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Black cohosh has central opioid activity in postmenopausal women: evidence from naloxone blockade and positron emission tomography neuroimaging

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Abstract

Objective: To test whether black cohosh (BC) exhibits an action on the central endogenous opioid system in postmenopausal women.

Design: This was a mechanistic study conducted in the same individuals of luteinizing hormone pulsatility with a saline/naloxone challenge (n = 6) and positron emission tomography with [^{11}C]carfentanil, a selective μ-opioid receptor radioligand (n = 5), before and after 12 weeks of unblinded treatment with a popular BC daily supplement.

Results: BC treatment for 12 weeks at a standard dose (Remifemin, 40 mg/day) had no effect on spontaneous luteinizing hormone pulsatility or estrogen concentrations. With naloxone blockade, there was an unexpected suppression of mean luteinizing hormone pulse frequency (saline vs naloxone = 9.0 ± 0.6 vs 6.0 ± 0.7 pulses/16 h; $P = 0.056$), especially during sleep when the mean interpulse interval was prolonged by approximately 90 minutes (saline night interpulse interval = 103 ± 9 min vs naloxone night interpulse interval = 191 ± 31 min, $P = 0.03$). There were significant increases in μ-opioid receptor binding potential in the posterior and subgenual cingulate, temporal and orbitofrontal cortex, thalamus, and nucleus accumbens ranging from 10% to 61% across brain regions involved in emotional and cognitive function. In contrast, binding potential reductions of lesser magnitude were observed in regions known to be involved in the placebo response (anterior cingulate and anterior insular cortex).

Conclusions: Using two different challenge paradigms for the examination of central opioid function, a neuropharmacologic action of BC treatment was demonstrated in postmenopausal women.

Key Words: Luteinizing hormone pulsatility – Opioid peptides – Menopause – Positron emission tomography.

Although estrogen therapy is the most effective modality for the relief of menopausal hot flashes and the sequelae of insomnia and impaired quality of life, evidence from the Women's Health Initiative linking long-term hormone therapy to heart attack, stroke, and breast cancer has left millions of postmenopausal women and breast cancer survivors with few satisfactory treatment alternatives. Hot flash sufferers are resorting to a variety of herbal supplements such as soy and red clover with presumed estrogen activity, but most trials show little support for efficacy over placebos.[1,2]

Preparations made from the rhizomes of the herbal plant black cohosh (BC) (*Cimicifuga racemosa*) are the most widely studied botanical therapies for relieving hot flashes, with well-designed laboratory studies supporting a favorable safety profile for human use.[3-5] Despite its popularity, clinical trial results from around the world have been mixed with respect to any benefit greater than that of placebo.[6-12] Some of the discrepant findings may be due to the high placebo response, typically 30% to 50%, as well as to the variety of BC compounds, doses, and plant sources used as test agents. The most rigorously tested compound in both US and European studies is the commercially prepared isopropranoloic/ethanolic extract Remifemin (Schaper and Brummer, GmBH, Salzgitter, Germany). Among its components thought to have an active therapeutic role (possibly through metabolic activation of estrogen pathways) are the triterpene glycosides, including actein, 27-deoxyactein, and cimifugoside, molecules structurally related to steroids.[13]

The mechanism of action of this herbal compound is poorly understood. Studies conducted after 1990 using purified BC free of estrogenic adulterants[14] have generally

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not demonstrated classic estrogen-like effects on target cell activity in various animal or in vitro models[15-19] or in clinical studies,[9,20] although modest protective effects on bone in postmenopausal women have been reported.[11] Moreover, estrogen receptor (ER) binding studies[21-24] have generally failed to reveal any interactions with ligands of the ER in breast and uterine cells. Paradoxically, BC extract has been shown to inhibit the estrogen-dependent MCF-7 mammary tumor cell proliferation and to enhance inhibition with tamoxifen, suggesting an estrogen antagonist, antitumor effect on the breast.[19,25,26]

In a series of studies in ovariectomized rats, BC extract improved bone mineral density, reduced abdominal fat deposition, and dampened luteinizing hormone (LH) pulsatility, but failed to increase uterine weight or ERβ gene expression.[27-29] In humans, no change in gonadotropin secretion has been reported with BC,[9,30] although no studies to date have incorporated frequent serial blood sampling to detect dynamic hypothalamic-pituitary-ovarian axis function.

At the same time, a review of 15 animal and 15 in vitro mechanistic studies concluded that *C. racemosa* possesses central neurotransmitter activity instead of a direct hormonal action.[31] Two recent in vitro studies from the same laboratory provide strong evidence that BC exhibits partial agonist activity in both serotonin[15] and opioid[32] systems. In the ovariectomized rat, several BC extracts failed to demonstrate any estrogenic action on uterine weight, but exhibited strong specific binding to the 5-HT1A and 5-HT7 receptors,[15] serotonin subtypes found predominantly in the hypothalamus, the key area for regulation of body temperature.[33] Additionally, in a Chinese hamster ovary cell system transfected with human μ-opioid receptors, BC served as an effective competitive ligand and activator.[32]

Central endogenous opioid peptides are known to mediate some of the estrogenic effects on the gonadotropin-releasing hormone (GnRH) system as well as play a role in thermoregulation and the placebo response, making this system a reasonable target for clinical studies of BC mechanisms in women. We, therefore, studied for the first time central parameters of endogenous opioid peptide function in postmenopausal women before and after treatment with a popular BC dietary supplement. We hypothesized that if BC is acting as an opioid ligand in the hypothalamus, it would suppress GnRH activity under basal conditions and stimulate GnRH secretion during opioid blockade. Using state-of-the-art molecular neuroimaging techniques to directly assess regional changes in binding activity of the μ-opioid receptor in the living brain,[34,35] we conducted parallel imaging studies of this neurotransmitter system using positron electron tomography (PET) in a subset of these same individuals.

METHODS

Participants

After permission was obtained from the hospital institutional review board and the Radiation Safety Committee for research with human subjects, healthy volunteers willing to undergo BC therapy exclusively for 12 weeks for the treatment of their hot flash symptoms were recruited by electronic, print, and television advertisement and from the University of Michigan Women's Health Registry.[36,37] All volunteers provided written informed consent.

The inclusion criteria were as follows: spontaneous amenorrhea for at least 12 months in conjunction with a screening follicle-stimulating hormone value of more than 40 mIU/mL and an estradiol value of less than 20 pg/mL, no hormone therapy use within 6 months of enrollment, and symptoms of estrogen deficiency (bothersome hot flashes, night sweats, or painful intercourse due to vaginal dryness). In all participants, the results of screening values for hemoglobin, hematocrit, liver function, glucose, and prolactin were in the expected ranges for healthy volunteers. All women had a normal endocrine screen, a body mass index of 20 to 30 kg/m^2 and reported none of the following: smoking; vegetarian diet; medical or psychiatric illness; medication use including oral contraceptives, benzodiazepines, antidepressants, psychostimulants or over-the-counter drugs or herbal supplements; pregnancy or breast-feeding in the past 6 months; sleep disorders; shift work; or dieting or excessive exercise or alcohol consumption. Those volunteers who underwent imaging studies had to be right-handed and not have received more than a total of 5 rad to a radiosensitive organ or 15 rad to the body as a whole during a 12-month period.

As a test of the full protocol, the first volunteer, who had previously demonstrated a postmenopausal pattern of elevated pulsatile LH secretion in an earlier protocol under similar conditions,[38] underwent the imaging studies before and after just 4 weeks of treatment with BC (as opposed to 12 wk in the remainder of the women), along with the naloxone/saline challenge at the end of treatment. As outcome data from this shortened protocol demonstrated significant changes,[39] they were included in the analysis of the entire study group.

Study protocol

At baseline, volunteers were admitted at noon to the inpatient division of the General Clinical Research Center of the University of Michigan Hospitals for insertion of a heparinized, indwelling IV catheter into a forearm vein. At 1:00 PM, a 5-mL blood sample was drawn for determinations of follicle-stimulating hormone, estradiol, and progesterone levels by the hospital ligand facility. Beginning at 3:00 PM, blood was collected every 10 minutes until 7:00 AM the next day (16 h). For each 10-minute sample, 2 mL of blood was drawn from the indwelling cannula, which was then flushed with heparinized saline.

The women were ambulatory at will during the day and early evening in a private room and then went to bed with lights turned out between 11:00 PM and 7:00 AM of the following day. Caffeinated beverages were restricted after 1:00 PM. The women were allowed to sleep on their back or

on the side opposite to the blood drawing arm, changing position at will.

Presumed sleep was measured by 16-hour records of motor activity, as recorded by an actigraph unit (Mini Mitter Co., Bend, OR) worn on the nondominant wrist.[40] Actigraphic recordings were analyzed for activity and nonactivity using commercially available computer software (Mini Mitter) according to the method used previously in sleep studies in menopausal women.[41] As a way to determine the impact of perceived hot flash incidence as a potential confound of sleep quality, and in turn LH pulse frequency, subjective hot flash recordings were obtained during the pulse studies using the actigraph event recorder, which permits manual event entry.

A 30-day supply of the study drug was provided by the study coordinator at each monthly visit. Beginning on the morning of discharge after completing the baseline study, the women were instructed to take one tablet of the study drug (one Remifemin 20 mg) twice daily with meals. Remifemin, contains BC extract (isopropyl alcohol, 40% by volume) equivalent to 20 mg of root per tablet. This brand and dose have been used previously in several clinical trials.[8,9,42] Compliance was monitored by pill counts at treatment weeks 4, 8, and 12.

During weeks 11 and 12 of treatment, participants returned to repeat the 16-hour LH pulse studies along with an 8-hour saline or naloxone infusion during the hours of 3:00 PM to 11:00 PM. To avoid interaction with the radioligand, the naloxone infusion was always administered at the second treatment visit (wk 12). For the challenge test, a second IV line was placed in the opposite arm for infusions of normal saline (0.9% NaCl; 20 mL/h) or naloxone for 8 hours at a constant rate (1 mg/m^2/h) from noon until 8:00 PM with the use of an infusion pump. Naloxone infusion at this dose and duration administered during waking hours elicits LH responsiveness for at least 8 hours after IV discontinuation.[43]

Imaging methods and analysis

Two 90-minute PET scans per woman (before and after treatment) were acquired with a Siemens (Knoxville, TN) HR$^+$ scanner in three-dimensional mode (reconstructed full-width at half-maximum resolution ~5.5 mm in plane and 5.0 mm axially), with septa retracted and scatter correction. Participants were positioned in the PET scanner gantry, and two IV (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. [^{11}C]Carfentanil was synthesized at high specific activity (>2000 Ci/mmol) by the reaction of [^{11}C]methyliodide and a nor-methyl precursor as previously described.[35] Ten to 15 mCi were administered in each of the scans, with a mass of carfentanil injected of less than 0.03 μg/kg per scan. This ensured that the compound was administered in tracer quantities, ie, subpharmacologic doses occupying less than 1% of the available receptors. Fifty percent of the radiotracer doses were administered as an initial bolus, and the remaining 50% by continuous infusion for the remainder of the study. This procedure compensates for the metabolism of the radiotracer, leading to constant plasma concentrations over time and more rapid equilibration between kinetic compartments. For each study, 21 sets of scans (frames) were acquired over 90 minutes with an increasing duration (30-s frames × 4, 1 min × 3, 2.5 min × 2, 5 min × 8, 10 min × 4).

Images were reconstructed using iterative algorithms (brain mode; Fourier rebinning with ordered subsets-expectation maximization, four iterations, 16 subsets; no smoothing) into a 128 × 128-pixel matrix in a 28.8-cm diameter field of view. Attenuation correction was performed through a 6-minute transmission scan (^{68}Ge source) obtained before the PET study, also with iterative reconstruction of the blank/transmission data followed by segmentation of the attenuation image. Small head motions during emission scans were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered with the same software.[44] Time points were then decay-corrected during reconstruction of the PET data.

Image data were then transformed on a voxel-by-voxel basis into two sets of parametric maps: (1) a tracer transport measure (K_1 ratio), and (2) a receptor-related measure (binding potential [BP]). To avoid the need for arterial blood sampling, these measures were calculated using a modified Logan graphical analysis[45] using the occipital cortex (an area devoid of μ-opioid receptors) as reference region. The slope of the Logan plot is equal to the $(f_2B_{max}/kd) + 1$ for this receptor site (receptor concentration divided by its affinity for the radiotracer), and it has been referred to as the distribution volume ratio; f_2B_{max}/kd (or distribution volume ratio − 1) is the receptor-related measure BP[46] or receptor availability in vivo. The term f_2 refers to the concentration of free radiotracer in the extracellular fluid and is considered to represent a constant and very small value.

Anatomical magnetic resonance imaging (MRI) scans were acquired before PET scanning on a 3-T scanner (General Electric, Milwaukee, WI). Acquisition sequences were axial spoiled gradient echo inverse recovery–prepared MRI scans (echo time = 3.4 ms, repetition time = 10.5 ms, inversion time = 200 ms, flip angle = 25 degrees, number of excitations = 1, using 124 contiguous images, 1.5-mm thickness). K_1 ratio and distribution of volume ratio images for each experimental period and MRI scans were coregistered both to each other and to the International Consortium for Brain Mapping stereotactic atlas orientation.[47] Statistical parametric maps of the differences between conditions were generated by anatomically standardizing the T1-spoiled gradient echo MRI scan of each woman to the International Consortium for Brain Mapping stereotactic atlas coordinates, with subsequent application of this transformation to the DA D_2/D_3 and μ-opioid receptor binding maps. The accuracy of coregistration and nonlinear warping algorithms was confirmed for each woman individually by comparing the transformed MRI and PET scans both to each other and to the International Consortium

TABLE 1. *Clinical characteristics of the study participants*

				Baseline study				Tx wk 11 (saline study)				Tx wk 12 (naloxone study)			
ID	Age, y	BMI	PM	E_2	FSH	% Sleep	HF	E_2	FSH	% Sleep	HF	E_2	FSH	% Sleep	HF
1	57	20.5	7	20	87	—	—	17	60	83.8	3	15	73	83.9	1
2	53	22.5	4	10	70	77.2	2	10	76	77.3	4	11	79	81.1	4
3	51	29.0	1	22	61	—	—	17	53	—		—	—	—	—
4	47	24.9	7	17	65	88.0	6	14	63	81.6	7	17	68	83.0	3
5	55	23.2	2	12	79	87.6	5	12	79	83.6	8	17	87	84.6	5
6	53	30.0	4	12	61	72.4	8	23	60	78.2	9	16	66	71.6	3
7	54	24.7	1.5	14	60	80.9	10	12	59	70.7	9	18	56	87.9	6

BMI, body mass index; PM, postmenopausal; Tx, treatment; E_2, estradiol (pg/mL); FSH, follicle-stimulating hormone (mIU/mL), hormone values determined from the 1:00 PM blood sample; HF, hot flash events recorded on actigraph; % Sleep, percentage of time spent sleeping from 11:00 PM to 7:00 AM calculated from actigraph data, unavailable for volunteers 1 and 3 (baseline).

for Brain Mapping atlas template. To compensate for small residual anatomic variations across the participants and to improve signal-to-noise ratios, a three-dimensional gaussian filter (full-width at half-maximum of 6 mm) was applied to each scan.

For each subtraction analysis, two-tailed, paired *t* statistic values were calculated for each voxel using the pooled variance across voxels[48] and the Statistical Parametric Mapping 2 package. In view of the small sample size of this pilot study, we accepted a *P* value of 0.002 uncorrected as the threshold of statistical significance.

Hormone assays

Plasma LH was measured using an automated chemiluminiscent Immulite system (Diagnostic Products Corp., Los Angeles, CA) as previously described.[49] As a test of the adequacy of the naloxone challenge to hypothalamic function, measures of cortisol and prolactin were determined by chemiluminescent assay using the automated Immulite system, obtained from the 3:00 PM, 11:00 PM, and 7:00 AM blood samples. All samples from a woman were measured in the same assay.

LH pulse analysis

Cluster analysis was used as the method for objective peak detection, as validated earlier.[50] The highest concentration in a pulse was designated the peak maximum, the lowest interpeak hormone concentration the nadir, the time (in minutes) separating consecutive peak maxima the interpulse interval, and the number of peaks per 8 hours the frequency.

Mean values for LH from the serial every 10-minute measures were determined across the 16-hour sampling interval of the three studies. The distributions of hormone values were assessed for normality, and natural log transformations were used to correct skew. Differences in mean concentrations and LH pulse characteristics across study days and conditions were determined by nonparametric tests for paired (Wilcoxon signed-rank test) and nonpaired (Mann-Whitney test) observations. The time blocks analyzed in each of the three LH pulse studies were 3:00 PM to 11:00 PM (day) and 11:00 PM to 7:00 AM (night), each providing 49 data points for analysis. LH pulse frequency is expressed as the number of LH pulses per 8 hours. For paired, within-subject comparisons of the day versus night conditions, a paired Student's *t* test was performed on transformed data. Values are reported as the mean ± SE.

RESULTS

Four of seven volunteers who fulfilled all screening criteria (volunteers 1, 2, 4, and 5) underwent the full protocol; two others declined to undergo the neuroimaging studies (volunteers 6 and 7) and another participant (volunteer 3) declined to complete the LH pulse studies. An eighth participant who reported her last menstrual period approximately 12 months before enrollment completed all studies but was dropped from the group analysis when she experienced vaginal spotting and presumptive ovulation (serum progesterone values >3 ng/mL) during treatment week 11. Thus, there were usable data from six participants for the LH pulse studies and from five participants for the imaging analysis.

The mean age of the cohort was 53.2 ± 1 years; the mean body mass index was 24.3 ± 1 kg/m^2, and mean years postmenopause was 3.7 ± 1 years. Table 1 presents the clinical characteristics of each participant during study. In all women, follicle-stimulating hormone concentrations were 60 mIU/mL or more, estradiol values were 25 pg/mL or less, and sleep efficiency rates exceeded 70%, a level previously demonstrated to be associated with differences in day-night LH pulsatility.[38] There were no significant differences in the mean number of recorded hot flash events across the three study days, although hot flash events were reduced in all cases during the naloxone challenge versus the saline infusion.

TABLE 2. *BC effects on spontaneous pulsatile LH secretion and with naloxone blockade*

	Baseline	BC saline (wk 11)	BC naloxone (wk 12)
Mean LH, mIU/mL	26.4 ± 8.4	27.7 ± 6.2	27.9 ± 7.3
Pulses/16 h[a]	9.0 ± 0.3	9.0 ± 0.6	6.0 ± 0.7
Amp, mIU/mL	7.9 ± 1.1	8.9 ± 1.7	8.4 ± 2.4
IPI, min[b]	96.6 ± 5.7	95.8 ± 7.2	142.8 ± 11.2

BC, black cohosh; LH, luteinizing hormone; Amp, LH pulse amplitude; IPI, interpulse interval.
[a] $P = 0.056$, naloxone vs saline.
[b] $P = 0.075$ naloxone vs saline.

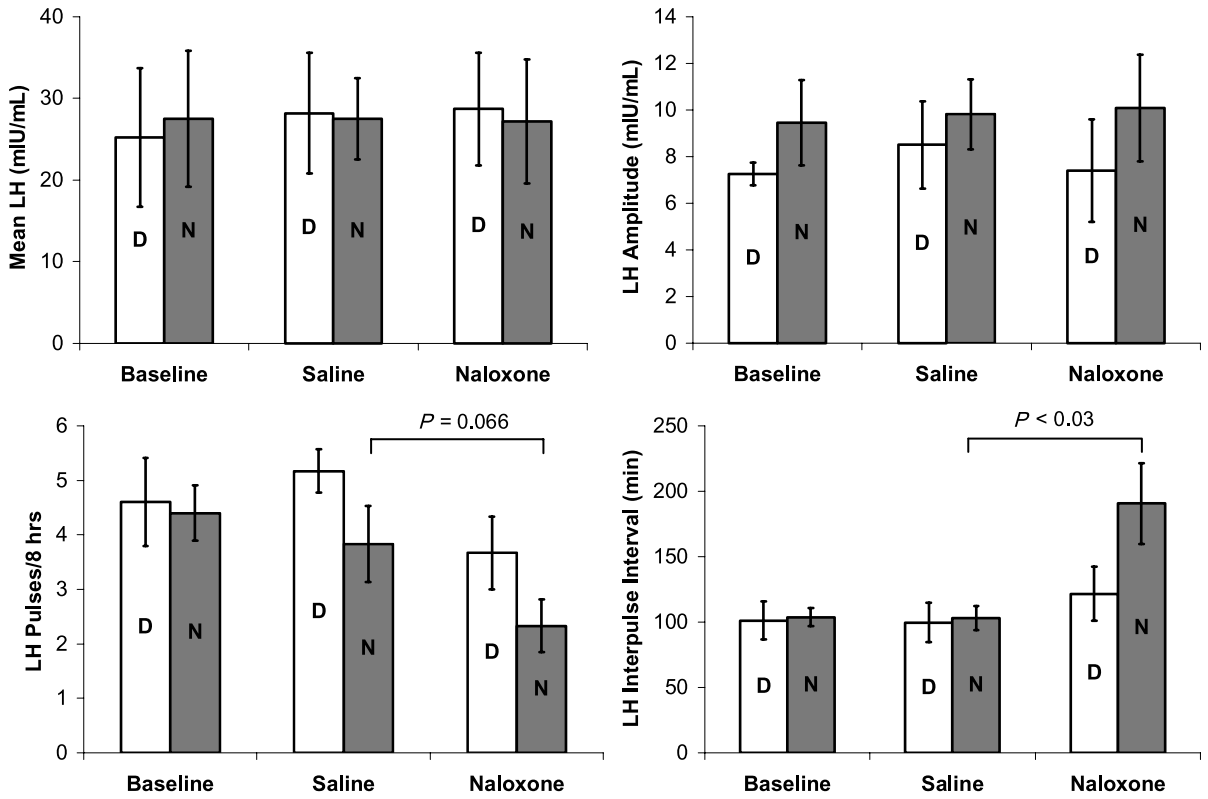

FIG. 1. Mean luteinizing hormone (LH) pulse features for the day (D) and night (N) at 8-hour intervals in six postmenopause women before (basal) and during treatment with black cohosh at week 11 (saline) and week 12 (naloxone) infusion studies.

LH pulse analysis

Baseline studies confirmed that the mean LH concentration and pulse pattern of the study group were typical of the postmenopause state (Table 2) and were similar during day and nighttime sampling (mean ± SEM day LH = 25.1 ± 8.5 mIU/mL vs mean ± SEM night LH = 27.6 ± 8.2 mIU/mL). After 11 weeks of BC treatment, no differences in group mean LH pulse parameters were observed compared with baseline. However, with the naloxone challenge, there was a trend for a significant suppression of mean LH pulse frequency and prolongation of the interpulse interval (IPI). When the 16-hour sampling period was divided into 8-hour day-night intervals (Fig. 1), the reduction in pulse frequency with naloxone infusion was most pronounced at night, resulting in a marked prolongation of the IPI by nearly 90 minutes compared with the saline study (saline night IPI = 103 ± 9 min vs naloxone night IPI = 191 ± 31 min, $P = 0.03$). In contrast, the expected increase in cortisol after naloxone versus saline was observed (mean ± SEM saline = 15.3 ± 1.5 vs mean ± SEM naloxone = 16.4 ± 0.7 ng/mL; $P = 0.05$), with a trend for an increase in prolactin (saline mean ± SEM = 9.9 ± 0.7 units vs naloxone mean ± SEM = 12.2 ± 1.1 units; $P = 0.08$). Figure 2 depicts a representative set of LH pulsatile patterns from the same individual across the 3 study days to demonstrate the effect observed in the group data.

Neuroimaging studies

Evidence of increases in μ-opioid receptor availability binding potential (BP) were obtained in the posterior and subgenual cingulate, orbitofrontal and temporal cortex, and

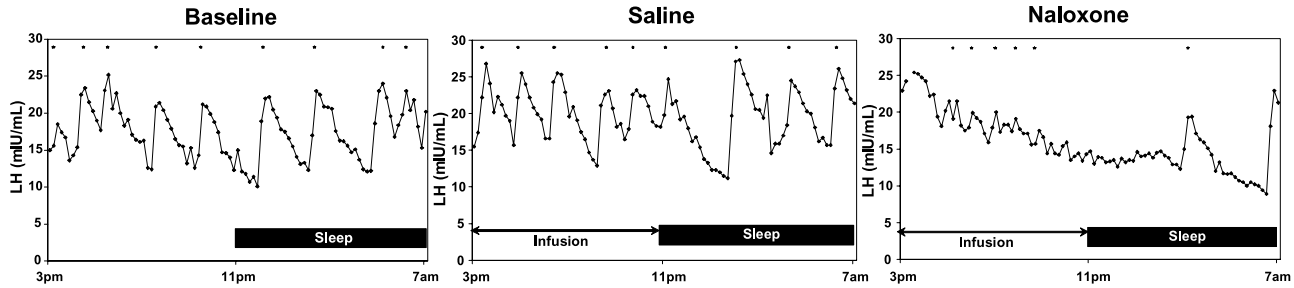

FIG. 2. Luteinizing hormone (LH) pulsatile patterns from the same individual at baseline and during black cohosh treatment at week 11 (saline study) and week 12 (naloxone) to demonstrate the suppressive effect of naloxone on LH mean secretion and pulse parameters. At treatment week 11, the pulsatile pattern was relatively unchanged versus baseline. In contrast, naloxone had a marked effect on pulse amplitude and frequency during both the awake (3:00 PM-11:00 PM) and sleep portions (11:00 PM-7:00 AM) of the study, resulting in a suppression in mean LH secretion.

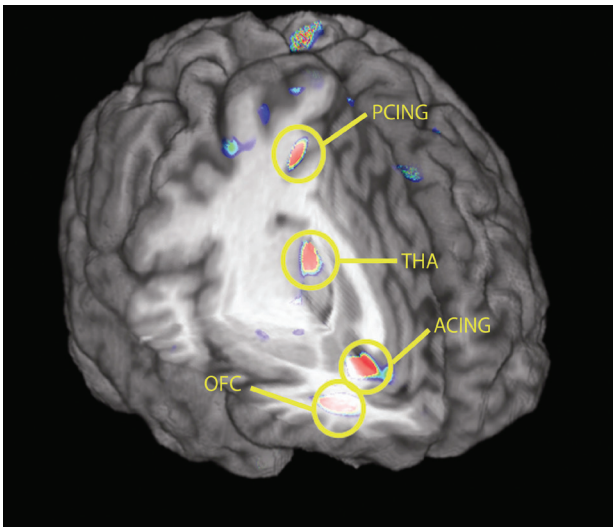

FIG. 3. Brain regions showing increases in μ-opioid receptor binding potential after black cohosh treatment, depicted in a three-dimensional view. Standardized *Z* scores of statistical significance are represented by a pseudocolor scale with the areas in red showing the most significant differences. ACING, anterior cingulate cortex; OFC, orbitofrontal cortex; PCING, posterior cingulate cortex; THA, thalamus.

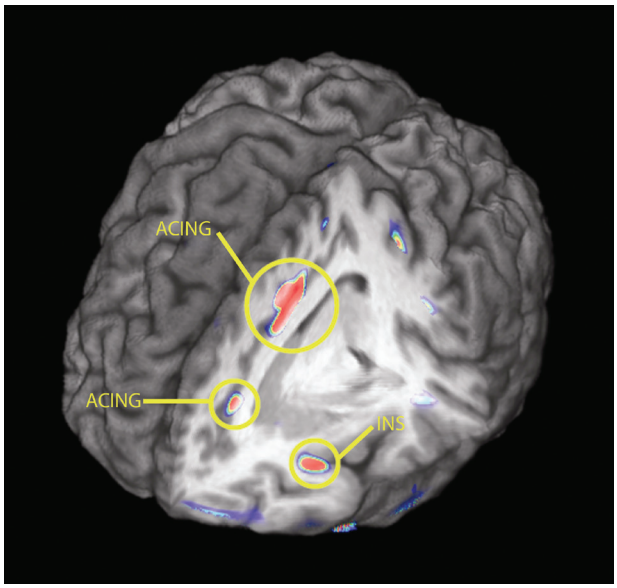

FIG. 4. Brain regions showing reductions in μ-opioid receptor binding potential after black cohosh treatment, depicted in a three-dimensional view. Standardized Z scores of statistical significance are represented by a pseudocolor scale with the areas in red showing the most significant differences. The most pronounced reductions took place in the anterior insular cortex and cingulate cortex. ACING, anterior cingulate cortex; INS, insular cortex.

the dorsomedial area of the thalamus (Fig. 3). Similar effects were obtained in an area that included the medial section of the nucleus accumbens, extending into the hypothalamus, although it did not reach statistical significance (Table 3). Reductions in μ-opioid receptor BP were also observed in some regions (Fig. 4); however, the average change for these areas was generally of lesser magnitude than the increases shown in Figure 3. Significant reductions in BP were obtained in the dorsal anterior cingulate (two peaks, one rostral and one dorsal). Changes in the same direction and of similar magnitude were also obtained in the anterior insular cortex, but they did not reach statistical significance (Table 4).

DISCUSSION

Predicated on the results of in vitro and animal work, and using our well-tested approaches, we undertook a pilot study directly assessing for the first time changes in LH pulsatility and μ-opioid receptor binding activity in postmenopausal women treated with BC. After 11 weeks of BC treatment, spontaneous pulsatile LH secretion was unchanged compared with baseline, suggesting a lack of estrogenic action on the hypothalamic-pituitary-ovarian axis. In contrast, naloxone infusion was associated with an unexpected suppression of LH pulsatility, which was more pronounced during sleep, again inconsistent with typical estrogen action. Furthermore, BC treatment resulted in bidirectional changes in μ-opioid receptor availability in vivo, varying by brain regions. Increases were observed in areas involved in cognitive and emotion processing, such as the posterior and subgenual cingulate, mesial temporal cortex, and dorsomedial region of the thalamus. Reductions in availability were registered in the rostral and dorsal anterior cingulate as well as in the anterior insula. Of interest, given the high placebo response in trials

TABLE 3. *Brain regions demonstrating significant increases in μ-opioid receptor binding potential (BP) after black cohosh treatment*

Region	Coordinates	Cluster size[a]	*Z* score	*P*[b]	% Change in BP
Subgenual anterior cingulate	5 26 −6	986	2.90	0.002	18
Left posterior cingulate	14 −13 50	196	2.88	0.002	61
Left orbitofrontal cortex	26 43 −3	558	3.01	0.001	26
Left temporal cortex	24 −44 −8	2,909	3.33	0.000	32
Medial thalamus	−4 −17 14	3,882	3.96	0.000	24
Right NAC/HYPOTH	−9 6 −6	19	2.26	0.012	10

NAC/HYPOTH, nucleus accumbens, extending medially into the hypothalamus.
[a]Coordinates, in millimeters, refer to the International Consortium for Brain Mapping (also known as Montreal Neurological Institute) *x*, *y*, *z* stereotactic coordinates. Cluster size is expressed in cubic millimeters for voxels with *P* values < 0.01 within the activated area.
[b]Voxel level, uncorrected.

TABLE 4. *Brain regions demonstrating significant reductions in μ-opioid receptor binding potential (BP) after black cohosh treatment*

Region	Coordinates	Cluster size[a]	*Z* score	*P*[b]	% Change in BP
Right dorsal anterior cingulate (1st peak)	−12 10 47	130	2.67	0.004	14
Right dorsal anterior cingulate (2nd peak)	−12 9 35	267	2.35	0.009	16
Right anterior insula	−38 22 −13	48	2.29	0.011	10

[a]Coordinates, in millimeters, refer to the International Consortium for Brain Mapping (also known as Montreal Neurological Institute) *x*, *y*, *z* stereotactic coordinates. Cluster size is expressed in cubic millimeters for voxels with *P* values < 0.01 within the activated area.
[b]Voxel level, uncorrected.

with BC,[51] reductions in μ-opioid receptor BP have previously been described in these regions during placebo administration.[52] These were interpreted as reflecting the release of endogenous opioids and were related to individual expectations and placebo-induced psychophysical effects in the areas of pain and affective state.[52,53]

From the perspective of the neuroendocrine effects of BC, earlier investigators failed to demonstrate any effect of naloxone on gonadotropin secretion or hot flashes in postmenopausal women who are estrogen depleted.[54,55] However, with estrogen therapy, naloxone blockade will provoke an increase in pulse frequency and an increase in mean LH secretion,[56,57] owing to the presumed action of estrogen to restore opioid activity on the hypothalamic GnRH system. Contrary to these typical effects, here we observed that naloxone administration in the context of BC treatment was associated with LH pulsatile suppression. Thus, a direct central estrogen-like effect of BC resulting in restoration of opioid activity was not identified in our study.

Our findings argue instead for a BC effect on other inhibitory neuromodulators of GnRH secretion, which is only unmasked in the absence of significant opioid tone. The unusual LH response to naloxone in postmenopausal women has previously been observed when sodium valproate, presumed to affect the GABAergic system, is given concomitantly with naloxone.[58] Alternatively, serotonergic pathways may link the opioid system and/or estrogen to inhibitory control of LH release.[59-62] Electrical stimulation of the dorsal raphe nucleus in the midbrain of ovariectomized rats inhibits episodic LH release, and this inhibitory influence can be reduced by depletion of brain levels of serotonin and inhibition or blockade of brain serotonin receptors.[63] In men, pretreatment with the serotonin reuptake inhibitor fenfluramine prevented the naloxone-induced increase in LH concentrations,[64] although later studies using fluoxetine and oral naltrexone for 7 days did not replicate this effect.[65]

Because this was not designed as a placebo-controlled efficacy trial, we did not obtain daily diaries of hot flash events at baseline and across the 12 weeks of treatment. However, as LH pulsatility and hot flashes are believed to be co-occurring epiphenomena,[66] we documented self-reported hot flash events during study days as a possible confound on sleep quality, and in turn LH pulsatility. Hot flashes were present during all three study days, with no differences between the incidence observed at baseline versus treatment week 11, suggesting the failure of BC treatment to ameliorate or protect against any stress-induced hot flash trigger brought on by the burden of the frequent blood sampling protocol. Whether the modest reduction in perceived hot flashes that occurred with the naloxone challenge (vs saline) was related to the decrease in LH pulses cannot be determined in the present study. Moreover, to what extent the unblinded nature of the protocol contributed to the reduction in symptoms is not known. Despite the presence of hot flashes during the study, sleep efficiency, although reduced as expected under the experimental conditions, was similar to that observed in healthy postmenopausal women without hot flashes undergoing a similar protocol.[67]

In parallel with the LH pulse studies, our neuroimaging studies provide evidence for the first time that BC has direct in vivo central effects that are mediated via alterations of the μ-opioid system. Specifically, BC treatment increased μ-opioid receptor availability in vivo in brain areas where estrogen effects in the same direction have been previously observed (thalamus and nucleus accumbens).[68] In addition, increases in μ-opioid receptor binding were also obtained in the mesial temporal and orbitofrontal cortex, and subgenual and posterior anterior cingulate, areas typically implicated in cognitive functions and cognitive-emotional integration.[69-75]

Unexpectedly, we also describe regional reductions in the BP measure, which under acute challenges are interpreted as indicating the release of endogenous opioid peptides and less availability of receptors to bind the radiotracer.[74,76] After the short-term treatment used in this study, these changes could represent an increase in endogenous opioid tone/release, a down-regulation of receptor sites, or both. The regions involved, the rostral and dorsal anterior cingulate and anterior insula, are typically implicated in responses to sensory stimuli, particularly those with emotional significance.[77,78] Brain regional activity in these areas is further modulated by the administration of placebos, presumably as a result of the expectations associated with the receipt of a potentially therapeutic agent.[79-81] At least in the context of the limited studies performed in humans examining these processes, the μ-opioid system appears to have a primary role in these effects.[52,82]

Given the high level of placebo responsiveness observed in BC trials for hot flashes, it is tempting to hypothesize that both pharmacologic and placebo effects are taking place simultaneously and affecting the same neurochemical substrate (the endogenous opioid system). Both effects, increases in μ-opioid receptor protein or affinity (BC pharmacologic effect) and endogenous opioid release (placebo effect), would potentiate the function of this neurotransmitter system.

The hypothalamus is believed to be central to thermoregulation and the hot flash trigger.[83] Our parallel neuroimaging studies only partially confirmed an opioid receptor effect of BC on the hypothalamus. Increases in the binding measure were obtained in this region below the statistically significant threshold used and extended into the medial section of the adjacent nucleus accumbens. Given the small size of this region, which would reduce the probability of finding significant effects, larger samples are required to answer this question.

Recent regional cerebral blood flow PET and functional MRI studies of thermoregulatory responses[84] and hot flashes[85] have reported increases in the activity of the anterior cingulate and the anterior insular cortex. These regions fully overlap with those in which the administration of BC was associated with a reduction in μ-opioid receptor availability. This may suggest that hot flashes may be associated with changes in endogenous opioid system function and that BC has an impact on the same regions and neurotransmitter system. However,

this hypothesis would have to be tested in a larger randomized, placebo-controlled study.

CONCLUSIONS

The strength of this pilot study is the comprehensive assessment of BC in the same women with both traditional neuroendocrine and neuroimaging approaches to evaluate the potential opioidergic effects of BC. The study is, however, limited by its preliminary nature in a small sample of participants, studied without placebo control or an objective measure of hot flashes. Furthermore, the effects obtained were complex at both the neuroendocrine and receptor levels. In the context of short-term administration, changes in receptor availability may represent effects on release, decreases or increases in endogenous opioid tone or, alternatively, changes in receptor protein or affinity. Although these data cannot be extrapolated to other study populations, the results obtained are nevertheless suggestive of neurobiological effects of BC affecting systems relevant to the pathophysiology of hot flashes and warrant further investigation.

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