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Early tolerance to the hypophagic effect of the cannabinoid receptor antagonist SR141716 does not impede blockade of an orexigenic stimulus

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Abstract

The cannabinoid CB_1 receptor antagonist SR141716 (Rimonabant®) is known to reduce food intake by central and peripheral mechanisms. Recently, SR141716 has been reported to block the orexigenic effect of ghrelin, a potent orexigenic peptide produced by the stomach.

This study investigated whether in rats, made tolerant to the hypophagic effect of SR141716, the drug was still capable to block the orexigenic activity of another non-natural (hypothalamic) peptide, i.e., the growth hormone releasing peptide (GHRP) hexarelin, a ghrelin mimetic.

In the acute experiments, each dose of SR141716 (1, 5 and 10 mg/kg i.p.) reduced food intake with respect to vehicle-treated rats, whereas hexarelin (160 μg/kg s.c.) markedly stimulated feeding. All doses of SR141716 were capable to reduce the orexigenic effect of the GHRP. A 15-day administration of SR141716 (10 mg/kg i.p.) reduced both food intake and body weight. Tolerance to the hypophagic effect of SR141716 developed within 5 days, but in contrast, body weight remained markedly below that of vehicle-treated group throughout the entire treatment period. Interestingly, despite development of tolerance to its hypophagic effect, SR141716 was capable to suppress the orexigenic effect of repeated hexarelin challenge tests performed throughout the chronic experiments.

In conclusion, the results of the present study confirm and broaden the existence of a functional relationship between ghrelin and endocannabinoids in the control of food intake, and bespeak the ability of a CB_1 receptor antagonist to suppress orexia caused by stimuli alien to direct stimulation of the cannabinoid system.
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Keywords: SR141716; Hexarelin; Food intake; Tolerance; Body weight

1. Introduction

Ghrelin, a 28-amino-acid peptide, is synthesised in the periphery (mainly in the stomach) and in the brain (Kojima et al., 1999; Ghigo et al., 2005). It acts as an endogenous ligand of the growth hormone secretagogue (GHS) receptor (Arvat et al., 2002). Additionally, recent studies have shown that ghrelin reliably stimulates food intake in many animal models, including humans (Van der Lely et al., 2004). Ghrelin seems to exert its hyperphagic effect through activation of specific hypothalamic areas linked to the regulation of feeding and metabolism, particularly the paraventricular nucleus (Kojima and Kangawa, 2005). Direct injection of ghrelin into this site generates a robust feeding response (Wren et al., 2001b). Ghrelin induces feeding by stimulating the orexigenic neuropeptide Y, orexin and agouti-related peptide pathways and, additionally, by inhibiting the anorexigenic cocaine and amphetamine-regulated transcript and peptides derived from pro-opiomelanocortin systems (Van der Lely et al., 2004). It may be reasonable, therefore, to anticipate functional interactions between ghrelin and other appetite- or energy-regulating systems.

It is now well established that appetite is modulated by central endogenous cannabinoid systems (Cota et al., 2003). Administration of cannabis compounds such as Δ^9-tetrahydrocannabinol (THC) stimulates appetite (Williams et al., 1998), while the cannabinoid CB_1 receptor antagonist SR141716 (Rimonabant®) reduces food intake after systemic administration (Colombo et al., 1998). Furthermore, recent studies have shown that appetite is increased by the endogenous cannabinoid ligands, 2-arachidonoylglycerol and anandamide (Williams and Kirkham, 1999; Kirkham et al., 2002).

As in the case of ghrelin, the paraventricular nucleus is also a sensitive site of action for the orexigenic actions of exogenous

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and endogenous cannabinoids, and systemic cannabinoid receptor agonist and antagonist drugs induce c-fos expression in the paraventricular nucleus (Wenger et al., 2003).

Recently, the CB_1 antagonist SR141716 has been reported to reverse the orexigenic effect of a single injection of ghrelin into the paraventricular nucleus (Tucci et al., 2004). This finding provides the first evidence for a functional interaction between hypothalamic ghrelin and the endocannabinoids.

Interestingly, even if SR141716 reduces dose-dependently both food intake and body weight in the early phase of a chronic treatment in the rat, tolerance to its hypophagic effect develops within 5 days, but despite this event, body weight remains markedly lower than that of vehicle-treated rats throughout the entire 14-day treatment period (Colombo et al., 1998). It is of note that tolerance is more rapid to occur in lean than in obese Zucker rats, suggesting that the obese state is more resistant to SR141716-induced tolerance (Vickers et al., 2003). The reasons of the maintenance of the body weight lowering effect of SR141716 are presently unknown, though some central and/or peripheral mechanisms have been invoked (Cota et al., 2003).

The aim of the present work was to evaluate whether in rats, made tolerant to the hypophagic effect of SR141716, the drug was still capable to block the strong orexigenic activity of a stimulus alien to the cannabinoid system, i.e., the growth hormone releasing peptide (GHRP) hexarelin, a ghrelin mimetic (Papotti et al., 2000). If this was the case, the potential therapeutic applications of Rimonabant® might be accrued.

2. Materials and methods

2.1. Animals

In either acute or chronic experiments (see below), Sprague–Dawley male rats (250–300 g; Charles-River, Calco, Italy) were used. They were singly housed in our facilities under controlled conditions (22±2 °C, 65% humidity, artificial light from 0600 to 2000 h). A standard dry pellet diet (Mucedola S.p.A., Milan, Italy) and water were available ad libitum. Animals were previously accustomed to be handled and to receive intraperitoneal injections (about 15 days), reproducing the same next experimental set. All the experiments were performed in accordance with the Italian Guidelines for the Use of Animals in Medical Research.

2.2. Drugs

N-piperidino-5(4 cholorophenyl)-1-(2,4-dicholophenyl)-4-methylpyrazole carboxamide (SR141716; Sanofi-Aventis Research Center, Montpellier, France) was suspended in 10% Tween 80 vehicle solution (Sigma-Aldrich, Milan, Italy). Hexarelin (His–D-2-methyl-Trp–Ala–Trp–D-Lys–NH_2, Euroopeptides, Argenteuil, France) was dissolved in isotonic saline.

2.3. Experimental procedure

2.3.1. Acute experiments

Animals were randomly allocated to 8 treatment groups of 9 animals each. On the test day, at 0900 h, each animal received an i.p. injection of SR141716 (1, 5 and 10 mg/kg) or vehicle. After 2 h, rats received a s.c. injection of hexarelin (160 μg/kg) or saline, and immediately after treatment were placed in their cages with a pre-weighed amount of food. Food intake was measured each hour in the ensuing 4 h interval by an electronic balance, with values approximating the first decimal of g. Any effort was made to control food spillage into cage bedding.

2.3.2. Chronic experiments

Thirty-six rats were randomly allocated to two experimental groups (vehicle or treated). They were given SR141716 (10 mg/kg i.p.) or vehicle once daily for a 15-day period. All weighing and dosing took place at approximately 0900 h each day. Body weight and 24 h food intake were recorded daily for 15 days.

Half of the animals of the vehicle and treated groups were exclusively used for hexarelin challenge test, which was performed on alternate days. Food intake post-hexarelin bolus was monitored with the same modalities and time intervals as referred previously for acute experiments. Baseline food intake during a 4-h period was evaluated in all animal groups before starting the chronic treatment.

2.4. Statistics

All values are expressed as mean±standard error. Data were analysed by two-way analysis of variance (ANOVA). Comparisons between individual groups were then made with Bonferroni's test. $P<0.05$ was considered significant.

To confirm the existence of a pharmacological antagonism between SR141716 and hexarelin, we compared the obtained data with these which would be arithmetically predicted from the opposing effects of the drugs when administered alone. The predicted outcome for the drug combination was calculated by subtracting the value of the decrease in (vehicle+saline) food intake produced by each dose of the CB_1 antagonist from food intake stimulated by the GHS. Were the decreases in food intake produced by the antagonist the only contributing factor to the antagonist-induced dose–effect curve on food intake stimulated by hexarelin, these predicted values would have substantially overlapped the obtained values.

3. Results

3.1. Acute experiments

In the acute experiments, the SR141716 (vehicle, 1, 5 and 10 mg/kg)×hexarelin (saline and 160 μg/kg) ANOVA yielded a significant main effect on food intake, for SR141716 [$df=3$; $F=23.31$; $P<0.001$], for hexarelin [$df=1$; $F=9.74$; $P<0.002$] and SR141716×hexarelin [$df=3$; $F=10.13$; $P<0.001$], indicating that food intake changed significantly under different treatments and that SR141716 and hexarelin significantly interacted.

Precisely, SR141716 reduced food intake in respect to vehicle-treated rats ($P<0.001$ for each dose vs. vehicle), whereas hexarelin markedly stimulated feeding ($P<0.002$ vs. saline). All doses of SR141716 were capable to reduce the