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Short communication

Anandamide modulates sleep and memory in rats

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Abstract

In this study we have assessed the effect of the intracerebroventricular administration of anandamide (ANA) as well as its precursor metabolite arachidonic acid (AA), on the sleep–wakefulness cycle, memory formation, locomotor activity and pain perception. Our results have indicated that ANA strikingly increases slow-wave sleep (SWS)2 and rapid-eye movement (REM) sleep at the expense of wakefulness (W); while deteriorating memory consolidation. ANA also increases locomotor activity but does not modify pain perception threshold. In contrast, AA increases W and reduces SWS2, while deteriorating memory consolidation and increasing locomotor activity. AA has no effect on pain perception. These results suggest that the brain cannabinoid system participates in the modulation of the vigilance states and mnemonic processes. Additionally, they suggest that the effect on pain perception may be a peripheral rather than a central effect.

Keywords: Arachidonic acid; Rapid-eye movement (REM) sleep; Pain; Locomotor activity

Anandamide (arachidonylethanolamide, ANA) [8] and *sn*-2 arachidonylglycerol [29] are recently described lipids that bind cannabinoid receptors [11]. Systemic ANA administration mimics the activity of Δ^9 tetrahydrocannabinol (THC), the most active biological component of marihuana [13], by inducing memory impairment [4,19,23] and motor incoordination [13] as well as analgesia [28,31]. At least two cannabinoid receptors have been described: CB1, with a distribution restricted to the central nervous system (CNS) [7,14,21] and CB2, with peripheral distribution [20,25].

Although it has been observed that the inhalation of marihuana, as well as the experimental administration of THC, induces sleep in humans [10,26], the actual effect of ANA on the sleep–wakefulness cycle has not been systematically studied.

Another lipid, oleamide (*cis*-9-10-octodecenoamide) that is obtained from the cerebrospinal fluid of sleep deprived cats [5,18] also possess potential cannabinoid activity. It has been observed that oleamide induces sleepiness in rats when administered systemically [5]. In addition, it has been observed that both lipids interact to produce some biological effects in vitro [2,6]. Due to this fact, we believe that anandamide may be capable of inducing sleep via a potential interaction with oleamide [22]. All of these observations postulate the existence of a cannabinoid system that may be participating in sleep regulation.

On the other hand, anandamide seems to have modulating effects on memory [4]. This potential effect is expected, since CB1 receptors are distributed in the hippocampus [14] and because marihuana usage disrupts memory formation [9]. In addition, arachidonic acid, which is an ANA precursor metabolite, seems to share this activity [1,3].

In this study we have attempted to further our understanding of the potential modulating effect of anandamide on the sleep–wakefulness cycle and on mnemonic processes. In order to test this hypothesis we designed the following experiments.

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For the sleep–wakefulness cycle study, 18 male Wistar rats (250–350 g, at the time of the surgery) were employed. Rats were implanted, under pentobarbital anesthesia, with electrodes for sleep recordings. The electroencephalogram (EEG) was recorded through two screw electrodes placed in the parietal bone over the hippocampus ($p = 4.0$, $L = 3.0$). Two wire electrodes were inserted into the neck musculature to record postural tone (EMG). An additional cannula (23 gauge) was implanted in the right lateral ventricle ($p = 0.8$, $L = 2.5$, $V = 3.0$) for the administration of the drugs. Rats were individually housed with water and food ad libitum. Light–dark cycle was controlled (12:12, lights on at 0800 h).

One week after the surgery the rats were acclimatized to the recording conditions for 3 days, then were divided into three groups ($n = 6$). The day of recording, rats belonging to a given group received the intracerebroventricular (i.c.v.) administration of one of the following treatments: alcohol in saline (5%, 5 μl, vehicle), ANA (3.6 nmol) or AA (3.6 nmol). Rats were continuously recorded for 4 h after the injection of the aforementioned treatments (from 0800 to 1200 h). They were observed for potential drug-induced behavioral changes through a one-way window.

Sleep recordings were visually inspected and four sleep–wakefulness cycle stages were determined: wakefulness (W), slow-wave sleep 1 (SWS1), slow-wave sleep 2 (SWS2) and rapid eye movement (REM) sleep. These stages were analyzed on the basis of the total time of occurrence during the 4 h. Latency to sleep as well as to REM sleep onset was determined. The frequency and duration of the four stages were also evaluated.

For the memory study, fifteen rats were stereotaxically implanted with a cannula into both hippocampal CA1 areas ($p = 4.0$, $L = 2.5$, $V = 2.0$). One week after the surgery, rats were trained in the footshock passive avoidance behavioral test (FSPABT), in order to evaluate short- and long-term memory (STM and LTM). The training apparatus was a trough-shaped alley (94 cm long, 25 cm wide and 30 cm deep) that was separated 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; while the shock compartment (62 cm) was dark and a V-shaped stainless-steel plates formed the floor and walls. This apparatus is equipped with 10 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 placed in a sound-attenuated, non-illuminated room. The photocells relay information to the computer about the rat's position within the chamber. The experimenter can view the rat directly as well as on-line through a caricature of the rat displayed on the computer screen. Once the rat was placed in the starting compartment, the computer allowed the rat to explore for 10 s, then opened the guillotine door. Immediately after the rat entered the shock compartment with all four paws, the computer closed the door and a footshock (0.8 mA) was delivered through the stainless-steel plates. Five seconds after the initiation of the footshock delivery, the door was opened and the rat was allowed to escape. Rats were allowed to stay in the starting compartment for 30 s, then removed and injected with the vehicle, ANA, or AA. Retention of the inhibitory avoidance training was assessed 15 min and 24 h after training. Each rat was placed in the starting compartment and 30 s later the computer opened the door. The latency to enter 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.

To evaluate locomotor activity, fifteen rats were implanted with a cannula aimed to the lateral ventricle, as previously described. Immediately after i.c.v. administration of either: the vehicle, ANA, or AA, they were placed in a photocell chamber for 10 min.

Pain perception was evaluated in fifteen rats implanted with lateral ventricle cannulae. To evaluate this behavior the tail-flick test was used. The rats were given i.c.v. injections of either: the vehicle, ANA or AA and later were evaluated 15 and 60 min later.

The statistical analysis of the sleep data was performed using an ANOVA and a post hoc Sheffé test. Memory evaluation was analyzed by Kruskal–Wallis and Mann–Whitney *U*-tests. We used these statistical tests due to the ceiling latency imposed by the experimenters (600 s) which results in a non-normal distribution of the sample.

Sleep–wakefulness cycle. Our results showed that ANA induced both SWS2 and REM sleep at the expense of wakefulness. SWS1 remained without change (Fig. 1). In contrast, AA increased W while reducing SWS2 (Fig. 1). In addition, AA increased SWS2 onset latency (control vs. ANA vs. AA, mean ± S.E.M. (min): 24.24 ± 9 vs. 26.36 ± 7.6 vs. 74.34 ± 10.5, $p < 0.05$), although REM sleep onset latency remained without change. On the other hand, AA increased the mean duration of W (control vs. ANA vs. AA, mean ± S.E.M. (min): 4.48 ± 1 vs. 3.98 ± 0.7 vs. 9.6 ± 2.6, $p < 0.05$). Mean duration of both SWS2 and REM sleep remained without change. However, the frequency of these stages were significantly changed by ANA (control vs. ANA vs. AA, mean ± S.E.M. (no. of events/4 h), SWS2: 29.16 ± 4.3 vs. 37.12 ± 0.1 vs. 21.5 ± 1.9, $p < 0.05$; REM sleep: 8.5 ± 2.5 vs. 15.62 ± 2.8 vs. 7.6 ± 1.5).

Memory. Results showed that vehicle administered immediately after training did not interfere with the performance of the inhibitory avoidance behavior 15 min and 24 h after training. In contrast, AA prevented the performance of this behavior at both time points. Interestingly, ANA did not modify the execution of this behavior at 15 min but definitively prevented it 24 h after training (Fig. 2).

Locomotor activity. Our results indicated that ANA readily increased this behavior during the 10 min following i.c.v. administration. This effect was moderately in-

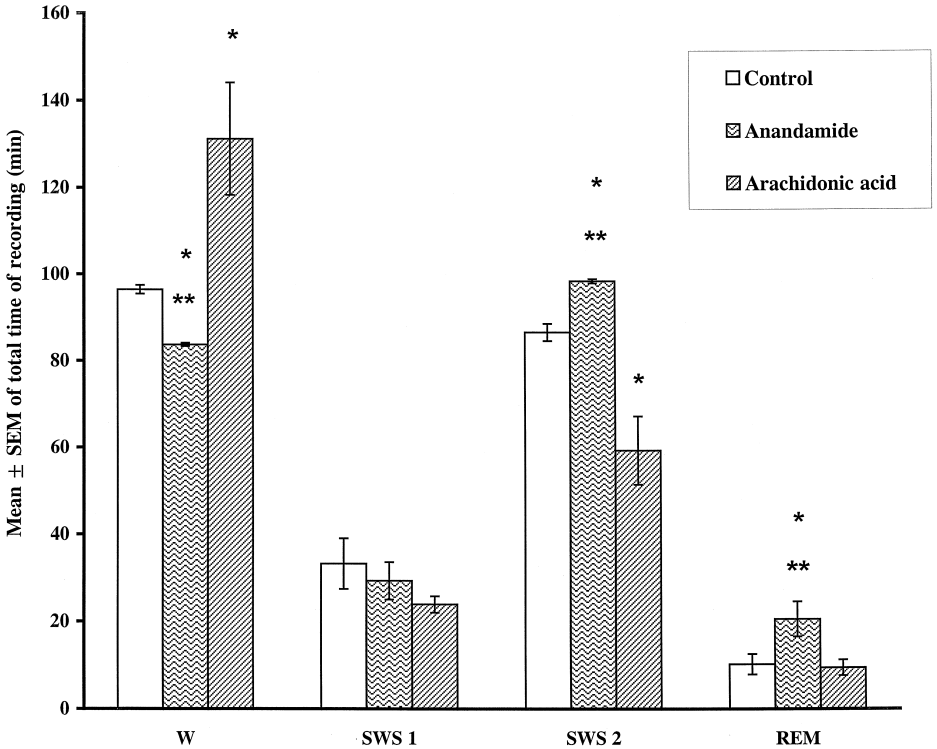

Fig. 1. This figure illustrates the effect of the i.c.v. administration of anandamide and arachidonic acid on the sleep–wakefulness cycle. A reduction in wakefulness, resulting in an increase of both SWS2 and REM is caused by anandamide. The opposite effect is induced by arachidonic acid, which increases wakefulness and decreases SWS2. $* p < 0.05$

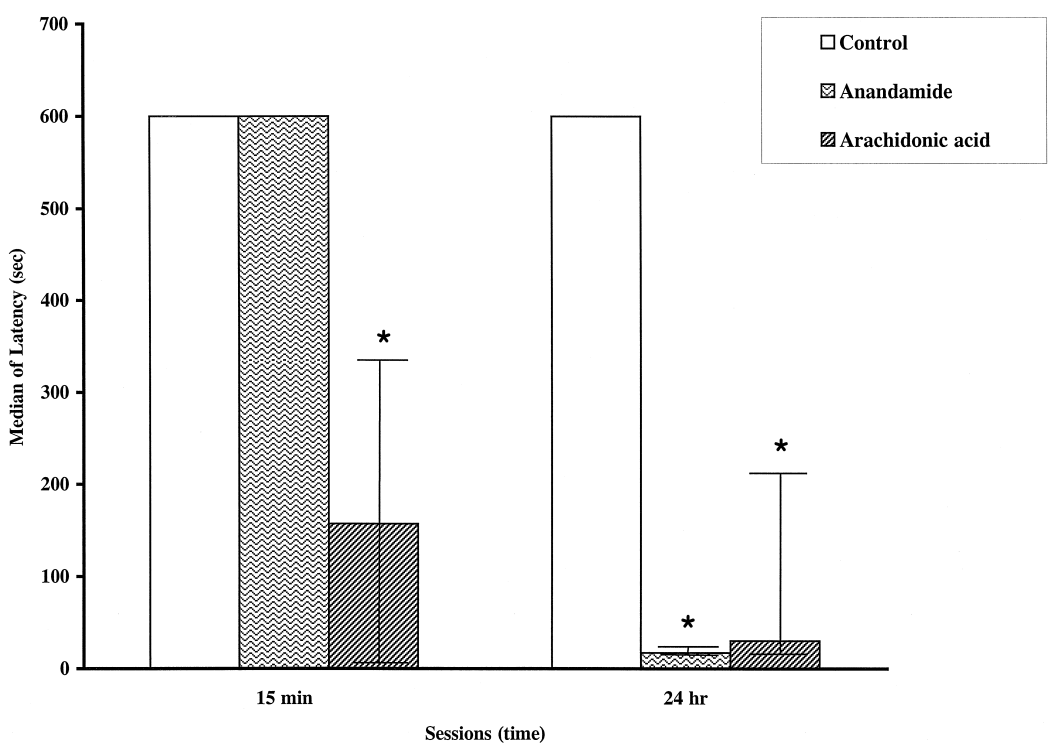

Fig. 2. This figure depicts the deleterious effect of both anandamide and arachidonic acid on memory retrieval. At 15 min, this graph shows the deleterious effect of AA on STM; while ANA has no effect. At 24 h ANA interferes with LTM. Data are expressed as median and interquartile range. $* p < 0.05$.

duced by AA compared to vehicle (control vs. ANA vs. AA, mean ± S.E.M. (counts/10 min): 2764.75 ± 520 vs. 18535.5 ± 734 vs. 5659.4 ± 997, $p < 0.05$).

Pain perception. ANA or AA induced no changes in pain perception in our animals.

In summary our results show that ANA is capable of modifying the sleep–wakefulness cycle, basically by inducing SWS2 and REM sleep. In contrast, AA induces an increase in W and a decrease in SWS2. In addition, ANA has deleterious effects on long-term memory but not on short term memory. In contrast, AA, the precursor metabolite, alters both short- and long-term memory. ANA increases locomotor activity during the entire period of evaluation (10 min), while AA only caused a moderate increase. In addition, a very interesting finding was the fact that both ANA and AA do not modify the pain perception threshold.

These findings suggest that ANA participates in the regulation of the level of alertness. This is not surprising since metabolites obtained from the Cannabis plant, i.e., THC, cause a similar effect [10]. It is very interesting that this lipid, ANA as well as other lipids derived from AA, such as prostaglandins, i.e., PGD_2 modulate the sleep–wakefulness cycle [12]. Also of interest is the increase on REM sleep induced by ANA, particularly since ANA also deteriorates memory consolidation. This is a contrasting effect, since a memory consolidating function has been attributed to REM sleep [27].

On the other hand, the precursor metabolite AA was able of modifying the sleep–wakefulness cycle by increasing W and decreasing SWS2 during the 4 h following the i.c.v. injection. Due to this fact, we believe that AA possess an effect by itself that is opposite to that induced by its metabolites ANA or PGD_2.

As for memory effects, ANA causes in our animals the previously reported deleterious effect. AA also produces impairment in memory retention. This is a controversial issue since there are a few reports suggesting an AA facilitating effect on memory. However, most of these studies have been performed in chicks [3,15,16]. In addition, there are also studies indicating that AA enhances long-term depression in rat hippocampal slices [1]. Also, the ability of mice to solve a maze was correlated with a reduction of AA [30]. In support of this finding, it has been reported that an increase in brain AA is associated with cognitive impairment, as shown by evaluating rats in the passive avoidance test [24]. Moreover, this increase was associated with tissue damage [24]. A potential role for AA in the cognitive abnormalities associated with Alzheimer's disease has also been claimed [17].

ANA effects on locomotor activity are quite contrasting with the effect on sleep, since we would expect a relaxing rather than an exciting action. However, this may be an acute and transient effect followed by the quiescence necessary for sleep onset as our sleep recordings show. As for memory evaluation, since ANA does not affect short-term memory but increases locomotor behavior, we believe that this latter effect did not influence this task.

Finally, the fact indicating that the i.c.v. administration of ANA does not modify pain perception threshold suggests that a peripheral rather than a central effect may mediate this action. Although to our knowledge, there is scant information about the role of peripheral cannabinoid receptors (CB2), these CB2 receptors may be participating in regulating behaviors different to those mediated by the central cannabinoid receptor CB1. In this way, CB2 receptors may be preferentially involved in pain regulation.

In conclusion, our study provides for the very first time evidence indicating that anandamide participates in the regulation of the sleep–wakefulness cycle and expands the studies suggesting that endogenous cannabinoids modulate memory. In addition, by administering ANA i.c.v. our results suggest that not all the ANA effects described are meditated by a central action.

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