

Neuropeptides in Human Reproduction

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I. BACKGROUND AND SIGNIFICANCE

Over the past decade, genetic studies in patients with gonadotropin-releasing hormone (GnRH) deficiency, a clinical syndrome defined by low gonadotropin and sex steroid levels (i.e. hypogonadotropic hypogonadism) due to a failure of hypothalamic GnRH secretion, have been instrumental in uncovering many new players involved in the neuroendocrine control of the human reproductive axis. In 2003, our group discovered that mutations in *KISS1R* (also known as *GPR54*), the receptor for kisspeptin, cause GnRH deficiency¹. Further characterization of kisspeptin revealed that it is expressed in the human infundibular (arcuate) nucleus² and that it is one of the most powerful stimulators of GnRH secretion studied to date³.

In 2009, human genetic studies led to the identification of another important regulator of human reproduction, neurokinin B, a neuropeptide of the tachykinin family⁴. The precise role of neurokinin B in the control of GnRH secretion is unclear, as studies have demonstrated either stimulatory or inhibitory effects depending on the hormonal milieu and/or species examined⁵. Intriguingly, the kisspeptin and neurokinin B neural pathways appear to intersect in their control over GnRH secretion as they have been shown to be colocalized in a subset of arcuate neurons across a wide range of species⁶⁻⁸. These arcuate neurons have been named “KNDy” neurons due to their co-expression of three neuropeptides: kisspeptin, neurokinin B, and dynorphin.

Dynorphin is an endogenous opioid peptide that binds to the kappa opioid receptor. Although mutations in dynorphin have not been found in patients with GnRH deficiency, physiologic studies in animal models have suggested that dynorphin is an important mediator of progesterone negative feedback on GnRH secretion. Administration of progesterone to ewes causes an increase in dynorphin levels in the CSF⁹, and administration of an antagonist specific to the kappa opioid receptor blocks the inhibitory effects of progesterone on LH pulse frequency in the ewe¹⁰. Furthermore, postmortem studies in humans demonstrated decreased prodynorphin mRNA expression in postmenopausal women, who lack recent progesterone exposure, compared with premenopausal women¹¹. Thus, kisspeptin, neurokinin B, and dynorphin appear to form a triumvirate controlling the human reproductive axis, yet it is unclear how they interact to orchestrate the pulsatile pattern of GnRH secretion.

We and other investigators have begun to tease apart the KNDy-GnRH network by administering physiologic doses of kisspeptin to healthy subjects¹²⁻¹⁸ and to patients with reproductive disorders. In the current protocol, we propose to use naloxone, an opioid receptor antagonist, to clarify dynorphin's role in modulating GnRH secretion in the human. Naloxone has served as a clinical research tool for a number of investigators, including those within our Unit, interested in understanding: 1) the significance of endogenous opioids in pubertal maturation¹⁹⁻²¹, 2) the neuroendocrine abnormalities in women with hypothalamic amenorrhea (HA)²¹⁻²⁵, and 3) the interface between opioids and sex-steroid feedback in healthy adult subjects^{26,27}.

By using the pharmacologic probe of naloxone, this protocol will enhance our understanding of normal reproductive endocrine physiology and the pathophysiology of reproductive disorders, including those caused by defects in neurokinin B or its receptor. The protocol selects study subjects with hypogonadotropic hypogonadism, to chart the role dynorphin signaling plays in reproduction. The protocol also explores how dynorphin modulates sex steroid feedback loops within the reproductive system.

II. SPECIFIC AIMS

Specific Aim: To determine dynorphin's role in the regulation of pulsatile GnRH secretion.

Hypothesis: Naloxone will increase LH levels in patients with reproductive disorders.

III. SUBJECT SELECTION

The goal of this protocol is to administer naloxone to individuals with reproductive disorders affecting GnRH secretion - hypogonadotropic hypogonadism (both congenital and acquired forms)

Enrollment goal: n=32, in order to have 16 complete the study

Inclusion Criteria

History:

- age 18-75 years
- confirmed diagnosis by either (1) a medical provider, supported by low sex steroids in the setting of low or inappropriately normal gonadotropins or (2) investigator review
- all medical conditions stable
- no condition (medical, mental, or behavioral) or current medication that, in the opinion of a study investigator, would likely interfere with participation in/completion of the protocol or with the interpretation of results
- no prescription medications that affect the opioid pathway (such as oxycodone or naltrexone)
- no active illicit drug use (not including marijuana)

Physical examination:

- systolic blood pressure < 140 mm Hg, diastolic < 90 mm Hg

Laboratory studies:

- negative urine drug screening panel
- hemoglobin
 - men on adequate testosterone replacement therapy: not below the male reference range
 - men off testosterone replacement therapy: no lower than 0.5 gm/dL below the lower limit of the reference range for normal women, as men with hypogonadism off treatment have low serum testosterone levels which causes hemoglobin concentrations to fall to the female range

IV. SUBJECT ENROLLMENT

Recruitment Methods

Participants will be recruited from multiple sources:

MGB (Mass General Brigham) Research Participant Data Registry (RPDR)

We may review identifiable medical records to determine if any patient within the MGB system is a candidate for study participation. Potential subjects who have not opted out of receiving Research Invitations will be contacted directly through letter, either via physical mail or Patient

Gateway. Research Invitations will be sent up to three times with at least two weeks between. A week after the initial Research Invitation is sent, we will call each patient who has not expressed disinterest to ask whether they would like to participate in our study.

Other REU Studies

We will review identifiable records of individuals who previously participated or expressed interest in other research studies in the REU. The study team may contact candidates (via phone, email, letter, or in-person) who have agreed to be contacted about future studies.

- A. If these individuals have an established clinical relationship with any physician investigator on this study, that physician may introduce the study, and recruitment of that candidate will be led by other study team members.
- B. If these potential subjects do not have a clinical relationship with one of the physician investigators, they will first be contacted via letter. Individuals who express interest in the study following the recruitment letter will be contacted by the study staff and recruited under guidance of MGB policies. At least ten business days after the letter is sent, we may call each individual (who did not choose to opt-out) to ask whether they would like to participate in our study.

Individuals seeking clinical care in the Reproductive Endocrine Associates (REA) clinic

We will review identifiable medical records to determine if patients are candidates for study participation. Candidates may be introduced to the study by their REA physician (via phone, email, letter, virtual visit or in-person) and will be recruited to the study by other study team members.

Referrals from non-REA clinical providers

Clinical providers both inside and outside of MGB may introduce candidates to this study orally and may share the study team's contact information with the candidate. With permission from the referred individual, the provider can share the candidate's contact information with the study team to proceed with recruitment.

Advertisements

We may use online and physical postings for recruitment including, but not limited to, MGB Rally, social media, and newsletters so that interested candidates may contact the study team.

Non-English Speaking Candidates

In the event a candidate who is non-English speaking expresses interest in the study, we will work with an MGB interpreter to conduct pre-screening. Provided the candidate passes pre-screening, we will proceed to obtain IRB approval for a full translation of the consent form into the appropriate language. Once that is complete, we will work with a remote or in-person MGB interpreter to conduct the consent process.

Pre-screening

Individuals recruited from MGB sources may have medical records reviewed for eligibility. In order to assess eligibility of all individuals, we may ask pre-screening questions via telephone or REDCap, and may request clinical notes and/or endocrine labs from their Endocrinologist, PCP, or other health care provider. If outside records are requested, candidates will be asked to complete medical record release forms. Identifiable information on individuals who do not enroll may be retained but will not be linked to any collected health information without permission of the individual.

Consent Process

Consent may be obtained either in-person (at Massachusetts General Hospital) or remotely. The consent form is provided for the subject to review prior to the consent process and study participation. Informed consent will be obtained by a licensed physician investigator or by non-physician investigators who are nurse practitioners or physician assistants with backup by a licensed physician.

The consent process will take place prior to the commencement of study procedures and will, in all instances, include a complete explanation of the study by one of the study medical professionals (physicians, physician assistants, or nurse practitioners). Study staff will go over the purpose of the study, study procedures, potential risks of participation, and alternatives to participation. Before signing an approved consent form, potential participants will be given the opportunity to ask any questions that they may have concerning the study. The voluntary nature of participation in these studies will be emphasized. It will be made clear that a decision not to participate will in no way affect the relationship of patient to medical professional, investigator, or institution regarding the ongoing provision of care.

When consent is obtained in-person, the subject will sign the consent form in the presence of the study medical professional after all parts of the consent process are completed. The study medical professional will also sign the consent form at the time of the visit as well.

When consent is obtained remotely, the study investigator will complete the consent process, as described above, over the phone. The study investigator will confirm subject's identity before signature is obtained. The subject and study medical professional will sign the consent form at this time.

REDCap eConsent will be used for all signatures. All electronic logging information and an electronic copy of the consent will be automatically saved by REDCap. Subjects may complete electronic signatures using either their own device or a research-owned iPad.

Finally, a copy of the signed consent form containing all the information outlined above, as well as contact information for the Principal Investigator, will be provided to the study participant in person, via mail, or electronically.

V. STUDY PROCEDURES

General Outline of Protocol

The goal of these studies is to assess the GnRH response to naloxone in patients with hypogonadotropic hypogonadism.

GnRH cannot be measured in the peripheral circulation, so luteinizing hormone (LH) will be measured as a surrogate marker of GnRH secretion. LH is secreted by the pituitary in direct response to GnRH, and there is a well-established one-to-one concordance between GnRH and LH pulses^{28,29}.

Protocol Details

1. Screening

The screening procedures will include a complete medical history, physical examination, and laboratory blood tests to ensure subjects meet the inclusion criteria. The total volume of blood drawn during screening is approximately 15 mL.

Male participants will be asked to fast overnight (at least 8 hours) before the screening blood draw, to ensure accurate testosterone values

Screening laboratory tests

- Reproductive hormones including, but not limited to: Luteinizing Hormone, Follicle Stimulating Hormone, Testosterone
- Hemoglobin (with study medical professional approval, can be based on lab result from medical record, within past 6 months)
- Urine drug screening panel (to ensure no opioid use)

All components of screening may be performed at the MGH REU or remotely. Therefore, some participants might not need an in-person screening visit at MGH.

- The screening medical history may be obtained by phone.
- Documentation of a physical examination performed by a non-study medical professional within the previous 12 months may be used in lieu of an in-person physical examination to determine eligibility.
 - All participants will undergo a physical examination by a study medical professional prior to frequent blood sampling.
- If non-MGB medical records will be obtained for documentation of eligibility, subjects will complete an MGH medical record release form.
- If applicable, remote screening laboratory studies will be performed at a local laboratory site (e.g., Quest Diagnostics, etc.); study funds will pay for all phlebotomy and shipping costs.

If all eligibility criteria are met, a member of the study team will contact the subject to schedule the study visits.

2. Pre-Study Visit Interventions

Sex-steroid and Medication Discontinuation (“washout”)

Subjects on hormonal medication (including oral contraceptives or hormone replacement) or non-hormonal medication that may modulate the reproductive axis may be asked to undergo a washout period appropriate to that particular medication and its method of administration as follows:

- Pulsatile GnRH therapy > 2 weeks
- Gonadotropin injections > 4 weeks
- Transdermal testosterone > 2 weeks
- Testosterone injections > 6 weeks
- If not specified above, a length of time > 5 half-lives of the drug

Subjects will be advised to discuss the washout with their prescribing physician prior to beginning washout. Any subject who experiences intolerable side effects as a result of medication washout will end participation in the study and be instructed to follow recommendations by their prescribing physician regarding resuming any medications. Subjects will be advised to consult with their prescribing physician to reinstate any medications after the study visit is complete.

Any active use of marijuana will be evaluated by a study medical professional to determine if it may impact study participation. Individuals who regularly use marijuana will be asked to abstain from use for two weeks prior to the study visit. ³⁰

3. Study Visit

Frequent blood sampling will be performed over 3 hours. The first 1.5 hours will assess the intrinsic activity of the reproductive axis (baseline), and the second 1.5-hour period will show the response to a naloxone bolus. The study visit may occur at the MGH Translational and Clinical Research Centers (TCRC) or at the Reproductive Endocrine Unit.

All visits will adhere to the following protocol:

- To ensure that measured testosterone values are accurate, male participants will be asked to fast overnight (at least 8 hours) and to remain fasted during the entirety of the study visit.
- Frequent blood sampling: blood will be drawn q10 minutes for three hours.
 - In the event that any sample is not collected at the expected timepoint, it may be skipped or drawn fewer than 10 minutes from another sample, not to exceed 2 samples within the same 10-minute period.
 - The total amount of blood drawn during the visit will be approximately 65mL.
- Naloxone will be given as a 10mg intravenous bolus.
- EMLA cream may be used to ease discomfort from needle insertion.
- Naloxone will be administered by TCRC nursing staff or a study investigator. Vital signs (HR, BP, RR, and T) will be measured prior to beginning frequent sampling.
- Vital signs will be measured within ten minutes following the naloxone bolus. Study procedures will be halted if any of the following criteria are met at any point in the study:
 - Blood pressure > 180 mmHg systolic or > 105 mmHg diastolic
 - Development or suspicion of an allergic reaction or serious adverse reaction
 - Positive pregnancy test
 - Temperature of $\geq 101.5^{\circ}\text{F}$.

Any subject who develops high blood pressure or an allergic reaction will receive medical care until the issue is resolved, with subsequent follow-up as needed.

Justification of Naloxone Dose

Under protocol 88-7174, "Exploration of Central Nervous System Modulators in Hypothalamic Amenorrhea (HA)," investigators in our Unit administered a continuous naloxone infusion (0.8 mg/hour x 6 hours) to 15 women with HA in the MGH Clinical Research Center and found that naloxone increased LH pulse frequency in 80% of the women.²⁴ Importantly, no subjects withdrew from participation in the study and there were no changes in vital signs or other side effects reported. A number of studies have confirmed the safety of naloxone over longer durations and/or at higher doses. Sauder et al.²¹ administered a 21-hour naloxone infusion (1.3-1.5 mg/hour) to women with HA and reported a significant increase in LH pulse frequency. In the same study, a shorter naloxone infusion was administered to 4 children, ages 11-16 years, producing an increase in LH in the pubertal, but not prepubertal, children. No adverse outcomes were reported in the HA or pediatric group. Even higher doses of naloxone (2 mg/hr x 24 hours or 10 mg IV bolus followed by 7 mg/hr x 12 hrs) have also been administered to healthy adult men and women and have been very well-tolerated³¹⁻³³. The maximum amount of naloxone to be given under the current protocol (10 mg bolus) is less than one eighth of the largest amount used in human studies in the literature to date. According to McMurray et al 1991, high-dose naloxone (94 mg = 10 mg bolus followed by infusion of 7 mg/hr x 12 hr) is well-tolerated by healthy adult subjects. There were no changes in vital signs during the infusion and no serious adverse events were

reported. Thus, naloxone has a strong safety record and has been shown to influence GnRH/LH secretion in diverse patient populations.

Incidental Findings, Return of Results, and Electronic Medical Record Documentation

If a study medical professional determines that any clinical test performed during screening has a concerning incidental finding (e.g.: hemoglobin is outside the sex-specific reference range), the study team will inform the subject and advise them to follow up with an appropriate clinical provider. Investigators will inform subjects that they can use the MGB Provider Hotline to obtain a provider if they do not have one.

Results of clinical testing performed for screening may be placed in the subject's medical record. If requested, results of all screening blood work may be provided directly to the subject or to the subject's clinical provider. This study will not generate any genetic information. Genetic sequencing information used in this study originates from the Mass General Brigham Biobank. This study will not release any genetic information to participants or place any genetic information into the medical record. Assay results from frequent sampling will not be provided to subjects. A notation that the subject is taking part in this research study may be made in their electronic medical record.

Use of Samples and Data

Any excess sera from frequent sampling will be coded and stored in the REU lab. Each sample will be given a unique identifying code and may be used for future research. Use of these specimens will be targeted at furthering our understanding of reproductive disorders and improving the management of subjects with these disorders and may include improving existing assays as well as developing new assays. IRB approval will be obtained if samples are used in the future for purposes not described in the protocol.

Samples may be shared with third parties, scientific collaborators, and laboratories. Study data may be shared with scientific collaborators at academic centers. Each collaboration will be driven by clear reproductive research questions and reviewed to ensure the proposed use is consistent with our consent form. When samples or data leave MGB, identifying information will be removed and samples/data will be coded using a unique identifying number. Identifiable data of subjects may be shared with other MGB investigators. Data will be sent to collaborators via secure methods such as secure email or secure file transfer.

Study data may be collected electronically (including via direct data entry into REDCap) or on paper. Electronic information will be stored on secure MGH provided storage systems. Passwords are required to access the information. Paper records are stored in the secure REU, which has restricted access, in double-locked file cabinets/rooms or stored at an offsite storage facility; paper records will also be scanned and stored electronically. After paper records are converted to electronic records the paper copy may be destroyed in compliance with MGB guidance on Electronic Storage of Research Documents. Ongoing access to data is restricted to members of the research team and access to identifiable information is restricted based on staff responsibilities.

Analysis performed under this study may be informed by data collected under other REU studies. All data from this study may be used for ongoing and future research and to better our understanding of reproductive disorders.

VI. BIOSTATISTICAL ANALYSIS

Assays

The assay methodologies employed to characterize the biochemical profile (both reproductive and metabolic) will be based on standard accepted methods as established by the MGH Pathology department and the Reproductive Endocrine Reference Laboratory. These methods may include immunoassays, measurement using standard laboratory platforms, radioimmunoassays, and mass spectroscopy. We anticipate that these methods will continue to evolve as the diagnostics field changes.

Data analysis

The hypothalamic-pituitary axis will be analyzed using the following parameters:

- a) Arithmetic mean levels for LH based on q10 min blood sampling. Pulsatile hormone secretion of LH may be assessed using a modification of the Santen and Bardin Method³⁰ augmented by deconvolution analysis³⁴. If evidence of endogenous LH secretion is present, LH pulse frequency may be determined by calculating the number of LH pulses during the sampling interval. LH pulse amplitude would be defined as the difference between the nadir and peak hormone levels.
- b) FSH and sex steroid levels may be determined using pools created from multiple concurrent timepoints.

To address whether naloxone increases LH levels in various study populations, we will compare mean LH levels before and after the naloxone bolus using paired t-tests.

Power calculation: Informed by the difference in mean LH levels in healthy men and women observed before and during naloxone infusion, we calculate that a sample size of 8 subjects would provide 90% power to detect a significant difference in mean LH before and after the naloxone bolus.

VII. RISKS AND DISCOMFORTS

The risks involved with this study are associated with the administration of study drug and frequent blood sampling.

Blood Draws and IV Sites:

There may be discomfort and/or pain associated with the placement of IV lines and a small risk of infection, bruising, thrombosis, and irritation at blood draw sites, including at sites of IV line placement. There is a slight chance that an IV line placed will fail. In the event this occurs, a new IV line will be placed. Participants might experience dizziness or faint in association with the placement of and blood drawing from IVs. IVs are generally covered/stabilized with some form of adhesive tape or dressing during the study visit. Participants might experience temporary skin irritation or discomfort from these adhesive products.

EMLA Cream

Repeated doses of EMLA Cream may increase blood levels of lidocaine and prilocaine, although systemic adverse reactions following appropriate use of EMLA Cream are unlikely due to the small dose absorbed. There have been reports of slight reddening, swelling, discoloration, rashes,

and hyperpigmentation at the application site, but these cases were generally mild and transient. No serious reactions have been reported.

Naloxone:

Naloxone has no known side effects in healthy adults. In opioid-dependent individuals, naloxone may produce symptoms of acute opioid withdrawal such as pain, agitation, diaphoresis, and hypertension. To prevent such a reaction, all subjects will be screened and excluded for evidence of illicit drug use. Subjects will be closely monitored after the naloxone bolus.

Medication Washout

Subjects who discontinue the use of their current medications may experience the return of symptoms that are controlled or mitigated by these medications. However, we do not expect our participants to experience a prolonged recurrence of symptoms, and we expect any short-term symptoms to be rapidly reversible. Subjects who experience intolerable side effects as a result of medication washout will end participation in the study.

VIII. POTENTIAL BENEFITS

There are no direct benefits to subjects that are anticipated from participating in this study. The only benefit comes from the altruistic contribution to furthering our understanding of the biology of human reproduction.

IX. MONITORING AND QUALITY ASSURANCEAdverse Event (AE) Documentation/Review:

The terminology from NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0 will be utilized for AE documentation. AEs that occur from enrollment (signing of consent form) until the end of study procedures will be recorded.

In addition to contemporaneous documentation and review of AEs by the Study Investigators as they occur, PI-initiated safety monitoring formally occurs at recurring quarterly internal meetings (4 or more times per year), when AEs are reviewed by the PI and multiple study Co-Investigators.

Adverse Event (AE) Reporting:

The Principal Investigator will report adverse events to the MGB IRB in accordance with MGB IRB guidelines.

Unanticipated Problem Reporting:

As per MGB IRB guidelines, the Investigator will report Any Unanticipated Problem Involving Risks to Subjects or Others (including related, unanticipated, Serious Adverse Events) to the MGB IRB by telephone, fax, or email within 5 working days/7 calendar days of becoming aware of the problem.

Monitoring:

The Principal Investigator is responsible for oversight of the trial. Clinical research coordinators and program managers regularly monitor collected source data and regulatory documentation for accuracy and completeness. All protocol deviations are documented and reported in accordance with MGB IRB guidelines. The PI will conduct interim monitoring of accumulated data from research activities to assure the continuing safety of participants, relevance of the research

question, appropriateness of the study design and statistical analysis, and integrity of the accumulating data.

The study will also be monitored by an independent Data Safety Monitoring Board.

The DSMB responsibilities will include:

- Review of the research protocol, informed consent documents and plans for safety and data monitoring of the study. This is to determine the risks and benefits to research subjects, protection and safety of the subjects and to offer suggestions for improving the study design.
- Review of all adverse events (expected, unexpected and serious), enrollment, and dropout rates and protocol deviations. This is conducted to ensure subject safety. The Board will be provided with reports of serious adverse events as they occur and may request additional information from the Investigator in the course of its review.
- Evaluation of the progress of the trial, including periodic assessments of data quality/completeness, recruitment goals, protocol adherence, accrual and retention of participants and other factors that may affect the study outcome. Protect confidentiality of the study participants, trial data and results of the monitoring.
- Weighing efficacy and adverse events to determine if the trial should continue as originally designed, should be changed or should be stopped based on the data.

A copy of the minutes will be distributed to the DSMB members, the Principal Investigator and the IRB. The minutes will reflect which of the following three determinations the DSMB may make as a result of its review: 1) the study may continue without modification/amendment; 2) the study should be amended; 3) the study should terminate.

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