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ORIGINAL INVESTIGATION

Grooming, scratching and feeding: role of response competition in acute anorectic response to rimonabant in male rats

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Abstract
Rationale Although the CB1 receptor antagonist/inverse agonist rimonabant acutely suppresses food intake in rodents, the behavioural specificity of this effect remains unclear.
Objectives To profile the behavioural effects of rimonabant in a free-feeding context.
Materials and methods Videoanalysis was employed to characterise the effects of acute rimonabant (1.5 and 3.0 mg/kg, IP) on the behaviour of non-deprived male rats exposed to palatable mash. Data were also collected on post-treatment weight gain, and, as prolonged appetite suppression has been found after single dosing with compounds of this series, rats were reassessed (drug-free) for food intake 7 days after initial testing.
Results Both doses of rimonabant not only decreased mash consumption (44–55%) but also reduced 24-h weight gain. Although videoanalysis confirmed the inhibitory effects of rimonabant on feeding behaviour, it also revealed concurrent reductions in locomotion, rearing and sniffing as well as substantial (up to tenfold) and dose-dependent increases in grooming and scratching. Timecourse analyses further revealed that rimonabant dose-dependently induced frequent episodes of atypical scratching that waned over the test but which were succeeded by prolonged and behaviourally disruptive grooming. Finally, as groups did not differ in mash consumption on retest, any prolonged anorectic effect of acute rimonabant dissipates within 7 days of treatment.
Conclusions The anorectic response to rimonabant in male rats would appear to be due largely to response competition. This parsimonious conclusion is supported by the less profound (although still significant) increases in scratching and grooming observed in rats treated with a sub-anorectic dose (0.5 mg/kg) of the compound.

Keywords Cannabinoids · CB1 receptors · Rimonabant · Appetite suppression · Food intake · Weight gain · Behavioural specificity · Response competition · Rats

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Introduction

Obesity is widely recognised as the largest and fastest growing public health problem in the developed world (World Health Organisation 2000; NIH Obesity Research Task Force 2004). In the UK and the USA, the prevalence of the disorder has more than tripled (both sexes) over the past two decades and this trend has been accompanied by an alarming rise in the incidence of childhood obesity (Rennie and Jebb 2005). The condition not only impairs general quality of life but also significantly increases morbidity and the risk of premature mortality (Macdonald 2000). While current treatments are limited in efficacy and sustainability, recent progress in our understanding of the neurobiology of appetite regulation/energy homeostasis has identified numerous targets of potential therapeutic significance (Carpino 2000; Chiesi et al. 2001; Clapham et al. 2001; Collins and Williams 2001; Bays and Dujovne 2002; Halford et al. 2003). Among these novel targets, the endocannabinoid system is currently attracting considerable scientific and clinical interest (for a review, see: DiMarzo and Matias 2005; Kirkham 2005; Cota et al. 2006).

Although the appetite-stimulating effects of cannabis (for review: Kirkham and Williams 2001) and its prinicpal active consituent Δ^9-tetrahydrocannabinol (THC; e.g.

Hollister 1971; Greenburg et al. 1976) have been recognised for quite some time, insight into the mechanisms underlying such effects is of much more recent origin. In this context, our general understanding of the behavioural pharmacology of cannabinoids was markedly enhanced by a series of events in the late 1980s/early 1990s; the discovery of two G-protein-coupled cannabinoid receptors (CB1—Devane et al. 1988; Matsuda et al. 1990; CB2—Munro et al. 1993), the identification of several endogenous cannabinoid receptor ligands (endocannabinoids; Devane et al. 1992; Mechoulam et al. 1995; Hanus et al. 2001), and the development of the CB1 receptor antagonist/inverse agonist SR141716A (rimonabant; Rinaldi-Carmona et al. 1994; 1995; Pertwee 2005). In a manner similar to THC (e.g. Brown et al. 1977; Williams et al. 1998; Koch and Matthews 2001), endocannabinoids such as anandamide and 2-AG have been found to exert hyperphagic effects even in pre-satiated animals (DiMarzo et al. 1998; Williams and Kirkham 1999; Hao et al. 2000; Williams and Kirkham 2002a). Consistent with the view that most behavioural effects of cannabinoids are mediated through CB1 receptor mechanisms (Adams and Martin 1996; Chaperon and Thiébot 1999; Kirkham and Williams 2001; Porter and Felder 2001), the hyperphagic effects of anandamide (Williams and Kirkham 1999) and Δ^9-THC (Williams and Kirkham 2002b) are blocked by rimonabant, but not by a CB2 (SR144528) receptor antagonist.

Initially assumed to occur exclusively via CB1 receptors in the central nervous system (CNS), cannabinoid regulation of energy homeostasis is now widely believed to involve both central and peripheral CB1 receptor mechanisms (Cota et al. 2003b; DiMarzo and Matias 2005; Kirkham 2005). Thus, short-term effects of cannabinoids on food consumption appear to be largely mediated via CB1 receptors in the basal forebrain (e.g. Jamshidi and Taylor 2001; Kirkham et al. 2002; Cota et al. 2003b) and on capsaicin-sensitive, CCK1 receptor-expressing vagal afferents originating from the stomach and duodenum (e.g. Gomez et al. 2002; Coutts and Izzo 2004; Pavon et al. 2006). In contrast, growing evidence suggests that longer-term effects on bodyweight (e.g. lipogenesis, fat accumulation, glucose metabolism) are mediated via CB1 receptors on adipocytes (Bensaid et al. 2003; Cota et al. 2003b), hepatocytes (Osei-Hyiaman et al. 2005), and pancreatic islets (Bermudez-Siva et al. 2006).

In addition to their importance in confirming the CB1 receptor as the molecular substrate of cannabinoid-induced hyperphagia, CB1 receptor antagonists/inverse agonists may have therapeutic value per se. Thus, when given alone to laboratory rats, acute rimonabant significantly and dose-dependently reduces the intake of sucrose and ethanol (e.g. Arnone et al. 1997; Freedland et al. 2000; Higgs et al. 2003) as well as normal laboratory chow (Colombo et al. 1998; Gomez et al. 2002; De Vry et al. 2004). Although similar anorectic effects in rodents have since been reported for other CB1 receptor antagonists/inverse agonists, such as AM251 (McLaughlin et al. 2003; Shearman et al. 2003; Chambers et al. 2004; Chambers et al. 2006), AM281 (Werner and Koch 2003) and AM1387 (McLaughlin et al. 2006), most work to date has been done with the prototypical compound rimonabant. Several studies have now confirmed that rimonabant inhibits to a similar extent the consumption of diets differing in nutrient composition, i.e. normal chow or diets high in either carbohydrate or fat (McLaughlin et al. 2003; Verty et al. 2004), and that single dosing with agents of this series can suppress food intake and weight gain for several days after treatment (e.g. McLaughlin et al. 2003; Chambers et al. 2004; McLaughlin et al. 2006; Chambers et al. 2006). With chronic treatment, tolerance quickly develops to the anorectic effects of rimonabant, whereas the drug remains effective in suppressing bodyweight for up to 28 days (Colombo et al. 1998; Vickers et al. 2003). This apparent dissociation in the effects of the compound on food intake and bodyweight is consistent with growing evidence in favour of dual behavioural *and* metabolic roles of the endocannabinoid system in energy homeostasis (Cota et al. 2003b; DiMarzo and Matias 2005; Kirkham 2005; however, see also Thornton-Jones et al. 2006).

In contrast to the large volume of evidence supporting an anorectic action of rimonabant, many authors have drawn attention to the relative dearth of information on the behavioural selectivity of this action (e.g. Cota et al. 2003a; Kirkham 2005; Cota et al. 2006; McLaughlin et al. 2006). Such concerns are exemplified by Tucci et al. (2006) who note that "although evidence supports the suggestion that the anorectic effect of rimonabant results from an attenuation of the rewarding properties of food, the contribution of drug-induced aversion/malaise or induction of behaviours that are incompatible with the expression of eating cannot be fully excluded" (p. 2674). Such views clearly recognise that food intake can be reduced by drug actions totally unrelated to the normal physiological regulation of appetite, actions such as sedation, nausea and hyperactivity (Halford et al. 1998). In this context, rimonabant is well-known to influence anxiety-like behaviour (e.g. Griebel et al. 2005), learning and memory processes (e.g. Cota et al. 2006) and the reinforcing effects of drugs of abuse (e.g. Carai et al. 2005; Tucci et al. 2006), as well as inducing compulsive motor responses (e.g. Aceto et al. 1996; De Fonseca et al. 1997; Navarro et al. 1997; Cook et al. 1998; Rubino et al. 1998; Darmani and Pandya 2000; Järbe et al. 2006; Pavon et al. 2006). As any one or a combination of these effects could interfere with feeding behaviour, the principal aim of the present study was to comprehensively profile the acute behavioural effects of

rimonabant in non-deprived male rats during a 1-h test with palatable food (e.g. Halford et al. 1998; Rodgers et al. 2001; Ishii et al. 2003, 2004, 2005).

The doses employed in the main study (1.5 and 3.0 mg/kg) were closely based on the existing literature and represent the lower end of the range (1–20 mg/kg) found to reliably suppress intake in rodents (Kirkham 2005). As recent reports have suggested prolonged anorectic activity after single dosing with CB1 receptor antagonists/inverse agonists (McLaughlin et al. 2003; Chambers et al. 2004, 2006; McLaughlin et al. 2006), these animals were retested (food intake only) in a drug-free state 1 week after treatment. Finally, in view of the results obtained in the main study, a second experiment was conducted using a lower dose (0.5 mg/kg) of the compound.

Materials and methods

Animals

All procedures were performed under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986. Animals were adult male Lister hooded rats (Charles River, UK), weighing 249.3±2.8g (N=30; experiment 1) and 205.0±2.2 g (N=10; experiment 2) on arrival in the laboratory. They were initially housed in groups of five for 1 week but, thereafter, were transferred to individual cages (cage size: 45×20×20 cm) for the remainder of the study. Individual housing was employed to facilitate both home cage familiarisation with the test diet and accurate bodyweight tracking. Animals were maintained on a 12-h reversed light/dark cycle (lights on: 1900 hours; lights off: 0700 hours) in an environment controlled for temperature (21±1°C) and humidity (50±2%). A reversed light cycle was employed to facilitate behavioural testing during the normally active (dark) phase of the cycle. Subjects were handled regularly for routine husbandry and, as detailed below, were fully habituated to all experimental procedures before drug testing. With the exception of the 30-min injection-test interval, when home cage food was removed, standard pelleted chow (Bantin & Kingman Universal Diet, UK) and tap water were freely available in the home cages. Bodyweights were recorded at the same time daily (0900 hours) throughout the study.

Drugs

Rimonabant (SR141716A; [N-piperidin-5-(4-chlorophhenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide]), kindly donated by Sanofi-Aventis (Chilly-Mazarin, France), was initially dissolved 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). A corresponding methylcellulose/DMSO solution was employed for vehicle control injections. All solutions had a final DMSO concentration of ≤1%. Test doses (experiment 1—1.5 and 3.0 mg/kg; experiment 2—0.5 mg/kg) were selected on the basis of recent findings in the literature (e.g. Gomez et al. 2002; Williams and Kirkham 2002b; Vickers et al. 2003; Chambers et al. 2004; Wiley et al. 2005). Solutions were freshly prepared on test days and administered intraperitoneally (IP) in a volume of 1 ml/kg 30 min before testing.

Apparatus

Feeding studies were conducted in a glass observation arena (60×30×45 cm) large enough to provide animals with the freedom to engage in a variety of behaviours. The arena floor was covered lightly in wood shavings, and a water bottle was suspended from one of the end walls. A preweighed glass food pot was placed in the centre of the test arena and secured to the floor by 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 & Kingman 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), while its consistency minimises spillage and hoarding (e.g. Halford et al. 1998). Two videocameras, one positioned vertically above the arena and the other horizontal to the front wall, were used to record test sessions for subsequent behavioural analysis. The resultant multi-angled view of the test arena facilitated scoring accuracy by avoiding the ambiguities that frequently arise from single perspectives. The cameras were linked to a monitor and DVD recorder via an image merger.

Procedure

All feeding test sessions were conducted under dim red light (2 lx) during the dark phase of the light/dark cycle (0800–1600 hours). As rats are nocturnal opportunistic scavengers, it was felt appropriate not only to maintain subjects under a reversed light cycle (vide supra), but also to test them in virtual darkness at a time when they are most active. Control food pots (two/day), positioned adjacent to but outside the test arena, were used to estimate loss of food mass through evaporation alone (average loss ≤0.2%). Two experiments were conducted: experiment 1 (main study) assessed the behavioural effects of 1.5 and 3.0 mg/kg rimonabant, while experiment 2 examined the effects of a lower dose of the compound (0.5 mg/kg). Both studies had

habituation and experimental phases, while experiment 1 additionally incorporated a 7-day drug-free retest.

Habituation phase

After 15 days acclimatisation to the novel laboratory conditions and personnel, rats were initially familiarised (3 h on 2 consecutive days) with mash in their home cages. The following week, each animal was exposed daily for 5 days to a pseudo-experimental procedure involving an IP injection of vehicle, return to the home cage (chow diet removed) for 30 min and finally 1-h exposure to the test arena with preweighed mash and ad libitum tap water. Mash consumption (accounting for any spillage) was accurately measured on each of these trials. The habituation phase not only fully familiarised rats with the test diet, test environment and injection procedures, but also facilitated the development of stable intake patterns and generated the data necessary for the formation of independent treatment groups (see below).

Experimental phase

Matched treatment groups were formed on the basis of food intake scores and bodyweights on the final habituation trial (experiment 1: three groups, $n=10$) or final basal intake trial (experiment 2: two groups, $n=5$). In each case, a between-subjects design was adopted in preference to a within-subjects design to avoid possible confounds arising from prolonged anorectic effects of acutely administered CB1 receptor antagonists/inverse agonists (e.g. McLaughlin et al. 2003; Chambers et al. 2004; McLaughlin et al. 2006; Chambers et al. 2006). Experimental testing began within 72 h of the final habituation trial, with test order counterbalanced for treatment both within and between test days. Animals were individually transported to a preparation room where they were treated (IP) with vehicle or rimonabant (experiment 1: 1.5 or 3.0 mg/kg; experiment 2: 0.5 mg/kg) and immediately returned to their home cages (chow diet removed). Thirty minutes later, they were transferred to a nearby laboratory and individually placed in the test arena with preweighed food (mash) and ad lib tap water in situ. Animals were left undisturbed for the 1-h digital video disc (DVD)-recorded test session, after which any spillage was carefully retrieved and food pots accurately reweighed.

Retest phase

In experiment 1 only, subjects were retested in a drug-free state 7 days after the acute drug challenge. Procedures were identical to those employed in the experimental phase with the exception that every animal received an IP injection of vehicle 30 min before the 1-h feeding test. As the sole aim of this phase was to detect any prolonged anorectic activity after single dosing with rimonabant, only food intake was measured on this occasion.

Bodyweights and post-treatment weight gain

Bodyweights were recorded at the same time daily (0900 hours) from day 1 of individual housing until 5 days after the retest phase. This procedure was used not only to confirm the equivalence of test-day bodyweights but also (experiment 1 only) to detect any prolonged effects of acute drug treatment on weight gain.

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 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 (Halford et al. 1998). Based on previous research (e.g. Halford et al. 1998; Rodgers et al. 2001; Ishii et al. 2005), measures recorded from DVD comprised *latency to locate food source* (time in seconds between the start of testing and first contact with the food pot) and *eat latency* (time in seconds 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 and genitals, stroking whiskers with paws, 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* (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 *freezing* (sudden and complete cessation of movement).

In addition to analysing treatment effects on 1-h total scores, each 60-min test period was divided into 12×5-min time bins and treatment effects on behavioural time courses were examined. Although testing during the active (dark) phase of the LD cycle naturally curtailed the display of resting behaviour, attention was paid to the behavioural satiety sequence (BSS), i.e. the temporal relationship between eating, grooming, and resting (Halford et al. 1998; Rodgers et al. 2001; Ishii et al. 2005).

Statistical analysis

The suitability of all datasets for parametric analysis was initially assessed using either Levene's Test for Homogeneity of Variance or Mauchly's Test of Sphericity. Datasets passing Levene's were analysed using independent *t* tests (experiment 2) or one-way independent analysis of variance (ANOVA; experiment 1) followed, where appropriate, by Bonferroni comparisons. Datasets failing this criterion were analysed by non-parametric Kruskal–Wallis and/or Mann–Whitney procedures. Where datasets involving repeated measures (one- or two-factor) failed Mauchly's test, Greenhouse–Geisser significance levels are reported. In all cases, findings were accepted as statistically significant where $P \leq 0.05$.

Habituation food intake data were analysed by one-way repeated-measures (ANOVA) followed by Bonferroni comparisons. For experiment 1, one-way independent parametric or non-parametric ANOVA were used (1) to confirm the initial matching of the three independent treatment groups as well as group equivalence in terms of test-day bodyweights, and (2) to assess treatment effects on 1-h food intake (experimental and retest phases), total 1-h behavioural scores, 7-day absolute post-treatment weight gain, and daily post-treatment weight gain expressed as a percentage of test-day bodyweight. For experiment 2, involving two treatment conditions only, these analyses were performed by independent *t* test or Mann–Whitney *U* test. To examine possible treatment effects on behavioural change over time, the full 60-min record for each main behavioural category was divided into 12×5-min time bins and analysed by two-way mixed ANOVA (treatment × time, repeated measures on time). Significant treatment × time interactions were initially explored (as appropriate) using one-way independent parametric or non-parametric ANOVA for each time period, followed (where indicated) by Bonferroni or Mann–Whitney comparisons.

Results

Experiment 1

Habituation phase and treatment group allocation

Mean bodyweight for the entire sample ($N=30$) was 249.3± 2.8 g on arrival, and 459.0±5.7 g at the end of the study. All animals remained healthy throughout the experiment. As would be expected, 1-h mash intake differed significantly over the five daily habituation trials (trial 1: 8.68±0.62 g; trial 2: 13.47±0.83 g; trial 3: 14.32±0.94 g; trial 4: 16.86± 0.93 g; trial 5: 15.15±0.45 g; [$F_{(4,\ 145)}=12.03$, $P<0.01$]). Although consumption on trial 1 was significantly lower than on trials 2–5 ($P \leq 0.01$), the development of a stable intake pattern was confirmed by the lack of difference in intake scores over trials 2–5. Three independent treatment groups ($n=10$) were formed on the basis of food intake and bodyweight measures taken on the final habituation trial. ANOVA confirmed that these groups did not differ significantly either on basal food intake (15.12±2.31 g, 15.15± 1.71 g, 15.19±1.55 g, [$F_{(2,\ 27)}=0.02$, NS]) or bodyweight (388.8±8.6 g, 390.4±6.0 g, 388.4±6.4 g, [$F_{(2,\ 27)}=0.00$, NS]).

Experimental phase: acute response to rimonabant (1.5–3.0 mg/kg)

Food intake ANOVA confirmed that groups did not differ in test-day bodyweight (vehicle: 408.0±9.9 g; 1.5 mg/kg: 410.2±5.8 g, 3.0 mg/kg: 409.1±8.1 g [$F_{(2,27)}=0.02$, NS]). Kruskal–Wallis analysis revealed that food intake was significantly altered by drug treatment [$\chi 2=15.73$, $df=2$, $P<0.001$; Fig. 1], with subsequent Mann–Whitney tests confirming a significant suppression of mash consumption by both doses of rimonabant ($p \leq 0.001$). Although some dose-dependency was evident (see Fig. 1), intake scores did not differ significantly between dose levels.

Total behavioural scores The effects of rimonabant (1.5–3.0 mg/kg) on total 1-h scores for ingestive and non-ingestive behaviours are summarised in Fig. 2. Significant treatment effects were found for the frequency [$\chi 2=6.91$, $df=2$, $P=0.032$] and duration [$F_{(2,\ 27)}=11.83$, $P<0.001$] of eating, with post-hoc comparisons showing that these measures were significantly suppressed by both doses of rimonabant ($P \leq 0.05$). Drug treatment did not significantly alter the time taken to locate the food source, eat latencies, or average duration of eating bouts (data not shown; [$F \leq$ 1.78, NS]). The vast majority of non-ingestive behaviours

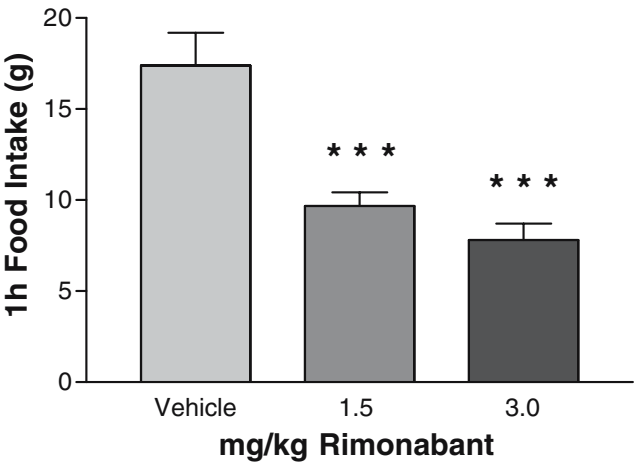

Fig. 1 Acute effect of rimonabant (1.5 and 3.0 mg/kg, IP) on food intake in male rats during a 1-h test with palatable mash. Data are expressed as mean (± SE) values. ***$P<0.001$ vs vehicle

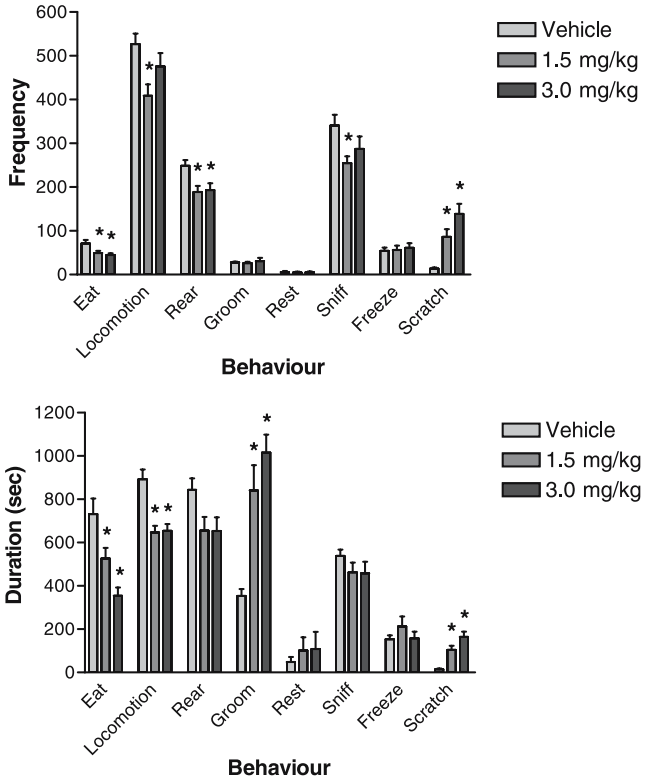

Fig. 2 Acute effects of rimonabant (1.5 and 3.0 mg/kg) on the frequency (*top panel*) and duration (*lower panel*) of eating and non-eating behaviours in male rats during a 1-h test with palatable mash. Data are expressed as mean (± SE) values. *$P \leq 0.05$ vs vehicle

were also significantly altered by acute rimonabant (1.5–3.0 mg/kg) treatment, including the frequency of locomotion, rearing and sniffing [$F_{(2,\ 27)} \geq 3.43$, $P \leq 0.05$]; the frequency of scratching [$\chi 2 = 20.22$, $df = 2$, $P < 0.001$]; the duration of locomotion and rearing [$F_{(2,\ 27)} \geq 3.36$, $P \leq 0.05$]; and the duration of grooming and scratching [$\chi 2 \geq 17.83$, $df = 2$, $P \leq 0.001$]. Post hoc analysis revealed that the lower dose of rimonabant significantly reduced the frequency of locomotion and sniffing ($P \leq 0.05$), whereas both doses significantly reduced the duration of locomotion and the frequency of rearing ($P \leq 0.05$). These symptoms of general behavioural suppression were accompanied by marked increases in the duration of grooming ($P \leq 0.001$) and in both the frequency and duration of scratching ($P \leq 0.02$). No significant treatment effects were found for the other behavioural measures recorded [$F_{(2,\ 27)} \leq 0.07$, NS; $\chi 2 \leq 0.62$, $df = 2$, NS].

Behavioural time courses and behavioural satiety sequence (BSS)

Two-way ANOVA revealed significant main effects of time for most behavioural variables ($F_{(11,\ 297)} \geq 2.52$, $P \leq 0.02$), except for groom frequency which remained fairly constant throughout the test session. These temporal patterns reflect well-documented reductions in active behaviour as the test session progresses (e.g. Ishii et al. 2003, 2004, 2005). However, it is noteworthy that the large increase in resting behaviour typically observed during the second half of the test session was not as marked as in previous experiments. This difference is undoubtedly due to procedural variation and, in particular, to dark phase testing under dim illumination (current study) vs light phase testing under normal laboratory illumination (previous studies). The only significant drug × time interactions obtained were for the frequency [$F_{(22,\ 297)} = 3.93$, $P < 0.001$] and duration [$F_{(22,\ 297)} = 3.08$, $P < 0.01$] of scratching behaviour. Follow-up comparisons showed that these measures were significantly ($P \leq 0.05$) enhanced (both doses) at all time points except the final 5 min of the test session. Figure 3 confirms that rimonabant (1.5 and 3.0 mg/kg) effects on scratching frequency and duration were not only dose-dependent but also most pronounced during the first half of the test when peak levels of feeding behaviour are normally observed.

The BSS concept describes the pattern of feeding behaviour in rats, whereby eating gives way to grooming and ultimately to resting. Treatment effects on the three components of the BSS are summarized in Fig. 4. For the control group (top panel), and as already noted, current test conditions (i.e. dark/active phase testing under dim illumination) precluded the display of high levels of resting even after the ingestion of a large meal (c. 17.5 g mash). Despite the absence of a clear BSS profile (i.e. eat-to-rest transition) in the control group, there did appear to be some evidence of a modest rimonabant-induced acceleration in the eat–rest transition at both dose levels (middle and bottom panels). However, what is most apparent from these charts is the dose-dependent and concurrent effects of drug treatment on feeding (suppression) and grooming (stimulation). While most marked during the early phase of testing, both effects clearly persist throughout the 1-h session.

Post-treatment bodyweight gain Although 7-day absolute bodyweight gain was not altered after single dose treatment with rimonabant [$F_{(2,\ 27)} = 2.47$, NS], animals receiving the 1.5 mg/kg dose gained the least weight over the period (vehicle: 29.08±2.03 g; 1.5 mg/kg: 22.63±2.32 g; 3.0 mg/kg: 27.08±1.94 g). However, when daily bodyweights were expressed as a percentage of testday bodyweight, animals treated with rimonabant (both doses) were significantly lighter 24 h post treatment than those that had received vehicle (data not shown: [$F_{(2,\ 27)} = 8.60$, $P < 0.001$]). Post hoc analysis confirmed that rimonabant actually reduced bodyweight (relative to test day baseline) over the first 24-h period ($P \leq 0.01$). No significant differences in percent bodyweight scores were apparent beyond this initial 24-h period.

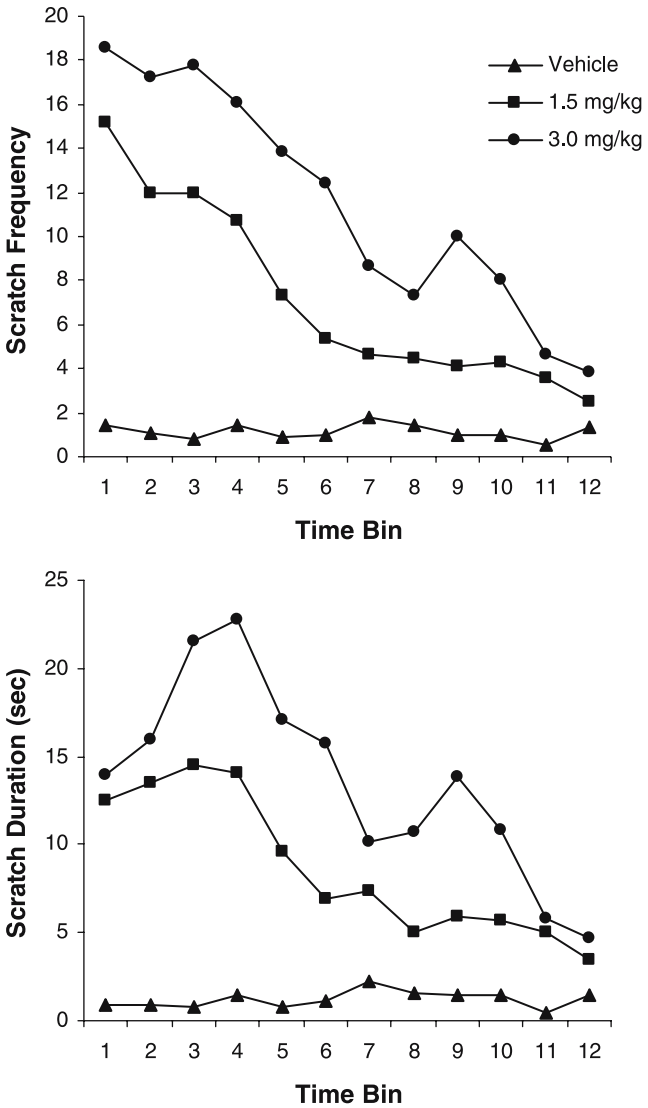

Fig. 3 Acute effects of rimonabant (1.5 and 3.0 mg/kg, IP) on the frequency (*upper panel*) and duration (*lower panel*) of scratching behaviour in male rats during a 1-h test with palatable mash. Data are expressed as mean values. Both doses significantly increased the frequency and duration of scratching except for the final 5 min of the test session: see text for details

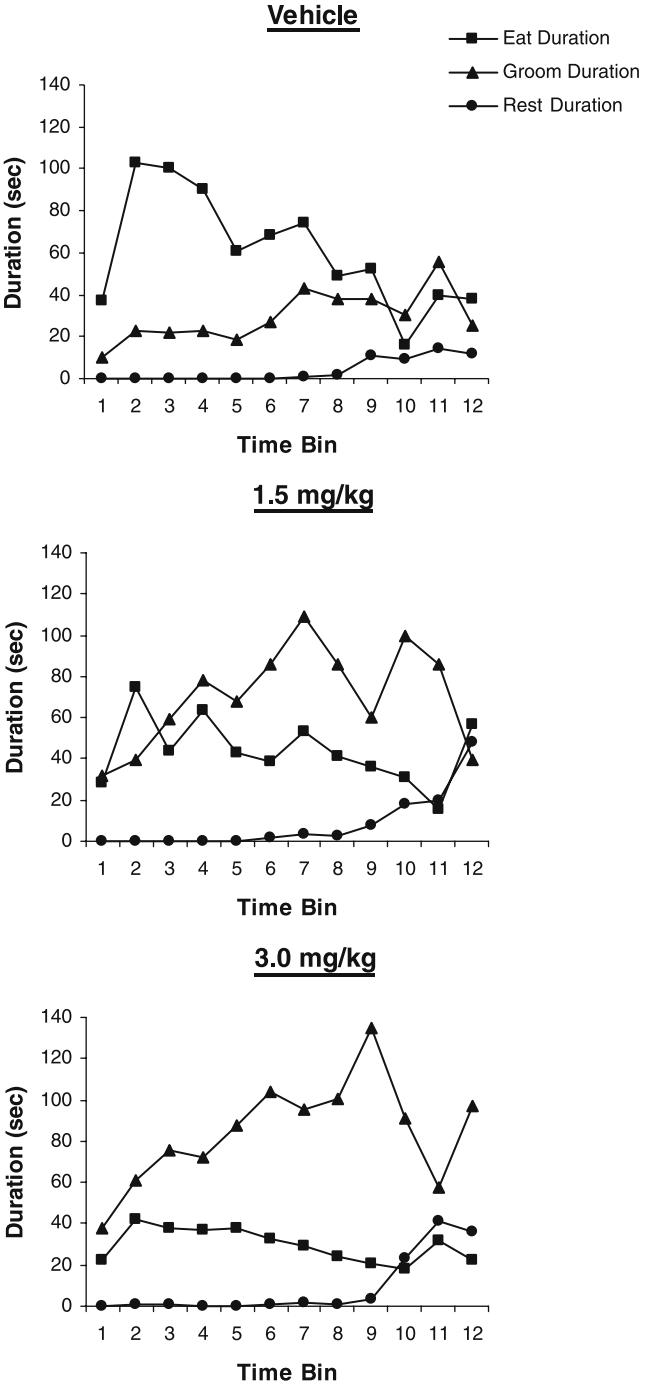

Fig. 4 Acute effects of rimonabant (1.5 and 3.0 mg/kg, IP) on the timecourse of eating, grooming and resting in male rats during a 1-h test with palatable mash. Data are expressed as the mean duration of each behaviour in each of 12×5-min timebins. Rimonabant dose-dependently reduced eating behaviour but concurrently exacerbated grooming behaviour: see text for details

Retest phase: food intake

When reassessed (drug-free) for food intake 7 days after initial testing, groups did not differ with respect either to bodyweight (vehicle: 437.1±10.7 g; 1.5 mg/kg: 433.0±6.1 g; 3.0 mg/kg: 436.2±8.9 g; [$F_{(2,\ 27)}$=0.59, NS]) or 1-h mash consumption (vehicle: 17.58±1.26 g; 1.5 mg/kg: 16.16± 1.50 g; 3.0 mg/kg: 15.64±1.55 g; [$F_{(2,\ 27)}$=0.48, NS]). The close correspondence in mash consumption by the vehicle control group during test (17.4 g) and retest (17.6 g) further confirms the stability of basal intake during the course of the experiment.

Experiment 2

Habituation phase and treatment group allocation

Mean bodyweight for the entire sample (N=10) was 205.0± 2.2 g on arrival and 487.6±9.1 g at the end of the study. All

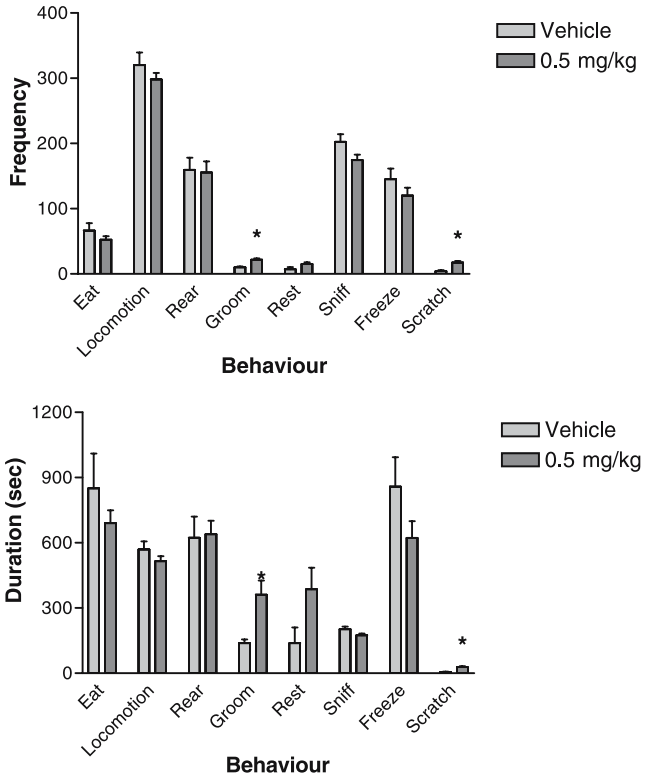

Fig. 5 Acute effects of rimonabant (0.5 mg/kg, IP) on the frequency (*top panel*) and duration (*lower panel*) of eating and non-eating behaviours in male rats during a 1-h test with palatable mash. Data are expressed as mean (± SE) values. *$P \leq 0.05$ vs vehicle

animals remained healthy throughout the experiment. Although food intake again varied significantly over the five daily habituation trials (rising from 15.28±1.33 g on trial 1 to 19.29±1.05 g on trial 5: [$F_{(4, 36)}=4.75$, $P=0.02$]), the absence of significant differences over trials 3 and 5 confirmed the development of stable intake patterns by the end of the habituation phase. After initial use in another feeding experiment, these animals were allowed a 1-week washout period before reassessment of basal intake. Normal levels of mash consumption were confirmed by a virtually identical intake score (19.36±1.94 g) to that recorded on the final habituation trial (vide supra). Two matched treatment groups ($n=5$) were formed on the basis of reassessment intake scores (19.02±1.98 g, 19.70±1.92 g; [$t(8)=-0.25$, $P>0.05$]) and corresponding bodyweights (486.1±10.7 g, 483.0±16.7 g; [$t(8)=0.16$, $P>0.05$]).

Experimental phase: acute response to rimonabant (0.5 mg/kg)

Food intake Groups did not differ significantly on testday bodyweight (vehicle: 489.4±22.3 g; 0.5 mg/kg: 484.1+ 36.8 g; [$t(8)=0.27$, $P>0.05$]). Furthermore, although somewhat reduced, 1-h mash consumption was not significantly altered by this low dose of rimonabant (vehicle: 21.84± 3.26 g; 0.5 mg/kg: 16.20±2.31 g; [$t(8)=1.42$, $P>0.05$]).

Behaviour Consistent with its lack of significant anorectic activity, rimonabant 0.5 mg/kg also failed to alter the time taken to locate the food source, eat latencies or the average duration of eating bouts (data not shown; [$t(8)\leq 1.11$, $P>0.05$]). Furthermore, as summarised in Fig. 5, treatment did not significantly influence the total frequency or duration of most behaviours displayed during the 1-h test, including feeding [$t(8)\leq 1.95$, $P>0.05$]. However, while the effects were less marked than observed at higher doses (compare Fig. 5 with Fig. 2), rimonabant 0.5 mg/kg significantly increased the frequency and duration of scratching and grooming [$t(8)\geq 3.33$, $P\leq 0.01$]. Despite the relatively small sample size, two-way ANOVA again confirmed significant main effects of time for most behavioural measures [$F_{(11, 88)}\geq 1.92$, $P\leq 0.05$], but a complete absence of treatment × time interactions [$F_{(11, 88)}\leq 1.53$, $P\geq 0.05$]. Although the BSS profiles (Fig. 6) show a relatively normal peak feeding response in animals treated with 0.5 mg/kg rimonabant, they

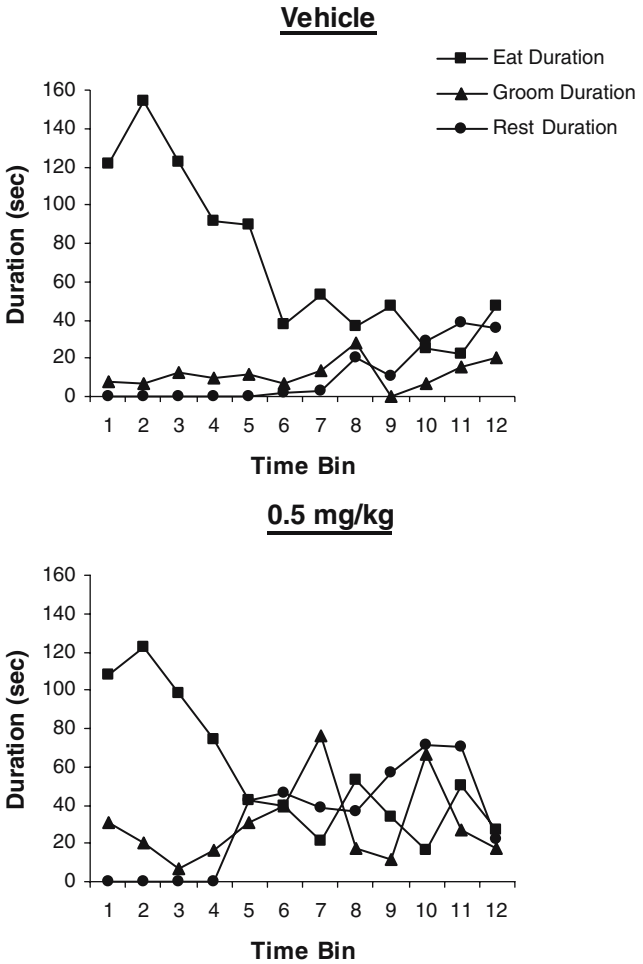

Fig. 6 Acute effects of rimonabant (0.5 mg/kg, IP) on the timecourse of eating, grooming and resting in male rats during a 1-h test with palatable mash. Data are expressed as the mean duration of each behaviour in each of 12×5-min timebins. At this low dose, rimonabant significantly increased time spent grooming but did not significantly influence time spent eating: see text for details

also illustrate the onset of a mild (although statistically significant) grooming syndrome at this dose level (compare with Fig. 4).

Discussion

Over the past decade, an extensive literature has amassed concerning the anorectic effects of the CB1 receptor antagonist/inverse agonist rimonabant. However, as very few drugs suppress appetite in a physiologically relevant or behaviourally specific manner (Chiesi et al. 2001; Clapham et al. 2001; Bays and Dujovne 2002; Halford et al. 2003), it is surprising that this literature pays comparatively little attention to the behavioural specificity of rimonabant-induced anorexia (for reviews, see: Kirkham and Williams 2001; Cota et al. 2003a; DiMarzo and Matias 2005; Kirkham 2005; Cota et al. 2006; Tucci et al. 2006). This gap in our knowledge assumes particular significance in view of the range of non-ingestive effects that have been reported for the compound, including alterations in emotionality, learning/memory and the reinforcing properties of many drugs of abuse (Carai et al. 2005; Griebel et al. 2005; Cota et al. 2006; Tucci et al. 2006). In this context, probably the most consistently reported behavioural effect of rimonabant in cannabinoid-naïve animals (rats, mice, shrews) is a vigorous scratching and grooming syndrome (Aceto et al. 1996; De Fonseca et al. 1997; Navarro et al. 1997; Aceto et al. 1998; Cook et al. 1998; Rubino et al. 1998; Darmani and Pandya 2000; Janoyan et al. 2002; Darmani et al. 2003; Järbe et al. 2002, 2006; Pavon et al. 2006). It is perhaps significant that none of these studies concerned appetite regulation, while, reciprocally, very few reports on rimonabant-induced anorexia have recorded anything other than food intake and/or bodyweight (Kirkham 2005). Our aim in the present study was to fill a perceived gap in the literature through detailed behavioural profiling of rimonabant in non-deprived rats presented with palatable food.

Consistent with previous findings (Arnone et al. 1997; Colombo et al. 1998; Gomez et al. 2002; McLaughlin et al. 2003; Shearman et al. 2003; Vickers et al. 2003; De Vry et al. 2004; Verty et al. 2004; Wiley et al. 2005; Thornton-Jones et al. 2006), the results of experiment 1 show that rimonabant (1.5 and 3.0 mg/kg) significantly suppressed mash intake when assessed 30 min post dosing. Furthermore, although a metabolic contribution cannot be excluded, the observed inhibition of weight gain over the initial 24 h after treatment is consistent with the reported enduring anorectic action of single doses of CB1 receptor antagonists/inverse agonists (McLaughlin et al. 2003; Chambers et al. 2004; Chambers et al. 2006; McLaughlin et al. 2006). However, as groups did not differ in consumption when retested (drug-free) 7 days after dosing, any persistent anorectic activity of single-dose rimonabant treatment clearly dissipates within this timeframe. Ethological analysis confirmed that acute rimonabant (1.5 and 3.0 mg/kg) significantly reduced both the frequency and duration of eating, without altering the time taken to locate the food source or to commence eating. Although the training history of the animals is not irrelevant in this context, the lack of a treatment effect on these two latency measures would argue against any disruption of basic perceptual, arousal and/or motivational mechanisms. These findings agree with the behavioural work of Webster et al. (2003) who, albeit in abstract form only, report that acute rimonabant (1–10 mg/kg) dose-dependently reduced mash intake and feeding behaviour in obese (*fa*/*fa*) Zucker rats.

A profile of reduced food intake, feeding behaviour and weight gain is entirely consistent with that expected of a selective anorectic agent (e.g. Halford et al. 1998, 2003). However, under present test conditions, rimonabant (1.5–3.0 mg/kg) also significantly reduced the frequency and/or duration of locomotion, rearing and sniffing. Although suggestive of behavioural non-specificity, these reductions in non-ingestive components of the behavioural repertoire would not per se be inconsistent with an acceleration in behavioural satiety, i.e. an earlier transition from feeding to resting can logically lead to reductions in general activity levels (e.g. Halford et al. 1998; Rodgers et al. 2001; Ishii et al. 2005). Despite the fact that testing under dim illumination during the active (dark) phase of the light cycle precluded a large post-meal increase in resting behaviour (vehicle controls), Fig. 4 suggests that rimonabant may indeed have produced an acceleration (a shift to the left) in behavioural satiety. However, close examination of the chart shows that such an effect is illusory, i.e. due almost entirely to the suppression of feeding rather than, as is normally seen with selective anorectics (e.g. Halford et al. 1998; Hewitt et al. 2002; Rodgers et al. 2002), an earlier *transition* from eating to resting.

If not displaying increased resting, what then were the rimonabant-treated animals doing during a finite test period when many other behaviours (including feeding) were suppressed? Consistent with a large body of literature on the basic behavioural pharmacology of the compound (Aceto et al. 1996; De Fonseca et al. 1997; Navarro et al. 1997; Aceto et al. 1998; Cook et al. 1998; Rubino et al. 1998; Darmani and Pandya 2000; Janoyan et al. 2002; Järbe et al. 2002; Darmani et al. 2003; Järbe et al. 2003, 2004, 2006; Pavon et al. 2006), our data show that rimonabant (1.5 and 3.0 mg/kg) markedly increased the duration of grooming and significantly increased both the frequency and duration of scratching. These profound effects are graphically illustrated in: Fig. 2, which emphasises the impact of treatment on the total time spent grooming (which, for 3 mg/kg, represents c.30% of the

entire test); Fig. 3, which confirms that this exacerbation of grooming was presaged and accompanied by frequent bouts of compulsive scratching; and Fig. 4, which shows that feeding was dose-dependently and completely displaced by grooming as the dominant behaviour during the 60-min test. Indeed, for both doses, the combined increases in time spent scratching and grooming (c. 600 and 800 s, respectively) very closely match the combined reductions in time spent feeding, locomoting, rearing and sniffing. Although the reverse relationship remains a theoretical possibility, it seems parsimonious to conclude that the former was in fact largely responsible for the latter, i.e. the excessive scratching and grooming induced by rimonabant (1.5–3.0 mg/kg) continually interrupted the flow of behaviour (including normal feeding).

To the present authors' knowledge, only two other *feeding* studies have reported rimonabant's effects on grooming and/or scratching. In a published conference abstract, Webster et al. (2003) found that rimonabant (3–10 mg/kg) produced a fourfold increase in the incidence of grooming with a consequent disruption of normal feeding patterns. Although Vickers et al. (2003) also noted that the drug's anorectic action was accompanied by three- to eightfold increases in the incidence of wet dog shakes, scratching and grooming, they argued against the possibility that the former was merely an artefact of the latter. The authors' argument was based on the observation, in obese (*fa*/*fa*) Zucker rats, that tolerance developed to the motor, but not to the anorectic, effects of the drug. However, it is pertinent to note that (a) while not statistically significant beyond the acute phase, the motor effects of rimonabant remained elevated throughout the entire 4-week study, and (b) the lack of tolerance to anorectic effect of rimonabant in these obese rats contrasts markedly with the swift development of tolerance to this action in non-obese rats (e.g. Colombo et al. 1998; Vickers et al. 2003).

Nevertheless, as the results of our first study could not exclude the possibility of a behaviourally selective anorectic effect at lower doses, a second experiment assessed the effects of 0.5 mg/kg rimonabant under virtually identical test conditions. Our findings show that, at this lower dose level, rimonabant failed to significantly reduce food intake, feeding behaviour or, indeed, most behaviours displayed during the 1-h test session. These data are entirely consistent with the vast majority of published studies on rimonabant anorexia, which point to a minimum effective dose of ≥1.0 mg/kg (Arnone et al. 1997; Colombo et al. 1998; Williams and Kirkham 1999; Freedland et al. 2000; Higgs et al. 2003; Vickers et al. 2003; Webster et al. 2003; De Vry et al. 2004; Wiley et al. 2005; Thornton-Jones et al. 2006; but see also Gomez et al. 2002; McLaughlin et al. 2003; Verty et al. 2004). However, despite its sub-anorectic profile, the 0.5 mg/kg dose of rimonabant still significantly (albeit less intensely than at higher doses) increased grooming and scratching. This finding indicates that the compound has more potent effects on scratching and grooming than on appetite, and seems to imply that a certain level of motor disturbance (e.g. levels of grooming ≥20% of total test time, as seen with 1.5 and 3.0 mg/kg) may be necessary before ingestive behaviour is significantly compromised. Overall, therefore, it would seem parsimonious to conclude that the acute anorectic response to rimonabant is most probably a consequence of response competition arising from excessive grooming and scratching.

Pertwee (2005) has recently outlined three basic mechanisms whereby CB1 receptor antagonists/ inverse agonists could produce intrinsic physiological and behavioural effects. These mechanisms are an inverse cannabimimetic action, competitive blockade of endocannabinoid tone (silent antagonism) and/or effects on non-cannabinoid neurotransmitter systems. In truth, it currently remains unclear as to which of these actions may be responsible for rimonabant-induced grooming and scratching (Wilson et al. 2006). However, as the syndrome is not completely abolished by CB1 receptor agonists (e.g. Järbe et al. 2002, 2003, 2004, 2006), mechanisms over and beyond cannabinoid receptors would appear to be involved. In this context, research on rimonabant-induced head-twitching and ear-scratching in mice has implicated downstream involvement of central 5-$HT_{2A/2C}$ receptor mechanisms (Darmani and Pandya 2000; Janoyan et al. 2002), a particularly intriguing finding in view of 5-HT_{2C} receptor involvement in the inhibitory regulation of appetite (e.g. Hewitt et al. 2002) and in neuropeptide-induced grooming (e.g. Duxon et al. 2001). Beyond these considerations, current evidence suggests that rimonabant-induced scratching and grooming could be initiated either centrally or peripherally. For example, cannabinoids are known to strongly influence basal ganglia function (Rubino et al. 1998; De Fonseca et al. 1998) and, as rimonabant increases unit activity in the substantia nigra (Gueudet et al. 1995) as well as c-Fos expression throughout the nigrostriatal system (De Fonseca et al. 1997), the observed syndrome could well be mediated via activation/disinhibition of neural circuits controlling these complex motor sequences.

Alternatively, CB1 receptor immunoreactivity is found in cutaneous nerve fibre bundles and mast cells (Ständer et al.2005) and, as CB1 receptor agonists have therapeutic value in pruritic conditions associated with chronic itching and scratching (Rukwied et al. 2003; Maekawa et al. 2006), rimonabant acting at CB1 receptors in the skin may create local irritation prompting compulsive scratching and grooming. At first glance, a recent study by Pavon et al. (2006) seems to support central as opposed to peripheral mediation of the scratching/grooming syndrome. These authors found that LH-21, a cannabinoid receptor antago-

nist that is poorly brain-penetrant compared with rimonabant, significantly inhibited food intake without inducing compulsive scratching and grooming. These new data would appear consistent with peripheral mediation of the anorectic response (see also Gomez et al. 2002) but central mediation of the scratching/grooming syndrome. However, this seemingly straightforward interpretation is complicated by the fact that LH-21 is not completely excluded from the CNS and, unlike rimonabant, is a silent CB1 receptor antagonist apparently devoid of inverse agonist activity.

In summary, the acute anorectic effect of rimonabant (1.5–3.0 mg/kg) under present test conditions appears to be behaviourally non-specific, most probably arising as a consequence of competing scratching and grooming responses. In view of ongoing debates regarding the principal mechanisms responsible for rimonabant-induced anorexia and weight loss in animals (e.g. Osei-Hyiaman et al. 2005; Bermudez-Siva et al. 2006; Thornton-Jones et al. 2006), and the relatively modest although sustained reductions in bodyweight recently reported in several clinical trials (Despres et al. 2005; Van Gaal et al. 2005; Pi-Sunyer et al. 2006), further detailed behavioural studies in animals are clearly warranted. In this context, it is certainly important to note that clinical trials have employed lower doses of rimonabant compared to most animal studies, and that the recent European authorisation of rimonabant (Acomplia®) is not as a pure appetite suppressant, but rather for the treatment of complicated obesity (metabolic syndrome/diabetes). Nevertheless, a more complete understanding of precisely how appetite is modulated by CB1 receptors will clearly depend on sophisticated preclinical work with a range of CB1 receptor antagonist/inverse agonists and silent antagonists, including ligands unable to cross the blood–brain barrier.

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