ELSEVIER

Neuroscience Letters 313 (2001) 61–64

Neuroscience Letters

www.elsevier.com/locate/neulet

Oleamide modulates memory in rats

Eric Murillo-Rodríguez[a], Magda Giordano[b], Rafael Cabeza[c], Steve J. Henriksen[d], Mónica Méndez Díaz[a], Luz Navarro[a], Oscar Prospéro-García[a,*]

[a]Grupo de Neurociencias, Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, A Postal 70-250, Mexico, D. F. 04510, Mexico
[b]Centro de Neurobiología. Universidad Nacional Autónoma de México, Mexico, D. F., Mexico
[c]Department of Biological Sciences, The University of Texas El Paso, El Paso, TX, USA
[d]Department of Neuropharmacology, TSRI, La Jolla, CA, USA

Received 22 May 2001; received in revised form 22 August 2001; accepted 30 August 2001

Abstract

Oleamide is a recently described lipid, obtained from the cerebrospinal fluid of sleep-deprived cats. It has been observed that oleamide possesses several biological effects, such as sleep induction, and immunological suppression as well as serotonin and gamma-aminobutyric acid receptors activation. In addition, oleamide also binds to the cannabinoid receptors. In this study, we have observed that oleamide facilitates memory extinction in a passive avoidance paradigm, reduces core temperature and pain perception, but does not affect significantly locomotion. These results suggest that oleamide modulates memory processes. However, we do not know if oleamide impairs the retrieval of the memory associated to the 'not go' behavior, or facilitates the fast re-learning of the 'go' behavior. In addition, since these effects are also induced by marijuana and anandamide, it is very likely that oleamide may be affecting the cerebral cannabinoid system to induce its effects. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Cannabinoids; Lipids; Gamma-aminobutyric acid; Serotonin; Passive avoidance

Memory is a highly complex process that involves several brain structures [21] as well as the role of several neurotransmitters and neuropeptides [19]. The effect of some biologically active lipids on memory mechanisms has been studied. For example, K-linolenic acid improves memory [22]; while arachidonic acid, and anandamide, a metabolite of arachidonic acid that binds to cannabinoid receptors and evokes most of the effects caused by marijuana [14], seem to impair memory processes [12].

Oleamide is a recently described lipid, isolated from cerebrospinal fluid (CSF) of sleep deprived cats [7]. Oleamide induces sleep in rats [3,11,15] and binds to the cannabinoid receptors [5]. Likewise, oleamide affects serotonin activity on 5HT1A, 5HT2A and 5HT7 receptors expressed in neurons [4]. Oleamide also affects the immunological response [9]. In addition, the fatty acid amide hydrolase (FAAH), the enzyme that degrades anandamide, degrades oleamide as well [6]. These facts suggest that oleamide may interact with the endogenous cannabinoid system.

Cannabinoid effects are mediated by at least two receptors, the CB1, central and the CB2, peripheral [2,14]. Oleamide seems to be able to bind to these receptors [5]. In fact, some of the effects on sleep induced by oleamide are blocked by a CB1 receptor antagonist [11]. In addition, it has been postulated that, since oleamide is degraded by FAAH as much as anandamide, the former may interfere with the degradation of the latter whereby facilitating anandamide action [10].

The widely recognized actions of marijuana and its more potent derivative Δ^9 tetrahydrocannabinol (Δ^9 THC) include effects on attention, memory, learning, sleep, pain perception, sexual behavior, and immune response, among others [2,16]. Most of these effects are also induced by anandamide, although with less potency [2,12]. In this study we have tested oleamide to determine if this lipid affects memory processes as evaluated by a passive avoidance paradigm. In addition, we evaluated the effect of oleamide on locomotor activity, core temperature and pain perception.

Male Wistar rats (250–300 g) were used in all the studies reported here. Different groups of rats were used for each

* Corresponding author. Tel.: +525-623-2509; fax: +525-623-22-41.
E-mail address: opg@servidor.unam.mx (O. Prospéro-García).

0304-3940/01/$ - see front matter © 2001 Published by Elsevier Science Ireland Ltd.
PII: S0304-3940(01)02256-X

test. Rats were maintained under a controlled dark-light cycle (12:12 h, lights on at 08:00 h) and were housed individually with free access to food and water.

Forty five rats were trained in the foot shock passive avoidance behavioral test, in order to evaluate long-term memory. The training apparatus was a trough-shaped alley (94 cm long, 25 cm wide and 30 cm tall) divided into two compartments by a guillotine door that retracted into the floor. The starting compartment (32 cm long) was illuminated by a 10-W lamp and the floor of this compartment was a grid made of aluminum bars. The shock compartment (62 cm) was dark and V-shaped stainless-steel plates formed the floor and walls. This apparatus is equipped with ten photocells distributed as follows: four in the starting compartment; and six in the shock compartment. The apparatus is controlled by a PC computer and is located in a sound-attenuated, non-illuminated room. The photocells relay information to the computer about the rat's position within the chamber. The experimenter may view the rat directly or on-line through an image of the rat displayed on the computer screen. Once the rat was placed in the starting compartment, it was allowed to explore for 10 s, after which time, the guillotine door was opened. Immediately after the rat entered the shock compartment with all four paws, the door was closed and a foot shock (0.8 mA) was delivered through the stainless-steel plates. After 5 s, the guillotine door was opened and the rat was allowed to escape. Rats remained in the starting compartment for 30 s. Immediately after the training, rats were randomly assigned to one of the following groups: control group ($n = 15$ rats), was injected with vehicle (vegetal oil, 1 ml, intraperitoneally (i.p.)). Oleamide-30 group ($n = 15$ rats) was injected with oleamide (30 mg/kg, i.p.), and oleamide-50 ($n = 15$ rats), was injected with oleamide (50 mg/kg, i.p.).

Memory retention test. The inhibitory avoidance task was tested 24 h after training. Each rat was placed in the starting compartment and 30 s later the door was opened. The latency to enter in the shock compartment with all four paws was recorded. Shock was not delivered. The rat was left in the starting compartment with the door open for a maximum of 600 s. In order to observe the extinction processes, rats were also tested daily for 4 additional days.

Locomotor activity. In this experiment, fifteen animals were randomly assigned to one of three groups: vehicle ($n = 5$); oleamide 30 mg/kg, i.p., ($n = 5$); and oleamide 50 mg/kg, i.p., ($n = 5$). Rats were evaluated immediately after the injection and over a period of 2 h. An automated system was used to monitor locomotor activity. Each activity monitor consists of an acrylic box (40.5 long, 40.5 cm wide and 30 cm tall) surrounded by two levels of infrared beams. Data were collected by an analyzer and transferred to a PC computer.

Body temperature. Ten rats were used in this part of the study. All of them were implanted with a telemetric transmitter placed into the abdominal cavity under halothane anesthesia (2–3%). One week after surgery, rats were placed in the chambers equipped with the receptor plate which relays the information to a PC computer. Rats were monitored until they had a reliable baseline then divided into two groups ($n = 5$). Either oleamide (30 mg/kg, i.p.) or vehicle was injected to each group and temperature was recorded for 5 h. Since the effect on this parameter was very robust, the dose of 50 mg/kg was not tested.

Pain perception – tail-flick test. Forty-five rats divided into three groups ($n = 15$) were evaluated with the tail flick test. Groups received an injection of either vehicle, oleamide (30 mg/kg, i.p.) or oleamide (50 mg/kg, i.p.). Fifteen and sixty minutes after administration, rats were tested. The tail flick apparatus was set to deliver a light beam at 55°C. Latency to move the tail to avoid the light was determined.

Pain perception – hot-plate test. Thirty different rats divided into three groups were also evaluated under the effect of vehicle or oleamide (30 or 50 mg/kg). Fifteen and sixty minutes after administration rats were tested. Hot-plate apparatus was set to keep the plate at 55°C. The behavior we considered as antinociceptive response was the latency elapsed from the time the rat was placed on the hot plate to the first overt expression of discomfort: licking of the hind paw, vocalization or jumping to escape.

Statistical significance was obtained by using different tests, depending on the behavior evaluated. For memory evaluation, we used Kruskal–Wallis and Mann–Whitney *U*-tests. These non-parametric tests were used due to the ceiling latency (600 s) we imposed, which results in a non-normal distribution of the sample. For locomotor activity, we used ANOVA and post-hoc Sheffé tests. For tail flick, hot-plate and core temperature, a two factors repeated measures ANOVA and post-hoc Bonferroni tests were used.

On the passive avoidance task, all groups had similar 24-h retention scores. However, on subsequent tests the oleamide-treated groups showed a rapid extinction of this behavior. This effect was dependent on the dose (see Fig. 1a).

On the other hand, oleamide caused only a tendency to reduce locomotor activity at the highest dose tested (50 mg/kg; $P < 0.09$) (data not shown).

Oleamide caused hypothermia (about 2°C) by the second hour after administration and went back to normothermia around 5 h later (see Fig. 1b).

Finally, oleamide (50 mg/kg) increased tail flick latency 15 min after administration and this effect faded away by the end of the first hour after administration, yet it was still significant (see Fig. 1c). In contrast, oleamide did not produce any observable effect on the hot-plate test (data not shown).

These results show that oleamide does not affect the retrieval of memory 24 h after training, yet it facilitates memory extinction. This effect was induced by the lowest dose, 30 mg/kg and was more prominent with the higher dose, 50 mg/kg. Although the interpretation of our observations is that oleamide deteriorates memory consolidation, these effects seem to be delayed. Basically, oleamide

fastened the extinction of memory by the second day after training. An alternative interpretation of these observations is that instead of impairing memory consolidation, oleamide facilitates the re-learning of the original behavior, which is 'to go' into the dark compartment of the chamber. If this were the case, our data is suggesting that the extinction of the 'not go' behavior is actually its faster substitution by the 'go' behavior. These findings are not easy to interpret in view of this delayed effect that makes us think that by the first day of evaluation, memory processes are normally working, but by the second day most of the rats are substituting their 'not go' for the 'go' strategy. More experiments exploring this intriguing effect ought to be performed, in order to clarify if this is a facilitation or deterioration of memory processes.

On the other hand, locomotor activity recorded for 2 h, although seem to be decreased by the dose of 50 mg/kg, it did not reach significance. We believe the effect on locomotor activity is unspecific, since other lipids, such as oleic acid, also induces immobility [8]. In addition, when we used a higher dose of oleamide (75 mg/kg), it produced a significant reduction in locomotor activity (data not shown). These observations indicate that memory processes are more sensitive to oleamide effect than motor control. As for core temperature, oleamide (30 mg/kg) reduced it by about 1.5°C within the first 2 h, returning to normal levels 5 h after administration. Since we observed that this dose was highly potent in reducing core temperature higher doses were not used. Finally, oleamide (50 mg/kg), increased the latency in the tail flick test which lasted about 1 h and had no effect on the hot-plate test. Differences in these two tests have been previously reported evaluating different drugs [20]. Actually, it has been suggested these two tests evaluate pain perception at two levels: hot-plate at supraspinal mechanisms [17] and tail-flick at spinal mechanisms [13].

Most of these effects are also produced by cannabinoids [14], which suggests that oleamide mimics cannabinoid activity. These findings are supported by the observation that oleamide binds to the cannabinoid receptors [5] and because it competes with anandamide as substrate for FAAH [6]. Therefore, oleamide's effects observed in our study may be a result of a facilitation of anandamide activity. An alternative explanation may consider that oleamide facilitates GABAA receptors activity [23], whereby impairing memory and locomotor activity. Pain perception was mainly affected at the level of spinal mechanisms, as it has been suggested by the tail-flick test. However, the supraspinal mechanisms were not affected, since the hot-

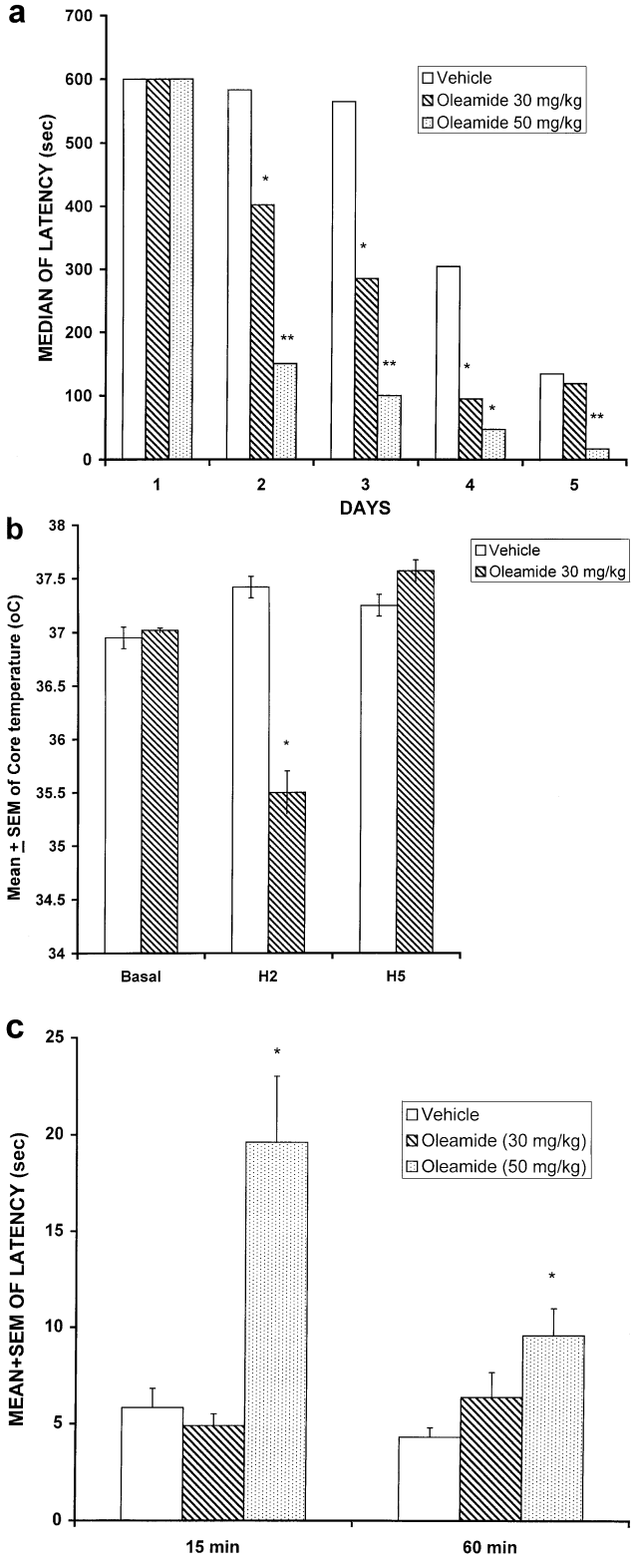

Fig. 1. (a) This graph illustrates the effect of two doses of oleamide on memory consolidation and extinction. All groups had similar 24 h retention scores yet oleamide, at both doses tested, hastened extinction of this behavior as shown by subsequent tests. *$P < 0.05$ compared to control (**compared to the other groups). (b) Average core temperature of rats treated with oleamide (30 mg/kg) or vehicle. Oleamide induced a decrease of about 2°C, 2 h after administration. This effect was cleared away 3 h later. *$P < 0.05$ compared to control. (c) Tail flick latency scores, determined 15 and 60 min after administration of oleamide (30 and 50 mg/kg) or vehicle. Latency was significantly increased 15 min, and the effect decays about 60 min after oleamide administration, yet significant. *$P < 0.05$ compared to control.

plate test was not affected. Temperature alterations in turn, may be a result of the interaction of oleamide with serotonin. It is known that oleamide facilitates serotonin activity on its receptors 5HT1A and 5HT2A, which seem to be involved in temperature regulation and pain perception [1]. An alternative possibility to be considered is that oleamide may have its own receptors.

Due to all these interactions and considering that oleamide's concentration in the CSF is increased during sleep deprivation in cats [7] and rats [3,11], we believe it may contribute to cause the very well known changes induced by sleep deprivation, such as hypothermia, hypolocomotion, hypoalgesia, memory impairment and drowsiness which are changes observed in most of the normal subjects after sleep deprivation [18].

In conclusion, our results suggest that oleamide is a lipid that affects several physiological parameters in a way similar to the manner marijuana and endogenous cannabinoid do. In addition, since its concentration in the CSF is increased during sleep deprivation, we believe it may mediate, at least in part, the syndrome caused by sleep deprivation.

This work was supported by the Grant IN233199 from DGAPA-UNAM to L.N. The authors wish to thank Dr Benjamin Cravatt for kindly providing oleamide. Mr Manuel Zarate for the care of the animals.

[1] Abdel-Fattah, A.F., Matsumoto, K., el-Hady, K.A. and Watanabe, H., 5-HT1A and 5-HT2 receptors mediate hypo- and hyperthermic effects of thryptophan in pargyline-pretreated rats, Pharmacol. Biochem. Behav., 52 (1995) 379–384.
[2] Ameri, A., The effect of cannabinoids on the brain, Prog. Neurobiol., 58 (1999) 315–348.
[3] Basile, A.S., Hanus, l. and Mendelson, W.B., Characterization of the hypnotic properties of oleamide, NeuroReport, 10 (1999) 947–951.
[4] Boger, D.L., Patterson, J.E. and Jin, Q., Structural requirements for 5-HT2A and 5-HT1A serotonin receptor potentiation by the biologically active lipid oleamide, Proc. Natl. Acad. Sci. USA, 95 (1998) 4102–4107.
[5] Cheers, J.F., Cadogan, A.K., Marsden, C.A., Fone, K.C. and Kendall, T.A., Modification of 5-HT2 receptor mediated behavior in the rat by oleamide and the role of cannabinoid receptors, Neuropharmacology, 4 (1999) 533–541.
[6] Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A. and Gilula, N.B., Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides, Nature, 384 (1996) 83–87.
[7] Cravatt, B.F., Prospéro-García, O., Siuzdak, G., Henriksen, S.J., Boger, D.L. and Lerner, R.A., Chemical characterization of a family of brain lipids that induce sleep, Science, 268 (1995) 1506–1509.
[8] Gobbi, M., Mennini, T., Valle, F.D., Cervo, L., Salmona, M. and Diomede, L., Oleamide-mediated sleep induction does not depend on perturbation of membrane homeoviscosity, FEBS Lett., 463 (1999) 281–284.
[9] Langstein, J., Hofstadter, F. and Schwarz, H., Cis-9, 10-octadecenoamide, an endogenous sleep-inducing CNS compound, inhibits lymphocyte proliferation, Res. Immunol., 147 (1996) 389–396.
[10] Mechoulam, R., Fride, E., Hanus, L., Sheskin, T., Bisogno, T., Di Marzo, V., Bayewitch, M. and Vogel, V., Anandamide may mediate sleep induction, Nature, 389 (1997) 25–26.
[11] Mendelson, W.B. and Basile, A.S., The hypnotic actions of oleamide are blocked by a cannabinoid receptor antagonist, NeuroReport, 10 (1999) 3237–3239.
[12] Murillo-Rodríguez, E., Sánchez-Alavez, M., Navarro, L., Martínez-González, D., Drucker-Colin, R. and Prospéro-García, O., Anandamide modulates sleep and memory in rats, Brain Res., 812 (1998) 270–274.
[13] Pastoriza, L.N., Morrow, T.J. and Casey, K.L., Medial frontal cortex lesions selectively attenuate the hot plate response – possible nocifensive apraxia in the rat, Pain, 64 (1996) 11–17.
[14] Piomelli, D., Giuffrida, A., Calignano, A. and Rodríguez de Fonseca, F., The endocannabinoid system as a target for therapeutic drugs, Trends Pharmacol. Sci., 21 (2000) 218–224.
[15] Prospéro-García, O., Cravatt, B.F., Siuzdak, G., Boger, D.L., Lerner, R.A. and Henriksen, S.J., cis-9, 10 Octadecenoamide: a novel natural lipid isolated from cat CSF with potential sleep-modulating properties, Sleep Res., 24 (1995) 50.
[16] Prospéro-García, O., Murillo-Rodríguez, E., Jiménez-Anguiano, A., Navarro, L., Sánchez-Alavez, M., Gómez-Chavarín, M., Martínez-González, M.D., Palomero-Rivero, M. and Drucker-Colín, R., Psychomimetic drugs - marijuana and 5-HT antagonists, In R. Lydic and H.A. Baghdoyan (Eds.), Handbook of Behavioral State Control: Cellular and Molecular Mechanisms, CRC Press, New York, 1999, pp. 433–442.
[17] Ramabradan, K. and Bansinath, M., A critical analysis of the experimental evaluation of nociceptive reactions in animals, Pharm. Res., 3 (1986) 263–270.
[18] Rechtshaffen, A., Current perspectives on the function of sleep, Perspect. Biol. Med., 41 (1998) 359–390.
[19] Steckler, T., Sahgal, A., Aggleton, J.P. and Drinkenburg, H.I.M., Recognition memory in rats - III. Neurochemical substrates, Prog. Neurobiol., 54 (1998) 333–348.
[20] South, S.M. and Smith, M.T., Apparent insensitivity of the hotplate latency test for detection of antinociception following intraperitoneal, intravenous or intracerebroventricular M6G administration to rats, J. Pharmacol, Exp. Ther., 286 (1998) 1326–1332.
[21] Woolf, N.J., A structural basis for memory storage in mammals, Prog. Neurobiol., 55 (1998) 59–77.
[22] Yehuda, S. and Carasso, R.L., Modulation of learning, pain threshold, and thermoregulation in the rat by preparations of free purified K-linolenic and linoleic acids: determination of the optimal C3-to-C6 ratio, Proc. Natl. Acad. Sci. USA, 90 (1993) 10345–10349.
[23] Yost, C.S., Hampson, A.J., Leonoudakis, D., Koblin, D.D., Bornheim, L.M. and Gray, A.T., Oleamide potentiates benzodiazepine-sensitive gamma-aminobutyric acid receptor activity but does not alter minimum alveolar anesthetic concentration, Anesthesiol. Analg., 86 (1998) 1294–1300.