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Additive effects of cannabinoid CB1 receptors blockade and cholecystokinin on feeding inhibition

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ABSTRACT

Cannabinoid CB1 receptor and cholecystokinin-1 (CCK_1) receptors are located in peripheral nerve terminals of the gut, where they mediate satiety signals. Here we describe a detailed analysis of the interaction of both receptors in the control of feeding of food-deprived rats. Male Wistar rats were deprived for food 24 h before testing. Rats were pre-treated with SR141716A (Rimonabant) or WIN 55,212-2 before CCK-8 sulphated administration and tested for food intake 60, 120 and 240 min after last drug injection. In parallel, the effect of Lorglumide – a CCK_1 receptor antagonist – pre-treatment was evaluated on feeding behaviour after SR141716A administration. Results show that SR141716A activates c-Fos expression in brainstem areas receiving vagal inputs. Blockade of CB1 receptors with SR141716A (1 mg/kg) reduces feeding and display additive satiety induction with the CCK_1 receptor agonist CCK-8 sulphated (5, 10, 25 μg/kg). The effect of SR141716A is not blocked by Lorglumide (10 mg/kg), indicating independent sites of action. Conversely, the administration of the CB1 agonist WIN 55,212-2 (2 mg/kg) reduced satiety induced by CCK-8. In conclusion, these results report additive anorectic actions for CCK1 activation and peripheral CB1 receptor blockade providing a framework for combined therapies in the treatment of eating disorders.

1. Introduction

It is presently well documented that the endocannabinoid system is a potent modulator of feeding behaviour. Both Δ9-tetrahydrocannabinol (THC, the psychoactive constituent of *Cannabis sativa*) and the endogenous cannabinoid anandamide (AEA) promote overeating in partially satiated rats by interaction with central cannabinoid CB1 receptors (Williams et al., 1998; Williams and Kirkham, 1999), and it is known that THC stimulates appetite also in humans (Berry and Mechoulam, 2002). Antagonism of CB1 receptors by SR141716A decreases food intake and counteracts the hyperphagia promoted by CB1 receptor activation (Arnone et al., 1997; Kirkham and Williams, 2001; Rowland et al., 2001). Despite the existence of central mechanism for the regulation of food intake by endocannabinoids (Williams and Kirkham, 1999; Jamshidi and Taylor, 2001) our laboratory has identified peripheral mechanisms responsible of their effects on appetite (Gomez et al., 2002). Anandamide and agonism of CB1 receptors by WIN55,212-2 promote hyperphagia through a peripheral mechanism, whereas CB1 receptor blockade suppresses feeding by acting on CB1 receptors located on capsaicin-sensitive sensory terminals innervating the gastrointestinal tract rather than after central administration (Gomez et al., 2002).

In the periphery, regulation of food intake is modulated by both stimulatory (orexigenic) and inhibitory (satiety) factors that stimulate nerve terminals innervating the gastrointestinal tract. The satiety hormone cholecistokinin (CCK) is a potent inhibitor of feeding that exerts its actions by binding to CCK_1 receptors located in vagal afferent neurons (Smith et al., 1981; Ritter and Ladenheim, 1985; Moran et al., 1990; McLaughlin et al., 1999; Broberger et al., 2001; Reidelberger et al., 2003, 2004). It is generally assumed that CCK_1 receptors are mainly distributed in the periphery and are responsible for the anorectic effects whereas a second-type receptor, the CCK_2 receptor, is widely localized in the brain mediating anxiety-related behaviours. There are also several molecular CCK forms in the brain and peripheral organs. The CCK_1 receptor exerts a relatively specific affinity for the biologically active CCK c-terminal octapeptide (CCK-8). Indeed, administration of the CCK-8 peripherally decreases meal size by a mechanism that requires the integrity of the vagus nerve (Smith et al., 1981).

A previous study has revealed that both, CCK_1 receptor and cannbinoid CB1 receptors are located in peripheral terminals of nodose ganglion (Burdyga et al., 2004). However, a detailed behavioural analysis of CB1-CCK_1 receptor interactions is still not available. Since both endocannabinoids and CCK appear to exert a role mediating food consumption by interaction with peripheral CB1 and CCK_1 receptors

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respectively, the goal of our study was to determine in vivo the interaction between CB1 receptors and CCK in the mediation of satiety. We used food-deprived rats for analyzing the satiety-inducing properties of CB1 antagonism (Gomez et al., 2002) and partially-satiated rats as a known model of cannabinoid CB1 receptor agonist-induced overfeeding (Williams et al., 1998; Williams and Kirkham, 1999). The induction of c-Fos expression by systemic administration of SR141716A was used as a correlate of the pharmacological interaction. We show here that SR141716A activates c-Fos expression in brainstem areas receiving vagal inputs. SR141716A has additive effects on CCK-induced feeding suppression and pretreatment with lorglumide did not modify the anorectic effects of CB1 antagonism. Conversely, cannabinoid CB1 agonism counteracts the feeding suppression induced by CCK in vivo. Our results suggest that CB1 antagonism inhibits feeding behaviour by peripheral mechanisms that do not involve CCK_1 receptors.

2. Methods

2.1. Animals and general protocol used

Male wistar rats (350 ± 50 g, Harlan Iberica, Barcelona, Spain) were housed individually in conditions of constant temperature (21 ± 2 °C) and a 12 h light/dark cycle (lights on: 07:00 am) with food and water available ad libitum except when restriction was required. Animals were deprived for food (but not water) during 24 h before testing the drugs and habituated to handling. Partially satiated animals were food-deprived for 24 h and allowed to eat for 60 min before drug testing. Animals were returned to their home cage containing now a can with a measured amount of food (30–40 g) 15 min after drug administration. Food pellets and food spillage were weighted at 60, 120 and 240 min after starting the test, and the amount of food was recorded.

Both the maintenance of the animals as well as the experimental procedures were in accordance with the European Communities Council Directive (86/609/EEC).

2.2. Drug administration

WIN 55,212-2 (Tocris) was dissolved in a mixed vehicle (ethanol/tween 80/saline, 1:1:18 vol/vol) and administered i.p. in a volume of 1 ml/kg. SR141716A (Rimonabant) was generously donated by Sanofi Research (Montpellier, France); it was dissolved in a mixed vehicle (ethanol/tween 80/saline, 1:1:18) and injected i.p. in a volume of 1 ml/kg. Pre-treatment time was 30 min. CCK-8 sulphated (Tocris) was dissolved in saline and administered i.p. in a volume of 1 ml/kg. Lorglumide (Tocris) was dissolved in the mixed (ethanol/tween 80/saline) vehicle and injected i.p. in a volume of 10 mg/kg 30 min before SR141716A treatment. Treatments and doses used were published elsewhere (Gomez et al., 2002; Ritter and Ladenheim, 1985).

2.3. c-Fos immunostaining

In order to explore if brain nuclei related to peripheral sensory system or hypothalamic appetite control displayed c-Fos expression after cannabinoid CB1 receptor antagonist treatment, we reanalyzed the quantitative data on the study previously published by our group (Rodriguez de Fonseca et al., 1997). Briefly, animals were handled for a week, injected once a day i.p. with sterile 0.9% saline along three consecutive days and then treated with vehicle or SR141716A (0.3–3.0 mg/kg, i.p.) Three hours after cannabinoid antagonist treatment, rats were quickly perfused with 0.9% saline followed by 2% paraformaldehyde in isotonic sodium phosphate buffer (PBS, pH 7.4). Brains were removed, fixed in the perfusion buffer for 24 h, stored for 3 to 7 days in a 30% solution of sucrose in PBS, sliced in 40-μm sections (Cryocut 1800; Leica, Foster City, CA), and collected in PBS. The Fos protein was quantified by immunohistochemistry analysis with affinity-purified rabbit antibodies to a peptide corresponding to human Fos amino acid residues 3 to 16 (Santa Cruz Biotechnology, Santa Cruz, CA) that was not reactive to Fos-B and Fra-1 proteins. Sections were incubated with goat antiserum to rabbit antibody in 0.3% Triton X-100 in PBS solution for 2 h at room temperature, followed by Fos antiserum (diluted 1:1000) in 0.3% Triton X-100 containing 0.1% bovine serum albumin in PBS for 20 h at 4 °C. Cell counting was performed manually at 100× magnification and assigned to brain structures accordingly to the Atlas of Paxinos and Watson (1986). Questionable cells were checked for nuclear localization of staining using magnification of 400×. The animals and the slides were coded so that the person counting the cells was unaware of the animal´s treatment. For each brain region from one individual animal the maximum number of Fos-positive cells per slice was used as a single data point and analyzed using ANOVA. Details on the method have been previously published (Rodriguez de Fonseca et al., 1997).

2.4. Data analysis and statistical procedures

Data from food intake studies were analyzed by two-way ANOVA using a between subject design, followed by Bonferroni post-test when a significant F-value was found. Data from c-Fos expression were analyzed by one-way ANOVA followed by Newman–Keuls multiple comparison test when a significant F-value was obtained. All statistic analyses were performed using GraphPad Prism (version 5.0) software.

3. Results

3.1. c-Fos expression in the brain after systemic SR141716A treatment

The quantitative cell counts of the 1997 experiments (Rodriguez de Fonseca et al., 1997) were used to map c-Fos levels after systemic administration of SR141716A, in an attempt of identifying brain pathways engaged during SR141716a-evoked hypophagia. Fig. 1 shows the pattern of c-Fos expression in brain nuclei related to either peripheral sensory system or hypothalamic circuitry 3 h after i.p. SR141716A administration. C-Fos expression was upregulated in the area postrema, paraventricular nucleus of the hypothalamus, nucleus of the solitary tract and central nucleus of the amygdala 60 min post-injection. The effect was more evident with low doses of SR141716A (0.3 mg/kg) and was attenuated after a higher dose of 3.0 mg/kg.

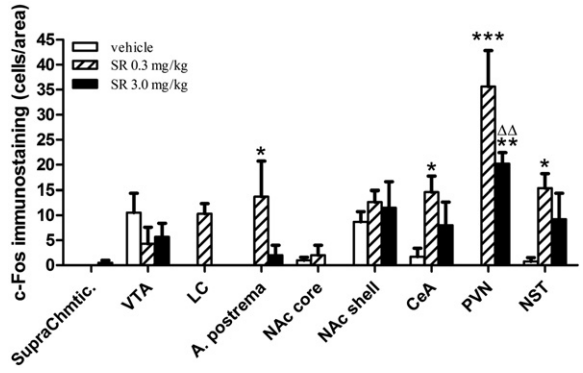

Fig. 1. c-Fos immunostaining in the brain after SR141716A intraperitoneal treatment. There is a marked increase in c-Fos expression after SR141716A (0.3-3.0 mg/kg, i.p.) treatment in areas related with the peripheral nervous system as the area postrema, nucleus paraventricular and nucleus of the solitary tract. There is also a significant induction of c-Fos in central nucleus of the amygdala. The c-Fos expression is dependent of the SR141716A dose. Different from vehicle-injected animals: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$. Different from SR141716 treatment: $^{\Delta\Delta}p<0.01$. One-way ANOVA followed by Newman–Keuls test. SCN: suprachiasmatic nucleus; VTA: ventral tegmental area; LC: locus coeruleus; A. postrema: area postrema; NAc core: nucleus accumbens core; NAc shell: nucleus accumbens shell; CeA: central nucleus of the amygdala; PVN: paraventricular nucleus; NST: nucleus of the solitary tract.

3.2. Interaction between CCK-8 and SR141716A in feeding behaviour

Fig. 2 represents the interaction between CCK-8 and SR141716A at different CCK-8 doses. Note that a, b and c panels are results from the same experiment. Results have been graphed in three panels to make easier the visualization of results for the reader. Two-way ANOVA found an interaction between curves ($F_{14,192}=2.53$; $p=0.0025$) and main effects of treatment ($F_{7,192}=43.95$; $p<0.0001$) and time ($F_{7,192}=195.1$; $p<0.0001$). Post-hoc analysis revealed that SR141716A reduced food intake compared with vehicle-injected animals and there were also reductions in food intake after CCK-8 treatment at the doses of 10 and 25 μg/kg (Fig. 2B,C, central and right panels respectively). Administration of SR141716A (30 min pre-treatment time) and CCK-8 has an additive effect in the suppression of food intake observed after 5, 10 and 25 μg/kg of CCK-8 (Fig. 2B,C; central and right panels) and SR141716A alone (Fig. 2A,B,C; left, central and right panels).

3.3. Effect of pretreatment with Lorglumide, a CCK_1 receptor antagonist, on feeding behaviour after SR141716A administration

Statistical analysis for data in Fig. 3A (upper panel) revealed that there was an effect of treatment ($F_{3,96}=12.12$; $p<0.0001$) and time ($F_{2,96}=16.30$; $p<0.0001$). SR141716A treatment reduced feeding 60 min, 120 min and 240 min post-injection (Fig. 3A) but there was no significant effect with the pre-treatment lorglumide at any time. Specific analysis of the data at the chosen time of 60 min (Fig. 3B, bottom panel) revealed that SR141716A reduces food intake in vehicle-injected animals and there was no lorglumide pre-treatment effect ($F_{1,32}=0.54$; $p=0.47$).

3.4. Interaction between CCK-8 and WIN 55,212-2 in feeding behaviour

Two-way ANOVA found a general effect of treatment ($F_{7,284}=25.82$; $p<0.0001$) and time ($F_{3,284}=83.89$; $p<0.0001$) in partially satiated animals (Fig. 4, upper panels). There were also a general effect of treatment ($F_{7,288}=24.67$; $p<0.0001$) and time ($F_{3,288}=75.31$; $p<0.0001$) in the curves of full deprived animals (Fig. 4, bottom panels). Post-hoc analysis revealed that CCK-8 treatment reduced food intake in partially satiated animals at dose of 100 μg/kg during 60, 120, 180 and 240 min post-treatment (Fig. 4C; upper right panel) and in full food-deprived animals at doses of 25 μg/kg during 60, 180 and 240 min post-treatment (Fig. 4E, bottom central panel) and at CCK-8 dose of 100 μg/kg during all times post-treatment tested (Fig. 4F, bottom right panel). Pre-treatment with the cannabinoid CB1 receptor agonist WIN 55,212-2 (30 min before)

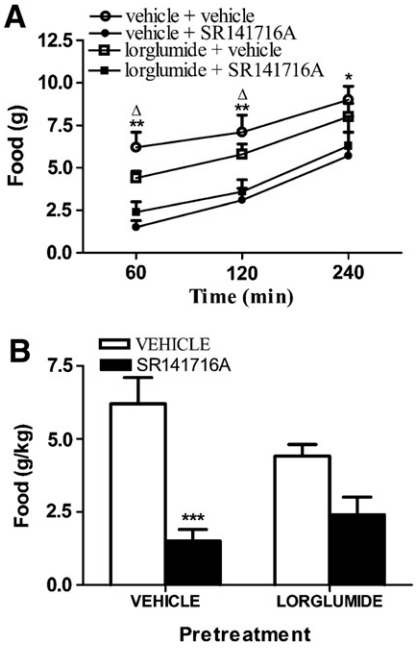

Fig. 3. Effect of CCK_1 receptor antagonist Lorglumide pre-treatment on the SR141716A-induced modulation of feeding behaviour. A) Effect of pre-treatment with lorglumide (10 mg/kg, i.p., 30 min pre-treatment time) on feeding behaviour after SR141716A along the time; B) Effect of lorglumide (10 mg/kg, i.p., 30 min pre-treatment time) on feeding 60 min after vehicle or SR141716A treatment. The CB1 receptor antagonist SR141716A (3 mg/kg, i.p.) decreases food intake in deprived animals 60 min, 120 min and 240 min after injection compared with vehicle-injected animals: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.01$. Different between vehicle-injected animals and lorglumide + SR141716A treatment: $^{\Delta}p<0.05$. Two-way ANOVA followed by Bonferroni post-test. There is no effect of pre-treatment with lorglumide at any time [i.e., $F(1, 32)=0.54$; $p>0.05$, n.s., 60 min post-treatment, one-way ANOVA followed by Newman–Keuls test]. Results shown as means ± SEM, $n=9$.

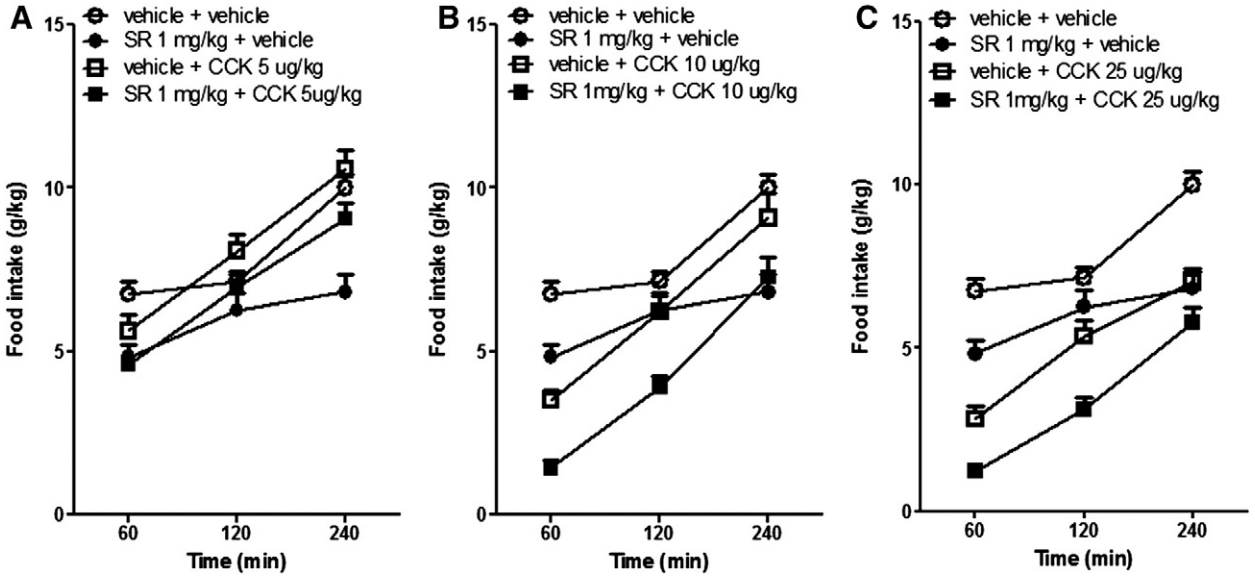

Fig. 2. Pre-treatment with SR141716A enhances feeding suppression induced by CCK-8.Administration of the CB1 receptor antagonist SR141716A (1 mg/kg, i.p., 30 min before CCK-8) induces feeding suppression in deprived animals and enhances satiety induced by CCK-8 (5, 10 and 25 μg/kg, i.p.). Results in a, b and c panels are part of the same experiment. Two-way ANOVA found an interaction and main effects of treatment and time at all doses of CCK-8. Results shown as means ± SEM, $n=9$.

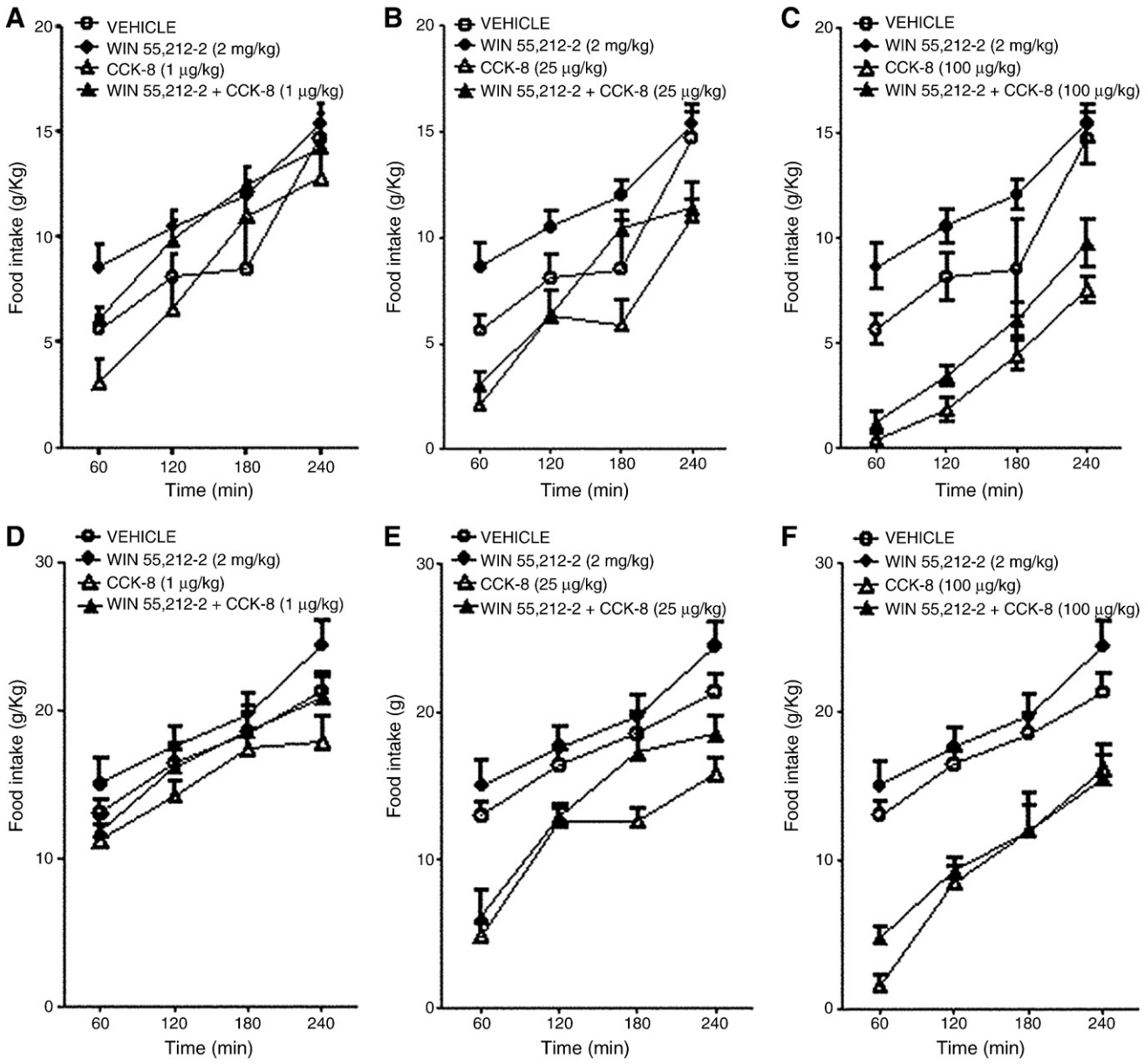

Fig. 4. Pre-treatment with WIN 55,212-2 reduces satiety induced by CCK-8.The cannabinoid CB1 receptor agonist WIN 55,212-2 (2 mg/kg, i.p., 30 min before CCK-8) counteracts feeding suppression induced by CCK-8 (1, 25 and 100 μg/kg, i.p.) in partially satiated (upper pannels) and deprived (lower panels) animals 60 min, 120 min and 240 min post-injection. Results in A, B, and C upper panels conform one experiment (partially satiated animals) and results in D, E and F lower panels conform other experiement (full-deprived animals). Two-way ANOVA found an effect of treatment and time at every dose of CCK-8 in both partially satiated and deprived conditions. Results shown as means ± SEM, $n = 10$.

partially counteracted the feeding suppression induced by CCK-8 at 25 μg/kg (180 min).

The general impression in the curves is that pre-treatment with CB1 agonist partially counteract the feeding suppression induced by CCK-8. The effect appears at early times and is overcome by elevated doses of CCK-8.

3.5. Interaction between SR141716A or WIN 55,212-2 and different doses of CCK-8 on feeding behaviour 60 min post-treatment

Fig. 5A (left panel) shows the interaction between WIN 55,212-2 and different doses of CCK-8 at one time chosen (60 min) for partially satiated animals. The analysis of the curves by two-way ANOVA indicated that there was a significant effect of WIN 55,212-2 pre-treatment ($F_{1,72} = 12{,}90$, $p < 0.0006$) and a significant effect of CCK-8 dose ($F_{3,72} = 26.43$ $p < 0.0001$). Post-hoc analysis revealed that WIN 55,212-2 increases food intake in vehicle-injected animals and in animals treated at the lowest dose of CCK-8 (1 μg/kg). At higher doses of CCK-8 (25 and 100 μg/kg) the effect of WIN 55,212-2 is no longer significant.

Fig. 5B (right panel) represents the interaction between SR141716A and different doses of CCK-8 60 min post-treatment. Two-way ANOVA found a main effect of SR141716A pre-treatment ($F_{1,64} = 48.08$; $p < 0.0001$) and a main effect of CCK-8 dose ($F_{3,64} = 63.48$; $p < 0.0001$). Post-hoc analysis revealed that pre-treatment with SR141716A reduces food intake compared with-vehicle injected animals in control conditions and there are also significant reductions with medium and high CCK-8 doses. The curve patterns for vehicle- and SR141716A-injected animals are similar indicating that both compounds have additive effects (rather than synergic effects).

4. Discussion

The present results show that: 1) systemic administration of cannabinoid CB1 antagonist activate c-Fos expression in brainstem

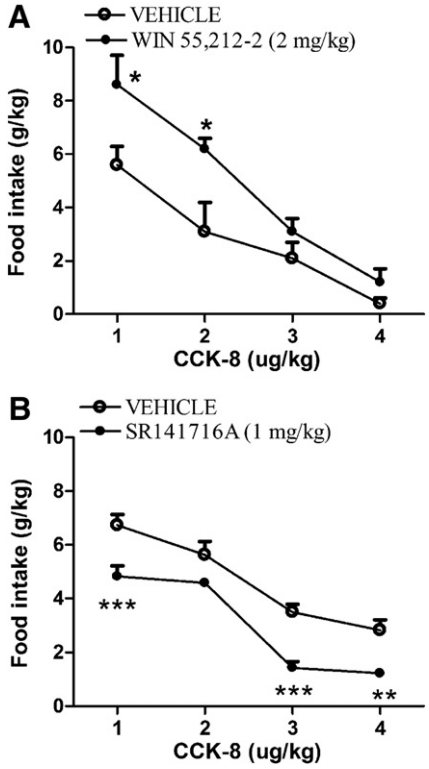

Fig. 5. Effect of pre-treatment with WIN 55,212-2 or SR141716A on the modulation of feeding behaviour induced by CCK-8 60 min post-treatment.WIN 55,212-2 (2 mg/kg, i.p.) or SR141716A (1 mg/kg, i.p.) were administered 30 min before CCK-8 injection (1-100 μg/kg, i.p.) and animals were tested for food intake 60 min post-treatment. WIN 55,212-2 counteracts feeding suppression induced by CCK-8, whereas the effects of SR141716A and CCK-8 are additive in the suppression of food intake. Results shown as means ± SEM, $n = 9$-10. Different from vehicle-injected animals: $^{*}p < 0.05$, $^{**}p < 0.01$; $^{***}p < 0.001$. Two-way ANOVA followed by Bonferroni post-test.

areas receiving vagal inputs; 2) systemically administered cannabinoid CB1 antagonists and CCK have additive effects as feeding suppressors; 3) the actions of CB1 antagonist on food consumption are not dependent on CCK_1 receptors; 4) the effects of cannabinoid CB1 agonists counteract those of CCK, a major feeding inhibitor in the gut.

Previous investigations in our laboratory allowed the identification of peripheral mechanisms for the endogenous cannabinoid anandamide and its natural analogue oleyletahnolamide (which does not bind CB1 receptors) in the regulation of feeding (Gomez et al., 2002; Rodriguez de Fonseca et al., 2001). While anandamide promotes overfeeding, oleylethanolamide induce satiety and pre-treatment with the CB1 antagonist SR141716A enhances the inhibition of feeding induced by intraperitoneal administration of oleyletahnolamide. Our present study reproduces the satiety effect of CB1 receptor blockade after intraperitoneal administration of SR141716A observed previously (Gomez et al., 2002). It is known that food intake has a peripheral control system based on highly complex interactions in the gut-brain axis. Peripheral inputs related to ingestive behaviour recruit CNS structures localized in the brainstem and hypothalamus (Schwartz, 2000; Schwartz et al., 2000). Here, to identify brain pathways engaged during SR141716A-evoked hypophagia, we mapped protein levels for the activity regulated gene c-Fos by immunohistochemistry after systemic administration of 0.3 mg/kg or 3.0 mg/kg SR141716A. The low dose of SR141716A evoked a highly localized increase in c-Fos levels in the PVN, area postrema, NST and central nucleus of the amygdala, compared with controls. The enhancement of c-Fos expression observed in the NST and area postrema indicates activation of the peripheral nervous system since both brainstem areas receive vagal inputs (Schwartz et al., 2000). Additionally, the PVN is a hypothalamic nucleus involved in feeding control where the coordination of catabolic signals in the SNC takes place (Schwartz et al., 2000). Similarly, it is widely accepted that the anorectic effect of CCK is mediated by the vagus nerve involving brainstem areas which receive vagal inputs, such as the NST and area postrema, as well as hypothalamic nuclei (Cano et al., 2003; Crawley and Corwin, 1994; Day et al., 1994). It is to note that others have shown a reduction or an increase on food intake after central administration of SR141716A (Verty et al., 2004) or CP55940 (Miller et al., 2004). The net central contribution of the endogenous cannabinoid system is of complex evaluation because it has been recently described that there are cannabinoid-mediated pathways for both, feeding stimulation and feeding inhibition, mediated by excitatory glutamatergic pathways or inhibitory GABAergic neurons (Bellocchio et al., 2010). Thus, systemic administration of a cannabinoid agonist/antagonist may have a net central effect masked by the equilibrium in between stimulatory or inhibitory actions. Although our results indicates a major peripheral component for cannabinoid modulation of feeding, the existence of a central component cannot be discarded and at least there is a report (Madsen et al., 2009) showing that SR141716A suppressed feeding when given at a high dose (10 mg/kg) in surgically vagotomized animals. Here, we observed also a dose-dependency on brain CB1 blockade since SR141716A effects on c-Fos expression were more evident with low doses of the CB1 antagonist (0.3 mg/kg) and were attenuated after a higher dose of 3.0 mg/kg. We believe that this differential pattern of c-Fos expression might be related to the dose-dependent central versus peripheral actions of SR141716A. Thus, the effects of the lower doses are related probably with blockade of CB1 receptors located on peripheral sensory terminals since we have described that SR141716A has a poor to intermediate penetration into the brain (Pavon et al., 2006). Blockade of the effects of centrally administered cannabinoid CB1 receptor agonists is not achieved by SR141716A at doses lower than 1 mg/kg, being optimal at 3 mg/kg (Gomez et al., 2002).

In this study we observed that whereas systemic administration of cannabinoid CB1 antagonists and CCK has additive effects on feeding suppression, the actions of SR141716A on food consumption are not dependent on CCK_1 receptors. A recent study (Burdyga et al., 2004) provides the framework for the additive effects observed between systemic CB1 antagonism and CCK_1 activation in our present study in vivo. Burdyga et al. (2004) demonstrated that vagal afferent neurons expressing CCK_1 receptors also express cannabinoid CB1 receptors, and that the expression of CB1 receptors in the nodose ganglia is increased by fasting and inhibited by cholecystokinin. Additionally, the CCK_1 antagonist lorglumide blocks the loss of CB1 receptor expression in afferent neurons after refeeding (Burdyga et al., 2004). In line with these results is tempting to speculate that the peripheral action of endogenous cannabinoids in stimulating the appetite is not mediated by interaction with peripheral CCK_1 receptor activation but may be mediated by CCK-regulated expression of CB1 receptors. These results emphasize the hypothesis that SR141716A and CCK share the same mechanism of action regarding suppression of feeding, showing together an anorectic additive effect due to the convergence of both intracellular pathways in the same physical location as suggested by CB1 and CCK_1 receptor colocalization.

As expected, pre-treatment with the CB1 agonist WIN55,212-2 counteracted the feeding suppression induced by CCK. The effect was more evident in partially-satiated animals, which was used as a model for CB1 agonist-induced overfeeding (Williams et al., 1998; Williams and Kirkham, 1999). Physiologically, peripheral CB1 receptors have a primary role as regulators of food intake by regulation of intestinal motility and gastric emptying. CB1 receptor activation is generally inhibitory and therefore CB1 agonists reduce gastric emptying and gastrointestinal transit, an effect that is counteracted by SR141716A (Di Marzo et al., 2008; Landi et al., 2002). Similarly, an enhanced gastric distention may contribute to the satiety effect of peripheral CCK (Miceli, 1985). The effects on food intake observed after WIN55,212-2 and SR141716A administration are likely produced by binding to CB1

receptors located in peripheral sensory terminals. Although additional cannabinoid receptors have been identified (Baker et al., 2006), the actual pharmacology of SR141716A or WIN55,212-2 on these receptors is not fully understood and its implications for the peripheral control of feeding remains to be determined.

The additive effects of CB1 antagonist and CCK_1 agonist acting peripherally in the suppression of feeding may offer an alternative for combined pharmacotherapy, avoiding central undesirable effects. Possible interactions between CCK and CB1 expression in the CNS are still unexplored. However, unlike CCK_2 receptors, CCK_1 receptor activation appears not to mediate anxiety-like behaviours (Rex and Fink, 1998; Cohen et al., 2004; Bertoglio and Zangrossi, 2005; Yang et al., 2006). The CCK_2 receptor is distributed through the brain and concentrated in brain areas related with anxiety, whereas CCK_1 receptor distribution is restricted to peripheral organs and brain areas related with the peripheral system, playing a major role in the modulation of feeding (Smith et al., 1981; Crawley and Corwin, 1994; Niehoff, 1989; Noble et al., 1999; Liu et al., 1994; Mercer et al., 1996). It is noteworthy that the existence of central undesirable effects as depression and anxiety in patients has recently outweighed the beneficial effects of the CB1 antagonist therapy against obesity (for a review see Lee et al., 2009; Moreira and Crippa, 2009). Our group demonstrated that a cannabinoid CB1 antagonist with poor penetration into the brain reduces feeding without producing anxiety (Pavon et al., 2006; Pavón et al., 2008). In this context, CCK_1 receptor agonism may be additive to the peripheral actions of CB1 antagonists as supressors of feeding allowing a reduction in the effective CB1 antagonist doses and consequently reducing undesirable central effects. Clearly, exploration of CB1 antagonist and CCK_1 agonist interactions deserves further investigation as possible combined therapy against obesity.

In conclusion, we show here that the anorectic effect of blocking CB1 receptors is accompanied by c-Fos activation of central brain structures related with the peripheral nervous system and is not mediated by CCK_1 receptor activation. Systemic CCK_1 agonist administration has additive effects on cannabinoid CB1 antagonist-induced feeding suppression whereas agonism of CB1 receptors counteracts the anorectic effects of CCK. Results together emphasize the importance of peripheral modulation of feeding by the endocannabinoid system, without discarding the existence of a central component, and provide a framework to study combined therapies for the treatment of eating disorders.

Conflict of interest

None.

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