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ScienceDirect
Behavioural Brain Research 182 (2007) 88–94
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Neuropharmacological effects of oleamide in male and female mice

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Received 6 March 2007; received in revised form 29 April 2007; accepted 8 May 2007
Available online 22 May 2007

Abstract

Oleamide, a fatty acid amide accumulates selectively in the cerebrospinal fluid of sleep deprived cats and rats. Oleamide has been reported to have effects on a wide range of receptors and neurotransmitter systems especially the centrally acting ones for example, dopamine acetylcholine, serotonin, gamma aminobutyric acid (GABA), cannabinoid and vanilloid among others. This suggests a wide range of central nervous system effects of the compound. The effects of intraperitoneal administered oleamide on Novelty-induced behaviours, learning and memory and forced swimming-induced depression were studied. The relative effects of the compound on the male and female mice were also noted. Oleamide dose-dependently reduced ($p < 0.05$) novelty induced rearing, grooming and locomotion. The effects on the all NIBs started within the first 10 min of the test and the peak of the effects was observed during the third 10 min period of the test. Effect of oleamide on short-term working memory was significantly ($p < 0.05$) affected only with the dose of 5 mg/kg while the other dose of 10 mg/kg had no effect. In the forced swimming test, acute triple intraperitoneal administration of oleamide at 10 mg/kg induced a significant reduction in the immobility duration in mice signifying an antidepressant effect. Sex differences in the effects of oleamide (10 mg/kg, i.p.) were clearly evident in active behaviours in FST. These results confirm the multiplicity of central nervous system receptors and neurotransmitters that Oleamide interacts with hence its numerous and diverse neuropharmacological effects. Most importantly, the present study suggests that oleamide has antidepressant-like property.

Keywords: Oleamide; Antidepressant; Locomotor; Grooming; Rearing; Mice

1. Introduction

Oleamide (OA) is a fatty acid amide identified as cis-9-octadeceneamide, a member of a family of lipid signaling molecules found to accumulate selectively in the cerebrospinal fluid of sleep deprived cats [34] and rats [32]. It induces electroencephalographically measured sleep when administered intracerebroventricularly [32]. It has been observed that OA possesses several biological activities like sleep induction, immunological suppression as well as serotonin and GABA receptor activation. Oleamide has also been reported to bind to the cannabinoid receptor [34]. The catabolic enzyme for OA is a membrane bound enzymatic activity that hydrolyses OA to its acid, oleic acid [19], which happens to be the same enzyme that metabolizes an earlier discovered endocannabinoid—anandamide found in various brain regions and peripherally in the spleen, lungs, kidney and testes [5].

Oleamide has been called the substance obtained from the cerebrospinal fluid of sleep-deprived cats [12,30]. Interestingly, this same compound had been found in a plant. It was discovered to be the active principle of a cholineacetyl transferase (ChAT) activator plant; *Zizyphus jujuba* (Family: Rhamnaceae), which is an edible Korean plant with anti-ageing and neuronal stabilization effects [24]. Oleamide was initially called cerebrodiene [30] of which subsequent structural analysis identified it as cis-9-octadeceamide [12].

Oleamide inhibits agonist binding to CB_1 receptor with potency only 3-fold lower than seen for Anandamide [4]. Although, OA does not appear to directly interact with Cannabinoid receptors [3,31], it raises the concentration of the endogenous CB_1 agonist (AEA) *in vitro*, possibly by interfering with its hydrolysis [31] or its cellular uptake [22]. Oleamide share most of the cannabinoid effect produced by AEA. Both OA and AEA are metabolized by the fatty acid amide hydrolase (FAAH) to arachidonic acid [19] and it seems that the enzyme accepts OA more than AEA as a substrate since it has been previously suggested that Oleamide effect result from competitive inhibition of FAAH catabolism of AEA [31]. The brain concentration of OA

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doi:10.1016/j.bbr.2007.05.006

required for this competitive inhibition is ≥5 μM and homeoviscosity data indirectly imply that high concentration as this is likely to be reached 30 min after sleep-inducing doses [21].

Oleamide was observed to facilitate memory extinction in a passive avoidance paradigm in rats. Even though, experimental results showed that Oleamide did not affect the retrieval of memory 24 h after the training. Although, this was interpreted to mean that Oleamide deteriorated memory consolidation, its effect was delayed. This may also be interpreted to mean that oleamide facilitates the re-learning of the original behaviour. The results obtained thus far indicate that more experiments exploring this effect are needed in order to clarify if this is facilitation or a deterioration of memory processes [34].

OA has been reported to modulate at least three major receptor systems that affect sleep (5-HT, $GABA_A$ and CB_1) [32] therefore, oleamide can modulate sleep-wake states and may be exerting its central effects through these receptors. According to many authors, oleamide has either a direct or an indirect interaction with the following receptor systems *in vivo* and *in vitro*: dopamine D_2 [18,25,45], G-protein receptors [45], vanilloid [29], serotonin [5,25]; $GABA_A$ [48], cannabinoid (CB_1) [11,29]. Lastly, there has been growing interest in the role of related family of Omega-3-polyunsaturated fatty acids in normal and abnormal central nervous system function, including a possible relationship to human affective disorders [23,36]. 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 [5]. Thus, the possibility remains open that an understanding of the interactions of OA with 5-HT receptors may be useful in future investigations of depression or the development of antidepressants [32]. The aim of this present study was to investigate oleamide effect on depression, novelty-induced behaviours and to confirm its influence on spatial working memory. The influence of sex variation was also examined, as both male and female animals were used in the investigations.

2. Method and materials

2.1. Animals

Adult male and female Albino mice (Vom strain) weighing between 17.1 and 25.0 g were obtained from the animal house of the Faculty of Health Sciences, Obafemi Awolowo University (O.A.U), Ile-Ife. The mice were kept in plastic cages in the Animal house of Pharmacology department, O.A.U., Ile-Ife. The animals were fed a standard laboratory diet and tap water *ad libitum*. All experiments were carried out in accordance with NIH guide for the care and use of laboratory animals. After one week of habituation, animals were subjected to the experiments. Females were caged separately from the males to prevent mating. Equal number of males and females were used for each of the experiments (as much as possible) and each animal was tested only once. All tests were carried out between 9.00 a.m and 2.00 p.m. All efforts were made to minimize animal suffering.

2.2. Drugs

Oleamide (Sigma Chemical Co., St. Louis, MO), Tween 80, Alcohol 10%, v/v (Sigma Chemical Co., St. Louis, MO).

2.3. Drug preparation

Twelve milligram (12 mg) of oleamide was weighed on a Metler balance and dispersed into 0.2 ml Tween 80 in a test tube with the aid of Vortex (stirring) equipment. This mixture was then made up to 2 ml with 10%, v/v ethanol (SIGMA). This was used for the calculated volume for the 20 mg/kg dose that was diluted to obtain 10 and 5 mg/kg doses. Solution of the drug was made fresh daily in vehicle and was administered via intraperitoneal injection in a 0.2 ml/30 g body weight injection volume.

2.4. Novelty induced behaviours

Behavioural analyses used were as described previously [1,9]. Six 10–min epochs of each of the following behavioural states: locomotion, grooming and rearing were observed and scored. This was to characterize drug-induced behavioural alterations in mice. For each test session, mice were allowed to acclimatize to the testing environment (a quiet well-ventilated room) for 30 min. All behavioural testings were carried out between 9.00 a.m and 2.00 p.m. Mice were injected intraperitoneally in an individual sequence with a volume of vehicle (0.2 ml/30 g body weight, $n = 8$) and oleamide at different doses of 5 or 10 mg/kg ($n = 8$ per dose) and placed in an open field arena. Briefly, the structure consists of a rectangular arena composed of a hardboard floor ($36 \times 36\ cm^2$) with a surrounding wall 30-cm height, both made of white painted wood. The floor was divided by permanent red marker into squares of $9\ cm^2$ at the bottom (on the external surface). Generally, spontaneous motor activity was monitored for 60 min in the modified open field method, as described by Ajayi and Ukponmwan [1]. Immediately after injection, each mouse was introduced into a cage and total locomotion (number of floor units entered—the floor of the squares crossed with all paws), frequency of grooming (the number of body cleaning with paws picking of the body and pubis with mouth and face-washing actions) and rearing frequency (number of times the animal stood on its hind legs or with its forearm against the wall of the observation cage or in the free air [1]) all these behavioural parameters were counted every 10 min during 60 min after drug administration or vehicle administration. Before introducing each animal, the arena was cleaned with 5% alcohol to eliminate the possible bias due to the odour that could be left by the previous animals.

2.5. Learning and memory test (Y-maze)

It is well known that that spontaneous alternation is a measure of spatial working memory. To alternate among spatial locations, a mouse must remember its previous location. The Y-maze can be used as a measure for short-term memory, general locomotor activity and stereotypic behaviour. Therefore, spontaneous alternation performance was assessed using a Y-maze composed of three equally spaced arms (120°; 41-cm long × 15-cm high). The floor of each arm consists of wood (5-cm wide). This test was carried out using this apparatus to obtain results for spontaneous alternation performance (memory) and locomotor activity. Each group of animals was tested after administration of vehicle (control) and different doses of oleamide (5 or 10 mg/kg). Each mouse was placed in one of the arm compartments and was allowed to move freely for 6 min without reinforcers. An arm entry is defined as the body of a mouse except for its tail completely entering into an arm compartment. The sequence of arm entries is manually recorded. An alternation is defined as an entry into all three arms on consecutive choices. For instance, each alternation is followed by a comma in the following sequence of arm entries (each arm is labelled A, B, or C): ACB, CA, B, C, A, CAB, C, A,. In this example, the mice entered 13 arms, eight of which are alternations. The number of maximum spontaneous alternations is then the total number of arms entered minus two, and the percent alternation is calculated as (actual alternations/maximum alternations) × 100. The apparatus is cleaned with 70% ethyl alcohol between sessions to eliminate the ordor from the previous animal.

2.6. Antidepressant test

The forced swimming test (FST) is a behavioural test widely used to screen new potent antidepressant drugs in rats and mice [37]. This test is sensitive and specific to all major classes of antidepressant drugs including tricyclic