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κ Agonists as a novel therapy for menopausal hot flashes

Amy E. Oakley, PhD,[1] Robert A. Steiner, PhD,[1,2] Charles Chavkin, PhD,[3] Donald K. Clifton, PhD,[2] Laura K. Ferrara, MA,[2] and Susan D. Reed, MD, MPH[2]

Abstract

Objective: The etiology of postmenopausal hot flashes is poorly understood, making it difficult to develop and target ideal therapies. A network of hypothalamic estrogen-sensitive neurons producing kisspeptin, neurokinin B and dynorphin—called KNDy neurons—are located adjacent to the thermoregulatory center. KNDy neurons regulate pulsatile secretion of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH). Dynorphin may inhibit this system by binding κ opioid receptors within the vicinity of KNDy neurons. We hypothesize that hot flashes are reduced by KNDy neuron manipulation.

Methods: A double-blind, cross-over, placebo-controlled pilot study evaluated the effects of a κ agonist. Hot flash frequency was the primary outcome. Twelve healthy postmenopausal women with moderate to severe hot flashes (aged 48-60 y) were randomized. Eight women with sufficient baseline hot flashes for statistical analysis completed all three interventions: placebo, standard-dose pentazocine/naloxone (50/0.5 mg), or low-dose pentazocine/naloxone (25/0.25 mg). In an inpatient research setting, each participant received the three interventions, in randomized order, on three separate days. On each day, an intravenous catheter was inserted for LH blood sampling, and skin conductance and Holter monitors were placed. Subjective hot flash frequency and severity were recorded.

Results: The mean (SEM) hot flash frequency 2 to 7 hours after therapy initiation was lower than that for placebo (standard-dose κ agonist, 4.75 [0.67] hot flashes per 5 h; low-dose κ agonist, 4.50 [0.57] hot flashes per 5 h; placebo, 5.94 [0.78] hot flashes per 5 h; $P = 0.025$). Hot flash intensity did not vary between interventions. LH pulsatility mirrored objective hot flashes in some—but not all—women.

Conclusions: This pilot study suggests that κ agonists may affect menopausal vasomotor symptoms.

Key Words: Hot flash etiology – Luteinizing hormone pulses – Kisspeptin – κ Agonist – KNDy neurons.

Neuroendocrine factors that trigger hot flashes are not well understood, but estrogen-sensitive circuits in the brain generate these phenomena.[1] Research in the late 1970s suggested that hot flashes temporally correlate with pulses of luteinizing hormone (LH); it was hypothesized that the same mechanism driving hot flashes also triggers episodic LH secretion.[2] Pulsatile LH secretion from the pituitary is driven by intermittent secretion of gonadotropin-releasing hormone (GnRH) from the brain.[3,4] However, hot flashes are clearly not dependent on pulsatile LH because they occur even in its absence.[5] Rather, it seems that the same upstream afferent input responsible for driving GnRH neurons is responsible for triggering hot flashes. We hypothesize that hot flashes and LH pulses have the same source generator but are not causally linked to each other.

Recent evidence suggests that pulsatile GnRH secretion is governed by a network of estrogen-sensitive neurons in the hypothalamic arcuate (infundibular) nucleus, which express kisspeptin, neurokinin B (NKB), and dynorphin.[6,7] These so-called KNDy (kisspeptin, NKB, and dynorphin) neurons also express the key isoform of the estrogen receptor (estrogen receptor-α),[8] and KNDy neurons are primary targets for the estrogen-dependent regulation of GnRH and gonadotropin secretion.[9-11] Moreover, it seems probable that KNDy neurons drive the pulsatile secretion of GnRH and LH, as evidenced by the fact that blockade of kisspeptin signaling in the brain inhibits GnRH pulses.[12] If bursts of KNDy neuronal activity evoke the pulsatile release of GnRH and LH, it is plausible that they also drive hot flashes.[13,14]

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From the [1]Department of Physiology and Biophysics, University of Washington, Seattle, WA; [2]Department of Obstetrics and Gynecology, University of Washington, Seattle, WA; and [3]Department of Pharmacology, University of Washington, Seattle, WA.

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Address correspondence to: Susan D. Reed, MD, MPH, Department of Obstetrics and Gynecology, University of Washington, Box 356460, Seattle, WA 98195. E-mail: reeds@uw.edu

Pulsatile activity of KNDy neurons is controlled by an interplay of NKB and dynorphin (the principal endogenous hypothalamic κ agonist) acting in a reciprocal fashion to generate pulsatile events—intermittently stimulated by NKB and suppressed by dynorphin (Fig. 1).[15-18] Several recent lines of evidence support this model. First, NKB provides stimulatory regenerative feedback via NK3R (the NKB receptor) located on KNDy neurons, forcing rapid activation of GnRH and LH.[17] Second, κ agonists bind the dynorphin receptor (also referred to as κ opioid receptor [KOR]) and inhibit LH pulses, presumably by interfering with kisspeptin and GnRH signaling.[15] Third, KOR antagonists stimulate LH pulse frequency, probably mediated through increased kisspeptin and GnRH release.[19-21] In addition, we have observed clinically that women on long-term opioid (such as methadone) treatment are amenorrheic and rarely complain of hot flashes at menopause. Importantly, the network of KNDy neurons governing pulsatile GnRH secretion also projects to hypothalamic areas containing circuits that control thermoregulation.[14,22] Thus, it is conceivable that if KNDy neurons were in a state of super-activation (as is the case in menopause),[23] they could disrupt baseline thermoregulation and trigger hot flashes, as has been proposed.[14,24] If kisspeptin neurons trigger hot flashes, it might be possible to inhibit hot flashes pharmacologically with κ agonists by inhibiting the neuronal activity of KNDy neurons.[15]

Unfortunately, pure κ agonists—when administered systemically to humans—cause dysphoria and nausea,[25] making them suboptimal choices for the treatment of hot flashes. However, fortunately, a new class of peripherally restricted κ agonists (PRKAs), whose access to the brain is restricted by the blood-brain barrier,[26] are undergoing development for the management of peripheral pain disorders. KNDy neurons reside in the arcuate (or infundibular) nucleus, an area of the hypothalamus that lies at the junction of the blood-brain barrier.[27] Thus, it may be possible to extinguish KNDy neuronal activity (and hot flashes) with PRKAs and avoid unwanted central adverse effects.[28] However, these agents are not yet approved by the Food and Drug Administration (FDA). Several PRKAs are undergoing phase 2 trials for irritable bowel syndrome[29] and postsurgical pain management[30,31] and display excellent adverse effect profiles but were not yet available for our pilot study.

As an alternative, we investigated whether pentazocine (a pure K agonist for pain) could reduce hot flashes in postmenopausal women. Although a pure κ agonist is not an ideal treatment for women with menopausal symptoms owing to adverse effects, a decrease in the frequency of hot flashes in women treated with a κ agonist would provide a proof of concept that κ signaling is important in the regulation of human thermoregulation and that blockade of κ receptors with a more ideal molecule (eg, PRKA) might well decrease hot flashes without untoward adverse effects. Using an inpatient, double-blind, randomized, placebo-controlled crossover pilot study, we administered a low-dose κ agonist, standard-dose κ agonist, or placebo to women with hot flashes.

METHODS

Study design, inclusions, exclusions, and primary endpoint

Women were eligible for the study if they were healthy (with no major medical illnesses), were aged between 45 and 60 years, had more than 12 months of amenorrhea, intact

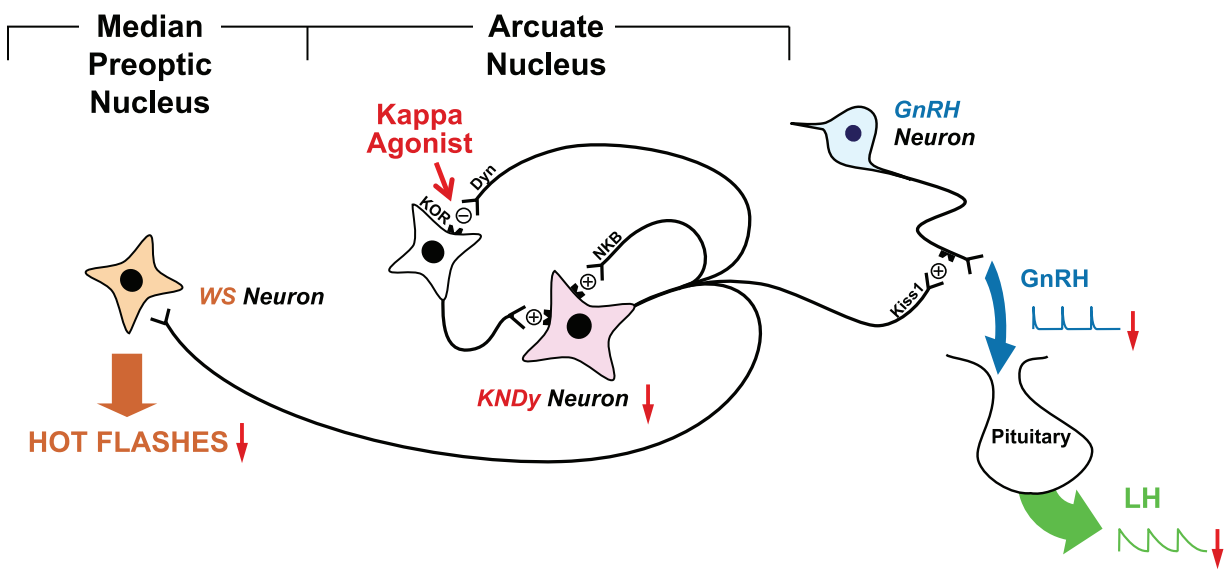

FIG. 1. A model of gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) pulse generator comprising kisspeptin, neurokinin B and dynorphin (KNDy) and other neurons within the arcuate nucleus. Based on this model, KNDy neurons also connect to warm-sensing (WS) neurons in the median preoptic nucleus to drive hot flashes. Activation of κ opioid receptor (KOR) signaling in the arcuate nucleus with a κ agonist could block GnRH/LH pulses and hot flashes. Dyn, dynorphin; Kiss1, kisspeptin; NKB, neurokinin B.

uterus and ovaries, 56 or more moderate to severe hot flashes during 1 week of baseline monitoring using daily diaries, and had a family member or friend available to drive them home after clinic visits. Women were excluded if they were using hormonal prescription medications or supplements for vasomotor symptoms; had taken narcotics, serotoninergic medications, gabapentin, monoamine oxidase inhibitors, antiepileptics, anticholinergics, or sedatives; had a history of polycystic ovarian syndrome; had depression; or had any chronic or acute medical illnesses (including renal diseases, hepatic diseases, pulmonary diseases, or seizures). They were excluded if they had follicle-stimulating hormone (FSH) levels lower than 20 mIU/mL, current substance abuse, severe corn allergy, or known allergic reaction to pentazocine or naloxone. Data collected from women who were accepted in the study were excluded from analysis if zero to two hot flashes occurred during 7 hours of monitoring on the day of placebo treatment. The primary endpoint was the frequency of hot flashes between 2 and 7 hours after ingestion of the first dose of the study drug.

Study recruitment

Women were recruited via flyers placed at community centers, local markets, gymnasiums, and the University of Washington Medical Center (after University of Washington institutional review board approval). Interested women called the study hotline and scheduled a screening visit with the study coordinator (Fig. 2). At telephone screening, verbal consent was obtained and demographic information (including date of birth, sex, and race) was recorded. The study coordinator received calls from 65 interested women. Of those, 39 women were potentially eligible and were mailed hot flash diaries to complete at home.

Consent and randomization

A written informed consent form was obtained from each woman on the day of her first study visit, and her treatments were randomized to pentazocine/naloxone 50/0.5 mg (standard dose), pentazocine/naloxone 25/0.25 mg (low dose), or placebo in a 1:1:1 ratio. Each participant served as her own control, and the study drugs were given in random order on three separate days. Randomization was achieved using a scheme developed by the University of Washington Investigational Drug Service with the Excel random number generator. Access to the randomization code was strictly controlled by the research pharmacy, and packaging and labeling of test and control treatments were identical to maintain double-blind conditions. Randomization occurred between January 1, 2013 and June 1, 2013.

Study drug

An Investigational New Drug application was filed with the FDA and approved with a treatment indication of menopausal hot flashes (IND number 116715). Pentazocine 50 mg/naloxone 0.5 mg (generic Talwin NX), manufactured by Watson Laboratories (Corona, CA), is used as an oral analgesic. For

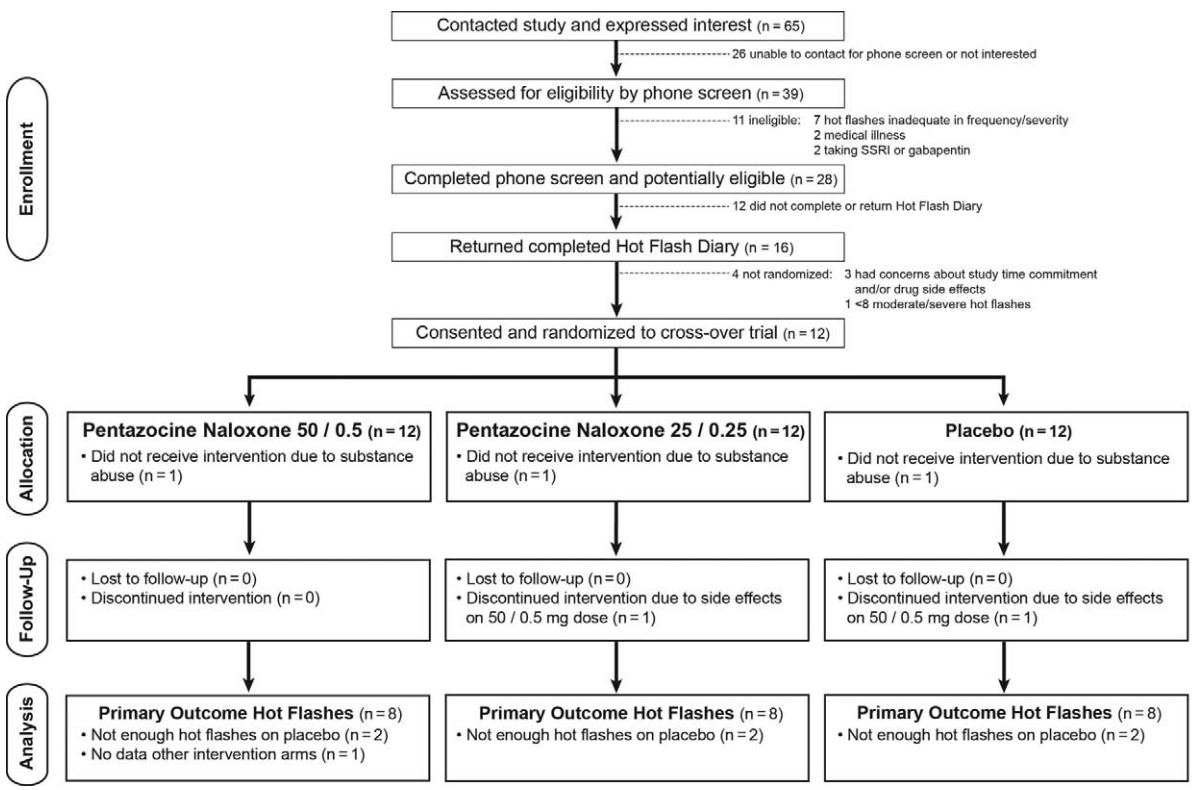

FIG. 2. Consolidated Standards of Reporting Trials flow diagram. SSRI, selective serotonin reuptake inhibitor.

blinding purposes, the pentazocine/naloxone tablet or half tablet was overencapsulated by the University of Washington Investigational Drug Service in an opaque gelatin capsule (Gallipot capsule, size 1, green). The capsules containing placebo or half tablet were backfilled with cornstarch NF. Analgesic onset is 15 to 30 minutes after oral administration, with a mean plasma half-life of 3.6 hours. Typical doses for pain are one to two tablets (50/0.5 mg) every 3 to 4 hours. Peak concentrations are observed 1 to 3 hours after administration.

Data collection

Demographic and health data were collected during eligibility screening. Blood pressure, heart rate, temperature, height, weight, and blood (for FSH and estradiol serum concentrations) were obtained at the first study visit. Participants were monitored in an inpatient research clinic setting on three separate days for each intervention (mean [SEM], 6.8 [2.2] d between visits 1 and 2; 4.6 [0.9] d between visits 2 and 3; overall range, 1-21 d between visits). At each study visit, an intravenous catheter was inserted, blood samples were drawn (3 mL), and a Bahr skin conductance monitor[32] and a Holter monitor were placed. The first drug dose (active or placebo) was administered (mean [SEM], 8.1 [12] min after arrival), and blood samples (24 samples at 1.5 mL each) were collected for LH measurements at 20-minute intervals. A second dose of the same study drug initially administered was given approximately 3.5 hours after the first dose. In addition, each participant pushed a button on the Bahr monitor when she sensed a hot flash, and she recorded the occurrence and severity of the flash in a diary. Adverse events were recorded by the registered nurse and also reported by the study participant at each subsequent visit or via telephone message to the study coordinator.

Outcomes

Primary outcome: hot flash frequency

Hot flash occurrences were assigned by investigators (A.E.O., S.D.R., and R.A.S.) without knowledge of the intervention arm. Only the last 5 hours of hot flash data during the sampling window was analyzed, as it was assumed that 2 hours would be required for the study drug to reach steady state after the first treatment (based on pharmacokinetics). A hot flash was defined by a combination of subjective and objective criteria. If no subjective indication was identified on the diary but there was a clear increase in skin conductance, we assumed that the participant either fell asleep or failed to report the hot flash. If there was a presumed hot flash by defined subjective and objective criteria but the investigators were not unanimous in the assignment of a hot flash, additional evidence on LH pulse occurrence was used in deliberations.

Secondary outcomes: LH pulse frequency and hot flash intensity

LH samples were taken during the 7-hour study period, and frequency of pulses was analyzed during the 2- to 7-hour period after the study drug was given. The DC3 algorithm was used to detect the occurrences of LH pulses.[33] Hot flash intensity was rated by the participant on the hot flash diary at the time as the hot flash occurrence, using a scale of 0 to 3 (0, *not at all*; 1, *a little*; 2, *moderately*; 3, *a lot*).

Radioimmunoassays

All hormone assays were performed in duplicate at the University of Virginia Ligand Assay Laboratory (Charlottesville, VA) using kits from Siemens Medical Solutions Diagnostics (Los Angeles, CA): (1) IMMULITE Human LH (catalog number LKLH5); (2) IMMULITE Human FSH (catalog number LKFS1); and (3) Radioimmunoassay Human Estradiol (catalog number TKE21). Reportable human hormone assay ranges were as follows: LH, 0.1 to 200 mIU/mL; intra-assay coefficient of variation, 4% (three assays); FSH, 0.1 to 170 mIU/mL; estradiol, 12.3 to 1,700.0 pg/mL.

Statistical analysis

Because hot flashes and LH pulses are discrete events, their frequencies during short intervals cannot be expected to be normally distributed. To circumvent this problem, we analyzed the results using Friedman analysis of variance, which provides a nonparametric repeated-measures test across treatments. We tested the effects of generic pentazocine/naloxone on the frequency of hot flashes and frequency of LH pulses. Standard parametric repeated-measures analysis of variance was used to test for the effects of generic pentazocine/naloxone treatment on hot flash intensity and mean levels of LH. $P < 0.05$ was considered statistically significant.

RESULTS

Sixteen women completed hot flash diaries for a week and mailed them back to the study coordinator (Fig. 2). Three of 16 women did not enroll because of study time commitment or concern about adverse effects of the drug. One of 16 women was ineligible because she did not meet the inclusion criterion of 56 or more moderate to severe hot flashes per week. Of the 12 randomized women, one was found to be ineligible after consent and randomization (only after consent did the research nurse have permission to look at her medical records, which revealed substance abuse). Two women provided data on study drug tolerance but were not included in the hot flash analysis because of low baseline hot flashes on placebo. A fourth woman provided data for one study visit but withdrew because of adverse effects from the study drug, leaving eight women who completed the three interventions (placebo, low-dose pentazocine/naloxone, and standard-dose pentazocine/naloxone) and had sufficient baseline hot flashes for analyses while on placebo.

Among the eight eligible and randomized women who completed all visits, the mean age was 55 years (range, 50-60 y); 50% of the women were white, and all were postmenopausal (>12 mo of amenorrhea and FSH >35 mIU/mL; mean [SEM], 77 [10] mIU/mL). Seven women had estradiol levels below the detectable limit of the assay (12.3 pg/mL),

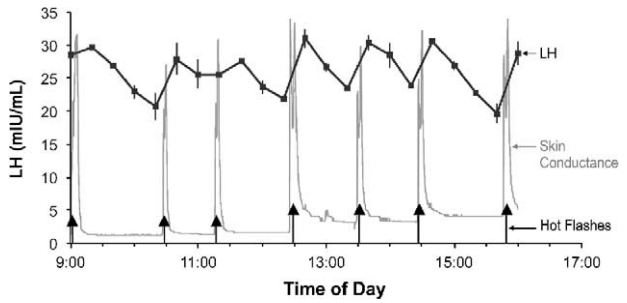

FIG. 3. Luteinizing hormone (LH) pulse patterns with skin conductance (objective) hot flashes in a postmenopausal woman treated with placebo. Filled squares, 20-minute blood samples assayed for LH; arrows, subjective hot flashes.

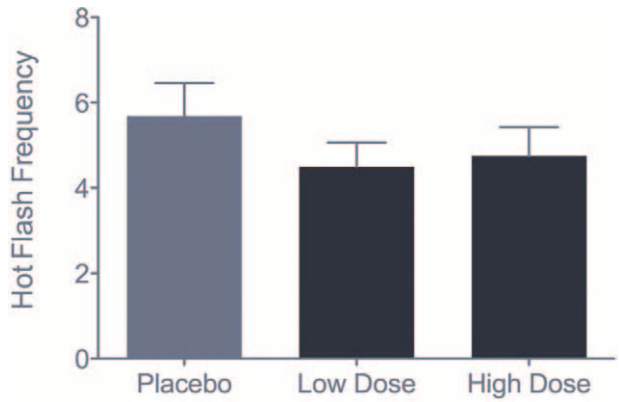

FIG. 4. Mean (SEM) hot flash frequency 2 to 7 hours after initiation of generic Talwin NX (κ agonist) treatment among postmenopausal women with moderate to severe hot flashes. Frequencies of hot flashes differed between placebo, low-dose, and standard-dose κ agonist groups ($P = 0.025$). Analyses were performed using Friedman analysis of variance.

and one woman had an estradiol level of 17.0 pg/mL. Baseline LH level was 24.6 mIU/mL (SE, 3.4 mIU/mL). The median body mass index (BMI) was 27.4 kg/m^2 (range, 22.9-52.3 kg/m^2).

There was a strong association between increased skin conductance and subjective occurrence of a hot flash, and these parameters were frequently associated with pulses of LH in some—but not all—women (Fig. 3). In some women, particularly those with very high LH levels and/or very frequent hot flashes, the putative association between LH pulses and hot flashes was not apparent. Treatment with pentazocine/naloxone significantly reduced the frequency of hot flashes by approximately one hot flash per 5 hours (~20%) relative to placebo ($P = 0.025$) in the 2- to 7-hour period after ingestion of either the low-dose study drug or the standard-dose study drug (Fig. 4). Pooled analyses of all women taking pentazocine/naloxone showed benefit of hot flash frequency over placebo ($P < 0.05$). Pentazocine/naloxone had no significant effects on the mean LH serum concentration or the reported intensity of hot flashes (data not shown). Treatment effect, LH baseline, and LH pulsatility did not seem to vary by age, number of years from the final menstrual period, or BMI, although conclusions cannot be drawn with such small numbers.

Adverse effects of pentazocine/naloxone were noted in some women, but five of six adverse events occurred in association with the standard dose. Emesis and dysphoria were the most common adverse effects among women with lower BMI. Low-dose generic pentazocine/naloxone was relatively well-tolerated, with only one woman reporting mild headache.

DISCUSSION

We demonstrated that a brief exposure to pentazocine/naloxone (a κ agonist) seems to reduce hot flash frequency in postmenopausal women, providing an empirical rationale that PRKAs—a new class of drugs not yet approved by the FDA—may provide relief for menopausal hot flashes. We propose that PRKAs, particularly when given for a longer duration of time than the κ agonists used in this study, may prove beneficial for menopausal hot flashes.

Administration of a κ agonist seems to immediately reduce hot flashes; the effect was modest (20% reduction) yet statistically significant within the 2- to 7-hour time frame from the time of the initial dose. One would not anticipate an immediate large effect because it takes standard-dose estrogen therapy (the gold standard for hot flash treatment) several weeks to reduce hot flashes by 60% to 80%. Full effect for lower doses is not achieved for at least 5 to 6 weeks, sometimes even up to 8 weeks.[34] Selective serotonin reuptake inhibitors may act more rapidly but have lower efficacy than standard-dose hormone therapy.[35] We would infer that a more prolonged treatment with any κ agonist might likewise be necessary to exert its full potency. Some of the participants in our study had hot flashes for up to 5 years. Such women can be presumed to have hypertrophied KNDy neurons[23]; by inference, sustained inhibition across days (or weeks) may be required to diminish hot flashes under these circumstances.

We do not promote the use of pure κ agonists for hot flashes because adverse effects preclude their use. However, untoward effects from PRKAs, in human studies thus far, seem few. Comparatively, nonhormonal medications for treating hot flashes, such as clonidine, selective serotonin norepinephrine reuptake inhibitors (eg, paroxetine, venlafaxine, desvenlafaxine, citalopram, and escitalopram), gabapentin, and pregabalin, may elicit intolerable adverse effects (including drowsiness, nausea, diarrhea, headaches, dizziness, sleep disturbances, agitation or anxiety, and fatigue), leading to discontinuation in up to 5% of women.[1,36-41] However, in nondepressed postmenopausal women, recent trials of venlafaxine and escitalopram did not show adverse effect profiles statistically different from that for placebo.[35,42]

We anticipated finding reduced LH pulse frequency associated with pentazocine/naloxone treatment in all women; however, that was not the case. There was a strong association between increased skin conductance and subjective occurrence of a hot flash, and these parameters were frequently associated with pulses of LH in some—but not all—women,

as reported previously.[43] This apparent coupling either was obscured by a higher LH baseline or simply did not exist. Given the relatively small number of participants studied, we may have lacked sufficient statistical power to identify an effect. It is also conceivable that in women with high-frequency hot flashes, there may have been insufficient time to observe hormone decay given the half-life of LH (20 min). In addition, it may be difficult to immediately suppress LH levels in individuals in whom there have been unrestrained gonadotropin secretion and gonadotropic hypertrophy. Finally, although we propose that the primary driver of menopausal hot flashes occurs via the KNDy neuron pathway, as with other complex physiologic processes, there may be several pathways governing this process.

There are strengths and limitations to this pilot study. The study was rigorously executed with hypotheses, outcomes, inclusion and exclusion criteria, and power determined before analysis. Pentazocine is a relatively pure selective KOR agonist. Naloxone is added to all FDA-approved formulations of oral pentazocine to discourage inappropriate intravenous injection abuse.[44] Naloxone, a μ-opioid receptor antagonist, produces a rapid onset of withdrawal symptoms and perhaps menopausal hot flashes[45] when delivered intravenously. However, when delivered at the oral doses used in this study, it is essentially biologically inert. Unlike intravenous naloxone, which has central nervous system effects, the effects of oral naloxone are primarily limited to the gut because marked metabolism of oral naloxone during hepatic first pass results in low systemic bioavailability[46] and precludes a central nervous system effect. Randomization was securely executed, and blinding of participants, study nurses, and analysts to treatment arms was assured. The use of modern hot flash monitoring techniques and serum LH sampling at 20-minute intervals in eight postmenopausal women permitted corroboration of previously published reports that hot flashes frequently occur in association with pulses of LH secretion[2,5]; LH sampling at 10-minute intervals might have improved detection of LH pulsatility. Subjective and objective hot flashes did not always correlate, suggesting either insensitivity of the monitor or lack of hot flash recognition by the participant; however, assignment of whether a hot flash occurred was performed blinded to treatment arm. If misclassification occurred, it would have been unbiased. Importantly, the study was performed only during the day; thus, effects on night sweats were not assessed and the duration was short (7 h)—a period far short of the weeks required by the FDA to show the clinical efficacy of medications for hot flashes.[34] Although our results are promising and indeed surprising, it is unclear whether a longer treatment duration would achieve the desired effect of at least a 60% (and preferably 80%) reduction in bothersome hot flash frequency. Ideally, all randomized women in our study would be included in the analyses, regardless of hot flash frequency on placebo day; however, given that this was a pilot study, including women without hot flashes at baseline might have diminished our ability to detect a signal, if it existed. A post hoc analysis including all women did not change our findings. The small sample size may have precluded the ability to detect an effect of the κ agonist on LH secretion. Poor tolerance of the standard dose of the study drug resulted in the dropout of one participant. Lastly, we chose a pure κ agonist, but evidence suggests that even "pure" κ agonists have μ effects.

CONCLUSIONS

The physiologic effects of currently used nonhormone therapies on thermoregulation are poorly understood, and their effects on hot flashes are modest, at best, in studies up to 12 weeks. Hormone therapies for hot flashes preclude use for certain populations of at-risk women. We propose a model (Fig. 1) that weaves the finding that LH pulses are coincident with hot flashes in some women together with the emerging idea that KNDy neurons form an oscillatory neuronal network that drives GnRH and LH neuron pulsatility and perhaps affects postmenopausal hot flashes via cross-talk with the adjacent thermoregulatory center. Efforts to fully understand the etiology of postmenopausal hot flashes, which guide the development of other highly targeted nonhormonal agents that can inhibit hot flashes without adverse effects, should be engaged. PRKAs might have adequate efficacy, present relatively few adverse effects, and add to the therapeutic arsenal for hot flash treatments.

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