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Effects of amylin and bupropion/naltrexone on food intake and body weight are interactive in rodent models ☆

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ABSTRACT

Antagonism of opioid systems (e.g., with naltrexone) has been explored as an anti-obesity strategy, and is particularly effective when co-administered with dual inhibitors of dopamine and norepinephrine reuptake (e.g., bupropion). Previously, we demonstrated that amylin enhances the food intake lowering and weight loss effects of neurohormonal (e.g., leptin, cholecystokinin, melanocortins) and small molecule (e.g., phentermine, sibutramine) agents. Here, we sought to characterize the interaction of amylin with naltrexone/bupropion on energy balance. Wild-type and amylin knockout mice were similarly responsive to the food intake lowering effects of either naltrexone (1 mg/kg, subcutaneous) or bupropion (50 mg/kg, subcutaneous) suggesting that they act independently of amylinergic systems and could interact additively when given in combination with amylin. To test this, diet-induced obese rats were treated (for 11 days) with vehicle, rat amylin (50 μg/kg/d, infused subcutaneously), naltrexone/bupropion (1 and 20 mg/kg, respectively by twice daily subcutaneous injection) or their combination. We found that amylin+naltrexone/bupropion combination therapy exerted additive effects to reduce cumulative food intake, body weight and fat mass. In a separate study, the effects of amylin and naltrexone/bupropion administered at the same doses (for 14 days) were compared to a pair-fed group. Although the combination and pair-fed groups lost a similar amount of body weight, rats treated with the combination lost 68% more fat and better maintained their lean mass. These findings support the strategy of combined amylin agonism with opioid and catecholaminergic signaling systems for the treatment of obesity.

1. Introduction

Recent successes in pharmacotherapy for weight loss focus on drug combinations that engage multiple molecular targets and regulatory systems involved in the regulation of appetite and metabolism (Heal et al., 2012). Bupropion, a dual norepinephrine and dopamine reuptake inhibitor approved as an antidepressant and smoking cessation aid (Ascher et al., 1995; Foley et al., 2006), causes mild weight loss (5%) in humans (Anderson et al., 2002; Gadde and Xiong, 2007). The proposed mechanism of bupropion is through combined actions on both norepinephrine and dopamine reuptake inhibition and selective inhibition of these transporters additively reduced body weight in mice (Billes and Cowley, 2007; 2008). Naltrexone is a selective opioid receptor antagonist approved for the treatment of opiate and alcohol abuse. In rodents, naltrexone reduces food intake and body weight (De Tomasi and Juarez, 2011) but is only associated with minimal weight loss in humans (Atkinson et al., 1985; Malcolm et al., 1985). Electrophysiology studies demonstrate that the combination of bupropion and naltrexone prolongs the activation of anorexigenic pro-opiomelanocortin neurons in the arcuate nucleus of the hypothalamus and in mice elicited a synergistic inhibitory effect on food intake (Greenway et al., 2009b). In humans, sustained release formulation of the combination causes significant weight loss (5–15%) that is greater than either agent alone (Greenway et al., 2009a, 2010; Wadden et al., 2011).

Amylin is a naturally occurring 37 amino acid peptide that is co-released with insulin from pancreatic beta cells during a meal. In animals and humans, amylin or its analogue pramlintide causes meal-ending satiation and weight loss (Lutz, 2012). In preclinical models amylin has been shown to interact with both neurohormonal (e.g., leptin, cholesystokinin and melanocortins) and small molecule (e.g., sibutramine and phentermine) agents to additively or synergistically reduce food intake and body weight (Bhavsar et al., 1998; Roth et al., 2012, 2008a, 2008b; Trevaskis et al., 2010). Moreover, in a 24 week clinical study, subjects treated with pramlintide in combination with either sibutramine or

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phentermine achieved greater total weight loss than subjects treated with pramlintide alone (Aronne et al., 2010). These findings led us to explore whether amylin would exert additive effects with other small molecule agents currently in development for the treatment of obesity.

The potential utility of combining amylin and opioid-modifying agents has not been assessed. Beyond the aforementioned reports of amylin agonism with phentermine or sibutramine the interaction of amylin with catecholaminergic agents has only been minimally explored. Hence, we investigated the interactive effects of these systems by: (1) exploring the anorexigenic effects of naltrexone and bupropion in an amylin-deficient state, (2) examining their combinatorial potential in a rodent model of obesity, and (3) evaluating whether the combination exerted effects beyond those attributable to reduced food intake.

2. Materials and methods

2.1. Animals, housing, diet and drug

All studies were approved by the Institutional Animal Care and Use Committee at Amylin Pharmaceuticals, LLC., in accordance with the Animal Welfare Act guidelines. Animals were housed individually in standard caging (mice, $6.06 \times 12.13 \times 5.33$ in.; rat, $12.13 \times 12.13 \times 7.37$ in.) at 22 °C. Mice were maintained in a 12-h light, 12-h dark cycle (lights on, 6 am–6 pm) and rats were on a reverse light cycle (lights on, 9 pm–9 am).

Acute food intake studies were performed in male amylin knockout mice or their wild-type littermates (~9–15 weeks old). The amylin knockout line was established on a mixed 129Ola/B6 background and backcrossed at least six generations onto a C57Bl/6J strain (as described in (Turek et al., 2010)). Amylin knockout and wild-type littermate controls were genotyped at weaning (Taconic, Hudson, NY, USA). All mice were maintained ad libitum on standard chow (7012; Harlan Teklad, Madison, WI, USA).

For acute and chronic diet-induced obese (DIO) rat studies, male Sprague-Dawley rats from Charles River Laboratories (CRL: CD rats; Wilmington, MA, USA) were maintained ad libitum on a high fat diet (D1226B; Research Diets) containing 32% kcal from fat for 15–20 weeks before and after treatment to induce a DIO state (~20–25 weeks old at the start of the study).

Rat amylin was synthesized at Amylin Pharmaceuticals, LLC, (San Diego, CA, USA) and was dissolved in 50% dimethyl sulfoxide/sterile water. Naltrexone was obtained from Sigma-Aldrich (St. Louis, MO, USA) and bupropion was from Sigma-Aldrich or Akaal Organics (Long Beach, CA, USA) and both were dissolved in 13% ethanol/20% propylene glycol/67% saline and injected subcutaneously at 6 or 1 ml/kg in mice and rats, respectively (Greenway et al., 2009b). For combination treatment groups, naltrexone and bupropion were co-formulated and delivered in the same solution.

2.2. Study 1: Acute effects of naltrexone and/or bupropion on food intake in amylin knockout mice

Male wild-type and amylin knockout mice were fasted overnight and injected subcutaneously with either vehicle, naltrexone (1 mg/kg) or bupropion (50 mg/kg). Mice were sorted into groups of similar body weight (in grams; wild-type/vehicle, 25.9 ± 0.4; wild-type/naltrexone, 25.3 ± 0.6; wild-type/bupropion, 25.1 ± 1.0; knockout/vehicle, 24.9 ± 0.7; knockout/naltrexone, 24.4 ± 0.7; knockout/bupropion, 25.0 ± 0.8; $n=8$/ group). Mice were re-fed immediately after dosing and cumulative food intake (corrected for spillage) was measured manually in their home cages at 30 and 60 min.

2.3. Study2: Acute effects of naltrexone and/or bupropion on food intake in DIO rats

Overnight fasted DIO rats were injected subcutaneously with vehicle, naltrexone (1 mg/kg), bupropion (20 mg/kg), or naltrexone+bupropion (1 and 20 mg/kg, respectively) and sorted into groups of similar body weight (in grams; vehicle, 505 ± 15; naltrexone, 514 ± 15; bupropion, 514 ± 18; naltrexone+bupropion, 515 ± 18; $n=5$/group). Immediately after drug administration the rats were re-fed and cumulative food intake (corrected for spillage) was measured manually in their home cages after 60 min.

2.4. Study 3: Effects of amylin, naltrexone and bupropion co-administration on 11-day food intake, body weight and body composition in DIO rats

This study was designed to test the interaction of amylin and naltrexone/bupropion on food intake, body weight and body mass composition over an 11-day treatment in DIO rats. Amylin was delivered by surgically implanted subcutaneous osmotic minipumps (Durect Corporation, Cupertino, CA, USA) at a dose (50 μg/kg/d) that has reliably shown to interact in an additive/synergistic manner with a variety of test agents (Roth et al., 2012, 2008b; Trevaskis et al., 2008). All rats were implanted with an osmotic pump that delivered either vehicle or amylin. Each rat also received twice daily injections, 60 min before the dark cycle and 6 h later, of either vehicle or naltrexone/bupropion (1 and 20 mg/kg, respectively). At the start of the study the rats were sorted into treatment groups of similar body weight (in grams; vehicle, 557 ± 11; amylin, 556 ± 9; naltrexone/bupropion, 559 ± 13; amylin+naltrexone/bupropion (combination), 560 ± 13; $n=6$/group). Food intake (corrected for spillage) and body weight was recorded daily. Body composition was measured at baseline and at termination using a nuclear magnetic resonance (NMR) instrument as described previously (Roth et al., 2006).

2.5. Study 4: Effects of combined amylin+naltrexone/bupropion treatment relative to pair-feeding on 14-day body weight, body composition and gene expression profiles in DIO rats

This study tested the effects of pair feeding on body weight, body composition and gene expression in DIO rats. Rats were sorted into treatment groups of similar bodyweight (vehicle, 621 ± 19; amylin, 622 ± 22; naltrexone/bupropion, 625 ± 20; amylin+naltrexone/bupropion (combination), 624 ± 19; pair-fed, 624 ± 17; $n=7$/group) and were dosed the same as described in study 3. The pair-fed group was restricted to the mean daily food intake of the amylin+naltrexone/bupropion combination group. Food was presented to the pair-fed group approximately 60 min before the onset of the dark cycle. After 14 days of treatment, non-fasted rats were euthanized by isoflurane overdose and tissues (hypothalamus and brown adipose tissue) were dissected and snap-frozen in liquid nitrogen for later analysis. Total RNA from hypothalamus and brown adipose tissue was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, USA) and cDNA was synthesized using the High Capacity cDNA Reverse Transcription system (Applied Biosystems, Foster City, CA, USA). Quantitative real-time polymerase chain reaction (RT-PCR) was performed in duplicate using TaqMan gene expression Assays-on-Demand and Universal PCR Master Mix in 384-well format using β-actin as an endogenous control (Applied Biosystems, Rn00667869_m1) with an ABI PRISM 7900 sequence detection system (Applied Biosystems). Assays for gene expression were pro-opiomelanocortin (POMC), Rn00595020_m1; melanocortin 4 receptor (MC4R), Rn01491866_s1; leptin receptor (LepR), Rn00561465_m1; neuropeptide Y (NPY), Rn01410145_m1; agouti-related peptide (AgRP), Rn01431703_g1; pro-melanin-concentrating