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GnRH analogues—agonists and antagonists☆

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Abstract

GnRH analogues have achieved widespread clinical use for the control of reproduction in animals. Over 2000 analogues of GnRH have been developed and tested over the last 30 years. Paradoxical anti-fertility effects are seen when the more potent agonists are delivered continuously to animals. The evaluation of agonist potency depends largely on the model used and wide varying potencies are reported for the same agonist. The design of analogues has centered on improving the receptor-binding and subsequent activation for agonists. Antagonists have been produced with strong receptor binding but without activation. Deslorelin is classified as a superagonist, with a potency perhaps 100 times that of GnRH. The interactions between agonist potency, dose and duration of treatment largely determine whether pro- or anti-fertility effects are induced. Due to the peptide nature of the synthetic analogues oral administration and potential gastrointestinal enzymatic degradation poor bioavailability results necessitating a parenteral delivery system. Some GnRH antagonists have been associated with significant histamine release, inhibiting their widespread use. More recently, antagonists have been developed that avoid this side effect without compromising potency. However the GnRH antagonist development has lagged behind that of the agonists, in part related to their high cost of production. In conclusion, GnRH agonists have achieved widespread clinical use in animals for controlling reproduction in either pro- or anti-fertility roles, yet antagonist development has been slower.

Keywords: GnRH agonist; GnRH antagonist; Deslorelin; GnRH potency; GnRH analogue

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1. Introduction

GnRH is the master hormone controlling mammalian reproductive physiology. Following the elucidation of the amino acid sequence of porcine GnRH (Schally et al., 1971) and the subsequent realisation that the decapeptide sequence was conserved across all mammals, interest then centered on developing modifications to the sequence in the expectation of greater potency and significant pro-fertility effects. The paradoxical anti-fertility effects of these more potent analogues were soon discovered and described. Agonists with the strongest receptor binding and activation along with slower degradation rates yielded the greatest anti-fertility effects. Antagonists with strong receptor-binding properties but without receptor activation became useful in avoiding the initial acute release ('flare effect') of gonadotrophins intrinsically associated with the agonists. Clarification of the biological properties of the GnRH agonists therefore required definition of the interactions between agonist potency, dose and duration of treatment. It is these attributes, which largely determine whether pro- or anti-fertility effects are induced.

2. Historical perspectives

The development of more potent GnRH analogues (both agonist and subsequently antagonist) depended largely on the improvements made in the science of peptide chemistry, notably in the area of peptide synthesis. Technological advances in solid phase peptide synthesis allowed for the automated production of short peptides (Merrifield, 1966). It has been estimated that over 2000 different analogs of GnRH have subsequently been synthesised (Karten and Rivier, 1986). The development has centered on producing agonists with a high affinity for the GnRH receptor and increased resistance to degradation or elimination. These potent 'superagonists' were subsequently discovered to have anti-reproductive effects. The vast amount of information available on GnRH agonist development has already been concisely reviewed (Karten and Rivier, 1986). With the GnRH receptor tertiary structure more recently elucidated, some of the receptor interactions have also been reviewed (Sealfon et al., 1997).

3. Potency evaluation models for GnRH analogues

To assess the biological response of an agonist requires consideration of its receptor affinity, in vivo absorption, distribution, and resistance to degradation and elimination profile. Consequently, the method used to ascertain an agonist's potency should be appreciated when considering comparison with other agonists. Assay systems are usually based on either in vivo or in vitro assessments of the agonist and these models have been reviewed in detail (Karten and Rivier, 1986; Hahn et al., 1984a; Schally et al., 1980).

The features of the commonly used GnRH potency assay systems will be reviewed here with particular reference to assessment of deslorelin where available. Comparison of data from different sources may only be valid if the same assay systems have been utilised precisely (Hahn et al., 1981).

In vivo (Table 1) assays for agonist activity may be based on induction of ovulation in either Nembutal® treated pro-oestrous cycling female rats, diestrous rats, or androgen sterilised female rats. Species variations exist between rats, hamsters and mice across these assays, with diestrous rats being most sensitive (Hahn et al., 1984a). LH release in immature male rats has also been utilised (Schally et al., 1980). Anti-fertility effects can be evaluated by monitoring the rat oestrous cycle via vaginal cytology during twice daily agonist administration. Other parameters, such as the delay in mating and the return to fertility have been evaluated, although reported to be less sensitive than ovulation-induction systems (Hahn et al., 1984a). Interference with established pregnancy can be used with assay systems described in the rabbit, hamster and rat. The mouse appears to be resistant to these effects (Hahn et al., 1984a). Significant effects of injection vehicle have been observed to contribute to the variation seen in response to in vivo assay (Hahn et al., 1981).

In vitro (Table 1) assay systems are commonly based on measuring the amount of LH and FSH released from either cultured female rat pituitary cells or pituitary homogenates (Schally et al., 1980). Standardised culture conditions are essential. GnRH receptor radioligand binding affinity may also be assessed using these tissues, noting that agonist and antagonist properties will not be distinguishable (Hahn et al., 1984a).

Antagonists are commonly evaluated for the minimum dose that is required to inhibit spontaneous ovulation in the rat anti-ovulatory assay (Hahn et al., 1981). Species sensitivity between rabbits, hamsters and rats is evident with this assay. Dispersed cell culture evaluation systems for antagonists consistently result in much greater potencies than intact pituitary methods (Hahn et al., 1981). Short-term (3 day) treatment of immature mice results in a dose-related increase in uterine weight that parallels those obtained for GnRH and has been used to compare potencies (Hahn et al., 1984b).

Potency evaluations for [NEt-des-Gly^{10}]GnRH (GnRH ethylamide) and [D-Ala^{6},NEt-des-Gly^{10}]GnRH or [D-Leu^{6},NEt-des-Gly^{10}]GnRH have been conducted in sheep (Kinder et al., 1976). The approach used peak serum LH and FSH, effectiveness of induction of ovulation and Area Under the Curve (AUC). A similar approach has been used in cattle (Chenault et al., 1990; Nawito et al., 1977). Statistical analyses of the latter study included AUC, $\log_2$(AUC), peak concentration and time to peak concentration (Chenault et al., 1990).

A new gene based assay system for GnRH analogs has recently been described (Beckers et al., 1997). The assay is based upon measuring the intracellular activation of the inositol-phospholipid pathway that culminates in the induction of c-fos genes. Since c-fos transcription peaks at high levels after receptor activation with GnRH, the approach to using a reporter gene based assay was feasible. The benefits of this assay system are that it is very sensitive, simple, and can be performed in a microtire plate. The authors considered that it will facilitate the screening and functional characterisation of GnRH analogs (Beckers et al., 1997).

A novel biological assay system for evaluating the efficacy of synthetic GnRH in the male bovine has been described (Kesler et al., 1999). The assay system was designed to evaluate batches of synthetic GnRH in vivo. In an attempt to minimise variation due to pulsatile testosterone release, bulls were administered norgestomet subcutaneous implants. As the dose of norgestomet was increased, the variability in testosterone decreased in a linear response. The assay system was able to significantly distinguish between 0, 1 and 5 μg; but as the dose of GnRH was increased between 5 and 40 μg, no increase in testosterone release

Table 1
Summary of published studies that use either in vivo or in vitro assay systems to estimate GnRH-agonist potency relative to GnRH.

Assay and reference	Triptorelin	Deslorelin	Buserelin
In vivo			
Mouse uterine wt increase (Hahn et al., 1984b)		55	
Rat oestrous suppression (Nestor et al., 1982)	100	100	
Rat induction of ovulation (Konig et al., 1975)			140
Rat induction of ovulation (Dutta et al., 1978)		15	
Immature male rat LH release (Coy et al., 1976)	13	7	
Bovine LH release Area Under Curve and peak LH (Chenault et al., 1990)			50
Bovine peak LH and Area Under Curve (Nawito et al., 1977)			60
In vitro			
LH release from rat pituitary homogenate (Reeves et al., 1980)			50
LH release from rat pituitary cell culture (Loumaye et al., 1982)			60.2
LH release from rat pituitary cell culture (Fujino et al., 1974)	36 (26–50)	144 (84–198)	
LH release from rat pituitary homogenate (Coy et al., 1975)	100		15
GnRH radioligand binding, whole cells, 37 °C (Loumaye et al., 1982)			56.8
GnRH radioligand binding, whole cells, 0 °C (Loumaye et al., 1982)			22
GnRH radioligand binding, membranes, 0 °C (Perrin et al., 1980)	10 (7–16)	15 (10–26)	
GnRH radioligand binding, membranes, 0 °C (Reeves et al., 1980)			60
GnRH radioligand binding, membranes, 0 °C (Clayton and Catt, 1980)			7.3
GnRH radioligand binding, membranes, 0 °C (Barron et al., 1984)	40	76	

was discernable. They concluded by stating that their assay system was more sensitive on a per kg bodyweight basis than other described biological assay systems. By comparison with the highly sensitive hamster ovulation assay (0.110 μg per kg bodyweight), the norgestomet treated bull assay (0.008 μg per kg bodyweight) was more sensitive when based on dose per bodyweight unit. The main advantage was the rapid turnaround time (5 h versus 2 days; bull versus hamster assay) for potency evaluation, along with the repeated usage of the same animals (Kesler et al., 1999).

The United States Food and Drug Administration (FDA) has produced recommendations for the testing and evaluation of GnRH analogues (Raheja and Jordan, 1994). Interestingly, the recommendations state that it is important to demonstrate that cessation of treatment will result in an eventual return to fertility. Rats are the experimental species mentioned in the paper, and the reversibility of pituitary suppression in this species seems to be high (Vickery and McRae, 1980) when compared to the sheep (Dobson, 1985). Consequently, using rats may underestimate the reversibility of a GnRH analog when it is used in other species.

4. Structural and functional correlates

The primary structure of mammalian GnRH is given in Fig. 1. A fundamental feature of agonists is the substitution of L-isomers with D-isomers. Receptor-binding and activation (agonist) are properties of the NH_2- and COOH-terminal domains; although both are involved in binding. The terminal NH_2-domain residues are predominantly responsible for receptor activation (His^2 and Trp^3, especially). Substitution of residues outside of the NH_2-terminal domain can affect receptor activation, possibly through the altered peptide conformation induced (antagonists) (Sealfon et al., 1997).

It is necessary to appreciate the tertiary structure of GnRH (Fig. 2) when considering the structure-activity relationship of agonists. Modifications to the primary structure of GnRH initially targeted the carboxy-terminus end. Replacement of the carboxy-terminal glycine amide-terminus with alkyl amines produced nonapeptides with greater ovulation-inducing potency [Pro^9-ethylamide (NEt)]-GnRH. The alkylamide-terminus alone did not increase potency; yet the Pro^9-alkylamine moeity produced a prolonged duration of action combined with increased potency (Karten and Rivier, 1986).

Substitution of Gly^6 with a D-Ala residue increased potency 350–400%, while the corresponding L-Ala^6 modification had only 4% the potency of GnRH (Karten and Rivier, 1986). This substitution probably stabilised the β-II-type bend in this position, and bulky hydrophobic side chains were also tolerated. It was postulated that the large side chains might interact with nearby residues in the receptor and further enhance binding (Sealfon et al., 1997).

The overwhelming evidence supports the multiplicative effects of simultaneous substitutions of D-Trp^6 and Pro^9-NEt on analog potency. These aromatic D-amino acid modifi-

$pGlu^1$–His^2–Trp^3–Ser^4–Tyr^5–Gly^6–Leu^7–Arg^8–Pro^9–Gly^{10}–NH_2

Fig. 1. Amino acid sequence of mammalian GnRH.

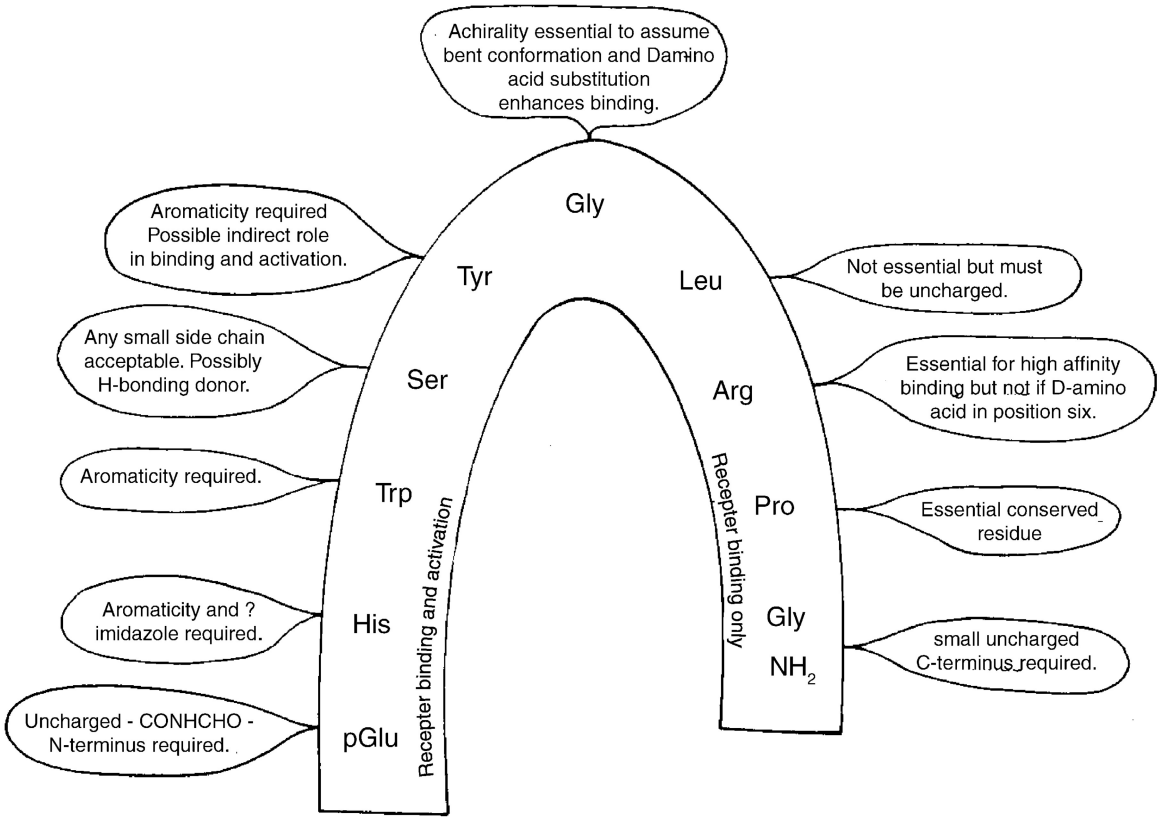

Fig. 2. Schematic drawing of the GnRH peptide indicating the structural and functional properties of individual amino acid residues (Sealfon et al., 1997).

cations produced lesser effects on potency when applied individually (Karten and Rivier, 1986).

Increasing the lipophilicity of amino acid substitutions was suggested to be associated with greater retention of the drug in the body and to prolong the duration of action (Karten and Rivier, 1986). This may be a result of enhanced renal reabsorption or fat storage. Protection is also possible by increasing plasma protein binding. Associated delayed renal clearance appears to be a feature of increasing the hydrophobicity of a series of analogs. These hypotheses regarding hydrophobicity and potency are not always consistent (Karten and Rivier, 1986).

In a series of α-aza-Gly^{10}(–NHNHCO–)-terminal modifications combined with a D^{6}-amino acid, it was found that potency could be increased to 5 times (rat ovulation-induction assay) that above buserelin [D-Ser(bu^{t})^{6},Pro^{9}-NEt]GnRH (Karten and Rivier, 1986). The authors commented that Buserelin had received the most attention of all analogs synthesised up to that point (Karten and Rivier, 1986).

Leu^{7} substitutions with variably sized, uncharged L-amino acids are generally well tolerated, without significant effects on potency (Sealfon et al., 1997). pGlu^{1}, His^{2} and Trp^{3} are considered essential for binding. Substitutions at these positions may be associated with binding but without receptor activation (per antagonist). These properties have been exploited in the development of more potent GnRH antagonists (Karten and Rivier, 1986).

Resistance to brain and pituitary enzyme degradation (proglutamate aminopeptidase, endopeptidase and post-proline cleaving enzyme) is another feature of the superagonists (Flouret et al., 1984). Some confusion exists as to the exact positions in the peptide where cleavage occurs (Karten and Rivier, 1986), although multiple D-amino acids offer substantial protection to peptidases.

5. Deslorelin—a GnRH superagonist

Peptides are often cited in journal publications, but non-uniformity in naming can lead to confusion as to the precise molecular structure. The correct nomenclature for expressing peptides has been briefly reviewed (Grant, 1995). Nomenclature, as recommended by The International Union of Pure and Applied Chemists (IUPAC–IUB, 1985) aims to prevent development of ambiguous peptide names. The prefix *des-A*^{b} refers to the deletion of an amino acid residue (A) at a particular position in the peptide (^{b}). In the case of linear peptides, the convention is to express the peptide left to right from the amino-terminus to the carboxy-terminus; to avoid ambiguity, the state of the termini is often indicated. Amino acids that are changed are placed in brackets [ ] before the name.

When evaluated in the immature rat bioassay, [D-Trp^{6}, Pro^{9}—des-Gly^{10}–NH_2]GnRH-ethylamide (deslorelin) induced release of 7 times more LH than GnRH (Coy and Schally, 1978), yet in this same assay with D-Leu^{6} or D-Ala^{6} in place of D-Trp^{6}, 15 and 17 times as much LH was released respectively. The addition of the Pro^{9}-ethylamide group increases potency by fourfold over the single D-Trp^{6} modification (Karten and Rivier, 1986). It would appear that the in vitro assays for deslorelin yield substantially higher potencies than the biological assays. When the rat oestrous suppression bioassay was used, both [D-Trp^{6}]GnRH and [D-Trp^{6}, Pro^{9}-NEt]GnRH were equipotent. Post-coital rat comparisons also showed equipotency (Karten and Rivier, 1986). In humans, [D-Trp^{6}]GnRH was cleared from plasma 3 times slower than GnRH, taking 18 min to reach half initial plasma concentrations (Barron et al., 1982).

Deslorelin appears to be particularly stable in solution, with an estimated life greater than 20 years when stored at 6 °C. Only minor degradation was noticed after 5 years when stored at higher temperatures (25, 40 and 50 °C) (Wood et al., 1997). Thermal stability is also a feature of GnRH.

The conflicting potency data for (deslorelin) across multiple studies is presented in Table 1. From these studies it may be concluded that the full biological potency of deslorelin lies somewhere between 10 and 144 times that of GnRH.

6. Agonist delivery systems

The interactions between GnRH agonist potency, dose and duration of treatment largely determine whether pro- or anti-fertility effects are induced. The general approaches to controlled peptide delivery have been reviewed (Pitt, 1987). Long-term continuous delivery has been achieved in cattle through the use of mini-osmotic pumps (Gong et al., 1996), biocompatible cholesterol implants (D'Occhio et al., 2002, 1996; Herschler and Vickery,

1981), polymer coated matrices (D'Occhio et al., 1996; McCleod et al., 1991), biodegradable microspheres (Roberts et al., 1989) and continuous intravenous pump infusion (Vizcarra et al., 1997).

7. Route of administration

Early studies of GnRH agonists primarily used parenteral routes of administration (i.v., s.c., i.m.). GnRH agonists are susceptible to gastrointestinal peptidase degradation, making oral administration unsuitable with only 0.1% bioavailability (Conn and Crowley, 1991; Chrisp and Goa, 1990). Intra-nasal administration is relatively inefficient and variable, with only 4–21% being available relative to s.c. or i.v. injection (Chrisp and Goa, 1990; Gudmundsson et al., 1984), necessitating frequent large doses (buserelin, 100–300 μg TID; nafarelin (Synarel®, Searle; 200–500 μg BID); although the intra-nasal route is well tolerated in humans. Vaginal absorption has been investigated in rats and was found to result in 20% bioavailability following combination with organic acids (Okadam et al., 1982). The effect of a 5% citric acid solution on enhancing vaginal absorption of both a dye and leuprolide was significant in a later study (Okada et al., 1983).

8. Future directions for agonist development

The application of GnRH agonists to the treatment of human prostate cancer has proved to be beneficial with prolonged patient survival. Prostate cancer is one of the most common tumours of men and the pharmaceutical therapeutic market is estimated to be in the billions of dollar range. This market along with the control of stimulation programs for human assisted reproduction has led to the commercialisation of many agonists (Table 2).

The stimulatory actions of the agonists on the pituitary induce an initial marked release of LH and FSH, which acts to increase plasma testosterone levels until downregulation has occurred and eventual castrate levels of testosterone are reached. This acute 'flare' effect can result in a temporary worsening of existing signs with increased bone pain in up to 20% of patients (Schally et al., 1993). Pre-treatment with an anti-androgens such as flutamide counteracts these effects and inhibits adrenal origin androgens from perpetuating signs of disease (Labrie, 1993; Labrie et al., 1986). The flare effect can be problematical in short-term human ovarian stimulation protocols, being associated with subsequent insufficient ovarian response with only one follicle and low oestrogen levels (Gordon and Hodgen, 1992; Diedrich and Bauer, 1990). Consequently, GnRH antagonists offer substantial advantages in avoiding the initial 'flare' and rapid desensitization. The future of analog development now appears to be focused on producing more potent and safer antagonists.

9. GnRH antagonist overview

The development of highly potent antagonists has lagged behind that of the progress made with the superagonists. The essential features required of antagonists are a high affinity

Table 2
Short-form peptide nomenclature, commonly used name and examples of some commercial GnRH agonist preparations

Peptide nomenclature	Common name	Commercial product
GnRH	Gonadorelin	Fertagyl®, 50 μg/ml, Intervet, Australia; Cystorelin®, 50 μg/ml, Merial, USA; Factrel®, 50 μg/ml, Fort Dodge Lab., USA.
[D-Ser(But)^6,Pro^9-NEt]GnRH	Buserelin	Receptal®, 4 μg/ml, Aventis (formerly Hoechst-Roussel, France), USA.
[D-Trp^6,Pro^9-NEt]GnRH	Deslorelin	Ovuplant®, 2.1 mg, absorbable s.c. implant, Peptech Animal Health, Australia.
[des-Gly^{10}-NEt]GnRH	Fertirelin	Ovalyse®; Pharmacia Upjohn, Mexico.
[D-Ser(But)^6,Aza-Gly^{10}]GnRH	Goserelin	Zoladex®, 10.8 mg s.c. implant, I.C.I. Pty Ltd, Australia.
[D-Leu^6,Pro^9-NEt]GnRH	Leuprolide	Lucrin Depot®, 7.5 mg, injectable microshperes, Abbott Australasia, Sydney, Australia; Lucrin®, 14 mg, aqueous injectable solution, Abbott Australasia, Sydney, Australia.
[D-Nal(2)^6]GnRH	Nafarelin	Synarel®, 2 mg/ml, nasal spray, Searle (now Pharamcia-Upjohn), USA.
[D-Trp^6]GnRH	Triptorelin	Decapeptyl®, 3.75 mg injectable microspheres, Debio Recherche Pharmaceutique, Switzerland.

for the GnRH receptor (binding but without activation), low histaminergic properties and resistance to enzymatic degradation (Karten and Rivier, 1986). Evaluation of the inhibitory actions of the antagonists can be achieved using similar systems to the agonists. Pituitary cell cultures can be used to assay for a lack of response to GnRH and to evaluate receptor-binding affinity. The immature male rat bioassay is based on the ability of antagonists to inhibit LH and FSH release following challenge with 200 ng of GnRH as measured by plasma LH concentrations. The pro-estrous rat anti-ovulatory assay has been used widely; antagonists are given at 12:00 noon on the day of pro-estrus along with untreated controls. Comparisons are made on the percentage of rats prevented from ovulating in relation to a certain dose. Inhibition of induced ovulation in the rabbit has also been utilised in antagonist bioassay systems (Schally et al., 1980).

Initial modifications involved substitutions at positions 2, 3 and 6. Introduction of D-Arg^6 increased the peptide potency and hydrophilicity. The concepts of combining aromaticity, basicity and hydrophilicity led to the introduction of heterocyclic amino acids into positions 3 and 6. Conformational related receptor-binding effects have been considered during antagonist development. Unfortunately, the more potent antagonists with the D-Arg^6 substitution were also found to be potent histamine releasing agents. The side effects of oedema of the face and extremities were noticed and some anaphylactoid reactions reported. Assay systems to assess the histamine releasing potential were utilised based on in vivo assessment of oedematogenic effects and direct measurement of histamine release from in vitro cultures of rat peritoneal mast cells (Bajusz et al., 1988). Current structural modifications are aimed at reducing the histamine releasing potential while maintaining or increasing potency (Karten and Rivier, 1986).

A promising new 3rd generation antagonist has recently been produced. Cetrorelix (SB-75; [Ac-D-Nal(2)^1, D-Phe(4Cl)^2, D-Pal(3)^3, D-Cit^6, D-Ala^{10}]GnRH) was completely free of

toxic effects while inhibiting ovulation in 75% of pro-estrous rats given 1.5 μg (Bajusz et al., 1988). Complete reversibility following daily treatment for 60 days with cetrorelix has been reported (Bokser et al., 1991) and longer acting microsphere formulations containing 3.58 mg of cetrorelix reversibly suppressed testosterone to castrate levels in rats for at least 164 days (Pinski et al., 1993). Cetrorelix, effectively prevented plasma FSH levels from showing typical wave like patterns (Fike et al., 1997) and inhibited pulsatile LH release (Peters et al., 1994) when used at a dose of 10 μg/kg in cattle every 24 h.

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References

Bajusz, S., Csernus, V.J., Janaky, T., Bokser, L., Fekete, M., Schally, A.V., 1988. New antagonists of LHRH. II. Inhibition and potentiation of LHRH by closely related analogues. Int. J. Pept. Prot. Res. 32, 425–435.

Barron, F.L., Millar, R.P., Searle, D., 1982. Metabolic clearance and plasma half-disappearance time of D-Trp6 and exogenous luteinsing hormone-releasing hormone. Clin. Endocr. Metab. 54, 1169–1173.

Barron, J., Griffths, E., Tsalacopoulos, G., Millar, R.P., 1984. Metabolism of [D-Trp6]LHRH. In: Vickery, B. (Ed.), LHRH and its Analogs. MTP Press, Lancaster, pp. 411–419.

Beckers, T., Reilander, H., Hilgard, P., 1997. Characterisation of gonadotropin-releasing hormone analogs based on a sensitive cellular luciferase reporter gene assay. Anal. Biochem. 251, 17–23.

Bokser, L., Srkalovic, G., Szepeshazi, K., Schally, A., 1991. Recovery of pituitary-gonadal function in male and female rats after prolonged administration of a potent antagonist of luteinsing hormone releasing hormone (SB-75). Neuroendocrinology 54, 136–145.

Chenault, J.R., Kratzer, D.D., Rzepkowski, R.A., Goodwin, M.C., 1990. LH and FSH response of holstein heifers to fertirelin actetate, gonadorelin and buserelin. Theriogenology 34, 81–98.

Chrisp, P., Goa, K.L., 1990. Nafarelin. A review of its pharmacodynamic and pharmacokinetic properties, and clinical potential in sex hormone-related conditions. Drugs 39, 523–551.

Clayton, R.N., Catt, K.J., 1980. Receptor-binding affinity of gonadotropin-releasing hormone analogs: analysis by radioligand-receptor assay. Endocrinology 106, 1154–1159.

Conn, P.M., Crowley, W.F., 1991. Gonadotropin-releasing hormone and its analogues. N. Engl. J. Med. 324, 93–103.

Coy, D.H., Labrie, F., Savary, M., Coy, E.J., Schally, A.V., 1975. LH-releasing activity of potent LH–RH analogs IN VITRO. Biochem. Biophys. Res. Commun. 67, 576–582.

Coy, D.H., Schally, A.V., 1978. Gonadotrophin releasing hormone analogues. Ann. Clin. Res. 10, 139–144.

Coy, D.H., Vilchez-Martinez, J.A., Coy, E.J., Schally, A.V., 1976. Analogs of luteinising hormone releasing hormone with increased biological activity produced by D-amino acid substitution in position 6. J. Med. Chem. 19, 423–425.

Diedrich, K., Bauer, O., 1990. Advantages and disadvantages in the use of GnRH analogues in an IVF program. In: Proceedings GnRH Analogues in Obstetrics and Gynaecology, Geneva, pp. 21–26.

Dobson, H., 1985. Effects of chronic treatment with a GnRH agonist on oestrous behaviour and on the secretion of LH and progesterone in the ewe. Theriogenology 24, 1–11.

D'Occhio, M.J., Aspden, W.J., Whyte, T.R., 1996. Controlled, reversible suppression of estrous cycles in beef heifers and cows using agonists of gonadotropin-releasing hormone. J. Anim. Sci. 74, 218–225.

D'Occhio, M.J., Fordyce, G., Whyte, T.R., Jubb, T.F., Fitzpatrick, L.A., Cooper, N.J., Aspden, W.J., Bolam, M.J., Trigg, T.E., 2002. Use of GnRH agonist implants for long-term suppression of fertility in extensively managed heifers and cows. Anim. Reprod. Sci. 74, 151–162.

Dutta, A.S., Furr, B.J.A., Giles, M.B., Valaccia, B., Walpole, A.L., 1978. Potent agonist and antagonist analogues of luliberin containing an azaglycine residue in position 10. Biochem. Biophys. Res. Commun. 81, 382–390.

Fike, K.E., Bergfeld, E.G., Cupp, A.S., Kojima, F.N., Mariscal, V., Sanchez, T., Wehrman, M.E., Grotjan, W.H., Hamernik, D.L., Kittok, R.J., Kinder, J.E., 1997. Gonadotropin secretion and development of ovarian follicles during oestrous cycles in heifers treated with luteinizing hormone releasing hormone antagonist. Anim. Reprod. Sci. 49, 83–100.

Flouret, G., Stetler-Stevenson, M.A., Carone, F.A., Peterson, D.R., 1984. Enzymatic degradation of LHRH and analogs. In: Hafez, E. (Ed.), LHRH and Its Analogs: Contraceptive and Therapeutic Applications. MTP Press, Hingham, MA, pp. 397–410.

Fujino, M., Yamazaki, I., Kobayashi, S., Fukuda, T., Shinagawa, S., Nakayama, R., 1974. Some analogs of luteinizing hormone releasing hormone (LH–RH) having intense ovulation inducing activity. Biochem. Biophys. Res. Commun. 57, 1248–1256.

Gong, J.G., Campbell, B.K., Bramley, T.A., Gutierrez, C.G., Peters, A.R., Webb, R., 1996. Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicle development in heifers continuously infused with a gonadotropin-releasing hormone agonist. Biol. Reprod. 55, 68–74.

Gordon, K., Hodgen, G., 1992. Evolving role of gonadotropin-releasing hormone antagonists. Trends Endocr. Metab. 3, 259–263.

Grant, G., 1995. Peptides, Synthetic. In: Meyers, R. (Ed.), Molecular Biology and Biotechnology: A Comprehensive Desk Reference. Wiley and Sons, New York, pp. 654–658.

Gudmundsson, J.A., Nillius, S.J., Bergquist, C., 1984. Inhibition of ovulation by intranasal nafarelin, a new superactive agonist of GnRH. Contraception 30, 107–114.

Hahn, D.W., McGuire, J.L., Vale, W., Rivier, J., 1984a. Biological assays utilized to characterize LHRH and its analogs. In: Hafez, E. (Ed.), LHRH and its Analogs. MTP press, Hingham, USA, pp. 49–60.

Hahn, D.W., Phillips, A., Lai, M.T., Klimek, S., McGuire, J.L., 1984b. Potency and activity variation of LHRH analogs in different models and species. Endocr. Res. 10, 123–138.

Hahn, D.W., Phillps-Probst, A., McGuire, J.L., 1981. The need for controlling variables invloved in biologic assays of LHRH. In: Sciarra, J. (Ed.), LHRH Peptides as Female and Male Contraceptives. Harper & Row, Philadelphia, pp. 366–373.

Herschler, R.C., Vickery, B.H., 1981. Effects of (DTrp6-Des-Gly10-ProNH29)-luteinizing hormone-releasing hormone ethylamide on the estrous cycle, weight gain, and feed efficiency in feedlot heifers. Am. J. Vet. Res. 42, 1405–1408.

IUPAC–IUB, 1985. Nomenclature and symbolism for amino acids and peptides—recommendations, 1983. J. Biol. Chem. 260, 14–42.

Karten, M.J., Rivier, J.E., 1986. Gonadotropin-releasing hormone analog design. Structure-function studies toward the development of agonists and antagonists: rationale and perspective. Endocr. Rev. 7, 44–66.

Kesler, D.J., Summers, R.N., Peterson, C.A., Steckler, T.L., 1999. Gonadorelin-induced testosterone release: a biological assay for quality assurance of gonadorelin in veterinary medicine. Drug Dev. Ind. Pharm. 25, 315–321.

Kinder, J.E., Adams, T.E., Nett, T.M., Coy, D.H., Schally, A.V., Reeves, J.J., 1976. Serum gonadotropin concentrations and ovarian response in ewes treated with analogs to LH–RH/FSH–RH. J. Anim. Sci. 42, 1220–1226.

Konig, W., Sandow, J., Geiger, R. (Eds.), 1975. Struture–function Relationships of LH–RH/FSH–RH. Peptides: Chemistry, Structure, Biology. Proceedings of the 4th American Peptide Symposium.

Labrie, F., 1993. GnRH agonists in prostate cancer. In: Proceedings GnRH Analogues in Cancer and Human Reproduction, Geneva, pp. 103–121.

Labrie, F., Dupont, A., Belanger, A., St-Arnaud, R., Giguere, M., Lacourciere, Y., Emond, J., Monfette, G., 1986. Treatment of prostate cancer with gonadotropin-releasing hormone agonists. Endocr. Rev. 7, 67–74.

Loumaye, E., Naor, Z., Catt, K., 1982. Binding affinity and biological activity of gonadotropin-releasing hormone agonists in isolated pituitary cells. Endocrinology 111, 730–736.

McCleod, B.J., Dodson, S.E., Peters, A.R., Lamming, G.E., 1991. Effects of a GnRH agonist (Buserelin) on LH secretion in post-partum beef cows. Anim. Reprod. Sci. 24, 1–11.

Merrifield, R.B., 1966. The instrument for automated synthesis of peptides. Anal. Chem. 38, 1905.

Nawito, M., Schallenberger, E., Schams, D., 1977. Release of lutropin (LH) and follitropin (FSH) in cattle after administration of a new gonadoliberin (GnRH) analogue in comparison with the gonadoliberin decapeptide. Theriogenology 7, 277–291.

Nestor, J., Ho, T., Simpson, R., Horner, B., Jones, G., McCrae, G., Vickery, B., 1982. Synthesis and biological activity of some very hydrophobic superagonist analogues of luteinizing hormone releasing hormone. J. Med. Chem. 25, 795–801.

Okadam, H., Yamazakim, I., Ogawam, Y., Hiraim, S., Yashiki, M.T., Mima, H., 1982. Vaginal absorption of a potent luteinizing hormone releasing hormone analag (leuprolide) in rats. I. Absorption by various routes and absorption enhancement. J. Pharm. Sci. 71, 1367–1371.

Okada, H., Yamazaki, I., Yashiki, T., Mima, H., 1983. Vaginal absorption of a potent luteinizing hormone releasing hormone analog (leuprolide) in rats. II. Mechanism of absorption enhancement with organic acids. J. Pharm. Sci. 72, 75–78.

Perrin, M.H., Vaughan, J.M., Rivier, J.E., Vale, W.W., 1980. High affinity GNRH binding to testicular membrane homogenates. Life Sci. 26, 2251–2255.

Peters, K.E., Bergfeld, E.G., Cupp, A.S., Kojima, F.N., Mariscal, V., Sanchez, T., Wehrman, M.E., Grotjan, H.E., Hamernik, D.L., Kittok, R.J., Kinder, J.E., 1994. Luteinizing hormone has a role in development of fully functional corpora lutea (CL) but is not required to maintain CL function in heifers. Biol. Reprod. 51, 1248–1254.

Pinski, J., Yano, T., Szeoeshazi, K., Groot, K., Schally, A.V., 1993. Recovery of pituitary-gonadal function in male rats after long term suppression induced by a single injection of microcapsules of LH–RH antagonist cetrorelix (SB-75). J. Androl. 14, 164–169.

Pitt, C.G., 1987. The controlled delivery of polypeptides including LHRH analogs. In: Nestor, J. (Ed.), LHRH and Its Analogs: Part 2. MTP Press, Lancaster, England, pp. 557–576.

Raheja, K.L., Jordan, A., 1994. FDA recommendations for preclinical testing of gonadotropin releasing hormone (GnRH) analogues. Regul. Toxicol. Pharmacol. 19, 168–175.

Reeves, J., Seguin, C., Lefebvre, F.-A., Kelly, P., Labrie, F., 1980. Similar luteinising hormone-releasing binding sites in rat anterior pituitary and ovary. Proc. Natl. Acad. Sci., U.S.A. 77, 5567–5571.

Roberts, A.J., Chang, C.F., Schally, A.V., Reeves, J.J., 1989. Luteinizing hormone concentrations in serum of postpartum beef cows injected with microencapsulated luteinizing hormone-releasing hormone analog. J. Anim. Sci. 67, 2730–2734.

Schally, A.V., Arimura, A., Cow, D.H., 1980. Recent approaches to fertility control based on derivatives of LH–RH. Vitam. Horm. Res. 38, 258–323.

Schally, A.V., Arimura, A., Kastin, A.J., Matuso, H., Baba, Y., Redding, T.W., Nair, R.M.G., Debeljuk, L., 1971. Gonadotropin releasing hormone: one polypeptide regulates secretion of luteinizing and follicle stimulating hormones. Science 173, 1036–1038.

Schally, A.V., Comaru-Schally, A.M., Hollander, V., 1993. Hypothalamic and other peptide hormones. In: Holland, J. (Ed.), Cancer Medicine, vol. 1. Lea & Febiger, Philadelphia, pp. 827–840.

Sealfon, S., Weinstein, H., Millar, R., 1997. Molecular mechanisms of ligand interaction with the gonadotropin-releasing hormone receptor. Endocr. Rev. 18, 180–205.

Vickery, B.H., McRae, G.I., 1980. Synchronization of oestrus in adult female rats by utilizing the paradoxical effects of an LH–RH agonist. J. Reprod. Fertil. 60, 399–402.

Vizcarra, J.A., Wettemann, R.P., Braden, T.D., Turzillo, A.M., Nett, T.M., 1997. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows. Endocrinology 138, 594–601.

Wood, G.C., Dass, C., Iyer, M.R., Fleischner, A.M., Sheth, B.B., 1997. Stability of deslorelin injection. PDA J. Pharm. Sci. Technol. 51, 176–180.