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Dose-related actions of GnRH II analog in the cycling rhesus monkey☆

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Abstract

Introduction: Gonadotropin-releasing hormone (GnRH) II expression, specific high-affinity receptors for GnRH II and its potent bioactivity in human and baboon tissues led us to hypothesize that GnRH II is a bioactive peptide in primates. We recently demonstrated the contraceptive activity of GnRH II analog in rhesus monkeys. In the present experiment, we extended those studies to the dose-related action of this analog on parameters of luteal function and conception.

Methods: GnRH II analog (0–32 μg/day) or saline was administered via osmotic minipumps for 6 days (Days 1–6 postovulation) to regularly cycling rhesus monkeys mated with fertile males around the time of ovulation. Cycle dynamics was monitored through circulating luteinizing hormone, progesterone and estradiol. Pregnancy was determined by circulating chorionic gonadotropin concentrations.

Results: Progesterone production (Days 3–11) was significantly less ($p<.05$) for animals treated with 2, 4 or 8 μg/mL GnRH II analog than for controls, yet with higher doses of GnRH II analog (i.e., 16 or 32 μg/day), luteal progesterone was not different from that of saline-treated controls. The length of the luteal phase in all treated groups was similar to that of controls. In 18 animals mated at the time of ovulation and then treated with GnRH II analog (2–32 μg/day), no pregnancies resulted. In saline-treated controls, five of eight animals (62.5%) became pregnant. Thus, the contraceptive activity of this GnRH II analog did not correlate with luteal progesterone inhibition.

Conclusions: These data demonstrate a dose-related action of GnRH II analog on luteal progesterone and establish the contraceptive activity of 2–32 μg/day GnRH II analog administered postovulation.

Keywords: GnRH II; Progesterone; Conception; Pregnancy; Implantation

1. Introduction

The existence and activity of multiple forms of gonadotropin-releasing hormone (GnRH) in nonmammalian vertebrates have been recognized for many years [1–3]. However, it was not until the 1990s that it was demonstrated that GnRH II (originally named chicken II GnRH) is expressed in vertebrates such as the tree and musk shrew and mole [4–6]. Shortly thereafter, the expression of mRNA for GnRH II in the primate brain was reported [7–9]. We have demonstrated the production of GnRH II by human placenta [10] and baboon ovary [11]. We have also shown that GnRH II and its analogs bind at high affinity to specific chorionic and ovarian GnRH II receptors and effect potent biologic activities. In addition, the expression of mRNA for GnRH II in the human endometrium [12], as well as in human granulosa cells in vitro [13], has been reported.

GnRH II was found to have less than one tenth to one third of the luteinizing-hormone (LH)-releasing activity of GnRH I on the pituitary of rat [14], sheep [15] or monkey [16]. On the other hand, our data demonstrated a potent activity for GnRH II and its analog on the inhibition of ovarian progesterone and the stimulation of placental human chorionic gonadotropin (hCG) production. Similarly, Kang et al. [13] have shown an inhibition of progesterone production from human granulosa cells in vitro. We have also observed that GnRH II binds to two distinct receptors in

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the ovary and placenta. Competition studies for GnRH II binding sites demonstrate that this high-affinity receptor does not bind buserelin or antide (GnRH I agonist and antagonist, respectively), while the low-affinity site binds both GnRH II and I agonists [10]. These findings led us to propose that a specific GnRH II receptor is active in human and nonhuman primates. This hypothesis is supported by Grundker et al. [17], who reported on the potent and specific response to GnRH II in ovarian and endometrial carcinoma cell lines, and by Mongiat et al. [18], who described different GnRH I and II response sites in rat ovarian and pituitary cells. An mRNA for the GnRH II receptor was reported in marmoset [19] and green monkey [20] and in human sperm [21]. However, in humans, the expressed mRNA for the GnRH II receptor has a frame shift and a stop codon in the coding sequence [22]; thus, it is unclear how it is translated.

Our findings of low potency for GnRH II in the direct regulation of pituitary LH, in the presence of specific high-affinity binding for GnRH II in the placenta and ovary, and in the potent action of GnRH II on hormone production in these tissues led us to propose that GnRH II functions in extrapituitary tissues through a specific GnRH II receptor. We propose that GnRH II affects reproductive function without disturbing pituitary function or menstrual cyclicity. To test this hypothesis, we designed a GnRH II analog, which is stable in the presence of blood, ovarian, endometrial and chorionic peptidases [23,24]; avidly binds the GnRH II receptor in the baboon ovary and placenta; and regulates ovarian progesterone and chorionic hCG production [10,11]. Using this GnRH II analog, we have demonstrated its contraceptive activity when administered around the time of ovulation [25]. In the present experiment, we report the dose–response effects of GnRH II analog on luteal function and contraceptive activity in mated cycling rhesus monkeys. The dose-related activity of GnRH II analog (using 0, 2, 4, 8, 16 or 32 μg/day GnRH II analog administered for 6 days on Days 1–6 of the luteal phase) on cycle length, luteinization, implantation and pregnancy outcome was determined.

2. Materials and methods

2.1. Animal procedures

The experiments were performed at the Xinye Primate Breeding Center in Henan province. The protocol was approved by the Academic Committee for Animal Experimentation of the Institute of Zoology of the Chinese Academy of Sciences. Thirty-two rhesus monkeys (*Macaca mulatta*) 5–7 years of age, which regularly cycled (as determined from at least two recorded cyclic bleeds), were used in the experiment. The monkeys were housed in individual cages, fed pelleted feed in the morning and evening, and fed vegetables at noon. On the day before ovulation (Day −1), a female monkey was weighed, introduced for mating into a cage with a reproductive adult male monkey and removed after 48 h (Day +1). Females were randomly and blindly implanted with an osmotic minipump, which delivered either 0, 2, 4, 8, 16 or 32 μg/day GnRH II analog [D-Arg(6)-GnRH II-aza-Gly(10)-NH_2] for 6 days. The primed osmotic pump (Durect Corp., Cupertino, CA, USA) was implanted subcutaneously in the upper back under light ketamine anesthesia. Primed pumps were prepared from vials containing dried aliquots of phosphate buffer or GnRH II analog in phosphate buffer and resuspended with sterile saline to deliver 0, 2, 4, 8, 16 or 32 μg/day GnRH II analog. Minipumps were loaded with GnRH II analog or with saline solution and allowed to prime overnight (16 h) in sterile phosphate-buffered saline, such that implanted pumps would deliver analog or saline at the desired dose starting from the time of implantation and would continue for the next 6 days. Dose-related GnRH II analog delivery was first confirmed in vitro. Primed equilibrated pumps were implanted after a basal blood sample had been collected. The pumps delivered the drug for 6 days, but were left in place for 18 days to limit procedures during the luteal phase or during early pregnancy. Blood samples were collected every other day during those 18 days then every week until menses have returned or until pregnancy has been confirmed. The samples were collected in EDTA (4 mM/mL blood)–bacitracin (50 U/mL blood), processed as plasma and frozen at −20°C until used for hormone assays, as described below. Throughout the experiment, the activities of the monkeys, including cycles and pregnancies, were monitored. Thirty animals were used in data analysis; that is, animals were implanted on Day +1 with osmotic pumps delivering saline ($n=12$), or with pumps delivering GnRH II analog at 2 μg/day ($n=4$), 4 μg/day ($n=3$), 8 μg/day ($n=2$), 16 μg/day ($n=5$) or 32 μg/day ($n=4$) for 6 days.

2.2. Hormonal analyses

Progesterone was measured in monkey plasma using an immunoradiometric Coat-a-Count assay for progesterone (Diagnostic Products, Los Angeles, CA, USA). The standard and samples (50 μL in duplicate) were analyzed according to the described procedure and counted to 2% efficiency using a Packard COBRA gamma counter. The data were calculated using COBRA software, and results were expressed as nanograms per milliliter. The sensitivity of the assay was 0.2 ng/mL, and the coefficient of variation within and between assays was 3.9% and 6.8%, respectively.

Estradiol was measured in monkey plasma using an immunoradiometric Coat-a-Count procedure for estradiol (Diagnostic Product). The standard and samples (100 μL in duplicate) were analyzed according to the described procedure and counted to 2% efficiency using a Packard COBRA gamma counter. The data were calculated using COBRA software, and results were expressed at picograms per milliliter. The sensitivity of the assay was 20 pg/mL, and