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

A.J. Tallett, J.E. Blundell, R.J. Rodgers *

Behavioural Neuroscience Laboratory, Institute of Psychological Sciences, University of Leeds, Leeds LS2 9JT, UK

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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

* Corresponding author. Institute of Psychological Sciences, University of Leeds, Leeds LS2 9JT, UK. Tel.: +44 113 343 5745; fax: +44 113 343 5749.
E-mail address: r.j.rodgers@leeds.ac.uk (R.J. Rodgers).

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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