

Melanocortin receptor-4 mediates the anorectic effect induced by the nucleus tractus solitarius injection of Glucagon-like Peptide-2 in fasted rats

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Abstract

Glucagon-like peptide-2 (GLP-2) is secreted from enteroendocrine L-type cells of the gut and also released from preproglucagonergic (PPG) neurons in the nucleus tractus solitarius (NTS) and adjacent medial reticular nucleus of the brain stem. The neurons in the NTS express GLP-2, and the neurons send extensive projections to the hypothalamus. Recent studies show that the intracerebroventricular administration of GLP-2 significantly suppresses food intake in animals and some evidence suggest that the melanocortin receptor-4 (MC4-R) signaling in the hypothalamus is required for intracerebroventricular GLP-2-mediated inhibition of feeding. There is proopiomelanocortin (POMC) positive neurons expressing MC4-R in the NTS. Suppression of MC4-R expressing neurons in the brain stem inhibits gastric emptying. In this study, we tested the effects of NTS GLP-2R activation and blockade on feeding behavior and evaluated the endogenous melanocortin system's role in the NTS in mediating effects of GLP-2 on feeding behavior in fed and fasted rats. Our results demonstrated that microinjection of GLP-2 into the NTS suppressed food intake in fasted-refeeding rats but did not affect food intake in free-feeding rats, and this inhibition was blocked by pretreatment of either Exendin₍₉₋₃₉₎ or SHU 9119, suggesting the GLP-2 system in the NTS exerts an inhibitory action on food intake. MC4-R mediates this action in the NTS.

Keywords: food intake; nucleus tractus solitarius (NTS); neurotransmitter; Exendin₍₉₋₃₉₎; SHU 9119

1. Introduction

Glucagon-like peptide-2 (GLP-2), one of the proglucagon-derived peptides, is secreted from enteroendocrine L-type cells of the gut together with glucagon-like peptide-1 (GLP-1) in response to dietary nutrients (Yusta et al. 2000; Holst 2007). GLP-2 is also released from preproglucagonergic (PPG) neurons in the nucleus tractus solitarius (NTS) and adjacent medial reticular nucleus of the brain stem (Kieffer and Habener 1999; Vrang et al. 2007). GLP-2 plays an important role in regulating energy absorption and maintenance of mucosal morphology, function, and integrity of the gut (Drucker and Yusta 2014). In addition to enterotrophic role, it also regulates gastrointestinal motility in humans and rodents. In fact, GLP-2 decreases gastric emptying (Wojdemann et al. 1998; Nagell et al. 2004) and inhibits gastric fundic tone leading to increased gastric capacity (Amato et al. 2009). Gastric emptying is a critical process for the short-term control of food intake and maybe a target for appetite modulation (Holst 2007). Taken together, the inhibition of gastric emptying is closely related to GLP-2 anorexigenic effects (Guan et al. 2012; Wismann et al. 2018). Moreover, the GLP-2-expressing PPG neurons of the NTS send extensive projections to the hypothalamic paraventricular nucleus (PVN) and dorsomedial hypothalamic nucleus (DMH), where both regions are involved in the regulation of feeding behavior (Larsen et al. 1997; Merchenthaler, Lane, and Shughrue 1999; Rinaman 1999; Vrang et al. 2007). Recent studies have suggested that GLP-2 inhibits ingestion behavior in animals, including rodents, when administered centrally or peripherally (Tang-Christensen et al. 2000; Lovshin et al. 2001; Baldassano et al. 2012). To date, GLP-2 has been proposed as a neurotransmitter in controlling feeding

behavior (Baldassano et al. 2012) and may mediate PPG neuron-induced synaptic transmission linking the hypothalamus and the brain stem (Larsen et al. 1997; Rinaman 1999; Vrang et al. 2007).

The actions of GLP-2 are mediated by the interaction with a specific GLP-2 receptor (GLP-2R), which is mainly expressed in the gut enteroendocrine cells, the neurons of the enteric nervous system, and the central nervous system (CNS) (Estall and Drucker 2006; Guan et al. 2012; Pedersen et al. 2015; Yusta et al. 2019). In the CNS, the GLP-2R is localized mainly in key regions of the brain for energy balance, including the hypothalamic nucleus and the NTS of the brain stem (Munroe et al. 1999; Yusta et al. 2000; Lovshin et al. 2004; Vrang and Larsen 2010; Shi et al. 2013). A few studies have been carried out about the consequences of GLP-2R activation within the brain (Shi et al. 2011; Shi et al. 2013), and the activation of the receptor in the hypothalamic nucleus has been considered to mediate the inhibitory effects of central GLP-2 administration on feeding behavior (Guan et al. 2012; Dalvi and Belsham 2012).

Interestingly, recent evidences suggest that the melanocortin receptor-4 (MC4-R) signaling in the hypothalamus is required for intracerebroventricular GLP-2-mediated inhibition of both gastric emptying and food intake (Guan et al. 2012). GLP-2 is predominantly produced from PPG neurons in the NTS, where GLP-2R is expressed. Moreover, like the hypothalamus, there are also proopiomelanocortin (POMC) positive neurons expressing MC4-R in the NTS, and suppression of MC4-R expressing neurons in the brain stem would decrease excitatory input to the gastrointestinal tract and inhibit gastric emptying (Fan et al. 2004; Richardson et al. 2013). Therefore, it is reasonable that the interaction between GLP-2 and GLP-2R in

the NTS may play a role in the inhibition of food intake, and the activities of the GLP-2 system in the NTS are at least partially mediated by the MC4-R signaling pathway.

In this study, using the microinjection and behavioral methods, we tested the effects of NTS GLP-2R activation and blockade on feeding behavior. We evaluated whether the endogenous melanocortin system in the NTS mediates the effect of GLP-2 on feeding behavior in fed and fasted rats.

2. Materials and method

2.1 Animals

46 Sprague-Dawley (SD) adult male rats weighed 270 ± 20 g (the Medical Experimental Animal Center of Xi'an Jiaotong University) were housed individually in cages with free access to standard chow diet and tap water at room temperature. They were randomly divided into a free-feeding group (n=13) and fasted-refeeding group (n=13), and another two groups (n=10 each) for the injection of the antagonists, SHU9119, and Exendin (9-39). The ethics committee approval number is 2019-1153. All protocols followed the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, 1996).

2.2 Stereotaxic surgery and microinjection of the drugs

All rats were implanted unilaterally guide cannulas into the NTS through stereotaxic surgery, according to previous studies (Lam et al. 2010; Wei et al. 2015). After the surgery, rats were returned to cages for one-week habituation and recovery.

The room lights were automatically controlled with 8:30 off and 20:30 on cycles for the free-feeding group and 18:00 off and 6:00 on for the fasted-refeeding group. There was also an overnight food deprivation (16:30-8:30) for the fasted-refeeding group before each specific experiment. We used a changed dark/ light circle for free-feeding group i.e. 8:30 off and 20:30 on, similar to our previous studies (Sun et al, 2013; Zhao et al, 2012) so that we could do all the measurement during the daytime after surgery and administration of the drugs. To maximum reduce the potential influence of this circadian rhythm change on the food intake and energy expenditure of animals, we allowed two weeks for the rats to adapt this changed new light cycle according to our previous similar study (Wei et al, 2015). During this adapting period of time, we measured the body-weights and compared them with those in normal dark/light cycle. There were no differences shown and both groups weighted as 270 ± 20 g. Therefore we can easily carry out food intake measurements in this created “dark time” with full confidence of less/no effects from this changed “dark/light” cycle.

Due to the technical difficulties in our experiments, such as the difficulties to guarantee the injection location on NTS if using bilaterally injection (data not shown), we decided to use unilaterally injection, rather than those done by Lam et al, 2010. Unilateral injections into the NTS were administered using 1- μ L Hamilton syringes connected by PE-10 polyethylene tubing to 30-gauge injection cannulas according to our previous study (Wei et al, 2015). The verification of injections into the NTS was also carried out after the experiments. Details are provided as supplementary documents.

2.3 Drugs

GLP-2₍₁₋₃₃₎ and Exendin₍₉₋₃₉₎ was purchased from Sigma and SHU 9119 from Tocris (Tocris Bioscience, UK). All drugs were dissolved in 0.9% sodium chloride immediately before the experiments. Accordingly, the 0.9% saline solution was used as a control vehicle. The dose of Exendin₍₉₋₃₉₎ (10 µg/0.5 µl) and SHU 9119 (0.5 nmol/0.5 µl) was used to test their effects on blocking GLP-2 inhibition on food intake according to previous studies (Tang-Christensen et al. 2000; Guan et al. 2012). The experiments were designed by employing a counterbalanced design to minimize any effect from the dose usage of drugs and the time points of food intake measurement on the effect of drug treatment on the rats. Details see the experimental protocol in the supplementary materials.

2.4 Measurements on food intake

Food intake was measured by calculating the differences in the weight of the total foods collected immediately before starting the experiment and after the measurement time points. Measurements were recorded to the nearest 0.1 g (corrected for spillage).

2.5 Data Analysis

Two-way RM ANOVA followed by Bonferroni's tests post hoc multiple comparisons were used to analyze the cumulative food intake in different groups and at the different measurement times with Prism 6.0 software (GraphPad Software, USA). The significance value was set at $P < 0.05$.

3. Results

3.1 Analysis of microinjections in the NTS

Fig. 1 showed the representative image of microinjection into the NTS. The right cannula placement showed that microinjection was correctly delivered into the causal part of NTS with 0 mm from the occipital crest suture, according to the description previously done (Lam et al. 2010; Paxinos et al. 2007; Wei et al, 2015). The injections were localized within all NTS areas. Histological analysis showed that half of the total 46 injections were correctly made into the NTS (Fig. 1). The other half were misplaced either too deep, in lateral, or too shallow (Supplementary Table 1).

For the free-feeding group, 5 rats were microinjected in NTS in place, 8 were outside the NTS; for the fasted-refeeding group, 6 rats in place, 7 outside the place; for SHU 9119 experiments, 6 in place while 4 outside the place; and for Exendin ⁽⁹⁻³⁹⁾ experiments, 6 in place and 4 outside the place. Therefore, we are confident that 50% of the successful injection rate was still statistically satisfactory to analyze the relevant experimental data.

3.2 GLP-2 inhibits food intake in fasted-refeeding rats but not in free-feeding rats

We compared separately the cumulative food intake of all rats where injections were found in the NTS and those outside of the NTS. We measured the cumulative food intake with different GLP-2 microinjection doses at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, and 24 h post-injection for free-feeding rats and fasted-refeeding rats.

For in-place injections, our results showed that GLP-2 had no effect on food intake across the measurement time points for all concentrations of GLP-2 used for free-feeding rats (n=5, Fig. 2A). For the fasted-refeeding rats (n=6), GLP-2 showed

inhibition effects on food intake in a dose and time-dependent manner (Fig. 2B). There was no difference in food intake at an initial 3 h of post-injection for all doses of GLP-2 used (Fig. 2B). However, when compared with 4 h and 6 h post-injection, there was a significant decrease in food intakes for 10 μ g of GLP-2 microinjection compared to the control (0 μ g of GLP-2) (Fig. 2B). While 1 μ g and 5 μ g of GLP-2 microinjection did not affect food intake when compared to the control at the same time point of measurements (Fig. 2B). Above all, our results showed that after 4-6 h microinjection of 10 μ g of GLP-2 into NTS, there was a significant decrease in food intake in fasted-refeeding rats while there was no difference in free-feeding rats.

For misplace injections, there was no significant difference in food intake of GLP-2 with different doses and at different measurement time points for both the free-feeding rats (n=8) and the fasted-refeeding rats (n=7) (Supplementary Table 1). These data indirectly confirmed that the microinjection of GLP-2 indeed caused the inhibition of food intake into NTS.

3.3 The inhibitory effect of GLP-2 on food intake in fasted-refeeding rats could be blocked by SHU 9119 or Exendin ⁽⁹⁻³⁹⁾

To understand further about the inhibitory effect of GLP-2 on food intake in NTS, we used GLP-1 receptor antagonist, Exendin ⁽⁹⁻³⁹⁾ (Tang-Christensen et al. 2000), and Melanocortin-4 receptor antagonist, SHU 9119 (Guan et al. 2012), as central GLP-2 receptor antagonist to test if this inhibitory effect can be blocked. We used the same experimental design with a combinational intervention of 10 μ g GLP-2 and Exendin ⁽⁹⁻³⁹⁾ or SHU 9119. The dose used was according to the previous study (Tang-Christensen et al. 2000; Guan et al. 2012).

For in-placed injection, as expected, we found no difference in food intake at initial 3 hours of post-injection for all combination of drugs, but at 4 h and 6 h post-injection, there was a significant decrease in both food intakes with 10 μ g of GLP-2 compared to the control (0 μ g of GLP-2), (Fig. 3A). This is in line with our above results (Fig. 2B), but this inhibitory effect can be nearly completely blocked by pre-applying SHU 9119 at 4 h, and 6 h post microinject. Besides, pre-injection of SHU 9119 did not cause any effect on food intake for all rats as SHU9119 + saline group, and SHU 9119 + GLP-2 group showed no effects on food intake at all post-injection measurement time points compared to the control (saline + saline group, Fig. 3A). These results further confirmed that the inhibitory role of GLP-2 on food intake in NTS in fasted-refeeding rats could be blocked by SHU 9119.

We also tested the blocking effects of Exendin₍₉₋₃₉₎ on the inhibitory effect of GLP-2. Similarly, our data showed that there was no difference on food intake at the initial 3 h of post injection. Nevertheless, when we compared the food intake at 4 h and 6 h post-injection, there were significant decrease in both food intakes for 10 μ g of GLP-2 microinjection compared to the control (0 μ g of GLP-2) (Fig. 3B). This was in agreement with the previous results (Fig. 2B), but this inhibitory effect can be nearly completely blocked by pre-applying Exendin₍₉₋₃₉₎ (Fig. 3B). Like SHU9119, pre-injection of Exendin₍₉₋₃₉₎ did not cause any effect on food intake for all rats as Exendin₍₉₋₃₉₎ + saline group and Exendin₍₉₋₃₉₎ + GLP-2 group showed no effects on food intake at all post-injection compared to the control (saline + saline group, Fig. 3B). These results further confirmed that the inhibitory role of GLP-2 on food intake in NTS in fasted-refeeding rats could be blocked by Exendin₍₉₋₃₉₎.

Above all, our data indicated that this inhibitory effect of GLP-2 on food intake might function through the GLP-1 receptor and the melanocortin-4 receptor in NTS.

Because the GLP-2 receptor is not available, Exendin (9-39) (Tang-Christensen et al. 2000) and SHU 9119 (Guan et al. 2012) were usually chosen to validate the blocking effect of GLP-2.

For misplaced NTS injection, there was no significant difference shown in all groups (Supplementary Table1).

All the above results revealed that the inhibitory effect of GLP-2 in NTS on food intake in fasted-refeeding rats could be blocked by GLP-1 receptor or Melanocortin-4 receptor antagonist in NTS.

4.Discussion

The GLP system's roles in the regulation of feeding behavior have been intensively investigated previously (Tang-Christensen et al. 1996; Turton et al. 1996; Tang-Christensen et al. 2000; Tang-Christensen, Vrang, and Larsen 2001). It has been clear that GLP-1 plays a potent role in controlling food intake when injected intracerebroventricularly (*icv*) in the animal model (Turton et al. 1996; Dakin et al. 2001) as well as when peripherally administered in both rodents and humans (Flint et al. 1998; Verdich et al. 2001; Dakin et al. 2004; Liu et al. 2010). However, the mechanism of action responsible for the GLP-2 dependent modulation of feeding still remains largely uncertain (Tang-Christensen et al. 2000; Lovshin et al. 2001;

Sørensen et al. 2003; Schmidt et al. 2003). Several investigations had suggested that the GLP-2 may act as an inhibitor of feeding behavior in rodents and chicks when *icv* administered (Tang-Christensen et al. 2000; Lovshin et al. 2001; Honda et al. 2015). In contrast, this peptide is not effective in long-term experiments when peripherally administered (Tsai et al. 1997; Scott et al. 1998). Moreover, in healthy humans, GLP-2 does not influence ingestion behavior or postprandial feeling of satiety (Sørensen et al. 2003; Schmidt et al. 2003).

In this study, we firstly examined the effects of microinjection of GLP-2 (1, 5, 10 μg) into the NTS on food intake in free-feeding rats and fasted-refeeding rats.

Unexpectedly, we found that GLP-2 microinjections did not significantly affect cumulative food intake in free-feeding rats (Fig. 3A). This observation is not in agreement with previous reports in rodents (Tang-Christensen et al. 2000; Lovshin et al. 2001; Dalvi and Belsham 2012). Tang-Christensen *et al.* discovered that central injection of 10 μg of GLP-2 caused a significant decrease in 2-h food intake than vehicles in free-feeding rats (Tang-Christensen et al. 2000). Lovshin *et al.* demonstrated that the central administration of pharmacological doses of GLP-2 powerfully inhibited short-term food intake in free-feeding mice (Lovshin et al. 2001). The data from Dalvi *et al.* also showed that *icv* 5 μg of GLP-2 remarkably suppressed food intake in free-feeding mice (Dalvi and Belsham 2012). It is somewhat difficult to explain the discrepancy of their results with ours from the free-feeding animals. The difference might be due to the administration route (*icv* vs intra-NTS.) and, subsequently, different sites of action of the GLP-2. Notably, all three studies mentioned above-injected GLP-2 into either the lateral ventricle or the third cerebral ventricle to focus on the interactions of GLP-2 with the major hypothalamic

nuclei that lie in the vicinity of the third or lateral ventricle. However, our data derived from the anorexigenic action of GLP-2 when injected directly into the NTS.

In contrast, our results showed at 4-6 h post injections of 10 µg of GLP-2 in NTS. The food intake was significantly reduced by 21.7% and 32.3%, respectively, compared with saline injection in fasted rats (Fig. 3B), implicating that the inhibition is a short-term action. The pretreatment completely diminished the anorectic effect elicited by GLP-2 in fasted rats with Exendin (9-39), an antagonist for GLP-1, also used as a blocker for GLP-2 (Tang-Christensen et al. 2000). Because the NTS has been suggested to express GLP-2R (Tang-Christensen et al. 2000; Lovshin et al. 2004), our result indicates that the anorectic effect of GLP-2 injection is induced by the interaction of GLP-2 with its receptor, the GLP-2R in the NTS. The data also showed that Exendin (9-39) alone did not affect food intake than vehicle-treated rats, suggesting that GLP-2R in the NTS is not topically activated. Interestingly, the NTS also expresses GLP-1R (Merchenthaler, Lane, and Shughrue 1999). Thus, it might be possible that there is cross-reactivity of GLP-2 with GLP-1R in the NTS. However, it has been suggested that GLP-2 can activate only the cells with GLP-2R expression but not those expressing GLP-1R (Lovshin et al. 2001). Moreover, data from a previous study showed that the effects of GLP-2 on food intake in GLP-1R knockout mice were not decreased by disruption of GLP-1R signaling but rather increased, suggesting that GLP-2 does not mediate its effects on feeding through GLP-1R (Lovshin et al. 2001; Lovshin et al. 2004).

Our results demonstrate that the NTS may be a potential target for regulating food intake by GLP-2 in the fasted animal. This expectation was supported by our

additional observation in which the misplaced injections of GLP-2 into the areas out of NTS did not inhibit food intake (Supplement Table 1).

With its transmission at the MC4-R, the brain melanocortin system has been demonstrated to mediate very potent effects on food intake and energy expenditure induced by neuronal and humoral feedback signals (Fan et al. 1997; Thiele et al. 1998; Grill et al. 1998). Our results showed that the inhibition of food intake induced by intra-NTS injection of GLP-2 was blocked by pretreatment with SHU 9119, an antagonist for MC4-R, in the fasted-refeeding rats (Fig. 4A), suggesting that MC4-R in the NTS plays an essential role in the inhibition produced by intra-NTS administration of GLP-2. Unexpectedly, we did not find any effect on food intake induced by SHU 9119 itself. This result is, to some extent, consistent with an earlier study (Tang-Christensen et al. 2000), in which pretreatment with SHU 9119 blocked the suppression induced by *icv* injection of GLP-2 in the free-feeding rats. It has been demonstrated that the POMC neurons are located in the arcuate hypothalamic nucleus (ARC) and NTS (Huo, Grill, and Bjorbaek 2006). Therefore, α -MSH axons may potentially originate from these two distinct nuclei. However, projections from NTS POMC neurons are mainly distributed in the caudal brainstem, whereas the forebrain is predominantly innervated by ARC POMC neurons (Wang et al. 2015). There are reports to show the presence of MC4R in the brain stem dorsal vagal complex (DVC, including NTS plus DMV) (Gautron et al. 2010; Kishi et al. 2003) and MC4R signaling in the brain stem on food intake (Williams, Kaplan, and Grill 2000; Zheng et al. 2005). Therefore, we speculate that the blocking effect on suppressing feeding behavior induced by intra-NTS injection of SHU 9119 might be from interactions neuronal circuits within the NTS. However, the present results cannot explain the

distinct mechanism(s) underlying the interactions involved, which needs further investigations.

The results from our study showed that GLP-2 microinjections did not significantly affect cumulative food intake in free-feeding rats (Fig. 2A), which is different from previous results in rodents which showed inhibitory effects (Tang-Christensen et al. 2000; Lovshin et al. 2001; Dalvi and Belsham 2012). We cannot explain what caused these differences for free-fed animals. We certainly took different injection route from previous studies (icv vs. intra-NTS.) and therefore GLP-2 functioned differently. Our experiment measured the anorexigenic action of GLP-2 injected directly into the NTS, while others with injected GLP-2 into either the lateral ventricle or the third cerebral ventricle to focus on the interactions of GLP-2 with the major hypothalamic nuclei that lie in the vicinity of the third or lateral ventricle.

Another possibility we would like to point is that the GLP2 injections as well as those of GLP and MC4R antagonists in our study were all made unilaterally into the NST, but not bilaterally due to the reason for the guaranteed accuracy of NTS injections. We suspect that bilaterally injection may have different effects from the unilaterally inject, but we are not sure what kind of difference it might be and how it would affect the GLP2 induced response. Indeed one can speculate that a monolateral injection of GLP2 in the NST is not sufficient to alter feeding in free feeding rats or that this is the reason why GLP2 induces only a modest response as that shown in our paper in fasted re-feeding rats. This is something worth to further investigate, such as using increased amount of GLP2 injection but it is not our focus in this study.

It is worth noting that the expression of appetite regulatory peptides/hormones is known to be changed by fasting or food deprivation (Yuan et al. 2014; Johansson et al. 2008). Thus, the condition that influences anorectic or orexigenic hormones and nutritional signals can be reduced or increased. In the present study, the anorectic effect was only detected in the fasted rats, suggesting that the effect of intra-NTS injection of GLP-2 may be related to animals' nutritional state.

Our results also showed reduced food intake occurring at later time (4-6 hours after the injection) compared with immediate effects taking place in the first hours (Baldassano et al. 2012) or 2-hours (Tang-Christensen et al. 2000). Same as the above, the reason for these differences could be multi-factors, such as different animal species (C57BL/J mice, Wistar rats), different routes of GLP-2 application (i.p., centrally administered, lateral ventricle ICV), and other conditions. SD rats were used in this study and GLP-2 was injected directly into NTS. Interestingly, another study of intracerebroventricular injection of h[Gly2] GLP-2 into mice (Lovshin et al. 2001) also recorded reduced food intake at the first four hours, similar to ours.

There are two main forms of GLP-2 in blood circulation: active GLP-2(1-33) and inactive GLP-2(3-33). GLP-2(3-33) is secreted from the intact GLP-2, namely GLP-2(1-33), which is rapidly hydrolyzed by dipeptidyl peptidase IV to form GLP-2(3-33) (Hansen L, et al. 2007). The half-life of GLP-2 in vivo is very short, only about 7 min in human (Hartmann B, et al. 2000). This may explain no effects on food intake by peripheral administration (subcutaneously, intravenous, vein infusion) of GLP-2 in human. GLP-2 is rapidly degraded (Jeppensen et al. 2001; Schmidt et al. 2003; Sorensen et al. 2003). Therefore, the long acting form of GLP-2 such as [Gly2]-GLP2

(teduglutide), the degradation-resistant analog of GLP-2 with increased efficacy, has been compared with native GLP-2 by several groups. Recent data have shown that intraperitoneal injection of [Gly2]-GLP-2 can significantly inhibit the feeding behavior of mice, and this action was longer than GLP-2 and detectable after 4 h (Baldassano et al. 2012). However, intracerebroventricular [Gly2]-GLP-2 did not inhibit food intake in the first hour after peptide injection in mice (Lovshin et al., 2001). [Gly2]-GLP-2 is currently used in clinical trials as a therapeutic for a variety of intestinal insufficiencies and diseases (Rowland KJ, 2011). More recently, Glepaglutide, a novel long-acting GLP-2 analogue with an effective plasma half-life of approx. 50 h giving this analogue the potential for less than once daily dosing, is in clinical trial for the short bowel syndrome (Naimi et al., 2019). We certainly would like to test the long active form of GLP-2 on our model to see their direct injection in the dorsomedial hypothalamic nucleus. However, this is beyond the scope of our current study.

In conclusion, our study showed that GLP-2 in NTS significantly inhibited food intake in fasted-refeeding rats, and this inhibition effect can be blocked by either Exendin (9-39) or SHU 9119. These findings suggest that the GLP-2 system in the NTS plays some role in decreasing food intake and that MC4-R in the NTS mediates this inhibition.

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Legends

Fig. 1 Representative image of unilateral injection in the NTS. An arrow pointing to the injection entering into NTS. AP, area postrema; NTS, nucleus tractus solitaries; CC, central canal. Scale bar: 1 mm.

Fig. 2 Effects on food intake with GLP-2 microinjection (0, 1, 5, and 10 μg) unilaterally into NTS at post-injection of 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, and 24 h for free-feeding rats (A) (n = 5) and fasted-refeeding rats (B) (n = 6). No effects of GLP-2 on food intake in free-feeding rats while GLP-2 decreased food intake in fasted-refeeding rats after 3 hours of microinjection of 10 μg of GLP-2. *compared to the control (0), Values as mean \pm S.E.M., $P < 0.05$.

Fig. 3 Unilaterally microinjection of (A) SHU 9119 (0.5 nmol) and (B) Exendin₍₉₋₃₉₎ (10 μg), respectively, into the NTS 15 min prior to microinjection of 10 μg GLP-2 blocked the intra-NTS GLP-2-induced inhibition on food intake in fasted-refeeding rats. The cumulative food intake was recorded at 0.5 h, 1 h, 2 h, 3 h, 4 h, and 6 h after injection. *compared to saline + saline; #compared to SHU9119 + saline; ^ compared to SHU9119 + GLP-2 (A). *compared to saline + saline; #compared to Exendin₍₉₋₃₉₎ + saline; ^compared to Exendin₍₉₋₃₉₎ + GLP-2 (B). Values as mean \pm S.E.M., n = 6/group, $P < 0.05$.