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Bench to bedside in appetite research: lost in translation?

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Abstract

Despite substantial progress in our understanding of the complex bio-machinery involved in the regulation of appetite and energy homeostasis, few weight loss drugs are currently government-approved in the USA or Europe. While acknowledging novel drug monotherapies (such as Belviq® & Saxenda®), this review focuses on the various drug polytherapies that are currently attracting so much research interest. Unfortunately, however, the dependent variables in these new studies remain firmly rooted in outcome measures i.e. reduced food intake and bodyweight. Such evidence is clearly essential, as are physiological data bearing upon potential 'off-target' effects of any new treatment. However, as emphasised by many authors, this profiling has to be matched by sophisticated behavioural analysis addressing fundamental 'process' questions such as how such reductions in intake and/or bodyweight have been achieved. The value of behavioural analysis is exemplified, and it is argued that such a process-led approach should optimise the translation from preclinical to clinical development of candidate drugs, and avoid yet further expensive blind alleys.

Key words: Obesity - Treatment options – Anti-obesity drugs – Novel molecular targets – Monotherapy vs polytherapy – Appetite suppression - Behavioural selectivity – Behavioural Satiety Sequence

Contents

1. Context: the obesity pandemic

Stroll down your high street, or simply observe folk in bus and rail stations, ferry terminals, airports, swimming pools and on beaches, and you cannot be fail to be disturbed by the sheer bulk of many of your fellow humans. Your observations will confirm two other facets of modern life; namely, that we are constantly bombarded with visual and olfactory inticements to consume cheap, energy-dense foods, preferably in 'large' portions, yet do not really have to exert ourselves in order to acquire such delights. For many of us, there is really only one possible outcome to this imbalance in the energy equation, i.e. weight gain.

Obesity, the excessive accumulation of body fat, is most frequently diagnosed using the body mass index or BMI (kg/m^2). People with a BMI $\geq$ 25 are considered overweight, while scores of 25.00-29.99, $\geq$ 30, 30.00-34.99, 35.00-39.99, and $\geq$ 40 define pre-obesity, obesity, and obesity classes I-III, respectively (Chugh and Sharma, 2012; Nuffer et al., 2016). It should be noted that there are potential differences in BMI definition as a function of ethnicity (National Obesity Observatory, 2011), and that more accurate indices of obesity exist (e.g. body composition analysis). With these caveats in mind, it is generally accepted that obesity has now reached pandemic proportions with some 1.9 billion adults overweight, more than 600 million adults obese, and over 40 million under-fives obese (WHO, 2015). Childhood and adolescent obesity is of particular concern in view of the serious long-term consequences for physical and mental health (Adair, 2008; Franks et al., 2010; Reilly and Kelly, 2011). Not only can early exposure to unhealthy eating habits lead to a greater risk of obesity in later life (Anzman et al., 2010), but the 'developmental origin hypothesis' (Volkow and O'Brien, 2007) holds that high-fat or high-sugar exposure in the womb can alter how brain and body develop in anticipation of future environments, including patterns of nutrient selection (e.g. Ong and Muhlhausler, 2011; Teegarden et al., 2009). More intriguingly still, recent research has suggested that rodent maternal obesity at conception can program brain reward circuitry in offspring by

dramatically altering the expression of opioid peptides and their receptors (Grissom et al., 2014), while human paternal and grand-paternal obesity may influence metabolic function in future generations via epigenetic re-modelling of sperm DNA methylation (Cropley et al., 2016; Donkin et al., 2016). Other important recent developments, the full ramifications of which have yet to be appreciated, concern (i) the role played by gut microbiota in the regulation of bodyweight and metabolism (Cryan and Dinan, 2012; Ridaura et al., 2013), with growing evidence that emulsifiers in processed foods significantly contribute to low-grade intestinal inflammation, obesity and the metabolic syndrome (e.g. Chassaing et al., 2015), and (ii) the therapeutic potential of pharmacologically converting potentially harmful white adipose tissue (WAT; energy storage) into physiologically more beneficial brown adipose tissue (BAT; energy dissipation) (for recent review: Giordano et al., 2016).

The health consequences of obesity not only impose serious restrictions on quality of life, they can also be life-threatening. The obese experience day-to-day problems with osteoarthritis, back pain and mobility (Lean et al., 1998) as well as breathing difficulties caused by fat store-induced reductions in lung volume (Kopelman, 2007). Furthermore, obesity is a major risk factor in the development of chronic disorders such as type-2 diabetes, hypertension, heart disease and stroke, sleep apnea and certain cancers (Kissbeah et al., 1989), and can reduce life expectancy by up to 20 years (Fontaine et al., 2003). In addition to these health costs, obesity is associated with major economic costs (e.g. Speakman and O'Rahilly, 2012). In the U.K., the annual cost of obesity and its consequences has been estimated at around £3.5 billion, a figure that doubles when overweight patients are included in the calculation. As this spend approximates 2.5% of the annual National Health Service budget (House of Commons, 2004), the clinical need for safe and effective interventions is obvious.

2. Treatment Options

Although prevention through early education and/or later retraining is a major goal, therapeutic interventions are essential for those who are currently significantly overweight or obese. Even modest reductions in bodyweight (e.g. 1-year weight loss of 5kg) can have significant health benefits including improvements in insulin sensitivity, glycaemic control and blood pressure (e.g. Goldstein, 1992). Current treatment options comprise lifestyle change, surgery and pharmacology (for review: Wyatt, 2013). Although the focus of the present review is on pharmacotherapy, it is nevertheless appropriate to briefly comment upon the other approaches - particularly since lifestyle change and surgery are very relevant to current thinking about optimal drug treatment strategies.

Lifestyle modification, including dietetic, exercise and psychological interventions, are the cornerstones of successful weight management programmes. This strategy encourages a negative energy balance, whereby calories are restricted (i.e. dieting) and/or energy expenditure increased (i.e. exercise), and has repeatedly been shown to reduce obesity and associated risk factors (Brown et al., 2009; Wadden et al., 2005). However, by itself, lifestyle modification is usually effective only in the short- to medium-term, with most patients regaining lost weight over longer timeframes (Anderson et al., 2001). As such, medication is now normally recommended as an adjunct therapy alongside or following successful lifestyle intervention (e.g. Bray, 2013; Patel, 2015; Wadden et al., 2005; 2013). Bariatric surgery, such as Roux-en-Y bypass or gastric banding, is much more effective than non-surgical interventions for weight loss and diabetes remission (Gloy et al., 2013; Stefater et al., 2013), and is currently recommended for adults with Type 2 diabetes and a BMI $\geq$ 35 (National Institute for Health and Care Excellence, 2014). Although this approach is not without significant risk (e.g. perioperative death, anastomotic leak, infection, need for re-operation; e.g. Puzziferri et al., 2014), the impact of bariatric surgery on gut hormone release, and the importance of these

biochemical alterations in promoting appetite suppression and weight loss, has instigated an exciting new era of anti-obesity drug development based on gut peptide combinations (see Section 4).

3. 'Magic bullets' in 20th Century

Drug treatment for obesity generally falls into one of three (non-mutually exclusive) categories: appetite suppressants, inhibitors of fat absorption, and/or agents that increase energy expenditure and thermogenesis (Li and Cheung, 2009). However, as detailed in many recent reviews (e.g. Adan, 2013; Bray and Greenway, 2007; Colon-Gonzalez et al., 2013; Heal et al., 2012; Jones and Bloom, 2015; Krentz et al., 2016; Rodgers et al., 2012), the record of anti-obesity drug development since the beginning of the twentieth century (the search for so-called 'magic bullets') has for the most part been far from glorious. Many treatments have been tried, tested, government-approved and introduced to clinical practice, only to be subsequently withdrawn in the face of significant adverse ('off-target') effects. In brief, agents that succumbed to this rather ignominious fate during C20 include sheep thyroid extract (cardiovascular risk), dinitrophenol (potentially fatal hyperthermia), *dex*-amphetamine and closely related compounds (addiction potential & cardiovascular risk), serotonin releasers such as *dex*-fenfluramine/Redux® (pulmonary hypertension), and a combination of fenfluramine and the sympathomimetic drug phentermine, Pondimin® (cardiac valvulopathy). A similar fate has more recently befallen the cannabinoid CB1 receptor antagonist/inverse agonist rimonabant/ Acomplia® (psychiatric risk) and the dual noradarenaline/serotonin reuptake inhibitor sibutramine/ Merida®/Reductil® (cardiovascular risk).

Until very recently, therefore, European clinicians have been left with but a single approved anti-obesity medication; the pancreatic lipase inhibitor, orlistat (Xenical®). Weight loss with this compound tends to be modest (circa 3kg in 12 months) but of sufficient magnitude to have beneficial effects on cardiovascular risk (e.g. Torgerson et al., 2004). Although relatively mild by

comparison with other agents, adverse effects of reduced fat absorption include diarrhoea, flatulence, bloating, abdominal pain and dyspepsia (Bray and Greenway, 2007). Despite this bleak state of affairs, major advances in our understanding of the multiplicity of central and peripheral signaling mechanisms regulating appetite and energy homeostasis (e.g. Broberger, 2005; Sohn et al., 2013; Stuber and Wise, 2016; Williams and Elmquist, 2012) have very recently led to the formal approval of several new anti-obesity drugs. These include the serotonin 5-HT_{2C} receptor agonist, lorcaserin (Belviq®), a compound formally approved in the USA by the Food and Drug Administration (FDA) in 2012 but for which the European marketing application was withdrawn in May 2013 due to remaining concerns about potential carcinogenic, cardiovascular and psychiatric risk: http://www.ema.europa.eu/docs/en_GB/document_library/Medicine_QA/2013/05/WC500143811.pdf . Another compound recently approved both by the FDA (2014) and the European Medicines Agency (EMA; 2015) is the glucagon-like peptide-1 (GLP-1) receptor agonist, liraglutide (Saxenda®) (for recent oveview: see Adan, 2013; Colon-Gonzalez et al., 2013; Heal et al., 2012; Huffer et al., 2016; Jones and Bloom, 2015; Krentz et al., 2016; Patel, 2015). However, it is noticeable that new approved drug monotherapies have actually been rather thin on the ground in recent times. One explanation is that single agents, effective in producing weight loss in the short-term, are unable to counter metabolic adaptations in order for that weight loss to be maintained (e.g. Roth et al., 2010). Another significant factor here is that novel single target drugs are more likely to have unsuspected off-target effects than combinations of existing treatments where there already is substantial clinical experience with the constituent agents.

4. The modern era: drug combinations

The historically poor clinical track-record of drug monotherapies has led to growing interest in the potential advantages of drug combinations (a.k.a. combination therapy or drug polytherapy). This approach, which is designed to simultaneously engage multiple molecular targets in CNS and/or

periphery, may ultimately prove more effective in maintaining weight loss and improvements in comorbidities (Rodgers et al., 2012). The potential advantages of polytherapy include the use of lower drug doses, fewer and less serious unwanted (or 'off-target') effects, additive if not synergistic weight loss, and reduced potential for counter-regulation (Finan et al., 2015b; Roth et al., 2010). As reviewed below, combination therapy is already beginning to yield successes at both preclinical and clinical level (Field et al., 2009; Heal et al., 2012; Jones and Bloom, 2015). In this context, it is very interesting to note that, in drug development programmes, polytherapies currently have a substantially higher 'transition probability' than monotherapies (40% vs 4.74%), i.e. the probability of moving from Phase I to Phase II testing, or from Phase II to Phase III testing (Hussain et al., 2015). Nevertheless, it should be emphasised that not all low-dose anorectic drug combinations produce additive effects (e.g. GLP-1 + cholecystokinin-33 (CCK-33), Gutzwiller et al., 2004; pancreatic polypeptide (PP) + peptide YY3-36 (PYY_{3-36}), Neary et al., 2008; rimonbant + sibutramine, Tallett et al., 2010a), with some even producing sub-additive effects (e.g. naloxone + sibutramine, Tallett et al., 2010b; topiramate + metformin, Toplak et al., 2007). And, in view of the nature of commerce and of scientific publication, there are probably many other ineffective combinations that have not reached the light of day. Therefore, no matter how well founded, assumptions about the potential efficacy of treatment combinations cannot and must not replace careful empirical research.

Tables 1-4

With this important caveat in mind, Tables 1-4 list treatment combinations that have been shown to exert additive or synergistic suppressant effects on food intake and/or bodyweight in preclinical and/or early clinical research. Some have also shown improvements in comorbidities such as insulin sensitivity, glycaemic control, dyslipidaemia and/or hypertension. Following a scheme initially

presented by Roth and colleagues (2010), combination treatments can involve: small molecule combinations (Table 1), small molecule-peptide combinations (Table 2), or small molecule-leptin, peptide-leptin or peptide-peptide combinations (Table 3). Furthermore, as summarised in Table 4, exciting recent advances have resulted in the creation of chemically-linked peptides ('phybrids') with a dual action, as well as novel single molecules ('chimeras') acting either as co-agonists or tri-agonists at two or three independent targets, respectively (Finan et al., 2015a; Tschöp et al., 2016).

It is clearly beyond the scope of this review to provide detailed profiles for all listed treatments; such information can be readily obtained from the primary sources given in Tables 1-4 and/or a host of excellent reviews published over the last decade (Adan, 2013; Bray and Greenway, 2007; Chugh and Sharma, 2012; Colon-Gonzalez et al., 2013; Field et al., 2009; Halford et al., 2010; Harrold et al., 2012; Heal et al, 2012; Jones and Bloom, 2015; Kennett and Clifton, 2010; Krentz et al., 2016: Li and Cheung, 2009; Manning et al., 2014; Nuffer et al., 2016; Patel, 2015; Rodgers et al., 2012). However, it is worth noting that the specific rationales underlying polytherapies have rapidly evolved in a short space of time - from almost pure pragmatism to physiological sophistication. For example, some combinations seem to have involved simply putting together low doses of two established anorectic agents, while others have combined agents believed to exert their effects by, for example, simultaneously targeting receptor populations involved in satiety signalling to the brain as well as forebrain reward-related mechanisms. More sophisticated still is the thinking behind the development of 'leptin sensitisers', Contrave®/Mysimba®, and the more recently developed peptide phybrids and chimeras.

It has long been recognised that the phenomenon of leptin resistance in the obese renders this molecule per se an ineffective therapy for weight loss. However, researchers have recently begun to use 'sensitising pharmacology' to unleash the weight-lowering properties of leptin. As summarised

by Quarta and colleagues (2016), compounds that appear to reinstate leptin sensitivity include amylin, exendin-4, GLP-1/glucagon, PYY_{3-36}, and CCK (see also Table 3). In addition to this potentially valuable development, both the FDA and EMA have recently (2014) given formal approval to the combination of bupropion (an atypical antidepressant) and naltrexone (an opioid receptor antagonist) as a treatment for obesity: in the USA, this combination is known as Contrave® and, in Europe, as Mysimba®. The rationale underlying this particular drug combination is based on the argument (Greenway et al., 2009, 2010; Wadden et al., 2011) that the limited anorectic/weight loss effect of bupropion is the result of a negative feedback loop whereby bupropion-induced activation of hypothalamic pro-opiomelanocortin (POMC) neurons results in a μ-opioid receptor-mediated inhibition of these very same neurons. As confirmed in electrophysiological and behavioural experiments, this negative feedback loop is inactivated by the addition of naltrexone (a broad spectrum opioid receptor antagonist), leading to a disinhibition of POMC neurons and a stronger anorectic response to bupropion.

The development of peptide phybrids and chimeras is particularly exciting for at least two reasons. Firstly, the sometimes intense nausea associated with anorectic dose levels of individual peptides (such as PYY_{3-36}, CCK, pramlintide, oxyntomodulin, exenatide and liraglutide) limits their clinical usefulness due to narrow therapeutic windows (Field et al., 2009). And, secondly, research showing that bariatric surgery (such as Roux-en-Y bypass), widely acknowledged as the most effective treatment for obesity (Stefater et al., 2013), alters the secretion pattern of several gut hormones (Kellum et al., 1990; Le Roux et al., 2006; Moringo et al., 2006). As this altered pattern of peptide secretion is thought to be largely responsible for the surgically-induced weight loss, simultaneous treatment with (lower doses of) two or more gut peptides is an entirely logical development. To exemplify the thinking behind peptide chimeras, Day and colleagues (2009) have reported on a novel peptide with dual agonism at glucagon and GLP-1 receptors (see also Pocai et al., 2009). The

pancreatic hormone, glucagon, has well-established thermogenic, anorectic, and weight loss effects in animals, whereas GLP-1 receptor agonists (such as exenatide & liraglutide) are known to improve glycaemic control and weight loss in humans with type 2 diabetes. Day et al. (2009) reasoned, and subsequently confirmed, that the antihyperglycaemic effect of GLP-1 receptor agonism could minimise any diabetogenic risk of excessive glucagon agonism, and further argued that the lipophilic and thermogenic propreties of glucagon as well as the satiating effects of GLP-1 agonism provide a very strong scientific basis for the development of a synergistic co-agonist peptide. A similar rationale underlies the very recent development of a peptide tri-agonist which simultaneously stimulates glucagon, GLP-1 and gastric inhibitory polypeptide (GIP) receptors (Finan et al., 2015b; Tschöp et al., 2016).

5. The importance of behaviour

Despite these encouraging recent developments, it must not be forgotten that behaviour is the interface between the organism and its environment. Whether rodent or human, behaviour is the means whereby food is located and ingested; and feeding behaviour can be suppressed by a host of factors, some specific but many much less so. Thus, in addition to vitally important controls for cardiovascular, teratological and carcinogenic risk, anti-obesity drug development programs should (but rarely do) include a variety of preclinical tests to assess potential adverse behavioural effects of treatment. Such negative 'off-target' effects could offer more parsimonious interpretations of reductions in food intake and bodyweight through, for example, pain, nausea, sedation, or response competition. Early recognition of such indirect forms of appetite suppression could save both time and money which could, in turn, be invested in more promising candidate molecules (e.g. Rodgers et al., 2010, 2012). However, despite repeated calls over the past 30 years for much more research attention to behaviours (ingestive and non-ingestive) displayed during feeding tests, the field as a whole remains somewhat 'hard of hearing' (e.g. Blundell et al., 1985; Halford et al., 1998, 2010;

Higgs et al., 2016; Rodgers et al., 2010, 2012; Vickers et al., 2011; Vickers and Clifton, 2012). With apologies for mixed metaphors, such tunnel vision undoubtedly contributed to the premature approval of the CB1 receptor antagonist/inverse agonist Acomplia® as an anti-obesity treatment in Europe, its subsequent withdrawal from the market, and the cessation (or at least moth-balling) of several related drug development programs (McLaughlin, 2012; Vickers et al., 2011). The major problem here was that of psychiatric risk linked to an unacceptably high incidence of anxious and depressive symptomatology. However, had sufficient attention been paid to the broader behavioural pharmacology of CB1 receptor ligands, including known effects in animal tests of anxiety and cognition, this particular debacle could surely have been avoided.

Krentz et al (2016) have recently commented that limitations in the translation of the pharmacological effects of anti-obesity drugs from animals to humans have historically been evident for both efficacy (e.g. species differences in metabolic regulation) and toxicity (e.g. valvulopathy). In response to such issues, new FDA approvals must now include a risk evaluation and mitigation strategy (REMS), stipulation of post-marketing safety trials (e.g. cardiovascular risk), and clear rules for the discontinuation of treatment. A vitally important related issue concerns the behavioural specificity of new (and, indeed, existing) treatments. As reviewed in some depth by Vickers and colleagues (Vickers and Clifton, 2012; Vickers et al., 2011), animal models actually have excellent predictive validity in appetite research whereby drug-induced weight loss in rodents subsequently translates into weight loss in humans. In this context, numerous animal tests have been developed to explore short- and long-term treatment effects on food intake in lean and (genetic or diet-induced) obese rodents. Such tests range from the simple study of food intake per se, through analyses of meal patterning and microstructure (i.e. meal size, meal frequency, inter-meal intervals), to the detailed study of the various behaviours displayed during feeding tests and control experiments for drug-induced malaise and aversion (i.e. tests of conditioned taste aversion, pica,

and taste reactivity). Of course, one of the most salient questions about drug-induced appetite suppression concerns behavioural specificity. As noted many years ago by Blundell and McArthur (1981), with their concept of 'behavioural flux', feeding behaviour does not exist in isolation but rather is embedded in a constellation of related behaviours. One obvious way to at least get an initial handle on the question of the behavioural specificity of anorectic drug action is to assess treatment effects not just on the target behaviour (feeding) but also on all the other behaviours displayed during the feeding test, such as drinking, grooming, sniffing, locomotion, rearing and resting. Such comprehensive behavioural profiling, when used in conjunction with dose-response analysis and videorecording, greatly facilitates the assessment of behavioural selectivity. Furthermore, this approach also permits detailed study of the normal structure of feeding behaviour itself by focusing on the temporal transition from feeding through grooming to resting, a pattern most commonly referred to as the behavioural satiety sequence (BSS; Antin et al., 1975; Blundell et al., 1985; Montgomery and Willner, 1988).

In brief (see comprehensive reviews by Halford et al., 1998; Rodgers et al., 2010), several parametric calibration studies have shown that natural influences on appetite temporally advance (preloading) or delay (fasting) the BSS without altering its basic structure. In contrast, manipulations that either induce nausea (lithium) or adulterate the taste of food (quinine) disrupt the basic structure of the sequence as well as its temporal profile. These behavioural 'signatures' of selectivity and non-selectivity, respectively, can in turn be used both to profile established anorectics and to assess the effects of agents in development (Vickers and Clifton, 2012; Vickers et al., 2011). Rodgers and colleagues (2010) detail the results of studies to have employed this methodology between 1975 and 2010. Although the vast bulk of that review concerned drug monotherapies, it did touch upon some of the then very recent data on the effects of certain drug combinations. Suffice it to say that, in the interim, and despite its obvious advantages in helping to differentiate selective and non-

selective anorectic drug action (*'separating the wheat from the chaff'*; Rodgers et al., 2010), only a handful of studies have employed BSS methodology (or indeed, any detailed behavioural methodology) to examine the effects of novel drug combinations.

The following section summarises recent work in the Leeds laboratory that has looked at the acute effects of various drug combinations on food intake, ingestive behaviour and a variety of non-ingestive behaviours in well-habituated adult male hooded rats presented with palatable mash. The basic methodology is detailed in Rodgers et al. (2010), with compound- and combination- specific information given in the individual publications cited below. In brief, to accommodate well-known individual variation in intake and behaviour, our studies almost always employ a within-subjects design with treatment-appropriate washout periods between successive tests. In view of the rationale for combination therapy (see Section 4 above), drug doses are carefully selected on the basis of initial in-house dose-response studies with each of the agents to be tested. The finally selected doses are specifically chosen on the basis of their individually sub-anorectic and/or sub-maximally anorectic profiles. All experimental feeding sessions tests are videorecorded and subsequently analysed (continuous observation) by an observer blind to treatment condition. Treatment codes are broken only after all video materials have been fully analysed.

6. Effects of drug combinations on intake, ingestive and non-ingestive behaviour, and the BSS

Over the past decade, we have assessed in detail the behavioural effects of the following the anorectic agents, both on their own and in combination: the broad-spectrum opioid receptor antagonists naloxone and naltrexone; the cannabinoid CB1 receptor antagonist/inverse agonists rimonabant and AM251; the noradrenaline/ serotonin reuptake inhibitor sibutramine; the noradrenaline/dopamine reuptake inhibitor bupropion; the 5-$HT_{2C/1B}$ receptor agonist *m*-

chlorophenylpiperazine (*m*CPP); and the glucagon-like peptide-1 (GLP-1) receptor agonist, exendin-4. Interested readers are referred to the cited publications for detailed literature reviews on each agent and/or combination of agents. However, a brief summary of the acute intrinsic dose-response profiles of all compounds is warranted prior to a discussion of our drug combination studies.

6.1 Initial acute dose-response studies

In our laboratory, the opioid receptor antagonists naloxone (1.0-5.0 mg/kg IP; Tallett et al., 2008a) and naltrexone (0.1-3.0 mg/kg IP; Wright and Rodgers, 2013a) dose-dependently suppressed food intake and feeding behaviour and accelerated, but did not disrupt, the BSS. In other words, the inhibitory effects of opioid receptor antagonists on intake appear to be behaviourally-selective. Somewhat similarly, ethological analysis revealed few behavioural effects of the dual noradrenaline and serotonin reuptake inbititor sibutramine (0.5-3.0 mg/kg IP; Tallett et al., 2009a), except for dose-dependent reductions in food intake, time spent feeding and post-treatment weight gain. This profile of behavioural selectivity was supported by timebin analysis which confirmed the structural integrity the BSS but also its temporal acceleration. In contrast to these profiles, the CB1 receptor antagonist/inverse agonists rimonabant (1.5-3.0 mg/kg IP; Tallett et al., 2007a) and AM-251 (1.5-3.0 mg/kg IP; Tallett et al., 2007b) not only dose-dependently suppressed food intake and feeding behaviour, but also markedly and dose-dependently stimulated scratching and grooming. Indeed, at the higher dose level of each compound, compulsive grooming so dominated the behavioural repertoire as to completely disrupt the BSS - suggesting that the anorectic action may be indirectly mediated via response competition (grooming/scratching syndrome).

With recent FDA and/or EMA approvals, considerable interest is currently focused on the 5-HT_{2C} receptor agonist lorcaserin (Belviq®), the combination of bupropion and naltrexone (Contrave®/ Mysimba®), and the GLP-1 receptor agonist liraglutide (Saxenda®). Early research with 5-$HT_{2C/1B}$

agonists, such as 1-(3-chlorophenyl) piperazine (*m*CPP), was essential in paving the way for the more selective 5-HT_{2C} receptor agonist, lorcaserin. However, those early studies had not only reported dose-dependent suppressions of food intake and weight gain but had also raised doubts about behavioural selectivity - with evidence of excessive grooming, nausea, anxiety and hypoactivity. Work in our laboratory (0.1-3.0 mg/kg IP; Wright and Rodgers, 2014a) confirmed the anorectic efficacy of *m*CPP with robust effects on intake and feeding-related measures at the highest dose tested. However, this dose also significantly increased grooming, inhibited locomotion and sniffing, and disrupted the BSS. Similar problems may occur at anorectic dose levels of more selective 5-HT_{2C} receptor agonists, including lorcaserin (e.g. Higgins et al., 2012; 2013; Serafine et al., 2015).

Structurally related to amphetamine, bupropion acts primarily as a dual noradrenaline and dopamine reuptake inhibitor and, in clinical studies on depression, has been found to induce weight loss. In our hands (10-40 mg/kg IP; Wright and Rodgers, 2013a), bupropion produced a very modest reduction in food intake and time spent feeding at the highest dose tested (40mg/kg), an effect associated with substantial psychomotor stimulation. The latter resulted in complete disruption of the BSS leading to the conclusion that the mild anorectic response to this agent may also be secondary to 'response competition' (general behavioural stimulation). Finally, a burgeoning literature exists concerning the anti-diabetic, anorectic and weight loss efffects of glucagon-like peptide 1 (GLP-1) receptor agonists. While GLP-1 itself is metabolically unstable, more recently developed agents, such as liraglutide and exenatide (exendin-4), have longer biological half-lives and have proven to be more clinically valuable in the management of diabetes. In our laboratory, we have assessed the behavioural effects of exendin-4 (0.025-2.5 μg/kg IP; Wright and Rodgers, 2014b) in tests of palatable food consumption. Although we were able to confirm a dose-dependent suppression of intake and feeding behaviour, these effects occurred at dose levels that inhibited all

active behaviours and which disrupted the BSS. As for many of the agents we have examined over the past decade, the behavioural signature of exendin-4 is suggestive of behavioural non-specificity.

6.2 Drug combination studies

The above summary of our dose-response analyses raises significant doubts about the behavioural selectivity of the anorectic response to the vast majority of compounds tested. Indeed, the only drugs for which no real doubt exists are the opioid receptor antagonists naloxone and naltrexone, and the dual noradrenaline/serotonin reuptake inhibitor sibutramine. In view of these findings, and consistent with the philosophy of drug combination therapy (see Section 4), we have since conducted a series of studies in which we have looked in detail at the behavioural effects of co-treatment with low (sub-anorectic and/or submaximally anorectic) doses of many of these compounds. Would such combinations result in statistically-significant additive or synergistic effects on food intake and feeding behaviour and, if so, would they be devoid of adverse 'off-target' effects? In confirmation of the need for empirical investigation in this field, not all of these studies revealed clinically-relevant positive interactions (section 4 above). Other drug combinations, however, revealed some intriguing patterns of interaction which, in view of their potential clinical implications, are discussed in more detail below.

<u>CB1 and opioid receptor antagonist co-treatment</u>

Our initial work in this area stemmed from concern about the compulsive grooming and scratching syndrome seen with anorectic doses of CB1 receptor antagonist/inverse agonists, an off-target effect also commonly reported in human studies (e.g. Addy et al., 2008). We hoped to minimise this syndrome, yet achieve a significant reduction in food intake and feeding behaviour, by combining low doses of these agents with a low doses of the opioid receptor antagonist naloxone. Our results confirmed that, when given alone, lower doses of rimonabant (0.25, 0.75 mg/kg; Tallett et al., 2008b)

and AM-251 (0.5, 1.0 mg/kg; Tallett et al., 2009b) failed to significantly suppress appetite and induced a lower intensity grooming/scratching response to that seen with higher doses of these drugs. To our pleasant surprise, not only did the addition of low dose naloxone (0.1 mg/kg) to CB1 antagonist treatment result in a significant additive effect on food intake and feeding behaviour, it also significantly attenuated the compulsive grooming and scratching syndrome. In a more recent study (Wright and Rodgers ,2013b), we used the anti-pruritic efficacy of naloxone to directly test the 'response competition' hypothesis of rimonabant-induced anorexia (section 6.1). More specifically, we argued that if the anorectic response to rimonabant (1.5mg/kg) were due to response competition from grooming and scratching, then blocking the latter with low dose naloxone should attenuate if not eliminate the former. In the event, our findings unequivocally rejected the response competition hypothesis in that naloxone completely blocked the pruritic, but not the anorectic, response to rimonabant. It would therefore appear that the suppression of food intake and the induction of grooming/scratching, while concurrent, are actually independent effects of CB1 receptor antagonist/inverse agonists.

Bupropion and naltrexone co-treatment

The novel anti-obesity agent Contrave® (a.k.a. Mysimba®) is a combination of the atypical antidepressant bupropion and the opioid receptor antagonist naltrexone. The scientific rationale for this specific combination is discussed in Section 4 above, with proof-of-concept demonstrated in both animal and human studies (Greenway et al., 2009, 2010; Wadden et al., 2011). However, as seen so many times before, research on this treatment has focused almost entirely upon food intake and weight gain with little attention to behaviour. Of particular concern was the significant psychomotor stimulation observed in rodents treated with bupropion alone (Section 6.1), an off-target effect consistent with the agitation and insomnia observed with this uptake blocker in humans (British National Formulary, 2013). We have recently employed BSS methodology to

examine the effects of acute co-treatment with a sub-maximally anorectic dose of bupropion (20 mg/kg) and either a sub-anorectic (0.1 mg/kg) or a sub-maximally anorectic (1.0 mg/kg) dose of naltrexone (Wright and Rodgers, 2013a). Co-administration of these agents not only produced an additive anorectic profile, but the addition of the opioid receptor antagonist also attenuated the psychomotor stimulant response to bupropion as well as its disruptive effects on the BSS. These findings not only confirm existing reports of a positive anorectic interaction between these two agents but also provide evidence that co-treatment with naltrexone may counter the 'off-target' psychostimulant effects of bupropion.

Exendin-4 and naltrexone co-treatment

As reviewed in section 6.1 above, the GLP-1 receptor agonist, exendin-4, dose-dependently suppressed food intake and feeding behaviour in male rats. However, these effects (& especially those seen at the highest dose tested, 2.5 µg/kg) were accompanied by significant reductions in all active behaviours. In view of the above encouraging results with opioid receptor antagonist co-treatment, we sought to determine the behavioural effects of combining a sub-maximally anorectic dose of naltrexone (0.1 mg/kg) with either a sub-anorectic (0.025 µg/kg) or a sub-maximally anorectic (0.25 µg/kg) dose of exendin-4 (Wright and Rodgers, 2014b). However, our results showed that, while naltrexone and the higher dose of exendin-4 each produced a significant suppression of intake and feeding behaviour (plus an acceleration in the BSS), co-treatment failed to produce stronger effects than those seen in response to either treatment alone.

This lack of anorectic interaction between naltrexone and exendin-4 contrasts with our other negative combination treatments (Tallett et al., 2010a, 2010b; Wright and Rodgers, 2014a) in that the latter only included sub-anorectic doses of each agent. In the Rodgers and Wright (2014b) study, however, even the combination of (albeit sub-maximally) anorectic doses of naltrexone and exendin-

4 failed to produce an effect greater than either agent alone. Of relevance in this context is a report by Liang and colleagues (2013) in which an additive anorectic profile for (similar doses of) naltrexone and exendin-4 was described. As ever, there were are large number of methodological differences between the studies that may have accounted for the differing outcomes. However, perhaps the most salient difference concerned the method of drug administration. Thus, whereas we administered the agents as two injections spaced 15min apart, Liang et al (2013) gave the two agents as a single injection. Our spaced injection methodology may therefore have inadvertently resulted in an unpredictable interaction whereby the agent injected first (exendin-4) somehow interefered with the pharmacokinetics and/or pharmacodynamics of the agent given second (naltrexone). This working hypothesis remains to be empirically tested. Nevertheless, as Liang et al (2013) reported that the co-treatment not only produced an additive anorectic response but also intensified the aversive effects of exendin-4 (assessed by conditioned taste aversion), neither their results nor ours would support this drug combination in the clinical management of obesity.

7. Conclusions and future directions

'Bench to bedside in appetite research: lost in translation?' is so entitled in order to emphasise the quite marked discrepancy between our detailed understanding of the neurobiology of appetite and the paucity of drugs currently licensed for the treatment of obesity. This discrepancy is not, however, due to a lack of preclinical research on the behavioural pharmacology of appetite. Rather, it is to a significant degree due to the inadequacy of that research base. In other words, there is a world of a difference between describing an anorectic drug effect in terms of reduced food intake and/or bodyweight (outcome) and understanding how such effects have been produced (process). Of course, in some cases, the process involves a reduction in nutrient absorption; in other cases, it entails increases in energy expenditure. However, in the vast majority of cases, process involves changes in some aspect of behaviour. It is our contention that, at the preclinical level at least, such

changes can only be characterised through detailed behavioural analysis. This article has concentrated on just one such approach, a detailed analysis of behaviours (non-ingestive as well as ingestive) displayed during tests of food intake, including the BSS. Other forms of behavioural analysis are of course available and, in many cases, essential as follow-ups to BSS analysis, e.g. analysis of meal patterning, conditioned taste aversion, taste reactivity, and progressive ratio responding. Similarly, more detailed pharmacological studies (e.g. isobolographic analyses; Roth et al., 2010) would be essential in order to define optimum dose ratios for any combination therapy.

Of the 8 drugs reviewed above, only three (naloxone, naltrexone, sibutramine) suppressed appetite and feeding behaviour without concurrently compromising other behaviours and/or disrupting the BSS. Initial dose-response studies parsimoniously suggested that the anorectic efficacy of CB1 receptor antagonists (rimonabant; AM-251) was due to response competition from excessive grooming and scratching while that of the atypical antidepressant bupropion was due to response competition from psychomotor stimulation. However, in the event, neither hypothesis was supported in follow-up co-treatment studies with opioid receptor antagonists. Those experiments were designed to assess whether sub-anorectic dose combinations would interact to produce a significant suppression of appetite. This was indeed achieved for rimonabant + naloxone, AM-251 + naloxone, and bupropion + naltrexone. The unexpected bonus, and as further evidence of the value of behavioural analyses, was the finding that the addition of an opioid receptor antagonist markedly reduced 'off-target' effects of both the CB1 receptor antagonist/inverse agonists (i.e. pruritus) and bupropion (i.e. psychomotor stimulation). The former result would suggest that any pruritic activity of CB1 receptor ligands still in development could be attenuated by opioid receptor antagonists, whereas an additional benefit of adding naltrexone to bupropion (as in Contrave®) might be the attenuation of any tendency of the latter to induce psychomotor stimulation. These insights would

not have been possible without detailed behavioural analysis - which is precisely what is missing (lost) in the attempted translation from bench to bedside in appetite research.

As commented by Jones and Bloom (2015), we have very recently witnessed the introduction of the first novel anti-obesity agents for more than a decade. However, tempering current enthusiasm surrounding such developments, they go on to argue that knowledge of the off-target effects of many of these agents remains incomplete. The latter of course is of crucial importance to the eventual status of the new kids on the block, and must surely incorporate the type of behavioural analyses advocated in the current article. This message, although obvious and straightforward, needs to be repeatedly emphasised. Otherwise, this field will continue to experience problems in the journey from bench to bedside.

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Table 1 Small molecule combinations that exert additive or synergistic suppressant effects on food intake and/or bodyweight. The table is not intended as an exhaustive listing. See text for detail.

Combination	Principal effect of each component	References
Bupropion/naltrexone (Contrave®; Mysimba®)[1]	NA & DA reuptake blocker/opioid receptor antagonist	*Greenway et al (2009, 2010); Wadden et al (2011); Billes et al (2014); Wright & Rodgers (2013a)*
Bupropion/zonisamide (Empatic®)[2]	NA & DA reuptake blocker/anticonvulsant	*Orexigen Therapeutics (2009)*
Phentermine/d-fenfluramine (Pondimin®)[3]	Sympathomimetic/serotonin releaser	*Grottick et al (2015); Roth & Rowland (1999); Weintraub et al (1992)*
Phentermine/lorcaserin or AR630	Sympathomimetic/5-HT2C receptor agonist	*Grottick et al (2015)*
Rimonabant or AM251/naloxone or naltrexone	Cannabinoid CB1 receptor antagonist/ opioid receptor antagonist	*Chen et al, (2004); Kirkham & Williams (2001); Lockie et al (2011); Lockie et al (2015); Rowland et al (2001); Tallett et al (2008b; 2009b); Wright & Rodgers (2013b)*
R**imonabant/*m*CPP**	CB1 receptor antagonist/ 5-$HT_{2C/1B}$ receptor agonist	*Ward et al (2008)*
Rimonabant/d-fenfluramine	CB1 receptor antagonist/5-HT releaser	*Rowland et al (2001)*
Rimonabant/Snap-94847	CB1 receptor antagonist/Melanin Concentrating Hormone antagonist	*Verty et al (2013)*
Naloxone/5-hydroxytrptophan (5-HTP)	Opioid receptor antagonist/5-HT precursor	*Fernandez-Tome et al (1988)*
Naloxone/fluoxetine	Opioid receptor antagonist/5-HT reuptake blocker (SSRI)	*Hagan et al (1997)*
Topiramate/phentermine (Qsymia®; Qnexa®; Qsiva®)[4]	Anticonvulsant/sympathomimetic	*Vivus 2010*
Topiramate/*m*CPP	Anticonvulsant/5-$HT_{2C/1B}$ receptor agonist	*Ward et al (2008)*
Experimental compounds	NPY1 receptor antagonist/NPY5 receptor antagonist	*Mashiko et al (2009)*
Linagliptin/GLP-1	DPP-IV inhibitor/GLP-1 receptor agonist	*Hansen et al (2014)*

DA = dopamine; DPP-IV = dipeptidyl peptidase IV; GLP-1 = glucagon-like peptide1 ; 5-HT = 5-hydroxytryptamine; NA = noradrenaline; NPY = neuropeptide Y; SSRI = serotonin selective reuptake inhibitor

[1]FDA/EMA approved 2014; [2]Phase III planning 2016; [3]withdrawn 1997; [4]FDA (but not EMA) approved 2012

Table 2 **Small molecule-peptide combinations** that exert additive or synergistic suppressant effects on food intake and/or bodyweight. The table is not intended as an exhaustive listing. See text for detail.

Combination	Principal effect of each component	References
Phentermine/amylin or pramlintide	Sympathomimetic/pancreatic hormone or analogue	*Aronne et al (2010); Roth et al (2008b)*
Sibutramine/amylin or pramlintide	NA & 5-HT reuptake blocker/pancreatic hormone/analogue	*Aronne et al (2010); Roth et al (2008b)*
Topiramate/amylin	Anticonvulsant/pancreatic hormone or analogue	*Lalonde et al (2004)*
Rimonabant/amylin	Cannabinoid CB1 receptor antagonist/pancreatic hormone or analogue	*Boustany-Kari et al (2011)*
Bupropion+naltrexone/amylin	NA & DA reuptake blocker + opioid receptor antagonist/pancreatic hormone or analogue	*Clapper et al (2013)*
Rimonabant or AM251/ GLP-1 analogues **Rimonabant or AM251/GIP antagonist**	Cannabinoid receptor antagonist/GLP1 receptor agonists CB1 receptor antagonist/ gastric inhibitory polypeptide antagonist	*Bojanowska & Radziszewska(2011); Patel et al (2014); Radziszewska et al (2014)* *Irwin et al (2008)*
Naltrexone/GLP-1 analogues	Opioid receptor antagonist/GLP1 receptor agonists	*Liang et al (2013); but see Wright & Rodgers (2014b)*
RM-493/liraglutide	Melanocortin-4 receptor agonist/GLP-1 receptor agonist	*Clemmensen et al (2015)*
GLP-1 analogue/steroid hormone (conjugate)	GLP-1 receptor agonist/oestrogen	*Finan et al (2012)*

DA = dopamine; GIP = gastric inhibitory polypeptide; GLP-1 = glucagon-like peptide1 ; 5-HT = 5-hydroxytryptamine; NA = noradrenaline

Table 3 **Other combined treatments (small molecule/leptin; peptide/leptin; peptide/peptide*)** that exert additive or synergistic suppressant effects on food intake and/or bodyweight. The table is not intended as an exhaustive listing. * see also Table 4 for novel peptidergic phybrid and chimeric compounds. See text for detail.

Combination	Principal effect of each component	References
Small molecule/leptin		
Rimonabant or AM-251/leptin	Cannabinoid CB1 receptor antagonists/adiposity signal	*Boustany-Kari et al (2011); Wierucka-Rybak et al.(2014)*
Sibutramine/leptin	NA & 5-HT reuptake blocker/adiposity signal	*Boozer et al (2001)*
Topiramate/leptin	Anticonvulsant/adiposity signal	*Lalonde et al (2004)*
Liraglutide/leptin	GLP1 receptor agonist/adiposity signal	*Kanowski et al (2015)*
Peptide/leptin		
Amylin/leptin	Pancreatic hormone/adiposity signal	*Roth et al (2008a)*
Pramlintide/leptin	Amylin analogue/adiposity signal	*Ravussin et al (2009)*
PYY_{3-36}/leptin	Gut peptide/adiposity signal	*Trevaskis et al (2008)*
GLP-1 analogues/leptin	Glucagon-like peptide 1 receptor agonists/adiposity signal	*Bojanowska & Nowak (2007); Reidelberger et al (2011a)*
CCK/leptin	Original gut satiety peptide/adiposity signal	*Emond et al (1999); Trevaskis et al (2010)*
Peptide/peptide		
CCK/bombesin & glucagon	Original gut satiety peptide/gut peptide & pancreatic hormone	*Hinton et al (1986)*
CCK/amylin	Original gut satiety peptide/pancreatic hormone	*Bhavsar et al (1998)*
Amylin/PYY_{3-36}	Pancreatic hormone/gut peptide	*Roth et al (2007)*
Amylin/GLP analogues	Pancreatic hormone/GLP-1 receptor agonists	*Roth et al (2012)*
GLP-1 analogues/calcitonin	GLP1 receptor agonists/thyroid hormone	*Bello et al (2010)*
GLP-1 analogues/PYY_{3-36}	GLP1 receptor agonists/gut peptide	*Neary et al (2005); Paulik et al (2011); Reidelberger et al (2011b); Talsania et al (2005); Steinert et al (2010)*

CCK = cholecystokinin; GLP-1 = glucagon-like peptide1 ; 5-HT = 5-hydroxytryptamine; NA = noradrenaline; PYY_{3-36} = peptide YY_{3-36}

Table 4 **Novel phybrid and chimeric combinations** that exert additive or synergistic suppressant effects on food intake and/or bodyweight. The table is not intended as an exhaustive listing. See text for detail.

Combination	Principal effect of each component	References
Phybrids (linked peptides)		
Amylin/PYY3-36	Pancreatic hormones/gut peptide	*Roth et al (2010)*
Exenatide/davalintide	GLP-1 receptor agonist/amylin analogue	*Trevaskis et al (2013)*
CCK-8/GLP-1 analogue	Original gut satiety peptide/GLP-1 receptor agonist	*Irwin et al (2015)*
Chimeras		
Single molecules with co-agonism at:	GLP-1 and glucagon receptors	*Paulik et al (2011); Reidelberger et al (2011b);Day et al (2009); Pocai et al (2009); Clemmensen et al (2014)*
	GLP-1 and GIP receptors	*Finan et al (2013)*
Single molecule with tri-agonism at:	GLP-1, GIP and glucagon receptors	*Finan et al (2015b)*

CCK = cholecystokinin; GIP = gastric inhibitory polypeptide; GLP-1 = glucagon-like peptide1 ; PYY_{3-36} = peptide YY_{3-36}