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Amylin receptor activation in the ventral tegmental area reduces motivated ingestive behavior

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ABSTRACT

Amylin is produced in the pancreas and the brain, and acts centrally to reduce feeding and body weight. Recent data show that amylin can act in the ventral tegmental area (VTA) to reduce palatable food intake and promote negative energy balance, but the behavioral mechanisms by which these effects occur are not fully understood. The ability of VTA amylin signaling to reduce intake of specific palatable macronutrients (fat or carbohydrate) was tested in rats in several paradigms, including one-bottle acceptance tests, two-bottle choice tests, and a free-choice diet. Data show that VTA amylin receptor activation with the amylin receptor agonist salmon calcitonin (sCT) preferentially and potently reduces intake of fat, with more variable suppression of sucrose intake. Intake of a non-nutritive sweetener is also decreased by intra-VTA administration of sCT. As several feeding-related signals that act in the mesolimbic system also impact motivated behaviors besides feeding, we tested the hypothesis that the suppressive effects of amylin signaling in the VTA extend to other motivationally relevant stimuli. Results show that intra-VTA sCT reduces water intake in response to central administration of the dipsogenic peptide angiotensin II, but has no effect on *ad libitum* water intake in the absence of food. Importantly, open field and social interaction studies show that VTA amylin signaling does not produce anxiety-like behaviors. Collectively, these findings reveal a novel ability of VTA amylin receptor activation to alter palatable macronutrient intake, and also demonstrate a broader role of VTA amylin signaling for the control of motivated ingestive behaviors beyond feeding.

1. Introduction

Amylin is a pancreatic- and brain-derived peptide that reduces food intake and body weight by promoting satiation (Dobolyi, 2009; Li et al., 2015; Lutz, 2005; Lutz et al., 1995b). These energy balance effects of amylin are mediated through actions in the central nervous system (CNS) (Lutz et al., 1995a). Research on amylin's feeding effects has largely focused on the hindbrain (Lutz et al., 1998; Mollet et al., 2004; Potes et al., 2010) and hypothalamus (Chance et al., 1991; Dunn-Meynell et al., 2016; Turek et al., 2010), but recent findings support the ventral tegmental area (VTA) of the mesolimbic reward system as a novel and important nucleus that mediates the energy balance effects of amylin. Amylin receptor activation in the VTA is both physiologically and pharmacologically relevant for the control of food intake and body weight gain (Mietlicki-Baase et al., 2015a, 2015b, 2013), but the behavioral mechanisms underlying these effects are unresolved. As amylin-based pharmacotherapies are thought

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to be promising for treating obesity (Jorsal et al., 2016; Sadry and Drucker, 2013), a comprehensive understanding of the mechanisms by which VTA amylin signaling suppresses feeding is critical.

Human obesity is driven in part by the overconsumption of energy-dense, highly palatable foods (Meye and Adan, 2014; Rolls, 2007). Interestingly, VTA amylin signaling has particularly potent suppressive effects on the intake of palatable foods. For example, acute administration of an amylin receptor agonist directly into the VTA suppresses intake of a high-fat diet (HFD) at lower doses than those required for suppression of chow intake (Mietlicki-Baase et al., 2013, 2015b). Furthermore, rats with AAV-mediated knockdown of VTA amylin receptors exhibit chronic hyperphagia and weight gain when maintained on HFD, with minimal to no effect on food intake or body weight in chow-fed rats (Mietlicki-Baase et al., 2015b). As HFD is a mixed-macronutrient diet that is elevated in both fat and refined carbohydrate (sucrose), it remains unclear whether amylin may affect intake of HFD based on reduced incentive salience for one or both of these macronutrients. Indeed, previous work suggests that systemic amylin can impact intakes of isolated macronutrients (Michel et al., 2007). However, this possibility has not been empirically tested.

Here, the effect of VTA amylin receptor activation on macronutrient intake is investigated in a series of one-bottle acceptance and two-bottle choice tests in which rats have access to carbohydrate and/or fat solutions. A free-choice diet model (la Fleur et al., 2010) is also used to evaluate the impact of VTA amylin signaling on food selection when animals have a simultaneous choice of pure fat, pure carbohydrate, and chow. To test the possibility that VTA amylin receptor activation suppresses other motivated ingestive behaviors, water intake as well as intake of a non-nutritive sweetener are evaluated after VTA injection of an amylin receptor agonist. Finally, an important consideration is that some feeding-related peptides can induce anxiety-like behaviors in rodents (Anderberg et al., 2016; Gulec et al., 2010; To and Bagdy, 1999), and hypophagia can be induced by anxiety-like responses (Blasio et al., 2014; Strongman, 1965). Therefore, to rule out the possibility that the suppression of feeding/motivated behaviors by VTA amylin is due to anxiogenesis, the effect of VTA amylin receptor activation on anxiety-like behaviors is tested in two separate paradigms. Collectively, the results of these studies suggest that amylin signaling in the VTA has more potent suppressive effects on fat intake versus carbohydrate intake, and that motivated fluid intake is reduced by VTA amylin receptor activation. These suppressive effects on motivated ingestive behavior following VTA amylin receptor activation occur without producing anxiety-like behaviors, collectively revealing a more expansive role for mesolimbic amylin receptor signaling in modulating motivated behaviors beyond feeding.

2. Material and methods

2.1. Animals

Adult male Sprague-Dawley rats (Charles River) were individually housed in hanging wire cages in a temperature- and humidity-controlled environment, under a 12 h-12 h light cycle. Except where noted, rodent chow (Purina 5001) and water were available *ad libitum*. Procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Experiments were conducted using a counterbalanced within-subjects design, except where noted. Treatments were separated by at least 48 h.

2.2. Drugs

Salmon calcitonin (sCT; Bachem) was dissolved in artificial cerebrospinal fluid (aCSF; Harvard Apparatus) for central injections and in sterile 0.9% saline for peripheral injections. Angiotensin II (AngII; Bachem) and AC187 (Tocris) were dissolved in aCSF for central injections. Behaviorally relevant doses were selected from the literature (Daniels et al., 2005; Mietlicki et al., 2009; Mietlicki-Baase et al., 2013, 2015b).

2.3. Surgeries

After one week of acclimatization, rats were anesthetized via IM injection of a cocktail of ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (0.64 mg/kg) and placed into a stereotaxic apparatus. Rats were surgically implanted with a bilateral guide cannula (Plastics One; 26-ga) aimed at the VTA (guide cannula coordinates: ±0.5 mm lateral to midline, 6.8 mm posterior to bregma, 6.6 mm ventral to skull; internal cannula aimed 8.6 mm ventral to skull). For AngII experiments, rats also were implanted with a single guide cannula aimed at the lateral ventricle (LV; guide cannula coordinates: 0.9 mm posterior to bregma, 1.6 mm lateral to midline, 2.8 mm ventral to skull; internal cannula extended 1.5–2.5 mm beyond guide cannula, based on functional verification). Analgesia was provided for all surgical procedures (2 mg/kg meloxicam, SC).

2.4. Verification of cannula placements

LV cannula placement was verified prior to behavioral testing by injecting a dipsogenic dose of AngII (10 ng in 1 μl aCSF) and measuring subsequent water intake. Animals passed this functional verification if they drank at least 5 ml of water in the 30min post-injection period (Mietlicki et al., 2009). VTA cannula placements were verified post-mortem via injection of 100 nl pontamine sky blue ink. A representative image of VTA cannula placement is shown in Fig. 1A, based on coordinates of Paxinos and Watson (1998). Only data from animals with correct cannula placements were included in analyses.

2.5. Intake of fat and carbohydrate solutions

Rats were trained to consume macronutrient solution(s) for at least 4 days prior to initial testing. During training and testing, rats were deprived of food and water for 1.5 h prior to a 60min access bout to macronutrient solution(s). No other food or water were available during this access bout. Buret access began 30min after lights on. On test days, rats received a unilateral intra-VTA injection of sCT (0.04 μg) or vehicle (100 nl aCSF) just before macronutrient access, and intakes of the solution(s) were measured every 10min for the 60min access period. The dose of sCT was selected from our previous work and is subthreshold for prolonged effects on feeding when delivered directly into the cerebroventricular system (Mietlicki-Baase et al., 2013). Fat solution consisted of Intralipid® (Baxter; soybean oil-based emulsion), while sucrose was used for the carbohydrate solution. Sucrose was made with deionized water (DIW), and Intralipid® was diluted with DIW as needed. Due to the within-subjects design of the experiments, it was ensured that at least one training (e.g., non-injection) day always preceded the next test day. Separate groups of rats were used for each of these fat/carbohydrate intake studies except where noted.

First, rats (n = 7) had simultaneous access to two burets, one of which contained 20% Intralipid® (2 kcal/ml) and the other, 10% sucrose (0.4 kcal/ml). On test days, intakes of Intralipid® and sucrose were measured to the nearest 0.1 ml. To evaluate the effect on