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Perspective

Effects of Gonadotropin Releasing Hormone Agonists and Antagonists on Reproductive Functions†

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Introduction

Gonadotropin-releasing hormone (GnRH) is a decapeptide originally isolated and characterized from porcine and ovine hypothalami as pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2.[1,2] GnRH stimulates the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH), induces ovulation, increases sperm production, stimulates steroidogenesis, and may act directly on the brain to increase sexual behavior in various species, including human beings (review in ref 3).

The essential role played by endogenous GnRH in regulating reproductive functions has been demonstrated by studies of active or passive immunization, which results in a marked inhibition of gonadotropin and steroid secretion, as well as atrophy of the gonads.[4-6] The physiological control exerted by endogenous GnRH on fertility parameters has also been investigated through its agonistic analogues, which can be applied to the study of the effects of chronic exposure to the releasing factor, and its antagonistic analogues, which can be used to examine the effects of preventing the actions of the endogenous peptide. As a result of these studies over the last decade, our understanding of the mechanisms that regulate reproductive functions has expanded and has also led to the potential use of GnRH analogues as therapeutic agents. At the present time, both GnRH agonists and antagonists are available which are potent enough to be used in long-term experiments, and the objective of this paper is to describe the effects of the prolonged exposure to both types of analogues on reproductive functions in the rat.

Overview of the Chemistry and Physiology of GnRH Analogues

Studies in which each residue of the native molecule GnRH, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, was systematically deleted or replaced by glycine, alanine, the appropriate D-amino acid, or another amino acid have provided insight into the roles of various regions of GnRH necessary for binding affinity and intrinsic activity (review ref 7-12). Potent GnRH agonists have been obtained with the modification of three amino acids: Gly^6, Leu^7, and Gly^{10}-NH_2.[13-15] One of the most potent GnRH analogues reported to date is a peptide in which Gly^6 is replaced by $DTrp^6$ and with the C-terminal modification des-Gly^{10} ([Pro^9-NEt]GnRH).[15,16] On the other hand, modifications involving the three N-terminal residues in GnRH have resulted in analogues that behave as competitive antago-

† Abbreviations used are: LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; LRF, luteinizing hormone-releasing factor; GnRH, gonadotropin releasing hormone; LH-RH, luteinizing hormone-releasing hormone.

(1) Matsuo, H.; Baba, Y.; Nair, R.; Arimura, A.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1334-1339.
(2) Burgus, R.; Butcher, M.; Amoss, M.; Ling, N.; Monahan, M.; Rivier, J.; Fellows, R.; Blackwell, R.; Vale, W.; Guillemin, R. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 278-282.
(3) Vale, W.; Rivier, C.; Brown, M. *Annu. Rev. Physiol.* **1977**, *39*, 473-527.
(4) Arimura, A.; Sato, H.; Kumasaka, T.; Worobec, R. B.; Debeljuk, L.; Dunn, J.; Schally, A. V. *Endocrinology* **1973**, *93*, 1092-1103.
(5) Nishi, N.; Arimura, A.; de la Cruz, K. G.; Schally, A. V. *Endocrinology (Baltimore)* **1976**, *98*, 1024-1030.
(6) Kerdelhue, B.; Catin, S.; Kordon, C.; Jutisz, M. *Endocrinology (Baltimore)* **1976**, *98*, 1539-1549.
(7) Vale, W.; Rivier, C.; Brown, M.; Leppaluoto, Y.; Ling, N.; Monahan, M.; Rivier, J. *Clin. Endocrinol.* **1976**, *5*, 261s-273s.
(8) Vale, W.; Rivier, C.; Brown, M.; Rivier, J. *Adv. Exp. Med. Biol.* **1977**, *106*, 123-156.
(9) Vale, W.; Rivier, C.; Brown, M. *Anu. Rev. Physiol.* **1977**, *29*, 473-527.
(10) Rivier, J.; Brown, M.; Rivier, C.; Ling, N.; Vale, W. "PPtides", Proceedings of the European Peptide Synposium, 14th, Wepion, Belgium, Apr 11-17, 1976, Loffet, A., Ed.; Editions de l'Universite de Bruxelles: Belgium, 1976; pp 427-451.
(11) Rivier, J.; Rivier, C.; Perrin, M.; Porter, J.; Vale, W. In "LHRH Peptides as Female and Male Contraceptives"; Zatuchni, G. I.; Shelton, J. D.; Sciarra, J. J., Eds.; Harper & Row: Philadelphia, 1981, p 13-23.
(12) Rivier, J.; Rivier, C.; Perrin, M.; Porter, J.; Vale, W. In "LHRH and It's Analogs—A New Class of Contraceptive and Therapeutic Agents"; Vickery, B. H.; Nestor, J. J., Jr.; Hafez, E. S. E., Eds.; MTP Press: Lancaster, Boston, The Hague, in press.
(13) Monahan, M.; Amoss, M.; Anderson, H.; Vale, W. *Biochemistry* **1973**, *12*, 4616-4620.
(14) Ling, N.; Vale, W. *Biochem. Biophys. Res. Commun.* **1975**, *63*, 801-806.
(15) Fujino, M.; Yamasaki, I.; Frobayashi, S.; Fukada, T.; Schinagawa, S.; Nakayana, R. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 1248-1256.
(16) Rivier, J.; Ling, M.; Monahan, M.; Rivier, C.; Brown, M.; Vale, W. In "Peptides: Chemistry, Structure and Biology", Proceedings of the American Peptide Symposium, 4th, New York, June 1-6, 1975; Wlater, R.; Meinhofer, J., Eds.; Ann Arbor Science Publishers: Ann Arbor, MI, 1975; pp 863-870.

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nists. At the present time, one of the most potent GnRH antagonists is [Ac-DNal(2)[1],4FDPhe[2],DTrp[3],DArg[6]]-GnRH.[12,17]

The acute intravenous administration of GnRH agonists results in a several-fold increase in plasma LH, FSH, and steroid levels.[8] The potent analogue [DTrp[6],Pro[9]-NEt]-GnRH is not only ca. 150 times more potent than GnRH[8] itself to release gonadotropin but also exhibits prolonged activity in vivo. This agonist can therefore be used in long-term studies in which one daily depot injection of the peptide in corn oil results in its continuous delivery over several hours.

While, as mentioned above, GnRH agonists are very active in acutely inducing LH and FSH secretion, their long-term administration is paradoxically associated with a marked inhibition of reproductive functions in both male and female rats. In the adult male, daily treatment with GnRH agonists causes a rapidly occurring blunting of the LH and, even more markedly, the FSH responses: inhibition of steroid secretion, lowering of sex organ weights, and disruption of spermatogenesis.[8,18-28] In the female rat, the agonists block the estrous cycle and terminate pregnancy.[20,29-37]

The injection of GnRH antagonists also results in lowered gonadotropin and steroid release (review in ref 8), with a resulting blockade of ovulation[38-40] and, on a long-term basis, atrophy of the gonads, arrest of spermatogenesis,[41,42] and termination of pregnancy.[43-47] The effects of the antagonists on gonadotropin secretion are concise in that they are observed less than 10 min after the first injection of the peptides and remain consistent throughout the treatment.[45]

Table I. Effect of a GnRH Agonist and the Onset of Puberty in the Female Rat

treatment	N	day of vaginal opening
control[a]	10	36.9 ± 0.84
2.5 μg/kg of agonist[b]	10	63.4 ± 1.52

[a] Treatments were administered once daily from day 24 through 60. [b] Agonist = [DHis[6](imBzl),Pro[9]-NEt]GnRH.

Table II. Effect of GnRH Antagonists on the Onset of Puberty in the Female Rat

treatment	N	day of vaginal opening
control[a]	10	35.6 ± 0.81
5 mg/kg of antagonist I[b]	10	49.4 ± 0.95
control	12	37.6 ± 0.83
1 mg/kf of antagonist II	12	48.5 ± 0.86

[a] Treatments were administered once daily from day 24 until the day of vaginal opening. [b] Antagonist I = [Ac-Δ[3]Pro[1],4ClDPhe[2],DTrp[3,6]]GnRH; antagonist II = [Ac-DNal(2)[1],4FDPhe[2],DTrp[3],DArg[6]]GnRH.

Thus, the long-term administration of both GnRH agonists and antagonists is deleterious to reproductive functions in the rat. The mechanisms through which both classes of analogues induce these changes are, however, significantly different. The antireproductive effects of GnRH agonists are presently believed to result from a combination of modes of action, including desensitization at the pituitary and gonadal levels, resulting in lowered gonadotropin and steroid release,[22,28,48-50] as well as direct effects on the gonads themselves.[51,52] By contrast, GnRH antagonists lower gonadotropin release by blocking the stimulatory action of endogenous GnRH on the pituitary.[53]

One essential difference between the modes of action of the two types of analogues resides in the observation that, while on a long-term basis both agonists and antagonists inhibit reproductive functions, each daily injection of the

(17) Rivier, C.; Rivier, J.; Perrin, M.; Vale, W. *Biol. Reprod.* **1983**, 19.
(18) Pelletier, G.; Cusan, L.; Auclair, C.; Kelly, P. A.; Desy, L.; Labrie, F. *Endocrinology (Baltimore)* **1978**, *103*, 641–643.
(19) Sandow, J.; Von Rechenberg, W.; Jerzabek, G.; Stoll, W. *Fertil. Steril.* **1978**, *30*, 205–209.
(20) Cusan, L.; Auclair, C.; Belanger, A.; Ferland, L.; Kelly, P. A.; Seguin, C.; Labrie, F. *Endocrinology* **1979**, *104*, 1369–1376.
(21) Belanger, A.; Cusan, L.; Auclair, C.; Seguin, C.; Caron, S.; Labrie, F. *Biol. Reprod.* **1980**, *22*, 1094–1101.
(22) Rivier, C.; Rivier, J.; Vale, W. *Endocrinology* **1979**, *105*, 1191–1201.
(23) Rivier, C.; Rivier, J.; Vale, W. *Int. J. Fertil.* **1980**, *25*(3), 145–150.
(24) Rivier, C.; Vale, W. W. *Life Sci.* **1979**, *25*, 1065–1074.
(25) Vale, W.; Rivier, C. Perrin, M.; Rivier, J. In "Peptides: Structure and Biological Function", Proceedings of the American Peptide Symposium, 6th, Georgetown University, Washington, DC, Jan 17–22, 1979; Gross, E.; Meienhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, 1979; pp 781–793.
(26) Rivier, C.; Vale, W. *Life Sci.* **1981**, *29*, 1523–1529.
(27) Fraser, H. M. *J. Reprod. Fertil.* **1982**, *64*, 503–515.
(28) Labrie, F.; Godbont, M.; Zelanger, A.; Lefebure, F-A.; Seguin, C.; Pelletier, Z.; Cusan, L.; Kelly, P. R.; Reeves, J. J. In "LHRH Peptides as Female and Male Contraceptives"; Zatuchni, G. I.; Shelton, J. D.; Sciarra, J. J., Eds.; Harper and Row: New York, 1981; pp 246–260.
(29) Johnson, E. S.; Gendrich, R. L.; White, W. F. *Fertil. Steril.* **1976**, *27*, 853–860.
(30) Banik, U. K.; Givner, M. L. *Fertil. Steril.* **1976**, *27*, 1078–1084.
(31) Humphrey, R. R.; Windsor, B. L.; Jones, D. C.; Reel, J. R.; Edgren, R. A. *Biol. Reprod.* **1978**, *19*, 84–91.
(32) Humphrey, R. R.; Windsor, B. L.; Reel, J. R.; Edgren, R. A. *Biol. Reprod.* **1977**, *16*, 614–621.
(33) Corbin, A.; Beattie, C. W.; Rees, R.; Yardley, J.; Foell, T. J.; Chai, S. Y.; McGregor, H.; Garsky, V.; Sarantakis, D.; McKinley, W. A. *Fertil. Steril.* **1977**, *28*, 471–476.
(34) Corbin, A.; Bex, F. J.; Yardley, J. P.; Rees, R. W.; Foell, T. J.; Sarantakis, D. *Endocr. Res. Commun.* **1979**, *6*, 1–14.
(35) Corbin, A.; Beattie, C. W.; Jones, R.; Bex, F. *Int. J. Gynaecol. Obstet.* **1979**, *16*, 359–372.
(36) Rivier, C.; Rivier, J.; Vale, W. *Endocrinology (Baltimore)* **1978**, *103*, 2299–2305.
(37) Bex, F. J.; Corbin, A. *Endocrinology (Baltimore)* **1979**, *105*, 139–145.
(38) Beattie, C. W.; Corbin, A.; Foell, T. J.; Garsky, V.; McKinley, W. A.; Rees, R. W. A.; Sarantakis, D.; Yardley, J. P. *J. Med. Chem.* **1975**, *18*, 1247–1250.
(39) de la Cruz, A.; Coy, D. H.; Vilchez-Martinez, J. A.; Arimura, A.; Schally, A. V. *Science* **1976**, *191*, 195–196.
(40) Bowers, C. Y.; Humphries, J.; Wasiak, T.; Folkers, K.; Reynolds, G. A.; Reichert, L. E. *Endocrinology (Baltimore)* **1980**, *106*, 674–683.
(41) Rivier, C.; Rivier, J.; Vale, W. *Science* **1980**, *210*, 93–95.
(42) Rivier, C.; Rivier, J.; Vale, W. *Endocrinology (Baltimore)* **1981**, *108*, 1998–2001.
(43) Rivier, C.; Rivier, J.; Vale, W. *Contraception* **1979**, *19*, 185–190.
(44) Rivier, C.; Vale, W. *Endocrinology (Baltimore)* **1982**, *110*, 347–351.
(45) Rivier, C.; Rivier, J.; Vale, W. *Endocrinology (Baltimore)* **1981**, *108*, 1425–1430.
(46) Rivier, C.; Vale, W. *Biol. Reprod.* **1981**, *24*, 1061–1067.
(47) Rivier, C.; Vale, W. *Endocrinology (Baltimore)* **1982**, *110*, 347–351.
(48) Catt, K. J.; Baukal, A. J.; Davies, T. F.; Dufau, M. L. *Endocrinology (Baltimore)* **1979**, *104*, 17–25.
(49) Harwood, J. P.; Clayton, R. N.; Catt, K. J. *Endocrinology (Baltimore)* **1980**, *107*, 407–413.
(50) Reddy, P. V.; Azhar, S.; Menon, K. M. J. *Endocrinology (Baltimore)* **1980**, *107*, 930–936.
(51) Hsueh, A. J. W.; Jones, P. B. C. *Endocr. Rev.* **1981**, *2*, 437–461.
(52) Sharpe, R. M. *J. Reprod. Fertil.* **1982**, *64*, 517–527.
(53) Monahan, M.; Rivier, J.; Vale, W.; Guillemin, R.; Burgus, R. *Biochem. Biophys. Res. Commun.* **1972**, *47*, 551–556.

agonists causes a certain degree of pituitary stimulation that, though progressively blunted, still results in periodic elevations of plasma gonadotropin levels above control values.[22] The resulting desensitization to trophic signals at the gonadal level, which represents an important component of the antifertility effect of GnRH agonists, does not occur during administration of GnRH antagonists.

Since both GnRH agonists and antagonists represent potential means of regulating fertility, as well as controlling steroid-dependent tumors, we thought it of interest to compare their long-term effects in similar paradigms and, in particular, to evaluate the degree of inhibition of reproductive functions produced by each class of peptides, as well as the dose of analogue necessary to reach it.

Effect of GnRH Analogues on Reproductive Functions in the Rat

Effect of GnRH Analogues on Puberty in the Rat. (a) GnRH Agonists. The daily administration of GnRH to immature female rats delays puberty (as assessed by the onset of vaginal opening) for the duration of the treatment.[8,45,54,55] As shown in Table I, 1 μg of a GnRH agonist administered daily from day 24 through day 60 prevented vaginal opening in all treated animals. Upon cessation of the treatment, vaginal opening was observed 3–8 days after the last injection, and the subsequent three estrous cycles appeared normal. GnRH agonists have also been reported to also be effective in inhibiting maturational changes in the male rat.[56]

(b) GnRH Antagonists. The effect of GnRH antagonists on puberty is illustrated in Table II. When the antagonists were administered to female rats from day 24 of age, vaginal opening was delayed by 10–14 days, but eventually occurred despite continuing administration of the analogue. These data are also consistent with studies showing that passive immunization to endogenous GnRH only delayed puberty in female rats, which did, however, achieve fertility.[57]

At the present time, we do not know whether GnRH agonist treated animals would have eventually exhibited sexual maturation. The observation that some antagonist-treated female rats showed vaginal opening as early as 38 days of age (i.e., only 3 days later than most controls), while no agonist-treated animals reached puberty during the course of the treatment, would, however, suggest that the agonist was more effective than the antagonist to interfere with normal sexual maturation.

GnRH antagonists have been demonstrated to be more powerful to inhibit LH than FSH release. We (C. Rivier and W. Vale, unpublished), as well as other groups (M. C. Charlesworth et al., submitted), have indeed repeatedly observed that even large doses of these antagonists would not lower plasma FSH levels by more than 50%. Since an increase in FSH, but not LH, secretion has been reported to represent the principal stimulus for puberty,[58] it is possible that the "escape" seen in antagonist-treated rats may have been at least partially due to the inability of these analogues to totally inhibit FSH release. By contrast, the total absence of FSH stimulation in the presence of agonist,[24] as well as the reported gonadal desensitization occurring during prolonged exposure to such analogues,[21,22,48] may have been responsible for the higher effectiveness of agonist.

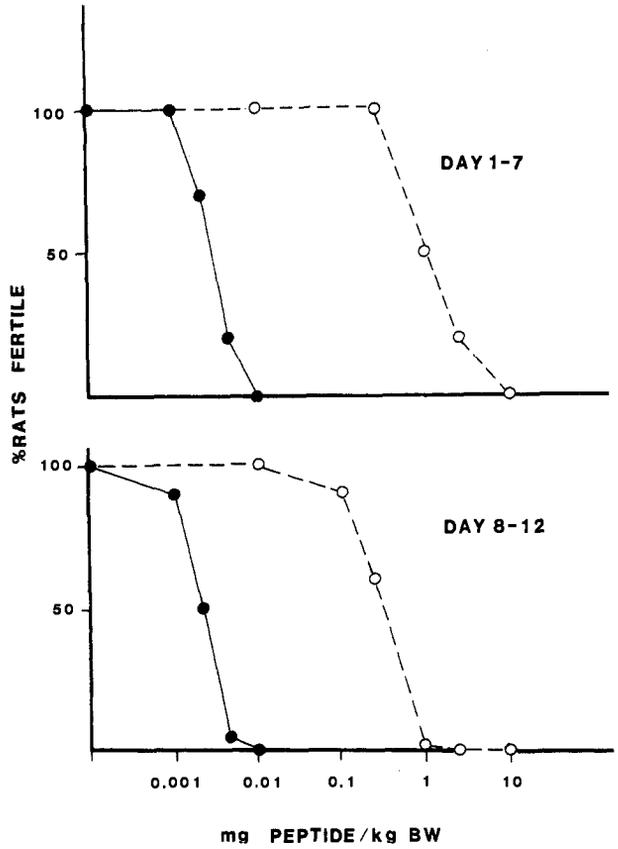

Figure 1. Comparison between the effects of the GnRH agonist [DTrp^6,Pro^9-NEt]GnRH (●) and the GnRH antagonist [Ac-DNal(2)^1,4FDPhe^2,DTrp^3,DArg^6]GnRH (○) on pregnancy in the rat. Each point represents the mean of 10 rats. Data from analogue-injected animals are expressed as percent control rats, which received corn oil only. Day 1 corresponds to the day of mating, as assessed by the presence of a vaginal plug in the bedding and of spermatozoa in the vaginal lavage. The analogues were dissolved in corn oil and injected sc once daily.

Effect of GnRH Analogues on Ovulation and the Estrous Cycle. (a) GnRH Agonists. Daily administration of a GnRH agonist to regularly cycling female rats, starting on diestrus-II, interrupted cyclicity (and ovulation) for the duration of the treatment. Regular cycles were resumed 5–8 days after the last injection.[36]

(b) GnRH Antagonists. Studies on the antiovulatory effects of GnRH antagonists have shown that 0.5 μg of the most potent analogues could block ovulation when administered at noon on proestrus, while larger doses were necessary to be effective when injected on diestrus-II.[17] Daily administration of the antagonist [Ac-Δ^{3}Pro^1,4ClDPhe^2,DTrp^{3,6},NMeLeu^7]GnRH completely blocked the estrous cycle, which resumed 6–9 days after cessation of the treatment.[45]

As illustrated by these experiments, both GnRH agonists and antagonists can effectively interfere with normal cyclicity in the female rat, and this effect is totally reversible.

Effect of GnRH Analogues on Pregnancy. (a) GnRH Agonists. It is well established that administration of GnRH agonists will interfere with gestation in the rat.[20,29-37] We have observed that GnRH agonists would terminate pregnancy when injected either from day 1 through 7, or day 8 through 12 after mating, with the

(54) Vilchez-Martinez, J. A.; Pedroza, E.; Arimura, A.; Schally, A. V. *Fertil. Steril.* **1979**, *31*, 677–682.
(55) Nekola, M. V.; Pedroza, E.; Schally, A. V. *Biol. Reprod.* **1981**, *24*, 505–511.
(56) Sharpe, R. M.; Fraser, H. M. *J. Reprod. Fertil.* **1980**, *60*, 359–368.
(57) Bercu, B. B.; Jackson, I. M. D.; Reichlin, S. *Proc. Soc. Exp. Biol. Med.* **1979**, *160*, 123–125.
(58) Critchlow, W.; Bar-Sela, M. E. *Neuroendocrinology* **1967**, *2*, 101–162.

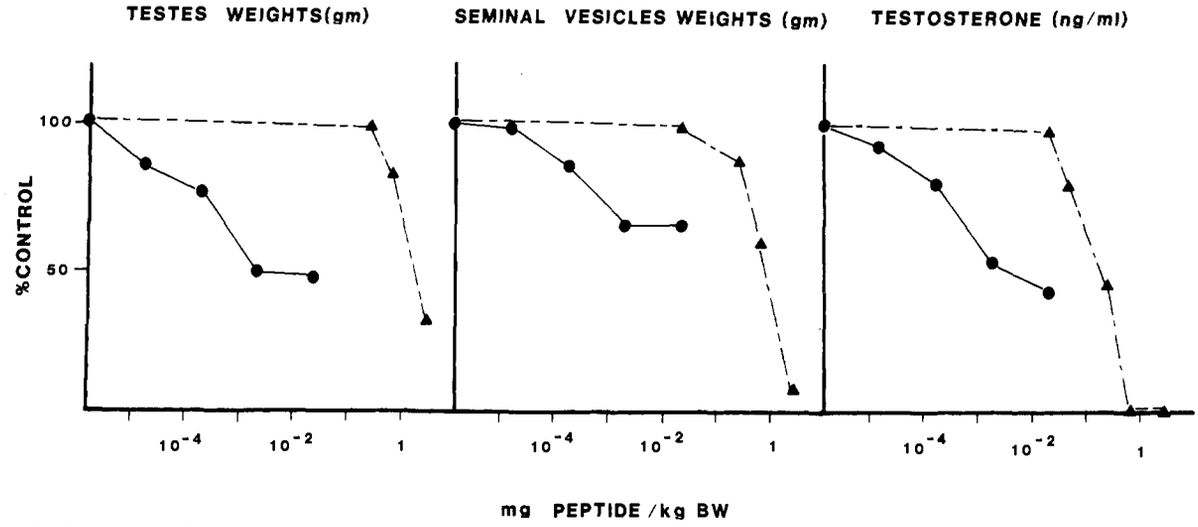

Figure 2. Comparison between the effects of the GnRH agonist [DTrp[6],Pro[9]-NEt]GnRH (●) and the GnRH antagonist [Ac-DNal-(2)[1],4FDPhe[2],DTrp[3],DArg[6]]GnRH (▲) on sex organ weights and testosterone secretion. Each point represents 10 rats. Data from analogue-injected animals are expressed as percent control rats, which received corn oil only. The analogues were dissolved in corn oil and injected sc once daily. The animals were sacrificed by decapitation 24 h after the last injection.

second regimen being slightly more effective (Figure 1). GnRH agonists can also terminate pregnancy when administered after the LH-dependent time of gestation (day 12), an effect probably due to their extra-pituitary actions.[59]

(b) GnRH Antagonists. GnRH antagonists will also disrupt gestation, but their ability to do so varies with the stage of pregnancy.[45-47] As illustrated in Figure 1, much larger doses of the antagonist were required during the 1st week than in the 2nd week after mating. In the experiment illustrated here, 10 mg/kg of antagonist was necessary to terminate pregnancy during days 1–7, while 1.0 mg/kg was 100% effective during days 8–12. By contrast, no effect of antagonist was observed after day 12 (C. Rivier and W. Vale, unpublished).

These data indicate that there are at least two differences between the antigestational effects of GnRH agonists and antagonists in the female rat: the dose-dependent effectiveness (within each class of analogues) of the peptides to terminate pregnancy and the temporal relationship between the abortifacient effects of both classes of peptides.

We have observed that exogenous administration of progesterone, PRL, or hCG during the 1st week of pregnancy could reverse the deleterious effect of GnRH agonists, while during the 2nd week only progesterone and hCG were effective.[47] These data suggested that during the 1st week after mating the antigestational properties of the agonists were at least partially mediated through inhibition of PRL secretion, a hormone that plays an essential luteotropic role in the rat. By contrast, since exogenous PRL was unable to prevent the abortifacient action of GnRH antagonists, it is probable that the effects of these analogues were not mediated through inhibition of PRL secretion. These data had led us to suggest that during the 1st week of gestation the differential ability of GnRH agonists and antagonists to modify PRL release were related to their relative efficacy to terminate pregnancy.[47]

Results from replacement therapy during the 2nd week of gestation indicate that the main mediator of both types of analogues' effects during that time is inhibition of LH release. We have indeed reported that there is a striking parallelism between maximal antireproductive effects of GnRH antagonists and the time of LH dependency of the corpus luteum,[47] an observation that is in agreement with previously published studies on the effect of immunoneutralization of endogenous GnRH in pregnant rats.[5] GnRH agonists, through their ability to cause pituitary desensitization, also induce abortion by depriving the corpus luteum of its trophic signal.

Finally, as previously mentioned, only GnRH agonists (but not antagonists) can terminate pregnancy when administered during the 3rd week after mating.[59] Since the agonists are abortifacient in pregnant rats hypophysectomized at day 13 of gestation, they most probably act through an extrapituitary site of action, namely, at the ovarian level, to inhibit luteal progesterone production. On the other hand, while GnRH antagonists have also been reported to exert extrapituitary effects,[60] we have been unable to demonstrate any antigestational action of these analogues during the 3rd week of pregnancy. Therefore, the effectiveness of GnRH agonists and the lack of effectiveness of GnRH antagonists during the last week of gestation can probably be attributed to the relative importance of the agonists' extrapituitary effect in mediating these antireproductive effects.

Effect of GnRH Analogues in the Adult Male Rat. (a) GnRH Agonists. The chronic administration of GnRH agonists to intact adult male rats induced a dose-related decrease in testicular and accessory organ weights, diminished production of testosterone, and disrupted tubular morphology, indicative of suppression of spermatogenesis.[8,18-27] This treatment also caused transient elevations of plasma progesterone levels[24] and a marked reduction of PRL secretion,[19] as well as a significant blunting of the gonadotropin responses.[22] During the long-term exposure to the agonists, the analogue-induced FSH release is actually even more obliterated than the LH release, which results in an elevated LH/FSH ratio.[24]

As illustrated in Figure 2, the reduction in testicular weights of agonist-injected male rats reached a plateau at ca. 50%, while plasma testosterone fell to a maximum of ca. 45% of control values at 1–10 μg/kg of agonist. However, despite such marked disruptions of reproductive

(59) Bex, F. J.; Corbin, A. *Endocrinology (Baltimore)* **1981**, *108*, 273–280.

(60) Hsueh, A. J. W.; Ling, N. C. *Life Sci.* **1979**, *25*, 1223–1230.

Table III. Effect of a GnRH Agonist on Fertility in the Adult Male Rat

treatment	N	% rats fertile at month of treatment					
		1	2	3	4	5	6
control	15	100	100	100	100	100	100
1 μg of agonist qd[a]	15	73	87	71	60	52	65

[a] Agonist = [DTrp^6,Pro^9-NEt]GnRH.

parameters, a significant number of male rats remained fertile (Table III), even during prolonged exposure to the agonist.

Cessation of the treatment was followed by a prompt restoration of steroid secretion, while testicular weights returned to control values within 6 weeks.[24]

(b) GnRH Antagonists. The long-term administration of GnRH antagonists to adult male rats resulted in decreased testosterone production and sexual organ weights, as well as total arrest of spermatogenesis.[41] As illustrated in Figure 2, while the doses of the antagonist necessary to disrupt reproductive functions were much larger than those of the agonist, the degree of testicular weight and testosterone release inhibition was significantly higher. As seen from this figure, 1 mg/kg of antagonist produced a 70% drop in testes weights and a >95% inhibition of androgen secretion, while the maximum inhibition observed in the presence of the agonist was ca. 45% in both cases.

No mating occurred in adult male rats treated with GnRH antagonists, but the concomitant administration of low doses of testosterone propionate was able to restore active sexual behavior, but not fertility.[42]

Thus, the comparison of the effects of GnRH agonists and antagonists in the male rat indicates a number of differences in the long-term effects of these peptides. As mentioned above, the degree of inhibition of reproductive function is significantly higher for the antagonists than it is for the agonists, even though at the present time much larger doses of antagonists vs. agonists are required. Furthermore, the antagonists cause a total arrest of spermatogenesis, while chronic exposure to the agonists is still accompanied by a certain degree of fertility.

Extrapituitary Effects of GnRH Analogues. Apart from causing desensitization at the level of the pituitary (see above), GnRH and its analogs have been reported to also exert extra-pituitary effects. These effects, which are readily demonstrable in the rat,[51] but not in all species tested,[61,86] mainly take place at the level of the gonads. The issue of their relative importance in mediating the anti-reproductive activity of GnRH analogs has not yet been determined. However, the presence of gonadal GnRH binding sites, as well as the characterization of GnRH-like molecules outside the central nervous system (see 51 for review), suggest that at least some of the extrapituitary effects of GnRH and its analogs may have physiological relevance.[62]

(a) GnRH Agonists. The ability of GnRH and its agonists to inhibit steroidogenesis by a direct effect on the gonads has been recently reviewed.[51,52] In particular, GnRH agonists will reverse the stimulatory action of LH and FSH on steroid production by granulosa or Sertoli cells maintained in culture, and will induce marked losses in FSH, PRL and LH gonadal binding sites and inhibit steroidogenesis in hypophysectomized rats receiving exogenous gonadotropins.[51,52,63-66] GnRH agonists can also terminate pregnancy at a time when gestation is independent of the pituitary, an effect mediated by inhibition of luteal progesterone production.[59] Other extrapituitary effects of these analogs include the stimulation of prostaglandin accumulation in rat granulosa cells *in vitro*,[67] antisteroidal activity at the level of the kidney,[68] a claudogenic effect in the pregnant rat uterine horn,[69] inhibition of the uterotropic acitivity of estradiol in ovariectomized rats,[70] induction of luteolysis in humans,[71] as well as centrally mediated sex behavioral effects.[72]

(b) GnRH Antagonists. Extrapituitary effects have also been described for GnRH antagonists. In vitro, these analogues will prevent the GnRH-mediated inhibition of granulosa cell function.[60] In vivo, we have observed (Figure 3) that the administration of the GnRH antagonist [Ac-Δ^3Pro^1,4ClDPhe^2,DTrp^{3,6}]GnRH for 7 days will reverse the testosterone release induced by hCG in hypophysectomized male rats. These results suggest that GnRH antagonists can compete for the GnRH binding sites that have been described at the level of the gonads, as well as counteract the effect of gonadotropins on the testes and the ovaries.

Conclusion and Future Developments

It is apparent that the long-term administration of both GnRH agonists and antagonists disrupts reproductive functions in the rat. While the mechanisms of action of the antagonists appear to primarily involve competitive blockade of pituitary GnRH receptors with a subsequent inhibition of gonadotropin (in particular LH) and steroid secretion, the effects of GnRH agonists are more complex, including desensitization to GnRH at the pituitary level (which is more marked for FSH than for LH), a loss of LH gonadal receptors, a decrease in PRL release, and direct (extrapituitary) gonadal actions. Furthermore, an important difference between the effects of GnRH agonists and antagonists resides in the fact that the administration of the antagonists is consistently accompanied by a marked decrease in plasma LH (and, to a lesser degree, FSH) levels, while each daily injection of the agonists causes a measurable (though blunted) increase in LH (but usually not FSH) release.

The time period during which GnRH analogues need to be present in the circulation to significantly alter reproductive parameters represents another element that differentiates their mode of action: GnRH antagonists need to occupy pituitary receptors on a continuous basis (and, therefore, be consistently present in the circulation) in order to be effective; on the other hand, since a large portion of the deleterious effects of GnRH agonists appears to be mediated through pituitary and gonadal desensiti-

(61) Asch, R. H.; Eddy, C. A.; Schally, A. V. *Biol. Reprod.* **1981**, *25*, 963–968.
(62) Segun, C.; Belanger, A.; Cusan, L.; Pelletier, G.; Reeves, J. J.; Lefebvre, F.-A.; Kelly, P. A.; Labrie, F. *Biol. Reprod.* **1981**, *24*, 889–901.
(63) Bambino, T. H.; Schreiber, J. R.; Hsueh, A. J. W. *Endocrinology (Baltimore)* **1980**, *107*, 908–917.
(64) Jones, P. B. C.; Hsueh, A. J. W. *Endocrinology (Baltimore)* **1980**, *107*, 1930–1936.
(65) Hseuh, A. J. W.; Erickson, G. F. *Nature (London)* **1979**, *281*, 66–67.
(66) Jones, P. B. C.; Hsueh, A. J. W. *Biol. Reprod.* **1981**, *24*, 747–759.
(67) Clark, M. R.; Thibier, C.; Marsh, J. M.; LeMaire, W. J. *Endocrinology* **1980**, *107*, 17–23.
(68) Lecomte, P.; Wang, N.-G.; Sundaram, K.; Rivier, J.; Vale, W.; Bardin, C. W. *Endocrinology (Baltimore)* **1982**, *110*, 1–6.
(69) Jones, R. C. *Contraception* **1979**, *20*, 569–578.
(70) Sundaram, K.; Cao, Y.-Q.; Wang, N.-G.; Bardin, C. W.; Rivier, J.; Vale, W. *Life Sci.* **1981**, *28*, 83–88.
(71) Casper, R. F.; Yen, S. S. C. *Science* **1979**, *205*, 408–410.
(72) Dudley, C. A.; Vale, W.; Rivier, J.; Moss, R. L. *Peptides* **1981**, *2*, 393–396.

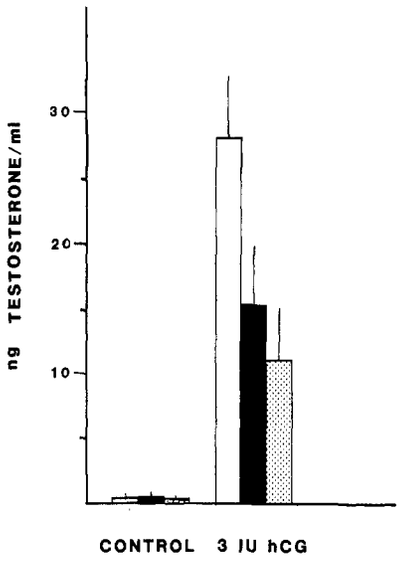

Figure 3. Effect of 1.0 (■) and 3 mg/kg (□) of GnRH antagonist [Ac-Δ^3Pro^1,4ClDPhe^2,DTrp^{3,6}]GnRH injected qd for 7 days to hypophysectomized male rats on hCG-induced testosterone secretion. Each point represents the mean ± SEM of eight animals.

zation, periodic exposures to the peptide are sufficient to disrupt reproductive functions. This may explain at least in part why much larger doses of the antagonists than the agonists are required to be effective.

Over the past years, considerable progress has been made in the design of new GnRH antagonists, and future improvements in potencies may lead to peptides that will be active at much lower doses than those presently available. It may therefore be possible that (even though their antifertility properties are still comparatively low) because GnRH antagonists do not cause the periodic stimulation of gonadotropin and steroid release observed with GnRH agonists, they will represent a major tool in regulating reproductive functions.

At the present time, both GnRH agonists and antagonists have been tested in clinical trials, particularly in situations where a marked lowering of steroid secretion is desired. It should be emphasized that the evaluation of the applicability of what has been learned from animal studies to the control of fertility in humans is still the question of considerable debate. The observation of significant species variability makes it difficult to extrapolate effects observed in laboratory animals to man. Various agonists, delivered subcutaneously or in nasal sprays to human subjects, have been used to block ovulation and induce luteolysis,[73-77] reverse precocious puberty,[78] and to participate in the treatment of the polycystic ovarian syndrome[79] and endometriosis.[80] It should be noted that one of the main possible side effects encountered in the use of GnRH agonists as anovulatory agents in humans is the potential danger of unopposed estrogen, and obviously a large number of additional clinical trials are necessary to evaluate this problem.

In the human male, pulsatile administration of GnRH itself has been successfully applied to the treatment of hypogonadotropic hypogonadism in the human male.[81] On the other hand, the long-term use of GnRH agonists as fertility regulators in man has so far been disappointing, since the (partial) arrest of the spermatogenesis that they induce is usually accompanied by loss of libido.[82,83] The possibility, however, that androgen replacement therapy may overcome this problem has been reported.[84]

It therefore appears that though optimism seems to be shared by most investigators working in this field,[87] one of the main problems that still needs to be solved is the desirability and/or acceptability of providing some combination of steroid therapy with GnRH agonists.

In terms of GnRH antagonists, clinical trials are still few, but the ability of one of the most potent antagonists presently available to significantly suppress gonadotropin release in hypogonadotropic hypogonadal women[85] suggests that such antagonists will also be of use in the regulation of human fertility. One of the main advantages of GnRH antagonists is the very high degree of reproductive function inhibition that they provide, particularly in the male, as well as the absence of the initial stimulatory phase observed with the agonists. It is therefore conceivable that these analogues may represent significant tools in the control of fertility, provided progress is made in terms of their potency.

As can be gathered by the data presented in this paper, the availability of GnRH agonists and antagonists has not only allowed a significantly better understanding of the physiological role played by endogenous GnRH in controlling reproductive functions but has also provided therapeutic tools that may be applied to the control of fertility, as well as to the management of various pathological conditions connected with reproductive functions. At the present time, the advantages offered by one class of analogues over the other in clinical studies are not yet clear, but the continuous improvement in the potency and effectiveness of GnRH analogues strongly suggests that they will represent major tools as regulators of reproductive functions.

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Registry No. LH-RH, 9034-40-6; testosterone, 58-22-0; [DTrp^6,Pro^9-NEt]GnRH, 57773-65-6; [Ac-DNal-(2)^1,4FDPhe^2,DTrp^3,DArg^6]GnRH, 86855-16-5; [Ac-Δ^3-Pro^1,4ClDPhe^2,DTrp^{3,6}]GnRH, 80152-22-3.

(73) Lemay, A.; Labrie, F.; Belanger, A.; Raynaud, J.-P. *Fertil. Steril.* **1979**, *32*, 646–651.
(74) Bergquist, C.; Nillius, S. J.; Wide, L. *Contraception* **1979**, *19*, 497–506.
(75) Lemay, A.; Laure, N.; Labrie, F. In ref 28; pp 184–198.
(76) Schmidt-Gollwitzer, M.; Hardt, W.; Schmidt-Gollwitzer, N.; Vonderone, M. In ref 28; pp 199–215.
(77) Sheehan, K. L.; Yen, S. S. C. In ref 28; pp 237–2141.
(78) Comite, F.; Cutler, G. B., Jr.; Rivier, J.; Vale, W. W.; Loriaux, D. L.; Crowley, W. F., Jr. *N. Engl. J. Med.* **1982**, *305*, 1546–1550.
(79) Chang, J.; Laufer, L.; Meldrum, D.; DeFazio, J.; Lu, J.; Vale, W.; Rivier, J.; Judd, H. *Soc. Gynecol. Invest.* **1981**, 165 (Abstr 287), Proceedings of 1981 Annual Meeting of the Society for Gynecologic Investigations.
(80) Meldrum, D.; Chang, J.; Lu, J.; Vale, W.; Rivier, J.; Judd, H. *Soc. Gynecol. Invest.* 3, **1981**, 3 (Abstr 4).
(81) Crowley, W. F.; Vale, W. W.; Rivier, J.; McArthur, J. In ref 28, pp 321–333.
(82) Rabin, D.; Linde, R.; Doelle, G.; Alexander, N. In ref 28, pp 296–306.
(83) Doelle, G.; Linde, R.; Alexander, N.; Kirchner, F.; Vale, W.; Rivier, J.; Rabin, D. *Int. J. Fertil.* **1982**, *27*(4), 234–237.
(84) Doelle, G. C.; Alexander, A. N.; Evans, R. M.; Linde, R.; Rivier, J.; Vale, W.; Rabin, D. *J. Androl.*, in press.
(85) Cetel, N. S.; Rivier, J.; Vale, W.; Yen, S. S. C. *J. Clin. Endocrinol. Metab.* **1983**, *57*, 62–65.
(86) Wang, N. G.; Sundaram, K.; Pavlou, S.; Rivier, J.; Vale, W.; Bardin, C. W. *Endocrinology (Baltimore)* **1983**, *112*, 331–335.
(87) Steinberger, Z. In ref 28; pp 376–381.