



Research article

Antagonistic interaction between central glucagon-like Peptide-1 and oxytocin on diet-induced obesity mice



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ABSTRACT

Glucagon-like peptide-1 (GLP-1), whose agonists are widely prescribed, is a peptide proven effective in reducing obesity. Similarly, oxytocin (OXT) is a peptide known to increase satiety and help reduce body weight. In the present study, we aimed to examine the metabolic effects of co-administration of GLP-1 and OXT in diet-induced obesity (DIO) mice to elucidate their functions and interactions in the central nervous system. To this end, 40 DIO mice were subjected to stereotaxic surgery for the installation of an osmotic minipump and intracerebroventricular administration of GLP-1, OXT, or both. Initially, it was anticipated that co-administration of these anorexigenic peptides would be as effective as, if not more than, either GLP-1 or OXT alone in providing metabolic benefits to the obese mice. Interestingly, co-administration of OXT and GLP-1 offset the reductions in body weight and food intake promoted by either peptide alone. Co-administration also negated the decrease in fat and increase in lean mass produced by either peptide alone. Moreover, co-administration showed an equivalent calorimetric benefit as either peptide alone. Therefore, these results suggest antagonistic, rather than synergistic or additive, effects of centrally administered GLP-1 and OXT that attenuate the metabolic benefits of either peptide.

1. Introduction

The development of safe, viable anti-obesity drugs has become an important goal for scientists and clinicians alike. Receptor agonists of glucagon-like peptide-1 (GLP-1), an incretin, are some of the newest prescription drugs available against obesity [1]. These incretin-like substances decrease body weight between 2.9 and 5.4 kg [2]. Because the effects of GLP-1 are not as significant as those of definite weight-loss therapies such as bariatric surgery [3], studies are currently underway to investigate the interaction effects of GLP-1 and other anorexigenic peptides to identify agents that can effectively augment the effects of GLP-1 receptor agonists [4, 5].

Meanwhile, oxytocin (OXT), a nonapeptide released from the paraventricular nucleus (PVN) neurons, has been found to reduce weight and enhance lipolysis in a dose-dependent manner [6]. When administered

into the central nervous system (CNS) directly, both acute and chronic OXT treatments decrease body weight and food intake in rodents [7, 8]. Mechanistically, OXT has been reported to act as a neuromodulator on the arcuate nucleus (ARC) [9, 10]. Similarly, a recent study described how fast-acting satiety glutamate neurons complement pro-opiomelanocortin (POMC) neurons from the ARC to PVN and express oxytocin receptor (OXTR) in their soma [9].

Interestingly, GLP-1 and OXT seem to be related in terms of regulation and function. *In vivo* or *in vitro* addition of GLP-1 changes OXT concentrations in different ways [11], and the effects of OXT are known to be associated with GLP-1 receptor signaling in the CNS [12]. Thus, OXT is a reasonable candidate for augmenting the metabolic benefits of GLP-1. The present study investigated the metabolic effects of GLP-1 and OXT co-administration in diet-induced obesity (DIO) mice to understand their interactions in the CNS.

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2. Methods

2.1. Animals

Forty 5 to 6-week-old male C57BL6/J mice (purchased from Japan SLC, Inc.) were maintained in individually ventilated cages. The animals were fed with 60% calories in fat (Research Diets Inc., D12492, New Brunswick, NJ) *ad libitum* to generate DIO mice. After 9 weeks, each animal was caged separately, and the body weight (BW) and food intake (FI) of each animal were measured daily. BW was measured as the percent of BW on the day of operation (OP), and FI was calculated as the weight difference between the fresh chow and the leftovers. Any animals that did not survive the full course of the study were excluded. All materials were sterile, and all procedures were approved by and conformed to the ethics and standards of Seoul National University Hospital Institutional Animal Care and Use Committee.

2.2. Osmotic pump preparation

Micro-osmotic pumps (mean pumping rate, 0.19 $\mu\text{l/h}$; mean fill volume, 100.3 μl ; Alzet 1002D, Durect Corporation, Cupertino, CA) were used with 0.9% saline, 11.58 mg/ml GLP-1 (equivalent to 16.01 nmol/d), 0.28 mg/ml OXT (lyophilized powder diluted in 0.9% saline, equivalent to 1.28 nmol/d), or a 1:1 mixture of both peptides. All the materials were freshly prepared on the day of operation. The GLP-1 dose was based on the results of our previous study [4]; the OXT dose, which is expected to last up to at least 26 days [8], was derived from previous studies on rats [6, 7, 8] by adjusting for differences in the average BWs of rats and mice. The pumps were primed at 37 °C with 0.9% saline for at least 4 h before delivery.

2.3. Stereotaxic operation and cannulation

On OP, each animal was injected intraperitoneally with 0.01 mg/g anesthesia (28% ketamine and 8.6% xylazine) and firmly placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). A micro-osmotic pump was placed in a dorsal subcutaneous pouch, and a 28G cannula with a polycarbonate elbow stop (Durect Corporation, Cupertino, CA) was placed 1.79mm caudal to bregma to target the third ventricle [4]. The cannula was fixed into the cranium with acrylic dental cement and stainless steel screw. The wound was sutured and applied with 9.6% lidocaine ointment. All animals postoperatively received 10 min of infrared radiation (IF 700 Gold, Harell Inc.) and a 0.16 ml subcutaneous injection of 30 mg/kg cefazolin solution.

2.4. Body composition

On OP and postoperative day (POD) 7, each animal's body composition was analyzed using a Minispec magnetic resonance system (LF50-BCA Analyzer, Bruker Inc., Billerica, MA). To reduce within-subject variability, all measurements were repeated in triplicate to obtain a mean value for each animal. Next, the percent differences in weight between OP and POD7 were determined.

2.5. Indirect calorimetry

From POD 7 to 9, each animal was placed in a home cage with indirect calorimetry system called Comprehensive Lab Animal Monitoring System (CLAMS, Columbia Instruments Inc.), to measure its calorimetric indices, such as V_{O_2} and V_{CO_2} . Energy expenditure (EE) was calculated as follows in kilocalories per hour: $EE \text{ (kcal/h)} = (3.815 * V_{O_2} + 1.232 * V_{CO_2}) * bw / 1000 * 1 / 1000$ [13], where V_{O_2} and V_{CO_2} are measured in ml/kg/h and body weight (bw) in grams. A 20-hour of adjustment period was followed by a 24-hour data collection period. The

day period was defined as 8am to 8pm, and the night period was defined as from 8pm to 8am. For each index, a 60-minute average was calculated for analysis. Outliers were defined as those with at least half of data points that are more than 1.5 interquartile ranges below the first quartile or above the third quartile.

2.6. Statistical analyses

Data in this study were analyzed for statistical significance using SPSS Statistics for Windows, version 23.0. Raw data for indirect calorimetry were analyzed for any outliers using R Project for Statistical Computing 3.4.1.1. One-way or two-way analysis of variance (ANOVA) followed by a post-hoc Fisher's least significant difference test was used primarily. The "interaction effect" was defined when the effect of one hormone depends on the effect of another hormone and considered statistically significant when the interaction effect between GLP-1 and OXT was significant in two-way ANOVA. Three-way ANOVA was performed modeling for three factors (GLP1, OXT and time). All results were expressed as mean \pm SEM, and all graphs were plotted using GraphPad Prism 5.0. Significance was set at $P < 0.05$ and near-significance at $P < 0.10$.

3. Results

3.1. Co-administration of GLP-1 and OXT on body weight and food intake

The average weight of DIO mice in this study was 37.34 ± 0.60 g after 9 weeks of feeding, about 11g higher than the average weight of normally fed C57BL6/J (C57BL6/JJmsSlc, Japan SLC, Inc.). Before the operation, there was no significant difference in body weights among the treatment groups [$F(3,21) = 0.890, P = 0.462$] (Figure 1A).

Over the course of peptide administration, GLP-1 ($-5.81 \pm 0.53\%$, $-2.84 \pm 0.71\text{g}$, $P = 0.007$) and OXT ($-5.71 \pm 0.57\%$, $-2.89 \pm 0.27\text{g}$, $P = 0.013$) significantly reduced the BWs of mice, whereas BWs of mice co-administered with the two peptides ($-3.68 \pm 0.57\%$, $-1.59 \pm 0.54\text{g}$, $P = 0.847$) showed no significant difference from the controls ($-3.53 \pm 0.57\%$, $-2.10 \pm 0.21\text{g}$). Three-way ANOVA revealed a significant interaction effect between GLP-1 and OXT [$F(1,147) = 14.691, P < 0.001$]. The peptides also showed a near-significant interaction effect at POD 1 and POD 7 ($P = 0.075$ and $P = 0.053$, respectively) (Figure 1B). Moreover, GLP-1 ($1.44 \pm 0.089\text{g}$, $P = 0.043$) and OXT ($1.25 \pm 0.096\text{g}$, $P = 0.001$) significantly reduced FI, while no significant difference was observed between co-administration ($1.67 \pm 0.096\text{g}$, $P = 0.774$) and the controls ($1.70 \pm 0.096\text{g}$). Three-way ANOVA revealed a significant interaction effect between GLP-1 and OXT [$F(1,147) = 13.044, P < 0.001$], and two-way ANOVA at each POD revealed a significant interaction effect between the peptides at POD1 and POD5 ($P = 0.041$ and $P = 0.047$, respectively) (Figure 1C).

3.2. Co-administration of GLP-1 and OXT on body composition

After a week of peptide treatment, the body fat percentages were significantly different among the treatment groups [$F(3,8) = 5.243, P = 0.027$]. Specifically, mice treated with GLP-1 ($-4.04 \pm 0.34\%$, $P = 0.067$) showed a near-significant reduction in body fat, and those treated with OXT ($-6.38 \pm 1.35\%$, $P = 0.006$) showed a significant reduction. However, co-administration of the two peptides ($-2.58 \pm 0.92\%$, $P = 0.258$) did not result in any significant difference compared to controls ($-0.83 \pm 0.32\%$). Two-way ANOVA showed a significant interaction effect between GLP-1 and OXT [$F(1,8) = 12.603, P = 0.008$] (Figure 2A). Correspondingly, body lean mass percentages were significantly different among the treatment groups [$F(3,8) = 4.083, P = 0.050$]. Mice treated with GLP-1 ($3.51 \pm 0.75\%$, $P = 0.067$) showed a near-significant increase, and those treated with OXT ($5.38 \pm 1.47\%$, $P = 0.011$) showed a significant increase in lean mass percentage. Again, co-administration of

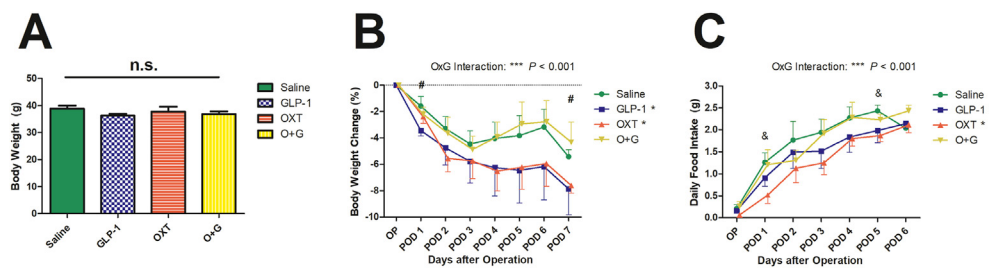


Figure 1. Effects of central GLP-1 and OXT on body weight and feeding. (a) The average body weights at the operation day (OP) are shown. The percent changes in (b) body weight measured each day from OP to the post-operation day (POD) 7 and (c) food intake from OP to POD 6 are then shown. N = 6–7 per treatment group. “GLP-1” refers to animals treated with only glucagon-like peptide-1, “OXT” refers to those treated with only oxytocin, and “O + G” refers to those treated with both OXT and GLP-1. “OxG interaction” refers to the interaction effect between OXT and GLP-1. Data are expressed as mean ± SEM (n.s. = not significant, *P < 0.05, **P < 0.01, and ***P < 0.001; for OxG interaction at a specific time point, #P < 0.10 and & P < 0.05).

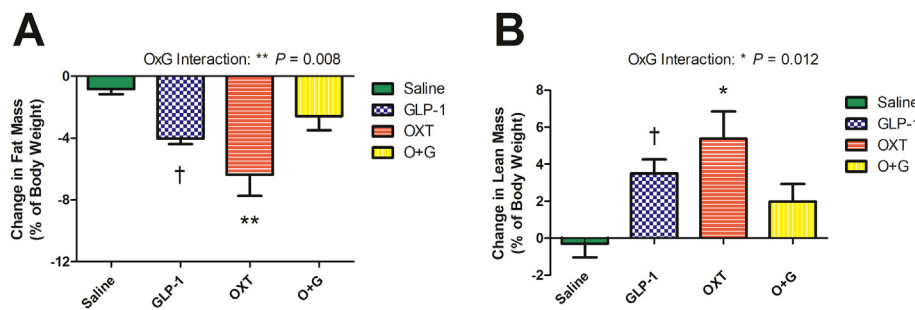


Figure 2. Effect of central administration of GLP-1 and OXT on animal body composition. Differences in (a) body fat mass percentage and (b) lean mass percentage between the operation day (OP) and post-operation day 7 (POD 7) were analyzed. N = 2–4 per treatment group. “GLP-1” refers to animals treated with only glucagon-like peptide-1, “OXT” refers to those treated with only oxytocin, and “O + G” refers to those treated with both OXT and GLP-1. “OxG interaction” refers to the interaction effect between OXT and GLP-1. Data are expressed as mean ± SEM (†P < 0.10, *P < 0.05, and **P < 0.01).

both peptides ($1.99 \pm 0.95\%$, $P = 0.258$) did not result in a significant difference from the controls ($-0.29 \pm 0.74\%$). Two-way ANOVA showed a significant interaction effect between GLP-1 and OXT [F (1,8) = 10.346, $P = 0.012$] (Figure 2B).

3.3. Co-administration of GLP-1 and OXT on calorimetric indices

The average energy expenditure data from the indirect calorimetry show a significant interaction effect between GLP-1 and OXT during the night [F (1,384) = 7.007, $P = 0.008$] (Figure 3A). The 24-hour profile of energy expenditure showed a significant difference among the treatment groups during the night [F (3,384) = 6.721, $P < 0.001$]. GLP-1 (average = 0.0158 kcal/h/kg, $P < 0.001$), OXT (average = 0.0158 kcal/h/kg, $P < 0.001$), and co-treatment (average = 0.0158 kcal/h/kg, $P < 0.001$) all increased the energy expenditure at night, compared to saline control (average = 0.0146 kcal/h/kg) (Figure 3B).

Moreover, the average V_{O_2} data from indirect calorimetry revealed a significant interaction effect between GLP-1 and OXT during the night [F (1,384) = 7.489, $P = 0.006$] (Figure 3C). The 24-hour profile of V_{O_2} revealed a significant difference among the treatment groups during the night [F (3,384) = 3.823, $P < 0.001$]. Specifically, GLP-1 (average = 3283 ml/kg/h, $P < 0.001$), OXT (average = 3237 ml/kg/h, $P = 0.001$), and co-treatment (average = 3265 ml/kg/h, $P < 0.001$) all increased V_{O_2} at night, compared to saline control (average = 3015 ml/kg/h) (Figure 3D).

Likewise, the average V_{CO_2} data demonstrated a significant interaction effect between GLP-1 and OXT during the night [F (1,384) = 4.919, $P = 0.027$] (Figure 3E). Again, the 24-hour profile of V_{CO_2} showed a significant difference among the treatment groups during the night [F (3,384) = 5.997, $P = 0.001$]. GLP-1 (average = 2571 ml/kg/h, $P = 0.006$), OXT (average = 2640 ml/kg/h, $P < 0.001$), and co-treatment (average = 2622 ml/kg/h, $P < 0.001$) all increased the V_{CO_2} at night, compared to saline control (average = 2423 ml/kg/h) (Figure 3F).

For the aforementioned indices, the raw data graphs of saline control, GLP-1, OXT, and co-administration groups showed no outliers. There were no significant differences for all of the indices during the day.

4. Discussion

The present study revealed that intracerebroventricular co-administration of GLP-1 and OXT surprisingly produced antagonistic interactions that attenuated the metabolic benefits of either peptide alone. The metabolic effects of administering each peptide alone found in this study are consistent with those of published literature. Previous studies reported that central administration of GLP-1 reduces FI and BW [14, 15, 16], decreases fat mass and increases lean mass [17], and increases energy expenditure [18]. Similarly, several studies revealed that centrally administered OXT reduces FI and BW [6, 19], decreases fat mass and increases lean mass [6, 8], and increases energy expenditure [6, 8, 19, 20].

To explain this unexpected result, we hypothesize that central co-administration of GLP-1 and OXT excessively activated the second-order satiety neurons in the PVN and caused “glutamate-induced excitotoxicity.” Rapid-acting satiety neurons from the ARC to PVN that express OXTR were found to activate second-order satiety neurons via glutamate, whereas slow-acting neurons that secrete alpha-melanocyte-stimulating hormone (α -MSH) were found to complement the satiety circuit by increasing glutamatergic synaptic activity [9] (Figure 4A). A recent study revealed that the subset of PVN neurons that express the GLP-1 receptor is indispensable to satiety [21]. The GLP-1 receptor belongs to the same class as the α -MSH receptor [22] and might play a similar role; thus, centrally administered GLP-1 might have increased glutamatergic transmission in the second-order satiety neurons. Therefore, co-administration of the peptides might have disproportionately stimulated glutamate receptors on postsynaptically potentiated PVN

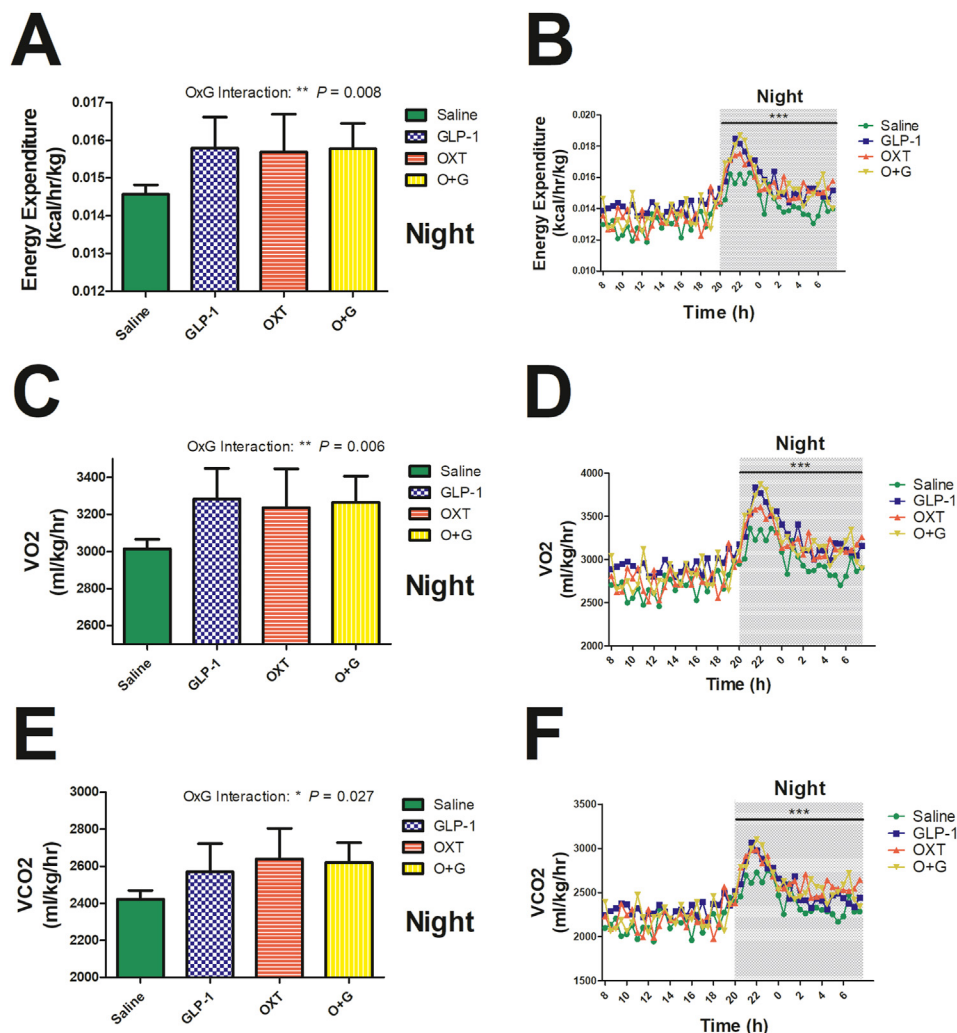


Figure 3. Effect of central GLP-1 and OXT on calorimetric indices. The average values during the night for (a) energy expenditure, (c) V_{O_2} , and (e) V_{CO_2} are shown. The 24h profiles of (b) energy expenditure, (d) V_{O_2} , and (f) V_{CO_2} are then shown; each data point represents 30-minute average, and nighttime (8pm to 8am) is represented with a shaded box. Data expressed in means \pm SEM. $N = 4-6$ per treatment group. “GLP-1” refers to animals treated with only glucagon-like peptide-1, “OXT” refers to those treated with only oxytocin, and “O + G” refers to those treated with both OXT and GLP-1. “OxG interaction” refers to the interaction effect between OXT and GLP-1. Data are expressed as mean \pm SEM ($\dagger P < 0.10$, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

satiety neurons causing excessive calcium influx (Figure 4B). This could lead to neuronal dysfunction and negate the metabolic benefits of either peptide [23].

Another interesting hypothesis for the antagonistic mechanism of the present study is the possibility of protein-protein interaction. These two peptides with biochemically similar structures might have effectively bound and sequestered each other in solution (Figure 4C). OXT has a sequence of 9 amino acids (CYIQNCPLG), with the first six in a ring structure [24], whereas GLP-1 has a sequence of 30 amino acids (HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR) [25]. Although the peptide-peptide interaction between two endogenous occurring peptides is rare, this is certainly a possibility that would have to be tested in vivo, by co-administering GLP-1 and non-peptide agonists of OXTR [26], or in silico, using resources such as PepSite [27].

The results of the present study may raise some important questions under scrutiny. For instance, about 5% decrease in body weight in all treatment groups has been observed after the operation; because this decrease has also been observed in a previous study with rats [8], we believe that the initial drop in body weight was due to the operation. Moreover, the gradual increase of food intake from POD 1 to POD 7 reflects recovery from post-operation stress.

Thus, a number of follow-up studies will be needed to verify the

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antagonistic interaction and the above hypotheses. As this study used a single dose for each peptide based on the results from the previous studies [4, 8], a study that tests combinations of multiple doses will have to be conducted. An electrophysiology study like an already published study [9] can investigate the connectivity of rapidly acting OXTR neurons to second-order GLP-1R neurons in PVN. Also, a quantitative pharmacological study [28] or a computerized simulation [29] will provide a better understanding of the dose-dependent effect and circuitry modulation behind this apparent antagonism. Moreover, the present study included only male mice to control for any sex-dependent effect of the peptides. Because the findings in this study are expected to be replicated in female mice, another study that uses female mice as study subjects should be conducted. Because central chronic infusion of OXT is known to increase OXT synthesis and release and affect the peripheral lipid metabolism [6], it will be also interesting to analyze the plasma levels of the peptides after the co-administration. Also, a study with bone mass measurements will expand the role of GLP-1 and oxytocin on bone metabolism.

In conclusion, central GLP-1 and OXT offer metabolic benefits, while the peptides used simultaneously do not seem to do so. These results, when verified, will provide a guidance for obesity combination pharmacotherapy development. More studies to elucidate the interactions

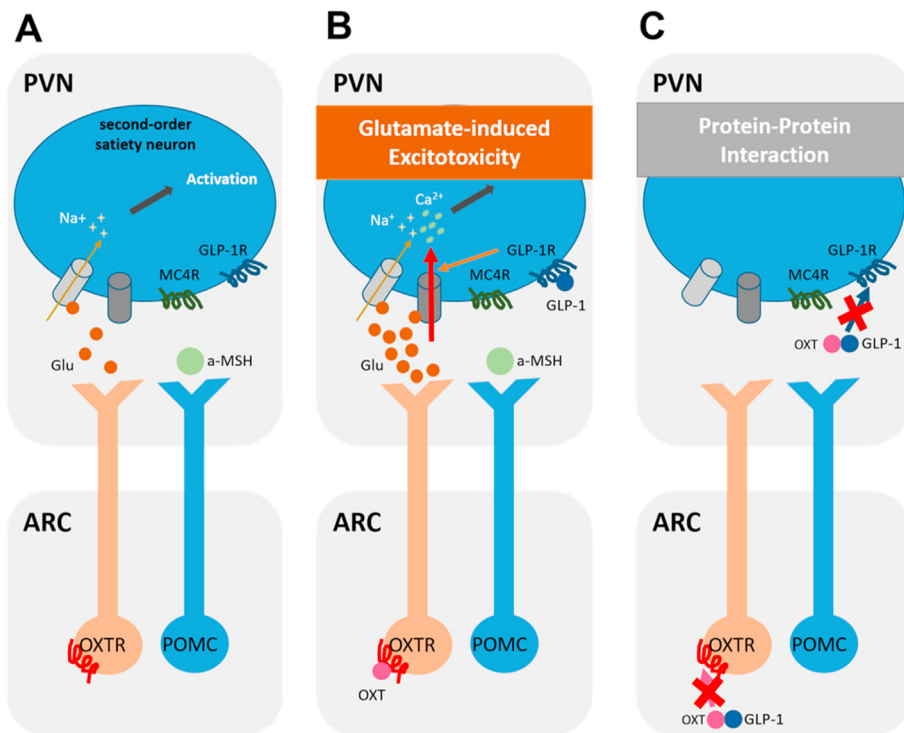


Figure 4. Hypotheses for antagonistic interaction between glucagon-like protein-1 (GLP-1) and oxytocin (OXT) in hypothalamus. (a) Current view of the satiety neuron circuitry from the arcuate nucleus (ARC) to paraventricular nucleus (PVN). The neuron labelled as OXTR refers to the rapidly-acting satiety neuron that secretes glutamate upon activation, and the neuron labelled as POMC refers to the slowly-acting satiety neuron that secretes pro-opiomelanocortin upon activation. Glutamate opens sodium channels to activate the second-order satiety neuron in PVN. (b) GLP-1 and OXT bind to their respective receptors, and excessive glutamate to the susceptible second-order satiety neuron causes calcium influx and subsequently, glutamate-induced excitotoxicity. (c) The peptide molecules of OXT and GLP-1 sequester each other, preventing the peptides from binding to their respective receptors. Abbreviations used in this figure are as follows: GLP-1R, glucagon-like protein-1 receptor; OXTR, oxytocin receptor; MC4R, melanocortin 4 receptor; Glu, glutamate; a-MSH, α -melanocyte-stimulating hormone.

between commonly used drugs should be conducted in the near future to provide new strategies against obesity.

Declarations

Author contribution statement

Jeonghoon Lee: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Haneul Moon: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Hyunji Lee: Performed the experiments; Analyzed and interpreted the data.

Yunkyeong Oh: Conceived and designed the experiments; Performed the experiments.

Changyeon Kim: Performed the experiments; Analyzed and interpreted the data.

Young Hee Lee, Min Sun Kim, Cheryl NamKoong, Hee Won Lee: Contributed reagents, materials, analysis tools or data.

Jung Hee Kim: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Hyung Jin Choi: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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