recently, i.e., [Ac-D-Phe(4-Cl)^{1,2}, D-Trp^3, D-Arg^6, D-Ala^{10}] LHRH (ORG-30276) (Coy, et al., Endocrinology, 100, 1445, 1982) and [Ac-D-Nal(2), D-Phe(4-F)^2, D-Trp^3, D-Arg^6] LHRH (ORF 18260) (Rivier, et al., In: Vickery, B. H., Nestor, Jr., J. J. Hafez, E. S. E. (eds.), LHRH and Its Analogs, pp. 11-22, MPT Press, Lancaster, UK, 1984), and are found to exert similarly high inhibitory activities both in vitro and in vivo, but, unlike to the control peptides, not to produce the in vivo edematous effects.

Hormonal activities in vitro are compared in superfused rat pituitary cell systems (S. Vigh and A. V. Schally, Peptides. 5 suppl. 1: 241-247, 1984) in which the effectiveness of LHRH (and other releasing hormones) can be accurately evaluated since the amount of LH (or other pituitary hormones) secreted into the effluent medium is not only proportional to the hormone-releasing potency of the peptide applied but also measurable readily by well-characterized radioimmunoassays.

To determine the potency of an LHRH antagonist, mixtures containing LHRH in a constant concentration (usually 1 nM) and the antagonist in varying concentrations are used for the superfusion in order to determine the molecular ratio of the antagonist to LHRH at which the action of LHRH is completely blocked. These ratios are about 5 for both peptides of the present invention and the control peptides when the rat pituitary cell system is preincubated with antagonists for 9 minutes.

In an antiovulatory in vivo assay (A. Corbin and C. W. Beattie; Endocr. Res. Commun. 2, 1-23, 1975; D. H. Coy, et al., Endrocrinology, 100, 1445, 1982), the peptides of the present invention are also found to be about equipotent to the control antagonist, namely, 87.5-100% blockage of ovulation can be observed at a subcutaneous dose of 1-3 ug/rat for each peptide.

In the edematogenic test of Schmidt, et al. (Contraception, 29, 283-289, 1984), however, a marked difference can be found between the control peptides and the peptides of the present invention. The control peptides produce edema of the face and extremities when administered subcutaneously in rats at doses of 0.75 or 1.25 mg/kg. No such reaction can be observed with the peptides of the present invention when given at a subcutaneous dose of 1.5 mg/kg.

In the tests as run the rats were assigned to three groups of five rats per group per compound tested. Comparison with made with a known prior art compound designated ORG 30276 namely (N-Ac-D-p-Cl-Phe[1,2], D-Trp[3], D-Arg[6], D-Ala[10])-LHRH. The groups were injected subcutaneously once a day on two consecutive days with the LHRH antagonists at a dose level of 1.5 mg/kg. One control group was injected with diluent only. The rats were observed during five hours each day. Reactions of the rats were classified as follows: NR no apparent reaction, PR partial responders: edema of the nasal and paranasal area, FR full responders: facial edema with edematous extremities. These results are summarized in Table 1 below.

TABLE 1

LHRH	1st Day			2nd Day		
Antagonist	NR	PR	FR	NR	PR	FR
ORG 30276	4	5	0	1	2	6
ORG 30276	1	3	0	0	1	3
Control	9	0	0	9	0	0
EX III	8	0	0			
EX V	9	0	0	8	1*	0
EX IV	9	0	0	9	0	0
EX I	8	0	0	8	0	0

All peptides shown are completely effective to block LHRH secretion in vitro at some reasonable concentration, altough most are slighdy less potent than the present standard in vitro; however, these peptides are much more potent in vivo.

This was shown by a test on histamine release in vitro from peritoneal mast cells carried out in accordance with the procedure of Morgan et al (Int. Archs. Allergy appl. Immun. 80, 70 1986).

HISTAMINE RELEASE IN VITRO

In this test rats were anesthetized with ether and peritoneal exudate cell were harvested by washing with 12 ml. of mast cell medium (MCM) (150 m M NaCl; 3.7 m M KCl; 3.0 m M Na_2HPO_4; 3.5 m M KH_2PO_4, 0.98 m M CaCl; 5.6 m M dextrose; 0.1% bovine serum albumin; 0.1% gelatin and 10 units/ml heparin) [9]. Cells from 4 or 5 rats were pooled, centrifuged at 120 g, resuspended with MCM to a concentration of 0.5×10^6 ml and 1 ml was aliquoted into 12×75 mm polyethylene tubes. Tubes were equilibrated to 37° C. for 15 min and incubated alone (background histamine release), with 48/80 (positive control) (Sigma Chemicals, St. Louis, Mo.), or with appropriate concentrations (1 ng through 10 ug/ml) of LHRH antagonists for 60 min. The reaction was terminated by cooling the tubes to 4° C. Tubes were centrifuged; supernatants were recovered and stored at −20° C. until assayed for histamine. Assays were performed in duplicate. Total cell histamine was determined by boiling for 10 min. Histamine released in reponse to antagonist was expressed as a percentage of total release. That concentration that released 50% of total mast cell histamine (HRD_{50} ug/ml) was determined for each antagonist. The results are summarized in FIG. 1

All of the peptides are considered to be effective to prevent ovulation of female mammals at very low dosages. The peptides of the invention are often administered in the form of pharmaceutically acceptable, nontoxic salts, such as acid addition salts. Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, fumarate, gluconate, tannate, maleate, acetate, citrate, benzoate, succinate, alginate, pamoate, malate, ascorbate, tartrate, and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a pharmaceutically acceptable diluent which includes a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid and a lubricant, such as magnesium stearate.

If administration in liquid form is desired, sweetening and/or flavoring may be used as part of the pharmaceutically-acceptable diluent, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected.

The pharmaceutical compositions will usually contain the peptide in conjunction with a conventional, pharmaceutically-acceptable carrier. Usually, the dosage will be from about 1 to about 100 micrograms of the peptide per kilogram of the body weight of the host when given intravenously; oral dosages will be higher. Overall, treatment of subjects with these peptides is generally carried out in the same manner as the clinical treatment using other antagonists of LHRH.

These peptides can be administered to mammals intravenously, subcutaneously, intramuscularly, orally, intranasally or intravaginally to achieve fertility inhibition and/or control and also in applications calling for reversible suppression of gonadal activity, such as for the management of precocious puberty or during radiation- or chemo-therapy. Effective dosages will vary with the form of administration and the particular species of mammal being treated. An example of one typical dosage form is a physiological saline solution containing the peptide which solution is administered to provide a dose in the range of about 0.1 to 2.5 mg/kg of body weight. Oral administration of the peptide may be given in either solid form or liquid form.

Although the invention has been described with regard to its preferred embodiments, it should be understood that changes and modifications obvious to one having the ordinary skill in his art may be made without departing from the scope of the invention, which is set forth in the claims which are appended thereto. Substitutions known in the art which do not significantly detract from its effectiveness may be employed in the invention.

EXAMPLE I

The synthesis of an analog of the formula:

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Arg-Pro-D-Ala-NH_2

was commenced with the preparation of the intermediate peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-Lys-Leu-Arg-Pro-D-AlaNH_2. The intermediate peptide was built step by step on a benzhydrylamine resin containing about 0.6 m.equiv. NH_2/g (from BACHEM) on a Beckman 990 synthesizer starting with the Boc-Gly in accordance with the procedures set forth below.

Coupling is carried out in accordance with Schedule A as follows:

SCHEDULE A

Reagent	Mixing Time (mins)
1. Boc Amino Acid (0.5–1.2 m mole/g. resin) + equiv. amt. of DIC	60–90
2. MeOH (twice)	1

-continued

SCHEDULE A

Reagent	Mixing Time (mins)
3. CH_2Cl_2 (twice)	1

Deblocking is carried out in accordance with Schedule B as follows:

SCHEDULE B

Reagent	Mixing Time (mins)
4. 50% TFA/1% ethanedithiol in CH_2Cl_2 (x2)	15 & 15
5. IpOH/1% ethane dithiol	1
6. 10% TEA in CH_2Cl_2	2
7. MeOH	1
8. 10% TEA in CH_2Cl_2	2
9. MeOH (x2)	1 & 1
10. CH_2Cl_2 (x2)	1 & 1

The phenolic hydroxyl group of Tyr is protected with 2,6-dichlorobenzyl (DCB).

Briefly, Boc is used for N-terminal protection. Tos is used to protect the guanidino group of Arg. Z(2-Cl) is used as the protecting group for the D-Lys side chain. Bzl for the OH group of Ser and Tyr is protected with DCB.

One and a half to two-fold excess of protected amino acid is used based on the NH_2-content of the benzhydrylamine-resin, plus one equivalent of DIC in CH_2Cl_2 or 10–50% DMF/CH_2Cl_2, depending on the solubility of Boc-amino acid, for two hours.

N-Terminal acetylation is performed with a 50-fold excess of acetic anhydride in CH_2Cl_2 for 0.5 hours. The protected intermediate peptide thus obtained has the following composition: Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser(X^4)-Tyr-(X^5)D-Lys(X^6)-Leu-Arg(X^8)-Pro-D-Ala-NH-X^{10} wherein X^4 is Bzl and X^5 is DCB, X^6 is Z(2-Cl), X^8 is Tos, and X^{10} is a benzhydryl group incorporated into the resin.

In order to cleave and deprotect the protected peptide-resin, it is treated with 1.4 ml. m-cresole and 15 ml. hydrogen fluoride per gram of peptide-resin for 0.5 hours at 0° and 0.5 hours at room temperature. After elimination of hydrogen fluoride under high vacuum, the resin-peptide is washed with diethyl ether and the peptide is then extracted with DMF and separated from the resin by filtration. The DMF solution is concentrated to a small volume under high vacuum, then triturated with diethyl ether. The crude product thus obtained is purified by preparative HPLC as described below, to give the pure free intermediate peptide having the above-mentioned structure wherein X^4, X^5, X^6, X^8 and X^{10} are hydrogen.

The free D-Lys^6-containing intermediate peptide is then reacted with potassium cyanate in 80% aqueous DMF solution (81 mg. KCNO/ml) at ambient temperature for 24 hours. The reaction mixture, after evaporation under high vacuum, is subjected to purification by preparative HPLC to yield the desired D-Hci-containing peptide. The peptide is judged to be substantially (95%) pure by using HPLC. HPLC analyses are carried out in a Hewlett-Packard 1090A gradient liquid chromatographic system on a C18 column (VYDAC 218TP546) eluted with solvents A: 0.1% TFA, B: 0.1% TFA in 70% CH_3CN with a gradient of 30–60% in 30 minutes. The intermediate peptide and the desired peptide have retention times of 25.5 minutes and 28.2 minutes, respectively.

Purification of peptides is carried out on a Beckman Prep-350 gradient liquid chromatograph using a 41.4×250 mm preparative reversed phase DYNEMAX C18 cartridge (300A, 12 um) with solvents A: 0.1% TFA and B: 0.1% TFA in 70% CH_3CN and using a gradient of 45–60% in 30 minutes.

EXAMPLE II

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Nal(2)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2 described in Example I, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°–10° for 10 hours. Retention time for the desired peptide is 30.8 min.

EXAMPLE III

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH^2 is conducted as described in Example I with the exception that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z-(2-Cl)] in position 6 of the intermediate peptide to afford another intermediate peptide having the formula Ac-D-Nal(2)-D-Phe (4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have HPLC retention times of 25.5 min. and 27.8 min., respectively.

EXAMPLE IV

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Nal(2)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example III, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°–10° for 10 hours. Retention time for the desired peptide is min.

EXAMPLE V

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe(4-Cl) is incorporated in place of Boc-D-Nal(2) in position 1 of the intermediate peptide to give another intermediate peptide having the formula Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have retention times of 24.0 min. and 26.6 min., respectively.

EXAMPLE VI

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Phe(4-Cl)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2 described in Example V with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°–10° for 10 hours. Retention time for the desired peptide is 29.2 min.

EXAMPLE VII

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-

NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe(4-Cl) is incorporated in place of Boc-D-Nal(2) in position 1 and that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z(2-Cl)] in position 6 of the intermediate peptide to yield another intermediate peptide having the formula Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have retention times of 24.0 min. and 26.3 min., respectively.

EXAMPLE VIII

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example VII, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 28.6 min.

EXAMPLE IX

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-Gly-NH_2 is conducted as described in Example I to afford another intermediate peptide having the formula Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have HPLC retention times of 24.8 min. and 27.4 min., respectively.

EXAMPLE X

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-Gly-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Nal(2)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2 described in Example IX with N-ethylisocyanate in DMF 0.1 mg. in 0.01 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 30.0 min.

EXAMPLE XI

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I with the exception that Boc-Pro is incorporated in place of Boc-D-Nal(2) in position 1 of the intermediate peptide to afford another intermediate peptide having the formula Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have retention times of 16.8 min. and 19.3 min., respectively.

EXAMPLE XII

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Pro-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2 described in Example XI, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 22.0 min.

EXAMPLE XIII

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-Pro is incorporated in place of Boc-D-Nal(2) in position 1 and that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z(2-Cl)] in position 6 of the intermediate peptide to yield another intermediate peptide having the formula Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2. This intermediate peptide and the desired peptide have retention times of 16.85 min. and 18.8 min., respectively.

EXAMPLE XIV

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example VI, with the exception that the intermediate peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example XIII is reacted with N-ethylisocyanate. The desired peptide has a retention time of 24.9 min.

EXAMPLE XV

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe is incorporated in place of Boc-D-Nal(2) in position 1 of the intermediate peptide to yield another intermediate peptide having the formula Ac-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have HPLC retention times of 20.8 min. and 23.4 min., respectively.

EXAMPLE XVI

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2 described in Example XV, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 26.0 min.

EXAMPLE XVII

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe is incorporated in place of Boc-D-Nal(2) in position 1 and that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z(2-Cl)] in position 6 of the intermediate peptide to yield another intermediate peptide having the formula Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2. This intermediate peptide and the desired peptide have retention times of 21.0 min. and 23.1 min., respectively.

EXAMPLE XVIII

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example XVII, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of

intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 25.4 min.

Similarly, there may be prepared the acid addition salts of the other peptides analogous to LHRH, described herein.

EXAMPLE XIX

A solution of 0.1 g. of the hydrogen fluoride salt of Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 (See Example I) is dissolved in 5 ml of water and passed through a column of 5 g. Dowex 3 anion exchange resin which had previously been equilibrated with acetic acid and washed with deionized water and the effluent is lyophilized to yield the corresponding acetic acid salt of Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2.

Repeating the above, substituting other acids of acetic acid during the equilibration of the resin, there may be obtained, for example, the corresponding salts with hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, benzoic acid, and the like. EXAMPLE XX

Tablet formulation for buccal (e.g., sublingual) administration:

1. LHRH Antagonist 10.0 mg.
   Compressible Sugar, USP 86.0 mg.
   Calcium Stearate 4.0 mg.
2. LHRH Antagonist 10.0 mg.
   Compressible Sugar, USP 88.5 mg.
   Magnesium Stearate 1.5 mg.
3. LHRH Antagonist 5.0 mg.
   Mannitol, USP 83.5 mg.
   Magnesium Starch, USP 1.5 mg.
4. LHRH Antagonist 10.0 mg.
   Pregelatinized Starch, USP 10.0 mg.
   Lactose, USP 74.5 mg.
   Pregelatinized Starch, USP 15.0 mg.
   Magnesium Stearate, USP 1.5 mg.

Method A. LHRH Antagonist is dissolved in a sufficient quantity of water to form a wet granulation when mixed with the sugar portion of the excipients. After complete mixing the granulation is dried in a tray of fluid-bed dryer. The dry granulation is then screened to break up any large aggregates and then mixed with the remaining components. The granulation is then compressed on a standard tableting machine to the specific tablet weight.

Method B. In this manufacturing method, all formulations would include 0.01% gelatin, USP. The gelatin would be first dissolved in the aqueous granulation solvent followed by the LHRH analog. The remaining steps are as in (a) above.

EXAMPLE XXI

Long Acting intramuscular injectable formulation

Long Acting IM. Injectable-Sesame Oil Gel
LHRH Antagonist 10.0 mg.
Aluminum Monostearate, USP 20.0 mg.
Sesame oil g.s. ad 1.0 ml.

The aluminum monostearate is combined with the sesame oil and heated to 125° C. with stirring until a clear yellow solution forms. This mixture is then autoclaved for sterility and allowed to cool. The LHRH antagonist is then added aseptically with trituration. Particularly preferred LHRH antagonists are salts of low solubility, e.g., zinc salts, zinc tannate salts, pamoate salts, and the like. These exhibit exceptionally long duration of activity.

EXAMPLE XXII

Long Acting IM Injectable-Biodegradable Polymer Microcapsules

LHRH Antagonist 1%
25/75 glycolide/lactide copolymer (0.5 intrinsic viscosity) 99%
Microcapsules (0°-150°) of above formulation suspended in:
Dextrose 5.0%
CMC, sodium 0.5%
Benzyl alcohol 0.9%
Tween 80 0.1%
Water, purified q.s. 100.0%
25 mg. of microcapsules are suspended in 1.0 ml. of vehicle.

EXAMPLE XXIII

Aqueous Solution for Intramuscular Injection

LHRH Antagonist 500 mg.
Gelatin, nonantigenic 5 mg.
Water for injection q.s. ad 100 ml.

The gelatin and LHRH antagonist are dissolved in water for injection, then the solution is sterile filtered.

EXAMPLE XXV

Formulation for Rectal Administration

Suppository Vehicle for Rectal Administration

LHRH Antagonist 5.0 mg.
Witepsol H15 20.0 mg.

The LHRH antagonist is combined with the molten Witepsol H15, mixed with and poured into 2 gm. molds.

We claim:

1. A peptide selected from the group of peptides having the formula:

$$X\text{-}R^1\text{-}R^2\text{-}R^3\text{-Ser-Tyr-}R^6\text{-Leu-Arg-Pro-}R^{10}\text{-}NH_2$$

wherein

X is an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms,
R^1 is D- or L-Pro, D- or L-A^1-Pro, D-Phe, D-Phe(4-Hl), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal (2),
R^2 is D-Phe or D-Phe(4-Hl),
R^3 is D-Trp, D-Phe, D-Pal, D-Nal(1) or D-Nal (2),
R^6 is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) and
R^{10} is Gly or D-Ala,
where Q2 is lower alkyl of 1-3 carbon atoms and H1 is fluoro, chloro or bromo,
and the pharmaceutically acceptable acid addition salts thereof.

2. A peptide of claim 1 wherein
R^1 is D- or L-Pro, D-Phe, D-Phe(4-Cl), D-Nal (2),
R^2 is D-Phe(4-F) or D-Phe(4-Cl), and
R^3 is D-Trp.

3. A peptide selected from the group of peptides having the formula:

$$X\text{-}R^1\text{-}R^2\text{-}R^3\text{-Ser}(X^5)\text{-Tyr}(X^5)\text{-}R^6(X^6)\text{-Leu-Arg}(X^6)\text{-Pro-}R^{10}\text{-NH-}X^{10}$$

wherein

X is an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms, t-Boc or hydrogen,
X^4 is hydrogen or a protecting group for the Ser hydroxyl group,
X^5 is hydrogen or a protecting group for the Tyr phenolic hydroxyl group,
X^6 is hydrogen or a protecting group for the Lys or Orn side chain amino group,
X^8 is hydrogen or a protecting group for the Arg guanidino group,
X^{10} is hydrogen or a resin support containing benzhydryl or methylbenzhydryl groups,
R^1 is D- or L-Pro, D- or LA^1-Pro, D-Phe, D-Phe(4-H1), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal (2),
R^2 is D-Phe or D-Phe(4-H1),
R^3 is D-Trp, D-Phe, D-Pal, D-Nal(1) or D-Nal (2),
R^6 is D-Lys or D-Orn and R^{10} is Gly or D-Ala,
where H1 is fluoro, chloro or bromo, provided that where X is t-Boc or hydrogen, X^4, X^5, X^6, and X^8 must be other than hydrogen.

4. A Peptide of claim 3 wherein
X^4 is hydrogen or 2,6-dichloro-benzyl,
X^5 is hydrogen or Z(2-Br) or 2,6-dichlorobenzyl,
X^6 is hydrogen or Z(2-Cl) and
X^8 is hydrogen, nitro, Tos, methyl-(t-butylbenzyl)sulfonyl or 4-methoxy-2,3,6-trimethyl benzylsulfonyl.

5. A Peptide of claim 4 wherein
X^4 and X^5 are hydrogen or benzyl,
X^6 is hydrogen or Z(2Cl), and
X^8 is hydrogen or Tos.

6. A peptide of claim 2 wherein X is acetyl.

7. A peptide of claim 6 wherein R^1 is Pro.

8. A peptide of claim 6 wherein R^1 is D-Nal(2).

9. A peptide of claim 6 wherein R^1 is D-Phe(4-Cl).

10. A peptide of claim 6 wherein R^1 is D-Phe.

11. A peptide of claim 7 wherein R^6 is D-Cit or D-Cit(Et).

12. A peptide of claim 7 wherein R^6 is D-Hci or D-Hci(Et).

13. A peptide of claim 8 wherein R^6 is D-Hci or D-Cit.

14. A peptide of claim 9 wherein R^6 is D-Hci or D-Cit.

15. A peptide of claim 10 wherein R^6 is D-Cit or D-Cit(Et).

16. A peptide of claim 10 wherein R^6 is D-Hci or D-Hci(Et).

17. A pharmaceutical composition for reducing the physiological availability of pituitary gonadotropins in a mammal which comprises a reductively effective amount of a compound of claim 1 and a pharmaceutically acceptable carrier.

18. A method of reducing the physiological availability of pituitary gonadotropins in mammals in need of such reduction which comprises administering thereto a reductively effective amount of a compound of claim 1.

19. A method of claim 18 wherein the amount is between 0.1 and 2.5 mg/kg body weight per day.

* * * * *

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United States Patent [19]

Schally et al.

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[54] LHRH ANTAGONISTS

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[*] Notice: The portion of the term of this patent subsequent to Jan. 24, 2006 has been disclaimed.

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[58] Field of Search ..... 530/313, 328; 514/15, 514/800; 930/110, 120, DIG. 803

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[57] ABSTRACT

The present invention deals with LHRH antagonists which possess improved water solubility and while having the high antagonist potency of the basic peptides, are free of the edematogenic effects. These compounds are highly potent in inhibiting the release of gonadotropins from the pituitary gland in mammals, including humans.

The compounds of this invention are represented by the formula

$$X—R^1—R^2—R^3—Ser—Tyr—R^6—Leu—Arg—Pro—R^{10}—NH_2$$

wherein

X is an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms, or $H_2N—CO$,

R^1 is D— or L—Pro, D— or L—Δ^3—Pro, D—Phe, D—Phe(4—H1), D—Ser, D—Thr, D—Ala, D—Nal(1) or D—Nal (2),

R^2 is D—Phe or D—Phe(4—C1)

R^3 is D—Trp, D—Phe, D—Pal(3), D—Nal(1) or D—Nal(2),

R^6 is D—Cit, D—Hci, D—Cit(Q) or D—Hci(Q) and

R^{10} is Gly or D—Ala

where Q is lower alkyl of 1–3 carbon atoms and H1 is fluoro, chloro or bromo, and the pharmaceutically acceptable acid addition salts thereof and methods of use pertaining to these compounds.

2 Claims, No Drawings

LHRH ANTAGONISTS

This invention was made with Government support under Grant Nos. CA40003 and 40004, awarded by the N.C.I. (NIH). The U.S. Government has certain rights in this application.

RELATED APPLICATIONS

This application is a continuation-in-part of copending application, Ser. No. 074,126, filed Jul. 17, 1987, now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates to novel peptides which inhibit the release of gonadotropins by the pituitary gland in mammals without inducing edematous reactions. More specifically, the present invention relates to analogs of the luteinizing hormone releasing hormone (LHRH), which has the structure:

p—Glu—His—Trp—Ser—Tyr—Gly—Leu—Arg—Pro—Gly—NH_2,

salts thereof, and to pharmaceutical compositions and methods of use pertaining to these analogs.

DISCUSSION OF THE PRIOR ART

For more than 15 years, investigators have been searching for selective, potent antagonists of the LHRH decapeptide (M. Karten and J. E. Rivier, Endocrine Reviews, 7, 44–66 (1986)). The high degree of interest in such antagonists is due to their usefulness in the fields of endocrinology, gynecology, contraception and cancer. A large number of compounds have been prepared as potential LHRH antagonists. The most interesting antagonists to date have been compounds whose structure is a modification of the structure of LHRH.

The first series of potent antagonists was obtained by introduction of aromatic amino acid residues into positions 1, 2, 3 and 6, or, 2, 3, and 6. The compounds are expressed as LHRH modified by replacement of the original amino acid residues by others at the position indicated by the superscript numbers. The known antagonists include:

[Ac-D-Phe(4-Cl)^{1,2}, D-Trp^{3,6}] LHRH (D. H Coy, et al., In: Gross, E. and Meienhofer, J. (eds) Peptides, Proceedings of the 6th. American Peptide Symposium, pp. 775–779, Pierce Chem. Co., Rockville Ill., 1979);

[Ac-Pro^1, D-Phe(4-Cl)^2 D-Nal(2)^{3,6}] LHRH (U.S. Pat. No. 4,419,347); and [Ac-Δ^3Pro^1,D-Phe(4-Cl)^2,D-Trp^{3,6}]LHRH (J. L. Pineda, et al., J. Clin. Endocrinol. Metab. 56, 420, 1983).

Later, in order to increase the water solubility of antagonists, basic amino acids, such as D-Arg, were introduced into position 6. For instance, [Ac-D-Phe(4-Cl)^{1,2}, D-Trp^3, D-Arg^6, D-Ala^{10}]LHRH (ORG-30276) (D. H. Coy, et al., Endocrinology, 100, 1445, 1982); and [Ac-D-Nal(2)^1, D-Phe(4-F)^2, D-Trp^3, D-Arg^6]LHRH (ORF-18260) (J. E. Rivier, et al., In: Vickery B. H., Nestor, Jr. J. J., Hafez, E.S.E. (eds), LHRH and Its Analogs, pp. 11–22, MTP Press, Lancaster, UK, 1984).

These analogs not only possessed the expected improved water solubility but also showed increased antagonistic activity. However, these highly potent, hydrophilic analogs containing D-Arg and other basic side chains at position 6 proved to produce transient edema of the face and extremities when administered subcutaneously in rats at 1.25 or 1.5 mg/kg (F. Schmidt, et al., Contraception, 29, 283, 1984; J. E. Morgan, et al., Int. Archs. Allergy Appl. Immun. 80, 70, (1986). Since the occurrence of edematogenic effects after administration of these antagonists to rats cast doubts on their safety for the use in humans and delayed the introduction of these drugs for clinical use, it is desirable to provide antagonistic peptides which are free of these side effects.

SUMMARY OF THE INVENTION

The present invention deals with LHRH antagonists which possess an improved water solubility and high antagonist potency of the basic peptides, and are free of the edematogenic effects. These compounds are highly potent in inhibiting the release of gonadotropins from the pituitary gland in mammals, including humans.

The compounds of this invention are represented by formula I

$$X—R^1—R^2—R^3—Ser—Tyr—R^6—Leu—Arg—Pro—R^{10}—NH_2 \quad I$$

wherein

X is an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms, or a carbamyl ($H_2N—CO$) group.

R^1 is D— or L—Pro, D— or L—Δ^3—Pro, D—Phe, D—Phe(4—Hl), D—Ser,

D—Thr, D—Ala, D—Nal(1) or D—Nal(2),

R^2 is D—Phe or D—Phe(4—Cl)

R^3 is D—Trp, D—Phe, D—Pal(3), D—Nal(1) or D—Nal(2),

R^6 is D—Cit, D—Hci, D—Cit(Q) or D—Hci(Q) and

R^{10} is Gly or D—Ala

where Q is lower alkyl of 1–3 carbon atoms and Hl is fluoro, chloro or bromo, and the pharmaceutically acceptable acid addition salts thereof.

The compounds of Formula I are synthesized by any suitable method. For example, exclusively solid-phase technique, partial solid-phase technique or by classical solution couplings Preferably, the compounds of Formula I are prepared by a known solid-phase technique. Such method provides intermediate peptides and/or intermediate peptide-resins of Formula II.

$$X^1—R^1—R^2—R^3—Ser(X^4)—Tyr(X^5)—R^6(X^6)—Leu—Arg(X^8)—Pro—R^{10}—NH—X^{10} \quad II$$

wherein

X^1 is an acyl group derived from straight and branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms, t-Boc, carbamyl or hydrogen,

X^4 is hydrogen or a protecting group for the Ser hydroxyl group,

X^5 is hydrogen or a protecting group for the Tyr phenolic hydroxyl group,

X^6 is hydrogen or a protecting group for the Lys or Orn side chain amino group,

X^8 is hydrogen or a protecting group for the Arg guanidino group,

X^{10} is hydrogen or a resin support containing benzhydryl or methylbenzhydryl groups

R^1 is D— or L—Pro, D— or L—Δ^3—Pro, D—Phe, D—Phe(4—Hl), D—Ser, D—Thr, D—Ala, D—Nal(1) or D—Nal(2),

R^2 is D—Phe or D—Phe(4—Cl),
R^3 is D—Trp, D—Phe, D—Pal(3), D—Nal(1) or D—Nal(2),
R^{*6} is D—Lys or D—Orn, D—Cit and D—Hci and R^{10} is Gly or D—Ala
where Hl is fluoro, chloro or bromo.

One process comprises reacting a peptide of Formula II wherein R^{*6} is D-Lys or D-Orn and X^6 is hydrogen, with a source of cyanate to yield a peptide of Formula III:

$$X^1—R^1—R^2—R^3—Ser(X^4)—Tyr(X^5)—R^{*6}—Leu—Arg(X^6)—Pro—R^{10}—NH—X^{10} \quad III$$

wherein

X^1, R^1, R^2, R^3, X^4, X^5, X^6, R^{10} and X^{10} are as defined above, and

R^{*6} is Cit or Hci. Suitably, the reaction is carried out when X^1 is acyl and all other X moieties are hydrogen. Suitable cyanate sources are alkali metal cyanates, e.g., potassium cyanate, or an N-alkyl isocyanate, e.g., N-ethyl-isocyanate. The peptide of Formula II are preferably synthesized by a known solid phase technique.

Alternatively and preferably, peptides of Formula I wherein X is an acyl or carbamyl group, are directly obtained by cleavage and deprotection of intermediate peptide-resins of Formula II wherein X^1 is an acyl or carbamyl group and R^{*6} is D-Cit or D-Hci. Peptides of Formula I wherein X is carbamyl ($H_2N—CO$) group are also obtained from peptide-resins of Formula II wherein X^1 is hydrogen or Boc by cleavage and deprotection followed by carbamoylation.

A gonadotropin antagonizing pharmaceutical composition is provided by admixing the compound of Formu'a I with a pharmaceutically acceptable carrier including microcapsules (microspheres) for delayed delivery.

There is also provided a method for relieving complications resulting from the physiological availability of amounts of pituitary gonadotropins in a mammal, in excess of the desired amount, which involves administering to the mammal a gonadotropin antagonizing dose of the compound of Formula I.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The nomenclature used to define the peptides is that specified by the IUPAC-IUB Commission on Biochemical Nomenclature (*European J. Biochem.*, 1984, 138, 9-37), wherein in accordance with conventional representation the amino groups at the N-terminus appears to the left and the carboxyl group at the C-terminus to the right. By natural amino acid is meant one of the common, naturally occurring amino acids found in proteins comprising Gly, Ala, Val, Leu, Ile, Ser, Thr, Lys, Arg, Asp, Asn, Glu, Gln, Cys, Met, Phe, Tyr, Pro, Trp and His. The abbreviations for the individual amino acid residues are based on the trivial name of the amino acid and are Ala, alanine; Arg, arginine; Cit, citrulline, Gly, glycine; Hci, homocitrulline; Leu, leucine; Lys, lysine; Pal(3), 3-(3-pyridyl)alanine; Nal(1), Nal(2), 3-(1-naphtyl)alinine, 3-(2-naphthyl)alanine; Orn, ornithine; Phe, phenylalanine; Phe(4-Cl), 4-chlorophenylalanine; Phe (4-F), 4-fluorophenylalanine; Pro, proline; Ser, serine; Trp, tryptophan and Tyr, tyrosine. All amino acids described herein are of the L-series unless stated otherwise, e.g., D-Trp represents D-tryptophan and D-Nal(2) represents 3-(2-naphthyl)-D-alanine.

Other abbreviations used are:

AcOH	acetic acid
AcOEt	ethyl acetate
Ac_2O	acetic anhydride
Boc-	tert.butyloxycarbonyl-
DIC	diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMF	dimethylformamide
HOBt	1-hydroxybenzotriazole hydrate
HPLC	high performance liquid chromatography
MeOH	methyl alcohol
TEA	triethylamine
DCC	dicyclohexylcarbodiimide
MeCN	acetonitrile
IpOH	isopropanol
Z(2-Cl)	2-chloro-benzyloxycarbonyl
DCB	2,6-dichlorobenzyl
Tos	p-toluenesulfonyl
TFA	trifluoroacetic acid
Z	benzyloxycarbonyl

Especially preferred are LHRH analogs of Formula I wherein:
X^1 is acetyl or carbamyl
R^1 is Pro, D—Phe, D—Phe(4—Cl) or D—Nal(2),
R^2 is D—Phe(4—Cl) or D—Phe(4—F),
R^3 is D—Trp or D—Pal(3)
R^6 is D—Cit or D—Hci, and
R^{10} is D—Ala.

The peptides are synthesized by a suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution phase synthesis (See M. Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984).

For example, the techniques of exclusively solid-phase synthesis are set forth in the textbook "Solid Phase Peptide Synthesis", J. M. Stewart and J. D. Young, Pierce Chem. Company, Rockford, Ill., 1984 (2nd. ed.), G. Barany and R. B. Merrifield, "The Peptides", Ch. 1, 1-285, pp. 1979, Academic Press, Inc.

Classical solution synthesis is described in detail in the treatise "Methoden der Organischen Chemie (Houben-Weyl): Synthese von Peptiden", E. Wunsch (editor) (1974) Georg Thieme Verlag, Stuttgart, W. Germany.

Common to such synthesis is the protection of the reactive side chain functional groups of the various amino acid moieties with suitable protecting groups which will prevent a chemical reaction from occurring at that site until the group is ultimately removed Usually also common is the protection of an alpha-amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha-amino protecting groups to allow subsequent reaction to take place at that location Accordingly, it is common that, as a step in the synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with side-chain protecting groups linked to the appropriate residues.

In Formula II:
R^1, R^2, and R^3 are as defined hereinabove.
X^1 is hydrogen or an acyl group derived from straight or branched chain aliphatic or alicyclic carboxylic acids having from 1 to 7 carbon atoms, or an alpha-amino protecting group. The alpha-amino protect-

ing groups contemplated by X^1 are those well known to be useful in the art of step-wise synthesis of polypeptides. Among the classes of alpha-amino protecting groups which may be employed as X^1 may be mentioned fluorenylmethyloxycarbonyl (Fmoc) or t-butyloxycarbonyl (Boc).

X^4 may be a suitable protecting group for the hydroxyl group of Ser such as benzyl (Bzl), and 2,6-dichloro-benzyl (DCB). The preferred protecting group is Bzl.

X^5 may be a suitable protecting group for the phenolic hydroxyl group of Tyr, such as Bzl, 2-Br-Z and 2,6-dichloro-benzyl (DCB). The preferred protecting group is DCB.

X^6 is a suitable protecting group for the side chain amino group of Lys or Orn. Illustrative of suitable side chain amino protecting groups are benzyloxycarbonyl (Z), and 2-chloro-benzyloxycarbonyl ((Z-(2-Cl)).

X^8 is a suitable protecting group for the guanidino group of Arg, such as nitro, Tos, methyl-(t-butyl benzene)-sulfonyl, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; Tos is the preferred group.

X^{10} is an amide protecting benzhydryl or methylbenzhydryl group incorporated into resin support; for the synthesis of peptide amides 98% styrene-2% divinylbenzene copolymers containing benzhydryl amine or methylbenzhydryl amine groups are preferred.

The selection of a side chain amino protecting group is not critical except that generally one is chosen which is not removed during deprotection of the alpha-amino groups during the synthesis.

The peptides of Formula I may be from intermediate peptide-resins of Formula II by procedures known in the art. The solid phase synthesis of intermediate peptide-resins of Formula II is essentially carried out as described by Merrifield, J. Am. Chem. Soc., 85, p. 2149 (1963). Solid phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected amino acid to a suitable resin Such a starting material can be prepared by attaching -amino protected Gly or D-Ala by an amide bond to a benzylhydrilamine resin. Such resin supports are commercially available and generally used when the desired polypeptide being synthesized has an carboxamide at the C-terminal.

The selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as a coupling reagent is N,N'-diisopropyl carbodiimide (DIC).

Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in about a two-three fold excess, and the coupling may be carried out in a medium of DMF:CH_2Cl_2 (1:1) or in CH_2Cl_2 alone. In cases where incomplete coupling occurs, the coupling procedure is repeated before removal of the alpha-amino protecting group prior to the coupling of the next amino acid. The success of the coupling reaction at each stage of the synthesis, is preferably monitored by the ninhydrin reaction, as described by E. Kaiser, et al., *Anal. Biochem.*, 34, 595 (1970).

After the desired amino acid sequence of intermediates B has been completed, the terminal Boc group is removed and if desired, N-terminal acylation carried out using the appropriate acyl anhydride or acid chloride in 50-fold excess in a halogenated hydrocarbon solvent; suitably, acetic anhydride in methylene chloride for 30 minutes. The intermediate peptide can be removed from the resin support by treatment with a reagent such as liquid hydrogen fluoride, which not only cleaves the peptide from the resin but also cleaves all remaining side chain protecting groups X^4, X^5, X^8, X^{10} and, if present, X^6.

When using hydrogen fluoride for cleaving, anisole or m-cresol, and, if desired, methylethyl sulfide are included as scaveagers in the reaction vessel.

Peptides of Formula II wherein R^{*6} is D-Lys or D-Orn and X^6 is hydrogen, are converted into peptides of Formula I by treatment with cyanate, suitably an alkali metal cyanate, preferably potassium cyanate, or an N-alkylisocyanate, for instance, N-ethylisocyanate, in DMF or aqueous DMF. The latter reaction, i.e., transformation of Orn/Lys-peptides into the corresponding Cit/Hci-peptides can be readily followed by HPLC using MeCN-aqueous TFA systems because of a characteristic 2.6±0.3 minutes increase of the retention times of Cit/Hci—and, for example, Cit(Et)/Hci(Et)-peptides relative to the corresponding Orn/Lys-peptides respectively.

When acylation is omitted, treatment of peptide-resins of Formula II with hydrogen fluoride yields decapeptides which have free omega-amino and/or alpha-amino groups and correspond to a Formula II where X^1, X^4, X^5, X^8, X^{10}, and, if present, X^6 are hydrogen. These free peptides are converted into peptides of Formula I wherein X is carbamyl by treatment with cyanate, suitably an alkali metal cyanate, preferably potassium cyanate. The latter reaction, i.e., transformation of H_2N into H_2N—CO—NH at the amino terminus of peptides and conversion of the Orn/Lys residues into the Cit/Hci residues, can be easily followed by HPLC using MeCN-aqueous TFA systems, because of a characteristic 2-3 min. increase of the retention times of carbamylated peptides, i.e., compounds with H_2N—CO—NH— group, relative to their congeners with H_2N group.

Alternatively and preferably, peptides of Formula I wherein X is an acyl or carbamyl group, are directly obtained by cleavage and deprotection of intermediate peptide-resins of Formula II, where X^1 is an acyl or carbamyl group and R^{*6} is D-Cit or D-Hci.

Although an exclusively solid-phase synthesis and a partially solid-phase synthesis of compounds of Formula I are disclosed herein, the preparation of the compounds also can be realized by classical solution-phase methods.

The synthetic peptides prepared as described in the Examples are compared with two of the most potent LHRH antagonists reported recently, i.e., [Ac-D-Phe(4-Cl)^{1,2}, D-Trp^3, D-Arg^6, D-Ala^{10}] LHRH (ORG-30276) (Coy, et al., Endocrinology, 100, 1445, 1982) and [Ac-D-Nal(2)^1, D-Phe(4-F)^2, D-Trp^3, D-Arg^6] LHRH (ORF 18260) (Rivier, et al., In: Vickery, B. H., Nestor, Jr., J. J. Hafez, E. S. E. (eds.), LHRH and Its Analogs, pp. 11-22, MTP Press, Lancaster, UK, 1984), and are found to exert similarly high inhibitory activities both in vitro and in vivo, but, unlike to the control peptides, not to produce the in vivo edematous effects.

Hormonal activities in vitro are compared in superfused rat pituitary cell systems (S. Vigh and A. V. Schally, Peptides. 5 suppl. 1: 241-247, 1984) in which the effectiveness of LHRH (and other releasing hormones) can be accurately evaluated since the amount of LH (or other pituitary hormones) secreted into the effluent medium is not only proportional to the hormone-releasing potency of the peptide applied but also

measurable readily by well-characterized radioimmunoassays.

To determine the potency of an LHRH antagonist, mixtures containing LHRH in a constant concentration (usually 1 nM) and the antagonist in varying concentrations are used for the superfusion in order to determine the molecular ratio of the antagonist to LHRH at which the action of LHRH is completely blocked. These ratios are about 5 for both peptides of the present invention and the control peptides when the rat pituitary cell system is preincubated with antagonists for 9 minutes.

In an antiovulatory in vivo assay (A. Corbin and C. W. Beattie; Endocr. Res. Commun. 2, 1-23, 1975; D. H. Coy, et al., Endocrinology, 100, 1445, 1982), the peptides of the present invention are also found to be about equipotent to the control antagonist, namely, 87.5-100% blockade of ovulation can be observed at a subcutaneous dose of 1-3 ug/rat for each peptide.

In the edematogenic test of Schmidt, et al. (Contraception, 29, 283-289, 1984), however, a marked difference can be found between the control peptides and the peptides of the present invention. The control administered subcutaneously in rats at doses of 1.25 or peptides produce edema of the face and extremities when 1.50 mg/kg. No such reaction can be observed with the peptides of the present invention when given at a subcutaneous dose of 1.5 mg/kg.

In the tests as run, the rats were assigned to three groups of five rats per group per compound tested. Comparison was made with a known prior art compound designated ORG 30276 namely (N-Ac-D-p-Cl-Phe^{1,2},D-Trp^{3}, D-Arg^{6},D-Ala^{10})-LHRH. The groups were injected subcutaneously once a day on two consecutive days with the LHRH antagonists at a dose level of 1.5 mg/kg. One control group was injected with diluent only. The rats were observed during five hours each day. Reactions of the rats were classified as follows: NR no apparent reaction, PR partial responders: edema of the nasal and paranasal area, FR full responders: facial edema with edematous extremities.

These results are summarized in Table 1 below.

TABLE 1

LHRH	1st Day			2nd Day		
Antagonist	NR	PR	FR	NR	PR	FR
ORG 30276	3	7	0	0	0	10
Control	9	0	0	9	0	0
EX III	8	0	0	8	0	0
EX V	9	0	0	8	1*	0
EX IV	9	0	0	9	0	0
EX I	8	0	0	8	0	0
EX XX	9	0	0	8	1*	0
EX XXI	9	0	0	9	0	0
EX XXVI	8	0	0	8	0	0
EX XXVII	9	0	0	9	0	0

*Very light edema of the face.

LHRH secretion in vitro at some reasonable concentration, although most are slightly less potent than the present standard in vitro; however, these peptides are much more potent in vivo.

This was shown by a test on histamine release in vitro from peritoneal mast cells carried out in accordance with the procedure of Morgan et al (Int. Archs. Allergy appl. Immun. 80, 70 1986).

Histamine Release In Vitro

In this test rats were anesthetized with ether and peritoneal exudate cell were harvested by washing with 12 ml. of mast cell medium (MCM) (150m M NaCl; 3.7m M KCl; 3.0m M Na_2HPO_4; 3.5m M KH_2PO_4, 0.98m M CaCl; 5.6m M dextrose; 0.1% bovine serum albumin; 0.1% gelatin and 10 units/ml heparin)[9]. Cells from 4 or 5 rats were pooled, centrifuged at 120 g, resuspended with MCM to a concentration of 0.5×10^6 ml and 1 ml was aliquoted into 12×75 mm polyethylene tubes. Tubes were equilibrated to 37° C. for 15 min and incubated alone (background histamine release), with 48/80 (positive control) (Sigma Chemicals, St. Louis, Mo.), or with appropriate concentrations (1 ng through 10 ug/ml) of LHRH antagonists for 60 min. The reaction was terminated by cooling the tubes to 4° C. Tubes were centrifuged; supernatants were recovered and stored at −20° C. until assayed for histamine. Assays were performed in duplicate. Total cell histamine was determined by boiling for 10 min. Histamine released in reponse to antagonist was expressed as a percentage of total release. That concentration that released 50% of total mast cell histamine (HRD_{50} ug/ml) was determined for each antagonist. The results are summarized in FIG. 1.

All of the peptides are considered to be effective to prevent ovulation of female mammals at very low dosages. The peptides of the invention are often administered in the form of pharmaceutically acceptable, nontoxic salts, such as acid addition salts. Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, fumarate, gluconate, tannate, maleate, acetate, citrate, benzonate, succinate, alginate, pamoate, malate, ascorbate, tartrate, and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a pharmaceutically acceptable diluent which includes a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid and a lubricant, such as magnesium stearate.

If administration in liquid form is desired, sweetening and/or flavoring may be used as part of the pharmaceutically-acceptable diluent, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected.

The pharmaceutical compositions will usually contain the peptide in conjunction with a conventional, pharmaceutically-acceptable carrier. Usually, the dosage will be from about 1 to about 100 micrograms of the peptide per kilogram of the body weight of the host when given intravenously; oral dosages will be higher. Overall, treatment of subjects with these peptides is generally carried out in the same manner as the clinical treatment using other antagonists of LHRH.

These peptides can be administered to mammals intravenously, subcutaneously, intramuscularly, orally, intranasally or intravaginally to achieve fertility inhibition and/or control and also in applications calling for reversible suppression of gonadal activity, such as for the management of precocious puberty or during radiation- or chemo-therapy. Effective dosages will vary with the form of administration and the particular species of mammal being treated. An example of one typical dosage form is a physiological saline solution containing the peptide which solution is administered to provide a dose in the range of about 0.1 to 2.5 mg/kg of body weight. Oral administration of the peptide may be given in either solid form or liquid form.

Although the invention has been described with regard to its preferred embodiments, it should be understood that changes and modifications obvious to one

having the ordinary skill in his art may be made without departing from the scope of the invention, which is set forth in the claims which are appended thereto. Substitutions known in the art which do not significantly detract from its effectiveness may be employed in the invention.

EXAMPLE I

The synthesis of an analog of the formula:

Ac—D—Nal(2)—D—Phe(4—Cl)—D—Trp—Ser—Tyr—D—Hci—Leu—Arg—Pro—D—Ala—NH_2

was commenced with the preparation of the intermediate peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2. The intermediate peptide was built step by step on a benzhydrylamine resin containing about 0.6 m. equiv. NH_2/g (from BACHEM) on a Beckman 990 synthesizer starting with the Boc-D-Ala in accordance with the procedures set forth below.

Coupling is carried out in accordance with Schedule A as follows:

SCHEDULE A

Reagent	Mixing Time (mins)
1. Boc Amino Acid (0.9–1.2m mole/g. resin) + equiv amt. of DIC	60–90
2. MeOH (twice)	1
3. CH_2Cl_2 (twice)	1

Deblocking is carried out in accordance with Schedule B as follows:

SCHEDULE B

Reagent	Mixing Time (mins)
4. 50% TFA/1% ethanedithiol in CH_2Cl_2 (twice)	15 & 15
5. IpOH/1% ethane dithiol	1
6. 10% TEA in CH_2Cl_2	2
7. MeOH	1
8. 10% TEA in CH_2Cl_2	2
9. MeOH (twice)	1 & 1
10. CH_2Cl_2 (twice)	1 & 1

Briefly, Boc is used for N-terminal protection. Tos is used fto protect the guanidino group of Arg, Z(2-Cl) is used as the protecting group for the D-Lys side chain, Bzl for the OH group of Ser and Tyr is protected with DCB.

One and a half to two-fold excess of protected amino acid is used based on the NH_2-content of the benzhydrylamine-resin, plus one equivalent of DIC in CH_2Cl_2 or 10–50% DMF/CH_2Cl_2, depending on the solubility of Boc-amino acid, for two hours.

N-Terminal acetylation is performed with a 50-fold excess of acetic anhydride in CH_2Cl_2 for 0.5 hours. The protected intermediate peptide thus obtained has the following composition:

Ac—D—Nal(2)—D—Phe(4—Cl)—D—Trp—Ser(X^4)—Trp—(X^5)D—LYS(X^6)—Leu—Arg(X^8)—Pro—D—Ala—NH—X^{10}

wherein

X^4 is Bzl and
X^5 is DCB, X^6 is Z(2-Cl),
X^8 is Tos, and
X^{10} is a benzhydryl group incorporated into the resin.

In order to cleave and deprotect the protected peptide-resin, it is treated with 1.4 ml. m-cresole and 15 ml. hydrogen fluoride per gram of peptide-resin for 0.5 hours at 0° and 0.5 hours at room temperature. After elimination of hydrogen fluoride under high vacuum, the resin-peptide is washed with diethyl ether and the peptide is then extracted with DMF and separated from the resin by filtration. The DMF solution is concentrated to a small volume under high vacuum, then triturated with diethyl ether. The crude product thus obtained is purified by preparative HPLC as described below, to give the pure free intermediate peptide having the above-mentioned structure wherein X^4, X^5, X^6, X^8 and X^{10} are hydrogen.

The free D-Lys^6-containing intermediate peptide is then reacted with potassium cyanate in 80% aqueous DMF solution (81 mg. KCNO/ml), at ambient temperature for 24 hours. The reaction mixture, after evaporation under high vacuum, is subjected to purification by preparative HPLC to yield the desired D-Hci-containing peptide. The peptide is judged to be substantially (95%) pure by using HPLC. HPLC analyses are carried out in a Hewlett-Packard 1090A gradient liquid chromatographic system on a C18 column (VYDAC 218TP546) eluted with solvents A: 0.1% TFA, B: 0.1% TFA in 70% CH_3CN with a gradient of 30–60% in 30 minutes. The intermediate peptide and the desired peptide has a retention times of 25.5 minutes and 28.2 minutes respectively.

Purification of peptides is carried out on a Beckman Prep-350 gradient liquid chromatograph using a 41.4×250 mm preparative reversed phase DYNEMAX C18 cartridge (300A, 12 um) with solvents A: 0.1% TFA and B: 0.1% TFA in 70% CH_3CN and using a gradient of 45–60% in 30 minutes. The pure peptide obtained as TFA salt, if desired, can be converted to the acetate form by passage through an AG3X (Bio-Rad) column in the acetate form followed by lyophilization.

EXAMPLE II

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH2 accomplished by reacting the the intermediate peptide Ac-D-Nal(2)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH2 described in Example I, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°–10° for 10 hours. Retention time for the desired peptide is 30.8 min.

EXAMPLE III

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH2 is conducted as described in Example I with the exception that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z-(2-Cl)] in position 6 of the intermediate peptide to afford another intermediate peptide having the formula Ac-D-Nal(2)-D-Phe (4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have HPLC retention times of 25.5 min. and 27.8 min., respectively.

EXAMPLE IV

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the the intermediate peptide Ac-D-Nal(2)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example III, with N-ethylisocyanate in DMF (0.1 mg. in 10 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 30.4 min.

EXAMPLE V

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe(4-Cl) is incorporated in place of Boc-D-Nal(2) in position 1 of the intermediate peptide to give another intermediate peptide having ther formula Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2, which in then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have retention times of 24.0 min. and 26.6 min., respectively.

EXAMPLE VI

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the the intermediate peptide Ac-D-Phe(4-Cl)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2 described in Example V with N-ethylisocyanate in DMF (0.1 mg. in 10 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 29.2 min.

EXAMPLE VII

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe(4-Cl) is incorporated in place of Boc-D-Nal(2) in position 1 and that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z(2-Cl)] in position 6 of the intermediate peptide to yield another intermediate peptide having the formula Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide This intermediate peptide and the desired peptide have retention times of 24.0 min. and 26.3 min., respectively.

EXAMPLE VIII

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the intermediate peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example VII, with N-ethylisocyanate in DMF[2] (0.1 mg. in 10 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 28.6 min.

EXAMPLE IX

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-Gly-NH_2 is conducted as described in Example I to afford another intermediate peptide having the formula Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have HPLC retention times of 24.8 min. and 27.4 min., respectively.

EXAMPLE X

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-Gly-NH_2 is accomplished by reacting the the intermediate peptide Ac-D-Nal(2)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2 described in Example IX with N-ethylisocyanate in DMF 0.1 mg. in 10 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 30.0 min.

EXAMPLE XI

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I with the exception that Boc-Pro is incorporated in place of Boc-D-Nal(2) in position 1 of the intermediate peptide to afford another intermediate peptide having the formula Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the desired peptide. This intermediate peptide and the desired peptide have retention times of 16.8 min. and 19.3 min., respectively.

EXAMPLE XII

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the the intermediate peptide Ac-D-Pro-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2 described in Example XI, with N-ethylisocyanate in DMF (0.1 mg. in 10 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 22.0 min.

EXAMPLE XIII

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-Pro is incorporated in place of Boc-D-Nal(2) in position 1 and that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z(2-Cl)] in position 6 of the intermediate peptide to yield another intermediate peptide having the formula Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2. This intermediate peptide and the desired peptide have retention times of 16.85 min. and 18.8 min., respectively.

EXAMPLE XIV

The synthesis of the peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example VI, with the exception that the intermediate peptide Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example XIII is reacted with N-ethylisocyanate The desired peptide has a retention time of 24.9 min.

EXAMPLE XV

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe is incorporated in place of Boc-D-Nal(2) in position 1 of the intermediate peptide to yield another intermediate peptide having the formula Ac-D-Phe-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2, which is then similarly converted to the de-

sired peptide This intermediate peptide and the desired peptide have HPLC retention times of 20.8 min. and 23.4 min., respectively.

EXAMPLE XVI

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the the intermediate peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2 described in Example XV, with N-ethylisocyanate in DMF (0.1 mg. in 10 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 26.0 min.

EXAMPLE XVII

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example I, with the exception that Boc-D-Phe is incorporated in place of Boc-D-Nal(2) in position 1 and that Boc-D-Orn(Z) is incorporated in place of Boc-D-Lys[Z(2-Cl)] in position 6 of the intermediate peptide to yield another intermediate peptide having the formula Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2. This intermediate peptide and the desired peptide have retention times of 21.0 min. and 23.1 min., respectively.

EXAMPLE XVIII

The synthesis of the peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(Et)-Leu-Arg-Pro-D-Ala-NH_2 is accomplished by reacting the the intermediate peptide Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH_2 described in Example XVII, with N-ethylisocyanate in DMF (0.1 mg. in 0.01 ml. per gm of intermediate) at 0°-10° for 10 hours. Retention time for the desired peptide is 25.4 min.

EXAMPLE XIX

The synthesis of an analog of the formula:

Ac—D—Nal(2)—D—Phe(4—Cl)—D—Trp—Ser—Tyr—D—Hci—Leu—Arg—Pro—D—Ala—NH_2

peptide was built step by step on a benzhydrylamine resin containing about 1 0 m. equiv. NH_2/g (from BACHEM) on a Beckman 990 synthesizer starting with the Boc-D-Ala in accordance with the procedures set forth below.

Coupling is carried out in accordance with Schedule C as follows:

SCHEDULE C

Reagent	Mixing Time (mins)
1. Boc Amino Acid (2-3m mole/g resin) – equiv amt. of DIC	60-90
2. MeOH (twice)	1
3. CH_2Cl_2 (twice)	1

Deblocking is carried out in accordance with Schedule B as follows:

SCHEDULE D

Reagent	Mixing Time (mins)
4. 50% TFA/1% ethanedithiol in CH_2Cl_2 (twice)	15 & 15
5. IpOH/1% ethane dithiol	1
6. 10% TEA in CH_2Cl_2	2
7. MeOH	1
8. 10% TEA in CH_2Cl_2	2
9. MeOH (twice)	1 & 1
10. CH_2Cl_2 (twice)	1 & 1

Briefly, Boc is used for the protection of the alpha-amino groups. Tos is used to protect the guanidino group of Arg. DCB is used as the protecting group for the phenolic hydroxyl group of Tyr, and the OH group of Ser is protected with Bzl. Two to three-fold excess of protected amino acid is used based on the NH_2-content of the benzhydryl-amine-resin, plus one equivalent of DIC in CH_2Cl_2 or 10–50% DMF/CH_2Cl_2, depending on the solubility of Boc-amino acid, for two hours.

N-Terminal acetylation is performed with a 50-fold excess of acetic anhydride in CH_2Cl_2 for 0.5 hours. The protected intermediate peptide thus obtained has the following composition:

Ac—D—Nal(2)—D—Phe(4—Cl)—D—Trp—Ser(X^4)—Tyr—(X^5)D—Hci—Leu—Arg(X^8)—Pro—D—Ala—NH—X^{10}

wherein
X^4 is Bzl and
X^5 is DCB,
X^8 is Tos, and X^{10} is a benzhydryl group incorporated into the resin.

In order to cleave and deprotect the protected peptide-resin, it is treated with 1.4 ml. m-cresole and 15 ml. hydrogen fluoride per gram of peptide-resin for 0.5 hours at 0° and 0.5 hours at room temperature. After elimination of hydrogen fluoride under high vacuum, the resin-peptide is washed with diethyl ether and the peptide is then extracted with DMF and separated from the resin by filtration. The DMF solution is concentrated to a small volume under high vacuum, then triturated with diethyl ether. The crude product thus obtained is purified by preparative HPLC as described below to yield the desired D-Hci-containing peptide. The peptide is judged to be substantially (95%) pure by using HPLC. HPLC analyses are carried out in a Hewlett-Packard 1090A gradient liquid chromatographic system on a "PHENOMENEX" (W-Pure: 5C18) column, eluted with solvents A: 0.1% TFA, B: 0.1% TFA in 70% CH_3CN with a gradient of 35–75% in 30 minutes. The desired peptide has retention time of 22.9 minutes.

Purification of peptides is carried out on a Beckman Prep-350 gradient liquid chromatograph using a 41.4×250 mm preparative reversed phase DYNAMAX C18 cartridge (300A, 12 um) with solvents A: 0.1% TFA and B: 0.1% TFA in 70% CH_3CN and using a gradient of 45–60% in 30 minutes. The pure peptide obtained as TFA salt, if desired, can be converted to the acetate form by passage through an AG3X (Bio-Rad) column in the acetate form followed by lyophilization.

EXAMPLE XX

The synthesis of the peptide H_2N-CO-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-

NH_2 is conducted as described in Example XIX, with the exception that H_2N-CO-D-Nal(2) is incorporated in place of Boc-D-Nal(2) in position 1, and the N-terminal acetylation is omitted to yield the desired peptide with retention time of 24.0 min.

EXAMPLE XXI

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example XIX, with the exception that Boc-D-Cit is incorporated in place of Boc-D-Hci in position 6 to give the desired peptide with a retention time of 22.5 min.

EXAMPLE XXII

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example XIX with the exception that BOC-D-Phe(4-Cl) is incorporated in place of Boc-D-Nal(2) in position 1 to give the desired peptide with a retention time of 24.0 min.

EXAMPLE XXIII

The synthesis of the peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example XIX, with the exception that Boc-D-Phe(4-Cl) is incorporated in place of Boc-D-Nal(2) in position 1 and that Boc-D-Cit is incorporated in place of Boc-D-Hci in position 6 to yield the desired peptide having a HPLC retention time of 20.8 min.

EXAMPLE XXIV

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-Gly-NH_2 is conducted as described in Example XIX, with the exception that Bcc-Gly is incorporated in place of Boc-D-Ala in position 10. The desired peptide thus obtained has a HPLC retention time of 22.4 min.

EXAMPLE XXV

The synthesis of the peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example XIX with the exception that Boc-D-Pal(3) is incorporated in place of Boc-D-Trp in position 3. The desired peptide has an HPLC retention time of 13.6 min.

EXAMPLE XXVI

The synthesis of the peptide Ac-D-Nal-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-AlaNH$_2$ is conducted as described in Example XIX, with the exception that Boc-D-Cit is incorporated in place of D-Hci in position 6 and that Boc-D-Pal(3) is incorporated in place of Boc-D-Trp in position 3. The desired peptide has an HPLC retention time of 13.3 min.

EXAMPLE XXVII

The synthesis of the peptide H N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example XIX, with the exception that Boc-D-Cit is incorporated in place of Boc-D-Hci in position 6, that Boc-D-Pal(3) is incorporated in place of Boc-D-Trp in position 3, and that N-terminal acetylation is omitted to yield the intermediate peptide H-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2. The free peptide thus obtained is then reacted with potassium cyanate in 80% aqueous DMF (81 mg. KOCN/300 mg. peptide/ml.) at ambient temperature for 24 hours. The reaction mixture, after evaporation under high vacuum, is subjected to purification by preparative HPLC to yield the desired peptide having HPLC retention time of 14.4 min.

EXAMPLE XXVIII

The synthesis of the peptide H N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is conducted as described in Example XIX, with the exception that Boc-D-Pal(3) is incorporated in place of Boc-D-Trp in position 3 and that N-terminal acetylation is omitted to yield the intermediate peptide H-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2. The free peptide thus obtained is then reacted with potassium cyanate in aqueous DMF (81 mg. KOCN/300 mg. peptide/ml.) at ambient temperature for 24 hours. The reaction mixture, after evaporation under high vacuum, is subjected to purification by preparative HPLC to give the desired peptide having a HPLC retention time of 14.7 min.

EXAMPLE XXIX

The synthesis of the peptide H N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2 is commenced with the preparation of intermediate peptide H-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2. The synthesis of the intermediate peptide is accomplished as described in Example XIX, with the exception that Boc-D-Orn(Z) is incorporated in place of Boc-D-Hci in position 6 and that N-terminal acetylation is omitted The free D-Orn^6-containing peptide is then reacted with potassium cyanate in 80% aqueous DMF (162 mg. KPCN/300 mg. peptide/ml.) at ambient temperature for 24 hours. The reaction mixture, after evaporation under high vacuum, is subjected to purification by preparative HPLC to yield the desired peptide with a HPLC retention time of 23.6 min.

EXAMPLE XXX

The synthesis of the peptide H_2N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH_2 is commenced with the preparation of intermediate peptide H-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2. The synthesis of the intermediate peptide is accomplished as described in Example XIX, with the exception that Boc-D-Lys[Z(2-Cl)] is incorporated in place of Boc-D-Hci in position 6 and that N-terminal acetylation is omitted. The free D-Lys^6-containing peptide is then reacted with potassium cyanate in 80% aqueous DMF *164 mg. KOCN/300 mg. peptide/ml.) at ambient temperature for 24 hours. The reaction mixture, after evaporation under high vacuum, is subjected to purification by preparative HPLC to yield the desired peptide with a HPLC retention time of 24.0 min.

EXAMPLE XXXI

Tablet formulation for buccal (e.g., sublingual) administration:

1. LHRH Antagonist 10.0 mg. Compressible Sugar, USP 86.0 mg. Calcium Stearate 4.0 mg.
2. LHRH Antagonist 10.0 mg. Compressible Sugar, USP 88.5 mg. Magnesium Stearate 1 5 mg.
3. LHRH Antagonist 5.0 mg. Mannitol, USP 83.5 mg. Magnesium Starch, USP 1.5 mg.

4. LHRH Antagonist 10.0 mg. Pregelatinized Starch, USP 10.0 mg. Lactose, USP 74.5 mg. Pregelatinized Starch, USP 15.0 mg. Magnesium Stearate, USP 1.5 mg.

Method A. LHRH Antagonist is dissolved in a sufficient quantity of water to form a wet granulation when mixed with the sugar portion of the excipients. After complete mixing the granulation is dried in a tray of fluid-bed dryer. The dry granulation is then screened to break up any large aggregates and then mixed with the remaining components. The granulation is then compressed on a standard tableting machine to the specific tablet weight.

Method B. In this manufacturing method, all formulations would include 0.01% gelatin, USP. The gelatin would be first dissolved in the aqueous granulation solvent followed by the LHRH analog. The remaining steps are as in (a) above.

EXAMPLE XXXII

Long Acting Intramuscular Injectable Formulation

Long Acting iM. Injectable—Sesame Oil Gel LHRH Antagonist 10.0 mg. Aluminum Monostearate, USP 20.0 mg. Sesame oil g.s. ad 1.0 ml.

The aluminum monostearate is combined with the sesame oil and heated to 125° C. with stirring until a clear yellow solution forms. This mixture is then autoclaved for sterility and allowed to cool. The LHRH antagonist is then added aseptically with trituration. Particularly preferred LHRH antagonists are salts of low solubility, e.g., zinc salts, zinc tannate salts, pamoate salts, and the like. These exhibit exceptionally long duration of activity.

EXAMPLE XXXIII

Long Acting IM Injectable—Biodegradable Polymer Microcapsules

LHRH Antagonists 1% 25/75 glycolide/lactide copolymer (0.5 intrinsic viscosity) 99%

Microcapsules (0°-150°) of above formulation suspended in:

Dextrose 5.0% CMC, sodium 0.5% Benzyl alcohol 0.9% Tween 80 0.1% Water, purified q.s. 100.0% 25 mg. of microcapsules are suspended in 1.0 ml. of vehicle.

EXAMPLE XXXIV

Aqueous Solution for Intramuscular Injection

LHRH Antagonist 500 mg. Gelatin, nonantigenic 5 mg. Water for injection g.s. ad 100 ml.

The gelatin and LHRH antagonist are dissolved in water for injection, then the solution is sterile filtered.

EXAMPLE XXXV

Formulation for Rectal Administration

Suppository Vehicle for Rectal Administration

LHRH Antagonist 5 0 mg. Witepsol H15 20.0 mg.

The LHRH antagonist is combined with the molten Witepsol H15, mixed with and poured into 2 gm. molds.

We claim:

1. A peptide having the formula:

$$X—R^1—R^2—R^3—Ser—Tyr—R^6—Leu—Arg—Pro—R^{10}—NH_2$$

wherein

X is acetyl,

R^1 is D-Nal(2),

R^2 is D-Phe(4Cl),

R^3 is D-Trp or D-Pal(3),

R^6 is D-Cit or D-Hci, and

R^{10} is D-Ala

and the pharmaceutically acceptable acid addition salts thereof.

2. A peptide of claim 1 wherein R^3 is D-Pal(3).

* * * * *

87229 PH

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,198,533
DATED : March 30, 1993
INVENTOR(S) : Andrew V. Schally, et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page, item [63], line 2, delete "abandoned", insert in place thereof -- now U. S. Patent 4,800,191 --.

Signed and Sealed this

First Day of September, 1998

Attest:

Attesting Officer

BRUCE LEHMAN

Commissioner of Patents and Trademarks

by akution of Brian Green
Regulatory Affairs Department
001978 851 7346 5
15/10/1999
enclosed the documents which I have announced in my e-mail
kind regards Achim

SHAL 3.0-004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Andrew V. Schally, et al.
Serial No.: 197,153
Patent No: 5,198,533
Granted: March 30, 1993
For: LHRH ANTAGONISTS

Edison, N. J. 08837
October 13, 1999

PETITION FOR ISSUANCE OF A CERTIFICATE OF CORRECTION

Hon. Commissioner of Patents and Trademarks
Washington, D. C. 20231

Sir:

The above-identified patent contains a Terminal Disclaimer over the original expiry term of its parent application U. S. patent 4,800,191.

In view of the change of the law allowing, for applications filed prior June 8, 1995 the choice of 17 year term from issuance, or a 20 year term from application, said patent 4,800,191 now has an expiration date of July 17, 2007.

It will be noted that the present application is a continuation-in-part of the parent and therefore, also has entitlement to a 20 year term from the original date of filing. Since the Terminal Disclaimer date as originally filed (on July 17, 1992) does not contain a date for expiry, but rather relates to the expiry date of the patent granted on the parent application, it is respectfully submitted that the above identified patent in fact, will expire on July 7th 2007. It is therefore requested that a Certificate of Correction noting this new expiry date be issued. x)

The appropriate fee of $130.00 is enclosed herewith.

Applicant notes that the previous Certificate of Correction requested on July 15, 1998 and received by the Patent Office on July 20, 1998 has not yet been acted upon. A follow-up on this matter is respectfully requested.

Respectfully submitted,

Tele: 732-494-5240
c:\wp51\shal\04c1.pet

Chris M. Bahn
Regis. No. 22,940

x) it must read July 17, 2007
we have already informed our attorney today!

BEST POSSIBLE COPY

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PLEASE REPLY TO EDISON

October 15, 1999

Mr. K. L. Dekker
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Germany

VIA TELEFAX
011-49-351-834-1945

RE: SHAL 3.0-004 - U. S. Patent 5,198,533; Our Ref.: PAT/DE 87229 PH

Dear Mr. Dekker:

With reference to the above-identified matter, please find enclosed herewith a copy of Amendment to Petition for Issuance of a Certificate of Correction.

Very truly yours,

Behr & Adams

By: Omri M. Behr /cg

Omri M. Behr

OMB/cg
encls.

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SHAL 3.0-004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Andrew V. Schally, et al.
Serial No.: 197,153
Patent No: 5,198,533
Granted: March 30, 1993
For: LHRH ANTAGONISTS

Edison, N. J. 08837
October 15, 1999

AMENDMENT TO
PETITION FOR ISSUANCE OF A CERTIFICATE OF CORRECTION

Hon. Commissioner of Patents and Trademarks
Washington, D. C. 20231

Sir:

On October 13, 1999, Applicant submitted a Petition for Issuance of a Certificate of Correction with respect to true expiry date. While the document for the Certificate of Correction bears the correct expiry date of July 17th, it was noted that by oversight the date requested on the actual Petition was given as July 7, 2007 rather than the correct date of July 17, 2007.

Please consider this as a request to allocate the correct expiry date.

Respectfully submitted,

Tele: 732-494-5240
c:\wp51\shal\04c2.pet

Daryl M. Behr
Reg. No. 22,940

EINGEGANGEN

14 Okt 1999

Patente

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PATENT AND
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MORRISTOWN OFFICE
10 PARK PLACE
MORRISTOWN, N.J. 07960
(973) 539-1110

ASSOCIATE OFFICE
WEISER & STAMLER
230 S. 15TH STREET
PHILADELPHIA, PA 19102
(215) 875-4541

PLEASE REPLY TO EDISON

October 13, 1999

Mr. K. L. Dekker
ASTA Medica AG
Patent Dept.
Radebeul
Meissner Str. 35
D-01445
Germany

VIA TELEFAX
011-49-351-834-1945

RE: SHAL 3.0-003 - U. S. Patent 4,800,191
SHAL 3.0-004 - U. S. Patent 5,198,533 - Your Ref.: PAT/DE 87229 PH

Dear Mr. Dekker:

Further to my telephone message to you, I will confirm that your view of the expiry date of the earlier patent is correct, that is to say, July 17, 2007. With respect to respect to U. S. patent 5,198,533, this patent has the right to a later expiration date, but does not receive it automatically. It is necessary to file for a Petition of Correction which, according to the official in that department to whom I spoke this morning, will automatically be granted.

As I soon as I have finished this letter to you, I will prepare and file such a Petition.

Very truly yours,

Behr & Adams

By: _____
Omri M. Behr

OMB/cg
c:\wp51\shal\03c5.fax

EXCLUSIVITY SUMMARY for NDA # 21-197 SUPPL # ____

Trade Name Cetrotide™

Generic Name cetrorelix acetate for injection

Applicant Name ASTA Medica HFD-580

Approval Date August 11, 2000

PART I: IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete Parts II and III of this Exclusivity Summary only if you answer "YES" to one or more of the following questions about the submission.

   a) Is it an original NDA? YES/_X__/ NO /___/

   b) Is it an effectiveness supplement? YES /___/ NO /_X__/

      If yes, what type(SE1, SE2, etc.)? _____

   c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "NO.")

      YES /X___/ NO /___/

      If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

      _____

      _____

      If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

      _____

d) Did the applicant request exclusivity?

YES /_X__/ NO /___/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

5 Years

e) Has pediatric exclusivity been granted for this Active Moiety?

YES /___/ NO /X___/

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule previously been approved by FDA for the same use? (Rx to OTC Switches should be answered No - Please indicate as such).

YES /___/ NO /_X__/

If yes, NDA # _____ Drug Name _____

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9.

3. Is this drug product or indication a DESI upgrade?

YES /___/ NO /X___/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9 (even if a study was required for the upgrade).

PART II: FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES
(Answer either #1 or #2, as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /___/ NO /X___/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____ _____

NDA # _____ _____

NDA # _____ _____

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /___/ NO /___/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____ _____

NDA # _____ _____

NDA # _____ _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9. IF "YES," GO TO PART III.

PART III: <u>THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS</u>

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2, was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis

for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

For the purposes of this section, studies comparing two products with the same ingredient(s) are considered to be bioavailability studies.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval **AND GO DIRECTLY TO SIGNATURE BLOCK ON Page 9:**

_____

_____

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO.

YES /___/ NO /___/

If yes, explain: _____

_____

NDA # _____ Study # _____
NDA # _____ Study # _____
NDA # _____ Study # _____

(b) For each investigation identified as "essential to the approval," does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

Investigation #3 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify the NDA in which a similar investigation was relied on:

NDA # _____ Study # _____

NDA # _____ Study # _____

NDA # _____ Study # _____

(c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

Investigation #__, Study # _____

Investigation #__, Study # _____

Investigation #__, Study # _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

(a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 157.1 as the sponsor?

Investigation #1

IND # _____ YES /___/ NO /___/ Explain: _____

_____

_____

Investigation #2

IND # _____ YES /___/ NO /___/ Explain: _____

_____

_____

(b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1

YES /___/ Explain _____ NO /___/ Explain _____

_____ _____

_____ _____

Investigation #2

YES /___/ Explain _____ NO /___/ Explain _____

_____ _____

_____ _____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES /___/ NO /___/

If yes, explain: _____

_____

_____

/S/ MSW MD
Signature of Preparer
Title: Regulatory Project Manager

8/11/00
Date

/S/ MMS
Signature of Office of Division Director

7/27/00
Date

cc:
Archival NDA
HFD- /Division File
HFD- /RPM
HFD-093/Mary Ann Holovac
HFD-104/PEDS/T.Crescenzi

Form OGD-011347
Revised 8/7/95; edited 8/8/95; revised 8/25/98, edited 3/6/00

ASTA MEDICA

ASTA Medica, Inc.

CETRORELIX ACETATE FOR INJECTION

Title: **Claimed Exclusivity**

In accordance with 314.108(b)(4), ASTA Medica, Inc. is claiming an exclusivity period of five years for CETROTIDE™ (cetrorelix acetate for injection).

This NDA is for 2 formulations (0.25 mg and 3 mg) for subcutaneous injection, to be used for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, followed by oocyte pick-up and assisted reproductive techniques.

The drug product, CETROTIDE™ 0.25 mg / 3 mg, containing the same active ingredient with the same conditions of approval, has not been previously approved.

A clinical development program for cetrorelix acetate was conducted by our parent company, ASTA Medica AG in Europe. Three of these studies (3010, 3020, and 3030) are adequate and well controlled studies which are essential to the approval of cetrorelix acetate for the prevention of premature ovulation in patients undergoing a controlled ovarian stimulation, followed by oocyte pick-up and assisted reproductive techniques.

The other clinical studies included in this NDA were sponsored, conducted and funded by our parent company ASTA Medica AG. A list of the clinical investigations other than bioavailability or bioequivalence studies, conducted by ASTA Medica AG, together with the location of the study in the Clinical Data Section of the NDA, is attached.

To the best of our knowledge, published studies are not sufficient to form the basis of a finding of substantial evidence of effectiveness for CETROTIDE™. There are no publications of studies which were not sponsored by ASTA Medica that meet FDA's definition of adequate and well-controlled studies which could be used to document the efficacy and safety of CETROTIDE.

Based on these facts, we conclude that the studies included in this submission are essential for the approval of CETROTIDE. As a result, we are requesting 5 years of exclusivity upon approval of this NDA.

Colin Stewart
President and CEO
ASTA Medica, Inc.

ASTA MEDICA

ASTA Medica, Inc.

CETRORELIX ACETATE FOR INJECTION

Study	Vol in Clinical Data Section
Study No. D-20761/0008: Efficiency of the GnRH-antagonist Cetrorelix (D-20671) in the controlled induction of ovulation for in-vitro-fertilization	Clin: 92 Stat: 133
Study No. D-20761/0009b: Efficacy and Safety of the GnRH-antagonist Cetrorelix (D-20761) in the prevention of spontaneous LH surge during controlled induction of ovulation	Clin: 93 Stat: 134
Study No. D-20761/0012: Suppression of the endogenous LH surge by the GnRH antagonist Cetrorelix in the controlled induction of ovulation	Clin: 94 Stat: 135
Study No. D-20761/IC93005: Efficacy of the GnRH antagonist Cetrorelix, 3mg, in the prevention of the spontaneous LH peak in the controlled induction of ovulation	Clin: 95-96 Stat: 136-137
Study No. D-20761/2986: Assessment of the minimal effective single dose of Cetrorelix for the prevention of spontaneous LH surge during controlled ovarian hyperstimulation prior to in vitro fertilization with embryo transfer	Clin: 97-98 Stat: 138-139
Study No. D-20761/2997: Dose-finding study to assess the efficacy of multiple doses of the GnRH-antagonist Cetrorelix to prevent premature LH surges in patients undergoing controlled ovarian superovulation (COS) for assisted reproduction techniques (ART)	Clin: 99-100 Stat: 140-141
Study No. D-20761/3097: Efficacy and safety of Cetrorelix (3 mg single dose s.c.) in the prevention of premature ovulation in patients undergoing COS/ART with two different stimulation procedures (recFSH/HMG)	Clin: 101-102 Stat: 142-143
Study No. D-20761/3010: Investigation into efficacy and safety of the LHRH-antagonist Cetrorelix and of the LHRH-agonist buserelin in patients to undergo controlled ovarian superovulation for assisted reproduction techniques (COS/ART)	Clin: 103-106 Stat: 144-147
Study No. D-20761/3030: Investigation into the efficacy and safety of the LHRH-antagonist Cetrorelix and of the LHRH-agonist triptorelin in patients to undergo controlled ovarian superovulation for assisted reproduction techniques (COS/ART)	Clin: 107-109 Stat: 148-150
Study No. D-20761/3020: Investigation into the efficacy and safety of the LHRH-antagonist Cetrorelix in patients to undergo controlled ovarian superovulation for assisted reproduction techniques (COS/ART)	Clin: 110-113 Stat: 151-154

ASTA MEDICA

ASTA Medica, Inc.

CETRORELIX ACETATE FOR INJECTION

Title: **Debarment Certification**

List of Convictions

The following is a list of all convictions, described in sections 306(a) and 306(b) of the Federal Food, Drug, and Cosmetic Act which occurred within the previous 5 years, of the applicant and affiliated persons responsible for the development or submission of this New Drug Application for CETROTIDE™ (cetrorelix acetate for injection).

Name	Offense	Date
None		

Debarment Certification

ASTA Medica Inc. hereby certifies that it did not and will not use in any capacity the services of any person debarred under section 306 of the Federal Food, Drug, and Cosmetic Act in connection with this New Drug Application for CETROTIDE™ (cetrorelix acetate for injection).

Colin Stewart
President and CEO
ASTA Medica, Inc.

APPEARS THIS WAY
ON ORIGINAL

ASTA MEDICA

NDA 21-197
Cetrotide™ (cetrorelix acetate for injection) 0.25 mg and 3 mg

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

August 11, 2000

ORIGINAL

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation III, CDER, FDA
Parklawn Building, Room 17B-45
5600 Fishers Lane
Rockville, MD 20857

DRUG AMENDMENT

BL

Re: Final Draft Package Insert

Dear Dr. Allen:

Reference is made to our New Drug Application for Cetrotide™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the inhibition of premature LH surges in patients undergoing controlled ovarian stimulation, identified as NDA 21-197. In addition, reference is made to an August 10, 2000 e-mail from the Agency containing labeling comments and discussions with the Division on August 10 and 11.

Enclosed please find the final version of the draft package insert, which was submitted via fax and e-mail to the Division earlier today. All of the Agency's requested changes have been incoporated. An electronic copy of this document is included on 3.5" floppy diskettes.

If you have any questions, please contact me at (978) 858-2553, or Roberta Tucker, RPh. Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED

CSO ACTION

☑ LETTER ☐ NOA ☐ MEMO

Approved 8/11/00

CSO

FAX

ASTA MEDICA

ASTA Medica, Inc.

ORIGINAL

FROM

Date: August 10, 2000

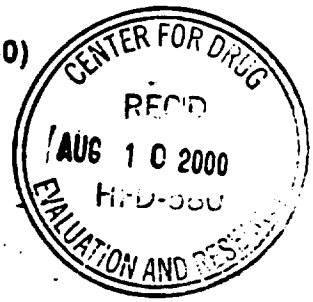
Brian A. Green
Phone: (978) 858-2553 Fax: (978) 851-5917
e-mail: bgreen@astamedica-usa.com

TO

Ms. Jeanine Best, Project Manager
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation II, CDER, FDA
Fax Number: (301) 827-4267

ORIG AMENDMENT

No of Pages: 3



RE: Copy of Table 2 from draft package insert

Dear Ms. Best:

Reference is made to our October 28, 1999 submission of a New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, for the inhibition of premature LH surges in patients undergoing controlled ovarian stimulation, which is identified as NDA 21-197.

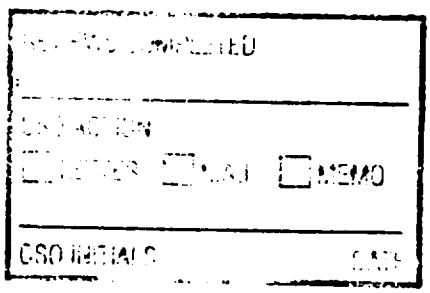
Attached please find a copy of a proposal for Table 2 in the package insert text. Also included is the version of table 2 included in the July 18, 2000 version of the PI, which was just prior to the request to delete the study numbers and to merge the results of the Cetrotide 0.25 mg studies.

I hope this information is useful. If you have any questions or comments, please feel free to contact me.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs



FAX

ORIGINAL

ASTA MEDICA

ASTA Medica, Inc.

FROM

Date: August 10, 2000

Brian A. Green
Phone: (978) 858-2553 Fax: (978) 851-5917
e-mail: bgreen@astamedica-usa.com

TO

Ms. Jeanine Best, Project Manager
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation II, CDER, FDA
Fax Number: (301) 827-4267

ORIG AMENDMENT

No of Pages: 3

RE: Copy of Table 17.3 from Study 3030

Dear Ms. Best:

Reference is made to our October 28, 1999 submission of a New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, for the inhibition of premature LH surges in patients undergoing controlled ovarian stimulation, which is identified as NDA 21-197.

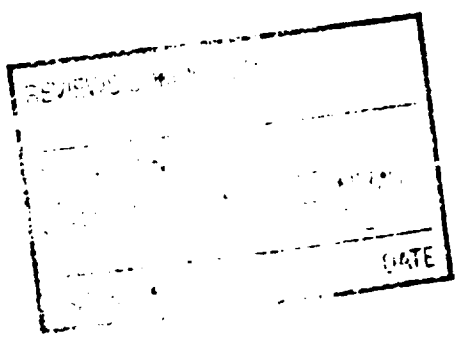
Attached please find a copy of Table 17.3 from the clinical trial report for Study 3030, which shows the frequency distribution of the first Cetrotide administration by HMG day. As this table shows, 7.0% of the initial Cetrotide administration occurred on or after HMG day 10, while 93.0% of the initial Cetrotide administration occurred on or before HMG day 9. This table was included in the original NDA in Volume 107, Page 124. I have included it in actual size and enlarged for easy reading.

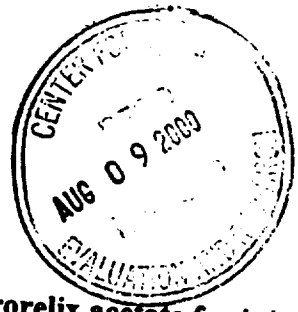
I hope this information is useful. If you have any questions or comments, please feel free to contact me.

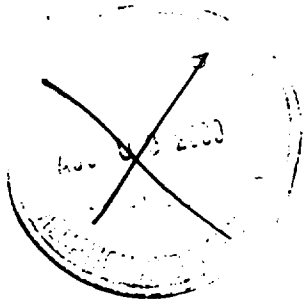
Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs







NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

August 8, 2000

ORIGINAL

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation III, CDER, FDA
Parklawn Building, Room 17B-45
5600 Fishers Lane
Rockville, MD 20857

ORIG AMENDMENT

Re: Revised Draft Package Insert

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the inhibition of premature LH surges in patients undergoing controlled ovarian stimulation, identified as NDA 21-197. In addition, reference is made to an August 4, 2000 e-mail and an August 8, 2000 fax from the Division containing labeling comments.

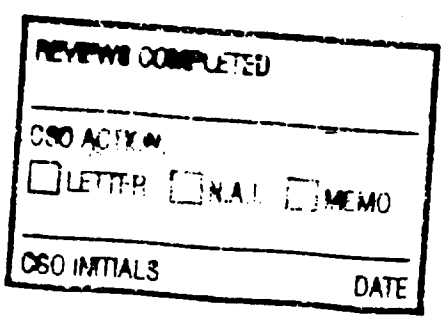
Enclosed please find a revised package insert. These documents were submitted via e-mail to the Division earlier today. The Division's changes were made in our draft, then subsequently marked up with our proposals. Deletions are noted with strike-out and additions are underlined. A document containing a list of the minor revisions and a justification for the deletion of E_2 measurements as a determination of the timing of Cetrotide™ 3 mg is included.

If you have any questions, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs



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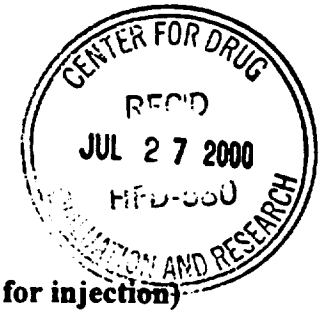


ASTA MEDICA

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

July 26, 2000

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation III, CDER, FDA
Parklawn Building, Room 17B-45
5600 Fishers Lane
Rockville, MD 20857

ORIG AMENDMENT

Re: Addendum to our July 25th Response to July 21, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the inhibition of premature LH surges in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a June 21, 2000 telephone conversation between myself and Ms. Freshnie Deguia from your Division, during which Ms. Deguia relayed a request from the Medical Officer to provide additional information concerning the fetal deaths for Patient 219 from Center 1 in Study 2997, Patient 2 from Center 26 in Study 3010 and Patient 13 from Center 8 in Study 3020.

On July 25, 2000, ASTA Medica provided a response to this request for information. In our response, we indicated that we had contacted the investigator from Center 8 in Study 3020 to obtain additional information for Patient 13. The investigator's reply was not available at the time of our response; enclosed please find a copy of the response from the investigator.

If you have any questions or require any additional information, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

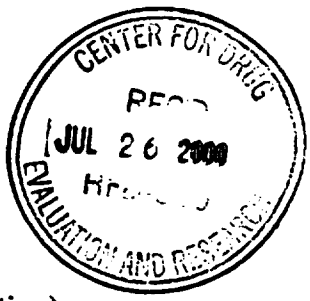
REVIEWS COMPLETED

CSO ACTION

☐ LETTER ☐ N.A.I. ☐ MEMO

CSO INITIALS _ DATE

ORIGINAL



ASTA MEDICA

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

July 25, 2000

ORIG AMENDMENT

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation III, CDER, FDA
Parklawn Building, Room 17B-45
5600 Fishers Lane
Rockville, MD 20857

Re: Response to July 21, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the inhibition of premature LH surges in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

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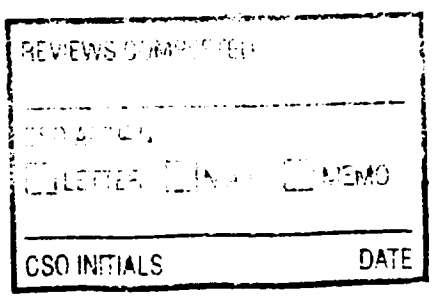
Enclosed please our response to this request for information. If you have any questions or require any additional information, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs



ORIGINAL

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

July 21, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496
Telephone 978.851.6981
Telefax 978.850.7346

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation III, CDER, FDA
Parklawn Building, Room 17B-45
5600 Fishers Lane
Rockville, MD 20857

ORIG AMENDMENT
B111

Re: Response to July 20, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a June 20, 2000 telephone conversation between myself and Ms. Freshnie Deguia from your Division, during which Ms. Deguia relayed a request from the Medical Officer to provide the Case Report Forms for Patients 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 22, 23, and 24 from Center 6 in Study 3020. In addition, the CRF for Patient 9 from Study 0008 was requested.

Enclosed please find copies of the requested CRF's as well as an explanation of the increased incidence of OHSS from Center 6 in Study 3020 and a brief case description of Patient 9 from Study 0008. If you have any questions or require any additional information, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED
CSO INITIALS
☐ LETTER ☐ N.A.I. ☐ MEMO
CSO INITIALS DATE

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

July 17, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT

BL

Re: Mock-ups of labeling

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature LH surges in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

Enclosed please find mock-ups of the vial labels, prefilled syringe labels, labels for the tray lids, and carton labels.

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

APPEARS THIS WAY
ON ORIGINAL

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

July 14, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT
BL 1

CENTER FOR DRUG EVALUATION AND RESEARCH
REC'D
JUL 17 2000
HFD-580

Re: ASTA Medica's Revised Draft Package Insert

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature LH surges in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a July 11, 2000 fax from the Division containing labeling comments. Enclosed please find a revised package insert with some minor revisions to the Division's draft, as discussed with Ms. Jeanine Best on July 13 and 14. The Division's changes were made in our draft, then subsequently marked up with our proposals. Deletions are noted with strike-out and additions are underlined. A document containing a list of the minor revisions is included. All files are being provided in MS Word on 3.5" diskettes.

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED

CSO ACTION:
☐ LETTER ☐ N.A.I. ☐ MEMO

CSO INITIALS DATE

FAX

ASTA MEDICA

ASTA Medica, Inc.

FROM

Brian A. Green
Phone: (978) 858-2553
e-mail: bgreen@astamedica-usa.com
Fax: (978) 851-5917

Date: July 11, 2000

TO

Ms. Jeanine Best, Project Manager
Division of Reproductive and Urologic Drug Products (HFD-580)
Office of Drug Evaluation II, CDER, FDA
Fax Number: (301) 827-4267

No of Pages: 4

RE: Copy of USAN Adoption Statement; Amendment of CAS number in NDA

Dear Ms. Best:

Reference is made to our October 28, 1999 submission of a New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, which is identified as NDA 21-197.

Attached please find a copy of a letter from the USAN Council informing ASTA Medica Inc. that ***cetrorelix acetate*** has been adopted as the USAN for CETROTIDE™. Please note that the unresolved issue in the second paragraph (concerning the inconsistency between the CAS number and the amount of acetate) has been resolved. The amount of acetate is unspecified; therefore, the appropriate CAS number (145672-81-7) will be listed. In addition, other minor modifications will be made to the adoption statement.

In Volume 1 (Page 358) and Volume 2 (Page 029), we provided the following CAS number: 130143-01-0. This CAS number is for the diacetate salt of cetrorelix. We would like to amend the NDA at this time to provide the correct CAS number for cetrorelix acetate, which is 145672-81-7.

I will submit a hardcopy of this information.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

ORIGINAL

ASTA MEDICA

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

June 30, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT

BP

Re: Final Preclinical MTD Finding Study Reports

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

Reference is also made to our submission of additional preclinical data on May 18, 2000. In that submission, we provided 11 preclinical study reports. In addition, we stated that reports for the additional four Maximum Tolerated Dose (MTD) finding studies (designated **Studies 7-10** in the May 18 submission) would be submitted in June.

Enclosed please find the four preclinical trial reports for the above-mentioned MTD finding studies and five related toxicokinetic reports for these studies. Please note that a summary of these studies was included in the May 18 submission on pages ii-iii. We have now submitted reports for all studies outlined in the May 18 submission.

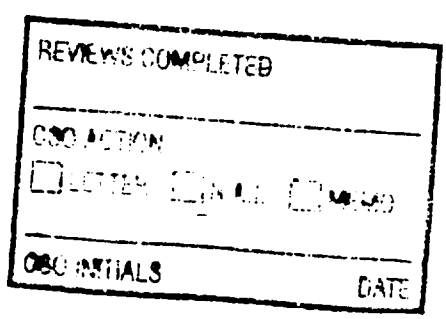
The results of the above referenced studies do not negatively impact the safety profile of CETROTIDE™ for the claimed indication and no revisions to the proposed package insert based on these studies is required.

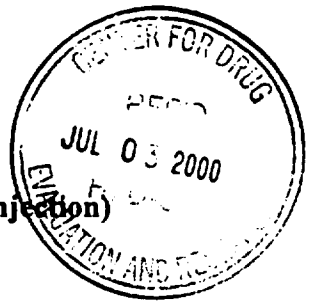
If you have any questions or require any additional information, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs





ASTA MEDICA

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

June 30, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
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Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIGINAL

Re: ASTA Medica's Revised Draft Package Insert

BL

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

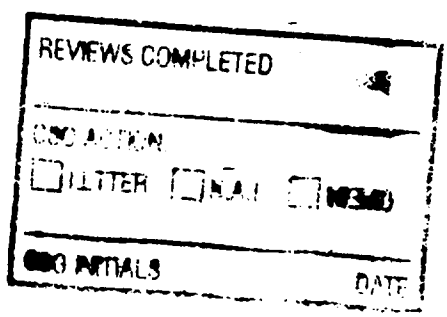
In addition, reference is made to a June 21, 2000 fax from the Division containing labeling comments. Enclosed please find a revised package insert with our proposed changes to the Division's draft. The Division's changes were made in our draft, then subsequently marked up with our proposals. Deletions are noted with strike-out and additions are underlined. A document containing a list of our proposed changes with justifications is included. All files are being provided in MS Word format 3.5" diskettes.

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs



ORIGINAL

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

June 29, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
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Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT

Re: Response to June 2, 2000 Request for Information

Dear Dr. Allen: BM

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a June 6, 2000 telephone conversation between myself and Ms. Jeanine Best from your Division, during which Ms. Best relayed a request from the Medical Officer to provide additional documentation to support the statement that increases in LFT and BUN are due to "... the effects and general impact of COS/ART procedures". Enclosed please find our response to this request for information.

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

ORIGINAL

ASTA MEDICA

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

June 29, 2000

Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT
JUN 30 2000
EVALUATION AND RESEARCH

BC

Re: Response to May 2, 2000 Request for Information (CMC); Amendment to Request for Shelf-Life

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a May 2, 2000 telephone conversation between myself and Ms. Jeanine Best from your Division, during which Ms. Best relayed a request from the Chemistry Reviewer to provide information concerning the effect of water having different pH values on the pH of reconstitued Cetrotide™. Enclosed please find an investigation report which shows that, regardless of the pH of the water, the pH of reconstituted Cetrotide™ is identical within each strength, and is within the specification of 4.0-6.0.

Further reference is made to a June 27, 2000 teleconference between ASTA Medica and the Division, during which several options were presented with respect to the expiration dating. We would like to amend our request for expiration dating as follows:

24 months for the 3 mg strength when stored at room temperature: 25 °C (77 °F)
24 months for the 0.25 mg strength when stored refrigerated: 2-8 °C (36-46 °F)

If you have any questions or require any additional information, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED

CSO ACTION

☐ LETTER ☐ N.A.I. ☐ MEMO

CSO INITIALS DATE

ORIGINAL

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

June 28, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT
BB

CENTER FOR DRUG EVALUATION AND RESEARCH
RECD
JUN 29 2000
HFD-580

Re: Response to June 6, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a June 6, 2000 telephone conversation between myself and Ms. Jeanine Best from your Division, during which Ms. Best relayed a request from the Biopharmaceutics Reviewer to perform a formal analysis on the relationship of the PK parameters AUC and C_{max} to demographics, specifically, body weight, age and race. Enclosed please find our response to this request for information.

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

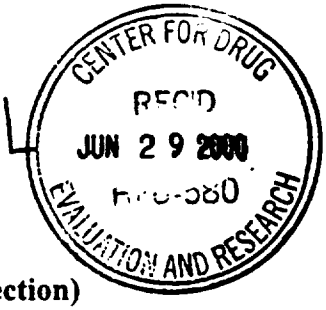
Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED
CSO ACTION
☐ LETTER ☐ N.A.I. ☐ MEMO
CSO INITIALS DATE

ORIGINAL



NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

June 28, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

DRUG AMENDMENT

Re: Response to June 8, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a June 8, 2000 telephone conversation between myself and Ms. Jeanine Best from your Division, during which Ms. Best relayed a request from the Medical Officer to provide additional clarification and information relating to a baby that was born with a "Syndrome de junction pyelocalicielle". In addition, the Medical Officer wanted to know if this syndrome is a hydronephrosis.

Enclosed please find our response to this request for information. If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED

CSO ACTION

☐ LETTER ☐ N.A.I. ☐ MEMO

CSO INITIALS DATE

ORIGINAL

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

June 28, 2000

Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT
B13

CENTER FOR DRUG EVALUATION AND RESEARCH
REC'D
JUN 29 2000
HFD-580

Re: Response to June 20, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a June 6, 2000 telephone conversation between myself and Ms. Jeanine Best from your Division, during which Ms. Best relayed a request from the Biopharmaceutics Reviewer to provide human in-vitro metabolism data and human in-vitro protein binding to human plasma data. This data is included in the original NDA:

Report D-20761/7094270011 - [U-Arg-^{14}C] Cetrorelix acetate salt: *In-vitro* protein binding in human albumin solution and human plasma. **Location: Volume 72, Page 296-309**

Report No. D-20761/FB20197 - Metabolism of cetrorelix in rats, dogs and man after subcutaneous single dose administration. **Location: Volume 72, Page 338-403**

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED
CSO ACTION:
☐ LETTER ☐ N.A.I. ☐ MEMO
CSO INITIALS DATE

ORIGINAL

ASTA MEDICA

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

June 19, 2000

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT

RP

Re: Response to June 15, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

Reference is also made to a telephone conversation on June 15, 2000 with Ms. Jeanine Best from your Division, during which she relayed a request from the Pharm/Tox reviewer to provide pharmacokinetic data for MTD studies 9134987 (in rats) and 915726 (in mice). These orientating MTD studies were submitted to the Division on May 18, 2000, and were designated Study 6 and Study 5 (respectively).

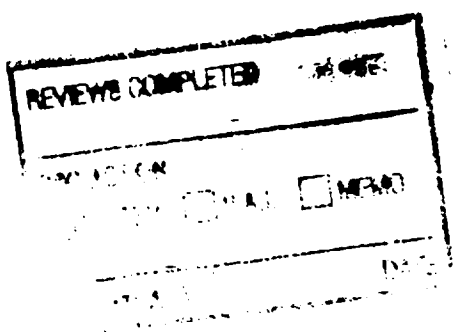
Enclosed please find a copy of the plasma concentration monitoring reports for the above referenced orientating MTD studies.

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

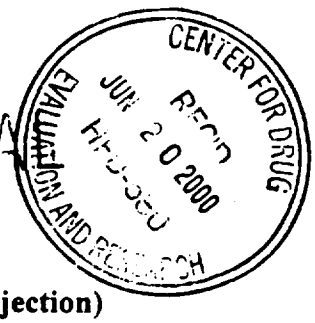
Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs



ORIGINAL



ASTA MEDICA

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

June 19, 2000

Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

CMC AMENDMENT
B I

Re: Response to Discipline Review Letter dated May 2, 2000

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a Discipline Review Letter from the Division dated May 2, 2000, which contained Microbiology comments. Enclosed please find a complete response to the Division's May 2, 2000 letter.

Since this product is manufactured outside the United States, the complete field copy of this submission will be submitted in parallel to Ms. Rochelle Kimmel, Division of Emergency Investigational Operations, as directed by the Boston District Office.

If you have any questions or require any additional information concerning the information in this submission, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs

REVIEWS COMPLETED
CSO ACTION
☐ LETTER ☐ N.A.I. ☐ MEMO
CSO INITIALS DATE

NDA 21-197
CETROTIDE™ (cetrorelix acetate for injection)

June 16, 2000

ORIGINAL

ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876-1496

Telephone 978.851.5981
Telefax 978.851.7346

Susan Allen, MD, Acting Director
Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation III, CDER, FDA
HFD-580
5600 Fishers Lane
Rockville, MD 20852

ORIG AMENDMENT

Re: Response to June 6, 2000 Request for Information

Dear Dr. Allen:

Reference is made to our New Drug Application for CETROTIDE™ (cetrorelix acetate for injection) 0.25 mg and 3 mg, submitted on October 28, 1999, for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation, identified as NDA 21-197.

In addition, reference is made to a June 6, 2000 telephone conversation between myself and Ms. Jeanine Best from your Division, during which Ms. Best relayed a request from the Medical Review Officer to respond to the following:

- Provide clarification of the subjects who make up the 13 WHO Grade II and III OHSS patients listed in Table 3 in labeling; this total number does not correlate to the total number of Grade II and III OHSS subjects listed in Table 11, Page 22 of the ISS.

- In Table 11 on Page 22 of the ISS, Study 2997 is listed as having 6 subjects not classified. However, in the report for Study 2997, Volume 99, Page 28, these patients were all listed as having WHO Grade II or III OHSS, and all 6 were hospitalized. Shouldn't these patients be listed in the Grade II and Grade III categories?

Enclosed please find our response to these questions. If you have any questions or require any additional information, please contact me at (978) 858-2553, or Roberta Tucker, RPh, Director of Regulatory Affairs, at (978) 858-2656.

Sincerely,

Brian A. Green

Brian A. Green
Manager
Regulatory Affairs