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The acute anorexic effect of liraglutide, a GLP-1 receptor agonist, does not require functional leptin receptor, serotonin, and hypothalamic POMC and CART activities in mice

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ABSTRACT

The acute anorexic effect of liraglutide, a GLP-1 receptor agonist, did not require functional leptin receptor, serotonin, and hypothalamic proopiomelanocortin and cocaine amphetamine regulated transcript activities in mice, although decrease in functional hypothalamic orexin activity might be involved in the acute anorexic effect of liraglutide.

1. Introduction

A high dose of liraglutide (3 mg), a human glucagon-like-peptide-1 (GLP-1) analog, which treats type 2 diabetes, was recently approved by the European Medicines Agency and United States Food and Drug Administration for weight reduction in human obesity [1].

Recent reports suggested that proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) in the arcuate nucleus of the hypothalamus largely mediate chronic administration of liraglutide-induced feeding suppression in rats or mice [2,3]. Mechanisms by which liraglutide acutely suppresses food intake, however, remains unclear.

To determine whether functional POMC and CART activities are essential for the acute anorexic effect of liraglutide, we examined the acute effect of liraglutide on food intake in db/db mice, with disturbed leptin receptor signaling, in which expression of hypothalamic POMC is decreased and expression of hypothalamic agouti-related protein (AGRP) is increased [4,5], and food-restricted KK mice, which decreased expression of hypothalamic POMC and CART and increased expression of hypothalamic orexin compared with normal fed KK mice [6]. KK mice develop mild obesity and type 2 diabetes.

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To determine the role of serotonin (5-HT) in the acute anorexic effect of liraglutide, we examined the effect of liraglutide on food intake in C57BL6J and db/db mice treated with the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA), which depletes 5-HT in vivo [7,8].

To determine the acute effect of liraglutide on the expression of hypothalamic genes involved in the critical regulation of feeding behavior, we examined the expression of hypothalamic POMC, CART, AGRP and orexin in C57BL6J mice.

2. Materials and methods

2.1. General procedures

Animals were purchased from Japan CLEA. The mice were individually housed in cages with free access to water and chow pellets in a light- and temperature-controlled environment (12 h on/12 h off, lights on at 08:00; 20–22 °C).

In exp 1, 6-week-old C57BL6J mice and db/db mice were intraperitoneally injected with saline or liraglutide (100 μg/kg) in the light cycle. Chow pellets were provided 30 min later. The intake of chow pellets was measured for the next 1 h and then 2 h.

In exp 2, 6-week-old C57BL6J mice and db/db mice were intraperitoneally injected with tryptophan hydroxylase inhibitor, PCPA (500 mg/kg), which depletes serotonin in vivo, once a day over 3 days as described previously [7,8]. The PCPA was dissolved in 0.2 ml 1% Tween saline. Then, animals were intraperitoneally injected with saline or liraglutide (100 μg/kg) in the light cycle. Chow pellets were provided 30 min later. The intake of chow pellets was measured for the next 1 h and then 2 h.

In exp 3, 6-week-old male KK mice were provided 3.5 g of chow pellets daily for 5 days (87.5% of the normal daily provision for KK mice), as described previously [6]. On day 6, animals were intraperitoneally injected with saline or liraglutide (100 μg/kg) in the light cycle. Chow pellets were provided 30 min later. The intake of chow pellets was measured for the next 1 h and then 2 h.

In exp 4, 6-week-old C57BL6J mice were intraperitoneally injected with saline or liraglutide (100 μg/kg) in the light cycle. One hour later, the animals were decapitated; animals were not fed. The hypothalamus was removed for RNA extraction, as described previously [6].

The dose of liraglutide (100 μg/kg) was selected based on evidence that liraglutide-induced hypophagia in mice [9]. Liraglutide was a kind gift from Novo Nordisk, Japan. The drug was dissolved in 0.2 ml 0.9% saline. The experiment was performed between 9:00 and 12:00. The animal studies were conducted in accordance with the institutional guidelines for animal experiments at the Tohoku University Graduate School of Medicine.

2.2. Real-time quantitative reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA was isolated from mouse hypothalamus using the RNeasy Midi kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNA synthesis was performed using a Super Script III First-Strand Synthesis System for RT-PCR Kit (Invitrogen, Rockville, MD) with 1 μg total RNA. cDNA synthesized from total RNA was evaluated in a real-time PCR quantitative system (LightCycler Nano Instrument Roche Diagnostics, Mannheim, Germany). The primers used were listed in Supplementary Table 1.

The relative amount of mRNA was calculated using β-actin mRNA as the invariant control. Data are shown as fold-change of the mean value of the control group, which received saline as described previously [6].

2.3. Statistical methods

Data are presented as mean ± SEM ($n = 6$). Comparisons between two groups were performed using Student's t test. A P value of less than 0.05 was considered statistically significant.

3. Results and discussion

Intraperitoneal injection of liraglutide (100 μg/kg) significantly suppressed food intake compared with saline controls for 2 h after injection in C57BL6J mice and db/db mice (Fig. 1A). In addition, intraperitoneal injection of liraglutide (100 μg/kg) significantly suppressed food intake compared with saline controls for 2 h after injection in C57BL6J mice and db/db mice which were treated with PCPA (Fig. 1B). These findings suggest that the acute anorexic effect of liraglutide does not require functional leptin receptor, 5-HT, or POMC and CART activities in the hypothalamus.

Intraperitoneal injection of liraglutide (100 μg/kg) significantly suppressed food intake compared with saline controls for 2 h after injection in normal fed KK mice while having no effect on food intake in food-restricted KK mice (Fig. 2). These findings suggest that the increased orexin activity in the hypothalamus might inhibit the acute anorexic effect of liraglutide in the food-restricted KK mice.

Moreover, intraperitoneal injection of liraglutide (100 μg/kg) did not affect the expression of hypothalamic POMC, CART, and AGRP at 1 h after injection in C57BL6J mice (Fig. 3), although intraperitoneal injection of 5-HT drugs which induce the acute anorexic effect, significantly increase the expression of hypothalamic POMC and CART [10–12].

On the other hand, liraglutide significantly decreased the expression of hypothalamic orexin compared with saline controls after injection in C57BL6J mice (Fig. 3). Genetic deletion of orexin induces feeding suppression in mice [13]. Together, these findings suggest that decrease in hypothalamic orexin activity contribute to the acute anorexic effect of liraglutide in mice.

Secher et al. determined the expression of POMC and CART in the arcuate nucleus after chronic administration of liraglutide (s.c., bidaily, 200 μg/kg) for 28 days in diet-induced obese rats [2]. In addition, the group determined the distribution of fluorescently labeled liraglutide (400 μg/kg) in the mouse brain at 6 hours after the administration following 4 day administration [2]. Barreto-Vianna et al. determined POMC and CART protein levels in mice fed high fat or standard chow after administration of liraglutide (s.c., bidaily,