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Short Communication

A study of the reciprocal relationship between the thermal and behavioral effects mediated by anandamide

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HIGHLIGHTS

- Endocannabinoids have an important role in thermoregulation and behavioral functions.
- Central AEA injection increases internal body temperature.
- Thermal effects mediated by central AEA injection may occur independent of locomotor activity.

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ABSTRACT

The endocannabinoid system plays an important role in thermal control and modulates several behaviors, such as locomotion and food intake (FI) that may affect the body temperature (T_b). To test whether the changes in T_b induced by anandamide (AEA) are related to behavioral changes, adult Wistar rats received an intracerebroventricular injection of AEA (0.1, 1.0 and 10.0 μg) and vehicle. Total FI was weighted daily, and T_b and spontaneous locomotor activity (SLA) were simultaneously and continuously recorded. AEA induced an increase in T_b without changing SLA and FI. For all doses tested, the T_b average in the post-injection period was higher than in the pre-injection period. The higher thermal effect was verified using a dose of 10.0 μg AEA, starting within the first hour post-injection, and was maintained for 8 h after treatment. A dose-dependent thermal effect was observed ($r = 0.953$; $p < 0.05$) at 1 h post-injection. Hypoactivity was verified only at a dose of 1.0 μg AEA. As expected, both the T_b and SLA values during the dark phase were always higher than during the light phase and were positively correlated ($r = 0.834$, $p < 0.001$); however, this correlation was inverted ($r = -0.852$, $p < 0.01$) after the rats received 10.0 μg AEA. In summary, our results suggest that brain AEA induces an increase in T_b, and that this effect may occur independently of changes in both locomotion and FI. Moreover, it is possible that the hypolocomotion induced by AEA could be an adaptive response to the increased T_b.

1. Introduction

The endocannabinoid system (ECS) is an endogenous signaling system composed of endocannabinoid receptors (CB1 and CB2), their endogenous ligands (endocannabinoids), and proteins involved in endocannabinoid synthesis and inactivation. Anandamide (AEA) was discovered first and is the most extensively studied endocannabinoid [1].

Rodents treated with cannabinoids display four characteristic symptoms, named the "tetrad of cannabinoid-induced effects": hypoactivity, catalepsy, antinociception and hypothermia. Thus, CB1 receptor activation is assumed when a given compound induces motor depression in an open field, catalepsy on an elevated ring, antinociception on a hot plate, and hypothermia [2].

The reduction in locomotor activity mediated by the peripheral administration of cannabinoid receptor agonists and the increased motor activity induced by their antagonists are well established in the literature [3–7]. However, despite the described hypothermia, a dual thermal effect of cannabinoids dependent on dose and route of administration has been accepted [8–10]. The peripheral administration of high doses of Δ^9-tetrahydrocannabinol (Δ^9-THC) induces a significant drop in internal body temperature (T_b), while lower doses increase rather than decrease T_b [8,9,11]; and systemic

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AEA administration also decreases T_b [3–6,12]. Centrally, microinjections of Δ^9-THC into the anterior hypothalamic/preoptic area or into the third and fourth cerebral ventricle induce hypothermia [13]. In contrast, intracerebroventricular (i.c.v.) or intrahypothalamic (i.h.) AEA injections induce hyperthermia [10].

It is important to mention that the T_b is directly influenced by circadian rhythms, which in turn, may be impacted by environmental (mainly ambient temperature and food availability) and biological factors (including locomotor activity, maturation and aging, body size and reproductive state). Because spontaneous locomotor activity (SLA) is also influenced by circadian rhythms, a direct relationship between T_b and SLA has been found; the locomotor activity results in heat production and a consequent increase in T_b [14,15].

Although several studies have demonstrated the thermal and behavioral effects induced by ECS, no data have verified whether the thermal effects mediated by ECS are due behavioral changes. Previous work that evaluated changes in locomotor activity or T_b mediated by ECS, used distinct and isolated trials, in which the values were not continuously and simultaneously recorded to observe these effects; thus the studies were unable to determine a possible relationship between them. Moreover, the behavioral tests and temperature measurements usually involve many different manipulations and the animals are exposed to unknown environments during experiments [3–6].

Thus, the aim of this study was to investigate whether the thermal effect and behavioral changes in SLA and food intake (FI) mediated by i.c.v. AEA injection are inter dependent.

2. Materials and methods

Adult male Wistar rats (8–9 weeks old) weighing 250–300 g from the CEBIO/UFMG were individually housed at an ambient temperature of 23 ± 2 °C under 14-h light/10-h dark cycles and had free access to water and rat chow.

First, they were submitted to surgical implantation of a guide cannula into the right cerebral ventricle and implantation of a telemetry sensor to measure their T_b and SLA. Following anesthetization with a mixture of ketamine (75 mg kg^{-1} bw; *ip*) and xylazine (10 mg kg^{-1} bw; *ip*), rats were fixed to a stereotaxic apparatus (David Kopf Instruments, M-900, Tujunga, CA, USA), and a guide cannula (22G) was positioned into the right lateral cerebral ventricle according to the following stereotaxic coordinates of the De Groot atlas: anteroposterior: −1.5 mm; lateral: −2.5 mm vertical, −3.0 mm above the base of the skull. Contact between the tip of the cannula and the ventricular space was indicated by a pressure drop in a saline-filled manometer attached to the cannula. The cannula was anchored to the skull with jeweler's screws fixed with acrylic cement, and protected with a protective cap. During the same surgical procedure, a sensor (G2 E-Mitter; Mini Mitter Company Inc., Sun River, OR, USA) was implanted into the peritoneal cavity through a small incision in the linea alba. The sensor was inserted and sutured to the abdominal muscle to prevent movement inside the cavity. After the abdominal muscle and skin were sutured, the rats received a single dose of analgesic (flunixin meglumine 1.0 mg kg^{-1} bw) and an antibiotic mixture (pentabiotic: 48,000 IU kg^{-1} bw).

Five days after the surgical procedures, the animals were submitted to the experimental protocol. They were weighed and taken to a room with an ambient temperature of 24 ± 1 °C and a photoperiod of 14-h light and 10-h dark (lights on at 6:00 a.m.). Each animal was placed in a cage on the receiver plate (ER-4000 Energizer/Receiver, Mini-Mitter Company, Sun River, OR, USA) connected to a computer containing the software for data acquisition (VitalView 4.0). The T_b and SLA values were continuously and simultaneously recorded every minute for 1 week. For the first two days, animals were familiarized to the experimental procedures related to drug injection to minimize any thermal effect during the experiments. A needle (30G) was placed inside the guide cannula with its tip protruding 0.3 mm for 2 minutes before removal. After the third day, every morning at 10:00 h, the animals underwent four different trials where they were treated with vehicle (1:1:8 ratio of ethanol:cremophor:saline) and drug (0.1, 1.0 and 10.0 μg of AEA, Cayman Chemical, Michigan, USA, see Table 1). The doses of AEA used were based on a previous study [10]. Using a polyethylene tube (PE-10), the needle was connected to a 5.0 μL Hamilton syringe containing the solution and introduced into the guide cannula. Then, 2 μL (i.c.v.) of vehicle or drug was slowly injected (over 1 min). The needle remained attached for 2 min before withdrawal to prevent the backflow of the injection fluid. At this moment, water and chow availability were assessed, and the chow was weighted. After the injections, the animals remained in the room without the influence of manipulations or the presence of experimenter.

Table 1
Schematic representation of the treatment days, showing the dose presented to each animal across experimental sessions.

Rat	Day 3	Day 4	Day 5	Day 6
01	Vehicle	0.1 μg	1.0 μg	10.0 μg
02	0.1 μg	1.0 μg	10.0 μg	Vehicle
03	1.0 μg	10.0 μg	Vehicle	0.1 μg
04	10.0 μg	Vehicle	0.1 μg	1.0 μg
05	Vehicle	0.1 μg	1.0 μg	10.0 μg
06	0.1 μg	1.0 μg	10.0 μg	Vehicle
07	1.0 μg	10.0 μg	Vehicle	0.1 μg
08	10.0 μg	Vehicle	0.1 μg	1.0 μg

All experimental procedures were approved by the Ethics Committee for the Care and Use of Laboratory Animals of the Federal University of Minas Gerais (protocol no. 132/2012) and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual.

The results are expressed as the mean ± standard error of the mean (S.E.M.) and the significance level was set at $p < 0.05$. The changes in the T_b and SLA during the light/dark cycles or during the first hour after injection were tested using a two-way analysis of variance (ANOVA) with repeated measures, followed by a *post hoc* Student–Newman–Keuls test. The effects of the various doses on the area under curve for T_b and SLA during the first hour after injection and the daily food intake were tested using a one-way ANOVA with repeated measures, followed by a *post hoc* Duncan's Multiple Range test and Student–Newman–Keuls test, respectively. The differences between the light (pre- and post-injection periods) and dark phases were tested using a one-way ANOVA with repeated measures followed by Tukey's test for T_b values and Student–Newman–Keuls test for SLA values. The correlations were assessed using Pearson's correlation coefficient.

3. Results

Intracerebroventricular AEA injection induced an increase in T_b in all trials. The dose of 0.1 μg AEA led to a slow increase in T_b, reaching a plateau from 13:00 to 17:00 h. A more pronounced long-lasting effect (from 11:00 to 18:00 h) was observed using a dose of 10.0 μg AEA, which attain a higher T_b at 11:00 h (Fig. 1A). No difference was found in SLA after injection of vehicle or AEA at the various doses (Fig. 1B).

The averages of the post-injection values during light phase revealed that all doses tested induced an increase in T_b compared with the pre-injection values; however, differences in the SLA were verified only for 1.0 μg AEA, where there was a reduction in SLA during the light phase. Due to the circadian oscillation of T_b and