Biochemical Pharmacology, Vol. 58, pp. 1807–1813, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(9?)00274-9

Allosteric Regulation by Oleamide of the Binding Properties of 5-Hydroxytryptamine$_7$ Receptors

Peter B. Hedlund, Monica J. Carson, J. Gregor Sutcliffe and Elizabeth A. Thomas*
Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, U.S.A.

ABSTRACT. Oleamide belongs to a family of amidated lipids with diverse biological activities, including sleep induction and signaling modulation of several 5-hydroxytryptamine (5-HT) receptor subtypes, including 5-HT$_{1A}$, 5-HT$_{2A/2C}$, and 5-HT$_7$. The 5-HT$_7$ receptor, predominantly localized in the hypothalamus, hippocampus, and frontal cortex, stimulates cyclic AMP formation and is thought to be involved in the regulation of sleep–wake cycles. Recently, it was proposed that oleamide acts at an allosteric site on the 5-HT$_7$ receptor to regulate cyclic AMP formation. We have further investigated the interaction between oleamide and 5-HT$_7$ receptors by performing radioligand binding assays with HeLa cells transfected with the 5-HT$_7$ receptor. Methiothepin, clozapine, and 5-HT all displaced specific [^{3}H]5-HT (100 nM) binding, with pK_D values of 7.55, 7.85, and 8.39, respectively. Oleamide also displaced [^{3}H]5-HT binding, but the maximum inhibition was only 40% of the binding. Taking allosteric (see below) cooperativity into account, a K_D of 2.69 nM was calculated for oleamide. In saturation binding experiments, oleamide caused a 3-fold decrease in the affinity of [^{3}H]5-HT for the 5-HT$_7$ receptor, without affecting the number of binding sites. A Schild analysis showed that the induced shift in affinity of [^{3}H]5-HT reached a plateau, unlike that of a competitive inhibitor, illustrating the allosteric nature of the interaction between oleamide and the 5-HT$_7$ receptor. Oleic acid, the product of oleamide hydrolysis, had a similar effect on [^{3}H]5-HT binding, whereas structural analogs of oleamide, *trans*-9,10-octadecenamide, *cis*-8,9-octadecenamide, and erucamide, did not alter [^{3}H]5-HT binding significantly. The findings support the hypothesis that oleamide acts via an allosteric site on the 5-HT$_7$ receptor regulating receptor affinity. BIOCHEM PHARMACOL **58**;11:1807–1813, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. oleamide; serotonin; receptor; allosteric; regulation; transfection

Oleamide is a member of a recently recognized family of amidated lipids found in the plasma and cerebrospinal fluid of mammals, including humans [1–3]. The diverse bioregulatory properties, including neuromodulatory effects, direct receptor-mediated mechanisms, and high biological activities of these fatty acid amide family members have gained increasing attention (for reviews, see Bezuglov *et al.* [4] and Boger *et al.* [5]). Included in this family are two endogenous ligands for the cannabinoid receptor, anandamide and palmitoylethanolamine, which have been shown to have not only direct actions on cannabinoid receptors, but also a variety of modulatory properties [6–8]. Additional fatty acid amide bioregulators include the compound olvanil, the oleamide derivative of capsaicin, which has been shown to be a selective agonist for the vanilloid receptor [9], and the simple fatty acid amide erucamide, which displays angiogenic properties [10].

In vivo, oleamide demonstrates a variety of physiological effects. Oleamide was initially reported to induce sleep after intraperitoneal injections in rats [2] and has since been shown to exhibit long-lasting hypothermic effects, also after intraperitoneal administration [11]. The mechanisms for these effects remain unknown; however, it has been shown recently that oleamide activates select populations of neurons in mouse cortex, thalamus, and hypothalamus, suggesting that there are distinct neuronal targets for oleamide [12].

In vitro, oleamide has been shown to modulate the signaling of several 5-HT† receptor subtypes, including 5-HT$_{1A}$, 5-HT$_{2A/2C}$, and 5-HT$_7$ [12–14]. These effects involve potentiation and/or inhibition of cyclic AMP and inositol phosphate pathways. Recently, functional studies indicated that oleamide acts at an apparent allosteric site on the 5-HT$_7$ receptor to regulate cyclic AMP formation [14]. Oleamide by itself induced a concentration-dependent increase in cyclic AMP formation that could not be inhibited by clozapine, suggesting that it acted at a site distinct from the primary 5-HT binding site. However, in the presence of 5-HT, oleamide had the opposite effect, antagonizing the cyclic AMP stimulating effect of 5-HT [14]. In addition, oleamide has been shown to activate 5-HT$_7$ neurons in mouse thalamus and hypothalamus, as indicated by c-fos induction after intraperitoneal adminis-

* Corresponding author: Dr. J. Gregor Sutcliffe, Department of Molecular Biology, MB10, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. Tel. (858) 784-8064; FAX (858) 784-2212; E-mail: gregor@scripps.edu
Received 25 January 1999; accepted 11 May 1999.

† *Abbreviation*: 5-HT, 5-hydroxytryptamine.

tration, further supporting the notion of serotonergic mechanisms in oleamide function [12].

The 5-HT_7 receptor has been shown to stimulate cyclic AMP formation [15, 16], possibly by acting via calmodulin-stimulated adenylyl cyclases [17]. The distribution of 5-HT_7 receptors in the brain has been described using *in situ* hybridization and radioligand binding studies [16, 18, 19]. These studies show that they are localized predominantly in the hypothalamus, hippocampus, and frontal cortex. As yet, selective ligands have not been available for these studies, although a recently described compound is thought to be a selective antagonist [20]. Pharmacological and cloning studies have revealed the existence of several splice variants of the 5-HT_7 receptor, resulting in slightly different lengths of their C-termini [21–23]. The different variants are all expressed in tissues expressing the 5-HT_7 receptor, and their functional coupling seems to be similar. Ever since its discovery, the 5-HT_7 receptor has been implicated in the regulation of sleep–wake cycles [16, 24]. The circadian phase regulation of the suprachiasmatic nucleus in response to 5-HT shows a pharmacological profile consistent exclusively with the 5-HT_7 receptor.

Taken together, these findings may indicate a regulatory mechanism involving oleamide and 5-HT that may be important for a number of physiological and pathological conditions, including sleep, appetite, alertness, thermoregulation, and psychiatric disturbances [25, 26]. In view of this, we have further evaluated in the present study the regulation of 5-HT_7 receptors by oleamide and its derivatives by examining the binding properties of 5-HT_7 receptors in transfected HeLa cells. The results support the hypothesis that oleamide regulates the 5-HT_7 receptor by acting at an allosteric site on the receptor.

MATERIALS AND METHODS

Materials

[^{3}H]5-HT (specific activity 21.7 Ci/mmol) was purchased from New England Nuclear. 5-HT, pargyline, and BSA were obtained from the Sigma Chemical Co. Clozapine and methiothepin were purchased from Research Biochemicals. Oleamide and its derivatives oleic acid, *trans*-9,10-octadecenamide, *cis*-8,9-octadecenamide, and erucamide were provided by Benjamin Cravatt (The Scripps Research Institute).

Cell Culture

HeLa cells were grown and cultured in OM5 medium [27] supplemented with 10% fetal bovine serum in a 5% CO_2 environment. The HeLa cells were transfected transiently with pCMV4REC20 (5-HT_7 receptor) [16] by using DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methyl sulphate; Boehringer Mannheim) lipotransfection as described by the vendor.

Membrane Preparation

The cells were used 72 hr after transfection, and all experiments were performed using fresh cell cultures. They were rinsed once with PBS and then incubated at 37° for 5 min with 5 mL of cell dissociation solution (Sigma). Then 5 mL of PBS was added, and the resulting cell suspension was transferred to a centrifuge tube and centrifuged for 10 min at 2200 rpm (1000 *g*). The supernatant was discarded, and the pellet was resuspended in 10 mL of buffer (50 mM Tris, 4 mM $CaCl_2$). The suspension was homogenized with a Teflon homogenizer, and then centrifuged for 10 min at 2200 rpm (1000 *g*). The pellet was resuspended in buffer as above, now also containing 0.1% BSA.

Receptor Binding

The membranes were incubated with [^{3}H]5-HT in 50 mM Tris buffer containing 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% BSA. The incubations were performed in 96-well plates with a final volume of 200 μL, for 1 hr at 25° under equilibrium conditions. The incubations were stopped by rapid filtration using a Packard cell harvester. Whatman GF-B filters pre-soaked in 0.1% polyethyleneimine for >3 hr were used. The filters were washed three times with deionized water. The filter radioactivity was detected using liquid scintillation in 4 mL of scintillation fluid. The final protein concentrations were determined by the Lowry method, using BSA as a standard, and were approximately 0.1 mg/well. The binding properties of the transfected 5-HT_7 receptors were initially characterized by evaluating the ability of 5-HT, clozapine, methiothepin, and oleamide to displace [^{3}H]5-HT binding. Competition curves were generated from experiments in which the drugs in ten concentrations ranging from 0.1 nM to 10 μM were allowed to compete with 100 nM [^{3}H]5-HT for its binding sites. Saturation curves were generated by incubating membranes with ten different concentrations of [^{3}H]5-HT ranging from 0.1 to 125 nM. Nonspecific binding was defined as the binding obtained in the presence of 10 μM 5-HT. The amount of specific binding obtained was at least 60% in all experiments. To evaluate any modulatory effects, the incubations were performed in the presence or absence of oleamide in concentrations ranging from 0.1 to 1000 nM. The effects of 300 nM concentrations of the oleamide derivatives oleic acid, *trans*-9,10-octadecenamide, *cis*-8,9-octadecenamide, and erucamide also were evaluated in similar saturation experiments.

Data Analysis

The binding parameters IC_{50} (drug concentration inhibiting 50% of the binding), B_{max} (maximum number of binding sites), and K_D (dissociation rate constant) were determined using iterative nonlinear, least-squares regression analysis [28]. Data points having absolute standard residual values >2 were excluded from the analysis. In the competition

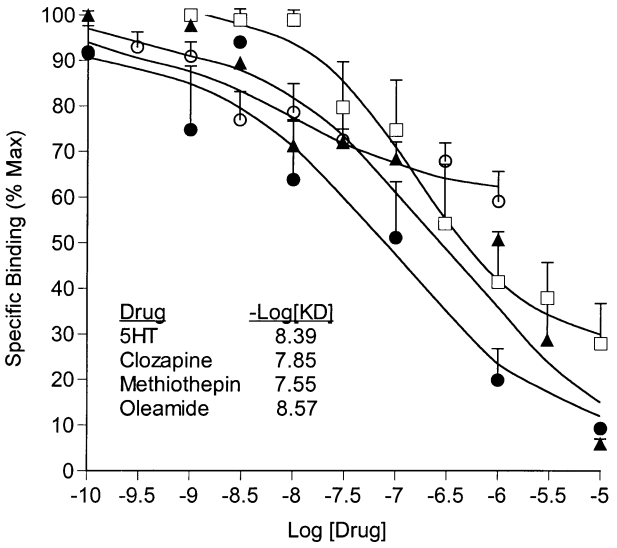

FIG. 1. Competition binding of [^{3}H]5-HT to membranes from HeLa cells transiently transfected with the 5-HT$_7$ receptor. Membrane preparations were incubated with [^{3}H]5-HT (100 nM), and the ability of 5-HT(●), clozapine (□), methiothepin (▲), and oleamide (○) to compete with the binding was evaluated at increasing concentrations. Data points are expressed as percent maximal specific binding, which was approximately 2500 cpm. The inset shows the calculated affinity constants as described in Materials and Methods. Values are expressed as means ± SEM; N = 5–8 independent experiments.

experiments, the K_D value for 5-HT was calculated using the equation $K_D = IC_{50} - L$ and the K_D value for the other competitors according to the equation $K_D = IC_{50}/(1 + (L/K_{D(5\text{-HT})}))$, where L represents the radioligand concentration. The K_D and cooperativity (α) values for oleamide were calculated as described previously [29] for allosteric agents.

Possible effects of the various treatments were evaluated using repeated measures ANOVA followed by Fisher's PLSD test.

RESULTS

To test for a direct interaction between oleamide and 5-HT$_7$ receptors, radioligand binding assays were performed on membranes from HeLa cells transfected with the 5-HT$_7$ receptor cDNA. In competition experiments with [^{3}H]5-HT (100 nM), methiothepin, clozapine, and 5-HT each displaced the specific [^{3}H]5-HT binding with corresponding pK_D values of 7.55, 7.85, and 8.39 (Fig. 1). Oleamide also displaced specific binding of [^{3}H]5-HT in a concentration-dependent manner with relatively high potency; however, the maximum effect of oleamide reached a plateau value at 59% of the total binding (Fig. 1), indicating an allosteric mechanism of action. From this plateau value, we have estimated a cooperativity value (α) for oleamide of 6.1 as described by Ehlert [29]. The resulting K_D value for oleamide, when considering this cooperativity, was calculated to be 2.69 nM (pK_D = 8.57) (see Ehlert [29]).

In saturation binding experiments, oleamide caused a 3-fold decrease in the affinity of [^{3}H]5-HT for the 5-HT$_7$ receptor. However, there was a limit to this affinity change at higher concentrations (>100 nM), indicating an allosteric mechanism of action. Significant effects were observed at 300 and 1000 nM (Fig. 2A, Table 1). Oleamide caused no significant alterations in average maximal binding (B_{max} values), with the possible exception of 1 nM oleamide. This exceptional value was probably due to fewer

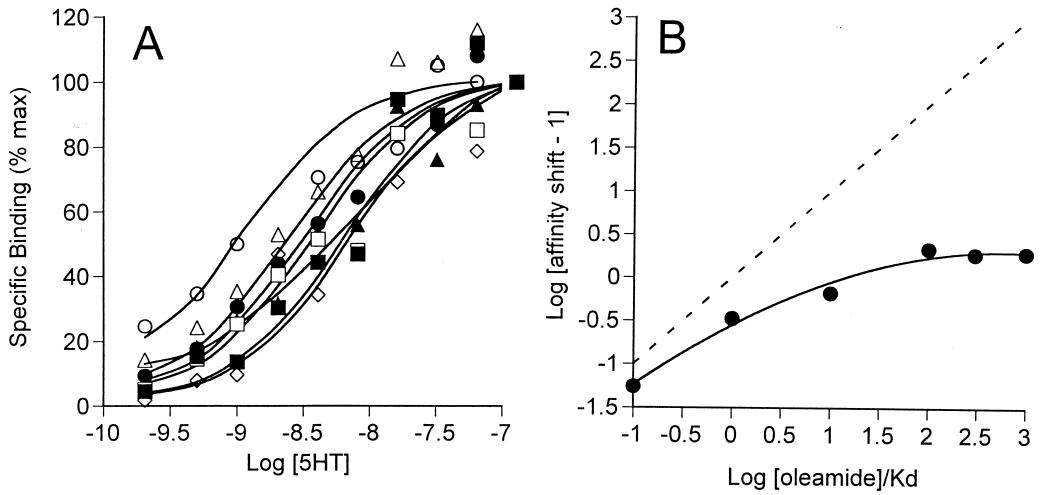

FIG. 2. Saturation binding of [^{3}H]5-HT to membranes from HeLa cells transiently transfected with the 5-HT$_7$ receptor. Membrane preparations were incubated with [^{3}H]5-HT (0.1 to 125 nM), in the absence or presence of increasing concentrations of oleamide. (A) Concentration–response effect as percent of B_{max} for each concentration from a representative experiment. The average maximal specific binding was 313 fmol/mg protein. The symbols represent control (○) and oleamide (0.1 nM, Δ; 10 nM,●; 30 nM, ▲; 100 nM, □; 300 nM, ■; 1000 nM, ◇). (B) Effect of oleamide on the EC_{50} value of [^{3}H]5-HT in the form of a Schild plot as an average for all experiments (N = 5–8 independent experiments). The dashed line shows the expected behavior of a competitive antagonist.

TABLE 1. Effects of oleamide on [^{3}H]5-HT binding to membranes from 5-HT$_7$ receptor-transfected HeLa cells

Oleamide (nM)	K_D (nM)	B_{max} (fmol/mg protein)
0	7.7 ± 2.5	345.3 ± 143.6
0.1	8.2 ± 3.2	275.8 ± 96.0
1	10.3 ± 4.8	50.7 ± 21.3*
10	12.8 ± 5.6	321.2 ± 100.7
100	24.3 ± 12.9	321.5 ± 91.6
300	22.7 ± 5.2†	366.2 ± 88.5
1000	22.5 ± 8.2†	255.1 ± 90.6

Membrane preparations from HeLa cells transiently transfected with the 5-HT$_7$ receptor were incubated with [^{3}H]5-HT (0.1 to 125 nM), in the absence or presence of increasing concentrations of oleamide. Values are expressed as means ± SEM; *N = 5–8 independent experiments.

*†Significantly different as determined by repeated measures ANOVA followed by Fisher's PLSD test: *$P < 0.01$, and †$P < 0.05$.

TABLE 2. Effects of oleamide and its derivatives on [^{3}H]5-HT binding to membranes from 5-HT$_7$ receptor-transfected HeLa cells

Compound	K_D (nM)	B_{max} (fmol/mg protein)
None	12.68 ± 2.02	172.2 ± 26.3
Oleamide	21.04 ± 2.61*	190.5 ± 39.1
Oleic acid	24.10 ± 6.16†	222.2 ± 23.5
*trans*9,10-Octadecenamide	18.26 ± 4.56	163.2 ± 23.5
cis-8,9-Octadecenamide	21.56 ± 6.86	172.9 ± 23.0
Erucamide	16.39 ± 4.67	173.0 ± 40.7

Membrane preparations from HeLa cells transiently transfected with the 5-HT$_7$ receptor were incubated with [^{3}H]5-HT (0.1 to 125 nM), in the absence and presence of oleamide and some of its derivatives (300 nM). Values are expressed as means ± SEM; n = 10 independent experiments.

*†Significantly different as determined by repeated measures ANOVA followed by Fisher's PLSD test:**P < 0.05, and †$P < 0.01$.

experiments performed with that particular concentration. Representation of these results by a Schild plot illustrates the allosteric nature of the interaction between oleamide and the 5-HT$_7$ receptor (Fig. 2B). The shift of affinity of [^{3}H]5-HT induced by oleamide reached a plateau, unlike that of a competitive inhibitor (theoretical dashed line in Fig. 2B).

To test for specificity of the effect of oleamide on [^{3}H]5-HT binding, three structural analogs of oleamide, which vary in the position and configuration of the double bond as well as in carbon backbone length (Fig. 3), were investigated in saturation binding experiments. In addition, oleic acid, the product of oleamide hydrolysis, also was tested (Fig. 3). Oleic acid had a similar effect to that of oleamide on the affinity of [^{3}H]5-HT for the 5-HT$_7$ receptor (Table 2). The other derivatives, *trans*-9,10-octadecenamide, *cis*-8,9-octadecenamide, and erucamide, did not alter the binding characteristics of [^{3}H]5-HT significantly, although a range of intermediate effects on the K_D values were observed (Table 2).

DISCUSSION

The major finding of the present study is that oleamide modulated the binding properties of 5-HT$_7$ receptors expressed *in vitro*. Characterization of the binding of [^{3}H]5-HT to 5-HT$_7$ receptors transiently transfected in HeLa cells showed that competitive drugs, such as methiothepin, clozapine, and 5-HT itself, displaced the [^{3}H]5-HT binding with a rank order of potency consistent with that previously described for 5-HT$_7$ receptors [16, 30]. Thus, it can be assumed that the transfections have resulted in expression of 5-HT$_7$ receptors and that we are not observing another, possibly endogenously expressed, 5-HT receptor subtype. Oleamide induced a 3-fold decrease in the apparent affinity of the 5-HT$_7$ receptor for [^{3}H]5-HT, without affecting maximal binding. The increase of the K_D value for the 5-HT$_7$ receptors reached a maximum at approximately 100 nM oleamide. A Schild analysis of the effects of oleamide indicated that with increasing concentrations a plateau

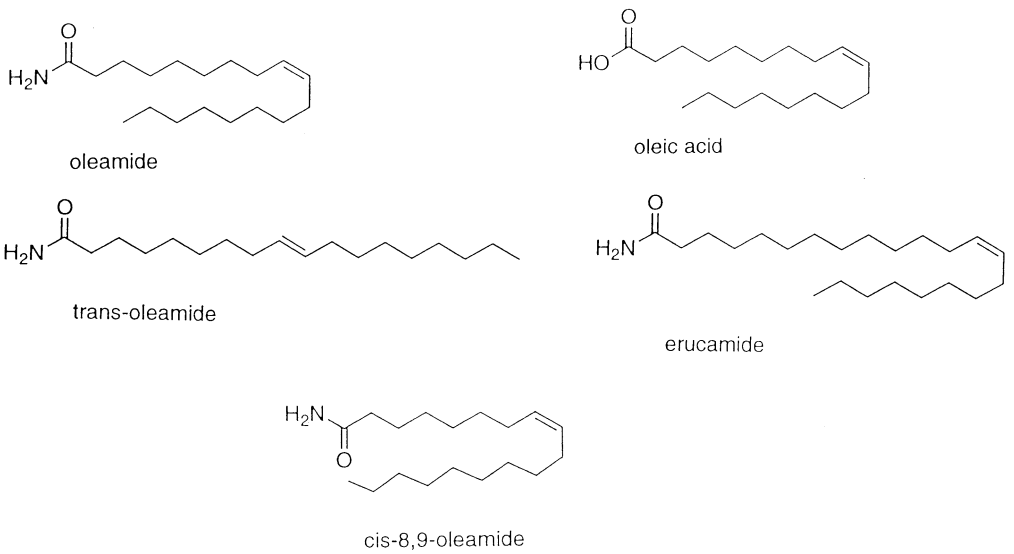

FIG. 3. Structures of oleamide and its derivatives.

value was reached where the shift in K_D could not be increased further. In addition, a maximally effective concentration of oleamide caused only a 40% displacement of [^{3}H]5-HT binding, indicating a non-competitive mechanism of action. This finding supports the hypothesis that oleamide acts via an allosteric site on the 5-HT$_7$ receptor, at which it induces a decrease in receptor affinity without influencing the number of binding sites. Such an allosteric interaction has been proposed based on findings that oleamide can regulate the cyclic AMP formation elicited by 5-HT$_7$ receptor activation [14]. There it was shown that oleamide could inhibit the cyclic AMP formation caused by 5-HT$_7$ receptor activation by acting at a site different from the primary 5-HT site, but a site present only in cells expressing the receptor.

Oleamide has been shown to interact with other 5-HT receptor subtypes, such as the 5-HT$_{2A/2C}$ and 5-HT$_{1A}$ subtypes [12–14]. However, little is known to date about unique binding sites for oleamide in the brain or other tissues. The presence of allosteric regulatory binding sites has been well established for ionotropic receptors, such as the NMDA (*N*-methyl-d-aspartate) glutamate receptor and the GABA$_A$ (γ-aminobutyric acid) receptor [31–33]. Ligands acting at the allosteric sites typically regulate the affinity of the endogenous ligands in a complex manner, but also have been shown to induce effects on channel function by themselves [31, 34]. Allosteric sites also have been described for G-protein coupled receptors, such as the acetylcholine, α-adrenergic, and 5-HT$_2$ receptors [34–37]. The 5-HT$_{2A}$ subtype has well defined allosteric sites that can inhibit or enhance 5-HT function [38–40]. For example, ketanserin and 2-bromo-lysergic acid diethylamide have been shown to inhibit 5-HT function at the 5-HT$_{2A}$ receptor in an allosteric manner [38, 39]. Conversely, ouabain has been shown to potentiate 5-HT-mediated contractions of rabbit ear artery by interacting with a less well defined allosteric site on the 5-HT$_{2A}$ receptor [40]. Recent studies by Massot *et al.* [41] have demonstrated that the 5-HT$_{1B/1D}$ receptor also can be regulated allosterically, in that case by a novel peptide, 5-HT-moduline. Radioligand binding studies in 5-HT$_{1B}$-transfected NIH3T3 cells demonstrated that 5-HT-moduline decreased maximal binding sites without affecting the K_d of the radioligand [41]. Given this information, it is likely that oleamide acts in a similar allosteric manner at these 5-HT$_1$ and 5-HT$_2$ receptor families.

While our results and those mentioned above are consistent with the notion that oleamide acts allosterically on the 5HT$_7$ receptor, the mechanism for this allostery is uncertain. Like other agents, oleamide may elicit a conformational change in the receptor that is necessary for signal activation. Alternatively, oleamide might interact at a unique site(s) near the protein–membrane interface that affects the conformation of membrane-bound proteins, including G proteins. Such effects could alter the affinity for 5-HT and/or modulate signaling activity. Recent studies have demonstrated functional dimerization of G protein-coupled receptors, which has been shown to affect cellular signaling [42–44]. Hebert and colleagues [44] have demonstrated that a peptide derived from the transmembrane domain of the β-adrenergic receptor could inhibit dimerization of this receptor, thus inhibiting agonist-induced stimulation of adenylyl cyclase activity. It is possible that oleamide affects receptor dimerization, thereby regulating downstream activation of effector systems, such as adenylate cyclase.

To test the specificity of oleamide, we also evaluated the ability of oleamide derivatives to modify the 5-HT$_7$ receptor binding. It was found that oleic acid could influence the K_D of [^{3}H]5-HT binding in a manner similar to oleamide, whereas none of the other derivatives tested had a significant K_D or B_{max} effect. Oleic acid is identical in structure to oleamide, except that it lacks the amide group on the primary carbon. Previous functional studies [13, 14] have suggested that the primary amide group is a structural moiety important for the activity of oleamide, since no effects on 5-HT$_{2A/2C}$ or 5-HT$_7$ receptor-mediated functional responses were observed with oleic acid. However, it may be hypothesized that oleic acid, and to some extent possibly the other derivatives, can act as an antagonist, binding to the same recognition site as oleamide. Since oleic acid lacks the primary amide group, it may not be capable of eliciting the proper conformational change in the receptor or associated protein(s) required for a full functional response. This would be in agreement with physiological studies that have demonstrated that oleic acid does not, for example, have the same sleep-inducing effect as oleamide. It should be noted, however, that with such a complex phenomenon as sleep, additional mechanisms most likely are involved.

The concept that G-proteins can be activated by ligands acting at an allosteric site is relatively new. It has been demonstrated that various typical allosteric agents can activate muscarinic receptors in the absence of a muscarinic receptor agonist [45]. The finding that oleamide could stimulate cyclic AMP formation in the absence of 5-HT in cells transfected with the 5-HT$_7$ receptor [14] gave further support to the notion of receptor activation by allosteric agents. Taken together with the present study, the hypothesis of an allosteric regulatory interaction between the endogenous lipid oleamide and the 5-HT$_7$ receptor is supported further. This interaction may be important for a number of physiological or pathological conditions, including regulation of sleep and body temperature.

We thank Dr. Benjamin Cravatt for supplying the oleamide and related compounds. This work was supported, in part, by a grant from the NIH (GM32355) and by fellowships from the Wenner-Gren Center Foundation (P.B.H.), Lundbeck Foundation (P.B.H.), and NARSAD (MEFA90TD, E.A.T.).

References

1. Arafat ES, Trimble JW, Andersen RN, Dass C and Desiderio DM, Identification of fatty acid amides in human plasma. *Life Sci* **45:** 1679–1687, 1989.
2. Cravatt BF, Prospero-Garcia O, Siuzdak G, Gilula NB, Henriksen SJ, Boger DL and Lerner RA, Chemical characterization of a family of brain lipids that induce sleep. *Science* **268:** 1506–1509, 1995.
3. Lerner RA, Siuzdak G, Prospero-Garcia O, Henriksen SJ, Boger DL and Cravatt BF, Cerebrodiene: A brain lipid isolated from sleep-deprived cats. *Proc Natl Acad Sci USA* **91**: 9505–9509, 1994.
4. Bezuglov VV, Bobrov MY and Archakov AV, Bioactive amides of fatty acids. *Biochemistry (Mosc)* **63:** 22–30, 1998.
5. Boger DL, Patterson JE and Jin Q, Structural requirements for 5-HT_{2A} and 5-HT_{1A} serotonin receptor potentiation by the biologically active lipid oleamide. *Proc Natl Acad Sci USA* **95:** 4102–4107, 1998.
6. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A and Mechoulam R, Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258:** 1946–1949, 1992.
7. Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD and Leon A, Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci USA* **92:** 3376–3380, 1995.
8. Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen BM and Leonoudakis DJ, Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J Neurochem* **70:** 671–676, 1998.
9. Liu L, Lo Y-C, Chen I-J and Simon SA, The responses of rat trigeminal ganglion neurons to capsaicin and two nonpungent vanilloid receptor agonists, olvanil and glyceryl nonamide. *J Neurosci* **17:** 4101–4111, 1997.
10. Wakamatsu K, Masaki T, Itoh F, Kondo K and Sudo K, Isolation of fatty acid amide as an angiogenic principle from bovine mesentery. *Biochem Biophys Res Commun* **168:** 423–429, 1990.
11. Horn TFW, Cravatt B, Patterson J, Boger D, Gombart L and Henriksen SJ, Effects of the sleep-inducing lipid oleamide on body temperature in rats. *Soc Neurosci Abstr* **24** (Part 1)**:** 696, 1998.
12. Thomas EA, Cravatt BF and Sutcliffe JG, The endogenous lipid, oleamide, activates 5-HT_7 neurons in mouse thalamus and hypothalamus. *J Neurochem* **72:** 2370–2378, 1999.
13. Huidobro-Toro JP and Harris RA, Brain lipids that induce sleep are novel modulators of 5-hydroxytryptamine receptors. *Proc Natl Acad Sci USA* **93:** 8078–8082, 1996.
14. Thomas EA, Carson MJ, Neal MJ and Sutcliffe JG, Unique allosteric regulation of 5-hydroxytryptamine receptor-mediated signal transduction by oleamide. *Proc Natl Acad Sci USA* **94:** 14115–14119, 1997.
15. Bard JA, Zgombick J, Adham N, Vaysse P, Branchek TA and Weinshank RL, Cloning of a novel human serotonin receptor (5-HT_7) positively linked to adenylate cyclase. *J Biol Chem* **268:** 23422–23426, 1993.
16. Lovenberg TW, Baron BM, de Lecea L, Miller JD, Prosser RA, Rea MA, Foye PE, Racke M, Slone AL, Siegel BW, Danielson PE, Sutcliffe JG and Erlander MG, A novel adenylyl cyclase-activating serotonin receptor (5-HT_7) implicated in the regulation of mammalian circadian rhythms. *Neuron* **11:** 449–458, 1993.
17. Baker LP, Nielsen MD, Impey S, Metcalf MA, Poser SW, Chan G, Obrietan K, Hamblin MW and Storm DR, Stimulation of type 1 and type 8 Ca^{2+}/calmodulin-sensitive adenylyl cyclases by the G_s-coupled 5-hydroxytryptamine subtype 5-HT_{7A} receptor. *J Biol Chem* **273:** 17469–17476, 1998.
18. Gustafson EL, Durkin MM, Bard JA, Zgombick J and Branchek TA, A receptor autoradiographic and *in situ* hybridization analysis of the distribution of the 5-ht_7 receptor in rat brain. *Br J Pharmacol* **117:** 657–666, 1996.
19. Mengod G, Vilaro MT, Raurich A, Lopez-Gimenez JF, Cortes R and Palacios JM, 5-HT receptors in mammalian brain: Receptor autoradiography and *in situ* hybridization studies of new ligands and newly identified receptors. *Histochem J* **28:** 747–758, 1996.
20. Forbes IT, Dabbs S, Duckworth DM, Jennings AJ, King FD, Lovell PJ, Brown AM, Collin L, Hagan JJ, Middlemiss DN, Riley GJ, Thomas DR and Upton N, (*R*)-3,*N*-Dimethyl-*N*-[1-methyl-3-(4-methyl-piperidin-1-yl) propyl]benzenesulfonamide: The first selective 5-HT_7 receptor antagonist. *J Med Chem* **41:** 655–657, 1998.
21. Heidmann DE, Metcalf MA, Kohen R and Hamblin MW, Four 5-hydroxytryptamine$_7$ (5-HT_7) receptor isoforms in human and rat produced by alternative splicing: Species differences due to altered intron-exon organization. *J Neurochem* **68:** 1372–1381, 1997.
22. Jasper JR, Kosaka A, To ZP, Chang DJ and Eglen RM, Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT_7 receptor (h5-$HT_{7(b)}$). *Br J Pharmacol* **122:** 126–132, 1997.
23. Stam NJ, Roesink C, Dijcks F, Garritsen A, van Herpen A, and Olijve W, Human serotonin 5-HT_7 receptor: Cloning and pharmacological characterisation of two receptor variants. *FEBS Lett* **413:** 489–494, 1997.
24. Cutrera RA, Saboureau M and Pevet P, Phase-shifting effect of 8-OH-DPAT, a 5-HT_{1A}/5-HT_7 receptor agonist, on locomotor activity in golden hamster in constant darkness. *Neurosci Lett* **210:** 1–4, 1996.
25. Brewerton TD, Toward a unified theory of serotonin dysregulation in eating and related disorders. *Psychoneuroendocrinology* **20:** 561–590, 1995.
26. Leonard BE, Serotonin receptors and their function in sleep, anxiety disorders and depression. *Psychother Psychosom* **65:** 66–75, 1996.
27. Raible DW and McMorris FA, Induction of oligodendrocyte differentiation by activators of adenylate cyclase. *J Neurosci Res* **27:** 43–46, 1990.
28. Hedlund PB and von Euler G, EasyBound—A user-friendly approach to nonlinear regression analysis of binding data. *Comput Methods Programs Biomed* **58:** 245–249, 1999.
29. Ehlert FJ, Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods. *Mol Pharmacol* **33:** 187–194, 1987.
30. Eglen RM, Jasper JR, Chang DJ and Martin GR, The 5-HT_7 receptor, Orphan found. *Trends Pharmacol Sci* **18:** 104–107, 1997.
31. Deutsch SI, Mastropaolo J and Hitri A, GABA-active steroids: Endogenous modulators of GABA-gated chloride ion conductance. *Clin Neuropharmacol* **15:** 352–364, 1992.
32. Reynolds IJ and Miller RJ, Allosteric modulation of *N*-methyl-d-aspartate receptors. *Adv Pharmacol* **21:** 101–126, 1990.
33. Wisden W and Seeburg PH, $GABA_A$ receptor channels, From subunits to functional entities. *Curr Opin Neurobiol* **2:** 263–269, 1992.
34. Costa E, Allosteric modulatory centers of transmitter amino acid receptors. *Neuropsychopharmacology* **2:** 167–174, 1989.
35. Jakubik J and Tucek S, Protection by alcuronium of muscarinic receptors against chemical inactivation and location of the allosteric binding site for alcuronium. *J Neurochem* **63:** 1932–1940, 1994.
36. Lee NH and El-Fakahany EE, Allosteric antagonists of the

muscarinic acetylcholine receptor. *Biochem Pharmacol* **42:** 199–205, 1991.
37. Xu Z and Purdy RE, Evidence for allosteric blockade of serotonergic receptors in rabbit thoracic aorta. *J Pharmacol Exp Ther* **248:** 1091–1095, 1989.
38. Burris KD and Sanders-Bush E, Unsurmountable antagonism of brain 5-hydroxytryptamine$_2$ receptors by (+)-lysergic acid diethylamide and bromo-lysergic acid diethylamide. *Mol Pharmacol* **42:** 826–830, 1992.
39. Frenken M and Kaumann AJ, Allosteric properties of the 5-HT$_2$ receptor system of the rat tail artery. Ritanserin and methysergide are not competitive 5-HT$_2$ receptor antagonists but allosteric modulators. *Naunyn Schmiedebergs Arch Pharmacol* **335:** 359–366, 1987.
40. Purdy RE, Prins BA, Weber MA, Bakhtiarian A, Smith JR, Kim MK, Nguyen TH and Weiler EW, Possible novel action of ouabain: Allosteric modulation of vascular serotonergic (5-HT$_2$) and angiotensinergic (AT$_1$) receptors. *J Pharmacol Exp Ther* **267:** 228–237, 1993.
41. Massot O, Rousselle JC, Fillion MP, Grimaldi B, Cloez-Tayarani I, Fugelli A, Prudhomme N, Seguin L, Rousseau B, Plantefol M, Hen R and Fillion G, 5-Hydroxytryptamine-moduline, a new endogenous cerebral peptide, controls the serotonergic activity via its specific interaction with 5-hydroxytryptamine$_{1B/1D}$ receptors. *Mol Pharmacol* **50:** 752–762, 1996.
42. Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemto R, Karschin A and Bettler B, GABA$_B$-receptor subtypes assemble into functional heterodimer complexes. *Nature* **396:** 683–687, 1998.
43. Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao W-J, Johnson M, Gunwaldsen C, Huang L-Y, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA and Gerald C, GABA$_B$ receptors function as a heteromeric assembly of the subunits GABA$_B$R1 and GABA$_B$R2. *Nature* **396:** 674–679, 1998.
44. Hebert TE, Moffett S, Morello J-P, Loisel TP, Bichet DG, Barret C and Bouvier M, A peptide derived from a β_2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. *J Biol Chem* **271:** 16384–16392, 1996.
45. Jakubík J, Bacáková L, Lisá V, El-Fakahany EE and Tucek S, Activation of muscarinic acetylcholine receptors via their allosteric binding sites. *Proc Natl Acad Sci USA* **93:** 8705–8709, 1996.