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Endogenous opioids and cannabinoids: System interactions in the regulation of appetite, grooming and scratching

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ABSTRACT

Growing evidence suggests substantial crosstalk between endogenous opioid and cannabinoid systems in the regulation of appetite. Not only is cannabinoid-induced hyperphagia abolished by opioid receptor antagonists (and vice versa), but several laboratories have reported supra-additive anorectic responses following co-administration of opioid and CB1 receptor antagonists. In the present study, videoanalysis has been used to characterise the acute effects of sub-anorectic doses of rimonabant (0.25, 0.75 mg/kg) and naloxone (0.1 mg/kg), alone and in combination, on mash intake, ingestive and non-ingestive behaviour, and post-treatment weight gain in male rats. The results confirmed that, when administered alone, none of these treatments significantly altered mash consumption, various measures of feeding behaviour, or weight gain. Although most non-ingestive behaviours were also unaffected, 0.75 mg/kg rimonabant induced compulsive scratching and grooming. However, when naloxone was given in combination with either dose of rimonabant, both food intake and time spent feeding were significantly decreased while the behavioural satiety sequence (BSS) was accelerated. On further analysis, the co-treatment reductions in food intake and feeding behaviour were found to be of an additive rather than supra-additive nature. Intriguingly, the co-administration of naloxone also virtually abolished the compulsive scratching response to the higher dose of rimonabant. Findings are discussed in relation to current views on the molecular bases of opioid–cannabinoid system interactions and the unexpected 'dual' advantage (reduction in appetite plus attenuation of side-effect) of low-dose combinations of opioid and cannabinoid CB1 receptor antagonists.

1. Introduction

It is now widely accepted that obesity (body mass index, BMI ≥ 30) impairs general quality of life and substantially enhances morbidity as well as the risk of premature mortality [7,21,28,48]. The markedly increased prevalence of the disorder (adults and children) over the past decade has led to its characterisation as the greatest health threat facing the developed and developing world [41,47,72,74]. Since currently licenced anti-obesity drugs (e.g. sibutramine, orlistat, phenteramine) are limited in tolerability, efficacy and sustainability [2,10,13,14], it is mandatory that an effective and safe pharmacotherapy becomes available in the very near future [25]. Fortunately, major advances in our understanding of the basic neurobiology of appetite regulation/energy homeostasis hold considerable promise for therapeutic innovation [26,67,69]. In this context, endocannabinoid mechanisms are currently attracting considerable attention both in their own right and as a consequence of recently reported, and (potentially) clinically invaluable, interactions with endogenous opioids [15,18,36].

The appetite-enhancing effects of opiates and opioids are well-documented as are the anorectic effects of broad-spectrum opioid receptor antagonists such as naloxone [5,16,73]. Similarly, the appetite-enhancing effects of cannabis, Δ^9-THC and endocannabinoids have also been well-documented as have the anorectic effects of cannabinoid CB1 receptor antagonist/inverse agonists [17,20,36,37,65]. However, there is a growing body of evidence to suggest that endogenous opioid and cannabinoid systems not only control appetite individually but that there is substantial crosstalk between these systems in the regulation of motivational processes. The possibility of opioid–cannabinoid system interactions first came to light in studies on nociception and drug reward [42,61] where, for example, it was shown that the antinociceptive and reinforcing effects of cannabinoids could be blocked not only by CB1 receptor antagonist/inverse agonists (e.g. rimonabant) but also by broad-spectrum opioid receptor antagonists (e.g. naloxone). Although initially interpreted in terms of cannabinoid-induced increases in the synthesis and release of endogenous opioids [42], the existence of reciprocal (and hence more complex) interactions soon became apparent. For instance, opiate self-administration is substantially reduced in mice by genetic deletion of the CB1 receptor [40] and, in rats and mice, by the CB1 receptor antagonist/inverse agonist rimonabant [45].

Early indications of possible opioid–cannabinoid interactions in the regulation of food intake included the observation that naloxone

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blocks both the facilitatory effect of Δ^9-THC on feeding elicited by electrical stimulation of the lateral hypothalamus [63] and the ability of CB1 receptor agonists to increase breakpoints in operant responding for palatable food [23]. However, unambiguous interpretation of the data is rendered difficult by the high (and potentially intrinsically anorectic) doses of naloxone used (1.0, 2.5 mg/kg) in these experiments. Similar interpretative difficulties arise when considering the results of two more recent studies. Thus, although Verty et al. [66] noted that the hyperphagic response to systemic or intra-hypothalamic morphine could be blocked by the CB1 receptor antagonist rimonabant, the antagonist per se had a substantial intrinsic anorectic activity at the most effective dose level (3.0 mg/kg). Using an elegant progressive ratio paradigm, Solinas and Goldberg [56] have more recently confirmed that breakpoints for food reinforcement are enhanced by Δ^9-THC and morphine and reduced by rimonabant and naloxone. Although the authors went on to demonstrate that the effect of Δ^9-THC in this motivational model is blocked by either rimonabant or naloxone while the effect of morphine is blocked by either naloxone or rimonabant, clear interpretation is once again clouded by the use of intrinsically anorectic doses of both antagonists (naloxone, 3.0 mg/kg; rimonabant, 1.0 and 3.0 mg/kg). However, dose selection was not a problem in the work of Williams and Kirkham [70,71] who demonstrated that the hyperphagic response to Δ^9-THC is blocked not only by sub-anorectic doses of rimonabant but also by sub-anorectic doses (as low as 0.1 mg/kg) of naloxone. Importantly, neither D-fenfluramine (established serotonergic anorectic) nor SR144528 (CB2 receptor antagonist) was effective in this regard.

The possibility of biologically-meaningful interactions between opioid and cannabinoid systems in the regulation of appetite has received most support from studies on the effects of co-administration of CB1 and opioid receptor antagonists in rodents. Although still rather few in number, these experiments provide evidence of supra-additive (i.e. synergistic) interactions between sub-anorectic doses of rimonabant and naloxone [38,49,54]. For example, a combination of 0.1 mg/kg doses of rimonabant and naloxone produces a 43% decrease in food intake in rats whereas a simple addition of their intrinsic effects would have yielded a reduction of only 6% [38]. Similar results were obtained using isobolographic analysis [54] which revealed a DI_{50} value of 0.6 mg/kg for the anorectic effect of a combination of rimonabant and naloxone compared to individual DI_{50} values of 1.8 mg/kg and 1.2 mg/kg. It is important to note that such findings are not limited to a particular species or to particular receptor ligands. Thus, evidence of synergistic anorectic interactions has also been reported in mice using the opioid antagonist nalmefene and the CB1 receptor antagonist/inverse agonist AM-251 [9]. However, while these data collectively suggest a mutual interdependence between opioid and cannabinoid mechanisms of appetite regulation, neither the acute anorectic response to AM-251 nor the ability of the compound to promote weight loss during chronic treatment is altered in mice completely lacking the μ-opioid receptor [8].

To date, pharmacological monotherapies for obesity have met with limited success [10,13,14,26,67] leading some researchers to argue in favour of polytherapy. For example, despite widespread problems in replicating the anorectic effects of low systemic doses of the gut peptide PYY_{3-36} [6,64], several research groups have recently reported synergistic success using combinations of this peptide together with extendin-4 [57], GLP-1(7–36) [46], or amylin [53]. In principle, polytherapies could have multiple advantages including low-dose efficacy, a greater and/or more sustained therapeutic response, and the potential for a reduction in and/or elimination of unwanted drug effects. In the present context, simultaneous blocking of cannabinoid and opioid receptor mechanisms could permit not only centrally-mediated reductions in the incentive value/palatability of food but also peripherally-mediated alterations in lipogenesis and glucose metabolism [15,17,18,20,36,37,65]. Furthermore, although the acute anorectic response to naloxone in rats is behaviourally-selective [58], that seen in response to cannabinoid CB1 receptor antagonist/inverse agonists such as rimonabant [59] and AM-251 [60] appears to be secondary to the induction of a compulsive scratching and grooming syndrome. Such unwanted (or side-) effects of CB1 receptor antagonist/inverse agonists might be avoided, yet anorectic efficacy retained, were they to be used at lower concentrations but in combination with an opioid receptor antagonist. In view of these considerations, the aim of the present study was to profile the behavioural effects of sub-anorectic doses of rimonabant and naloxone when given (alone and in combination) to non-deprived male rats presented with highly palatable food.

2. Materials and methods

2.1. Ethics

The experiment reported in this paper was conducted under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Subjects

Ten adult male Lister hooded rats, weighing 224.8±2.2 g on arrival from Charles River (UK), were housed 5/cage (46×26.5×26 cm) for one week and then transferred to individual cages (45×20×20 cm) for a further 2 weeks prior to any intervention. Individual housing was employed to facilitate bodyweight tracking and initial home cage familiarisation with the test diet. Animals were maintained on a 12-h reversed light cycle (lights off: 0700h) in a temperature (21±1 °C) and humidity (50±2%)-controlled environment. Reversed lighting was used to facilitate behavioural testing during the active phase of the light–dark cycle. Subjects were handled regularly for routine husbandry and were extensively habituated to all experimental procedures prior to drug testing. With the exception of the injection-test interval, during which home cage food was removed, standard pelleted food (Bantin & Kingman Universal Diet, UK; digestible energy value=14 KJ/g) and tap water were freely available in the home cages. Bodyweights were recorded at the same time daily (0900 h) throughout the experiment.

2.3. Drugs

Rimonabant ([*N*-piperidin-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide]), kindly donated by Sanofi-Aventis (Chilly-Mazarin, France), was initially suspended in a small volume of dimethyl sulfoxide (DMSO; Sigma-Aldrich, Poole, UK) and subsequently made up to required concentrations in 0.5% methylcellulose (Sigma-Aldrich). The final concentration of DMSO, including vehicle control solution, was ≤1%. Naloxone hydrochloride (Sigma-Aldrich) was dissolved in a vehicle of physiological (0.9%) saline which, alone, served for control injections. The doses of rimonabant (0.25 mg/kg and 0.75 mg/kg) and naloxone (0.1 mg/kg) were chosen on the basis of sub-anorectic profiles in our laboratory [58,59]. All vehicle and drug solutions were freshly prepared on test days and administered intra-peritoneally (IP) in a volume of 1 ml/kg. The injection-test interval for rimonabant (or vehicle) was 30 min and, for naloxone (or vehicle), 15 min.

2.4. Apparatus

Behavioural testing was conducted in a large glass vivarium (60×30×45 cm), the floor of which was covered with wood shavings [27,30,31,58–60]. A water bottle was suspended from one of the end-walls and a glass food pot, weighed immediately prior testing, was secured to the centre of the floor with an annular metal mounting. The test diet (mash) was prepared freshly each morning by adding water