Behavioural Brain Research 207 (2010) 174–181

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Sibutramine & naloxone: Infra-additive interaction in the regulation of appetite?

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ARTICLE INFO

Article history:
Received 30 July 2009
Received in revised form
28 September 2009
Accepted 2 October 2009
Available online 8 October 2009

Keywords:
Appetite
Food intake
Behavioural satiety sequence
Weight gain
Naloxone
Sibutramine
Monotherapy
Polytherapy
Rats

ABSTRACT

Sibutramine is one of a small number of clinically approved anti-obesity agents while naloxone not only has intrinsic anorectic efficacy but, in low doses, also produces additive/synergistic anorectic effects in combination with other compounds. In view of the potential advantages of drug polytherapy over conventional monotherapy, the present study explored the effects of acute low dose combinations of sibutramine (0.125, 0.25 mg/kg) and naloxone (0.1 mg/kg) on food intake, feeding and non-feeding behaviour, and post-treatment weight gain in male rats. Neither drug, alone or in combination, significantly affected weight gain. Naloxone *per se* modestly though significantly suppressed both food intake and time spent feeding without disrupting the behavioural satiety sequence (BSS). However, neither dose of sibutramine affected these measures nor did they further enhance the anorectic response to the opioid receptor antagonist. Indeed, the combination of naloxone and 0.25 mg/kg sibutramine produced effects on intake and feeding behaviour that were substantially lower than those predicted on the basis of the sum of the individual drug effects (i.e. an infra-additive profile). These data, which contrast directly with reported positive interactions between opioid receptor antagonists and other anorectic agents (e.g. rimonabant, bupropion), would not support naloxone-sibutramine polytherapy in the clinical management of obesity.

1. Introduction

Despite the current obesity pandemic [44,46,50,75] and the associated need for effective interventions [49], it is widely acknowledged that available drug treatments are limited in scope, efficacy and sustainability [2,4,9,12–14,26]. However, given the sheer multiplicity and partial redundancy of signalling mechanisms involved in the regulation of appetite and energy homeostasis (e.g. [58]), a cogent argument can be made for potential advantages of drug polytherapy over more traditional monotherapies [1,70]. For example, combinations of individually sub-effective doses of two compounds may produce additive/synergistic effects on appetite and/or weight loss, override the counter-regulatory mechanisms that frequently occur following monotherapy-induced weight loss, and/or ameliorate adverse side-effects associated with one or other compound [23].

Over the past 10 years, positive (additive &/or synergistic) interactions have been reported for the anorectic and/or weight-reducing effects of polytherapy with: D-fenfluramine (d-FEN) and both phenteramine [53] and phenylpropanolamine [71]; amylin and both CCK [8,69] and phenteramine [54]; PYY_{3-36} and amylin [52], extendin-4 [68] and GLP-1 (7–36) [45]. Recent work in our own laboratory has uncovered potentially useful interactions between the opioid receptor antagonist naloxone and CB1 receptor antagonist-inverse agonists (rimonabant & AM 251; [64,65]). Not only were additive interactions found for food intake and time spent feeding but naloxone also blocked the compulsive scratching syndrome typically seen with these CB1 receptor ligands. Such findings extend earlier work reporting interactions between naloxone and rimonabant [36,55] and between nalmefene and AM 251 [11]. Since Greenway and colleagues [23] have also recently reported positive interactions for naltrexone and bupropion (dual dopamine & noradrenaline reuptake inhibitor), novel therapies which incorporate an opioid receptor antagonist may be particularly effective in controlling appetite and facilitating weight loss.

In this context, sibutramine (Meridia®; Reductil®) has been licensed as an anti-obesity agent for more than a decade and is one of only a handful of agents currently available for the clinical management of the disorder [40,43,47]. Its anorectic and thermogenic effects are produced via two active metabolites that inhibit the reuptake of serotonin (5-HT) and noradrenaline (NA). The anorectic effects of the compound are mediated via central α1-adrenergic, β1-adrenergic and $5\text{-}HT_{2B/2C}$ receptor mechanisms [24,32], while its thermogenic effects are mediated by β3-adrenoceptors in brown adipose tissue [10,15,59]. Evidence suggests that sibutramine has a range of side-effects including dry mouth, headache, insomnia, nausea, constipation, tachycardia, hypertension and increased risk of coronary heart disease and stroke [40,47]. Another limitation

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doi:10.1016/j.bbr.2009.10.004

of sibutramine is the substantial (up to 55% within 18 months) regain of weight following cessation of treatment [3]. While low doses may well minimise or even eliminate side-effects, they are also likely to be less effective in achieving the desired clinical outcome. However, progress may be feasible through low-dose combination (polytherapy) with another anorectic agent. Given its positive anorectic interaction with agents targeting other signalling pathways involved in appetite regulation/energy homeostasis (e.g. CB1 receptors; [36,55,64,65]), the broad-spectrum opioid receptor antagonist naloxone appears to be a particularly promising candidate. In this context, it is also relevant to note that both naloxone and sibutramine have rather selective effects in accelerating behavioural satiety [63,66].

Although additive and synergistic interactions on food and/or alcohol consumption have been reported for co-treatment with opioid receptor antagonists and diverse agents that enhance 5-HT activity [5,18,20,25,33,38,51,77], these studies have failed to routinely assess feeding and non-feeding behaviour. This serious shortcoming raises uncertainty about the behavioural specificity of reported effects. As reviewed above, our earlier studies with low doses of naloxone and CB1 receptor antagonist/inverse agonists not only showed that the combination significantly reduced appetite but also that naloxone eliminated the compulsive scratching induced by the CB1 receptor ligands [64,65]. In this context, serotonergic compounds can also induce scratching [6,7,17,73], an effect blocked by naloxone [37,76]. Although sibutramine is not known to stimulate scratching (e.g. [66]), co-treatment with naloxone may have interesting consequences for non-ingestive as well as ingestive behaviours.

In view of the above considerations, the aim of the present study was to assess the effects of individual and combined treatment with low (sub-anorectic) doses of the general opioid receptor antagonist naloxone and the anti-obesity drug sibutramine. In addition to measures of food intake and weight gain, detailed behavioural analysis was performed to determine both the nature and behavioural specificity of any observed interactions.

2. Materials & methods

2.1. Subjects

Subjects were 10 adult male Lister hooded rats (Charles River, UK), weighing 218.5 ± 2.7 g on arrival in the laboratory. They were initially housed in groups of 5 for 1 week but, thereafter, were transferred to individual cages (cage size: 45 cm × 20 cm × 20 cm) for the remainder of the study. Animals were maintained on a 12 h reversed light/dark cycle (lights on: 19:00 h; lights off: 07:00 h) in an environment controlled for temperature (21 ± 1 °C) and humidity (50 ± 2%). They were handled regularly for routine husbandry and, as detailed below, fully habituated to all experimental procedures prior to drug testing. With the exception of the injection-test interval (home cage food was removed), standard pelleted chow (Bantin & Kingham Universal Diet, UK) and tap water were freely available in the home cages. Bodyweights were recorded (to the nearest g) at the same time daily (09:00 h) throughout the study. All procedures were conducted under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Drugs

Sibutramine hydrochloride (Tocris Bioscience, Bristol, UK) and naloxone hydrochloride (Sigma–Adrich, Poole, UK) were dissolved to required concentrations in physiological (0.9%) saline. Test doses were selected to be within the sub-anorectic range for each compound. In each case, dose selection was made on the basis of earlier dose-ranging studies under identical test conditions. For sibutramine, we have previously found significant anorectic activity with acute systemic doses as low as 0.5 mg/kg [66] and, as such, selected doses of 0.25 and 0.125 mg/kg for the present work. A single dose of 0.1 mg/kg naloxone was selected on the basis of previous work showing a lack of significant intrinsic anorectic activity under present conditions yet clear additive anorectic activity in combination with other agents [63–65]. All solutions were freshly prepared on test days and administered intraperitoneally (IP) in a volume of 1 ml/kg either 30 min (sibutramine) or 15 min (naloxone) prior to testing.

2.3. Apparatus

Feeding studies were conducted in a large glass arena (60 cm × 30 cm × 45 cm), the floor of which was covered lightly in wood shavings. A water bottle was suspended from one of the end-walls, and a preweighed glass food pot was secured to the centre of the floor using an annular metal mounting. The test diet (mash) was freshly made each morning by adding water to a powdered form of the maintenance diet (Bantin & Kingham Universal Diet, UK; 1 g dry = 3.125 g mash; digestible energy value of mash = 4.48 kJ/g); portions of mash were disbursed to individual glass pots, covered and refrigerated until required. Mash has the advantage of high palatability (obviating the need for prior food deprivation, e.g. [31]), while its consistency minimises spillage and hoarding [28]. Two videocameras, one positioned vertically above the arena and the other horizontal to the front wall, recorded test sessions for subsequent behavioural analysis. The cameras were linked, via an image merger, to a monitor and digital videodisk (DVD) recorder.

2.4. Procedure

All feeding sessions were conducted under dim red light (2 lux) during the dark phase of the light/dark cycle (0800–1600 h). Control food pots (2 per day), positioned adjacent to the test arena, were used to assess loss of food mass through evaporation alone (average 1 h weight loss = 0.14%). The study comprised both habituation and experimental phases.

2.4.1. Habituation phase

After 2 weeks acclimatisation to laboratory conditions and personnel, rats were initially familiarised with mash in their home cages (3 h on 2 consecutive days). The following week, each animal was exposed daily for 5 days to a pseudo-experimental procedure involving two IP injections of vehicle, one 30 min before and the other 15 min before a 1 h exposure to the test arena with preweighed mash and ad libitum tap water. Following each vehicle injection, animals were returned to their home cages (chow food removed). Mash consumption (accounting for any spillage) was accurately measured on each habituation trial. This phase of the study not only fully familiarised rats with the test diet, test environment and injection procedures, but also facilitated the development of stable basal intake patterns.

2.4.2. Experimental phase

The experimental phase commenced within 72 h of the final habituation trial and was conducted according to a Latin Square design. On different occasions (each separated by a 7-day washout), animals received one of the following treatment combinations: vehicle/vehicle (V–V); vehicle/naloxone 0.1 mg/kg (V–N); sibutramine 0.125 mg/kg + vehicle (SL–V); sibutramine 0.25 mg/kg + vehicle (SH–V); sibutramine 0.125 mg/kg + naloxone 0.1 mg/kg (SL–N); or sibutramine 0.25 mg/kg + naloxone 0.1 mg/kg (SH–N). The first injection (saline or sibutramine) was given 30 min prior to testing, and the second (saline or naloxone) 15 min before testing. Home cage food was removed after the first injection and replaced immediately following testing. Fifteen minutes after the second injection, animals were transferred to a nearby laboratory and individually placed in the test arena with pre-weighed food (mash) and ad lib tap water *in situ*. They were left undisturbed for the 1 h DVD-recorded test session, following which any mash spillage was carefully retrieved and food pots accurately reweighed.

2.5. Behavioural analysis

Test DVDs were scored blind by a highly trained observer (intra-rater reliability ≥0.8) using ethological analysis software ('Hindsight'; [72]) that permits the real-time scoring of behaviour by direct keyboard entry to a PC. A continuous monitoring technique was used in view of its documented advantages over various time-sampling techniques [28]. Based on previous research [28,31,61–67], measures recorded from DVD comprised *latency to locate food source* (food ID latency; defined as the time in sec between the start of testing and first contact with the food pot) and *eat latency* (defined as the time in sec between first contact with the food source and the first eating episode), together with frequency and duration scores for the following mutually exclusive behavioural categories: *eating* (biting, gnawing, or swallowing food from food pot or from front paws); *drinking* (licking the spout of the water bottle); *grooming* (licking of the body, feet & genitals, stroking whiskers with paws, biting the tail); *scratching* (repetitive ipsilateral hind paw scratching of flanks, neck & head); *sniffing* (rapid wrinkling of the nose/twitching of vibrissae at an aspect of the environment, head movements with rear limbs immobile); *locomotion* (walking around the cage or circling; movements involving all four limbs); *rearing* (front paws raised from the cage floor, either supported against a wall or free standing); *resting* (sitting or lying in a relaxed position with head curled to body or resting on the floor; animal inactive); and *stop* (sudden and complete cessation of movement; [65]). The above measures of eating behaviour were used to calculate the average length of feeding bouts (s; total duration of eating/total frequency of eating) and the average rate of eating (total food consumption in g/total duration of eating).

In addition to analysing treatment effects on 1 h total behavioural scores, each 60 min test period was divided into 12 × 5 min time-bins and treatment effects on behavioural timecourses were examined. Although testing during the active (dark)