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Chemistry

Structural requirements for 5-HT_{2A} and 5-HT_{1A} serotonin receptor potentiation by the biologically active lipid oleamide

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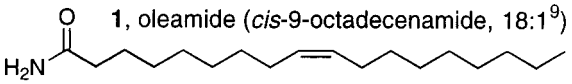
ABSTRACT **Oleamide is an endogenous fatty acid primary amide that possesses sleep-inducing properties in animals and that has been shown to effect serotonergic receptor responses and block gap junction communication. Herein, the potentiation of the 5-HT_{1A} receptor response is disclosed, and a study of the structural features of oleamide required for potentiation of the 5-HT_{2A} and 5-HT_{1A} response to serotonin (5-HT) is described. Of the naturally occurring fatty acids, the primary amide of oleic acid (oleamide) is the most effective at potentiating the 5-HT_{2A} receptor response. The structural features required for activity were found to be highly selective. The presence, position, and stereochemistry of the Δ^9-*cis* double bond is required, and even subtle structural variations reduce or eliminate activity. Secondary or tertiary amides may replace the primary amide but follow a well defined relationship requiring small amide substituents, suggesting that the carboxamide serves as a hydrogen bond acceptor but not donor. Alternative modifications at the carboxamide as well as modifications of the methyl terminus or the hydrocarbon region spanning the carboxamide and double bond typically eliminate activity. A less extensive study of the 5-HT_{1A} potentiation revealed that it is more tolerant and accommodates a wider range of structural modifications. An interesting set of analogs was identified that inhibit rather than potentiate the 5-HT_{2A}, but not the 5-HT_{1A}, receptor response, further suggesting that such analogs may permit the selective modulation of serotonin receptor subtypes and even have opposing effects on the different subtypes.**

Oleamide (**1**) is an endogenous fatty acid primary amide that accumulates in the cerebrospinal fluid under conditions of sleep deprivation (1–3) and induces physiological sleep in animals (1).



Structure **1**

Consistent with its role as a prototypical member of a new class of biological signaling molecules, enzymatic regulation of the endogenous concentrations of oleamide has been described (1, 4–7) or proposed (8). Fatty acid amide hydrolase (FAAH) is an integral membrane protein that degrades **1** to oleic acid, and potent inhibitors of the enzyme have been detailed (4, 9–11). The characterization and neuronal distribution of FAAH have been disclosed (5–7), and the enzyme was found to possess the ability to hydrolyze a range of fatty acid amides including anandamide, which serves as an endogenous ligand for the cannabinoid receptor (12, 13). Unlike anandamide, an appealing feature of this new class of biological signaling agents is the primary amide, suggesting that their storage and release may be controlled in a manner analogous to that of peptide hormones terminating in a primary amide (8).

Recent studies have shown the oleamide modulates serotonergic neurotransmission (14, 15). In the first disclosure of such effects, oleamide was shown to potentiate 5-HT_{2C} and 5-HT_{2A} receptor-mediated chloride currents in transfected frog oocytes but not those elicited by the 5-HT_3 ion-gated channel receptor or other G protein-coupled receptors (14). This potentiation was greatest for the 5-HT_{2C} receptor subtype where the effect was observed at concentrations as low as 1 nM and was maximal at 100 nM oleamide. Oleamide did not alter the serotonin (5-HT) EC_{50} but instead increased receptor efficacy. Similarly, oleamide has been reported to potentiate phosphoinositide hydrolysis in rat pituitary P11 cells expressing the 5-HT_2 receptor but to inhibit 5-HT_7 receptor-mediated stimulation of cAMP levels in HeLa cells transfected with the receptor (15). In these efforts, oleamide was shown to act as a weak agonist at the 5-HT_7 receptor but to behave as an unsurmountable antagonist in the presence of serotonin illustrating that it may act at an allosteric site. Thus, oleamide has been shown to enhance (5-HT_{2A}, 5-HT_{2C}), disrupt (5-HT_7), or have no effect (5-HT_3) on serotonergic signal transduction at various receptor subtypes. Serotonin receptors have been implicated in anxiety, depression, appetite, and thermoregulation as well as sleep and mood regulation, and strong links between 5-HT_1, 5-HT_2, and 5-HT_7 and the regulation of sleep have been disclosed (16, 17). Herein we describe a study that defines the features of oleamide required for potentiation of the 5-HT_{2A} receptor response and report the analogous but more tolerant potentiation of the 5-HT_{1A} receptor, which has not been previously examined. A set of analogs that inhibit rather than potentiate the 5-HT_{2A}, but not 5-HT_{1A}, receptor response was identified suggesting such agents may permit selective modulation of serotonin receptor subtypes or even have opposing effects on the different subtypes.

MATERIALS AND METHODS

Materials. The analogs examined were purchased (Sigma, Pfaltz & Bauer, Aldrich), prepared as described (3, 4, 18), or synthesized following protocols previously detailed (3, 4, 18, 19).

Methods. The assays were conducted with R-SAT kits (Receptor Technologies, Winooski, VT) containing NIH 3T3 cells expressing the rat 5-HT_{2A} receptor (20, 21) or RAT-1 cells expressing the human 5-HT_{1A} receptor (22, 23) cotransfected with the β-galactosidase gene and were performed according to the procedures provided (24, 25). The cells in DMEM containing serotonin (100 nM) and the analogs (500 nM) were incubated in a humidified 5% CO_2 incubator at 37°C for 4 or 5 days for the 5-HT_{2A} and 5-HT_{1A} transfected cells,

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Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); FAAH, fatty acid amide hydrolase.

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respectively. Levels of β-galactosidase were measured after incubation with the chromogenic substrate *o*-nitrophenyl-β-D-galactopyranoside (26) at 30°C in a humidified incubator for a recommended period of time, and the absorbance was measured at 405 nm. The results were normalized to 100% for oleamide (relative % potentiation) for ease of comparison and are the average of 2–8 determinations.

RESULTS

The effects of oleamide and its analogs on rat 5-HT_{2A} and human 5-HT_{1A} receptors were examined by using R-SAT-transfected cellular assays linked to a colorimetric β-galactosidase assay (24–26), which provide results identical to those derived from second messenger assays. Activation of the 5-HT_{2A} or 5-HT_{1A} receptors in 5-HT-dependent cell lines results in cell proliferation measured by the levels of β-galactosidase produced. Analogous to the findings of Huidobro-Toro and Harris (14), treatment with oleamide alone had no effect, but its coadministration with 5-HT provided a significant potentiation of the effect of 5-HT administration alone. A maximal response with the rat 5-HT_{2A} receptor was observed at 100 nM oleamide when assayed at 100 nM or 1 μM 5-HT, the concentrations at which the potentiation response (165 and 170%, respectively) was greatest. At higher concentrations of oleamide (1 μM), no additional potentiation was observed with the rat 5-HT_{2A} receptor (Table 1*A*). Similarly, the maximal potentiation for human 5-HT_{1A} was observed at 100 nM 5-HT (Fig. 1), and concentrations as low as 1–10 nM oleamide produced a measurable effect (Table 1*B*). The maximum effect was observed at concentrations of 100 nM 5-HT, and oleamide treatment provided a 370% (100 nM oleamide) or 560% (1 μM oleamide) potentiation approximating the magnitude observed with the 5-HT_{2C} receptor (14).

An extensive series of agents was tested at 500 nM for their ability to potentiate the rat 5-HT_{2A} or human 5-HT_{1A} receptor response to 100 nM 5-HT. This spans the concentration range (100 nM–1 μM) in which oleamide exhibits its greatest potentiation and employs a 5-HT concentration at which the effect was found to be largest. The concentrations employed in the study are well within physiologically relevant concentrations. Serotonin levels in human cerebrospinal fluid (3.3 ng/ml), plasma (3.4 ng/ml), and platelets (748 ng/10^9 platelets) translate into 10–20 nM concentrations (27), which could be much higher at the synapse, and oleamide levels in human plasma (31.7 μg/ml, 110 μM) (28) and mouse neuroblastoma $N_{18}TG_2$ cells (1.5 μg/10^9 cells, *ca*. 100× the concentration of anandamide) typically exceed those examined (29). There was no additional effect when the FAAH enzyme inhibitor phenylmethylsulfonyl fluoride (1) was included in the assay, suggesting that the agents susceptible to protease degradation are stable in the assay. The analog screening was conducted with the rat 5-HT_{2A} assay, and a subset of analogs was also examined in the human 5-HT_{1A} assay. The results reported are normalized to a 100% potentiation by oleamide for the ease of direct comparisons.

Table 1. 5-HT_{1A} and 5-HT_{2A} potentiation

5-HT conc. (μM)	Relative % response at oleamide conc.*			
	1 μM	0.1 μM	0.01 μM	0.001 μM
	A. 5-HT_{1A} potentiation			
0.01	170	235	120	105
0.1	560	370	150	120
1.0	210	145		
10	113	107		
100	103	94		
	B. 5-HT_{2A} potentiation			
0.01	150	120	100	105
0.1	140	165	100	120
1.0	170	170		
10	85	90		

*±8%.

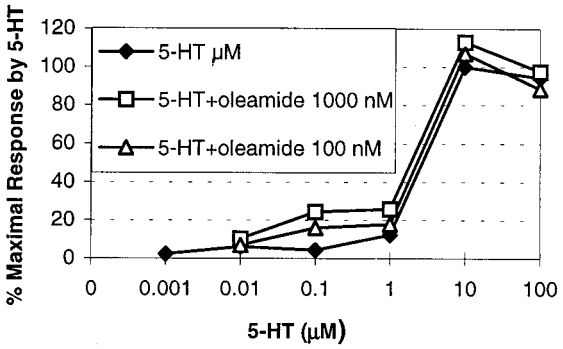


FIG. 1. Potentiation of the human 5-HT_{1A} receptor response observed in R-SAT assays in RAT-1 cells transfected with the receptor and linked to a colorimetric β-galactosidase assay.

Fatty Acid Primary Amides. The first series examined was the primary amides (3, 4, 30, 31) of the naturally occurring fatty acids and related synthetic analogs (Table 2). From these studies, important trends in structural requirements of the endogenous agent emerged. The most effective primary amide of the naturally occurring fatty acids was oleamide. In the 5-HT_{2A} assay and for agents that contain one double bond, the presence, position, and stereochemistry of the olefin as well as the chain length were found to have a pronounced effect. Removal of the Δ^9 double bond (18:0) or its replacement with a *trans* double bond (18:$1^{9\text{-}trans}$) resulted in no observable potentiation. Shortening the chain length to 14 or 16 carbons resulted in the loss of activity, providing weak inhibitors, whereas lengthening the chain substantially diminished the effectiveness. A Δ^9-*cis* olefin exhibited the strongest effect, and the potency sharply declined as its position was moved in either direction. This is especially clear in the oleamide series (18:1) where the potency sharply declined as the distance from the Δ^9 position increased. Although this is central to the structure of oleamide and potentially represents a relationship with either the carboxamide or methyl terminus, the inactivity of the primary amides of 20:1^9, 22:1^9, and 24:1^9 and the modest activity of 20:1^{13}, 22:1^{13}, and 24:1^{15} suggest that it may be the Δ^9 relationship with the methyl terminus that may be most important. Moreover, whereas extending the distance between the carboxamide and the double bond resulted in reduced or inactive compounds, shortening the length typically provided agents that displayed progressively more potent inhibition versus potentiation. Although the significance of this is not yet clear, it suggests the possibility that tightly regulated endogenous agents may serve to both potentiate or inhibit a serotonin receptor response.

With the polyunsaturated fatty acid primary amides, those containing two *cis* double bonds exhibited modest activity, and those containing 3–6 double bonds were typically less active. The exception to this generalization is γ-linolenamide $18:3^{6,9,12}$, which proved to be a more effective but still less potent agent. Arachidonamide containing four cis double bonds was ineffective.

The behavior of 18:0, 18:$1^{9\text{-}trans}$, 18:1^8, 18:1^{12}, and 18:$2^{9,12}$ proved analogous to the observations detailed by Huidobro-Toro and Harris (14) in studies with the 5-HT_{2C} receptor. These similar observations not only indicate that the R-SAT assay for assessment of the 5-HT_{2A} receptor potentiation provides observations analogous to second messenger assays but also implies that the oleamide structural features required for activity may be well conserved throughout the 5-HT_2 receptor subtypes.

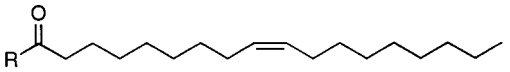
Table 2. Fatty acid primary amides

notation	rel % potentiation 5-HT_{2A}	5-HT_{1A}
18:0 (stearamide)	0%	32%
one double bond		
$14:1^{9}$ (myristoleamide)	-36%	66%
$16:1^{9}$ (palmitoleamide)	-16%	88%
$17:1^{8}$	28%	
$18:1^{6}$	-48%	
$18:1^{7}$	32%	
$18:1^{8}$	36%	27%
$18:1^{9}$ (oleamide)	100%	100%
$18:1^{9\text{-trans}}$ (elaidamide)	0%	20%
$18:1^{12}$	44%	
$18:1^{13}$	4%	
$18:1^{15}$	0%	
$19:1^{10}$	0%	
$20:1^{5}$	-60%	
$20:1^{8}$	0%	
$20:1^{9}$	0%	
$20:1^{11}$	0%	
$20:1^{13}$	32%	
$22:1^{9}$	0%	
$22:1^{13}$ (erucamide)	16%	
$24:1^{9}$	0%	
$24:1^{15}$ (nervonamide)	12%	
two double bonds		
$18:2^{9,12}$ (linoleamide)	44%	96%
$18:2^{9,12\text{-trans}}$ (linoelaidamide)	0%	
$20:2^{11,14}$	50%	
three double bonds		
$18:3^{6,9,12}$ (γ-linolenamide)	60%	
$18:3^{9,12,15}$ (α-linolenamide)	36%	
$20:3^{8,11,14}$	28%	
$20:3^{11,14,17}$	48%	
four double bonds		
$20:4^{5,8,11,14}$(arachidonamide)	0%	
five double bonds		
$20:5^{5,8,11,14,17}$	36%	
six double bonds		
$22:6^{4,7,10,13,16,19}$	25%	

In contrast, the 5-HT_{1A} receptor was found to be more tolerant of structural changes. Like the effects at the 5-HT_{2A} receptor, the saturated or *trans* fatty acid primary amides 18:0 and $18:1^{9\text{-}trans}$ as well as $18:1^{8}$ were less effective than oleamide, albeit not inactive, on the 5-HT_{1A} receptor. Similarly, $14:1^{9}$, $16:1^{9}$, and linoleamide $18:2^{9,12}$ were more effective on the 5-HT_{1A} receptor than 5-HT_{2A} with the latter two approaching the potency of oleamide.

Carboxamide Terminus. A study of the carboxamide terminus revealed well defined structural requirements. Not only was the primary amide of oleic acid capable of potentiating the 5-HT response, but secondary and tertiary amides also provided a comparable potentiation provided the amide substituents were small (Table 3). The activity smoothly progresses

Table 3. Carboxamide terminus



agent (R)	rel % potentiation 5-HT_{2A}	5-HT_{1A}
NH_2 (oleamide)	100%	100%
MeNH	133%	213%
Me_2N	156%	
EtNH	136%	
Et_2N	112%	
CH_2=$CHCH_2NH$	56%	
PrNH	0%	
i-PrNH	0%	100%
i-PrNMe	0%	
BuNH	-8%	
	0%	
PhNH	-4%	
	-72%	145%
HONH	81%	15%
MeONMe	68%	
NH_2NH	0%	

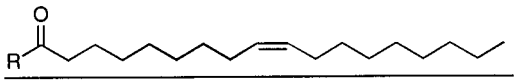
through the series with the maximum effect observed with the NMe_2 tertiary amide. As the amide substituents further increased in size, the effect diminished and ultimately provided inactive derivatives. Thus, the primary carboxamide is not required although its efficacy approximates that of the most potent amide. This suggests that the carboxamide may serve as an H-bond acceptor but need not serve as an H-bond donor. In addition, the cyclopropyl amide was uniquely effective at inhibiting the response to serotonin at the 5-HT_{2A} receptor. Although endogenous agents that may act similarly have not been identified, such allosteric inhibitors at 5-HT_{2A} may prove to be useful biochemical tools and potentially interesting therapeutics.

In contrast to the well defined effects on the 5-HT_{2A} receptor, both the isopropyl and cyclopropyl amides as well as the methyl amide were found to be effective at potentiating 5-HT_{1A} even though the first two were inactive or inhibitory on 5-HT_{2A}. Such distinctions suggest that derivatives of oleamide may be developed that not only possess greater potentiation effects but that can also selectively modulate the various serotonin receptor subtypes or even have opposing effects (i.e., cyclopropyl amide).

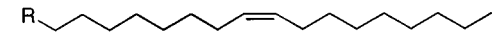
Alternative substitutions for the carboxamide including oleic acid itself, oleyl esters, alcohols, amines, aldehydes, acetals, and electrophilic ketones did not provide a comparable potentiation of 5-HT_{2A} but appear to be better tolerated with 5-HT_{1A} (Table 4). Of particular interest are oleyl aldehyde and the trifluoromethyl ketone (Table 4, R = H and CF_3). Both not only possess polarized carbonyls and can serve as H-bond acceptors, but both are potent inhibitors of FAAH, which is responsible for the degradation of oleamide (4). This dual activity suggests that they may not only potentiate the activity of oleamide by inhibiting its degradation but that they may also serve as oleamide agonists at the 5-HT_{1A} receptor.

Oleyl Ethanolamide, Anandamide, and Related Structures. An important subset of modified carboxamides is the ethanolamide derivatives (32–35), which include anandamide (12). Consequently, the ethanolamides and bis(ethanol)amides of oleic and arachidonic acid were examined (Table 5). Both derivatives of oleic acid were inactive, providing no effect on

Table 4. Carboxamide terminus



agent (R)	rel % potentiation	
	5-HT_{2A}	5-HT_{1A}
NH_2 (oleamide)	100%	100%
OH (oleic acid)	0%	16%
OMe	-28%	
OEt	-24%	
OPr	0%	
OCH_2CHMe_2	0%	
H	0%	81%
CF_3	8%	46%
CH_2Cl	12%	
CH_2Br	0%[a]	
CHN_2	-36%	



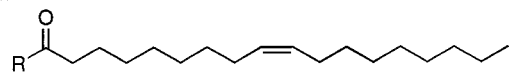
agent (R)	rel % potentiation
	5-HT_{2A}
CH_2OH	0%
CH_2OAc	0%
CH_2NH_2	0%
$CH(OMe)_2$	0%

[a]Toxic to cells.

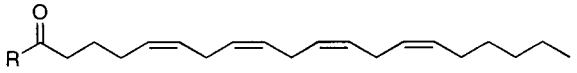
the 5-HT_{2A} receptor whereas those of arachidonic acid including anandamide were weakly inhibitory. Similarly, recent studies have implicated 2-arachidonyl glycerol as an endogenous ligand for the cannabinoid receptor (36), and diacylglycerols including 1-oleyl-2-acetylglycerol have been reported as inhibitors of Chinese hamster V79 cell gap junctions (31). This latter compound was examined, and it did not potentiate but rather weakly inhibited the 5-HT_{2A} receptor response.

Both oleyl ethanolamide and anandamide were found to potentiate the 5-HT_{1A} receptor response to 5-HT, and the former was more potent. Although this might be interpreted to suggest a special significance for the ethanolamides, oleyl propanolamide was equally effective. As such, the results are more consistent with the simpler interpretation that the 5-HT_{1A} receptor potentiation is more tolerant of modifications in the carboxamide terminus and accommodates a wider range of secondary or tertiary amides (*cf.* Tables 3 and 4).

Table 5. Ethanolamides



agent (R)	rel % potentiation	
	5-HT_{2A}	5-HT_{1A}
NH_2 (oleamide)	100%	100%
$HOCH_2CH_2NH$	0%	112%
$HOCH_2CH_2CH_2NH$	0%	106%
$(HOCH_2CH_2)_2N$	0%	
$HOCH_2CH(OAc)CH_2O$	-32%	



agent (R)	rel % potentiation	
	5-HT_{2A}	5-HT_{1A}
NH_2	0%	
$HOCH_2CH_2NH$	-36%	89%
$(HOCH_2CH_2)_2N$	-28%	

Putative Precursors and Related Agents. The potential that oleamide may be stored as a *N*-oleyl glycinamide derivative and released on α-hydroxylation of glycine by a peptidylglycine α-amidating monooxygenase (8) led to the examination of a set of *N*-oleyl glycine derivatives (Table 6). None of the derivatives potentiated the 5-HT_{2A} serotonin receptor response, and most proved to be weak inhibitors. This is consistent with their behavior as large secondary or tertiary amide derivatives, and their activity follows the prior trends (Table 3). In contrast, *N*-oleyl glycine was a weak potentiator of the 5-HT_{1A} receptor consistent with its more tolerant accommodation of modifications in the carboxamide terminus.

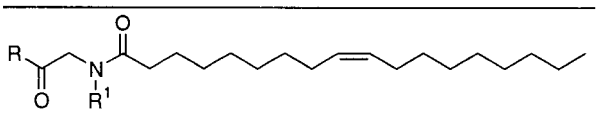
Methyl Terminus. The potentiation of 5-HT_{2A} or 5-HT_{1A} was especially sensitive to the structural characteristics at the methyl terminus (Table 7). Extending the length of the methyl terminus chain (*cf.* Table 2) and the incorporation of polar functional groups resulted in a loss of activity.

Modifications in the Double Bond. The agents **2–10** were examined to define the role of the olefin (Table 8). Analogous to the observations made with octadecanamide (18:0) and the *trans*-9-octadecenamide (18:$1^{9\text{-}trans}$), which were ineffective at potentiating 5-HT_{2A} (*cf.* Table 2), nearly all agents were ineffective. These include **7–10** for which a benzene ring was incorporated into the structure at a location that mimics the Δ^9 double bond as well as **5** and **6**, which mimic a hairpin conformation. Although it is difficult to draw specific conclusions from their inactivity, it highlights that the effects of oleamide at the 5-HT_{2A} receptor are surprisingly selective for the endogenous lipid. The exceptions include 9-octadecynamide (**2**), which was nearly equipotent with oleamide, and **4**, which was approximately 50% as effective. The observation that the former is so effective suggests that the appropriate presentation of a π-system in addition to the conformational effects of the *cis* double bond may be important. Consistent with this, **4** versus **3** proved surprisingly effective and may benefit from the partial π characteristics of the cyclopropane, which would allow it to mimic both the π and conformational characteristics of the *cis* double bond.

Similar observations with the 5-HT_{1A} receptor were made with **2** and **4**. In contrast to the 5-HT_{2A} results, both **9** and **10**, but not **8**, were effective at potentiating the 5-HT_{1A} receptor response and imply that an extended versus hairpin conformation of oleamide might be important at the 5-HT_{1A} receptor.

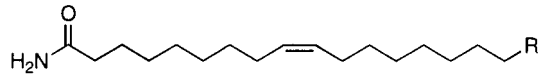
Modifications in the Linking Chain. Modifications in the seven-carbon chain linking the olefin and carboxamide were examined and found to have a detrimental effect with 5-HT_{2A} (Table 9). Substitution of the α-carbon or its replacement with a heteroatom resulted in a loss of activity or provided agents

Table 6. *N*-Oleyl glycine derivatives



agent		rel % potentiation	
R	R^1	5-HT_{2A}	5-HT_{1A}
OH	H	-20%	61%
NH_2	H	0%	
OEt	H	-32%	
OH	Me	-36%	
OEt	Me	-28%	

Table 7. Methyl terminus

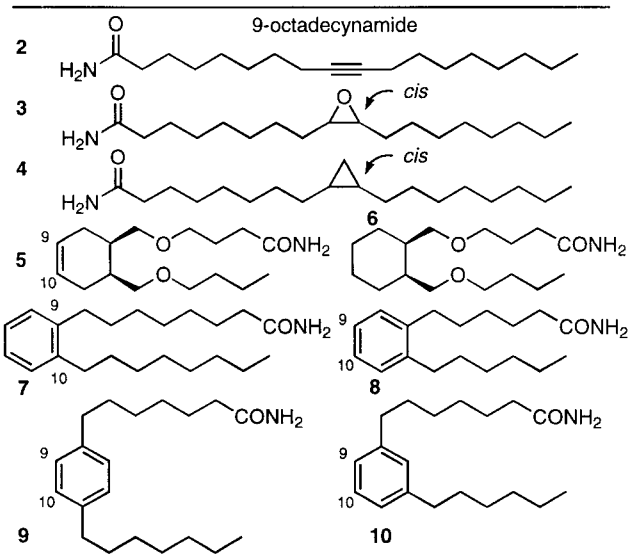


agent (R)	rel % potentiation	
	5-HT_{2A}	5-HT_{1A}
CH_3 (oleamide)	100%	100%
CH_2OCH_3	0%	36%
CH_2OH	8%	-20%
$CONH_2$	0%	-19%
CO_2H	0%	

that inhibited the 5-HT_{2A} response. Most notable are 2,2-dimethyloleamide as well as the urethane (X = NH), which proved to be potent inhibitors.

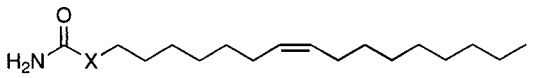
In contrast, most of the linking chain modifications did not adversely affect the 5-HT_{1A} potentiation, and this is significant in several respects. It is consistent with the greater tolerance for carboxamide modifications observed at the 5-HT_{1A} receptor and highlights again that many oleamide analogs may have distinguishing effects on the serotonin receptor subtypes. Moreover, the first seven entries in Table 9 constitute analogs that are more resistant to hydrolytic FAAH degradation and suggest an improved duration of effect would accompany their enhanced efficacy *in vivo*. In addition, the two α-ketoamides (X = CO, $COCH_2$) in Table 9 are potent inhibitors of FAAH (4) illustrating that they may serve to potentiate the effects of oleamide by inhibiting its hydrolysis and serve as oleamide agonists in their own right at the 5-HT_{1A} receptor.

Table 8. Olefin modifications and substitutions



agent	rel % potentiation	
	5-HT_{2A}	5-HT_{1A}
1 (oleamide)	100%	100%
2	88%	38%
3	0%	
4	48%	40%
5	0%	
6	0%	
7	0%	
8	-8%	19%
9	0%	82%
10	0%	70%

Table 9. Linker modifications



agent (X)	rel FAAH hydrolysis rate	rel % potentiation	
		5-HT_{2A}	5-HT_{1A}
CH_2 (oleamide)	1.0	100%	100%
$CH(CH_3)$	0.09	0%	79%
$C(CH_3)_2$	0.01	-82%	110%
O	0.1	24%	168%
NH	< 0.001	-86%	156%
CH(SH)		-32%	
CH(SAc)		-56%	
CH(OH)		0%	200%
CHCl		4%	
C(=O)		0%	84%
$C(=O)CH_2$		0%	70%
$NHCH_2CH_2$		44%	

DISCUSSION AND CONCLUSIONS

We have shown that the structural features of oleamide required for potentiation of the 5-HT_{2A} receptor response are well defined, supporting a selective site of action. Of the naturally occurring fatty acids, oleamide is the most effective, and other endogenous fatty acid amides including arachidonamide, anandamide, and oleyl ethanolamide were less active or ineffective. For oleamide, the presence, position, and stereochemistry of Δ^9-*cis* double bond is required, and even subtle structural variations reduce or eliminate activity. Secondary or tertiary amides but not acids, esters, aldehydes, alcohols, amines, acetals, or electrophilic or polarized ketones may replace the primary carboxamide. Even the amide substitutions follow a well defined relationship limited to small amide substituents. Modifications of the methyl terminus or in the hydrocarbon chain linking the carboxamide and *cis* double bond typically eliminate the activity. In contrast, the 5-HT_{1A} receptor was more tolerant of structural modifications especially at the carboxamide terminus.

The well defined structural features of oleamide required for potentiation of the 5-HT_{2A} or 5-HT_{1A} receptor response in the presence of serotonin provide the opportunity to correlate the properties with physiological states including sleep (38, 39). Such studies will clarify whether the serotonergic effects of oleamide and related agents may be responsible. Many of the well defined structural features of oleamide are tightly conserved among the 5-HT_{2A}, 5-HT_{1A}, and 5-HT_{2C} (14) receptors suggesting they may be well conserved throughout the 5-HT_1 and 5-HT_2 receptor subtypes. However, distinguishing structural effects were observed where the 5-HT_{1A} receptor was more tolerant of structural modifications in the carboxamide terminus of oleamide. In addition, several agents including a small set of naturally occurring fatty acid primary amides were identified that inhibited rather than potentiated the 5-HT_{2A} but not 5-HT_{1A} receptor response. Although the significance of these observations is not yet clear, it not only suggests the possibility that tightly regulated endogenous agents may serve to both potentiate and inhibit a serotonin response but that analogs of oleamide may permit the selective modulation of serotonin receptor subtypes and, in selected instances, even have opposing effects on the different receptor subtypes. The studies to date have demonstrated that at concentrations of 100 nM serotonin, 100 nM oleamide potentiates 5-HT_{2C} (365%) (14), 5-HT_{1A} (370%, results herein), and 5-HT_{2A} receptors [165% for results herein, 228% (15), and 260% (14)], inhibits 5-HT_7 receptors (−50%) (15), and has no effect on the

ion-gated channel 5-HT_3 receptor. The identification of such agents provides new biochemical tools for the study of serotonin receptors and may lead to therapeutic applications involving selective modulation of the serotonin response at the receptor subtypes.

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