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Glucagon directly interacts with vagal afferent nodose ganglion neurons to induce Ca^{2+} signaling via glucagon receptors

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ABSTRACT

Glucagon is released from the pancreatic islets postprandially and under hypoglycemic and cold conditions, and regulates glucose metabolism, feeding, energy expenditure and heat production, the functions partly controlled by the brain. Peripheral glucagon could signal to the brain via passing through the blood–brain barrier and/or acting on the vagal afferent. However, the latter remains to be determined. The present study aimed to clarify whether glucagon directly interacts with the nodose ganglion (NG) neurons of vagal afferent nerves in mice. *In vivo* study showed that intraperitoneal injection of glucagon induced phosphorylation of extracellular signal regulated kinase 1 and 2 (ERK1/2), cellular activation makers, in NG neurons. In fura-2 microfluorometric studies, glucagon increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single NG neurons. The glucagon-induced $[Ca^{2+}]_i$ increases were suppressed by a glucagon receptor antagonist, des-His^1-[Glu^9]-Glucagon (1–29) amide, and the glucagon receptor mRNA was expressed in NG neurons. The majority of glucagon-responsive NG neurons exhibited $[Ca^{2+}]_i$ responses to insulin and cholecystokinin-8, the hormones that are secreted postprandially and implicated in satiety. These results demonstrate that glucagon, by interacting with the glucagon receptor, directly activates vagal afferent nerves, possibly being relayed to the signaling to the brain and formation of satiety.

1. Introduction

Glucagon, a 29-amino acid peptide, is released from the pancreatic islets, intestine and stomach. Glucagon is released under hypoglycemic conditions and then elevates blood glucose levels, serving as a major counter hormone [1]. The regulation of glucose metabolism by glucagon is mediated by its direct action on the peripheral tissues such as the liver [1] and also by the brain [2,3]. Glucagon is also released postprandially in a transient manner [4–6], which was shown to be involved in inhibition of food intake via reduction of meal size [7–11]. Glucagon and insulin, both released postprandially, elicit an additive inhibition of food intake [12]. Furthermore, glucagon is secreted by cold exposure and plays a role in increasing energy expenditure and thermogenesis via activation of brown adipose tissue (BAT) [8]. Glucagon not only directly regulates BAT, but acts through the sympathetic nervous innervating BAT [8,13,14]. Thus, glucagon regulates glucose metabolism, feeding, energy expenditure and thermogenesis at least partly by influencing the brain. However, the route through which peripheral glucagon informs the brain is less defined, and the target site for possible cooperation between glucagon and insulin on feeding is not clear.

Peripheral substances influence the brain primarily via (1) entering the brain through blood–brain barrier (BBB) to act on the target cells in the brain and/or via (2) interacting with the vagal afferent nerves that connect peripheral organs to the medulla of brain stem [15,16]. It has been shown that the transfer of glucagon from the circulation to the brain is strongly restricted by BBB [17]. Among pancreatic islet hormones, insulin and pancreatic polypeptide have been shown to influence the brain functions such as satiety [18–20] and memory [21,22], and to activate the nodose ganglion (NG) neurons that compose the afferent vagal nerves [23,24]. Hence, glucagon could also act on the vagal afferent nerves.

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In the present study, we examined whether glucagon directly interacts with the vagal afferent NG neurons and whether glucagon and insulin target the common NG neurons in mice. We investigated the direct effect of glucagon on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single neurons isolated from NG. Effect of intraperitoneal (ip) injection of glucagon on NG neurons was assayed by phosphorylation of extracellular signal regulated kinase 1 and 2 (ERK1/2) (also known as mitogen-activated protein kinases), which are activated by membrane depolarization and Ca^{2+} influx in PC12 cells [25], brain neurons [26–28] and primary afferent (dorsal root ganglion) neurons [29]. We found that glucagon directly activates vagal afferent NG neurons via the glucagon receptor, and that glucagon and insulin act on the same NG neurons.

2. Materials and methods

2.1. Materials

Human glucagon and cholecystokinin-8 (CCK-8, 26–33, sulfated form) and were purchased from Peptide Institute (Osaka, Japan). Porcine insulin was obtained from Sigma (MO) and glucagon receptor antagonist, des-His^1-[Glu^9]-Glucagon (1–29) amide, from Tocris Bioscience (MO).

2.2. Animals

Male C57BL/6J mice aged 1–3 months were purchased from Japan SLC (Shizuoka, Japan). The animals were housed for at least 1 week under conditions of controlled temperature (23 ± 1 °C), humidity (55% ± 5%), and lighting (light on at 7:30 and off at 19:30). Food and water were available ad libitum. Animal experiments were carried out after receiving approval from the Institutional Animal Experiment Committee of the Jichi Medical University, and in accordance with the Institutional Regulation for Animal Experiments and Fundamental Guideline for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

2.3. Immunohistochemical detection of pERK1/2 in nodose ganglia

Saline or glucagon (100 nmol/kg, 10 ml/kg) was ip injected in the mice during 19:30 to 20:30 following fasting for 2 hours. At 15 min after injection, these mice were transcardially perfused with 4% paraformaldehyde under anesthesia. The nodose ganglia were collected, postfixed in the same fixative for 2 h at 4 °C, and incubated in phosphate buffer containing 25% sucrose for 48 h. Longitudinal sections (8 μm) of NGs were cut using a precision cryostat (Leica Microsystems, IL), collected at 48 μm intervals. For detection of pERK1/2, sections were incubated with rabbit polyclonal antibody to phospho-p44/42 MAPK (Thr202/Tyr204, pERK1/2) (1/500; #9101; Cell Signaling Technology, MA) for overnight at 4 °C. Sections were washed and incubated with Alexa 488-conjugated goat anti-rabbit IgG (1:500; A11008; Life technologies, MD) for 30 min at room temperature. Fluorescence images were acquired with a BX50 microscope and a DP50 digital camera (Olympus, Tokyo, Japan).

2.4. Reverse transcriptase (RT)-PCR

Total RNA of NGs and liver in the mouse was isolated using TRIzol (Life technologies). The first-strand cDNA was synthesized from 100 ng RNA using Verso cDNA Synthesis Kit (Thermo Scientific, MA). Expression of glucagon receptor was examined by PCR using REDExtract-N-Amp PCR ReadyMix (Sigma, 95 °C for 2 min, then 35 cycles of 94 °C for 15 s, 60 °C for 15 s and 72 °C for 1 min). The PCR products were electrophoresed on 2% agarose gel for the validation of proper product sizes. Primer sequences and the product length were as follows: glucagon receptor between exon 4–7 sense, 5′-CTACCTTGGTACCACAAAGTGC-3′, antisense, 5′-GAGCACAAAGGACGCAAACA-3′, 306 bp; glucagon receptor between exon 7–10 sense, 5′-TTGGTCATCGATTGGCTGCT-3′, antisense, 5′-ACGCAGGATCCACCAGAATC-3′, 351 bp.

2.5. Preparation of single neurons from nodose ganglia

Single neurons were isolated from mouse NGs as previously reported [30]. Briefly, NGs were treated 20 min at 37 °C with 0.1–0.5 mg/ml collagenase Ia (Sigma), 0.4–0.6 mg/ml dispase II (Roche, Basel, Swiss), 15 μg/ml DNase II type IV (Sigma), and 0.75 mg/ml bovine serum albumin (Sigma) in HEPES-buffered Krebs–Ringer bicarbonate buffer (HKRB) composed of (in mM) 4.7 KCl, 1.2 KH_2PO_4, 129 NaCl, 5 $KaHCO_3$, 1.2 $MgSO_4$, 1.8 $CaCl_2$, and 10 HEPES with pH adjusted at 7.4 using NaOH supplemented with 5.6 glucose. Single neurons were cultured in Eagle's minimal essential medium containing 5.6 mM glucose supplemented with 10% feral bovine serum, 100 μg/ml streptomycin, and 100 units/ml penicillin for 12–24 h.

2.6. Measurements of $[Ca^{2+}]_i$ in single nodose ganglion neurons

Measurements of $[Ca^{2+}]_i$ in primary cultured NG neurons were carried out as described previously [30]. Briefly, following incubating with 2 μM fura-2 AM (DOJINDO, Kumamoto, Japan) for 30 min at 37 °C, the cells were mounted in a chamber and superfused with HKRB containing 5.6 mM glucose at 1.3 ml/min at 30 °C. Fluorescence ratio images at 510 nm due to excitation at 340 and 380 nm were produced by an Aquacosmos ver. 2.5 (Hamamatsu Photonics, Shizuoka, Japan). When $[Ca^{2+}]_i$ changed within 5 min after addition of agents and their amplitudes were at least twice larger than the fluctuations of the baseline, they were considered responses. Only the neurons that responded to 55 mM KCl were analyzed.

2.7. Statistical analysis

All data were shown as means ± SEM. Statistical analysis was performed by unpaired *t*-test using the Prism 5 (GraphPad Software, CA). $p < 0.05$ was considered significant.

3. Results

3.1. Intraperitoneal (ip) injection of glucagon induces ERK1/2 phosphorylation in NG neurons

Effect of glucagon on NG neurons was assayed by phosphorylation of ERK1/2 (pERK1/2). In the control with saline injection, few NG neurons were labeled with pERK1/2 antibody (Fig. 1A). In contrast, ip injection of glucagon (100 nmol/kg) induced pERK1/2 in NG neurons at 15 min after injection (Fig. 1B). The incidence of pERK1/2-positive neurons with glucagon (approximately 8%) was significantly higher than that with saline (Fig. 1C). These data indicated that peripheral glucagon administration promptly activates NG neurons.

3.2. Glucagon directly interacts with and increases $[Ca^{2+}]_i$ in NG neurons via interacting with glucagon receptor

The direct effect of glucagon on vagal afferent neurons was investigated by measuring $[Ca^{2+}]_i$ in fura-2 loaded NG neurons. Glucagon at 10^{-10}–10^{-7} M were sequentially applied to NG neu-

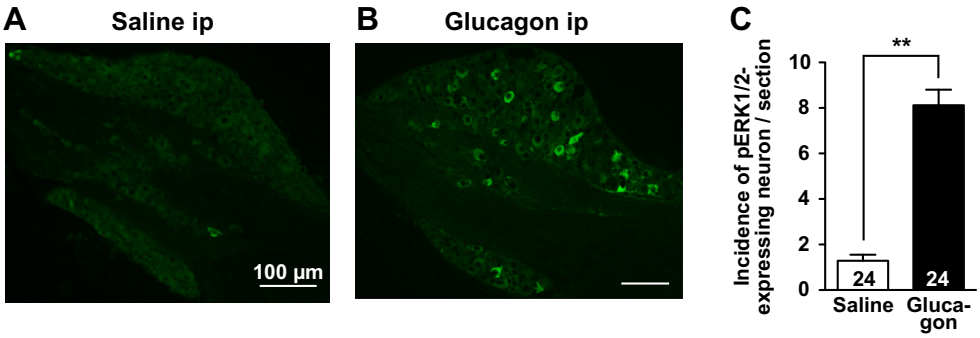

Fig. 1. Glucagon intraperitoneal (ip) injection induces ERK1/2 phosphorylation in NG neurons. (A) and (B) NG section immunostained for pERK1/2 at 15 min after ip injection of saline (A) and 100 nmol/kg glucagon (B). Scale bars indicate 100 μm. (C) Incidence of pERK1/2-experssing neurons in the NG section after ip saline (open bar) or 100 nmol/kg glucagon (filled bar). The number of sections is indicated in each bar (6 sections/mouse, 4 mice). $^{**}p < 0.01$ by unpaired *t*-test.

rons with treatment and washing periods of 4 and 8 min, respectively (Fig. 2A and B). Glucagon at 10^{-9}–10^{-7} M, but not 10^{-10} M, increased $[Ca^{2+}]_i$ in NG neurons. The incidence of $[Ca^{2+}]_i$ responses to glucagon showed a concentration-dependency, with a maximal value around 8% at 10^{-8} and 10^{-7} M (Fig. 1C). The amplitudes of $[Ca^{2+}]_i$ responses to glucagon took plateau at 10^{-9}–10^{-7} M (Fig. 2D).

Whether glucagon increases $[Ca^{2+}]_i$ in NG neurons via the glucagon receptor was examined. A repetitive administration of 10^{-8} M glucagon twice induced repeated $[Ca^{2+}]_i$ increases, whose amplitudes were comparable (Fig. 3C). In the presence of a glucagon receptor antagonist, des-His^1-[Glu^9]-Glucagon (1–29) amide (10^{-6} M), glucagon induced little increase in $[Ca^{2+}]_i$ and, after washing out the antagonist, second stimulation with glucagon increased $[Ca^{2+}]_i$ (Fig. 3B). Amplitude of $[Ca^{2+}]_i$ responses to 10^{-8} M glucagon was significantly smaller in the presence of the antagonist (Fig. 3C). Furthermore, expression of the glucagon receptor mRNA in NG neurons was examined by RT-PCR using two kind of primers against different regions (exon 4–7 and exon 7–10). Glucagon receptor mRNA was expressed abundantly in liver, confirming previous report [31]. The PCR products were also detected in NG, though the product intensity was weaker than in liver (Fig. 3D).

3.3. Glucagon-responsive NG neurons respond to insulin and CCK

Insulin and CCK are released postprandially and directly activate NG neurons [24,32,33]. As shown in Fig. 4A, among 21 NG neurons that responded to 10^{-7} M glucagon, 18 (85.7%) responded to insulin at 10^{-6} M. All of glucagon-responsive NG neurons

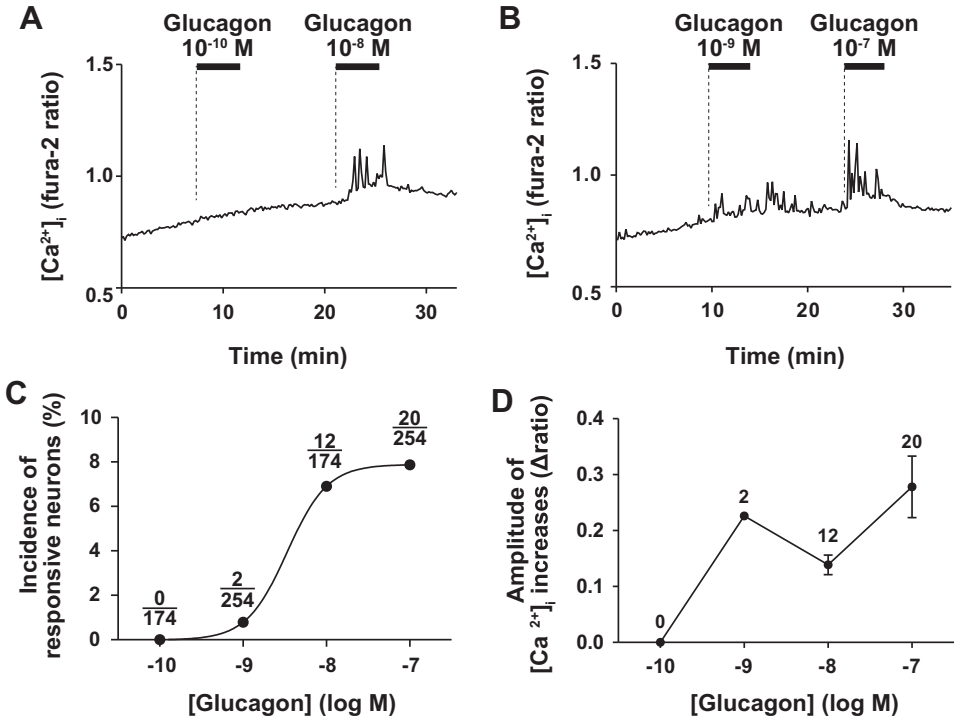

Fig. 2. Glucagon increases $[Ca^{2+}]_i$ in isolated single NG neurons. (A) and (B) Repetitive administration of glucagon at 10^{-10}–10^{-7} M concentration-dependently increased $[Ca^{2+}]_i$ in a single NG neurons. $[Ca^{2+}]_i$ is expressed as fura-2 ratio and bars above the tracings indicate the periods of agent administration. These $[Ca^{2+}]_i$ recordings are representative of the NG neurons that responded to glucagon at 10^{-8} M but not at 10^{-10} M ($n = 12$, A), and at 10^{-9} and 10^{-7} M ($n = 2$, B). (C) and (D) Concentration-dependent effects of glucagon to increase $[Ca^{2+}]_i$ in single NG neurons, examined by the protocol of repetitive administration shown in (A) and (B). In (C), numbers above each point indicate the number of neurons that responded to glucagon over that responded to 55 mM KCl. In (D), numbers above each point indicate the number of the glucagon-responsive NG neurons.

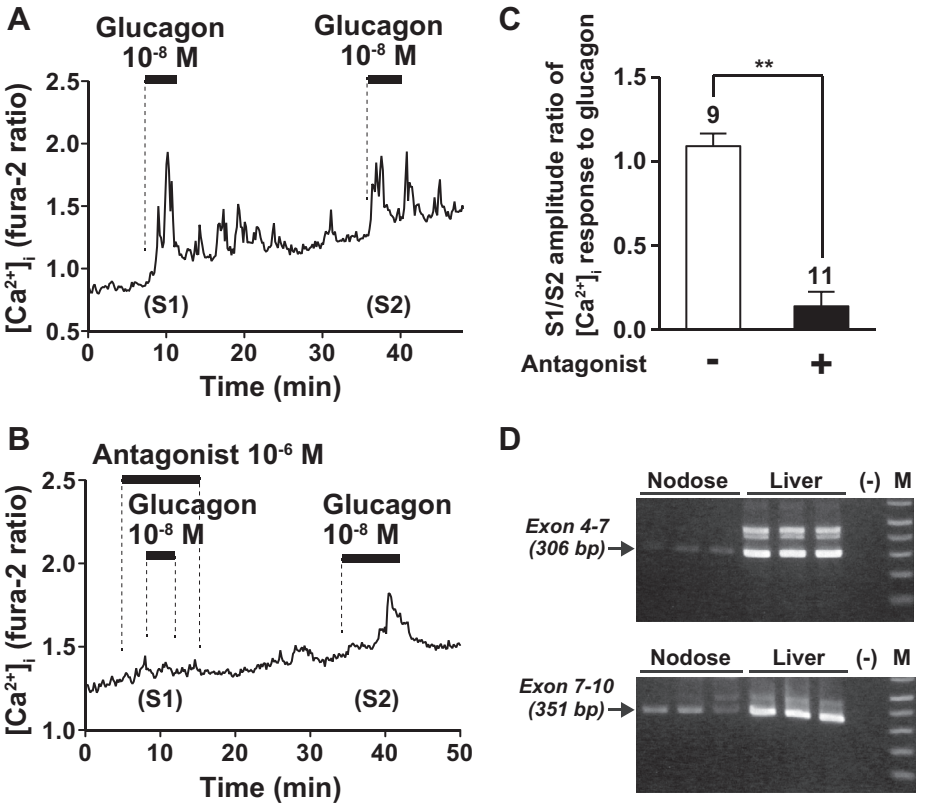

Fig. 3. Glucagon induces $[Ca^{2+}]_i$ increases via glucagon receptor in single NG neurons. (A) Administration of 10^{-8} M glucagon twice induced repeated $[Ca^{2+}]_i$ increases in single NG neurons ($n = 9$). (B) Glucagon-induced $[Ca^{2+}]_i$ increases were markedly inhibited by a glucagon receptor antagonist, des-His^1-[Glu^9]-Glucagon (1–29) amide (10^{-6} M) (shown as Antagonist, $n = 11$). (C) The ratio of the peak $[Ca^{2+}]_i$ response to the first glucagon stimulation (S1) over that to the second glucagon stimulation (S2) under control (A) and test conditions (B). The numbers on each bar indicate the numbers of glucagon-responsive NG neurons. $^{**}p < 0.01$ by unpaired t-test. (D) RT-PCR products of glucagon receptor mRNA in NG and liver. $M = 100$ bp ladder size maker, (-) = without template as a negative control.

responded to CCK-8 (Fig. 4B). Thus, the glucagon-responsive neurons overlapped with insulin- and CCK-responsive neurons.

4. Discussion

The present study has demonstrated that glucagon directly interacts with single NG neurons to induce $[Ca^{2+}]_i$ signaling via the glucagon receptor. Ip injection of glucagon induced pERK1/2 in NG as early as 15 min after injection. The incidence of the NG neurons with pERK1/2 *in vivo* and that of single NG neurons with $[Ca^{2+}]_i$ increases *in vitro* are comparable (approximately 8%) (Figs. 1C and 2C). These data suggest that the ip glucagon-induced activation of vagal afferents may be due to its direct action on the NG neurons. This finding of glucagon activation of vagal afferent neurons is in accordance with previous report that glucagon injection activates the neurons in the nucleus tractus solitarius (NTS) of the brainstem to which vagal afferents project [34].

In this study, relatively small fraction of NG neurons (8%) responded to glucagon and relatively high concentration of glucagon (10^{-8} M) was required. However, these properties appear to be reasonable in the light of the heterogeneity and role of vagal afferents. The terminals of vagal afferents innervate peripheral organs and sense the local information in/around the organs, including gastrointestinal hormones [16,35] and pancreatic insulin [24]. Gastrointestinal and pancreatic hormones exist in much higher concentrations in/around the organs releasing these hormones than in the peripheral circulation. In fact, the insulin concentration in the pancreatic vein is approximately 100-fold higher than that in the circulation [24]. Insulin at high concentrations activates the NG neurons innervating the pancreas with much higher incidence than other NG neurons [24]. In the present study, vagal afferent neurons were activated by glucagon at 10^{-9} M or higher (local concentration) but not 10^{-10} M (circulating concentration). Furthermore, the majority of glucagon-responsive NG neurons also responded to another pancreatic hormone, insulin. Taken together, the subpopulations of NG neurons innervating the pancreas and/or portal vein may sense the local glucagon that is present at a higher concentration.

It was previously proposed that glucagon acts in the liver, in which a signal is produced and relayed to the brain via vagal nerves [8,36,37]. The concept that the liver is the primary target site was supported by the studies reporting that the glucagon receptor is localized in the liver but not vagal nerves [37]. The present study showed that the glucagon receptor is expressed in vagal afferent nerves and that glucagon-induced $[Ca^{2+}]_i$ increases in vagal afferent NG neurons are inhibited by glucagon receptor antagonist. These results clearly demonstrate that glucagon directly interacts with the glucagon receptor to activate vagal afferent NG neurons.

The function of the glucagon-activated vagal afferent NG neurons remains to be clarified. In the present study, the vagal afferent NG neurons that responded to glucagon with $[Ca^{2+}]_i$ increases also responded to CCK and insulin. It is well known that CCK inhibits food intake by activating vagal afferent NG neurons [32,33,38]. We previously reported that insulin directly activates vagal afferent NG neurons and that this interaction is impaired in hyperphagic obese mice [24], suggesting that it may be involved in the anorexigenic action of insulin reported previously [12,39–41]. Both CCK and insulin are released postprandially. Hence, it is suggested that the postprandial, transient release of glucagon, possibly in

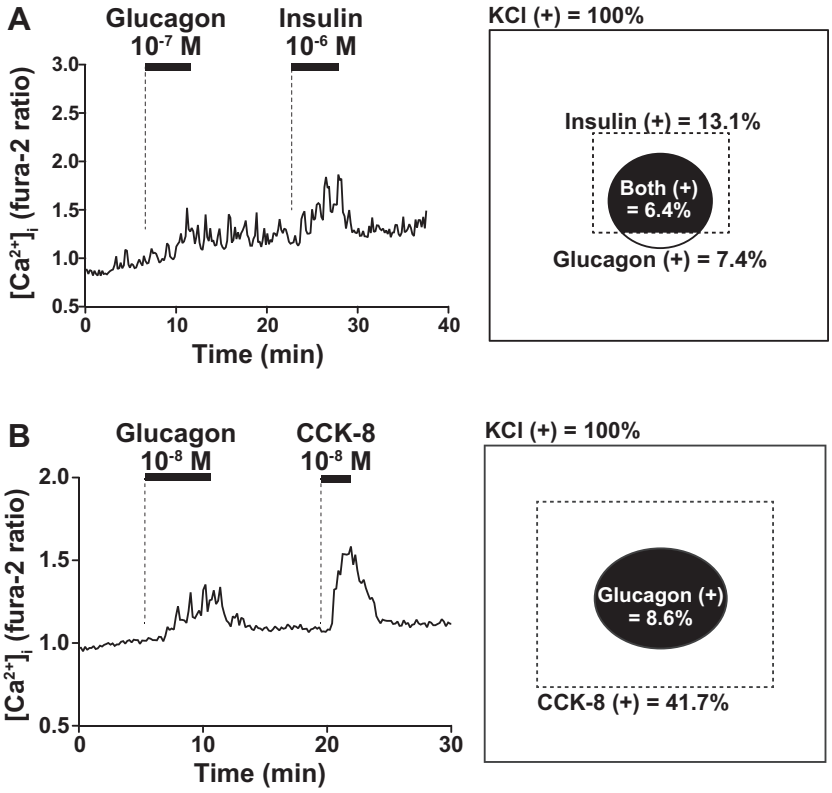

Fig. 4. Glucagon-responsive NG neurons overlap insulin- and CCK-8-responsive neurons. (A) Among 283 neurons examined, 21 neurons (7.4%) responded to 10^{-7} M glucagon. Among these 21 glucagon-responsive neurons, 18 responded to insulin (right panel). (B) Among 151 neurons examined, 13 neurons (8.6%) responded to 10^{-8} M glucagon. All of 13 glucagon-responsive neurons responded to CCK-8 (right panel).

collaboration with insulin and CCK, activates NG neurons, which may be relayed to the signaling to the brain and production of satiety. The glucagon activation of NG neurons could also be implicated in the action of this hormone to stimulate energy expenditure and heat production [8,13,14]. Glucagon is also released under hypoglycemic conditions. Hence, the activation of NG neurons by glucagon may also be involved in the recovery from hypoglycemia, a process known to be partly regulated by brain-mediated mechanisms [3,42]. However, further study is definitely required to clarify the physiological/pathophysiological functions of the activation of NG neurons by glucagon.

The present study has demonstrated that glucagon directly interacts with vagal afferent NG neurons to induce $[Ca^{2+}]_i$ signaling. This finding provides a clue to clarify the mechanisms for the diverse effects of glucagon, including glucose metabolism, satiety, energy expenditure and heat production.

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References

[1] G. Jiang, B.B. Zhang, Glucagon and regulation of glucose metabolism, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E671–E678.
[2] P.I. Mighiu, J.T. Yue, B.M. Filippi, M.A. Abraham, M. Chari, C.K. Lam, C.S. Yang, N.R. Christian, M.J. Charron, T.K. Lam, Hypothalamic glucagon signaling inhibits hepatic glucose production, Nat. Med. 19 (2013) 766–772.
[3] F. Lechin, B. Dijs, B. Pardey-Maldonado, Insulin versus glucagon crosstalk: central plus peripheral mechanisms, Am. J .Ther. 20 (2013) 349–362.
[4] W. Langhans, K. Pantel, W. Muller-Schell, E. Eggenberger, E. Scharrer, Hepatic handling of pancreatic glucagon and glucose during meals in rats, Am. J. Physiol. 247 (1984) R827–R832.
[5] A. de Jong, J.H. Strubbe, A.B. Steffens, Hypothalamic influence on insulin and glucagon release in the rat, Am. J. Physiol. 233 (1977) E380–E388.
[6] W.A. Muller, G.R. Faloona, R.H. Unger, The influence of the antecedent diet upon glucagon and insulin secretion, N. Engl. J. Med. 285 (1971) 1450–1454.
[7] N. Geary, Pancreatic glucagon signals postprandial satiety, Neurosci. Biobehav. Rev. 14 (1990) 323–338.
[8] K.M. Heppner, K.M. Habegger, J. Day, P.T. Pfluger, D. Perez-Tilve, B. Ward, V. Gelfanov, S.C. Woods, R. DiMarchi, M. Tschop, Glucagon regulation of energy metabolism, Physiol. Behav. 100 (2010) 545–548.
[9] W. Langhans, U. Zeiger, E. Scharrer, N. Geary, Stimulation of feeding in rats by intraperitoneal injection of antibodies to glucagon, Science 218 (1982) 894–896.
[10] J.R. Martin, D. Novin, Decreased feeding in rats following hepatic-portal infusion of glucagon, Physiol. Behav. 19 (1977) 461–466.
[11] A.J. Stunkard, T.B. Van Itallie, B.B. Reis, The mechanism of satiety: effect of glucagon on gastric hunger contractions in man, Proc. Soc. Exp. Biol. Med. 89 (1955) 258–261.
[12] N. Geary, L. Asarian, W. Langhans, The satiating potency of hepatic portal glucagon in rats and insulin or insulin antibodies, Physiol. Behav. 61 (1997) 199–208.
[13] C.J. Billington, T.J. Bartness, J. Briggs, A.S. Levine, J.E. Morley, Glucagon stimulation of brown adipose tissue growth and thermogenesis, Am. J. Physiol. 252 (1987) R160–R165.

[14] T. Heim, D. Hull, The effect of propranalol on the calorigenic response in brown adipose tissue of new-born rabbits to catecholamines, glucagon, corticotrophin and cold exposure, J. Physiol. 187 (1966) 271–283.
[15] W.A. Banks, The blood–brain barrier as a regulatory interface in the gut-brain axes, Physiol. Behav. 89 (2006) 472–476.
[16] Y. Iwasaki, T. Yada, Vagal afferents sense meal-associated gastrointestinal and pancreatic hormones: mechanism and physiological role, Neuropeptides 46 (2012) 291–297.
[17] W.A. Banks, J.B. Jaspan, W. Huang, A.J. Kastin, Transport of insulin across the blood–brain barrier: saturability at euglycemic doses of insulin, Peptides 18 (1997) 1423–1429.
[18] S.C. Woods, E.C. Lotter, L.D. McKay, D. Porte Jr., Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons, Nature 282 (1979) 503–505.
[19] D.M. Surina-Baumgartner, W. Langhans, N. Geary, Hepatic portal insulin antibody infusion increases, but insulin does not alter, spontaneous meal size in rats, Am. J. Physiol. 269 (1995) R978–R982.
[20] A. Asakawa, A. Inui, H. Yuzuriha, N. Ueno, G. Katsuura, M. Fujimiya, M.A. Fujino, A. Niijima, M.M. Meguid, M. Kasuga, Characterization of the effects of pancreatic polypeptide in the regulation of energy balance, Gastroenterology 124 (2003) 1325–1336.
[21] K. Anthony, L.J. Reed, J.T. Dunn, E. Bingham, D. Hopkins, P.K. Marsden, S.A. Amiel, Attenuation of insulin-evoked responses in brain networks controlling appetite and reward in insulin resistance: the cerebral basis for impaired control of food intake in metabolic syndrome?, Diabetes 55 (2006) 2986–2992
[22] Z. Kroner, The relationship between Alzheimer's disease and diabetes: type 3 diabetes?, Altern Med. Rev. 14 (2009) 373–379.
[23] Y. Iwasaki, M. Kakei, H. Nakabayashi, E.A. Ayush, M. Hirano-Kodaira, Y. Maejima, T. Yada, Pancreatic polypeptide and peptide YY_{3-36} induce Ca^{2+} signaling in nodose ganglion neurons, Neuropeptides 47 (2013) 19–23.
[24] Y. Iwasaki, K. Shimomura, D. Kohno, K. Dezaki, E.A. Ayush, H. Nakabayashi, N. Kubota, T. Kadowaki, M. Kakei, M. Nakata, T. Yada, Insulin activates vagal afferent neurons including those innervating pancreas via insulin cascade and Ca influx: its dysfunction in IRS2-KO mice with hyperphagic obesity, PLoS One 8 (2013) e67198.
[25] L.B. Rosen, D.D. Ginty, M.J. Weber, M.E. Greenberg, Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras, Neuron 12 (1994) 1207–1221.
[26] L.J. Chandler, G. Sutton, N.R. Dorairaj, D. Norwood, N-methyl D-aspartate receptor-mediated bidirectional control of extracellular signal-regulated kinase activity in cortical neuronal cultures, J. Biol. Chem. 276 (2001) 2627–2636.
[27] N. Lindgren, M. Goiny, M. Herrera-Marschitz, J.W. Haycock, T. Hokfelt, G. Fisone, Activation of extracellular signal-regulated kinases 1 and 2 by depolarization stimulates tyrosine hydroxylase phosphorylation and dopamine synthesis in rat brain, Eur. J. Neurosci. 15 (2002) 769–773.
[28] S. Baldassa, R. Zippel, E. Sturani, Depolarization-induced signaling to Ras, Rap1 and MAPKs in cortical neurons, Brain Res. Mol. Brain Res. 119 (2003) 111–122.
[29] Y. Dai, K. Iwata, T. Fukuoka, E. Kondo, A. Tokunaga, H. Yamanaka, T. Tachibana, Y. Liu, K. Noguchi, Phosphorylation of extracellular signal-regulated kinase in primary afferent neurons by noxious stimuli and its involvement in peripheral sensitization, J. Neurosci. 22 (2002) 7737–7745.
[30] Y. Iwasaki, H. Nakabayashi, M. Kakei, H. Shimizu, M. Mori, T. Yada, Nesfatin-1 evokes Ca^{2+} signaling in isolated vagal afferent neurons via Ca^{2+} influx through N-type channels, Biochem. Biophys. Res. Commun. 390 (2009) 958–962.
[31] L.H. Hansen, N. Abrahamsen, E. Nishimura, Glucagon receptor mRNA distribution in rat tissues, Peptides 16 (1995) 1163–1166.
[32] T.O. Lankisch, Y. Tsunoda, Y. Lu, C. Owyang, Characterization of CCK_A receptor affinity states and Ca^{2+} signal transduction in vagal nodose ganglia, Am. J. Physiol. Gastrointest. Liver Physiol. 282 (2002) G1002–G1008.
[33] S.M. Simasko, J. Wiens, A. Karpiel, M. Covasa, R.C. Ritter, Cholecystokinin increases cytosolic calcium in a subpopulation of cultured vagal afferent neurons, Am. J. Physiol. Regul. Integr. Comp. Physiol. 283 (2002) R1303–R1313.
[34] J.A. Parker, K.A. McCullough, B.C. Field, J.S. Minnion, N.M. Martin, M.A. Ghatei, S.R. Bloom, Glucagon and GLP-1 inhibit food intake and increase c-fos expression in similar appetite regulating centres in the brainstem and amygdala, Int. J. Obes. (Lond) 37 (2013) 1391–1398.
[35] J.H. Peters, R.C. Ritter, S.M. Simasko, Leptin and CCK selectively activate vagal afferent neurons innervating the stomach and duodenum, Am. J. Physiol. Regul. Integr. Comp. Physiol. 290 (2006) R1544–R1549.
[36] N. Geary, G.P. Smith, Selective hepatic vagotomy blocks pancreatic glucagon's satiety effect, Physiol. Behav. 31 (1983) 391–394.
[37] S.C. Woods, T.A. Lutz, N. Geary, W. Langhans, Pancreatic signals controlling food intake; insulin, glucagon and amylin, Philos. Trans. R. Soc. Lond. B Biol. Sci. 361 (2006) 1219–1235.
[38] G.P. Smith, C. Jerome, B.J. Cushin, R. Eterno, K.J. Simansky, Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat, Science 213 (1981) 1036–1037.
[39] K.D. Niswender, D.G. Baskin, M.W. Schwartz, Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis, Trends Endocrinol. Metab. 15 (2004) 362–369.
[40] D. Porte Jr., S.C. Woods, Regulation of food intake and body weight in insulin, Diabetologia 20 (3) (1981) 274–280.
[41] M.W. Schwartz, D. Porte Jr., Diabetes, obesity, and the brain, Science 307 (2005) 375–379.
[42] A.J. Verberne, A. Sabetghadam, W.S. Korim, Neural pathways that control the glucose counterregulatory response, Front. Neurosci. 8 (2014) 38.