SPECIAL ISSUE

Early preclinical work with gonadotropin-releasing hormone analogues

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In this article, I provide a narrative remembrance of the many early proof-of-concept studies that were performed at the Jones Institute for Reproductive Medicine in the late 1980s and early 1990s. A group, led by the late Dr. Gary Hodgen, piloted some of the ways gonadotropin-releasing hormone analogues are now being used clinically. We also put many different early peptide and small molecule (orally active) gonadotropin-releasing hormone antagonists through a battery of tests to explore their effects on male and female reproductive hormones. Most of the compounds we tested never reached the clinic because of various reasons. However, some have and are now making a difference in people's lives. (Fertil Steril Rep® 2023;■:■–■. ©2023 by American Society for Reproductive Medicine.)
Key Words: Nonhuman primate models, proof-of-concept, GnRH analogues, preclinical

Many of the early proof-of-concept studies with gonadotropin-releasing hormone (GnRH) analogues (both agonists and antagonists) were performed by Gary D. Hodgen, Ph.D., Robert F. Williams, Ph.D., and clinical fellows, initially at the National Institutes of Health and then more extensively at the Jones Institute for Reproductive Medicine at Eastern Virginia Medical School in Norfolk, Virginia. Within the Institute, Dr. Hodgen founded the Technology Development Center, which was focused on translating preclinical proof-of-concept studies using nonhuman primate models into patents that could then be licensed to pharmaceutical companies as part of technology transfer deals.

During his 17 years at Eastern Virginia Medical School, Dr. Hodgen was the principal investigator of $258 million worth of sponsored research, with his inventions providing $34 million of patent licensing income for the Jones Institute and the Medical School. He coined the terms first generation, second generation, third generation, and fourth generation GnRH antagonists (1). Unfortunately, he passed away in 2005 after a long illness (2) and could not witness the arrival of the fifth generation orally active GnRH antagonists launched recently.

THE NATIONAL INSTITUTES OF HEALTH DAYS

Early studies demonstrated medical hypophysectomy with daily administration of the GnRH antagonist ([Ac-pClPhe$_1$,pClDPhe$_2$,DTrp$_3$,DArg$_6$,DAla$_{10}$]-GnRH-HCl) specific to the gonadotropin axis (3). In 1986, Collins et al. (4) published research on the acute effects of GnRH antagonist on corpus luteum function and endometrial maintenance. This study confirmed that the GnRH antagonist regimen was uniformly effective in rapidly reducing gonadotropin pulsatility. This diminution in pituitary support led to a precipitous decline in episodic progesterone secretion from the monkey corpus luteum and subsequent induction of premature menstruation.

One early study demonstrated Dr. Hodgen's creative translational approach. He proposed that administering a short course of GnRH antagonist and measuring urinary calcium levels could identify women who lose calcium rapidly and, thus, have a higher risk of osteoporosis (5). He patented the method; however, to my knowledge, it has not been applied in practice.

THE JONES INSTITUTE AND THE TECHNOLOGY DEVELOPMENT CENTER

I joined the Jones Institute as a postdoctoral fellow in 1986 after completing my Ph.D. at Monash University in Melbourne, Australia. My thesis was on the role of β-endorphin in the lactational inhibition of reproduction in sheep and tammar wallaby. At the Jones Institute, my primary role was to work with Dr. Williams and Dr. Donald A Richardson, MD, to explore whether β-endorphin was similarly involved in suppressing reproduction in nursing nonhuman primates.

However, I was quickly pulled into Gary's gravitational field. As such, I

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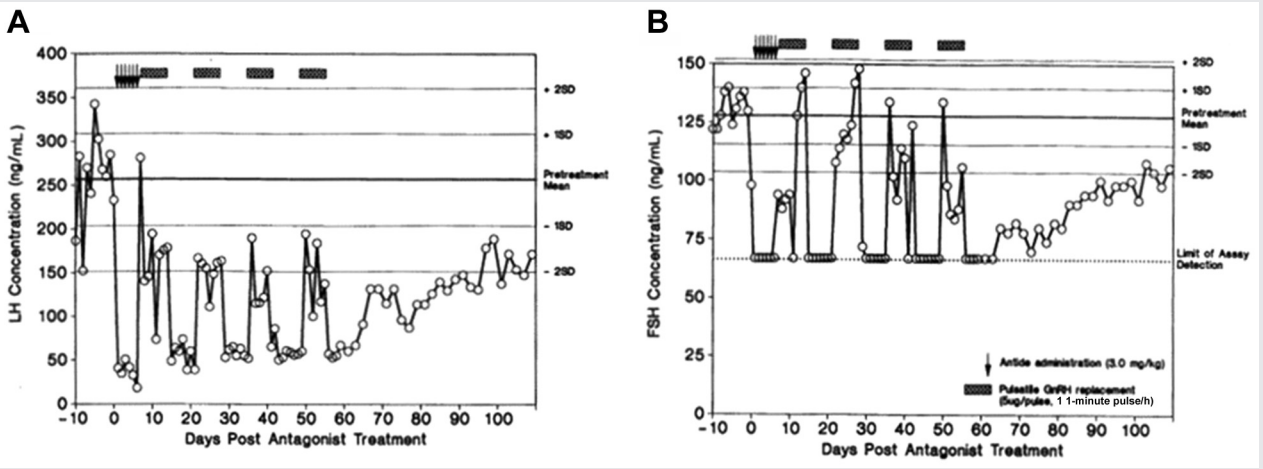
FIGURE 1



Circulating concentrations of (**A**) luteinizing hormone (LH) and (**B**) follicle-stimulating hormone (FSH) in a representative long-term ovariectomized monkey treated with a novel combination therapy of gonadotropin-releasing hormone (GnRH) antagonist (Antide, 3 mg/kg, SC) and pulsatile gonadotropin-releasing hormone (5 μg/pulse, 1 1-minute pulse/h).
SC = sub cutaneously. (From Gordon et al. [10]. Reprinted by permission of the publisher.)

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became a member of the team that conducted a wide variety of exploratory studies with GnRH analogues using the cynomolgus monkey as a model of human reproduction.

One of the first studies I remember was testing whether a recently synthesized GnRH antagonist, then known as Nal-Lys, would inhibit luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in castrated female monkeys. Initial studies were often performed on castrated/ovariectomized monkeys because the levels of LH and FSH rise quickly after removing the ovaries, allowing for easy measurement of the suppressive effects of compounds. The standard practice was to draw blood samples daily for a few days to establish the baseline, administer the test drug, then draw more samples for about a week to catch the inhibition and then recovery. The immunoassay laboratory quantified the FSH and LH levels.

The results were quite unexpected; after 7 days, levels of LH and FSH were still undetectable (1). We went back to the monkeys and drew additional blood samples and eventually documented recovery of LH and FSH levels at 40–50 days. From this, we hypothesized that the prolonged inhibition could be the result of the Nal-Lys GnRH antagonist sequestered in peripheral tissue and slowly released. The GnRH antagonist could be highly resistant to enzymatic degradation and, thus, extensively recycled in circulation or the antagonist may have a noxious effect on the anterior pituitary, the hypothalamus, or both. These results prompted further investigation to determine the mechanism behind such a prolonged duration of action.

Subsequently, Doug Danforth, Ph.D. led the effort to establish a method to measure circulating levels of Nal-Lys (now named Antide) so that we could assess its pharmacokinetic and pharmacodynamic properties. He was also tasked with determining whether there were noxious effects on the pituitary. I was tasked with exploring whether levels and effects changed on the basis of the timing of delivery in the menstrual cycle and whether Nal-Lys behaved more like a GnRH agonist and down-regulated the pituitary receptors.

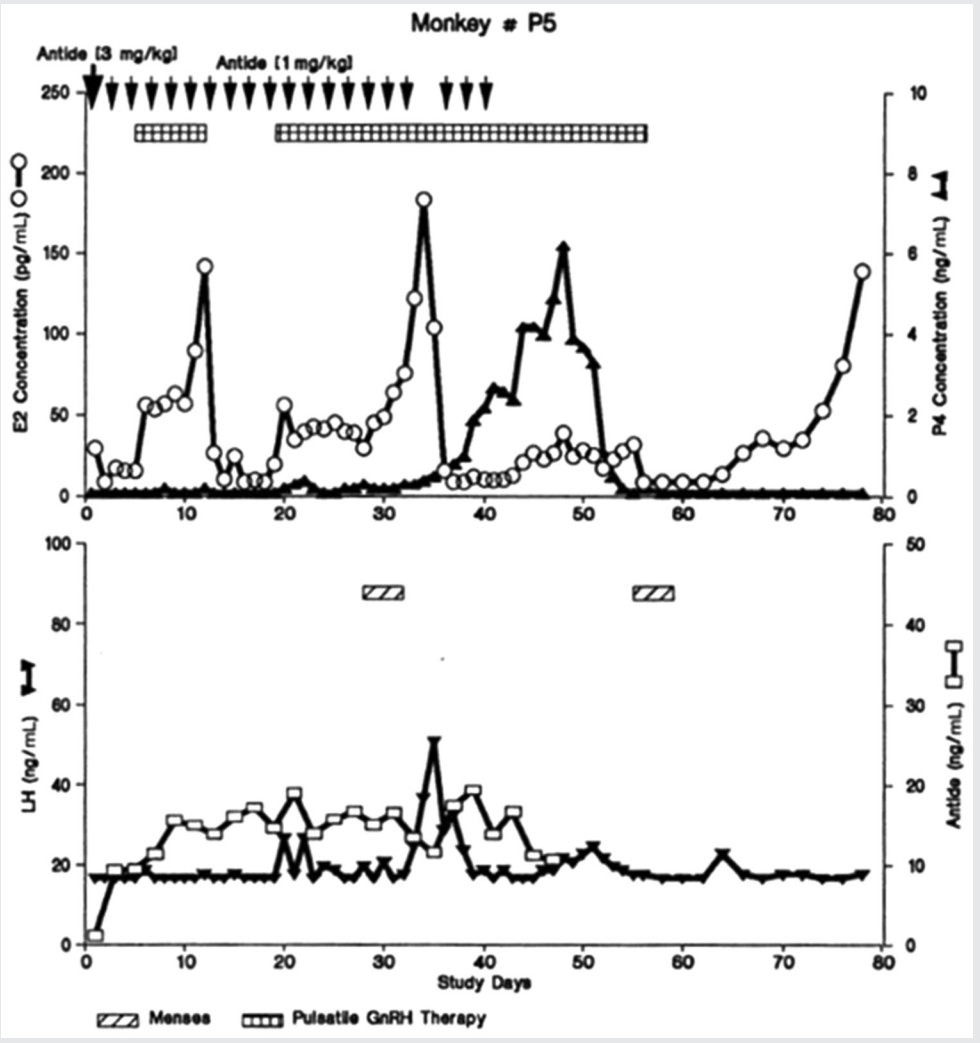
Dr. Danforth developed an in vitro method to examine the direct effects of Nal-Lys on pituitary gonadotrophs (6). He did not observe any evidence of toxic or noxious effects. When sufficient levels were present, the antagonist blocked the release of LH, and when it was removed, the gonadotrophs were immediately responsive to exogenous GnRH. He also adapted this method to quantify levels of Antide (7), and we went on to characterize the inverse pattern of circulating levels vs. serum LH and FSH levels. We discovered that although there was considerable interindividual variation, if levels fell below approximately10 ng/mL, pulsatile secretion of LH would resume.

We characterized the effects of Nal-Lys when given in the follicular and luteal phases (8–10). When administered in the early follicular phase at sufficient doses, it rapidly suppressed LH and FSH and truncated follicular growth. Resumption of folliculogenesis occurred at varying times associated with declines in its circulating levels.

When given in the luteal phase, Antide suppressed serum LH and caused immediate cessation of progesterone secretion associated with luteolysis, with endometrial shedding indistinguishable from menses. Resumption of cyclicity was delayed depending on the dose administered.

We also experimented with the balance between the inhibitory effects of the antagonist and the stimulatory effects of pulsatile exogenous GnRH. In a small series of studies, we demonstrated that serum LH and FSH levels in

FIGURE 2



Circulating concentrations of P4 (▲), E2 (○), Antide (□), and luteinizing hormone (LH) (▼) in a representative intact monkey treated with Antide plus pulsatile gonadotropin-releasing hormone (GnRH) in a sustaining dose regimen composed of an initial 3 mg/kg dose of Antide (↓) with subsequent 1 mg/kg doses administered on alternate days to achieve sustained levels of Antide. Pulsatile GnRH was given at a dose of 5 μg/pulse (1 1-minute pulse/h) from study day 5 until study day 26. Thereafter, pulsatile GnRH was given in a dose of 10 μg/pulse. E2 = estradiol; P4 = progesterone. (From Gordon et al. [10]. Reprinted by permission of the publisher.)

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ovariectomized monkeys could be repeatedly turned off and on when administering pulsatile GnRH after a large bolus of GnRH antagonist fully suppressed the pituitary (Fig 1) (10).

When we employed this approach in monkeys with intact ovaries, the initial inhibitory effects of GnRH antagonist on LH, FSH, estradiol, and progesterone were quickly reversed when we initiated pulsatile GnRH (10 μg/pulse, 1 1-minute pulse/h), resulting in the resumption of follicular growth. Serendipity played a part when the pulsatile GnRH was interrupted owing to technical difficulties and the monkey became fully suppressed again only to have the cycle reactivated once we reinitiated the pulsatile GnRH (Fig 2) (10).

We extrapolated these results and speculated that it may be possible to control the balance between FSH and LH by suppressing the endogenous drive and replacing it with pulsatile GnRH of varying frequencies, anticipating that slower pulses would lead to preferential secretion of FSH and faster pulses would favor LH. We speculated that this may provide a treatment for the LH/FSH imbalance in women with polycystic ovary syndrome.

During this time, the Jones Institute trained numerous reproductive endocrinology fellows, most of whom were required to work on their own research and on the clinical side. Many of the projects involved GnRH analogues in nonhuman primate models, and many of these fellows went on to become leaders in our field.

These include Kevin Winslow, M.D., who characterized the time needed to achieve down-regulation with a GnRH

TABLE 1

Therapeutic applications of gonadotropin-releasing hormone agonists and antagonists.

1. Prostatic carcinoma
2. Endometriosis
3. Precocious puberty
4. Ovulation induction, IVF, and GIFT adjuvant
5. Uterine leiomyomata fibroids
6. Menopausal diagnosis of osteoporosis
7. Chemotherapy prophylaxis
8. Contraception
9. Polycystic ovarian disease
10. Premenstrual syndrome

Note: GIDT = gamete Intra-fallopian transfer; IVF = in vitro fertilization.

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agonist and for the pituitary to recover (11). I still remember the joy on his face when he learned that the abstract he submitted to the American Society for Reproductive Medicine that year had been selected as one of the prize-winning papers.

There was Richard T Scott, M.D., who, among many other studies he completed, worked with me to characterize the pituitary response to a GnRH antagonist (ORG 30850) that Organon was developing and discovered the short-term hyperresponse that occurred when giving a GnRH bolus a few hours after the GnRH antagonist (12). Other fellows included Vish Karande, M.D., who characterized the distribution of radio-labelled Antide in rats (13); John Queenan Jr., M.D., who was involved in the proof-of-concept titration studies documenting that estradiol levels could be individually titrated into a target therapeutic threshold range (14); Frank Irianni, M.D., who studied various novel combinations of GnRH analogue for controlled ovarian stimulation (15); and Michael Edelstein, M.D., who examined the effects of a GnRH analogue in gonadal intact and castrated male primates, finding prolonged activity in males(16, 17).

From these studies, we speculated on the variety of hormonally dependent conditions GnRH antagonists could be applied to (Table 1) (18, 19).

During this time, we became interested in the possibility of titrating monkeys to a therapeutic range of estradiol levels between 30 and 50 pg/mL. This became Dr. Hodgen's "too much, too little, just right 'goldilocks' hypothesis" that would later be enshrined as the Barbieri hypothesis (20). To test this, we gave 5 intact monkeys 0.1 mg/kg/d of Antide. After 14 days, we measured the serum estradiol levels. If the level was <30 pg/mL, we maintained the dose. If the levels were >30 pg/mL, we increased the dose to 0.5 mg/kg/d. On day 14, 2 monkeys were already below the threshold of 30 pg/mL, whereas the other three had their doses increased to 0.5 mg/kg/d, which suppressed levels into the desired range. Levels were fairly stable and fluctuated around the 20–30 pg/mL range for the remaining duration of the study.

Initial results were presented at the Endocrine Society meeting in 1991 (21) but were never published in a full manuscript because of a technology transfer deal with a pharmaceutical company.

We then went on to initiate another very challenging study in 20 monkeys with surgically induced endometriosis in their peritoneal cavities. We quantified the position, size, and number of lesions in each; then, serum estradiol levels were titrated into the therapeutic range of 20–40 pg/mL and held there for six months before reassessing the size and number of endometriotic lesions. Although we never published the full results, it was clear that the study was successful, with lesions shrinking and, in some cases, disappearing. However, the required dose was quite variable, suggesting that it would be impractical in clinical practice. Nonetheless, we demonstrated proof of concept for the funding pharmaceutical company. We went on to test several other GnRH antagonists, including ORG 30850 (Organon, Oss, the Netherlands) (12); Azaline-B; various Takeda Abbot Pharmaceuticals (Chicago, Illinois) compounds, including A75998 (22, 23); and some very early orally active small molecules (24).

Although few of the individual compounds have made it to regulatory approval and clinical use, the concepts tested and their applications are now being successfully employed. It is very gratifying to see so many of these applications now making a difference in people's lives.

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