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Effect of Oleamide on Sleep and Its Relationship to Blood Pressure, Body Temperature, and Locomotor Activity in Rats

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Oleamide (*cis*-9,10-octadecenoamide) is a brain lipid that has recently been isolated from the cerebral fluid of sleep-deprived cats. Intracerebroventricular and intraperitoneal administration of oleamide induces sleep in rats. However, it is unclear whether oleamide's hypnogenic effects are mediated, in part, by its actions on blood pressure and core body temperature. Here we show that systemic administration of oleamide (10 and 20 mg/kg) in rats increased slow-wave sleep 2, without affecting blood pressure and heart rate. In addition, oleamide decreased body temperature and locomotor activity in a dose-dependent manner. These latter effects were not correlated in time with the observed increases in slow-wave sleep. These data suggest that the hypnogenic effects of oleamide are not related to changes in blood pressure, heart rate, or body temperature. © 2001 Academic Press

Key Words: **oleamide; sleep; body temperature; motor activity; blood pressure.**

INTRODUCTION

Identifying endogenous sleep-promoting substances has reemerged in recent years and there is increasing evidence for a role of a variety of endogenous agents in regulating physiological sleep in mammals. Indeed, hormones, cytokines, lipids, and other peptides have been implicated in triggering sleep (see (24, 42)). Recently, we (30) reported that a novel brain lipid isolated from the cerebrospinal fluid of sleep-deprived cats was found to accumulate and disappear under conditions of sleep deprivation and recovery, respectively. The substance was determined to be *cis*-9,10-octadecenoamide (oleamide) (Fig.1). Likewise, levels of oleamide in the cerebrospinal fluid (CSF) of sleep-deprived rats have been measured showing significant increments after 6 h of sleep deprivation (3).

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Oleamide and other alkane-chain fatty acid amides, including anandamide, the endogenous ligand for the cannabinoid receptor, constitute a novel group of amidated lipids that are normally found in the brain and blood of mammals, including humans (2, 7, 8, 30). Since its identification and isolation, oleamide has been involved in diverse cellular and physiological functions. *In vitro*, oleamide inhibits lymphocyte proliferation (26), potentiates the action of serotonin on some receptor subtypes (5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A}) (4, 23, 43,), modulates 5-HT_7 receptor-mediated effects (44), blocks gap junction communication (5, 17), and potentiates $GABA_A$ receptors (28, 29, 45, 46). On the other hand, studies characterizing the *in vivo* effects of oleamide in rats have shown that this amide produces hypomotility (3) and hypothermia. (Henriksen, unpublished studies). Likewise, we observed (7) that intraperitoneal (ip) and intracerebroventricular (icv) administration of oleamide induces sleep in rats, while other authors have reported that icv administration of oleamide only decreased sleep latency (3, 36). Moreover, one study reported no differences in the amount of total sleep after administration of oleamide (12).

It has been proposed that the hypnogenic properties of some putative sleep factors are related to changes in blood pressure (16). In view of the controversial effects of oleamide on sleep and the possibility that its effects could be mediated by disruptions in blood pressure (BP) and body temperature (Tb), we examined the effects of oleamide (ip) on sleep, BP, heart rate (HR), and Tb in rats.

METHODS

Animals and surgery. Sixteen male Sprague–Dawley rats (300–350 g) were implanted under halothane anesthesia (1–2%) with a standard set of stainless-steel screw electrodes for chronic sleep recordings. The electroencephalogram (EEG) was recorded from electrodes placed in the frontal and parietal bone over the hippocampus ($P = 4.0$; $L = 3.0$). A third EEG electrode was placed in the skull over the cerebellum and served to ground the animal to reduce signal artifacts. Two

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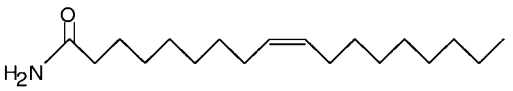

FIG. 1. Chemical structure of oleamide.

wire electrodes inserted in the neck musculature were used to record postural tone through electromyography activity (EMG). Insulated leads from the EEG and EMG electrodes were then soldered to a miniconnector that was cemented to the skull with dental acrylic. After surgery, all rats were allowed a 1-week recovery before of implantation of radiotelemetry transmitters to record Tb and nonspecific locomotor activity (LMA). After the rats were anesthetized with halothane (1–2%), the hair from the subxiphoid space to the pelvis was shaved and the area was scrubbed with iodine. A midline abdominal incision was made to allow for the implantation of miniature transmitters (Data Sciences International, Model TA10TA-F40) to monitor Tb and LMA. To record BP and HR, a separate group of 12 male Sprague–Dawley rats (300–350 g) was anesthetized and prepared for a midline abdominal incision as above described. The intestine was held back using saline-moistened gauze sponges and the bifurcation of the aorta to the renal arteries was visualized. The aorta was dissected and a silk suture was placed beneath the aorta, and traction was applied to the suture to restrict the blood flow. The aorta was punctured just cranial to the bifurcation and the tip of the pressure transmission catheter was implanted within the vessel. A tissue-adhesive cellulose patch was applied to seal the puncture site. Before closing the incision, the blood pressure sensor located within the body of the implant (Data Sciences International, Model TA11PA-C40) was sutured to the abdominal wall.

After surgery the rats were housed in individual Plexiglas recording cages placed in environmentally controlled chambers (Tech/Serv, Model EPC-010). For the next 14 days, the rats were habituated to handling and ip injection procedures at the same time as experimental injections were scheduled to occur. During this period and throughout the experiment the ambient temperature was controlled at 25 ± 1°C and a 12:12-h light–dark cycle was maintained. Food and water were available *ad libitum*.

Sleep recordings. Each recording cage contained a commutator with a flexible recording cable connected to the rat's head and allowed the animals unrestricted movement within the cage. A Grass Model 7D polygraph continuously amplified and displayed signals from the EEG and EMG electrodes. All signals were filtered between 1.0 and 35 Hz. Gain for all EEG signals was adjusted to yield a compatible signal for each animal and remained uniform for the duration of the study. The recording chambers contained a mini-video camera for continuously observing behavior during the recording session.

Tb, LMA, BP, and HR recordings. Tb, nonspecific LMA, BP, and HR were monitored by telemetry using DATAQUEST A.R.T. data collection software. Those variables were recorded and averaged as discrete events for 10 s every 5 min, by receivers (Data Science, Model RPC-1) located beneath each cage.

Experimental protocol. Rats implanted with recording electrodes for monitoring EEG and EMG, as well as Tb and LMA, were habituated to recording chambers for 24 h. Once the habituation period was completed, rats were randomly divided into three groups. Sleep–wake cycles were recorded in these groups for 4 h, while simultaneously, TB and LMA were recorded for up to 7 h. All recordings began 4 h after dark onset (CT-16; CT, circadian time; CT-0, lights on). All treatments were administered under dim red illumination observing the following schedule: on baseline day the animals remained undisturbed and the physiological monitoring began at CT-16. The following day rats were injected ip with 1 ml of peanut oil (vehicle) at CT-16 and recordings were continued with the same data acquisition schedule; on the third day the same rats were challenged at CT-16 with an ip administration of oleamide [2.5 mg/kg (n = 4), 10 mg/kg (n = 8), or 20 mg/kg (n = 4)].

The second cohort of rats implanted with transmitters uniquely for recording BP and HR was randomly divided into three groups (n = 4) and recorded for 7 h under the schedule and doses described above. Procedures for minimizing potential contamination by extraneous pyrogens were used for all drug solutions and vehicles.

Data analysis and statistics. The polygraphic results were analyzed visually and classified according to the following stages of vigilance: wakefulness (W), slow-wave sleep 1 (SWS1), slow-wave sleep 2 (SWS2), and rapid eye movement (REM) sleep, as previously described (7, 30).

Percentage of total time spent in W, SWS1, SWS2, and REM sleep was calculated. Likewise, to analyze the effects of oleamide across time, the total time of recording was divided in half and time spent in each vigilance stage throughout these recording periods was calculated. The latencies to sleep onset and to the first REM sleep episode onset, as well as frequency and duration of the individual episodes of SWS1, SWS2, and REM sleep, were also calculated. Likewise, the amount of LMA per hour, and the average Tb, BP, and HR across the recording period was calculated. Results were compared by a repeated-measures analysis of variance (ANOVA), with the Scheffé F test used for specific comparisons when indicated by ANOVA. Animal care, maintenance, and experimental procedures followed the National Institutes of Health Guide for

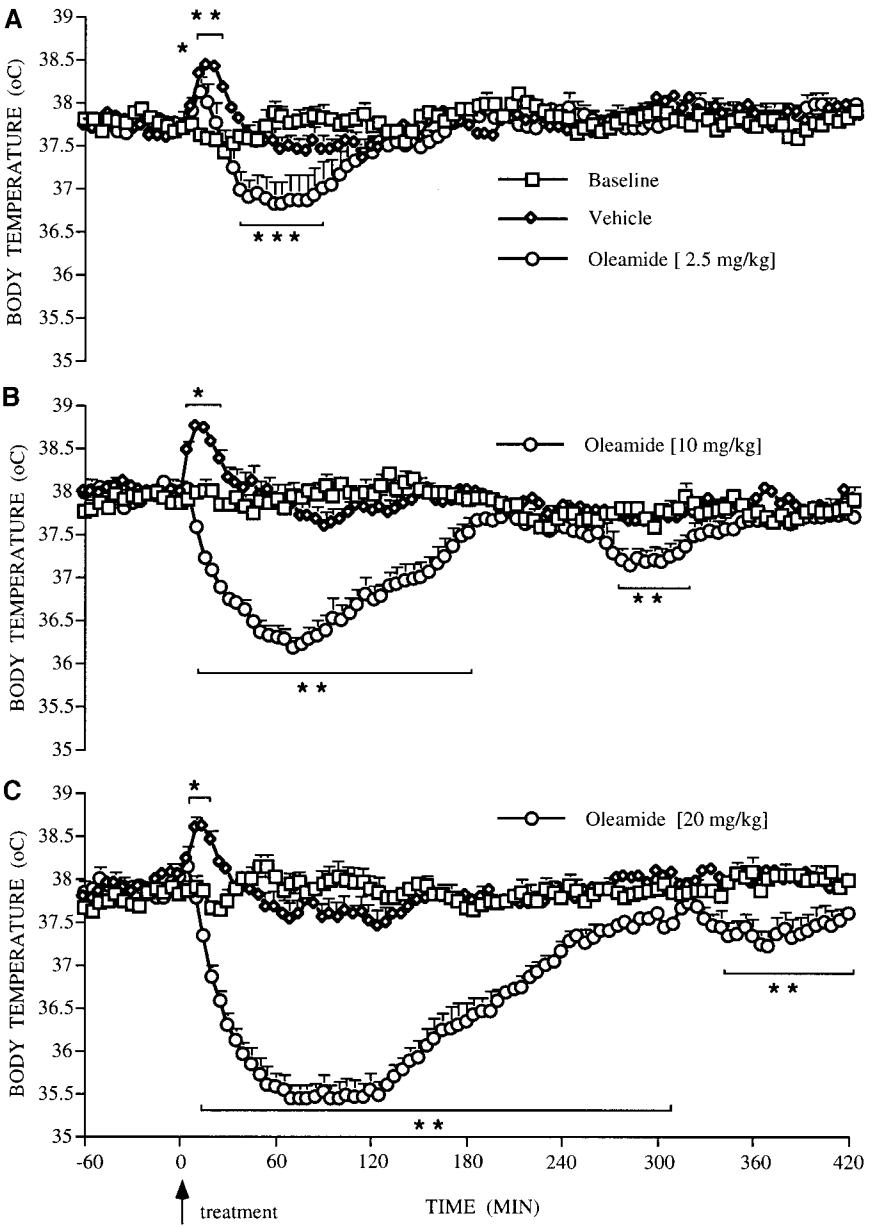

FIG. 2. Effects of oleamide on Tb. Handling animals during injections increased Tb. However, administration of oleamide suppressed this effect and induced hypothermia in a dose-dependent manner. Administration of 2.5 mg/kg oleamide decreased Tb 1.0°C within 35–100 min after injection (A), while 10 mg/kg (B) and 20 mg/kg (C) oleamide decreased temperature 1.75 and 2.25°C, respectively. Values (mean ± SEM) were compared using one-way ANOVA and Scheffé *F* test (see text for significance).

the Care and Use of Laboratory Animals and the Scripps Research Institute Animal Care and the Committee Standards.

RESULTS

Effects of ip injection of 2.5 mg/kg oleamide. Rats receiving oleamide (2.5 mg/kg) exhibited no significant differences in the amount of W, SWS1, SWS2, and REM sleep (data not shown) or in any of the sleep parameters studies compared to controls (Table 2). On the other hand, the handing of animals during injections with vehicle and oleamide significantly increased Tb [$F(2,9) = 7.8131$; $P < 0.01$, compared to baseline]. This effect was less pronounced, although still significant, in oleamide-treated rats [$F(2,9) = 4.0148$; $P < 0.05$, compared to vehicle]. Likewise, a decrease in Tb was observed after oleamide administration [$F(2,9) = 4.50$; $P < 0.05$, compared to baseline and vehicle; Fig. 2A].

The hourly analysis of LMA showed that the handling of the animals during vehicle and oleamide

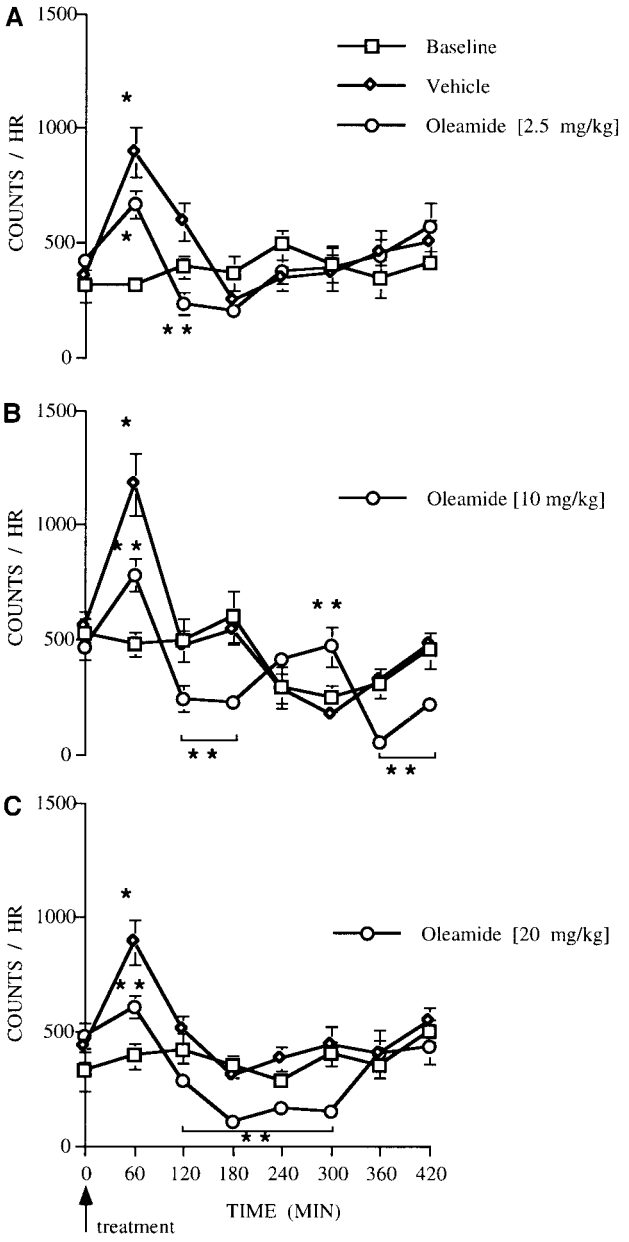

FIG. 3. Effects of oleamide on LMA. Animal manipulation and injection of vehicle increased LMA during the first hour after injection, compared to baseline. However, when rats were injected with oleamide the handling effect on LMA was less intense. A decrease in LMA was observed 120 min after the injection of 2.5 mg/kg oleamide, compared to vehicle (A). Administration of 10 mg/kg oleamide decreased LMA within 120–180 and at 360–420 min. At these doses a rebound in activity was observed 300 min after oleamide administration (B). Oleamide (20 mg/kg) decreased LMA at 120–330 min after treatment (C). Values (mean ± SEM) were compared using one-way ANOVA and Scheffé *F* tests (see text for significance).

injections transiently increased LMA [$F(2,9) = 15.1935$; $P < 0.01$, compared to baseline]. However, 2 h after oleamide injection LMA was significantly decreased [$F(2,9) = 8.2898$; $P < 0.01$, compared to vehicle; Fig. 3A].

Similar to the Tb and LMA results, the handling of animals during injections of oleamide and vehicle produced an increase in BP and HR [$F(2,9) = 6.58$; $P < 0.01$, compared to baseline]. However, after recovery of the handling effect, the administration of oleamide (2.5 mg/kg) did not alter BP or HR, compared to baseline or vehicle injections (Figs. 4A and 5A, respectively).

Effects of ip injection of 10 mg/kg oleamide. During the first 5 min after oleamide injection rats remained quiet showing smooth contractions of the abdominal muscles, without signs of discomfort. The administration of oleamide causes a significant increase in the total time spent in SWS2 [$F(2,21) = 4.1249$; $P < 0.05$, compared to baseline and vehicle; Fig. 6A]. This effect was due to the transient increase of SWS2 during the second half of recording [$F(2,21) = 4.068$; $P < 0.05$, compared to baseline and vehicle; Table 1]. Moreover, oleamide at this concentration increased the mean number of SWS2 episodes [$F(2,21) = 5.351$; $P < 0.01$] without changing the other sleep parameters measured in this study (Table 2).

On the other hand, the handling-induced increase in Tb following vehicle injection [$F(2,21) = 10.4828$; $P < 0.01$, compared to baseline] was not observed in oleam-

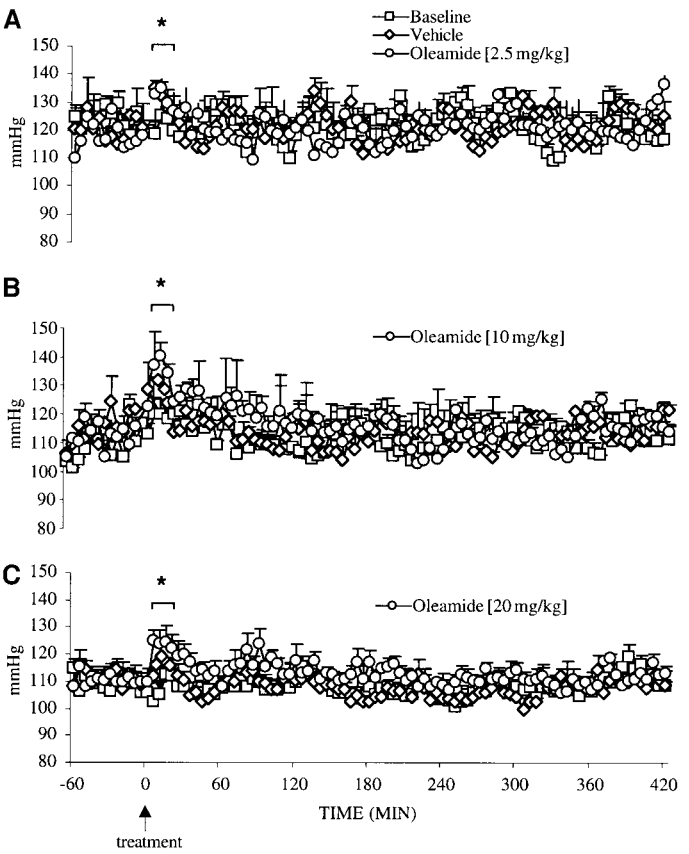

FIG. 4. Effects of oleamide on BP. Different doses of oleamide did not disrupt BP. Manipulation of the animals caused a significant increase in BP (mmHg; millimeters of mercury).

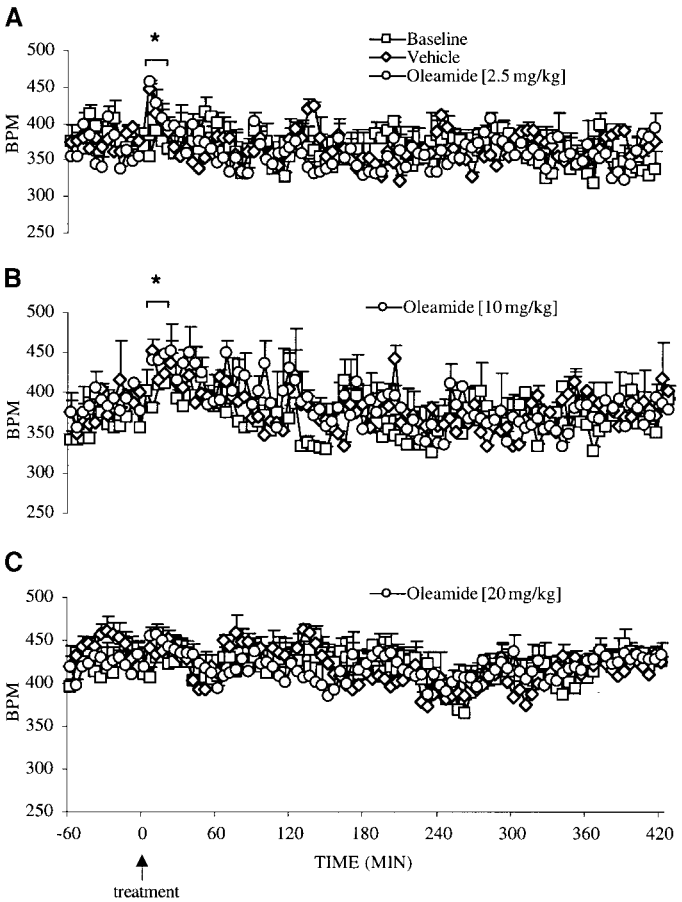

FIG. 5. Effects of oleamide on HR. With the exception of the handling effect that caused an small increase in HR, no significant differences were observed in HR with any of the doses of oleamide tested (BPM, beats per minute).

ide-treated rats showing a biphasic decrease in Tb [$F(2,21) = 5.1686$; $P < 0.01$, compared to baseline and vehicle; Fig. 2B].

A handling-induced increase in LMA was observed following vehicle injections [$F(2,21) = 13.7109$; $P < 0.001$, compared to baseline]. However, this effect was significantly attenuated in oleamide-treated rats and was still significantly different compared to baseline values [$F(2,21) = 13.7001$; $P < 0.001$]. In addition, at this dose oleamide also produced a biphasic decrease in LMA [$F(2,21) = 6.7498$; $P < 0.01$, compared to baseline and vehicle]. Moreover, a significant rebound in LMA was observed 300 min after oleamide administration [$F2,21 = 5.9706$; $P < 0.01$, compared to baseline and vehicle; Fig. 3B].

A handling-induced increase in BP an d HR [$F(2,9) = 6.241$; $P < 0.01$] was observed in the group of animals treated with oleamide (10 mg/kg). However, no alterations in BP and HR were observed after recovery of the handling effect (Figs. 4B and 5B, respectively).

Effects of ip injection of 20 mg/kg oleamide. Rats injected with this high dose of oleamide exhibited the same behavior observed in the animals injected with 10 mg/kg oleamide. The administration of 20 mg/kg oleamide caused a significant increase in the total time spent in SWS2 [$F(2,9) = 7.3835$; $P < 0.01$, compared to vehicle], as well as a significant decreased in the total time spent in REM sleep [$F(2,9) = 14.1762$; $P < 0.001$, compared to baseline and vehicle]. These effects were due to the significant increase in the time spent in SWS and REM sleep during the second half of recording [SWS: $F(2,9) = 5.659$; $P < 0.01$, compared to vehicle and REM sleep: $F(2,9) = 4.727$; $P < 0.05$, compared to baseline and vehicle; Table 1]. On the other hand, the frequency of SWS2 increased significantly, compared to baseline and vehicle [$F(2,9) = 9.0592$; $P < 0.01$], while the number of REM sleep periods decreased [$F(2,9) = 26.6471$; $P < 0.001$, compared to baseline and vehicle]. Finally, oleamide at this dose significantly increased REM sleep latency [$F(2,9) = 5.29$; $P < 0.05$] (Table 2).

Administration of oleamide (20 mg/kg) resulted in a pronounced hypothermic response that was observed during the entire recording period [$F(2,9) = 6.8$; $P < 0.01$; Fig 2C].

The handling-induced increase in LMA observed after vehicle injection [$F(2,9) = 12.3904$; $P < 0.001$, compared to baseline], was significantly decreased in rats injected with oleamide [$F(2,9) = 12.0119$; $P < 0.001$, compared to baseline and vehicle]. Furthermore, the injection of this dose of oleamide significantly decreased LMA [$F(2,9) = 4.9776$; $P < 0.05$, compared to baseline and vehicle; Fig. 3C].

On the other hand, except for the increase in BP due to the handling of the animals [$F(2,9) = 5.19$; $P < 0.05$], the injection of oleamide (20 mg/kg) did not produce significant changes in BP and HR (Fig. 4C and 5C, respectively).

DISCUSSION

The results obtained in the present study are consistent with previous reports indicating that oleamide increases SWS2 sleep (7), due to a significant increase in the number of SWS2 episodes. In addition, at the highest dose of oleamide (20 mg/kg) we observed a significantly reduced time spent in REM sleep and an increased latency. The reduction in REM sleep was due to a significant decrease in the number of episodes, while the mean duration of individual periods was unchanged. These results suggest that oleamide exerts a facilitating effect on the mechanisms related to SWS2, whereas high doses of oleamide may, in addition, inhibit mechanisms related to the generation and maintenance of REM sleep.

The results described above clearly show that oleamide has multiple effects on sleep states. Although the possible sleep-induced mechanisms of oleamide remain to be defined, it has been proposed that its somnogenic

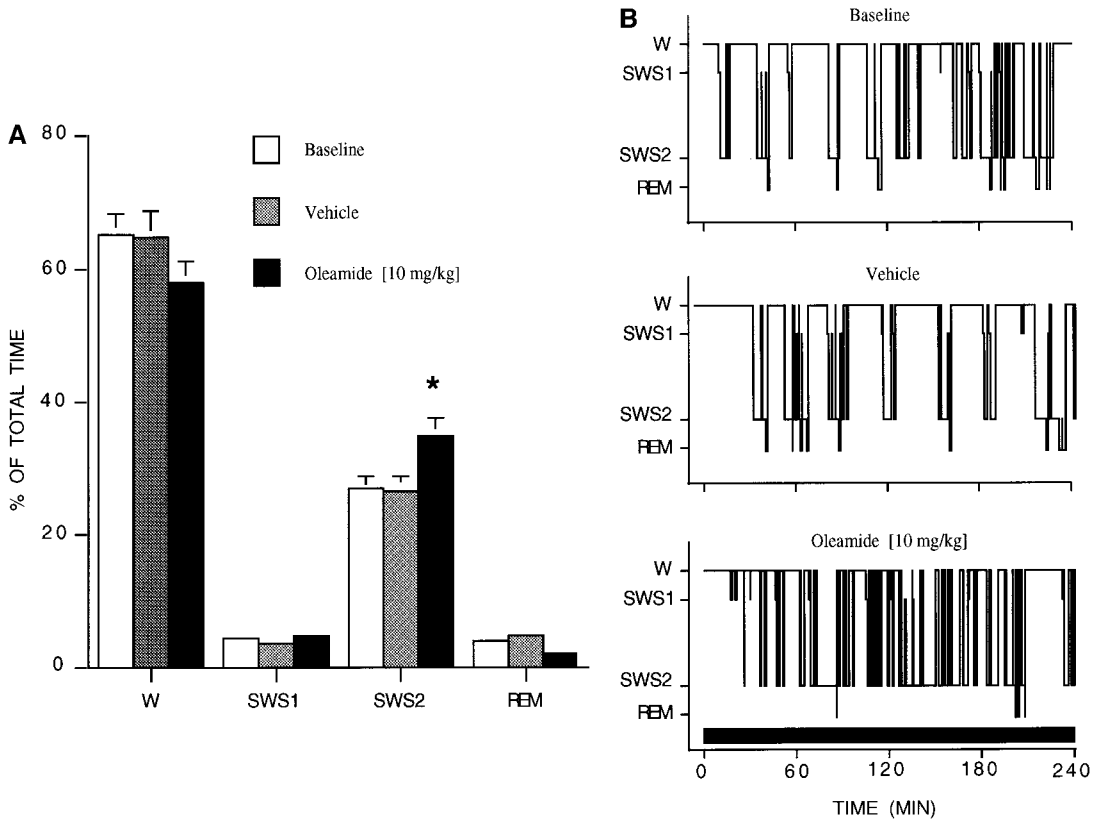

FIG. 6. Effects of 10 mg/kg oleamide on the total time of W, SWS1, SWS2, and REM sleep in 4 h of polygraphic recording (A). Oleamide significantly increased the total amount of SWS2 in rats [$F(2,21) = 6.057$; $P < 0.01$], compared to baseline and vehicle. The sleep architecture of a representative rat during treatments is shown (B). The increase in SWS2 as well as reduction in the number of REM sleep episodes following the administration of oleamide is evident. The bar at the bottom indicates dark cycle.

properties could be related to its ability to affect both serotonergic and GABAergic systems. This hypothesis has been supported by the fact that oleamide potentiates 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} receptors (4, 23, 43), and $GABA_A$ receptors (27, 28, 45, 46) *in vitro*. It is also supported by pharmacological studies showing that the stimulation or blocking of these receptors produces disruptions in sleep patterns in rats (11, 13, 29). In addition, it has been reported that systemic administration of agonists that potentiate $GABA_A$ receptor function, like barbiturates, benzodiazepines, nonbenzodiazepine hypnotics, and neuroactive steroids, also promotes non-REM sleep and decreases REM sleep. The effect of these agents on REM sleep is a result of a reduction in the number of REM episodes and an increase in REM sleep latency (14, 15, 19–21, 25, 33–35,

TABLE 1

Time Spent in SWS2 and REM Sleep (min) in 4 h of Recording (Mean ± SEM)

	SWS2 First half of recording	SWS2 Second half of recording	REM First half of recording	REM Second half of recording
Baseline	28.75 ± 4.82	37.57 ± 2.13	3.66 ± 1.42	3.75 ± 1.12
Vehicle	30.01 ± 4.90	34.50 ± 3.95	4.48 ± 1.47	6.46 ± 2.11
Oleamide (10 mg/kg)	32.52 ± 4.08	47.52 ± 3.73*	1.83 ± 0.85	3.28 ± 1.17
Baseline	29.20 ± 5.73	35.72 ± 4.46	5.62 ± 1.71	4.25 ± 0.32
Vehicle	28.10 ± 3.87	26.60 ± 0.81	6.15 ± 1.73	4.25 ± 1.39
Oleamide (20 mg/kg)	34.52 ± 3.97	42.40 ± 3.57*	1.00 ± 0.57	0.97 ± 0.46*

Note. To analyze the effects of oleamide on sleep across time, the total time of recording was divided in two halves of 2 h. Oleamide (10 mg/kg and 20 mg/kg) increased the amount of SWS2 mainly during the second part of recording. The injection of 20 mg/kg transiently decreased REM sleep but this only reached significance during the second part of recording. Values were statistically compared using a one-way ANOVA (see text for significance).

TABLE 2

Sleep Parameters (Mean ± SEM)

	TTSWS	TTREM	Sleep-L	REM-L	SWS1-F	SWS1-D	SWS2-F	SWS2-D	REM-F	REM-D
Baseline	61.62 ± 6.82	9.63 ± 1.93	14.85 ± 3.57	76.17 ± 40.77	22.00 ± 6.05	0.53 ± 0.02	43.75 ± 8.74	1.63 ± 0.23	10.00 ± 3.89	1.10 ± 0.13
Vehicle	60.10 ± 6.12	11.42 ± 2.72	16.57 ± 7.27	60.45 ± 31.33	24.75 ± 2.78	0.51 ± 0.01	37.75 ± 7.02	1.74 ± 0.19	9.00 ± 2.27	1.20 ± 0.05
Oleamide (2.5 mg/kg)	72.52 ± 1.34	8.55 ± 1.01	30.05 ± 8.79	96.6 ± 16.61	14.00 ± 4.49	0.43 ± 0.03	52.50 ± 7.59	1.51 ± 0.24	6.25 ± 0.94	1.37 ± 0.04
Baseline	66.32 ± 4.50	7.41 ± 2.43	19.87 ± 4.78	59.23 ± 23.08	21.25 ± 2.97	0.55 ± 0.02	34.25 ± 2.01	1.73 ± 0.19	7.50 ± 2.41	0.81 ± 0.15
Vehicle	64.51 ± 4.72	10.95 ± 2.73	34.68 ± 11.45	63.50 ± 17.88	22.50 ± 2.71	0.54 ± 0.02	34.25 ± 3.99	1.99 ± 0.19	9.75 ± 2.28	1.00 ± 0.16
Oleamide (10 mg/kg)	80.05 ± 4.74*	5.12 ± 1.49	30.40 ± 3.92	48.82 ± 17.84	22.28 ± 2.84	0.47 ± 0.02	44.37 ± 2.86*	1.83 ± 0.10	3.12 ± 1.27	0.61 ± 0.22
Baseline	64.92 ± 5.30	9.87 ± 1.59	26.40 ± 4.42	49.27 ± 4.58	13.5 ± 2.02	0.60 ± 0.02	36.25 ± 1.65	1.81 ± 0.19	11.00 ± 0.57	0.99 ± 0.10
Vehicle	54.70 ± 3.14	10.40 ± 1.52	30.83 ± 3.81	55.50 ± 4.35	12.5 ± 1.44	0.58 ± 0.02	31.25 ± 2.71	1.81 ± 0.21	9.66 ± 1.20	1.21 ± 0.22
Oleamide (20 mg/kg)	76.92 ± 3.53*	1.97 ± 0.70	34.15 ± 2.96	147.5 ± 40.93*	18.25 ± 1.31	0.56 ± 0.03	47.25 ± 3.47*	1.64 ± 0.07	3.33 ± 0.33*	0.67 ± 0.08

Note. Oleamide induced changes in some of the sleep parameters studies. Sleep-L and REM-L: mean sleep and REM sleep latency, respectively; SWS1-F, SWS2-F, and REM-F: mean number of SWS1, SWS2, and REM sleep episodes, respectively; SWS1-D, SWS2-D, and REM-D: mean duration of the individual SWS1, SWS2, and REM sleep episodes, respectively; TTSWS and TTREM: total time of SWS and REM sleep, respectively (see text for significance).

40, 41). Likewise, it has been reported that the administration of low doses of 8-hydroxy-2-(di-*n*-propilamino) tetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist, increases SWS2, decreases REM sleep, and increases REM sleep latency (38). Thus, it is probable that the effects of oleamide on sleep could be related to its pharmacology of potentiating 5-HT and $GABA_A$ receptor function. Although the oleamide-induced increase in sleep could be mediated by serotonergic and/or the GABAergic processes, the involvement of another receptor type, such as cannabinoid receptors, cannot be ruled out (4, 32, 36). Further studies aimed at understanding the complex relationship between oleamide, 5-HT receptors, $GABA_A$ receptors, cannabinoid receptors, and others and sleep are needed.

Some controversies exist regarding, the potentiating effect of oleamide on sleep. For example, Dugovic *et al.* reported no effect of oleamide on sleep in rats after ip injections of 30.0 mg/kg (12). These results compared to the finding reported here might be due to the fact that Dugovic *et al.* employed oleamide in injections at the beginning of the light (CT-0) or dark phases (CT-12), while in our study oleamide was injected 4 h after dark onset (CT-16). In support of this interpretation it has been suggested that the effects of sedative hypnotics in nocturnal rodents is both more sensitive and more reliable when treatments are performed several hours after lights-out (14). For example, it has been reported that several factors can mask drug effects when treatments are given close to the beginning of the light or dark phases (37), including day-to-day variability in SWS levels at the daily cusp of the circadian activity to rest transition, the polyphasic nature of sleep in rats, and the potential SWS ceiling effects, during the circadian rest phase (14, 37). In addition, Dugovic *et al.* do not specify which vehicle was used to dissolve oleamide. Therefore, it is not possible, with out further controls, to know if their vehicle might have had wake-promoting effects. In the present study, oleamide was dissolved in peanut oil, which has been shown to have no effect on either sleep-wakefulness cycle or other physiological functions like LMA (6).

In addition, recently in partial confirmation of our results, it was reported that oleamide (2.8 μg, icv) reduced sleep onset latency, but had no significant effects on the total amount of SWS and REM sleep (3, 36). Whereas our results show that oleamide (ip) had no effect on sleep latency, it did produce a significant increase in SWS2. These discrepancies may be due to several factors, including different routes of oleamide administration, different data acquisition methods, or a difference in the type of data analysis applied.

Feinberg and Campbell (16) have recently discussed the possibility that hemodynamic effects of some putative sleep factors could produce a pathological EEG slowing and therefore confound sleep scoring. Our results show that oleamide did not produce significant changes in either BP or HR, indicating that the oleamide-induced hypnogenic effect observed in this study may not be attributed to either transient or permanent EEG slowing caused by alterations in BP and/or HR.

In addition to its effects on sleep, we observed that systemic administration of oleamide elicits a dose-dependent hypothermic response. The mechanisms associated with this hypothermic effect are unknown, but we speculate that the decrement in Tb could be related to the effects of oleamide on sleep-initiating mechanisms. There is an observed close relationship between the sleep–wake cycle and Tb. It is well known that during wakefulness, Tb reaches the highest levels, whereas during sleep Tb decreases gradually, with slight increases occurring during REM sleep (10, 47). However, our results show that the lowest Tb observed following the administration of oleamide (between 60 and 120 min after its administration) were not correlated with the time when the highest levels of SWS2 were observed (between 180 and 240 min after injection). These results suggest that the mechanisms underlying oleamide effects on Tb and SWS2 are different. However, the ability of oleamide to potentiate

$GABA_A$ and 5-HT_{1A} receptors cannot be excluded as a possible explanation for the oleamide-induced decrease in Tb. In agreement with this proposal, it has been reported that the activation of $GABA_A$ receptors by ip injections of muscimol may induce hypothermia in rats (48), whereas the activation of 5-HT_{1A} receptors by systemic injection of low doses of 8-OH-DPAT decreases Tb in rats (1, 18, 22). Future studies will be needed to determine the mechanism by which oleamide alters Tb.

Likewise, our results show that ip administration of oleamide produces hypolocomotion in a dose-dependent manner. Similar results have been reported in other studies, where a decrement in activity has been observed after the ip administration of different doses of oleamide (3, 36). It may be argued that the oleamide-induced hypolocomotor effect could be a consequence of its hypothermic effects. However, consistent with our sleep data, the time where the minimum activity was observed does not correlate with the time when the lowest Tb values were recorded. Thus, the motor activity disruptions caused by oleamide do not appear to be a consequence of its hypothermic effect. It has been suggested that a decrease in LMA induced by oleamide could be mediated by oleamide-induced increases in anandamide levels, because this compound produces hypomotility in rodents (9). However, the hypomotility induced by oleamide is not prevented by pretreatment with SR 141716, a potent CB_1 receptor antagonist (3). Rather, these effects appear to be mediated by oleamide's ability to potentiate 5-HT_{1A} and 5-HT_{2C} receptors. In support of this hypothesis, it has been reported that the activation of 5-HT_{1A} receptors by 8-OH-DPAT induces hypolocomotion in rats, whereas the administration of meta-chlorophenylpiperazine (a 5-HT_{1A} and 5-HT_{2C} receptor agonist) decreases LMA (31, 39, 49).

Although it is not yet possible to determine the precise mechanism of action of oleamide on the sleep–wake cycle, the results of this study indicate that in addition to increasing SWS2 and decreasing REM sleep, oleamide causes hypothermia and hypomotility in a dose-dependent manner. Moreover, oleamide-induced disruptions in sleep, Tb, and LMA are not mediated by changes in blood pressure and/or heart rate. Further studies will aid in understanding the mechanisms underlying the properties of this new substance related to the endogenous fatty acid primary amides.

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