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Research report

Behaviourally-selective hypophagic effects of naloxone in non-deprived male rats presented with palatable food

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Abstract

Endogenous opioids have long been implicated in mechanisms of appetite control. A significant strand in the evidence base has been the hypophagic action of broad-spectrum opioid receptor antagonists (such as naloxone) in opiate-naïve animals. However, while much has been learned about sites of action, underlying receptor mechanisms and the role of taste hedonics, surprisingly little is known about the behavioural selectivity of naloxone-induced hypophagia. As such, two experiments employed detailed video analysis to profile the behavioural effects of naloxone (Experiment 1: 1.0–5.0 mg/kg; Experiment 2: 0.01–1.0 mg/kg) in non-deprived male rats during 1 h free-feeding tests with palatable mash. Results confirmed that, at doses ≥1.0 mg/kg, naloxone consistently suppresses food consumption and feeding behaviour but, congruent with its short biological half-life, had no carryover effects on post-treatment weight gain. Crucially, the anorectic doses of naloxone did not alter the time taken to find food or to commence feeding, the time spent feeding in the initial phase of testing, or the rate at which food was consumed. Furthermore, they neither interfered with non-ingestive components of the behavioural repertoire (e.g. locomotion, rearing) nor did they disrupt the normal structure of feeding behaviour (the behavioural satiety sequence, BSS). Rather, the principal effect of naloxone was to produce a shift to the left in (i.e. accelerate) the BSS. Findings are discussed in relation to the role of (μ) opioid receptor mechanisms in taste hedonics and the likelihood of a naloxone-induced reduction in the orosensory reward that would normally accompany/follow the ingestion of palatable food.

Keywords: Endogenous opioids; Opioid receptors; Naloxone; Appetite suppression; Food intake; Palatability; Weight gain; Behavioural Selectivity; Rats

1. Introduction

Although it has been known for over 40 years that low doses of plant-derived opiates can induce hyperphagia [62], it was the discovery of the enkephalins/endorphins in the early–mid 1970s [1,79] that paved the way for intensive research on opioids and appetite [10,21,90]. Indeed, the earliest evidence implicating endogenous opioids in the neurobiological regulation of ingestive behaviour came shortly after the initial discovery of opiate receptors [69,77,85], and comprised a report on the intrinsic anorectic action of systemic naloxone in food-deprived rats [35]. Since then, naloxone and other broad-spectrum opioid receptor antagonists (such as naltrexone, MR2266) have been found to significantly inhibit not only deprivation-induced hyperphagia [6,13,17,31,32,36,40,45,52,58,60,61,74,75], but also nocturnal free-feeding [11,17,38,47,58] as well as the hyperphagic responses to glucoprivation [52,58,67], stress [26,64,86], benzodiazepine receptor agonists [12,39,80], opioid receptor agonists [10,20,73], electrical stimulation of the lateral hypothalamus [16], and lesions of the ventromedial hypothalamus [42].

The anorectic effect of systemic naloxone in rodents typically lasts up to 2 h post-administration [17,44,60,75], a duration of action consistent with the relatively short biological half-life of the compound [84]. Furthermore, the appetite suppressant effects of such broad-spectrum opioid receptor antagonists are stereospecific [58,75], centrally-mediated [16,23,61,72], and detectable in invertebrates, birds and diverse mammalian species including humans [22,90]. More recent research, involving selective opioid receptor antagonists and/or genetically-modified animals [for review: 10], has focused on the opioid receptor subtype (i.e. μ, δ, κ) most heavily involved in appetite regulation. While acknowledging growing evidence for potentially important species differences [32,34], this work has tended to favour a major role for μ receptors,

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with somewhat lesser involvement of κ and δ receptors [3–5,15,38,52,54–56,65,78,87].

Even from the outset, it was apparent that opioid receptor antagonist-induced anorexia is quite sensitive to a number of non-pharmacological variables, including phenotype (obese animals > sensitive than lean animals: [59,63,76]), nutritional status (sated animals > sensitive than food-deprived animals: [6,13,57]), and test diet (palatable food > sensitive than regular chow: [2,6,22,24,29,30,31,34,74]). Indeed, as eloquently expressed by Hayward and Low [33], the current view is that the endogenous opioid system is 'involved predominantly in the hedonics of feeding rather than in the regulation of energy homeostasis' (p.17). This widely-held opinion [see also: 7, 19, 70, 90] is based partly on the fact that the anorectic action of opioid receptor antagonists is most easily obtained in sated animals presented with palatable food [6,57], an effect that can be overcome by prolonged food deprivation [6,13,31]. More direct evidence includes the findings that naloxone and/or naltrexone not only reduce food preferences and sham feeding in animals [18,19,44,48,49] but also decrease food pleasantness ratings in humans [90]. Furthermore, the ingestion of highly palatable food has been found to activate hypothalamic pools of β-endorphin [28], while direct microinfusions of selective μ receptor agonists (e.g. DAMGO) into the nucleus accumbens increase food intake (especially palatable foods) and enhance positive hedonic reactions in the taste reactivity test [68,91–93]. In this context, functional mapping studies have quite recently identified a 'hot spot' for μ opioid enhancement of taste hedonics in the ventral striatum [8,41,89].

Despite these impressive advances, however, several quite fundamental questions remain regarding the role of endogenous opioids in appetite. Thus, while some studies have looked at possibly selective effects of opioid receptor antagonists on macronutrient intake [52,60] and on meal patterning [31,47,63], few appear to have directly addressed the behavioural selectivity of opioid antagonist-induced anorexia. Aware of the crucial importance of this issue, many researchers have simply relied upon earlier demonstrations that, at doses of ≤10 mg/kg, opioid receptor antagonists such as naloxone do not generally influence spontaneous locomotor activity [e.g. 35] or motor coordination [e.g. 74]. Other authors have instead relied upon more direct evidence that anorectic doses of these compounds do not concomitantly alter feeding latencies, initial food consumption, or feeding rates [31,46,47]. To our knowledge, however, only one report has simultaneously assessed the effects of an opioid receptor antagonist on food intake, feeding behaviour and non-feeding behaviours. Using food-restricted rats tested for nocturnal chow consumption in the home cage environment, Kirkham and Blundell [45] confirmed that naloxone (2.5–5.0 mg/kg) reduced intake without significantly influencing feeding rate or locomotor activity. However, since the hypophagic response was accompanied by concurrent reductions in drinking, sniffing, grooming and rearing as well as increases in resting, it is clear that a lack of drug effect on locomotor activity and/or feeding rate is no guarantee of behavioural selectivity. Furthermore, although the authors concluded that naloxone facilitates the physiological consequences of food ingestion that normally lead to a termination of feeding, arguments were at least partly compromised by the absence of a statistically significant effect on feeding behaviour *per se*.

In view of renewed interest in appetite-modulating role of endogenous opioids, either alone [6,10] or in conjunction with manipulations of other relevant biological systems [8,19,25,43], the aim of the present study was to replicate and extend the pioneering work of Kirkham and Blundell [45] on the behavioural profile of naloxone in rats tested in a free-feeding context. Optimal test conditions were used, comprising sated animals presented with palatable food (vide supra), and all tests sessions were recorded on DVD for subsequent detailed behavioural analysis. Two independent experiments were conducted in which the effects of naloxone were successively profiled over the dose ranges 1.0–5.0 mg/kg and 0.01–1.0 mg/kg.

2. Materials and methods

2.1. Ethics

All procedures were conducted under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Subjects

Adult male Lister hooded rats (Charles River, UK), weighing 207.5 ± 3.2 g ($N = 10$; Experiment 1) and 205.0 ± 2.2 g ($N = 10$; Experiment 2) on arrival in the laboratory, were initially group-housed for 1 week (5/cage; 46 cm × 26.5 cm × 26 cm). They were then transferred to individual cages (cage size: 45 cm × 20 cm × 20 cm) and allowed to acclimatise for a further 2 weeks prior to any manipulation. Individual housing facilitated daily bodyweight tracking as well as initial familiarisation with the test diet in the home cage environment. Animals were maintained on a 12-h reversed light cycle (lights off: 07:00 h) in a temperature (21 ± 1 °C) and humidity (50 ± 2%) – controlled environment. A reversed light cycle was employed such that behavioural testing could be performed during the normally active (dark) phase of the cycle. Subjects were handled regularly for routine husbandry, and (see below) were fully habituated to all experimental procedures prior to drug testing. With the exception of the injection-test interval, when 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 (09:00 h), and naive animals were used in each experiment.

2.3. Drugs

Naloxone hydrochloride (Sigma–Aldrich, UK) was dissolved in physiological saline (0.9%) which, alone, served for control injections. The dose range employed in Experiment 1 (0, 1.0, 2.5 and 5.0 mg/kg) was derived from the literature [17,35,36,45–47,51,60,61,71], while the doses used in Experiment 2 (0, 0.01, 0.01 and 1.0 mg/kg) were based on the results of Experiment 1 as supported by more recent research [e.g. 6, 31]. Solutions were freshly prepared on test days and administered intraperitoneally (IP) in a volume of 1 ml/kg 15 min prior to testing.

2.4. Apparatus

In both experiments, detailed behavioural profiling was conducted in a glass observation arena (60 cm × 30 cm × 45 cm), large enough to provide animals with freedom to engage in a variety of behaviours [37,82,83]. The floor of the arena was covered with wood shavings, and a water bottle was suspended from one of the end-walls. A glass food pot, weighed immediately prior testing, was positioned in the centre of the arena and secured to the floor with an annular metal mounting. The test diet (mash) was prepared freshly each morning by simply