

The Hypnotic Actions of the Fatty Acid Amide, Oleamide

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Oleamide is an endogenous fatty acid amide which can be synthesized de novo in the mammalian nervous system, and has been detected in human plasma. It accumulates in the CSF of rats after six hours of sleep deprivation and induces sleep in naive rats and mice. Inhibition of the primary catabolic enzyme of oleamide (fatty acid amide hydrolase) by trifluoromethyl-octadecenone reduces sleep latency and increases total sleep time when given centrally to rats and peripherally to mice. While the mechanism of action of oleamide is unclear, it has been demonstrated to increase the amplitude of currents gated by 5-HT_{2a}, 5HT_{2c} and GABA_A receptors. Moreover, the action of oleamide most relevant to sleep induction involves, in part, cannabinergic

pathways, as evidenced by the ability of the cannabinoid antagonist SR 141716 to inhibit the hypnotic actions of OA. Nonetheless, enhancement of cannabinergic function may not be the only mechanism by which OA alters sleep, as it can act synergistically with subthreshold doses of triazolam (0.125 µg) to reduce sleep latency. These findings raise the possibility that OA may be representative of a group of compounds which might be developed into clinically-used hypnotics, and are discussed in the context of fatty acid derivatives as modulators of neuronal function. [Neuropsychopharmacology 25:S36–S39, 2001]
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This review will describe the pharmacology of the unsaturated fatty acid amide oleamide (OA), presenting data indicating that this substance is formed in the mammalian CNS and has significant sleep-inducing properties. In addition, potential mechanisms by which OA induces sleep will be addressed, including interactions with serotonergic and GABAergic neurotransmission, with a particular emphasis on cannabinergic systems. Finally, the implications of these findings for understanding the function of endogenous sleep-promoting substances will be considered.

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PHARMACOLOGY OF OLEAMIDE

There has been longstanding interest in long chain, polyunsaturated free fatty acids as modulators of neurotransmitter and voltage-gated ion channel activity (Meves 1994). The physiologic significance of these compounds is unclear, however, because the concentrations required to affect receptor function are in the range of 10^{-5} to 10^{-4} M (Witt and Nielsen 1994). In contrast, the related family of unsaturated fatty acids have similar abilities to modulate neurotransmitter gated ion channels, but with much higher potencies (10^{-9} to 10^{-7} M) (Devane et al. 1992; Huidobro-Toro and Harris 1996; Yost et al. 1998). Included in these compounds is oleamide (cis-9,10-octadecenamide), which is structurally related to sphingosine and sphinganine. OA, under its former name “cerebrodiene”, was detected in the CSF of sleep deprived cats (Lerner et al. 1994) and was later reported to suppress motor activity, while inducing the sedated behavior and eye closing suggestive of sleep in rats (Cravatt et al. 1995). Subsequently, the absolute

concentration of OA was found to increase more than 3-fold in the CSF of rats subjected to six hours or more of sleep deprivation. Administration of OA was also found to reduce motor activity in the open field, while inducing electroencephalographic indices of sleep (Basile et al. 1999).

Oleamide is found endogenously; it can be formed de novo in brain microsomes (Sugiura et al. 1996) and is detectable in human luteal phase plasma (Arafat et al. 1989). Fatty acid amide hydrolase, its major catabolic enzyme, has been isolated and localized in a number of brain regions (Thomas et al. 1997). OA does not directly affect ligand binding to serotonin and GABA receptors (Basile et al. 1999; Huidobro-Toro and Harris 1996). However, OA increases currents gated by 5-HT_{2a} and 5-HT_{2c} receptors at concentrations of 200 nM (Huidobro-Toro and Harris 1996) and it modulates GABA-induced chloride current amplitudes biphasically, enhancing currents in the hundreds of nanomolar range, but suppressing them at higher concentrations (Yost et al. 1998). Although OA does not appear to directly interact with cannabinoid receptors (Basile et al., 1999; Mechoulam et al., 1997), it raises concentrations of the endogenous CB-1 agonist anandamide (itself one of the family of unsaturated fatty acid amides) in vitro, possibly by interfering with hydrolysis (Mechoulam et al. 1997) or its cellular uptake (Hauer et al. 1998). Oleamide reportedly has little or no effect at muscarinic cholinergic, metabotropic glutamate, N-methyl-D-aspartate (NMDA), or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Huidobro-Toro and Harris 1996). The mechanism by which OA induces these receptor effects is uncertain, though based on the experience with long chain, polyunsaturated free fatty acids (Meves 1994), it activates protein kinases, which phosphorylate consensus sites on receptor-related ion channels, thus altering their function. Alternatively, some of the effects induced by OA may result from the liberation of ammonia during its catabolism (Cravatt et al. 1995; Ha and Basile 1996).

OLEAMIDE AS A SLEEP-INDUCING SUBSTANCE

Studies in rats indicate that oleamide enhances sleep (Table 1). Microinjection of 2.8 and 5.6 μ g intraventricularly during daytime recordings significantly shortens EEG sleep latency (Basile et al. 1999). Presumably because of its very rapid metabolism, no significant effect is seen on total sleep in rats under these conditions. In mice, 10 mg/kg given intraperitoneally significantly shortens sleep latency and increases total sleep time, primarily by increasing NONREM sleep, with no significant effect on REM (Mendelson and Basile 2000a).

When trifluoromethyl-octadecenone (LOH-1-151),

Table 1. Effects of Oleamide IP on Sleep in Mice (n = 6)

Sleep measure	Treatment	Value (min)	p <
Sleep onset latency	Vehicle	17.3 \pm 2.1	.01
	OA 10 mg/kg	6.6 \pm 1.2	
Total sleep time (min)	Vehicle	64.0 \pm 4.1	.01
	OA 10 mg/kg	82.6 \pm 3.9	
REM sleep time (min)	Vehicle	3.9 \pm 1.4	NS
	OA 10 mg/kg	3.6 \pm 1.6	
nonREM sleep time (min)	Vehicle	60.2 \pm 3.7	.01
	OA 10 mg/kg	78.7 \pm 4.2	
Wake time after sleep onset	Vehicle	35.9 \pm 2.4	NS
	OA 10 mg/kg	31.0 \pm 3.4	
REM sleep onset latency	Vehicle	64.7 \pm 9.2	NS
	OA 10 mg/kg	66.7 \pm 18.8	

Values represent mean \pm SEM minutes. NS = not statistically significant. Data are derived from two hour recordings in 6 male C57 BL-6 mice following administration of OA at 10:00 AM, in a 12:12 L:D condition such that lights come on at 8:00 AM.

an inhibitor of the major degradative enzyme for OA (fatty acyl amide hydrolase, type I) is administered intraventricularly to rats, however, sleep latency is reduced, and total sleep is increased (Mendelson et al. 2000c). A similar effect is seen when LOH-1-151 in a dose of 10 mg/kg is given intraperitoneally to mice, as seen in Figure 1 and Figure 2.

OLEAMIDE GIVEN IN COMBINATION WITH RECEPTOR BLOCKERS

One way to explore which receptors might mediate the sleep-inducing effects of oleamide would of course be to observe sleep after administering it in combination with blockers for the receptors it is known to affect in

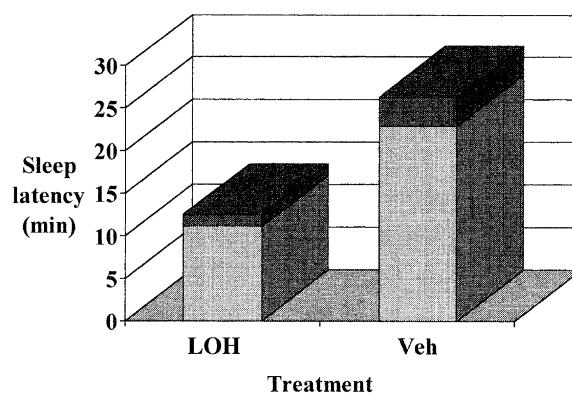


Figure 1. Effects of trifluoromethyl-octadecenone (LOH-1-151) 10 mg/kg IP on sleep latency in mice. Values represent mean \pm SEM minutes. Effect of treatment was significant ($p < .02$) by 2-tailed t -test. Data derived from a study in which 2-hour recordings were performed after injection at 10:00 A.M., under L:D conditions such that lights come on at 8:00 A.M.

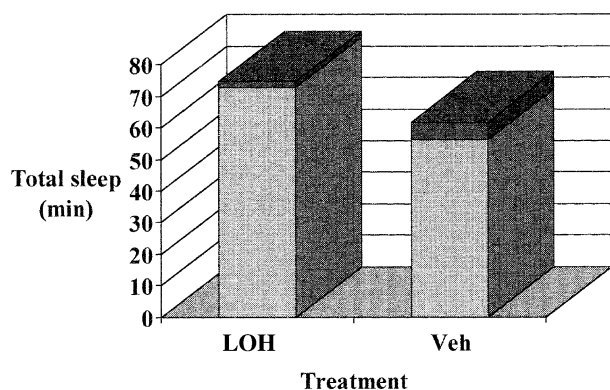


Figure 2. Effects of trifluoromethyl-octadecenone (LOH-1-151) 10 mg/kg IP on total sleep time in mice. Values represent mean \pm SEM minutes. Effect of treatment was significant ($p < .05$) by 2-tailed t -test. Treatment conditions are the same as in Figure 1.

vitro. One such agent is SR-141716, which selectively blocks the CB1 receptor with a $K_i \sim 5.6$ nM, and which prevents such cannabinoid actions as memory disruption, analgesia and hypothermia (Terranova et al. 1996). Although higher peripheral doses may increase wakefulness (Santucci et al. 1996), low intraventricular doses (3 μ g) were determined to have no effect on sleep in rats following daytime injections. When given in combination with 2.8 μ g OA, SR-141716 was found to prevent the significant reduction in sleep latency observed when OA was given by itself (Mendelson and Basile 1999).

To this author's knowledge, OA has not yet been given in combination with a benzodiazepine recognition site antagonist. When a dose of OA two orders of magnitude lower than those which induce sleep (0.0175 μ g) is given intraventricularly to rats, along with a very low dose of triazolam (0.125 μ g), neither agent alone alters sleep, but the combination has a potent hypnotic action (Mendelson and Basile 2000b).

CONCLUSIONS

In summary, OA is a substance which accumulates in the CSF of sleep-deprived animals, and which induces sleep when administered to rats and mice. The observation that its hypnotic effects are prevented by a cannabinoid-1 receptor blocker suggests that one of its mechanisms of action involves an alteration (probably indirect) of CB-1 receptor function. The observation that it has a synergistic action with triazolam leaves open the possibility that its interaction with GABA_A receptors has functional significance as well, or at least that they both affect a common downstream pathway. The possibility that OA may induce sleep via alteration of 5-HT receptors remains to be explored.

One implication of OA being a compound which is synthesized in the mammalian CNS and accumulates in the CSF during sleep deprivation, is of course that it is potentially a "hypnotoxin", an endogenous sleep regulatory substance. A number of authors have developed proposed sets of criteria for an endogenous sleep substance, e.g., that it should act at physiologic concentrations and that there is no evidence of tolerance. The challenge which awaits the proponents of any one potential sleep inducing factor is the large number of such substances (at last count, 30–40). If future studies lead to making a case for OA as such a substance, one of the intriguing aspects of OA is that it modulates at least three major receptor systems that affect sleep (5-HT, GABA_A and CB-1). It appears that it does not do so by directly altering ligand binding, for at concentrations up to 50 μ M, it had no effect on the binding of [³H]muscimol, [³H]flunitrazepam or [³H]ketanserin to GABA_A, benzodiazepine or 5-HT₂ receptors, respectively, as determined in rat brain membrane preparations (Basile et al. 1999). Alternatively, one possible mechanism by which it might do this is by activating protein kinases and hence changing the phosphorylation state of a variety of receptors. The GABA_A receptor subunits are characterized by various consensus phosphorylation sites for a number of kinases (Leidenheimer et al. 1991). It is possible, for instance, that a receptor system, let us say the GABA_A complex, might respond to GABA differently in the phosphorylated versus the dephosphorylated state. Protein phosphorylation at a tyrosine kinase-dependent site as well as at a distinct but uncharacterized site that is dephosphorylated by calcineurin have been reported to be necessary for GABA_A receptor function (Huang and Dillon 1998). Insofar as OA alters a range of receptor systems, it is possible that it might have a more fundamental organizing role in the regulation of sleep and waking.

In addition to offering a potential insight into physiologic sleep regulation, OA is of interest for at least three clinical reasons. The identification of an agent this potent in inducing sleep raises the possibility, of course, that it might be the prototype of a new form of clinical hypnotic medication. Second, oleamide is widely used as an industrial lubricant in polyolefin manufacturing, and is found in many types of food containers (Cooper et al. 1995). Finally, there has been growing interest in the role of the related family of omega-3 polyunsaturated fatty acids in normal and abnormal CNS function, including a possible relationship to human affective disorders (Hebbeln and Salem 1999; Peet et al. 1998; Maes and Smith 1998). One ongoing hypothesis is that an interaction of these compounds with depressive symptomatology may involve their effects on 5-HT receptors. Interestingly, the amides of these compounds have only 40–60% as much activity as oleamide (Bolger et al. 1998). Thus the possibility remains open that an

understanding of the interactions of oleamide with 5-HT receptors may be useful in future investigations of depression or the development of antidepressants.

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