Original article

MOLECULAR METABOLISM

Limiting glucocorticoid secretion increases the anorexigenic property of Exendin-4

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ABSTRACT

Objective: Glucagon-like peptide-1 (GLP-1) analogs are attractive options for the treatment of type II diabetes and obesity because of their incretin and anorexigenic effects. Peripheral administration of the GLP-1R agonist Exendin-4 (Ex-4) also increases glucocorticoid secretion in rodents and humans, but whether the released glucocorticoids interact with Ex-4's anorexigenic effect remains unclear.

Methods: To test this, we used two experimental approaches that suppress corticosterone secretion and then assessed Ex-4 effects on eating in adult male rats. First, we combined acute and chronic low dose dexamethasone treatment with Ex-4. Second, we ablated hindbrain catecholamine neurons projecting to the hypothalamus with anti-dopamine-β-hydroxylase-saporin (DSAP) to block Ex-4-induced corticosterone secretion.

Results: Combining dexamethasone and Ex-4 produced a larger acute anorexigenic effect than Ex-4 alone. Likewise, chronic dexamethasone and Ex-4 co-treatment produced a synergistic effect on eating and greater body weight loss in diet-induced obese rats than Ex-4 alone. DSAP lesions not only blunted Ex-4's ability to increase corticosterone secretion, but potentiated the anorexigenic effect of Ex-4, indicating that Ex-4-dependent corticosterone secretion opposes Ex-4's actions. Consistent with the enhancement of Ex-4's anorexigenic effect, DSAP lesion altered Ex-4-dependent changes in neuropeptide Y, preproglucagon, and corticotropin releasing hormone gene expression involved in glucocorticoid feedback.

Conclusions: Our findings demonstrate that limiting glucocorticoid secretion and actions with low dose dexamethasone or DSAP lesion increases Ex-4's ability to reduce food intake and body weight. Novel glucocorticoid receptor based mechanisms, therefore, may help enhance GLP-1-based obesity therapies.

Keywords GLP-1; Exendin-4; Corticosterone; Anti-dopamine-β-hydroxylase-saporin; Hypothalamus; Neuropeptide Y

1. INTRODUCTION

Endogenous glucagon-like peptide-1 (GLP-1) contributes to glycemic control by enhancing glucose-induced insulin secretion and inhibiting glucagon release [1,2]. In addition to its incretin action, GLP-1 inhibits eating and gastric emptying by activating peripheral and central mechanisms [3–7]. Therefore, GLP-1 and its receptor have become attractive targets for the treatment of type II diabetes (T2D) and obesity. Due to the short half-life (<2 min) of native GLP-1, longer acting GLP-1 analogs such as exenatide and liraglutide have been developed, and they have been approved for the treatment of T2D and, more recently, obesity [8–10]. GLP-1 analog treatments improve pancreatic β-cell functions and enhance insulin secretion, thus alleviating hyperglycemia in obese and diabetic patients [11]. The mechanisms of the food intake and body weight reducing effects of GLP-1 analogs are complex and appear to recruit central and peripheral GLP-1 receptors (GLP-1R) [12–15]. Recently, Sisley et al. showed that neuronal GLP-1R, but not GLP-1R in the visceral nerves, mediate the chronic eating inhibition by liraglutide [16], emphasizing the importance of central GLP-1R in the pharmacology of GLP-1 based therapy. The hypothalamic arcuate nucleus (ARC) and the area postrema (AP) in the hindbrain exhibited a particular high expression of fluorescent-tagged liraglutide and are regarded as the major brain sites mediating liraglutide-dependent weight loss [17].

GLP-1R agonist treatment, however, activates neurons in multiple brain areas implicated in the control of food intake, glucose metabolism, thermoregulation, and autonomic functions [18–20]. For instance, Exendin-4 (Ex-4) treatments activate catecholamine (CA) neurons in hindbrain regions including the AP, the nucleus tractus solitarii (NTS), and the ventrolateral medulla (VLM), which contribute to the regulation of heart rate and blood pressure [20,21]. Hindbrain CA neurons are also known to influence eating and glycemia in glucoprivation via two distinct pathways, i.e., the activation of 1) the sympathoadrenal response to increase circulating catecholamines, and 2) the hypothalamic-pituitary-adrenal (HPA) axis to increase corticosterone release [22,23]. In particular, a subset of CA neurons in the NTS

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and VLM projecting to the paraventricular hypothalamus (PVH) are required for glucoprivic challenges to stimulate eating and corticosterone secretion [24,25]. Neurotoxin-induced ablation of these neurons blunted the corticosterone release and eating response to the glucoprivic agent 2-deoxy-D-glucose (2DG).

CNS glucose sensing mechanisms also contribute to the eating inhibition by GLP-1 [26]. In fasting and after 2DG treatment, low glucose availability in the brain blunted the ability of GLP-1 to reduce food intake in rats, reiterating that GLP-1 is an important regulator of glucose homeostasis via eating control. Furthermore, acute peripheral administration of a pharmacological dose of Ex-4 not only produces a profound eating inhibition, but, paradoxically, also induces hyperglycemia by sympathetic nerve and adrenal activation in rats [27]. Indeed, peripheral administration of GLP-1 or Ex-4 increases HPA activity and circulating glucocorticoids in rodents (corticosterone) and human (cortisol) [28]. Because the adrenals do not appear to express GLP-1R [29], GLP-1 most likely activates a central pathway leading to HPA axis stimulation [30,31]. Therefore, it is reasonable to speculate that the activation of CA neurons by Ex-4 stimulates the HPA axis and thus counterbalances the anorexigenic property of GLP-1R activation when blood glucose levels are low. Identifying the central mechanism affecting the potency of GLP-1R agonist treatment will be important for the improvement of GLP-1-based drug therapies.

To understand the role of HPA axis activation in the eating inhibition by peripheral Ex-4 administration, we first administered Ex-4 after suppressing corticosterone secretion using low dose dexamethasone, a long acting synthetic corticoid, in rats fed standard chow diet. This manipulation increased the anorexigenic potency of Ex-4 and prompted us to assess the chronic effects of Ex-4 and dexamethasone co-administration on food intake, body weight, energy expenditure, and glucose tolerance in diet-induced obese (DIO) rats. Finally, to investigate the CNS mechanism mediating the Ex-4 effects on the HPA axis and food intake, we injected anti-dopamine-β-hydroxylase (DBH) saporin neurotoxin into the PVH to specifically ablate DBH expressing neurons projecting to the PVH. Our findings demonstrate that pharmacological and central lesion approaches to block the downstream actions of corticosterone can increase the potency of Ex-4 to reduce food intake, revealing an important balance between GLP-1 and corticosterone in the regulation of energy homeostasis.

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague Dawley (SD) rats were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed individually in a climate-controlled room (temperature: 23 ± 2 °C, humidity: 55 ± 5%). Rats were maintained on a 12 h light/dark cycle with ad libitum access to standard chow diet (No 3436, Provimi Kliba AG, Kaiseraugst, Switzerland) and tap water, except as noted. All procedures were approved by the Cantonal Veterinary Office of Zurich.

2.2. Acute dexamethasone and Exendin-4 treatment in lean rats

Twenty-four male SD rats (320–340 g) rats on standard chow diet were implanted with jugular vein catheters for blood sampling and recovered for 7 days. The rats were then divided into 4 groups (n = 6) and received dexamethasone (Sigma–Aldrich, St. Louis, USA, Cat # 31381, 50 μg/kg, s.c.) or PBS 90 min prior to Ex-4 (Bachem, Switzerland, H-8730, 1 μg/kg, i.p.) at dark onset. Blood was collected for baseline (90 min prior to dark onset), and at 0 (dark onset), 0.5, 1, 2, 4, and 24 h for the measurements of leptin, insulin, and corticosterone. Food intake, body weight, and energy expenditure were measured for 24 h in an open circuit calorimetry phenomaster system (TSE systems, Bad Homburg, Germany).

2.3. Chronic dexamethasone and Exendin-4 treatment in obese rats

Another cohort of twenty four male SD rats (320–340 g) were fed 60% high-fat diet (HFD, Ssniff Spezialdiäten GmbH, Soest, Cat # E15742-34) for 20 weeks and were monitored for body weight gain weekly. After 20 weeks on HFD, obese rats (600–800 g) were divided into 4 groups (n = 6) and received either dexamethasone (5 μg/kg, s.c.) and/or Ex-4 (3 μg/kg, s.c.) treatment at dark onset for 14 days. Food intake and body weight were measured daily. After 14 days, rats were subjected to indirect calorimetry and to an intraperitoneal glucose tolerance test. Blood was collected for leptin (fasted) and corticosterone (at the peak time of circadian rhythm) measurements by tail vein sampling.

2.4. Indirect calorimetry

Respiratory exchange ratio (RER) and heat production (EE) measurements were conducted in an open circuit calorimetry Phenomaster system (TSE) after 3 days of habituation. Data are presented as the average RER and EE values in dark, light, and total cycle.

2.5. Glucose tolerance test (IPGTT)

All rats were fasted overnight and treated with 2 g/kg glucose (i.p.). Tail vein blood was collected at 0, 15, 30, 60, 90, and 120 min after glucose bolus injection.

2.6. Stereotaxic surgeries

SD rats (320–340 g; pre-surgical body weight) were anesthetized by intraperitoneal injection of 2 mg/kg Xylazine (Rompun 2%, Provet AG, Lyssach, Switzerland) and 10 mg/kg BW Ketamin (Ketalar 50 mg/ml, Pfizer AG, Zurich, Switzerland) and positioned in the stereotaxic apparatus. Fluorogold (FG, Fluorochrome, LLC USA; 2.5% in PBS) was unilaterally injected into the PVH (1.8 mm caudal, 0.4 mm lateral to the bregma, and 7.7 mm ventral to dura) using a glass capillary micropipette connected with polyethylene (PE)-tubing to a microinjector (Picospritzer III, Parker Hannifin, Hollis, USA). All FG injected rats (n = 4) were implanted with intraperitoneal catheters for subsequent drug injections. Similarly, DSAP (Advanced Targeting Systems, San Diego, USA; 42 ng/200 nl in phosphate buffer, pH 7.4) or equimolar concentrations of unconjugated saporin were bilaterally injected into the PVH of 27 male SD rats. DSAP (n = 17) or SAP (n = 10) injected rats were implanted with jugular vein catheters for blood sampling. Previous studies [25,32] indicated that 2 weeks is adequate for the transport of the immunotoxin and degeneration of the affected neurons following the injection; therefore, behavioral and metabolic effects were assessed beginning approximately 3 weeks following DSAP injections.

2.6.1. 2DG test

To verify the lesion, a glucoprivic feeding test was performed using 2-deoxy-D-glucose (2DG, Sigma, 200 mg/kg, s.c.). Rats were injected with 2DG or saline before dark onset. Food intake was measured 4 h following the injection. Rats showing 30% increase in food intake (2DG-induced hyperphagia in SAP group) were eliminated from the analysis in DSAP group.

2.6.2. Food intake measurements

On the day of the experiment, all SAP and DSAP rats were fasted for 4 h and received either Ex-4 (0.3 and 1.0 μg/kg, i.p.) or saline at dark

