9852 J. Agric. Food Chem. 2006, 54, 9852–9857

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Black Cohosh (*Actaea racemosa*, *Cimicifuga racemosa*) Behaves as a Mixed Competitive Ligand and Partial Agonist at the Human μ Opiate Receptor

MEE-RA RHYU,[†,‡,§,#] JIAN LU,[§] DONNA E. WEBSTER,[†,‡,§] DANIEL S. FABRICANT,[†,‡] NORMAN R. FARNSWORTH,[†,‡] AND Z. JIM WANG*,[‡,§]

Department of Medicinal Chemistry and Pharmacognosy, UIC/NIH Center for Botanical Dietary Supplements Research and Program for Collaborative Research in the Pharmaceutical Sciences, and Department of Biopharmaceutical Sciences, College of Pharmacy, University of Illinois, Chicago, Illinois 60612

Black cohosh is a commonly used botanical dietary supplement for the treatment of climacteric complaints. Because the opiate system in the brain is intimately associated with mood, temperature, and sex hormonal levels, the activity of black cohosh extracts at the human μ opiate receptor (hMOR) expressed in Chinese hamster ovary cells was investigated. The 100% methanol, 75% ethanol, and 40% 2-propanol extracts of black cohosh effectively displaced the specific binding of [^{3}H]DAMGO to hMOR. Further studies of the clinically used ethanol extract indicated that black cohosh acted as a mixed competitive ligand, displacing 77 $\pm$ 4% [^{3}H]DAMGO to hMOR (K_i = 62.9 μg/mL). Using the [^{35}S]GTPγS assay, the action of black cohosh was found to be consistent with an agonist, with an EC_{50} of 68.8 $\pm$ 7.7 μg/mL. These results demonstrate for the first time that black cohosh contains active principle(s) that activate hMOR, supporting its beneficial role in alleviating menopausal symptoms.

KEYWORDS: Black cohosh; menopause; hot flashes; opiate; botanical dietary supplement

INTRODUCTION

Actaea racemosa L. or *Cimicifuga racemosa* (L.) Nutt. (Ranunculaceae), commonly known as black cohosh, has been traditionally used for gynecologic and pain disorders. The first medicinal use of black cohosh is generally attributed to Native Americans, who used black cohosh for the treatment of a variety of disorders, including various conditions unique to women, such as amenorrhea and menopause, and pain during menses and childbirth (*1*, *2*). Use of black cohosh was accepted and popularized by eclectic medical practitioners in the late 19th and early 20th centuries. Dr. John King made a significant contribution by publicizing the use of black cohosh or, as he referred to it, *Macrotys*. King claimed that *Macrotys* was a remedy of "abnormal conditions of the principal organs of reproduction in the female" and that the roots were "very efficacious in the treatment of chronic ovaritis, endometriosis, and menstrual derangements, such as amenorrhea, dysmenorrhea" (*1*). Black cohosh root was an official drug (under the name "black snakeroot") in the United States Pharmacopoeia (USP) from 1820 to 1926.

With a shift toward evidence-based modern medicine, the use of black cohosh in America faded by the 1930s. However, its medicinal use has gained popularity in Europe, especially in Germany, since the 1950s. Black cohosh is one of the main herbs recommended in Germany for menopause, PMS, and secondary amenorrhea (*3*). In Europe, black cohosh is commonly prescribed as an alternative to hormone replacement therapy for menopause (*4*). Over the past decade or so, black cohosh has also regained its popularity for alleviating menopausal symptoms in the United States. The North American Menopause Society (NAMS) recommends the short-term (less than 6 months) use of a black cohosh supplement for menopause (*5*). A number of clinical trials have been carried out to examine the efficacy and safety of black cohosh (*6*−*11*). Black cohosh appears to be effective in reducing hot flashes and depression, although more clinical evidence is still needed (*12*−*17*).

The mechanism for the effectiveness of black cohosh in treating menopausal symptoms is not entirely clear. Black cohosh was initially thought to contain formononetin, an estrogenic isoflavone (*18*−*20*). However, more recent studies have not found significant estrogenic constituents or activity (*21*−*25*). Because there are significant CNS manifestations in menopause and a tight connection between sex hormones and CNS receptors (*26*−*28*), it is plausible that black cohosh extracts

* Address correspondence to this author at the Department of Biopharmaceutical Sciences, University of Illinois, 833 S. Woods St., Chicago, IL 60612 [telephone (312) 996-0888; fax (312) 996-0098; e-mail zjwang@uic.edu].
[†] Department of Medicinal Chemistry and Pharmacognosy.
[‡] UIC/NIH Center for Botanical Dietary Supplements Research.
[§] Department of Biopharmaceutical Sciences.
[#] Visiting from The Food Function Research Division, Korea Food Research Institute, Gyeonggi-Do 463-420, Korea.

10.1021/jf062808u CCC: $33.50
Published on Web 12/07/2006

may interact with CNS systems for at least some of the beneficial effects in treating menopausal symptoms. Indeed, it has been reported that black cohosh acted as an agonist at the 5HT1A, 1D, and 7 receptors (*29*) and may also have affinity for the dopamine D2 receptor (*20*). Because the opioid system, especially the μ opiate receptor, is essential for temperature and hormone homeostasis, this may be one of the mechanisms for the action of black cohosh in menopause. In this study, we examined three different black cohosh extracts in μ opiate receptor binding and functional assays.

MATERIALS AND METHODS

Chemicals. Guanosine 5′-diphosphate (GDP), guanosine 5′-[γ-thio]-triphosphate (GTPγS), bovine serum albumin (BSA), dithiothreitol (DTT), disodium ethylenediaminetetraacetate (EDTA), and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid [*N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid, HEPES] were obtained from Sigma (St. Louis, MO). [D-Ala^2,*N*-MePhe^4-Gly-ol^5]enkephalin (DAMGO), and [^{3}H]DAMGO were from Multiple Peptide Systems (San Diego, CA). [^{35}S]GTPγS was purchased from Amersham Biosciences (Piscataway, NJ). All other chemicals were purchased from Sigma.

Plant Materials. Black cohosh extracts were prepared and characterized as previously described (*29*−*31*). Briefly, black cohosh rhizomes/roots were provided by PureWorld Botanicals, Inc. (South Hackensack, NJ; lot 9-1744) (*29*), and were botanically verified by the UIC/NIH Center for Botanical Dietary Supplements Research and characterized by PCR (*30*). A voucher specimen (BC001) has been deposited at the Program for Collaborative Research in the Pharmaceutical Sciences (PCRPS), University of Illinois at Chicago. Milled roots/rhizomes of black cohosh were separately extracted by percolation with 75% ethanol (CR-ethanol), 100% methanol (CR-methanol), or 40% 2-propanol (CR-isopropanol) and then dried to a powder in vacuo. These extracts were chemically characterized by HPLC and standardized to active triterpene glycosides (*31*). The HPLC method has since been adopted by the USP-NF. The 75% ethanol extract, chemically standardized to 5.6% active triterpene glycosides and biologically standardized to the 5HT7 receptor, is currently being used in a four-arm randomized double-blind and placebo-controlled phase II clinical trial that is being conducted by the UIC/NIH Center for Botanical Dietary Supplements Research. Extensive effort has been carried out to identify the active principles in black cohosh extracts, which have so far yielded a number of new compounds (*32*−*37*). For pharmacological assays, the dried extracts were dissolved in DMSO. The final DMSO concentrations in all assays were below 0.5%, which was found not to interfere with either the receptor binding or GTPS binding assay.

Cell Culture. Chinese hamster ovary (CHO) cells stably transfected with human μ opioid receptors (CHO-hMOR) were established as described previously (*38*). CHO-hMOR cells were cultured in Dulbecco's modified Eagle medium (DMEM) and Ham's F-12 medium (1:1) supplemented with 10% newborn calf serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin. To maintain stable selection, 200 μg/mL G418 was added to the growth medium. Cells were cultured in an incubator maintained at 37 °C with 5% CO_2 in humidified air.

Receptor Binding Assay. The receptor binding assay was carried out as previously described (*38*−*40*). Briefly, membranes from CHO-hMOR cells were prepared by Polytron homogenization at setting 6 for 2 min on ice, followed by centrifugation at 20000*g* for 30 min at 4 °C. Protein content was determined according to the Coomassie protein assay method (Pierce Biotechnology, Rockford, IL) using bovine serum albumin as the standard. For the receptor binding assay, the receptor membranes (50 μg of protein/reaction) were incubated with a series of concentrations of [^{3}H][D-Ala^2,*N*-Me-Phe^4-Gly^5-ol]enkephalin (DAMGO, 1 nM) and different concentrations of black cohosh extracts in 50 mM Tris buffer (pH 7.4) at 30 °C for 1 h. Nonspecific binding was determined in the presence of 20 μM unlabeled DAMGO. Reactions were terminated by rapid vacuum filtration through GF/B filters presoaked with 0.2% polyethylenimine. Filter-bound radioactivity was determined by liquid scintillation counting (Beckman Coulter Inc., Fullerton, CA). Results, expressed as mean ± SD, were analyzed using the Prism program (GraphPad Software, San Diego, CA).

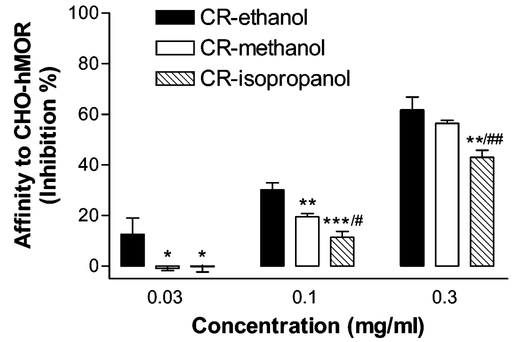

Figure 1. Affinity of black cohosh extracts for the human μ opiate receptor (hMOR) expressed in Chinese hamster ovary (CHO) cells. Three black cohosh extracts, 100% methanol (CR-methanol), 75% ethanol (CR-ethanol), and 40% 2-propanol (CR-isopropanol), were used to displace the binding of [^{3}H]DAMGO, a selective hMOR agonist, to CHO-hMOR cells. The affinities of these extracts, at three different concentrations (0.03, 0.1, and 0.3 mg/mL) were compared. The percentage inhibition of the specific radioactivity of [^{3}H]DAMGO bound to the CHO-hMOR cell membranes was shown. Data are the mean ± SD ($N = 3$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, compared with CR-ethanol. $^{\#}$, $P < 0.05$; $^{\#\#}$, $P < 0.01$, comparison between CR-methanol and CR-isopropanol.

Kinetics of Receptor Binding. The mode of receptor binding by black cohosh extract was further characterized to determine full versus partial and competitive/uncompetitive versus noncompetitive binding to the μ opiate receptor. To obtain K_d and K_i values, [^{3}H]DAMGO ranging from 0.1 to 4 nM and black cohosh ethanol extract ranging from 0 to 200 μg/mL were used in the receptor binding assay as described above. Data were further transformed into a double-reciprocal plot to determine the mechanism of receptor−ligand interaction.

[^{35}S]Guanosine 5′-[γ-Thio]triphosphate (GTPγS) Binding Assay. Because the μ opiate receptor is a G protein coupled receptor, we employed the GTPγS binding assay to determine the activation of the receptor. The [^{35}S]GTPγS binding assay was performed on the basis of the method previously described (*38*, *39*). Briefly, cell membranes (40 μg of protein) were incubated with 0.1 nM [^{35}S]GTPγS in the reaction buffer (50 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 5 mM magnesium chloride, 30 μM GDP, 1 mM DTT, and 0.1% BSA, pH 7.4) in the presence or absence of black cohosh extracts or DAMGO at 30 °C for 1 h. The basal level was defined as the amount of [^{35}S]GTPγS bound in the absence of any agonist. Nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. Reactions were terminated by rapid filtration, and filter-bound radioactivity was determined as described above.

Data and Statistical Analysis. Data, expressed as mean ± SD, were analyzed using the GraphPad Prism program to obtain IC_{50}, maximum inhibition, EC_{50}, and E_{max} values. The dissociation constant K_i was determined using the method of Cheng and Prusoff (*41*). Differences in responses between groups were determined using ANOVA followed by Student's *t* (two groups) or Dunnett's *t* (multiple groups) tests.

RESULTS

Affinity of Black Cohosh Extracts for the μ Opiate Receptor. Three different black cohosh extracts, a 75% ethanol extract, a 100% methanol extract, and a 75% isopropanol extract, were subjected to a receptor binding assay to determine their affinity for the human μ opiate receptor (hMOR). [^{3}H]DAMGO, a μ opiate receptor-specific radioligand that binds to the receptor with a high affinity (*38*), was used in the assay. All three extracts were able to displace the specific binding of [^{3}H]DAMGO to hMOR (**Figure 1**), indicating that black cohosh contains constituents with an affinity for the receptor. As expected, the affinity of black cohosh extracts is concentration-dependent. The 75% ethanol extract of black cohosh (CR-ethanol) appeared to be most effective. This is also the extract that is used in a phase

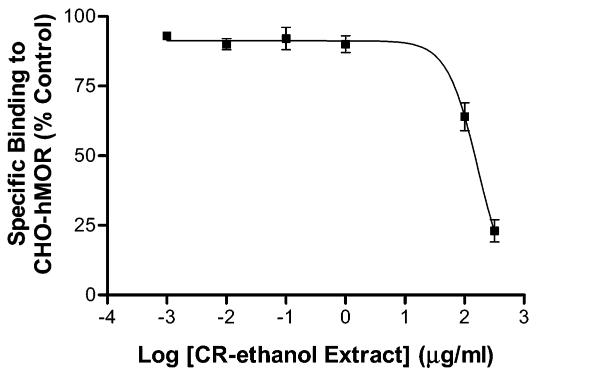

Figure 2. Displacement of [^{3}H]DAMGO binding by a clinically used 75% ethanol extract of black cohosh (CR-ethanol). CR-ethanol (0.01–300 μg/mL) was used to dose-dependently compete for the binding of [^{3}H]DAMGO (1 nM) to the CHO-hMOR cell membranes. The specific radioactivity of [^{3}H]DAMGO bound to hMOR in the absence of black cohosh was set to 100%. Each point represents the mean ± SD ($N = 3$). The K_i and IC_{50} values were estimated to be 62.9 and 165.9 μg/mL, respectively.

II clinical trial that is being conducted by the UIC/NIH Center for Botanical Dietary Supplements Research. We further characterized CR-ethanol in receptor binding and functional assays.

To obtain the dissociation rate constant (K_i), different concentrations of CR-ethanol, ranging from 0.001 to 300 μg/mL, were tested in the competition binding assay against [^{3}H]-DAMGO (1 nM). The extract dose-dependently displaced the specific binding of [^{3}H]DAMGO, with a K_i value of 62.9 μg/mL ($IC_{50} = 165.9$ μg/mL) (**Figure 2**). At the highest concentration used, the extract displaced 77 ± 4% of [^{3}H]DAMGO binding, indicating the black cohosh extract had a strong affinity for hMOR.

Mechanism of Receptor Binding. To investigate whether the black cohosh extract functions as a competitive, noncompetitive, uncompetitive, or mixed inhibitor of ligand binding to hMOR, a radioligand receptor saturation binding assay was carried out using [^{3}H]DAMGO (0.1−4 nM) in the absence or presence of 100 and 200 μg/mL of CR-ethanol. Black cohosh significantly inhibited the saturation binding of DAMGO to hMOR, and as a result, the maximum binding, B_{max}, was decreased by 41 and 68% with 100 and 200 μg/mL CR-ethanol, respectively (**Figure 3A**). The apparent K_d of DAMGO for hMOR was increased 1.2- and 4.4-fold in the presence of 100 and 200 μg/mL of CR-ethanol, respectively (**Table 1**). Further analysis using a double-reciprocal plot indicated that the data fit best with a mixed competition mode where the hMOR-bound constitutent(s) in black cohosh can not only bind to the receptor binding site of hMOR alone but can also bind in the presence of DAMGO. A schematic model for black cohosh acting as a mixed competitive ligand (I) for the binding of DAMGO to hMOR is illustrated in **Figure 4**.

Activation of the Human μ Opiate Receptor by Black Cohosh Extract. It is critical to ascertain if the black cohosh extracts contain agonistic or antagonistic opiate activity at the receptor. The μ opiate receptor belongs to the superfamily of G protein-coupled receptors. Activation of hMOR leads to the activation of G proteins and subsequent cellular signaling. The efficacy of a hMOR agonist in activating G-proteins, which can be measured by [^{35}S]GTPγS binding to the cytoplasmic membranes (*39*), can be used to determine the agonist's activity at the receptor. Therefore, we employed the GTPγS binding assay to determine the mode of action of CR-ethanol. The extract dose-dependently activated hMOR as revealed by the significant

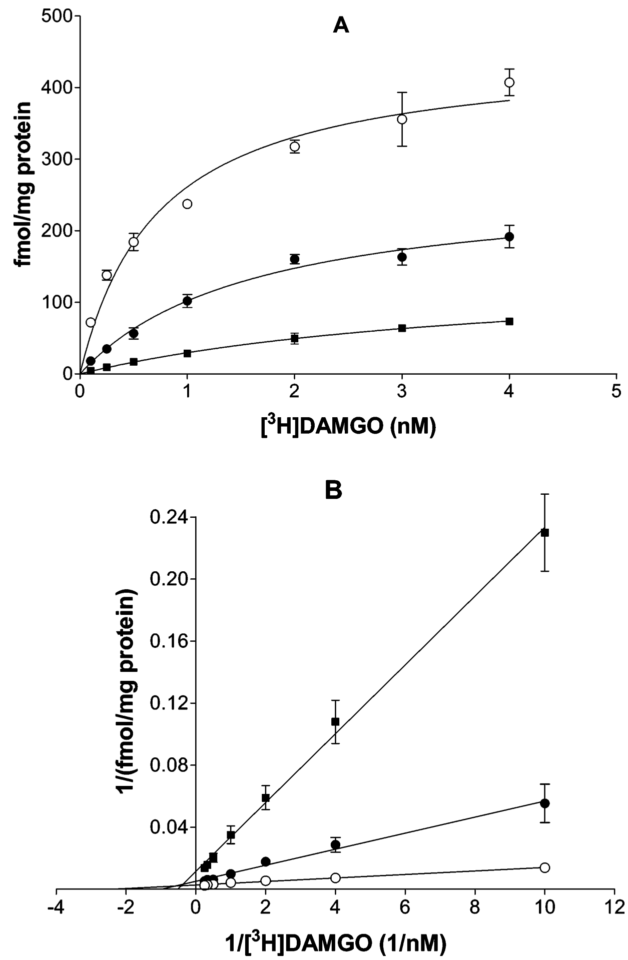

Figure 3. Inhibition of black cohosh on the specific binding of [^{3}H]DAMGO to hMOR: (**A**) saturation binding of [^{3}H]DAMGO to hMOR in the absence or presence of 100 or 200 μg/mL black cohosh; (**B**) reciprocal plots of the binding data of [^{3}H]DAMGO to hMOR; (○) no black cohosh; (●) 100 μg/mL black cohosh; (■) 200 μg/mL black cohosh. Each point represents the mean ± SD ($N = 3$).

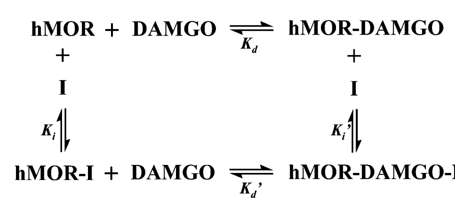

Figure 4. Scheme illustrating the mixed competitive action of black cohosh (I) on the binding of DAMGO to the human μ opiate receptor (hMOR). K_d, K_d', K_i, and K_i' are dissociation rate constants.

Table 1. [^{3}H]DAMGO Binding to the Human μ Opiate Receptor in the Absence or Presence of Black Cohosh 75% Ethanol Extract

	black cohosh (μg/mL)		
	0	100	200
K_d (nM)	0.73	1.62	3.94
B_{max} (fmol/mg of protein)	451.4	267.2	146.5

stimulation of GTPγS binding to CHO-hMOR (**Figure 5**). The maximum effect was 162.2 ± 14.7%, which was lower than that of DAMGO, a full agonist of hMOR ($P < 0.05$). The EC_{50} of CR-ethanol was estimated to be 68.8 ± 7.7 μg/mL ($N = 3$). These data suggest that the black cohosh extract contains active constituent(s) that act as an agonist at the human μ opiate

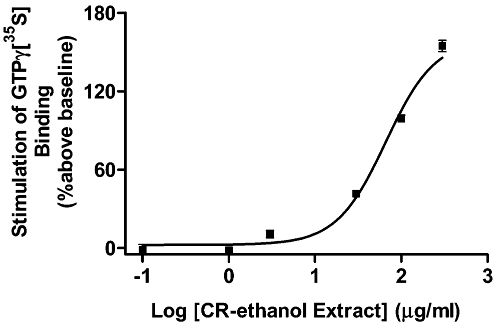

Figure 5. Stimulation of [^{35}S]GTPγS binding by a clinically used 75% ethanol extract of black cohosh (CR-ethanol). CR-ethanol stimulated [^{35}S]-GTPγS binding by 162.2 ± 14.7%. Each point represents the mean ± SD (N = 3). The EC_{50} value was estimated to be 68.8 ± 7.7 μg/mL.

receptor, which is consistent with its beneficial action in relieving at least some menopausal symptoms.

DISCUSSION

A large segment of the female population is affected by menopausal symptoms. Limited treatment options are available. Hormone replacement therapy is effective in treating hot flashes and other symptoms. However, the Women's Health Initiative and long-term follow-up from the Heart and Estrogen/progestin Replacement Study found an increased risk of cardiovascular disease, breast cancer, and stroke among women randomized to hormone therapy (*42*−*45*). Many women have turned to botanical dietary supplements for the relief of menopausal symptoms. Black cohosh is one of the most commonly used botanical dietary supplements for alleviating menopausal symptoms. Despite its long history and wide use, its mechanisms of action are still not entirely understood.

Early studies have examined the phytoestrogenic activity contained in black cohosh (*18*−*20*). However, more recent studies did not find significant estrogenic constituents or activity (*21*−*25*, *29*). Because there are significant CNS manifestations in menopause and a tight connection between sex hormones and the CNS receptors (*26*−*28*), it has been proposed that black cohosh may act centrally (*20*, *29*).

The current study has demonstrated for the first time that black cohosh extracts contained constituents that have significant affinity for the human μ opiate receptor. Moreover, a clinically used 75% ethanol extract of black cohosh acted as a partial agonist at the receptor.

The opiate receptor system affects several aspects of female reproductive neuroendocrinology, such as the control of sex hormones and the display of lordosis behavior (*28*, *46*−*51*). In fact, a decreased β-endorphin level is considered to be a hormonal marker for menopause (*27*). Hot flashes, a major complaint of menopausal women, are hypothesized to be caused by an erroneous setting of body core temperature (*52*). The CNS temperature control center is regulated by the endogenous opiate system as well as catecholamines (*26*, *27*, *53*). Opiates can therefore alter core temperature setting directly or indirectly by affecting the release and levels of catecholamines (*27*, *54*, *55*). Striking similarities exist between opiate withdrawal and menopausal hot flashes (*56*). Not surprisingly, opiate dependence withdrawal has been used as an animal model of menopausal hot flashes (*57*).

Therefore, botanical dietary supplements containing opiate activity are expected to have beneficial effects in relieving menopausal symptoms, including reducing hot flashes. The opiate agonistic activity of black cohosh may explain at least in part its efficacy in alleviating menopausal symptoms.

ABBREVIATIONS USED

CHO, Chinese hamster ovary; CR-ethanol, a 75% ethanol extract of black cohosh; CR-methanol, a 100% methanol extract of black cohosh; CR-isopropanol, a 40% 2-propanol extract of black cohosh; DAMGO, [D-Ala^2,*N*-Me-Phe^4-Gly^5-ol]enkephalin; DTT, dithiothreitol; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); hMOR, human μ opiate receptor.

LITERATURE CITED

(1) Foster, S. Black cohosh: *Cimicifuga racemosa*. A literature review. *HerbalGram* **1999**, *45*, 35−50.
(2) Borrelli, F.; Ernst, E. *Cimicifuga racemosa*: a systematic review of its clinical efficacy. *Eur. J. Clin. Pharmacol.* **2002**, *58*, 235−241.
(3) Blumenthal, M., et al. *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*; American Botanical Council: Integrative Medicine Communications: Austin, TX, 998; p 90.
(4) McKenna, D. J.; Jones, K.; Humphrey, S.; Hughes, K. Black cohosh: efficacy, safety, and use in clinical and preclinical applications. *Altern. Ther. Health Med.* **2001**, *7*, 93−100.
(5) NAMS. Treatment of menopause-associated vasomotor symptoms: position statement of The North American Menopause Society. *Menopause* **2004**, *11*, 11−33.
(6) Warnecke, G. Influence of a phytopharmaceutical on climacteric complaints. *Die Meizinische Welt* **1985**, *36*, 871−874.
(7) Stoll, W. Phytopharmacon influences atrophic vaginal epithelium: double-blind study—*Cimicifuga* vs estrogenic substances. *Therapeuticum* **1987**, *1*, 23−31.
(8) Lehmann-Willenbrock, E.; Riedel, H. H. [Clinical and endocrinologic studies of the treatment of ovarian insufficiency manifestations following hysterectomy with intact adnexa]. *Zentralbl. Gynakol.* **1988**, *110*, 611−618.
(9) Wuttke, W.; Seidlova-Wuttke, D.; Gorkow, C. The *Cimicifuga* preparation BNO 1055 vs. conjugated estrogens in a double-blind placebo-controlled study: effects on menopause symptoms and bone markers. *Maturitas* **2003**, *44* (Suppl. 1), S67−77.
(10) Osmers, R.; Friede, M.; Liske, E.; Schnitker, J.; Freudenstein, J.; et al. Efficacy and safety of isopropanolic black cohosh extract for climacteric symptoms. *Obstet. Gynecol.* **2005**, *105*, 1074−1083.
(11) Uebelhack, R.; Blohmer, J. U.; Graubaum, H. J.; Busch, R.; Gruenwald, J.; et al. Black cohosh and St. John's wort for climacteric complaints: a randomized trial. *Obstet. Gynecol.* **2006**, *107*, 247−255.
(12) Hardy, M. L. Herbs of special interest to women. *J. Am. Pharm. Assoc. (Wash.)* **2000**, *40*, 234−242, 327−329.
(13) Jacobson, J. S.; Troxel, A. B.; Evans, J.; Klaus, L.; Vahdat, L.; et al. Randomized trial of black cohosh for the treatment of hot flashes among women with a history of breast cancer. *J. Clin. Oncol.* **2001**, *19*, 2739−2745.
(14) Kang, H. J.; Ansbacher, R.; Hammoud, M. M. Use of alternative and complementary medicine in menopause. *Int. J. Gynaecol. Obstet.* **2002**, *79*, 195−207.
(15) Kronenberg, F.; Fugh-Berman, A. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. *Ann. Intern. Med.* **2002**, *137*, 805−813.
(16) Pockaj, B. A.; Loprinzi, C. L.; Sloan, J. A.; Novotny, P. J.; Barton, D. L.; et al. Pilot evaluation of black cohosh for the treatment of hot flashes in women. *Cancer Invest.* **2004**, *22*, 515−521.
(17) Nappi, R. E.; Malavasi, B.; Brundu, B.; Facchinetti, F. Efficacy of *Cimicifuga racemosa* on climacteric complaints: a randomized study versus low-dose transdermal estradiol. *Gynecol. Endocrinol.* **2005**, *20*, 30−35.

(18) Jarry, H.; Harnischfeger, G.; Duker, E. The endocrine effects of constituents of *Cimicifuga racemosa*. 2. In vitro binding of constituents to estrogen receptors. *Planta Med.* **1985**, 316−319.

(19) Duker, E. M.; Kopanski, L.; Jarry, H.; Wuttke, W. Effects of extracts from *Cimicifuga racemosa* on gonadotropin release in menopausal women and ovariectomized rats. *Planta Med.* **1991**, *57*, 420−424.

(20) Jarry, H.; Metten, M.; Spengler, B.; Christoffel, V.; Wuttke, W. In vitro effects of the *Cimicifuga racemosa* extract BNO 1055. *Maturitas* **2003**, *44* (Suppl. 1), S31−38.

(21) Struck, D.; Tegtmeier, M.; Harnischfeger, G. Flavones in extracts of *Cimicifuga racemosa*. *Planta Med.* **1997**, *63*, 289−290.

(22) Kennelly, E. J.; Baggett, S.; Nuntanakorn, P.; Ososki, A. L.; Mori, S. A.; et al. Analysis of thirteen populations of black cohosh for formononetin. *Phytomedicine* **2002**, *9*, 461−467.

(23) Liu, J.; Burdette, J. E.; Xu, H.; Gu, C.; van Breemen, R. B.; et al. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J. Agric. Food Chem.* **2001**, *49*, 2472−2479.

(24) Liske, E.; Hanggi, W.; Henneicke-von Zepelin, H. H.; Boblitz, N.; Wustenberg, P.; et al. Physiological investigation of a unique extract of black cohosh (*Cimicifuga racemosa* rhizoma): a 6-month clinical study demonstrates no systemic estrogenic effect. *J. Womens Health Gend. Based Med.* **2002**, *11*, 163−174.

(25) Borrelli, F.; Izzo, A. A.; Ernst, E. Pharmacological effects of *Cimicifuga racemosa*. *Life Sci.* **2003**, *73*, 1215−1229.

(26) Shanafelt, T. D.; Barton, D. L.; Adjei, A. A.; Loprinzi, C. L. Pathophysiology and treatment of hot flashes. *Mayo Clin. Proc.* **2002**, *77*, 1207−1218.

(27) Stomati, M.; Rubino, S.; Spinetti, A.; Parrini, D.; Luisi, S.; et al. Endocrine, neuroendocrine and behavioral effects of oral dehydroepiandrosterone sulfate supplementation in postmenopausal women. *Gynecol. Endocrinol.* **1999**, *13*, 15−25.

(28) Weiss, G.; Skurnick, J. H.; Goldsmith, L. T.; Santoro, N. F.; Park, S. J. Menopause and hypothalamic-pituitary sensitivity to estrogen. *JAMA−J. Am. Med. Assoc.* **2004**, *292*, 2991−2996.

(29) Burdette, J. E.; Liu, J.; Chen, S. N.; Fabricant, D. S.; Piersen, C. E.; et al. Black cohosh acts as a mixed competitive ligand and partial agonist of the serotonin receptor. *J. Agric. Food Chem.* **2003**, *51*, 5661−5670.

(30) Xu, H.; Fabricant, D. S.; Piersen, C. E.; Bolton, J. L.; Pezzuto, J. M.; et al. A preliminary RAPD-PCR analysis of *Cimicifuga* species and other botanicals used for women's health. *Phytomedicine* **2002**, *9*, 757−762.

(31) Li, W.; Chen, S.-N.; Fabricant, D.; Angerhofer, C. K.; Fong, H. H. S.; et al. High-performance liquid chromatographic analysis of black cohosh (*Cimicifuga racemosa*) constituents with in-line evaporative light scattering and photodiode array detection. *Anal. Chim. Acta* **2002**, *471*, 61−75.

(32) Chen, S. N.; Fabricant, D. S.; Pauli, G. F.; Fong, H. H.; Farnsworth, N. R. Synthesis of cimiracemate B, a phenylpropanoid found in *Cimicifuga racemosa*. *Nat. Prod. Res.* **2005**, *19*, 287−290.

(33) Fabricant, D. S.; Nikolic, D.; Lankin, D. C.; Chen, S. N.; Jaki, B. U.; et al. Cimipronidine, a cyclic guanidine alkaloid from *Cimicifuga racemosa*. *J. Nat. Prod.* **2005**, *68*, 1266−1270.

(34) Chen, S. N.; Fabricant, D. S.; Lu, Z. Z.; Fong, H. H.; Farnsworth, N. R. Cimiracemosides I−P, new 9,19-cyclolanostane triterpene glycosides from *Cimicifuga racemosa*. *J. Nat. Prod.* **2002**, *65*, 1391−1397.

(35) Chen, S. N.; Fabricant, D. S.; Lu, Z. Z.; Zhang, H.; Fong, H. H.; et al. Cimiracemates A−D, phenylpropanoid esters from the rhizomes of *Cimicifuga racemosa*. *Phytochemistry* **2002**, *61*, 409−413.

(36) Chen, S. N.; Li, W.; Fabricant, D. S.; Santarsiero, B. D.; Mesecar, A.; et al. Isolation, structure elucidation, and absolute configuration of 26-deoxyactein from *Cimicifuga racemosa* and clarification of nomenclature associated with 27-deoxyactein. *J. Nat. Prod.* **2002**, *65*, 601−605.

(37) Fabricant, D. S. Pharmacognostic investigation of black cohosh (*Cimicifuga racemosa* (L.) Nutt.). Ph.D. dissertation, Department of Pharmacognosy, University of Illinois at Chicago, Chicago, IL, 2006; 210 pp.

(38) Webster, D. E.; Lu, J.; Chen, S. N.; Farnsworth, N. R.; Wang, Z. J. Activation of the μ-opiate receptor by *Vitex agnus-castus* methanol extracts: implication for its use in PMS. *J. Ethnopharmacol.* **2006**, *106*, 216−221.

(39) Wang, Z.; Gardell, L. R.; Ossipov, M. H.; Vanderah, T. W.; Brennan, M. B.; et al. Pronociceptive actions of dynorphin maintain chronic neuropathic pain. *J. Neurosci.* **2001**, *21*, 1779−1786.

(40) Lu, J.; Jeon, E.; Lee, B. S.; Onyuksel, H.; Wang, Z. J. Targeted drug delivery crossing cytoplasmic membranes of intended cells via ligand-grafted sterically stabilized liposomes. *J. Controlled Release* **2006**, *110*, 505−513.

(41) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 percent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(42) Grady, D.; Wenger, N. K.; Herrington, D.; Khan, S.; Furberg, C.; et al. Postmenopausal hormone therapy increases risk for venous thromboembolic disease. The Heart and Estrogen/progestin Replacement Study. *Ann. Intern. Med.* **2000**, *132*, 689−696.

(43) Grady, D.; Herrington, D.; Bittner, V.; Blumenthal, R.; Davidson, M.; et al. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). *JAMA−J. Am. Med. Assoc.* **2002**, *288*, 49−57.

(44) Hulley, S.; Furberg, C.; Barrett-Connor, E.; Cauley, J.; Grady, D.; et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). *JAMA−J. Am. Med. Assoc.* **2002**, *288*, 58−66.

(45) Rossouw, J. E.; Anderson, G. L.; Prentice, R. L.; LaCroix, A. Z.; Kooperberg, C.; et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA−J. Am. Med. Assoc.* **2002**, *288*, 321−333.

(46) Kreek, M. J.; LaForge, K. S.; Butelman, E. Pharmacotherapy of addictions. *Nat. Rev. Drug Discov.* **2002**, *1*, 710−726.

(47) Delitala, G.; Trainer, P. J.; Oliva, O.; Fanciulli, G.; Grossman, A. B. Opioid peptide and α-adrenoceptor pathways in the regulation of the pituitary-adrenal axis in man. *J. Endocrinol.* **1994**, *141*, 163−168.

(48) Abs, R.; Verhelst, J.; Maeyaert, J.; Van Buyten, J. P.; Opsomer, F.; et al. Endocrine consequences of long-term intrathecal administration of opioids. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 2215−2222.

(49) Genazzani, A. R.; Petraglia, F. Opioid control of luteinizing hormone secretion in humans. *J. Steroid Biochem.* **1989**, *33*, 751−755.

(50) Acosta-Martinez, M.; Etgen, A. M. Estrogen modulation of mu-opioid receptor-stimulated [^{35}S]-GTP-γ-S binding in female rat brain visualized by in vitro autoradiography. *Neuroendocrinology* **2002**, *76*, 235−242.

(51) Acosta-Martinez, M.; Etgen, A. M. Activation of μ-opioid receptors inhibits lordosis behavior in estrogen and progesterone-primed female rats. *Horm. Behav.* **2002**, *41*, 88−100.

(52) Freedman, R. R. Pathophysiology and treatment of menopausal hot flashes. *Semin. Reprod. Med.* **2005**, *23*, 117−125.

(53) Kaufman, J. M.; Kesner, J. S.; Wilson, R. C.; Knobil, E. Electrophysiological manifestation of luteinizing hormone-releasing hormone pulse generator activity in the rhesus monkey: influence of α-adrenergic and dopaminergic blocking agents. *Endocrinology* **1985**, *116*, 1327−1333.

(54) Freedman, R. R.; Krell, W. Reduced thermoregulatory null zone in postmenopausal women with hot flashes. *Am. J. Obstet. Gynecol.* **1999**, *181*, 66−70.

(55) Kronenberg, F.; Downey, J. A. Thermoregulatory physiology of menopausal hot flashes: a review. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 1312−1324.

(56) Simpkins, J. W.; Katovich, M. J.; Song, I. C. Similarities between morphine withdrawal in the rat and the menopausal hot flush. *Life Sci.* **1983**, *32*, 1957−1966.

(57) Katovich, M. J.; Simpkins, J. W.; O'Meara, J. Effects of acute central LH-RH administration of the skin temperature response in morphine dependent rats. *Brain Res.* **1989**, *494*, 85−94.

Received for review October 1, 2006. Accepted October 24, 2006. This publication was funded by the following grants: AT003476 from the National Center for Complementary and Alternative Medicine (NCCAM) and the Office of Dietary Supplements (ODS); AT000155 jointly provided to the UIC/NIH Center for Botanical Dietary Supplements Research by the ODS, the NCCAM, the Office for Research on Women's Health, and the National Institute for General Medical Sciences; and DA005050 from the National Institute on Drug Abuse. D.E.W. is supported by an NIH predoctoral fellowship (F31AT002669). J.L. is a University Fellow. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NCCAM, NIDA, or the National Institutes of Health.

JF062808U