Effects of the anandamide uptake blocker AM404 on food intake depend on feeding status and route of administration

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A B S T R A C T

Endocannabinoids (anandamide and 2-AG) are relevant modulators of appetite and energy expenditure through their action on cannabinoid CB1 receptors. The actions of anandamide on feeding behavior are dependent both, on the anatomical location of CB1 receptors (central nervous system versus peripheral tissues) and the feeding status. Anandamide uptake into cells, prior to its degradation by specific enzymatic systems, is a necessary step for the regulation of its extracellular levels. The present study explores the route and feeding stimulus dependency of the effects of the anandamide uptake blocker AM404. Peripherally, AM404 reduced feeding in partially satiated animals through a PPARα-independent mechanism, but not in food deprived ones. When AM404 was injected into the cerebral ventricles of food deprived rats, it resulted in hyperphagia that was antagonized by the cannabinoid receptor inverse agonist SR141716A. These results support the multimodal action of endocannabinoid signaling in feeding regulation, which depends on the anatomical site and the feeding status of the animal.

1. Introduction

The endocannabinoid system (ECS) has been detected throughout the central nervous system as well as in peripheral tissues involved in the control of energy balance (Matias et al., 2006). This system comprises cannabinoid receptors (at least two types CB1 and CB2), their endogenous ligands generically named endocannabinoids and the enzymatic machinery for their synthesis and inactivation. The most studied endocannabinoids are anandamide (N-arachidonoylthetanolamine, AEA) and 2-arachidonoylglycerol (2-AG) and both are polyunsaturated fatty acid derivatives (Devane et al., 1992; Mechoulam et al., 1995). In particular, AEA belongs to a family of bioactive lipid mediators, N-arachidonolamines (NAE, fatty acid ethanolamides), that includes another important molecule related to food intake and energy balance, N-oleylethanolamine (OEA) (Rodríguez de Fonseca et al., 2001). NAEs are involved in a wide range of physiological activities, and even conflicting effects, through their variety in several targets (G protein-coupled receptors, nuclear receptors, channels…). In fact, despite the structural similarity of AEA and OEA, both NAEs exhibit an opposite role on feeding behavior. While OEA induces satiety via peroxisome proliferator-activated receptor alpha (PPARα), AEA displays an important appetite-inducing effect mainly via cannabinoid CB1 receptors (Capasso and Izzo, 2008; Fu et al., 2003; Pavón et al., 2010). Together with the rest of NAEs, AEA shares common biosynthetic and degradative mechanisms that have been characterized in different cells, particularly in neurons (Matias et al., 2007; Rodríguez de Fonseca et al., 2005).

The inactivation mechanism of AEA includes two independent steps: cellular reuptake, by a putative AEA transporter that has not been isolated or cloned yet; and hydrolysis, mediated mainly by a fatty acid amide hydrolase (FAAH) (Bassavarajappa, 2007; Piomelli, 2003). Focusing on AEA membrane transport, there are many drugs capable to inhibit such endocannabinoid transporter (e.g. AM404, VDM11, OMDM-1 or OMDM-2) (Beltramo et al., 1997; De Petrocellis et al., 2000; Lopez-Rodriguez et al., 2001; Ortar et al., 2003) in order to potentiate the activity of endogenous AEA, but little is known about the effects of this blockade on feeding behavior.

AM404 was originally identified as the first synthetic inhibitor for the AEA membrane transporter into neurons (Beltramo et al., 1997; Piomelli et al., 1999). However, AM404 also acts at multiple targets. It activates transient receptor potential vanilloid receptor 1 (TRPV1), inhibits FAAH-mediated hydrolysis of AEA, inhibits cyclooxygenase...
(COX)-1 and COX-2, and prostaglandin synthesis (Hogestatt et al., 2005). AM404 does not directly activate cannabinoid receptors, but it is able to potentiate and prolong several CB1-mediated actions through an increase of endocannabinoid content (Giuffrida et al., 2001). It has been reported that AM404 promotes the extinction of fear memories in rats (Bitencourt et al., 2008), produces anxiolytic- and antidepressive-like effects in rats and mice (Adamiczky et al., 2008; Bortolato et al., 2006; Moreira et al., 2007; Patel and Hillard, 2006), reduces neuronal damage on cerebral ischemia in gerbils (Zani et al., 2007), displays antinoceptive properties in rodents (Hasanein, 2009; La Rana et al., 2008) and modulates addiction-related behaviors in rodents (Cippitelli et al., 2007; Del Arco et al., 2002).

However, the effects of AM404 on food intake have not been extensively explored yet. We found only two studies which have reported results on feeding with this AEA clearance inhibitor injected peripherally in rodents. Cippitelli et al. (2007) have described that an intraperitoneal administration of AM404 does not alter food intake in 24 h fasted rats and, similarly another study shows no effects on feeding following a subcutaneous administration in free-feeding rats (Chu et al., 2010). Recently, it has been demonstrated that central injections in nucleus accumbens of another endocannabinoid uptake inhibitor (OMDM-1) stimulate appetite in the same magnitude that AEA (Soria-Gomez et al., 2007). Thus, AM404 might represent a useful pharmacological tool to increase for extended periods of time endogenous levels of AEA, modifying feeding behavior via peripheral or central treatment without acting directly at CB1.

It is well-known that ECS might regulate energy balance at several functional levels in brain (e.g. limbic/reward areas and hypothalamus) and periphery (gastrointestinal tract, pancreas, liver, adipose...) according to physiological and metabolic requirements under each feeding/nutritional status (Capasso and Izzo, 2008). However this signaling system is found to be dysregulated and overexpressed in eating disorders such as obesity (Engeli, 2008). For example, obese patients display increased levels of postprandial AEA in plasma compared to control patients (Gatta-Cheri et al., 2011). These findings related to elevated levels of AEA were previously reported in binge eating disorder, but also in anorexia and underweight patients (Monteleone et al., 2005). Therefore, a pharmacological modulation of endocannabinoid levels represents an interesting tool to further investigate the role of these lipid mediators in appetite and body weight regulation.

Taking into consideration that endocannabinoid actions are dependent on anatomical location of CB1 receptors and caloric status, the aim of this study was to test the relative efficacy of AEA transporter inhibitor AM404 on the regulation of feeding behavior. For that purpose, we evaluated the peripheral and central effects on food intake after acute administrations of AM404 under different nutritional conditions (both treatments under food deprivation and partial satiation).

2. Material and methods

2.1. Animals

Male Wistar rats (Charles Rivers Laboratories España, S.A., Barcelona, Spain) weighing 300–350 g at the beginning of the experiments were housed individually in a room with humidity and temperature control on a 12 h light/dark cycle (lights off 8:00 PM). Animals had ad libitum access to standard chow and water, except when restriction was required for experimental studies. In order to reduce stress associated with human contact, animals were habituated to handling at least 5 min once daily for 1 week prior to food intake experiments and/or intracranial surgeries by the experimenters.

Additional studies were performed on male mice weighing 25–30 g. Both wild-type (129S1/SvImJ, stock #002448) and PPARα-null (129S4/ SvJae-Ppara−/−

All animal procedures were performed in accordance with the European Communities Council Directive (86/609/EEC) and Spanish regulation (BOE 252/34367-91, 2005) for the care and use of laboratory animals.

2.2. Drugs

AM404 [N-(4-hydroxyphenyl)-arachidonoyl-ethanolamide; Tocris Bioscience, Bristol, UK] GW6471 [N-((2S)-2-((1Z)-1-Methyl-3-oxo-3-(4-(trifluoromethyl)phenyl)prop-1-enyl)amino)-3-(4-(2-(5-methyl-2-phenyl 1,3-oxazol-4-yl)ethoxy)phenyl)propyl]propanamide; Tocris Bioscience, Bristol, UK] and SR141716A [rimonabant or N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide; Sanofi, Paris, France] were dissolved in different vehicle solutions according to the route of administration. Drugs were administered by intraperitoneal (i.p.) injection in a vehicle solution consisting of 5% Tween® 20 in sterile saline in a volume of 1 mL/kg. AM404 was also administered by intracerebroventricular (i.c.v.) injection in 70% dimethylsulfoxide (DMSO) diluted in saline, in a total volume of 5 μL after a previous surgery procedure.

2.3. Surgery procedure

For i.c.v. injections, stainless steel guide cannulas aimed at the lateral ventricle (LV) were implanted in the rats. Animals were anesthetized with an isoflurane/oxygen vapor mixture (1.5–2.0%) and mounted on a stereotaxic apparatus for implantation of a guide cannula (7 mm, 23 gauge) (David Kopf Instruments, Tujunga, CA). The stereotaxic coordinates were relative to Bregma point: AP, +0.6 mm; L ±2.0 mm; DV –3.2 mm (from the surface of the skull) (Paxinos and Watson, 1998). The cannula was placed 1 mm above the LV and secured to the skull by 2 stainless steel screws and cranialplastic cement. Dummy stylet wires were inserted into cannulas to prevent occlusion. After 7 days postsurgical recovery period, cannula patency was confirmed by gravity flow of isotonic saline through a stainless steel injector (8 mm, 30 gauge) inserted within the guide to 1 mm beyond its tip. This procedure allowed the animals to become familiar with the injection technique.

2.4. Drugs administration

AM404 was administered peripherally (i.p.) 15 min before starting the feeding studies at doses of 0.4, 2 and 10 mg/kg in a volume of 1 mL/kg for rats, and 0.3, 3 and 30 mg/kg in 10 mL/kg for mice. For central (i.c.v.) administration of AM404 in rats, the stylet was removed from the guide cannula of each rat and an injector connected to 70 cm of calibrated polyethylene-10 tubing was lowered into the striatum. The tubing was then raised until flow began, and 5 μL of drug solution at doses of 0.4, 2 and 10 μg was infused over a 30–60 s period. The injector was left in the guide cannula for an additional 30 s and then removed, and the stylet was immediately replaced. Animals were tested in the corresponding food intake study 5 min after injections. The cannula placements were evaluated after each experiment by dye injection. Only rats with proper placements were included in the data analysis.

GW6471 and SR141716A were administered i.p. 30 min previously to food presentation in a volume of 1 mL/kg at a dose of 3 and 0.3–3 mg/kg respectively.

Pre-injection times were based upon previous studies with these feeding paradigms and cannabinoid drugs after their pharmacological characterization (Cani et al., 2004; Cippitelli et al., 2007; Chambers et al., 2004; Gomez et al., 2002; Pavon et al., 2006).
2.5. Food intake studies

The effects of these drugs on feeding behavior were analyzed in rats deprived of food for 24 h or in partially satiated animals (24 h food-deprived rats/mice allowed to eat for 60 min before drug testing) (Gomez et al., 2002; Williams and Kirkham, 1999).

To habituate the animals, 72 h before testing with drugs, animals were food-deprived for 24 h with ad libitum access to water. Then, the bedding material was removed from the cage and a small can containing food pellets was placed inside the cage for 4 h. When this initial test was finished, the rats were under a free-feeding period of 48 h. After this time, the animals were definitively food-deprived for 24 h with free access to water for food intake studies.

For partial satiation, 24 h fasted animals were allowed to eat from the can for 60 min and then, it was removed 60 min before food presentation.

In both cases, drugs were administered 30 min (i.p.), 15 min (i.p.) or 5 min (i.c.v.) before beginning the studies and therefore the food exposure. Animals were immediately returned to their home cage with no bedding material. Finally, a can with a measured amount of food (usually 30–40 g) and a bottle containing 250 mL of fresh water were placed in time 0. Food pellets and food spillage were weighed at (15, 30) 60, 120 and 240 min or only at 60 min (for i.c.v. administrations), and the amount of water consumed was also measured.

2.6. Statistical analysis

All data for graphs are expressed as the mean ± SEM. The different experiments included 8–10 animals per treatment according to the assay. Statistical analysis of results was performed using one- and two-way analysis of variance (ANOVA) followed by a post hoc test for multiple comparisons (Bonferroni post test). Specifically, for food intake studies at different times, a two-way ANOVA was used with time and treatment as factors to detect differences in cumulative food intake. One way ANOVA was used to detect differences in the rest of feeding experiments at 60 min. A p-value below 0.05 was considered statistically significant as compared to vehicle groups. All analysis was carried out by the computer program GraphPad Prism version 5.04 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Effects on food intake of AM404 administered peripherally

It is previously reported that i.p. administration of AEA had no effect in food-deprived rats. However, AEA elicited a prolonged and dose-dependent hyperphagia when it was administered in partially satiated rats (Gomez et al., 2002). In order to investigate the effect of AM404 in starvation and partial satiety, we injected different doses i.p. and registered the food consumed at three different times. Additionally, the amount of food eaten during 1 h in partially satiated rats was 4.57 ± 0.21 g (14.89 ± 0.83 g/kg).

As shown in Fig. 1A, a two-way ANOVA revealed that treatments had no effects on cumulative food intake (F9, 86 = 0.47; n.s.) in 24 h fasted rats with no interaction with time (F9, 86 = 0.55; n.s.). As expected, time affected the results significantly, increasing the cumulative food intake (F2, 86 = 44.85; p < 0.0001). However, peripheral treatments with AM404 in partially satiated rats (Fig. 1B) exhibited significant effects on food intake when the data were analyzed (F9, 91 = 8.95; p < 0.0001), but with no interaction between treatment and time (F9, 91 = 0.17; n.s.). Time resulted a factor significant on these results again (F2, 91 = 26.11; p < 0.0001). Therefore, when we compared cumulative food intake between AM404 and vehicle, we observed a dose-dependent inhibitory effect on feeding resulting in a significant decrease with 2 mg/kg (*p < 0.05, at 120 min) and 10 mg/kg of AM404 at all time tested (**p < 0.01 at 60 min; *p < 0.05 at 120 and 240 min) compared to vehicle-treated group. At dose of 0.4 mg/kg, a decrease on food intake was also observed although this dose never resulted significant. After 240 min the relative amount of food eaten by vehicle-treated rats was reduced by approximately 30% when partially satiety rats were treated with 2 and 10 mg/kg of AM404.

3.2. Effects on food intake of systemic AM404 after previous administration of GW6471

In order to know whether OEA via PPARα was involved in the anorectic properties displayed by AM404 in partially satiated rats, we injected a PPARα antagonist (GW6471, 3 mg/kg; i.p.) 15 min before an effective dose of AM404 (10 mg/kg; i.p.) (Fig. 2). The amount of food eaten during 1 h in these pre-satiated rats was 4.84 ± 0.60 g (15.63 ± 1.89 g/kg) before beginning the feeding studies.

A two-way ANOVA detected significant differences in partially satiated rats by treatment on cumulative food intake (F6, 84 = 10.64; p < 0.0001) with no interaction with time (F6, 84 = 1.52; n.s.). Time affected the results significantly, increasing the cumulative food intake (F2, 84 = 13.57; p < 0.0001) once again.

As previously described, an effective dose of AM404 (10 mg/kg; i.p.) in partially satiated rats induced a significant decrease in cumulative food intake when we compared to vehicle-treated group (**p < 0.001 at 60 min; *p < 0.05 at 120 min). However, GW6471 (3 mg/kg; i.p.) did not produce any significant effect on feeding, although an upward trend was observed at all time tested. With respect to the combined treatment with same doses of GW6471 and AM404,
there was a significant decrease in food intake versus vehicle group (**p < 0.001 at 60 min). GW6471 was not able to block the anorectic effect caused by AM404 during 120 min, although at 240 min this effect was not observed.

3.3. Effects on food intake of systemic AM404 in both PPARα-null and wild-type mice

Complementary to the previous feeding study in rats, we investigated whether the peripheral effect of AM404 in satiation could influence feeding through PPARα in a genetic model such as PPARα-null mice. To this end, the anorectic actions of AM404 were evaluated in partially satiated PPARα-null mice and wild-type control mice. The amounts of food eaten during 1 h after fasting in both partially satiated mice were: 0.65 ± 0.04 g (24.00 ± 1.32 g/kg), PPARα-null mice; and 0.58 ± 0.04 g (23.40 ± 1.39 g/kg) wild-type mice.

Different doses of AM404 (0.3, 3 and 30 mg/kg; i.p.) were tested on feeding behavior at different times during 240 min as depicted in Fig. 3. We detected no differences between both genotypes, and AM404 caused a dose-dependent decrease in food intake in partially satiated wild-type and PPARα-null mice.

A two-way ANOVA showed significant differences between treatments on cumulative food intake (F3, 140 = 45.83; p < 0.0001) in partially satiated wild-type mice. A significant interaction between treatment and time was detected (F12, 140 = 2.23; p = 0.0132), together with the significant increase in food consumption caused by time factor (F4, 140 = 147.56; p < 0.0001). Therefore, AM404-treated mice displayed a significant decrease in cumulative food intake with 30 mg/kg (**p < 0.01 at 15 min; ***p < 0.001 at 30, 60, 120 and 240 min) and 3 mg/kg (**p < 0.01 at 30 min; ***p < 0.001 at 60 min), whereas 0.3 mg/kg did not induce any significant effect compared to vehicle group (Fig. 3A).

In partially satiated PPARα-null mice, a dose-dependent effect was also observed and another two-way ANOVA detected that treatments caused significant effects on food intake (F3, 120 = 20.42; p < 0.0001) with no interaction between treatment and time (F12, 120 = 1.99; n.s.). As expected, time affected extremely these results (F4, 120 = 81.34; p < 0.0001). In this case, AM404-treated PPARα-null mice displayed a significant decrease in food consumption with 30 mg/kg (**p < 0.01 at 15 min; **p < 0.05 at 30 min; ***p < 0.001 at 120 min; ***p < 0.001 at 240 min) and 3 mg/kg (**p < 0.05 at 15 min), whereas 0.3 mg/kg did not induce any significant effect compared to vehicle group (Fig. 3B).

These data indicated that AM404 elicited an appetite-suppressing effect in partially satiated rodents through a PPARα-independent mechanism.

3.4. Effects on food intake of AM404 administered centrally

Central administration of cannabinoid agonists such as AEA has been reported to affect feeding behavior in rats, and even producing opposing effects on appetite at high doses (10 μg) according to experimental conditions (Gomez et al., 2002; Soria-Gomez et al., 2007). We tested different doses of AM404 (0.4, 2 and 10 μg) infused centrally on feeding to investigate the impact of this drug under starvation and satiety again.

One-way ANOVA revealed significant differences between treatments (F3, 28 = 3.11; p = 0.0053) on food intake in 24 h food-deprived rats (Fig. 4A). In fact, the highest dose of AM404 (10 μg) resulted in a significant increase in feeding behavior as compared to vehicle-treated group (**p < 0.01). After 60 min the relative amount of food ingested by vehicle-treated rats was increased by approximately 25% when these fasted rats were treated with 10 μg of AM404. However, the rest of doses tested showed no effects.

In contrast, AM404 exhibited no differences between treatments (F3, 36 = 0.18; n.s.) in partially satiated animals including 10 μg of AM404 as illustrated in Fig. 4B. In this case, the amount of food eaten during 1 h in pre-satiated rats was 4.93 ± 0.52 g (16.14 ± 1.62 g/kg) in order to induce partial satiation.

3.5. Effects on food intake of central AM404 after previous administration of SR141716A

Since peripheral injections of cannabinoid CB1 receptor blockers have been extensively reported to be sufficient to suppress appetite...
With vehicle-treated rats. The pharmacological increase of AEA induced by i.p. AM404 since we detected no changes on feeding behavior using three different doses. Probably, these elevated levels of intestinal AEA might overlap the pharmacological increase of AEA induced by i.p. AM404 since we detected no changes on feeding behavior using three different doses.

### Discussion

Endocannabinoids have a critical role in appetite and body weight regulation via global CB1 receptors that are abundantly expressed in many peripheral tissues involved in metabolic actions and cerebral areas related to energy balance and consummatory behavior. Therefore, such metabolic control involves a bidirectional communication between peripheral tissues and brain via afferent or efferent terminals with a prominent expression of ECS in these neural terminals, which permits a balanced regulation by inducing or suppressing appetite (Kunos and Tam, 2011). In the present study we show that the route of administration of the AEA uptake inhibitor AM404 affects its capability of modulation of feeding behavior, supporting the notion of a differential role for central and peripheral CB1 receptors in the control of appetite. Additionally, feeding status (food deprivation versus partial satiety) is a determinant factor of AM404 actions on food intake.

We report that a peripheral administration of AM404 elicits a different effect on food ingestion between food deprivation and partial satiation. While AM404 induced no effects on food intake in food-restricted rats, this drug produced appetite-suppressing effects in a dose-dependent manner. After 24 h starvation it has been reported in rats a huge accumulation of AEA (7-fold increase) in the small intestine in comparison with free-feeding animals which was reversed on re-feeding (Gomez et al., 2002). Probably, these elevated levels of intestinal AEA might overlap the pharmacological increase of AEA induced by i.p. AM404 since we detected no changes on feeding behavior using three different doses.
In another study, it has been reported that systemic administration of AM404 (10 mg/kg) caused a gradual increase of AEA in (2-fold increase) rat plasma, although the authors showed that the concentration reached by AEA in plasma (~10 nM) might be insufficient to activate cannabinoid CB1 receptors (Kd = 50 nM for CB1) (Giraffada et al., 2000; Pertwee, 1997). These observations could explain the lack of appetite-stimulating effects in our results.

However, AM404 induced a surprising dose-dependent decrease on feeding under partial satiation. Potential explanations for this action may include motor impairment induced by AM404, or the induction of anorexia through AEA-independent mechanisms. This later option is based on a potential increase on intestinal levels of OEA mediated by AM404 (Giraffada et al., 2000). OEA displays opposing actions in the peripheral control of food intake (Gomez et al., 2002; Rodriguez de Fonseca et al., 2001) and, while OEA levels are reduced in the small intestine during starvation, they increase immediately upon re-feeding (Fu et al., 2003; Rodriguez de Fonseca et al., 2001). Our data suggest that the reduction on feeding observed in partially satiated might be caused by a net promotion of OEA compared to AEA. In agreement with these interpretations, it has been described that orexigenic effects induced by exogenous administration of AEA (10 mg/kg; i.p.) in partially satiated rats are totally abolished by OEA (5 mg/kg; i.p.) resulting in anorectic effects and, therefore OEA actions prevail over AEA (Gomez et al., 2002). The accumulation of OEA due to AM404 might be possible because AM404 not only inhibits AEA transport (Beltramo et al., 1997) but also that of OEA, as revealed by the elevation of plasma levels of both NAEs found after peripheral administration of AM404 (Giraffada et al., 2000). We have further explored this hypothesis by investigating the role of PPARα receptors, a known target of OEA, on anorexia induced by AM404. To this end we used a PPARα antagonist that was administered to partially-satiated rats as well as PPARα-null mice which were used to reproduce the same experimental conditions in partial satiation. Although the dose-dependent decrease on food intake after AM404 administration was maintained in both rodent models, it was found to be independent of the functional presence of PPARα. Therefore, it can be proposed that if OEA is responsible of these anorectic actions, it might induce its appetite-suppressing effects through of PPARα-independent targets such as GPR55, GPR119, and TRPV1. Additionally it is important emphasize that AM404 can produce some of its effects directly. AM404 is able to bind to other different targets, including vanilloid TRPV1, receptors (Zygmunt et al., 2000). This co-activation of the cannabinoid receptors and TRPV1, often complicates the distinction between both pathways and will be considered in future studies.

Besides peripheral actions of AM404, we evaluated its effects on feeding behavior after its central administration. Several anatomical locations in hypothalamus, limbic forebrain and lower brainstem have been implicated in the orexigenic properties of endocannabinoids (Kunos and Tam, 2011). Thus, AEA level has been described to rise in the limbic forebrain in food-deprived animals, while it was not affected in other nutritional conditions such as free-feeding or satiation (Kirkham et al., 2002). Following this rationale, central infusion of drugs capable to inhibit endocannabinoid clearance such as the transport inhibitor OMDM-1 stimulates food intake in free-feeding rats (Soria-Gomez et al., 2007). In our experimental design, AM404 stimulated food intake after central infusions about 30%, and this finding is in accordance with this previous study. However, although we detected this hyperphagic effect in fasted rats, no effects were observed in partially-satiated rats. Thus, feeding status is also essential for the observation of AM404 effect. As it has been extensively reported, endocannabinoid levels are elevated in brain as well as in peripheral tissues after food-deprivation and, consequently high concentration of AM404 could be necessary to promote or prolong orexigenic actions of AEA through an inhibition of its cerebral reuptake.

By contrary, we observed no changes on food intake after central administration of the same dose of AM404 in partially-satiated rats. A possible explanation might be due to low levels of AEA reported during satiation (Kirkham et al., 2002) compared to free-feeding and starvation, AM404 could be unable to rise extracellular AEA concentrations by means of inhibiting its cellular transport with a predominance of other signals related to satiety such as OEA (Rodriguez de Fonseca et al., 2001).

In order to investigate whether this appetite-stimulating effect in 24 h food-deprived rats after AM404 infusion was CB1-mediated, a selective CB1 antagonist was peripherally given. While anorectic properties have been extensively observed when CB1 blockers (SR141716A, AM251, LH-21) are given peripherally, central infusion of these drugs has been described to be inactive in rodents (Gomez et al., 2002; Kirkham et al., 2002; Pavon et al., 2008; Soria-Gomez et al., 2007). Our data also showed an anorectic effect by systemic administration (i.p.) of high dose of SR141716A.

Interestingly, the increase of food intake observed with central AM404 in fasted rats was totally counteracted after a combined treatment with SR141716A, even using a subthreshold dose of this selective inverse agonist that avoids its intrinsic anorexigenic actions. Both CB1 blockade and activation by these drugs could balance the endocannabinoid tone, which restores feeding behavior to rats under caloric restriction.

In conclusion, AM404 displays a different profile on food intake depending on its route of administration and feeding status. We suggest that AM404 can reduce food intake acting peripherally after a partial meal, probably through an increase of OEA. However, it can also increase feeding behavior through AEA when its cerebral content is elevated in fasted subjects, supporting previous descriptions of AEA-dependent initiation of feeding (Jamshidi and Taylor, 2001). This particular pharmacological profile limits the potential use of anandamide uptake blockers for the treatment of appetite disorders.

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