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Effects of acute low-dose combined treatment with rimonabant and sibutramine on appetite and weight gain in rats

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ABSTRACT

In view of its potential advantages, drug polytherapy is currently attracting significant interest in the field of obesity research. In this context, concurrent manipulation of serotonergic and cannabinoid pathways in rodents has been found to reduce food and fluid intake in both an additive or synergistic manner. To further assess the value of this polytherapeutic approach, the current study examined the acute effects of low-dose combinations of the cannabinoid CB1 receptor antagonist/inverse agonist rimonabant (0.5 mg/kg) and the dual serotonin- and noradrenaline-reuptake inhibitor sibutramine (0.125 and 0.25 mg/kg) in male rats. Ethological analysis was used to generate comprehensive behavioural profiles, including the behavioural satiety sequence (BSS). Findings confirmed that, although neither drug given alone significantly altered food intake, feeding behaviour or weight gain, rimonabant *per se* tended to reduce consumption and time spent feeding while significantly increasing scratching and grooming responses. However, none of these effects of the CB1 receptor antagonist/inverse agonist was significantly altered by the presence of either dose of sibutramine. In striking contrast to recent reports of acute low-dose interactions (enhanced appetite suppression and reduced side-effects) between rimonabant and naloxone, present results would not appear to support the clinical potential of rimonabant/sibutramine polytherapy for obesity.

1. Introduction

Obesity is a major public health concern in the developed and developing world, having more than tripled in prevalence over the past two decades (World Health Organisation, 2000; NIH Obesity Research Task Force, 2004; Rennie and Jebb, 2005). It impairs quality of life, increases the risk of type 2 diabetes, various cancers, respiratory disease, coronary heart disease and hypertension (Pi-Sunyer, 1993; Mokdad et al., 1999; National Audit Office, 2001), and reduces life expectancy by 5–20 years (Fontaine et al., 2003). Despite an urgent need for effective therapeutic interventions (Padwal and Majumdar, 2007), it is widely acknowledged that current pharmacological monotherapies are limited in tolerability, efficacy and sustainability (Chiesi et al., 2001; Clapham et al., 2001; Collins and Williams, 2001; Bays and Dujovne, 2002; Halford et al., 2003; Korner and Aronne, 2004; Bray and Greenway, 2007). In this context, it has recently been argued that polytherapy (i.e. the simultaneous targeting of at least two signalling pathways involved in energy homeostasis) may be more successful in promoting weight loss and treating the metabolic syndrome. Indeed, the counter-regulatory mechanisms that follow drug-induced weight loss (e.g. increased appetite, reduced metabolic rate) may be easier to override with polytherapies (Adan et al., 2008; Vemuri et al., 2008). In principle, polytherapy permits the use of lower doses of individual compounds which, when used concurrently, might not only successfully reduce food intake and/or body weight but also minimise undesirable side-effects (Greenway et al., 2009).

The dose-addition model describes three possible types of drug interaction: where the combined drug effect is similar to the sum of each drug alone, the interaction is termed additive; where it is greater than the sum, it is termed supra-additive (or synergistic); and, where it is less than the sum, it is termed infra-additive (Wessinger, 1986). Over the past decade, additive and/or synergistic interactions have been reported for the anorectic and/or weight-reducing effects of: D-fenfluramine (d-FEN) combined with either phenteramine (Roth and Rowland, 1999) or phenylpropanolamine (Wellman et al., 1995), cannabinoid CB1 receptor antagonists/inverse agonists with naloxone (Kirkham and Williams, 2001; Rowland et al., 2001; Tallett et al., 2008b, 2009a); amylin with either CCK (Bhavsar et al., 2004; Thavanathan and Volkoff, 2006) or phenteramine (Roth et al., 2008); PYY_{3-36} with amylin (Roth et al., 2007), extendin-4 (Talsania et al., 2005) or GLP-1 (7–36) (Neary et al., 2005); and naltrexone combined with bupropion (Greenway et al., 2009).

Against this background, two of the major signalling pathways implicated in the regulation of appetite and energy homeostasis involve the indoleamine neurotransmitter serotonin (5-HT) and the more recently identified endocannabinoids (eCB). It has long been established that food intake and bodyweight are reduced by drugs

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that enhance central 5-HT transmission (e.g. Simansky, 1996; Blundell and Halford, 1998; Garfield and Heisler, 2009), while considerable current interest surrounds the similar profile of CB1 receptor antagonist/inverse agonists such as rimonabant (for reviews, see Cota et al., 2003; DiMarzo, 2008; Kirkham, 2009). As 5-HT mechanisms predominantly influence satiety whereas CB1 mechanisms affect both the rewarding effects of food and general metabolism, the possibility of system interaction seems entirely plausible. Consistent with this hypothesis, 5-HT and CB1 receptors are co-expressed in many brain areas (Hermann et al., 2002), CB1 receptors are expressed on 5-HT soma and terminals (Haring et al., 2007; Lau and Schloss, 2008), CB1 receptor agonists reduce 5-HT turnover in many brain areas (e.g. Molina-Holgado et al., 1993; Moranta et al., 2006), anandamide binds to 5-HT_2 receptors (Kimura et al., 1998), CB1 receptor knockout mice have impaired functioning of 5-HT_{1A} and 5-$HT_{2A/C}$ receptors (Mato et al., 2007), and stimulation of CB1 receptors directly decreases (Nakazi et al., 2000) while their blockade increases (Tzavara et al., 2003) 5-HT efflux in the cortex.

At the physiological/behavioural level, eCB–5-HT interactions have been reported for hypothermia (Malone and Taylor, 1998, 2001), analgesia (Racz et al., 2008), anxiety (Marco et al., 2004; Uriguen et al., 2004; Braida et al., 2007), and depression (Takahashi et al., 2008). However, only a few studies have thus far assessed potential eCB–5-HT interactions in the regulation of appetite, and these have produced somewhat inconsistent results. For example, even intrinsically anorectic doses of d-FEN are unable to reverse the hyperphagic effects of Δ^9-tetrahydrocannabinol (Williams and Kirkham, 2002). However, increased alcohol consumption following chronic treatment of mice with a CB_1 receptor agonist was prevented by chronic 5-HT_{1A} receptor blockade which, by itself, did not affect alcohol intake (Kela et al., 2006). Although an additive anorectic effect has been reported for the combination of rimonabant and d-FEN (Rowland et al., 2001), only food intake was measured and, as such, behavioural specificity remains unclear. Furthermore, despite a recently identified synergistic interaction between rimonabant and the 5-HT_{2C} receptor agonist *m*CPP in a progressive ratio study on feeding motivation in mice (Ward et al, 2008), a concurrent reduction in response rate is also suggestive of behavioural non-specificity.

The dual 5-HT and noradrenaline-reuptake inhibitor sibutramine (Meridia®, Reductil®) has been licensed as an anti-obesity treatment for more than a decade (McNeely and Goa, 1998; Nisoli and Carruba, 2000; Luque and Rey, 2002). Its ability to promote weight loss is believed to be a joint function of appetite suppression via central α1-adrenergic, β1-adrenergic and 5-$HT_{2B/2C}$ receptor mechanisms (Grignaschi et al., 1999; Jackson et al., 1997), and enhanced thermogenesis via β3-adrenoceptor mechanisms in brown adipose tissue (Connoley et al., 1999; Casado et al., 2003; Golozoubova et al., 2006). However, despite extensive clinical application, sibutramine has side-effects ranging from dry mouth, headaches, insomnia, nausea, and constipation to hypertension and the associated risk of heart disease and stroke (Nisoli and Carruba, 2000; Luque and Rey, 2002). The CB1 receptor antagonist/inverse agonist rimonabant also appears to promote weight loss through a dual action, suppressing appetite via central CB1 receptor mechanisms and enhancing metabolism via peripheral CB1 receptor mechanisms (Cota et al., 2003; DiMarzo, 2008; Kirkham, 2009). In animals, however, its acute anorectic action may be secondary to compulsive scratching and grooming (Tallett et al., 2007b, 2008b) while, in humans, chronic treatment is associated with a high incidence of psychiatric symptoms (Hill and Gorzalka, 2005; Van Gaal et al., 2005; Nissen et al., 2008). In view of these profiles, and current interest in the potential advantages of drug polytherapy (e.g. Adan et al., 2008; Vemuri et al., 2008; Greenway et al., 2009), our present aim was to examine in detail the combined low dose effects of sibutramine and rimonabant on food intake, behaviour, and weight gain in male rats. The design adopted has already proven valuable in demonstrating both additive (Tallett et al., 2008b, 2009a) and infra-additive (Tallett et al., 2010) interactions between anorectic agents of different classes.

2. Materials and methods

2.1. Subjects

Subjects were 10 adult male Lister hooded rats (238.2 ± 1.6 g on arrival) obtained from Charles River, U.K. They were housed 5/cage (46 × 26.5 × 26 cm) for one week following which they were transferred to individual cages (45 × 20 × 20 cm) for the remainder of the study. Single housing facilitated both initial familiarisation with the test diet and daily bodyweight tracking. Rats were maintained on a 12-h reversed light cycle (lights off: 0700 h) in an environment controlled for temperature (21 ± 1 °C) and humidity (50 ± 2%). The reversed light cycle allowed behavioural testing to be conducted during the active (dark) phase of the light–dark cycle. Animals were handled regularly during routine husbandry and were thoroughly habituated to all experimental procedures prior to drug testing. Pelleted chow (Bantin & Kingman Universal Diet, UK; digestible energy value = 14 kJ/g) and tap water were available ad libitum within the home cages, with the exception of the injection-test interval during which home cage food was removed. Bodyweights were recorded at the same time daily (0900 h) throughout the experiment. 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, UK) was dissolved in physiological saline (0.9%) which, alone, served as vehicle control. Rimonabant ([N-piperidin-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide]), kindly donated by Sanofi-Aventis (Chilly-Mazarin, France), was 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 was ≤1% for both drug and vehicle solutions. For each compound, sub-anorectic dose selection was made on the basis of earlier dose-ranging and/or interaction 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 (Tallett et al., 2009c) but not below (Tallett et al., 2010). As such, sub-anorectic doses of 0.25 and 0.125 mg/kg (calculated as the salt) were selected for the present work. A single dose of 0.5 mg/kg rimonabant was chosen on the basis of previous studies showing a lack of significant intrinsic anorectic activity under present test conditions yet clear additive anorectic activity in combination with several other agents (Tallett et al., 2007b, 2008b, 2009a). All solutions were freshly prepared on test days and administered intraperitoneally (IP) in a volume of 1 ml/kg 30 min (rimonabant or methyl cellulose) or 25 min (sibutramine or saline) prior to testing.

2.3. Apparatus

Behavioural testing was conducted in a glass observation arena (60 × 30 × 45 cm), large enough to provide animals with freedom to engage in a variety of behaviours (e.g. Ishii et al., 2003; Tallett et al., 2007a,b). The floor of the test arena was covered with 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 with an annular metal mounting. The test diet (mash) was prepared freshly each morning by adding water to a powdered form of the maintenance diet (Bantin & Kingman Universal Diet, UK; 1 g dry = 3.125 g mash; digestible energy value = 4.48 kJ/g). Portions of

mash were disbursed to individual pots, covered and refrigerated until shortly before use. Mash has the advantage of high palatability (obviating the need for prior food deprivation), while its consistency minimises spillage and hoarding (e.g. Ishii et al., 2003; Tallett et al., 2007a,b). Two video cameras, one positioned vertically above the arena and the other horizontal to the front wall, were used to record the test sessions for subsequent behavioural analysis. This multi-angled view of the test arena facilitated scoring accuracy. Camera signals were fed via an image merger to a nearby monitor and DVD recorder.

2.4. Procedure

All procedures were conducted during the dark phase of the light/dark cycle (0800–1600 h) under dim red light (2 lx). Each test day, 2 control food pots were positioned adjacent to the test arena to assess loss of food mass through evaporation alone (average loss = 0.14%; range 0.06–0.25%).

2.4.1. Habituation phase

After 2 weeks acclimatisation to laboratory conditions, rats were familiarised with mash in their home cages for 3 h on 2 consecutive days. The following week, they were individually exposed to a pseudo-experimental procedure daily for 5 days. This involved the removal of home cage food, IP injection of rimonabant vehicle (DMSO/methyl cellulose) and return to home cage for 5 min, followed by IP injection of sibutramine vehicle (saline) and return to home cage for 25 min. Animals were then placed in the test arena for 1 h, with preweighed mash and ad libitum tap water. Mash consumption (controlling for any spillage) was accurately measured after each of these trials, with animals returned to their home cages (chow reinstated). This habituation phase not only familiarised animals with the test diet, test environment and injection procedures, it also helped to ensure the development of stable mash consumption prior to the experimental phase.

2.4.2. Experimental phase

The experimental phase commenced within 3 days of the final habituation trial, and was conducted according to a within-subjects (crossover) design. A Latin Square was used to determine treatment order, with a washout period of 7 days between successive treatments. On test days, rats were individually transported to a preparation room where they were treated (IP) with either rimonabant (R; 0.5 mg/kg) or its vehicle (V; DMSO/methyl cellulose) and returned to their home cages (chow removed). 5 min later, animals were treated with either sibutramine (SL = 0.125 mg/kg; SH = 0.25 mg/kg) or its vehicle (saline) and returned to their home cages for a further 25 min prior to testing. Overall, there were 6 treatment conditions: V–V, V–R, SL–V, SL–R, SH–V, and SH–R. For testing, animals were transferred to an adjacent laboratory where they were individually placed in the test arena with preweighed mash and ad libitum tap water, and left undisturbed for the one-hour DVD-recorded test session. At the end of the test session, any spillage was carefully retrieved, food pots accurately reweighed, and animals returned to their home cages (chow reinstated).

2.5. Behavioural analysis

Test DVDs were scored blind by a highly trained observer (intra-rater reliability ≥ 0.8), using ethological analysis software ('Hindsight'; Weiss, 1995) that permits real-time scoring of behaviour by direct keyboard entry to a PC. A continuous observation method was employed due to its advantages over time-sampling techniques (Halford et al., 1998). Based on previous research (Ishii et al., 2003; Tallett et al., 2007a,b; 2008a,b; 2009a–c), measures recorded from DVD were: *latency to locate food source* (time in seconds between the start of testing and first contact with the food pot), and *latency to feed* (time in seconds between first contact with the food source and the first feeding episode), together with frequency and duration of the following mutually exclusive behavioural categories: — *feeding* (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 and genitals; stroking of face and whiskers with forepaws, biting the tail); *scratching* (repetitive ipsilateral hind paw scratching of flanks, neck and 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* (forepaws 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; Tallett et al., 2009a,c). Two further measures of feeding behaviour were derived from the recorded parameters: *average duration of feeding bouts* (total feeding duration in seconds divided by total feeding frequency), and *average feeding rate* (total food intake in grams divided by total feeding duration in minutes).

In addition to analysing treatment effects on total one-hour scores, each 60-minute test period was divided into 12 × 5-minute time bins to allow analysis of treatment effects over time. Although testing in virtual darkness during the active (dark) phase of the light/dark cycle curtailed the display of postprandial resting (see Tallett et al., 2009b), attention was nevertheless paid to the behavioural satiety sequence (BSS), i.e. the temporal relationship between eating, grooming, and resting.

2.6. Test-day bodyweight and post-treatment bodyweight gain

Bodyweights were recorded at the same time daily (0900 h) from day one of individual housing until 7 days post-dosing. This procedure was used not only to confirm the equivalence of test-day bodyweights across the different treatment conditions but also to detect any prolonged effects of acute drug treatment on weight gain. In addition to analysing treatment effects on 7-day absolute weight gain, finer-grain analysis was permitted by expressing bodyweights for each post-treatment day as a percentage of test-day bodyweight (where test day = 100%).

2.7. Statistical analysis

Food intake data over the habituation phase were analysed by one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni comparisons. Treatment effects on food intake, one-hour behaviour and 7-day absolute weight gain were analysed by two-way (3 × 2) repeated measures ANOVA (factor 1 = sibutramine; factor 2 = rimonabant) followed, as appropriate, by Bonferroni comparisons. Treatment effects on behavioural change over time within the test session (3 × 2 × 12), as well as on percentage bodyweight gain daily over the 7 days post-dosing (3 × 2 × 7), were analysed by three-way repeated measures ANOVA (factor 1 = sibutramine; factor 2 = rimonabant; factor 3 = timebin or day). Significant interactions were initially explored using two-way ANOVA for each time period/day followed, where significant, by Bonferroni tests. Where datasets failed Mauchly's Test of Sphericity, Greenhouse–Geisser significance levels are reported and, in all cases, findings were accepted as statistically significant when $p \leq 0.05$.

3. Results

3.1. Habituation

Mean bodyweight for the sample ($N = 10$) was 238.2 ± 1.6 g on arrival and 509.2 ± 12.2 g by the end of the study. All animals remained healthy throughout the experiment. As might be expected,

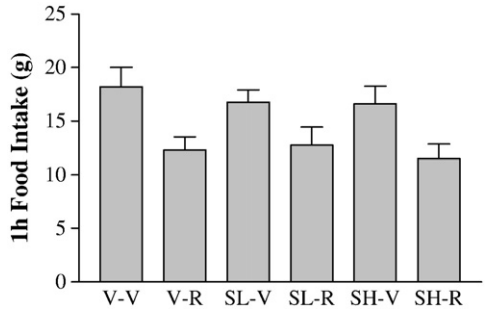

Fig. 1. Effects of sibutramine and rimonabant, alone and in combination, on food intake in non-deprived male rats exposed for 1 h to palatable mash. Data are mean values ± SEM. V = vehicle; R = rimonabant 0.5 mg/kg; SL = sibutramine 0.125 mg/kg; SH = sibutramine 0.25 mg/kg. No treatment conditions differed significantly from V–V control.

ANOVA revealed that mash intake differed significantly over the course of habituation (trial 1: 12.02 ± 0.79 g; trial 2: 9.27 ± 0.87 g; trial 3: 12.74 ± 1.01 g; trial 4: 13.99 ± 1.26 g; trial 5: 16.56 ± 1.12 g; [$F_{(4,36)} = 13.25$, $p < 0.001$]). Although consumption on trial 2 was significantly lower than on all other habituation trials ($p \leq 0.05$), stability of basal food intake was confirmed by the lack of significant difference in consumption across trials 3–5 ($p > 0.05$).

3.2. Effects of sibutramine and rimonabant, alone and in combination

3.2.1. Test-day bodyweight and food intake

Test-day bodyweights did not significantly differ across treatment conditions (V–V: 444.3 ± 15.7 g; V–R: 441.8 ± 18.2 g; SL–V: 438.8 ± 15.3 g; SL–R: 444.9 ± 13.1 g; SH–V: 436.9 ± 15.3 g; SH–R: 451.8 ± 15.8 g; [$F_{(5,45)} = 0.16$, NS]). There was a significant main effect of rimonabant on test-day food intake ($F_{(1,9)} = 65.49$, $p < 0.001$), but no significant main effect of sibutramine ($F_{(2,18)} = 0.95$, NS) nor a significant rimonabant × sibutramine interaction ($F_{(2,18)} = 0.20$, NS). Since post-hoc comparisons between the various treatment conditions failed to uncover any significant effects on intake versus V–V control (all $p > 0.05$; see Fig. 1), the significant main effect for rimonabant simply reflected the influence of much larger sample sizes when datasets are collapsed in main effects analysis (i.e. n = 30; V–R + SL–R + SH–R versus V–V + SL–V + SH–V). A more detailed inspection of the data confirmed that the non-significant reduction in intake seen in response to rimonabant remained largely unaltered in the presence of sibutramine. Thus, whereas rimonabant alone (V–R) suppressed intake by 32% relative to V–V control, very similar levels of suppression were seen in both SL–R (30%) and SH–R (37%) conditions. It should be noted that the suppression of intake by sibutramine alone (SL–V and SH–V) did not exceed 10% (Fig. 1).

3.2.2. Total behavioural scores

Data for feeding-related parameters (latency to locate food source, latency to feed, average duration of feeding bouts, and average rate of feeding) are summarised in Table 1, while treatment effects on the total frequency and duration of ingestive and non-ingestive behaviours are illustrated in Figs. 2 and 3.

There were no significant sibutramine × rimonabant interactions for any of the test variables ($F_{(2,18)} \leq 3.30$, NS) apart from average eating rate ($F_{(2,18)} = 3.96$, $p < 0.05$). Post-hoc tests revealed that eating rate was significantly slower in the SH–V condition compared to all other conditions (V–V, V–R, SL–V and SL–R; $p \leq 0.05$) except SH–R which, nevertheless, closely approached significance ($p = 0.06$); see Table 1.

Significant main effects of rimonabant were revealed for eat bout duration (Table 1) as well as the frequency and duration (Fig. 2) of feeding, locomotion, rearing, grooming, scratching and sniffing ($F_{(1,9)} \geq 8.22$, $p \leq 0.05$). In contrast, there were no significant main effects of rimonabant on eat rate, on the latency to identify the food source or to commence eating, or on the frequency or duration of resting, drinking or stop behaviour ($F_{(1,9)} \leq 4.62$, NS). Post-hoc analyses confirmed that most of the significant main effects of the CB1 receptor antagonist/inverse agonist, including effects on feeding-related parameters, were due to the atypically large sample sizes ($n = 30$) associated with main effects analysis. However, compared to V–V control, the frequency of rearing and the frequency and duration of locomotion were significantly suppressed by SH–R ($p < 0.05$), whereas the suppression of rear duration by SH–R closely approached significance (p = 0.051). These effects appeared to be primarily driven by rimonabant since (i) there were no differences in these measures between V–R and SH–R conditions, and (ii) there was a significant difference between SH–V and SH–R on measures of locomotion frequency and duration ($p \leq 0.05$). Furthermore, both the frequency and duration of scratching were significantly increased by V–R, SL–R and SH–R relative to V–V control ($p \leq 0.05$). As SH–R-induced scratching differed significantly from SH–V but not from V–R, the scratching syndrome was clearly due to rimonabant rather than sibutramine treatment. In parallel fashion, grooming duration was significantly increased by V–R and SH–R relative to V–V control ($p \leq 0.01$), whereas the increase in this measure in SL–R very closely approached significance ($p = 0.06$). This pattern of findings once again indicates that rimonabant enhanced grooming behaviour regardless of the presence or absence of sibutramine. Finally, post-hoc analyses failed to reveal any differences from V–V control for measures of groom frequency, or the frequency or duration of sniffing, resting, or stop behaviour (all $p > 0.05$; Fig. 2).

Significant main effects of sibutramine were found for the frequency and duration of locomotion and resting, as well as for the frequency of rearing ($F_{(2,18)} \geq 3.59$, $p < 0.05$), but not for the latency to locate food source, the latency to commence feeding, the average duration of feeding bouts, rear duration or the frequency or duration of feeding, grooming, sniffing, freezing, scratching or drinking behaviour ($F_{(2,18)} \leq 2.72$, NS). Although post-hoc analysis of the significant main effects revealed that the higher dose of sibutramine decreased locomotion frequency and duration ($p < 0.05$) and increased rest frequency (high dose only; $p < 0.05$), the absence of significant differences between individual treatment conditions and V–V control again indicates that these were relatively weak drug effects seen only with large sample sizes (Table 1 and Fig. 2).

Table 1
Effects of sibutramine and rimonabant, alone and in combination, on latency to locate food source, latency to commence feeding, average duration of feeding bouts and rate of feeding in non-deprived male rats presented with palatable mash. Data are given as mean values ± SEM. s = seconds; g = grams. V = vehicle; R = rimonabant 0.5 mg/kg; SL = sibutramine 0.125 mg/kg; SH = sibutramine 0.25 mg/kg. See text for details and Fig. 2 for complementary data.

Measure	V–V	V–R	SL–V	SL–R	SH–V	SH–R
Latency to locate food source (s)	8.91 ± 2.17	9.28 ± 1.19	8.51 ± 2.74	6.19 ± 1.30	9.75 ± 2.64	7.19 ± 1.28
Latency to commence feeding (s)	17.56 ± 4.79	28.34 ± 8.69	35.46 ± 9.30	18.45 ± 4.55	21.96 ± 5.68	21.87 ± 3.95
Average duration of feeding bouts (s)	10.21 ± 1.15	8.19 ± 0.53	10.17 ± 0.96	8.36 ± 0.54	10.32 ± 0.81	8.79 ± 0.79
Feeding rate (g/min)	1.57 ± 0.05	1.52 ± 0.12	1.43 ± 0.07	1.48 ± 0.08	1.09 ± 0.09**	1.40 ± 0.08

** $p < 0.01$ versus V–V control.

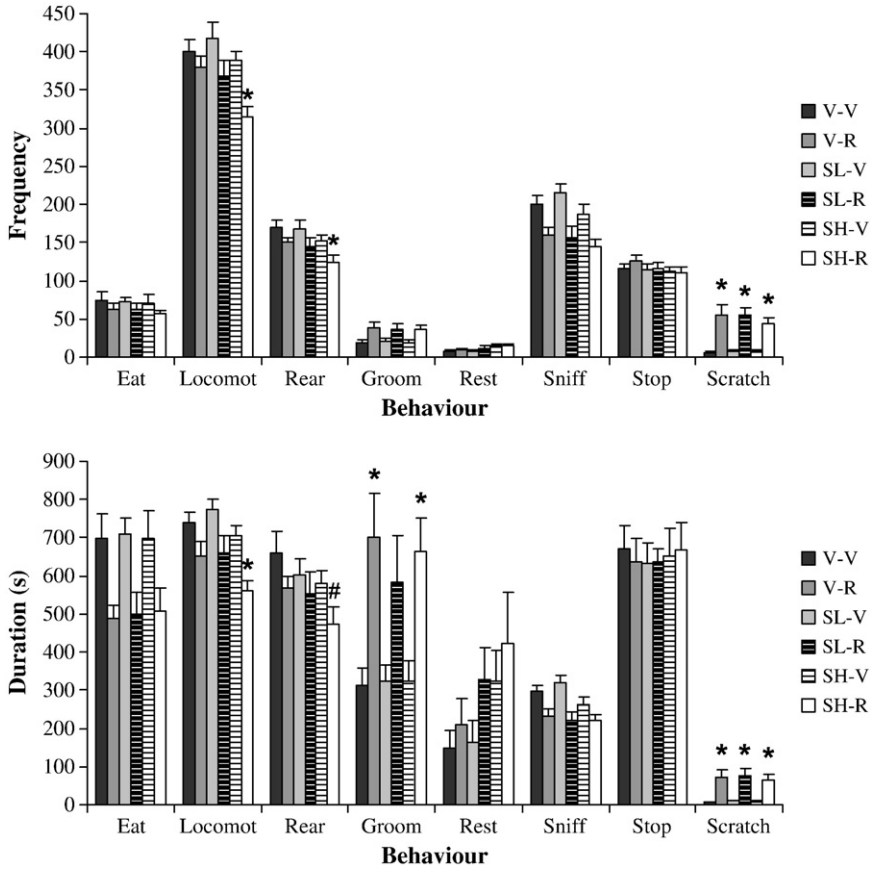

Fig. 2. Effects of sibutramine and rimonabant, alone and in combination, on the frequency (upper panel) and duration (lower panel) of feeding and non-ingestive behaviours in non-deprived male rats during one-hour tests with palatable mash. Data are mean values ± SEM. V = vehicle; R = rimonabant 0.5 mg/kg; SL = sibutramine 0.125 mg/kg; SH = sibutramine 0.25 mg/kg. *$p \leq 0.05$ versus V–V control; #p = 0.051 versus V–V control.

3.2.3. Behavioural timecourses and behavioural satiety sequence (BSS)

ANOVA failed to reveal any significant three-way interactions (time × sibutramine × rimonabant; [$F_{(22,198)} \leq 1.38$, NS]) or any significant time × sibutramine interactions for any of the test behaviours ($F_{(22,198)} \leq 2.25$, NS). There were no significant time × rimonabant interactions for the majority of behaviours ($F_{(11,99)} \leq 2.48$, NS) except stop frequency and the frequency and duration of scratching ($F_{(11,99)} \geq 2.55$, $p < 0.05$). A series of one-way ANOVAs within each time bin indicated a significant increase of rimonabant on stop frequency in time bins 1–4 only ($F_{(1,9)} \geq 7.54$, $p \leq 0.05$). However, for both the frequency and duration of scratching, there was a significant stimulatory effect of rimonabant in all time bins ($F_{(1,9)} \geq 7.96$, $p \leq 0.05$). This rimonabant-induced scratching syndrome is clearly illustrated in Fig. 3, as is its immunity to either dose of sibutramine (SL or SH). Finally, and as might be expected, there were significant main effects of time for most test behaviours ($F_{(11,99)} \geq 3.98$, $p < 0.05$), with the exception of groom frequency ($F_{(11,99)} = 1.08$, NS). These temporal patterns reflect well-documented decreases in active behaviour and increases in inactive behaviour over the course of the test session (e.g. Rodgers et al., 2001; Ishii et al., 2003; Tallett et al., 2007a).

Treatment effects on the behavioural satiety sequence (BSS) are summarised in Fig. 4. Consistent with previous work, the profile for the V–V control condition shows that the typical peak feeding response (first 20 min) gradually wanes as the test session progresses. Although resting is seen to increase over time, the absence of a clear transition (crossover) between eating and resting is consistent with the generally low levels of postprandial resting seen under current test conditions (Tallett et al., 2009b). The centre and lower panels on the left side of Fig. 4 clearly confirm that sibutramine treatment had very little effect on the peak feeding response or indeed the normal structure of feeding behaviour. However, all panels on the right side of the figure confirm the modest (non-significant) suppression of the peak feeding response by rimonabant (with or without sibutramine). Furthermore, in all rimonabant treatment conditions (i.e. V–R, SL–R and SH–R), it would appear that the eat-to-rest transition has been accelerated (shifted to the left; from final time bins to time bins 6–7) relative to all non-rimonabant conditions (i.e. V–V, SL–V and SH–V) conditions. Although these patterns initially suggest an earlier than normal expression of behavioural satiety, it is crucial to note that the high levels of grooming induced by rimonabant actually disrupted the BSS to such an extent that, at certain times during the test, it became the dominant behaviour (Fig. 4, right-hand panels). These findings urge extreme caution in attributing interpretative significance to the apparent rimonabant-induced acceleration in behavioural satiety.

3.2.4. Post-treatment bodyweight gain

ANOVA on 7-day absolute bodyweight gain failed to reveal a significant sibutramine × rimonabant interaction ($F_{(2,18)} = 0.92$, NS) or indeed significant main effect for either sibutramine ($F_{(2,18)} = 0.51$, NS) or rimonabant ($F_{(1,9)} = 0.08$, NS). However, it is interesting to note that, relative to V–V control, least weight was gained in the SH–R condition (V–V: 20.66 ± 2.28 g; V–R: 22.29 ± 1.12 g; SL–V: 20.51 ± 2.56 g; SL–R: 19.64 ± 1.25 g; SH–V: 20.87 ± 1.33 g; SH–R: 18.61 ± 1.94 g). This lack of treatment effect on weight gain was further confirmed by analyses of percent weight gain data (data not shown). ANOVA failed to reveal a three-way interaction ($F_{(12,108)} = 0.63$, NS), or any two-way interactions (sibutramine × rimonabant: [$F_{(2,18)} = 0.18$, NS]; day × sibutramine: [$F_{(12,108)} = 0.54$, NS]; day × rimonabant: [$F_{(6,54)} = 1.03$, NS]). Furthermore, there were no main effects of either sibutramine ($F_{(2,18)} = 0.50$,

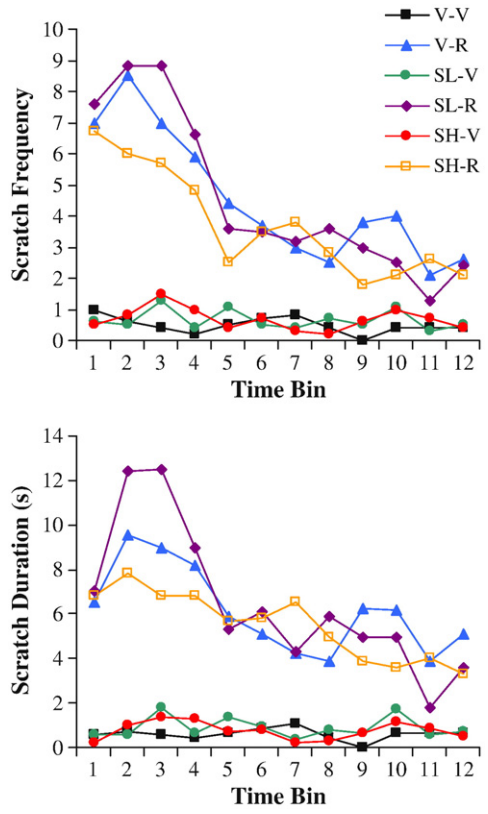

Fig. 3. Effects of sibutramine and rimonabant, alone and in combination, on the timecourse or scratch frequency (upper panel) and scratch duration (lower panel) in non-deprived male rats during one-hour tests with palatable mash. Data are presented as mean values. V = vehicle; R = rimonabant 0.5 mg/kg; SL = sibutramine 0.125 mg/kg; SH = sibutramine 0.25 mg/kg.

NS) or rimonabant ($F_{(1,9)} = 0.24$, NS). The highly significant main effect of day ($F_{(6,54)} = 162.79$, $p < 0.001$) simply reflected natural growth patterns in all conditions.

4. Discussion

In view of the potential therapeutic advantages of drug polytherapy for obesity (e.g. Adan et al., 2008; Vemuri et al., 2008; Greenway et al., 2009), the current experiment was designed to assess the combined effects of sub-anorectic doses sibutramine (0.125 and 0.25 mg/kg) and rimonabant (0.5 mg/kg) on food intake, feeding behaviour and post-treatment weight gain in male rats. Given recent findings with combinations of CB1 receptor antagonist/inverse agonists (rimonabant, AM251) and the opioid receptor antagonist naloxone (Tallett et al., 2008b; 2009a), it was of particular interest to determine whether acute co-treatment with sub-anorectic doses sibutramine and rimonabant would (a) significantly reduce food intake and feeding behaviour (via additive or synergistic interaction) and/or (b) lead to a reduction/elimination of rimonabant-induced scratching and grooming (via infra-additive/antagonistic interaction). Our results failed to support any of these outcomes, thereby suggesting that polytherapy with sibutramine and rimonabant (and possibly other CB1 receptor antagonist/inverse agonists) is unlikely to be a clinically useful strategy in the management of obesity.

Consistent with previous dose–response studies both in our laboratory (Halford et al., 1995; Tallett et al., 2009c; Tallett et al., 2010) and elsewhere (e.g. Jackson et al., 1997; Grignaschi et al., 1999), present findings confirm that, when administered alone, sibutramine (0.125 and 0.25 mg/kg) failed to significantly influence food intake, feeding behaviour or post-treatment weight gain when compared to vehicle (V–V) control. Nor did sibutramine significantly alter the normal structure of feeding behaviour (BSS). The only exception to this lack of effect on feeding-related parameters was a reduction in the average rate of eating observed at the higher dose level. Although an unexpected finding, especially since no such effect is seen at higher doses (0.5–3.0 mg/kg; Tallett et al., 2009c), this overall behavioural profile confirms that the doses of sibutramine selected for this study fall within the sub-anorectic range. Main effects analysis also showed that the higher dose of sibutramine modestly, though significantly, decreased the frequency/duration of locomotion and increased the frequency of resting. Although effects on locomotion and resting have previously been reported, they have typically been observed at much higher doses (e.g. Halford et al., 1995; Tallett et al, 2009c) suggesting that present findings are due mainly to the increased power of main effects analysis.

In contrast to the clear sub-anorectic profile of sibutramine, the results for rimonabant (0.5 mg/kg) suggest that this dose of the CB1 receptor antagonist/inverse agonist was close to the threshold for anorectic activity. Thus, while no significant differences in food intake, feeding behaviour or weight gain were observed between V–V and V–R treatment conditions, it is worth noting that (i) both mash consumption and feeding duration in V–R were reduced by approximately 30% relative to vehicle control, (ii) the peak feeding response observed during the first 20 min of the test was somewhat suppressed in all treatment conditions involving rimonabant, and (iii) main effects analysis (collapsed datasets and higher sample sizes) revealed significant overall suppressant effects of rimonabant on intake as well as the frequency and duration of feeding. Indeed, the latter analyses also showed that rimonabant significantly decreased the frequency and duration of locomotion, rearing, grooming, sniffing and scratching. Many of these effects of rimonabant have previously been reported but at higher dose levels (≥1.5 mg/kg; Tallett et al., 2007b), once again confirming the increased power of the larger sample sizes associated with main effects analysis. However, it is noteworthy that the only significant effects detected between the V–R and V–V conditions were an increase in the duration of grooming and in the frequency and duration of scratching. Previous work from our laboratory (Tallett et al., 2007b, 2008b, 2009a) has strongly suggested that the acute anorectic response to rimonabant and related compounds may be a secondary consequence of this well-known side-effect of CB1 receptor antagonist/inverse agonists (Darmani and Pandya, 2000; Jarbe et al., 2002, 2006). It is also clear from Fig. 4 that the rimonabant-induced escalation in grooming throughout the test session markedly disrupted the normal structure of feeding behaviour (BSS).

Although rimonabant (0.5 mg/kg) alone (albeit non-significantly) suppressed intake and feeding behaviour by approximately 30% relative to vehicle control, the addition of sibutramine (0.125 or 0.25 mg/kg) did not further enhance this effect i.e. the drug combinations did not result in a significant reduction in intake, feeding behaviour or, indeed, weight gain. The failure of co-treatment to significantly suppress consumption is also surprising given the potent anorectic response to individual treatment with slightly higher doses of both compounds (Tallett et al., 2007b, 2009c). Although inconsistent with reports of additive anorectic effects following co-treatment with rimonabant and the serotonin-releasing agent d-FEN (Rowland et al., 2001), and with recent reports of a synergistic interaction following co-treatment with rimonabant and the 5-HT_{2C} agonist *m*CPP (Ward et al., 2008), the behavioural specificity of these interactions is questionable. In the former study, no behavioural data were reported while, in the latter, reduced breakpoints in lever-pressing for food were accompanied by significantly reduced response rates. However, it is equally important to emphasise that the noted discrepancies could simply be due to the serotonergic specificity of d-FEN and *m*CPP relative to the dual efficacy of sibutramine in inhibiting the reuptake both of noradrenaline and 5-HT. In terms of other drug interaction studies involving sibutramine, an additive interaction has

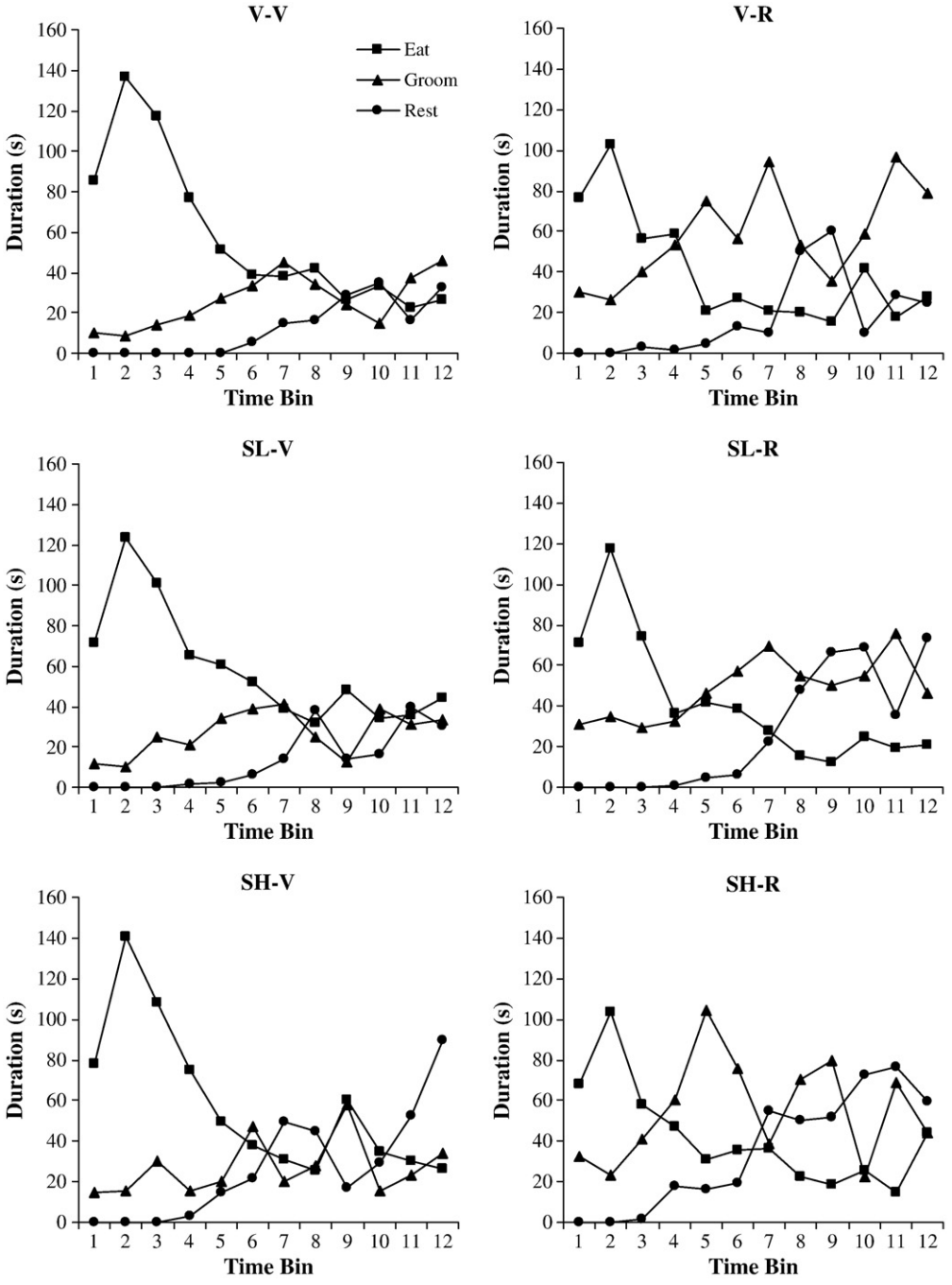

Fig. 4. Effects of sibutramine and rimonabant, alone and in combination, on the behavioural satiety sequence (BSS) in non-deprived male rats exposed for 1 h to palatable mash. Data are presented as mean duration scores in seconds. V = vehicle; R = rimonabant 0.5 mg/kg; SL = sibutramine 0.125 mg/kg; SH = sibutramine 0.25 mg/kg. The combination of SL–R and SH–R accelerated the BSS, i.e. produced a shift to the left in the temporal sequence of behaviour.

been reported when combined with amylin (Roth et al., 2008) but, surprisingly, not when combined with orlistat (e.g. Padwal et al., 2003; Kaya et al., 2004). The latter clinical finding is particularly interesting as it suggests that not all combinations of individually effective anti-obesity agents can be expected to have additive or synergistic effects.

In addition to a lack of anorectic interaction, the presence of sibutramine did not ameliorate rimonabant-induced scratching or grooming, nor the disruptive impact of the latter on the BSS. These findings, clearly illustrated in Figs. 3 and 4, stand in marked contrast to the efficacy of naloxone in this regard (Tallett et al., 2008b, 2009a) and is somewhat surprising for several reasons. First, since serotonergic agents *per se* induce scratching in rats and humans (Fjellner and Hägermark, 1979; Berendsen et al., 1990; Berendsen and Broekkamp, 1991; Eison et al., 1992; Weisshaar et al., 1997; Kuraishi et al., 2008), while rimonabant-induced scratching is partially blocked by a selective 5-$HT_{2A/2C}$ receptor antagonist (Darmani and Pandya, 2000), sibutramine might have been expected to exacerbate rimonabant-induced scratching. Second, recent research has revealed that selective serotonin reuptake inhibitors reduce pruritus in humans (Ständer et al., 2009) while, in rats, grooming is suppressed by increased 5-HT transmission (Kennett et al., 1997; Hewitt et al., 2002) and stimulated by 5-HT receptor antagonists (Marco et al., 2004). As such, sibutramine might have been expected to attenuate rimonabant-induced scratching and grooming. However, neither outcome was supported in the present study since, rather than being

intensified or attenuated, rimonabant-induced scratching and grooming remained largely unaltered by co-treatment with sibutramine.

Although present findings on combined treatment with sibutramine and rimonabant were largely negative, they were not completely so. For example, the higher dose of sibutramine (SH–V) significantly reduced the rate of eating relative to V–V control, an effect not seen when this dose was administered in conjunction with rimonabant (SH–R). The apparent ability of rimonabant to block the suppressant effect of sibutramine on eating rate is puzzling though may be a mathematical artefact related to the (albeit non-significant) inhibitory effects of the CB1 receptor antagonist/inverse agonist on mash intake and feeding duration (i.e. average eating rate = intake (g) divided by time spent eating (s)). Furthermore, as shown in Fig. 2, the frequency and duration of locomotion and rearing were significantly reduced by the combination of rimonabant and the higher dose of sibutramine (0.25 mg/kg). However, this appears to have been largely due to an action of rimonabant, since (i) these behaviours were non-significantly suppressed by individual treatment with rimonabant (V–R) but not by either dose of sibutramine (SL–V/SH–V), and (ii) at higher (anorectic) doses, treatment with rimonabant but not sibutramine significantly suppresses rearing and locomotion (Tallett et al., 2007b, 2009c). In fact, sibutramine (albeit at higher doses) has actually been shown to *increase* these behaviours (Golozoubova et al., 2006).

In summary, present findings demonstrate that acute combined treatment with sub-anorectic doses of rimonabant and sibutramine failed to significantly alter food intake, feeding behaviour or weight gain. While rimonabant *per se* significantly increased grooming and scratching, thereby disrupting the normal structure of feeding behaviour (BSS profiles), none of these effects was altered by the presence of sibutramine. Current data thus suggest that acute co-treatment with rimonabant and sibutramine may be a relatively unpromising pharmacological approach to obesity management, and that other avenues of drug polytherapy should be explored. However, there are several important caveats to this conclusion. Firstly, current findings are based on acute co-treatment with these agents whereas longer-term co-treatment (more comparable to the clinical situation) may have produced a different outcome. Secondly, present findings are also limited to the specific doses used. Although empirically selected as individually sub-anorectic, it remains theoretically possible that somewhat higher doses (particularly of sibutramine) may have yielded different results. Thus, despite effects on locomotion and resting, the maximal intrinsic anorectic effect of sibutramine (0.25 mg/kg) in the current study was only in the order of a 10% reduction relative to vehicle control. While such a modest effect could theoretically still have produced an additive or synergistic effect in combination with the near-threshold anorectic dose of rimonabant, future research should perhaps employ near-threshold doses of *both* agents and/or conduct technically more complex isobolographic analyses (e.g. Roth and Rowland, 1999; Rowland et al., 2001). Despite these caveats, however, it should not be overlooked that polytherapy with rimonabant and sibutramine is also unlikely to be a fruitful clinical strategy because inclusion of sibutramine failed to alleviate the non-specific (i.e. unwanted) behavioural effects of rimonabant.

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