

## Activation of the $\mu$ -opiate receptor by *Vitex agnus-castus* methanol extracts: Implication for its use in PMS

D.E. Webster<sup>a,b</sup>, J. Lu<sup>a</sup>, S.-N. Chen<sup>b</sup>, N.R. Farnsworth<sup>b</sup>, Z. Jim Wang<sup>a,b,\*</sup>

<sup>a</sup> Department of Biopharmaceutical Sciences, College of Pharmacy, 833 South Woods Street, Room 335, University of Illinois, Chicago, IL 60612, USA

<sup>b</sup> UIC/NIH Center for Botanical Dietary Supplements Research and Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois, Chicago, IL 60612, USA

Received 12 October 2005; accepted 19 December 2005

Available online 24 January 2006

### Abstract

The dried ripe fruit of *Vitex agnus-castus* L. (VAC) is widely used for the treatment of premenstrual syndrome (PMS). A previous study reported that extracts of VAC showed affinity to opiate receptors; however, functional activity was not determined. We tested two different VAC extracts in receptor binding and functional assays. Our objectives were: (1) to confirm the opiate affinity; (2) to rule out interference by free fatty acids (FFA); (3) to determine the mode of action of VAC at the  $\mu$ -opiate receptor. Methanol extracts of VAC were prepared either before (VAC-M1) or after (VAC-M2) extraction with petroleum ether to remove fatty acids. Both extracts showed significant affinities to the  $\mu$ -opiate receptor, as indicated by the concentration-dependent displacement of [<sup>3</sup>H]DAMGO binding in Chinese hamster ovary (CHO)-human  $\mu$ -opiate receptor (hMOR) cells. The IC<sub>50</sub> values were estimated to be 159.8  $\mu$ g/ml (VAC-M1) and 69.5  $\mu$ g/ml (VAC-M2). Since the defatted extract not only retained, but exhibited a higher affinity ( $p < 0.001$ ), it argued against significant interference by fatty acids. In an assay to determine receptor activation, VAC-M1 and VAC-M2 stimulated [<sup>35</sup>S]GTP $\gamma$ S binding by 41 and 61% ( $p < 0.001$ ), respectively. These results suggested for the first time that VAC acted as an agonist at the  $\mu$ -opiate receptor, supporting its beneficial action in PMS.

© 2005 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** *Vitex agnus-castus*; Opiate; CNS; Premenstrual syndrome; PMS;  $\beta$ -Endorphin

### 1. Introduction

The dried ripe fruit from *Vitex agnus-castus* L. (VAC) (Lamiaceae, formerly Verbenaceae), commonly known as chasteberry, is one of the most popular botanical dietary supplements for the treatment of premenstrual syndrome (PMS) (Upton, 2001). PMS refers to the cyclical occurrence of emotional, behavioral, and physical complaints occurring in the last 5–10 days of the luteal phase of the menstrual cycle. Symptoms include depressive moods, irritability, anxiety, confusion, breast tenderness (mastalgia), abdominal bloating, fatigue, headache, and others (Mortola, 1996). The etiology of PMS is not fully understood. PMS is often treated with conventional drugs including anti-inflammatory agents such as ibuprofen and psychotropic drugs

such as selective serotonin reuptake inhibitors (Freeman et al., 1999), or hormonal interventions such as progesterone therapy (Halbreich, 2002; Wyatt et al., 2002; Rapkin, 2003). Many women seek alternative therapies, including botanical dietary supplements, because conventional therapies do not help them, or they do not want to risk the side effects of hormonal or psychotropic drugs.

VAC, a small shrub or tree up to 6 ft tall, is native to the Mediterranean and western Asia. It is now cultivated all over the world, including the southern part of the United States (Upton, 2001). VAC has been used since ancient Greek times as a treatment for menstrual problems. In addition, it has been used to treat pain, swelling, inflammation, headaches, rheumatism, and sexual dysfunction (Upton, 2001). Treatment with VAC results in few unwanted side effects, and is therefore a popular alternative therapy for women who do not respond to or tolerate the hormonal or psychotropic drugs. The last decade has provided several successful clinical trials supporting the use of VAC for treatment of PMS (Lauritzen et al., 1997; Berger et al., 2000; Loch et al., 2000; Schellenberg, 2001; Atmaca et al., 2003).

**Abbreviations:** CHO, Chinese hamster ovary; FSH, follicle stimulating hormone; hMOR, human  $\mu$ -opiate receptor; LH, luteinizing hormone; PMS, premenstrual syndrome; VAC, *Vitex agnus-castus* L.

\* Corresponding author. Tel.: +1 312 355 1429; fax: +1 312 996 0098.

E-mail address: [zjwang@uic.edu](mailto:zjwang@uic.edu) (Z.J. Wang).

The mechanism for the actions of VAC in PMS is not entirely clear. The most thoroughly studied mechanism is through dopamine receptors in the anterior pituitary. Several studies have indicated that VAC acts on dopamine D2 receptors to decrease prolactin levels (Jarry et al., 1994; Hoberg, 1999; Berger et al., 2000; Meier et al., 2000; Wuttke et al., 2003). This mechanism is most likely responsible for alleviating symptoms of mastodynia and hyperprolactinemia (Milewicz et al., 1993; Meier and Hoberg, 1999). Several diterpenes, including rotundifuran and clerodadienols, found in a non-polar fraction of VAC exhibited dopamine D2 receptor agonist activity (Wuttke et al., 2003), and inhibit prolactin release in vivo (Meier et al., 2000; Wuttke et al., 2003). It has also been suggested that elements in a VAC extract may act on estrogen (ER- $\beta$ ) receptors, and cholinergic receptors (Berger et al., 2000; Liu et al., 2001; Upton, 2001). Apigenin was isolated from VAC and shown to be an ER- $\beta$  agonist (Wuttke et al., 2003). However, estrogenic activity may not be beneficial in PMS (Wyatt et al., 2002).

Another mechanism through which VAC may work is the opiate system, which consists of  $\mu$ ,  $\delta$ , and  $\kappa$  opiate receptors and endogenous opiate peptides such as  $\beta$ -endorphin. This peptide assists in regulating the menstrual cycle through inhibition of the hypothalamus–pituitary–adrenal axis (HPA), therefore decreasing the amount of gonadotropic releasing hormone (GnRH) acting on the pituitary, which decreases the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH, released from the pituitary, then enter into a complex feedback loop with progesterone and estrogen to regulate the menstrual cycle (Silberstein and Merriam, 2000). Levels of  $\beta$ -endorphin decrease along with estrogen in the late luteal phase of the menstrual cycle, which correlates with the appearance of symptoms of PMS (Giannini et al., 1984, 1990; Chuong and Coulam, 1988).

Meier and co-workers reported that an ethanolic VAC extract (Ze440) and several subfractions of a methanolic extract had affinity for opiate receptors. The binding affinity (IC<sub>50</sub>) was reported to be at 20–70  $\mu$ g/ml. However, it remains unknown if VAC extracts produce agonistic or antagonistic effects. The information is important since an absence of agonistic activity would not support the use of VAC in PMS through opiate mechanisms.

In this study, we examined two methanol extracts of VAC in  $\mu$ -opiate receptor binding and functional activity assays to determine if VAC is producing an effect at the receptor level. The  $\mu$ -opiate receptor was chosen in this first functional study because it is the primary receptor for the action of  $\beta$ -endorphin. The study aimed to achieve three goals: (1) to confirm the previous finding by Meier et al. (2000), (2) to rule out interference in binding assays by free fatty acids (FFA) contained in VAC extracts, and (3) to determine the mode of action of VAC extracts.

## 2. Materials and methods

### 2.1. Drugs and chemicals

Guanosine-5'-diphosphate (GDP), guanosine-5'-[ $\gamma$ -thio]triphosphate (GTP $\gamma$ S), bovine serum albumin (BSA), dithiothre-

itol (DTT), disodium ethylenediamine tetraacetate (EDTA), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (HEPES) were obtained from Sigma (St. Louis, MO, USA). Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-Cys (dermorphin), [D-Ala<sup>2</sup>,*N*-MePhe<sup>4</sup>-Gly-ol<sup>5</sup>]enkephalin (DAMGO), and [<sup>3</sup>H]DAMGO were from Multiple Peptide Systems (San Diego, CA, USA). [<sup>35</sup>S]GTP $\gamma$ S was purchased from Amersham Biosciences (Piscataway, NJ, USA).

### 2.2. Plant extract preparation

*Vitex agnus-castus* L. was provided by PureWorld Botanicals Inc. (South Hackensack, NJ, USA) from cultivated material grown in New Mexico (USA). Voucher specimens have been deposited at the Program for Collaborative Research in the Pharmaceutical Sciences (PCRPS) at the University of Illinois at Chicago. Extracts were prepared using macerated fruits of VAC. For the first extract, VAC-M1 3.125% (w/w), fruits of VAC were extracted directly with 90% MeOH. Since VAC may contain free fatty acids that can potentially interfere with binding assays, in the second preparation, fruits were first defatted with petroleum ether (PE). The marc was then extracted with 90% methanol to yield a defatted extract, VAC-M2 2.514% (w/w). Both extracts were evaporated in vacuo to obtain dry extracts. For pharmacological assays, the dried extracts were dissolved in DMSO and further diluted with water to appropriate concentrations. The final DMSO concentrations in all assays were below 0.5%, which we found to not interfere with either receptor binding or GTP $\gamma$ S binding assay.

### 2.3. Cell culture

Full-length human  $\mu$ -opiate receptor (Wang et al., 1994) was cloned into pcDNA3 using *Hind*III sites, and transfected into Chinese hamster ovary cells (CHO-K<sub>1</sub>, ATCC, Manassas, MD, USA) using the calcium phosphate method (Arden et al., 1995). G418 (400  $\mu$ g/ml) was used to select resistant clones. Several G418-resistant clonal cell lines were selected and screened using the receptor binding assay. One such cell line (CHO-hMOR) was selected and established based on receptor density, and used for the study. 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  $\mu$ g/ml streptomycin (Wang and Sadee, 2000). To maintain stable selection, 200  $\mu$ g/ml G418 was added. Cells were cultured at 37 °C with 5% CO<sub>2</sub> in humidified air.

### 2.4. Receptor binding assay

The receptor binding assay was performed as previously described (Arden et al., 1995; Wang et al., 2001). Briefly, receptor membranes were prepared from CHO-hMOR cells by polytron homogenization at setting 6 for 2 min on ice, followed by centrifugation at 20,000  $\times$  *g* for 30 min at 4 °C. Protein content was determined by the Coomassie protein assay method (Pierce Biotechnology, Rockford, IL, USA) using bovine serum albumin as the standard. For [<sup>3</sup>H]DAMGO saturation binding,

the receptor membranes (50  $\mu\text{g}$  protein/reaction) were incubated with varying concentrations of [ $^3\text{H}$ ]DAMGO in 50 mM Tris buffer (pH 7.4) at 30 °C for 1 h. The assay was conducted in triplicate. For receptor displacement assays, the receptor binding was conducted in triplicate in 50 mM Tris buffer (pH 7.4) containing 1 nM [ $^3\text{H}$ ]DAMGO at 30 °C for 1 h (50  $\mu\text{g}$  protein/reaction). Non-specific binding was determined in the presence of 20  $\mu\text{M}$  unlabeled DAMGO. Reactions were terminated by the rapid vacuum filtration through GF/B filters presoaked with 0.2% polyethylenimine. Filter-bound radioactivity was determined by liquid scintillation counting (Beckman, Fullerton, CA, USA). Binding data were analyzed with the aid of GraphPad Prism program (San Diego, CA, USA) to obtain  $B_{\text{max}}$ ,  $K_{\text{d}}$ ,  $\text{IC}_{50}$ , and maximum inhibition. The dissociation constant  $K_{\text{i}}$  values were determined by the method of Cheng and Prusoff (1973).

### 2.5. GTP $\gamma$ S binding assay

Determination of [ $^{35}\text{S}$ ]GTP $\gamma$ S binding in cell and brain membranes was performed based on the method previously described (Wang et al., 2001; Narita et al., 2003). Briefly, cell membranes were prepared as above. Membranes (40  $\mu\text{g}$  protein) were incubated with 0.1 nM [ $^{35}\text{S}$ ]GTP $\gamma$ S (1000 Ci/mmol) in the reaction buffer (50 mM HEPES, pH 7.4, 100 mM sodium chloride, 1 mM disodium ethylenediamine tetraacetate, 5 mM magnesium chloride, 30  $\mu\text{M}$  guanosine-5'-diphosphate, 1 mM dithiothreitol, and 0.1% BSA) in the presence or absence of VAC extracts or DAMGO, at 30 °C for 60 min. The basal level was defined as the amount of [ $^{35}\text{S}$ ]GTP $\gamma$ S bound in the absence of any agonist. Non-specific binding was determined in the presence of 10  $\mu\text{M}$  unlabeled GTP $\gamma$ S. Reactions were terminated by rapid filtration through Whatman GF/B filters, followed by three washes with ice-cold wash buffer (50 mM Tris, pH 7.4). The membrane-bound [ $^{35}\text{S}$ ]GTP $\gamma$ S was determined by liquid scintillation counting. Data were analyzed with the aid of GraphPad Prism program to obtain  $\text{EC}_{50}$ , and  $E_{\text{max}}$ .

### 2.6. Statistical analysis

Data, expressed as mean  $\pm$  standard error, were analyzed with the aid of GraphPad Prism program to obtain  $\text{IC}_{50}$ , maximum inhibition,  $\text{EC}_{50}$ , and  $E_{\text{max}}$  values. The dissociation constant  $K_{\text{i}}$  values were determined by the method of Cheng and Prusoff (1973). Differences in responses between groups were determined using ANOVA followed by Student's *t*-tests.

## 3. Results

Chinese hamster ovary cells were stably transfected with the cloned human  $\mu$ -opiate receptor (Wang et al., 1994) to establish a cell model for the study. The selected clonal cell line (CHO-hMOR) expressed a high level of hMOR, with an expected high affinity to DAMGO, a MOR selective ligand ( $K_{\text{d}} = 0.6$  nM; Fig. 1A). The number of binding sites per cell was estimated to be  $8.6 \times 10^4$  (Fig. 1A). As expected, morphine, a naturally occurring opiate agonist, competitively displaced the binding of

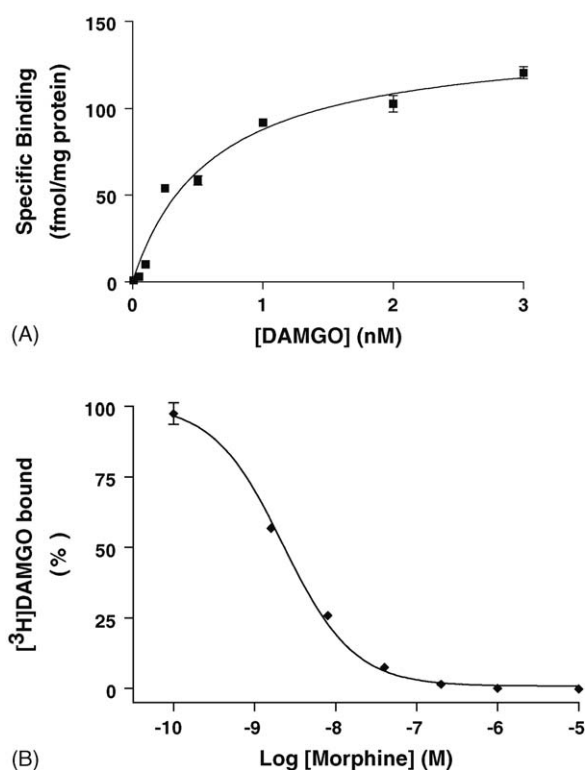


Fig. 1. Saturation binding of [ $^3\text{H}$ ]DAMGO (A), and displacement of [ $^3\text{H}$ ]DAMGO binding by the opiate agonist morphine (B) in CHO-hMOR cells. For [ $^3\text{H}$ ]DAMGO saturation binding, the receptor membranes were incubated with varying concentrations of [ $^3\text{H}$ ]DAMGO at 30 °C for 1 h. For displacement of [ $^3\text{H}$ ]DAMGO binding, the receptor membranes were incubated with 1 nM [ $^3\text{H}$ ]DAMGO and a series of different concentrations of morphine at 30 °C for 1 h, and the specific radioactivity of [ $^3\text{H}$ ]DAMGO bound to hMOR in the absence of morphine was set to 100%. Each point represents the mean  $\pm$  S.E.M. from three independent experiments. Morphine  $\text{IC}_{50}$  and  $K_{\text{i}}$  were estimated to be 2.3 and 0.8 nM, respectively.

[ $^3\text{H}$ ]DAMGO to the receptor (Fig. 1B). The  $\text{IC}_{50}$  and  $K_{\text{i}}$  were estimated to be 2.3 and 0.8 nM, respectively. This cell line and the same displacement binding assay were applied in the study to characterize the affinity of VAC extracts.

### 3.1. Receptor binding assay

Similar to morphine, the VAC methanol (90%) extract (VAC-M1) was able to competitively displace the binding of [ $^3\text{H}$ ]DAMGO, indicating the extract's affinity to hMOR (Fig. 2). The  $\text{IC}_{50}$  and  $K_{\text{i}}$  values were estimated to be 159.8 and 59.9  $\mu\text{g}/\text{ml}$ , respectively. At the highest concentration used (300  $\mu\text{g}/\text{ml}$ ), VAC-M1 was able to displace  $69.1 \pm 0.1\%$  specific binding.

It has been suggested that free fatty acids in VAC extracts may interfere with receptor binding assays (Ingkaninan et al., 1999; Liu et al., 2004). To address this issue, we defatted VAC with petroleum ether before the marc was extracted with 90% methanol (VAC-M2). VAC-M2 also showed significant affinity to hMOR with  $\text{IC}_{50}$  and  $K_{\text{i}}$  of 69.5 and 26.1  $\mu\text{g}/\text{ml}$ , respectively. At the highest concentration used (300  $\mu\text{g}/\text{ml}$ ), VAC-M2 displaced  $89.7 \pm 1.0\%$  specific binding (Fig. 2). Because extraction

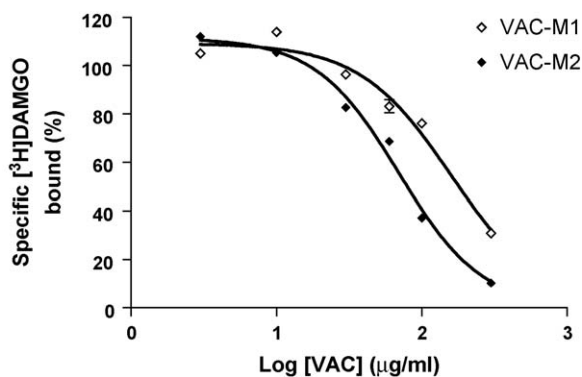


Fig. 2. Displacement of [<sup>3</sup>H]DAMGO (1 nM) binding by VAC extracts (VAC-M1 (◇) and VAC-M2 (◆)) in CHO-hMOR cells. Methanol extracts of VAC were prepared either before (VAC-M1) or after (VAC-M2) extraction with petroleum ether. The specific radioactivity of [<sup>3</sup>H]DAMGO bound to hMOR in the absence of VAC extracts was set to 100%. Each point represents the mean ± S.E.M. from three independent experiments. The IC<sub>50</sub> (*K*<sub>i</sub>) values were estimated to be 159.8 µg/ml (59.9 µg/ml) and 69.5 µg/ml (26.1 µg/ml) for VAC-M1 and VAC-M2, respectively.

with petroleum ether removes the majority of non-polar and less polar components including most of free fatty acids, our data argue against significant, if any, interference of MOR binding by FFA in VAC.

### 3.2. GTPγS binding assay

It is critical to ascertain if VAC methanol extracts contain agonistic or antagonistic opiate activity at the receptor. Since the  $\mu$ -opiate receptor belongs to the superfamily of G protein-coupled receptors, we employed the GTPγS binding assay to determine the mode of action of VAC extracts. Activation of MOR by its agonist such as DAMGO produced an increase in the binding of GTPγS to cell membranes (Wang et al., 2001; Narita et al., 2003). Under our assay condition, DAMGO, used as a positive control, produced a maximum stimulation of  $146.1 \pm 5.7\%$  (mean ± S.E.M.) over the baseline (Fig. 3A) and the EC<sub>50</sub> was 254 nM, which was similar to the values previously reported (Selley et al., 2001). Similar to DAMGO, both VAC-M1 and VAC-M2 produced a dose-dependent activation of G proteins, as indicated by GTPγS binding (Fig. 3B). The maximum effect was  $41.4 \pm 3.1$  and  $61.4 \pm 1.9\%$  above the baseline, for VAC-M1 and VAC-M2, respectively. The EC<sub>50</sub> values were estimated to be 2.8 µg/ml for VAC-M1 and 2.2 µg/ml for VAC-M2 (*N* = 3 for both extracts). These data demonstrated that VAC methanol extracts (VAC-M1 and VAC-M2), had agonistic activity at the  $\mu$ -opiate receptor.

## 4. Discussion

Although chaste berry has been used since ancient Greek times and has been shown clinically to be effective for the treatment of symptoms of PMS, the mechanisms of action are not completely understood. The purpose of this study was to investigate the  $\mu$ -opiate receptor pathway as a potential mechanism for VAC. The opiate system plays an essential role in regulating tonic pain perception, mood, appetite, and other functions.

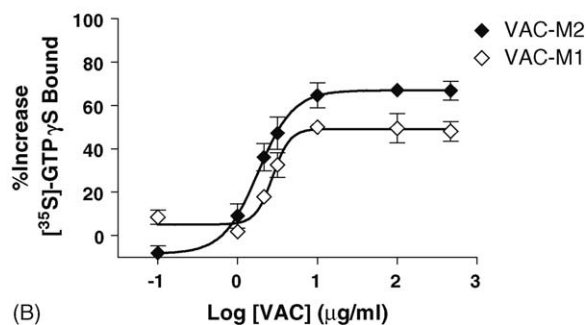
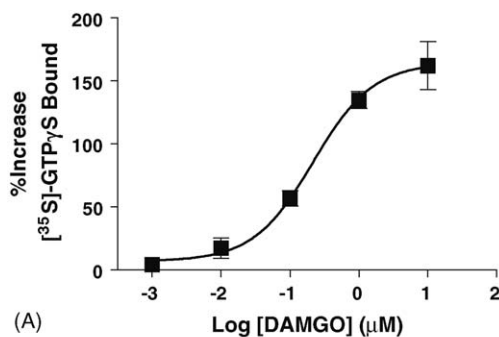


Fig. 3. Stimulation of [<sup>35</sup>S]GTPγS binding by the  $\mu$ -opiate receptor agonist DAMGO (A) and VAC extracts (VAC-M1 (◇) and VAC-M2 (◆)) (B) in CHO-hMOR cells. Like the positive control (A), both VAC extracts stimulated [<sup>35</sup>S]GTPγS binding, indicative of the presence of agonist activities in VAC-M1 and VAC-M2. Each point represents the mean ± S.E.M. from three independent experiments. The EC<sub>50</sub> values were estimated to be 2.8 µg/ml for VAC-M1 and 2.2 µg/ml for VAC-M2.

A reduction of opiate activity is a hallmark in PMS (Chuong et al., 1985; Tulenheimo et al., 1987; Giannini et al., 1988, 1990; Chuong and Hsi, 1994). The severity of symptoms such as anxiety, food cravings, and physical discomfort is inversely proportional to the amount of decline in  $\beta$ -endorphin levels in the luteal phase (Halbreich and Endicott, 1981; Giannini et al., 1984, 1994).  $\beta$ -Endorphin exerts a tonic inhibition (especially through  $\mu$ -opiate receptors) on the hypothalamus–pituitary–adrenal axis (HPA) (Delitala et al., 1994; Kreek et al., 2002). Such tonic inhibition is weakened significantly in patients with severe PMS (Facchinetti et al., 1989, 1994). Therefore, VAC may improve symptoms of PMS by activating  $\mu$ -opiate receptors to reverse the loss of opiate tonic inhibition or directly activating analgesic and mood regulatory pathways.

A previous report suggested that VAC extracts had affinity to opiate receptors (Meier et al., 2000). The current study confirmed that VAC methanol extracts had affinity to the  $\mu$ -opiate receptor. The  $\mu$ -opiate receptor is the primary action site for  $\beta$ -endorphin in vivo. In future studies, we will determine if similar affinity is found for  $\delta$  and  $\kappa$  opiate receptor, as has been suggested (Meier et al., 2000).

One confounding factor in interpreting the results from the previous study (Meier et al., 2000) was that the VAC extract may have contained free fatty acids, which may cause false positives in receptor binding experiments at high concentrations (Ingkaninan et al., 1999; Liu et al., 2004). To rule out this potential interference, we compared two different VAC methanol extracts. VAC-M2 was extracted first with petroleum

ether to remove fatty acids. We found that VAC-M2 retained significant binding affinity to the  $\mu$ -opiate receptor. In fact, the affinity of VAC-M2 was better than that of VAC-M1, suggesting that extraction with petroleum ether enriched opiate activity in VAC-M2. These data argue strongly against significant, if any, interference of MOR binding by free fatty acids in the VAC-M1 extract.

The specific action of VAC extracts at the  $\mu$ -opiate receptor was further supported by their activity in the GTP $\gamma$ S assays, although the primary purpose for these experiments was to determine the mode of action of VAC extracts. [<sup>35</sup>S]GTP $\gamma$ S binding assays directly determine the activation of G proteins by a G protein coupled receptor. The assay has been widely used to determine the activation of the  $\mu$ -opiate receptor by its agonists (Traynor and Nahorski, 1995; Hosohata et al., 1998; Selley et al., 2001). Both VAC-M1 and VAC-M2 stimulated GTP $\gamma$ S binding in CHO-hMOR cells, suggesting that the  $\mu$ -opiate receptor can be activated by these extract. Opiate agonistic, not antagonistic, activity from VAC is consistent with its therapeutic effect for the treatment of PMS. Therefore, these results suggested that affinity and activity of VAC at the  $\mu$ -opiate receptor can serve as a molecular mechanism for its effectiveness in treating PMS. In a separate study, we found free fatty acids did not stimulate [<sup>35</sup>S]GTP $\gamma$ S binding (Webster and Wang, unpublished results), further ruling out the interference of free fatty acids. We propose further studies to determine active fractions and compound(s) that are responsible for the opiate activity.

## 5. Conclusion

In summary, this study confirmed the previous finding that VAC extracts had affinity to the  $\mu$ -opiate receptor. By comparing the actions of two methanol extracts, we conclude that the observed receptor affinity was not due to interference by free fatty acids. Moreover, our studies for the first time identified agonistic activity exhibited by VAC extracts at the  $\mu$ -opiate receptor. The opiate activity may, therefore, be a plausible mechanism for the beneficial action of VAC in PMS.

## Acknowledgments

This publication was made possible by grants (AT002406 and AT000155) from the National Center for Complementary and Alternative Medicine (NCCAM) and a grant (DA05050) from the National Institute on Drug Abuse (NIDA). Donna Webster is supported by an NIH predoctoral fellowship (F31AT002669). Jian Lu 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.

## References

Arden, J., Segredo, V., Wang, Z., Lameh, J., Sadee, W., 1995. Phosphorylation and agonist-specific intracellular trafficking of an epitope-tagged mu-opioid receptor expressed in HEK 293 cells. *Journal of Neurochemistry* 65, 1636–1645.

- Atmaca, M., Kumru, S., Tezcan, E., 2003. Fluoxetine versus *Vitex agnus-castus* extract in the treatment of premenstrual dysphoric disorder. *Human Psychopharmacology* 18, 191–195.
- Berger, D., Schaffner, W., Schrader, E., Meier, B., Brattstrom, A., 2000. Efficacy of *Vitex agnus-castus* L. extract Ze 440 in patients with premenstrual syndrome (PMS). *Archives of Gynecology and Obstetrics* 264, 150–153.
- Cheng, Y., Prusoff, W., 1973. Relationship between the inhibition constant (K<sub>1</sub>) and the concentration of inhibitor which causes 50 percent inhibition (I<sub>50</sub>) of an enzymatic reaction. *Biochemical Pharmacology* 22, 3099–3108.
- Chuong, C., Coulam, C., 1988. Current view and beta-endorphin hypothesis. In: Gise, I.H.K.N., Berkowith, R.L. (Eds.), *The Premenstrual Syndrome*. Churchill Livingstone, New York, pp. 75–95.
- Chuong, C., Coulam, C., Kao, P., Bergstralh, E., Go, V., 1985. Neuropeptide levels in premenstrual syndrome. *Fertility and Sterility* 44, 760–765.
- Chuong, C.J., Hsi, B.P., 1994. Effect of naloxone on luteinizing hormone secretion in premenstrual syndrome. *Fertility and Sterility* 61, 1039–1044.
- Delitala, G., Trainer, P., Oliva, O., Fanciulli, G., Grossman, A., 1994. Opioid peptide and alpha-adrenoceptor pathways in the regulation of the pituitary-adrenal axis in man. *Journal of Endocrinology* 141, 163–168.
- Facchinetti, F., Fioroni, L., Martignoni, E., Sances, G., Costa, A., Genazzani, A.R., 1994. Changes of opioid modulation of the hypothalamo-pituitary-adrenal axis in patients with severe premenstrual syndrome. *Psychosomatic Medicine* 56, 418–422.
- Facchinetti, F., Martignoni, E., Nappi, G., Fioroni, L., Sances, G., Genazzani, A., 1989. Premenstrual failure of alpha-adrenergic stimulation on hypothalamus–pituitary responses in menstrual migraine. *Psychosomatic Medicine* 51, 550–558.
- Freeman, E.W., Rickels, K., Arrendondo, F., Kao, L.C., Pollack, S.E., Sondheimer, S.T., 1999. Full- or half-cycle treatment of severe premenstrual syndrome with a serotonergic antidepressant. *Journal of Clinical Psychopharmacology* 19, 3–8.
- Giannini, A., Martin, D., Turner, C., 1990. Beta-endorphin decline in late luteal phase dysphoric disorder. *International Journal of Psychiatry in Medicine* 20, 279–284.
- Giannini, A., Melemis, S., Martin, D., Folts, D., 1994. Symptoms of premenstrual syndrome as a function of beta-endorphin: two subtypes. *Progress in Neuropsychopharmacology and Biological Psychiatry* 18, 321–327.
- Giannini, A., Price, W.A., Loiselle, R.H., 1984. Beta-endorphin withdrawal: a possible cause of premenstrual tension syndrome. *International Journal of Psychophysiology* 1, 341–343.
- Giannini, A., Sullivan, B., Sarachene, J., Loiselle, R., 1988. Clonidine in the treatment of premenstrual syndrome: a subgroup study. *Journal of Clinical Psychiatry* 49, 62–63.
- Halbreich, U., Endicott, J., 1981. Possible involvement of endorphin withdrawal or imbalance in specific premenstrual syndromes and postpartum depression. *Medical Hypotheses* 7, 1045–1058.
- Halbreich, U., 2002. The pathophysiologic background for current treatments of premenstrual syndromes. *Current Psychiatry Reports* 4, 429–434.
- Hoberg, E., 1999. *Phytochemical and Analytical Investigations of Vitex agnus-castus* L. Swiss Federal Institute of Technology Zurich, Zurich, p. 129.
- Hosohata, K., Burkey, T., Alfaro-Lopez, J., Varga, E., Hruba, V., Roeske, W., Yamamura, H.I., 1998. Endomorphin-1 and endomorphin-2 are partial agonists at the human mu-opioid receptor. *European Journal of Pharmacology* 346, 111–114.
- Ingkaninan, K., von Frijtag Drabbe Kunzel, J.K., Ijzerman, A.P., Verpoorte, R., 1999. Interference of linoleic acid fraction in some receptor binding assays. *Journal of Natural Products* 62, 912–914.
- Jarry, H., Leonhardt, S., Gorkow, C., Wuttke, W., 1994. In vitro prolactin but not LH and FSH release is inhibited by compounds in extracts of *agnus-castus*: direct evidence for a dopaminergic principle by the dopamine receptor assay. *Experimental and Clinical Endocrinology* 102, 448–454.
- Kreek, M., LaForge, K., Butelman, E., 2002. Pharmacotherapy of addictions. *Natural Reviews Drug Discovery* 1, 710–726.
- Lauritzen, C., Reuter, H., Repges, R., Bohnert, K.-J., Schmidt, U., 1997. Treatment of premenstrual tension syndrome with *Vitex agnus-castus*:

- controlled, double-blind study versus pyridoxine. *Phytomedicine* 4, 183–189.
- Liu, J., Burdette, J.E., Sun, Y., Deng, S., Schlecht, S.M., Zheng, W., Nikolic, D., Mahady, G., van Breemen, R.B., Fong, H., Pezzuto, J.M., Bolton, J.L., Farnsworth, N.R., 2004. Isolation of linoleic acid as an estrogenic compound from the fruits of *Vitex agnus-castus* L. (chaste-berry). *Phytomedicine* 11, 18–23.
- Liu, J., Burdette, J.E., Xu, H., Gu, C., van Breemen, R.B., Bhat, K.P., Booth, N., Constantinou, A.I., Pezzuto, J.M., Fong, H.H., Farnsworth, N.R., Bolton, J.L., 2001. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *Journal of Agricultural and Food Chemistry* 49, 2472–2479.
- Loch, E.G., Selle, H., Boblitz, N., 2000. Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing *Vitex agnus-castus*. *Journal of Womens Health Gender Based Medicine* 9, 315–320.
- Meier, B., Berger, D., Hoberg, E., Sticher, O., Schaffner, W., 2000. Pharmacological activities of *Vitex agnus-castus* extracts in vitro. *Phytomedicine* 7, 373–381.
- Meier, B., Hoberg, E., 1999. *Agni-casti fructus*. *Zeitschrift fur Phytotherapie* 20, 140–158.
- Milewicz, A., Gejdel, E., Sworen, H., Sienkiewicz, K., Jedrzejak, J., Teucher, T., Schmitz, H., 1993. *Vitex agnus-castus* extract in the treatment of luteal phase defects due to latent hyperprolactinemia. Results of a randomized placebo-controlled double-blind study. *Arzneimittelforschung* 43, 752–756.
- Mortola, J., 1996. Premenstrual syndrome. *Trends in Endocrinology and Metabolism* 7, 184–189.
- Narita, M., Masami, S., Mizoguchi, H., Narita, M., Yajima, Y., Sakurada, S., Tseng, L., Suzuki, T., 2003. Up-regulation of  $\mu$ -opioid receptor-mediated G-protein activation in protein kinase C $\gamma$  knockout mice following repeated naloxone treatment. *Neuroscience Letters* 338, 103–106.
- Rapkin, A., 2003. A review of treatment of premenstrual syndrome and premenstrual dysphoric disorder. *Psychoneuroendocrinology* 28 (Suppl. 3), 39–53.
- Schellenberg, R., 2001. Treatment for the premenstrual syndrome with *agnus-castus* fruit extract: prospective, randomised, placebo controlled study. *BMJ* 322, 134–137.
- Selley, D., Cao, C., Sexton, T., Schwegel, J., Martin, T., Childers, S., 2001. Mu-opioid receptor-mediated G-protein activation by heroin metabolites: evidence for greater efficacy of 6-monoacetylmorphine compared with morphine. *Biochemistry and Pharmacology* 62, 447–455.
- Silberstein, S.D., Merriam, G.R., 2000. Physiology of the menstrual cycle. *Cephalalgia* 20, 148–154.
- Traynor, J.R., Nahorski, S.R., 1995. Modulation by mu-opioid agonists of guanosine-5'-O-(3-[35S]thio)triphosphate binding to membranes from human neuroblastoma SH-SY5Y cells. *Molecular Pharmacology* 47, 848–854.
- Tulenheimo, A., Laatikainen, T., Salminen, K., 1987. Plasma beta-endorphin immunoreactivity in premenstrual tension. *British Journal of Obstetrics and Gynaecology* 94, 26–29.
- Upton, R. (Ed.), 2001. Chaste Tree Fruit, *Vitex agnus-castus*: Standards of Analysis, Quality Control, and Therapeutics. American Herbal Pharmacopoeia, Santa Cruz, CA.
- Wang, Z., Gardell, L., Ossipov, M., Vanderah, T., Brennan, M., Hochgeschwender, U., Hruba, V., Malan, T., Lai, J., Porreca, F., 2001. Pronociceptive actions of dynorphin maintain chronic neuropathic pain. *Journal of Neuroscience* 21, 1779–1786.
- Wang, J., Johnson, P., Persico, A., Hawkins, A., Griffin, C., Uhl, G., 1994. Human mu-opiate receptor: cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. *FEBS Letters* 338, 217–222.
- Wang, Z., Sadee, W., 2000. Tolerance to morphine at the mu-opioid receptor differentially induced by cAMP-dependent protein kinase activation and morphine. *European Journal of Pharmacology* 389, 165–171.
- Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., Seidlova-Wuttke, D., 2003. Chaste tree (*Vitex agnus-castus*)—pharmacology and clinical indications. *Phytomedicine* 10, 348–357.