GLP-2 potentiates L-type Ca\(^{2+}\) channel activity associated with stimulated glucose uptake in hippocampal neurons

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GLP-2 potentiates L-type Ca\(^{2+}\) channel activity associated with stimulated glucose uptake in hippocampal neurons. Am J Physiol Endocrinol Metab 298: E156–E166, 2010. First published November 17, 2009; doi:10.1152/ajpendo.00585.2009.—Glucagon-like peptide-2 (GLP-2) is a neuropeptide secreted from endocrine cells in the gut and neurons in the brain. GLP-2 stimulates intestinal crypt cell proliferation and mucosal blood flow while decreasing gastric emptying and gut motility. However, a GLP-2-mediated signaling network has not been fully established in primary cells. Since the GLP-2 receptor mRNA and protein were highly expressed in the mouse hippocampus, we further characterized that human \(^{125}\)I-labeled GLP-2, specifically bound to cultured hippocampal neurons with \(K_d=0.48\) nM, and GLP-2 acutely induced subcellular translocalization of the early gene c-Fos. Using the whole cell patch clamp, we recorded barium currents \((I_{\text{Ba}})\) flowing through voltage-gated Ca\(^{2+}\) channels (VGCC) in those neurons in the presence of GLP-2 with and without inhibitors. We showed that GLP-2 (20 nM) enhanced the whole cell \(I_{\text{Ba}}\) mediated by L-type VGCC that was defined using an L-type Ca\(^{2+}\) channel blocker (nifedipine, 10 \(\mu\)M). Moreover, GLP-2-potentiation of L-type VGCC was abolished in neurons pretreated with a PKA inhibitor (PKI\(_{14-22}\), 1 \(\mu\)M). Finally, using a fluorescent nonmetabolized glucose analog (6-NBDG) imaging, we showed that glucose was taken up directly by cultured neurons. GLP-2 increased 2-deoxy-D-\([\text{3H}]\)glucose uptake that was dependent upon dosage, activation of PKA, and potentiation of L-type VGCC. We conclude that GLP-2 potentiates L-type VGCC activity through activating PKA signaling, partially stimulating glucose uptake by primary cultured hippocampal neurons. The potentiation of L-type VGCC may be physiologically relevant to GLP-2-induced neuroendocrine modulation of neurotransmitter release and hormone secretion.

GLUCAGON-LIKE PEPTIDE-2 (GLP-2) is a neuropeptide secreted from endocrine cells in the gut and neurons in the brain. Through a specific G protein-coupled receptor, GLP-2R, GLP-2 stimulates intestinal crypt cell proliferation and mucosal blood flow to promote nutrient absorption while decreasing gastric emptying and gut motility to inhibit food intake (38, 58). The GLP-2R is expressed not only in distinct gastrointestinal cells (such as enteric neurons, enteroendocrine cells, subepithelial myofibroblasts, and pancreatic \(\alpha\)-cells) but also in specific regions of the central nervous system (CNS, such as the hippocampus and hypothalamus) (38, 58). Currently, there is much interest in the central effects of GLP-1/2 in the neuroendocrine control of food intake. Besides the hypothalamus, the hippocampus is sensitive to hunger and satiety signals, thus influencing appetitive behavior through inhibitory learning and memory processes (22, 23, 67). Moreover, neuroendocrine hypothalamic circuits are integrated for the control of energy homeostasis with high-order hippocampal learning and memory processes (22). However, the GLP-2-mediated cellular action and signaling network are largely unknown in the CNS.

Calcium entry through voltage-gated Ca\(^{2+}\) channels (VGCC) in hippocampal neurons is important for neurotransmitter release, synaptic plasticity, gene transcription, and neuronal survival (3). Increases in intracellular Ca\(^{2+}\) concentrations can activate key kinases or phosphatases, which modulate ion channels, transcription factors, and regulatory proteins that are involved in synaptic plasticity and memory formation (13). For example, Ca\(^{2+}\) acting on the synaptic vesicle protein synaptotagmin I triggers rapid exocytosis of neurotransmitters (15, 16). In addition, Ca\(^{2+}\) entry through L-type VGCC induces long-term potentiation in the hippocampal pyramidal neurons, which is an important form of neuronal plasticity in learning and memory processing (61).

The evidence that GLP-2R is expressed exclusively in these excitable cells suggests that GLP-2 signal transduction may mediate electrical activity of the plasma membrane, thus inducing neurotransmitter release from neurons and hormone secretion from endocrine cells. For example, GLP-2 stimulates glucagon secretion from pancreatic \(\alpha\)-cells through unidentified molecular mechanisms (43). Furthermore, GLP-1, coproduced with GLP-2 from neurons or endocrine cells, augments barium currents flowing through L-type VGCC through a Ca\(^{2+}\)-dependent mechanism in pancreatic \(\beta\)-cells (40, 57). This may play a crucial role in inducing insulin secretion and neurotransmitter release. Moreover, the activity of the L-type VGCC is enhanced in neurons by cAMP-dependent protein kinase A (PKA). It is well established that GLP-2R activation increases intracellular cAMP concentration and enhances PKA-dependent signaling in hippocampal neurons (39). However, it is unknown whether GLP-2 potentiates L-type VGCC activity in hippocampal neurons.

In the mammalian brain, ~50% of the total energy consumption is associated with neural signaling, including maintenance of resting potentials and neurotransmitter recycling (2, 46). In the brain, glucose is the predominant substrate for energy metabolism. Delivery of glucose from the blood to the brain requires transport across the endothelial cells of the blood-brain barrier and the plasma membranes of neurons and...
glia. Previous studies show that GLP-2 enhances glucose uptake by enterocytes through promoting cellular translocation of ATP-dependent Na+/glucose cotransporter 1 (17, 25). However, it is unknown whether GLP-2 affects glucose uptake by neurons where ATP-independent, facilitative glucose transporters (such as GLUT3) are expressed. A recent study indicates that Ca2⁺ influx is an important modulator of insulin-mediated glucose uptake through Ca2⁺/calmodulin, which acts in GLUT4 translocation to the plasma membrane (35, 62). Therefore, we wanted to determine whether GLP-2 facilitates glucose uptake by enhanced activity of L-type VGCC in neurons.

Our present study shows that GLP-2 potentiates the activation of L-type Ca2⁺ channels associated with stimulated glucose uptake in primary cultured hippocampal neurons. This L-type Ca2⁺ channel activation may play an important role in triggering neurotransmitter release and hormone secretion from GLP-2R-positive neurons and endocrine cells, respectively.

METHODS

All experiments were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. The C57BL/6J mice were fed ad libitum and given free access to water.

Localization of GLP-2R. The expression of glp2r mRNA in the mouse brain was performed by in situ hybridization (36) and quantified by an automated image processing technique (11). The cellular distribution of glp2r mRNA abundance was denoted by a digital false-color map representing the original expression pattern, on which it was painted in yellow, blue, and red, respectively, for the weak, moderate, and strong expressions. The localization of the GLP-2R protein was confirmed by immunohistochemistry and defined on cultured hippocampal neurons by use of immunocytochemistry. Methods in details are provided in Supplementary materials (Supplementary materials are found in the online version of this paper.).

Primary culture of hippocampal neurons. Neurons were obtained from 1-day-old neonatal mice. The hippocampus was dissected in ice-cold sterilized PBS containing 10% FBS, 0.1 mM L-glutamine, 10 mM HEPES (pH 7.4 adjusted with NaOH), osmolarity 300-320 mOsm.

2-deoxy-d-[3H]glucose uptake. 2-Deoxy-d-[3H]glucose ([3H]2-DG) uptake was determined as described (7). Hippocampal neurons on DIV 6 were stained with serum-free NeurobasalA medium for 4 h and exposed to GLP-2 at 20 nM for 30 min at 37°C in KRH buffer (125 mM NaCl, 5 mM KCl, 1.8 mM CaCl2, 2.6 mM MgSO4, 5 mM HEPES, pH 7.4) containing 500 µM 2-DG and [1,2-3H]-2-DG (30–60 Ci/mmol, MP Biomedicals). When tested, 10 µM nifedipine (Sigma) or 1 µM PKA inhibitor amide 14–22 (PKI, Calbiochem) was added 30 min prior to GLP-2 treatment. Cytochalasin B (50 µM) was added for the background subtraction of nonspecific glucose uptake. Glucose uptake by 6-NBDG tracing. Fluorescent nonmetabolized 6-NBDG [6-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-6-deoxyglucose] was used in place of [3H]2-DG (64, 65). Neurons on coverslips were loaded with 300 µM 6-NBDG for 5 (or 15) min, washed out, incubated in KRH buffer for 15 min, washed, and incubated in KRH buffer for 15 min. Labeled living neurons were detected under a fluorescence confocal microscopy.

Statistical analysis. Data were analyzed by ANOVA (SAS version 9.1; SAS Institute, Cary, NC). Data are expressed as means ± SE. P values < 0.05 or 0.01 were considered statistically significant.

RESULTS

GLP-2R functionally expressed in primary hippocampal neurons. To identify spatial expression patterns of glp2r mRNA in the brain, we performed in situ hybridization at a cellular resolution. We found that the glp2r mRNA was most abundantly expressed in the hippocampus (Fig. 1A, c–f) compared with the cortex, cerebellum, hypothalamus, and brainstem regions (data not shown), where glp2r mRNA is localized in rat and mouse brain (38, 39, 58). This glp2r-specific expression pattern might indicate that GLP-2R plays a distinct role in the regulation of spatial metabolism and synaptic transmission. By immunohistochemistry, GLP-2R protein was expressed in the hippocampus (Fig. 1B, a). At the cellular level, GLP-2R (in green in Fig. 1B, b) was expressed on the surface of cellular membranes and colocalized with neurons [labeled by a neuron-specific protein, i.e., protein gene product 9.5 (PGP9.5), as shown in red in Fig. 1B, a] and merged in yellow in Fig. 1B, c). Furthermore, GLP-2R was expressed mainly in neuronal soma (in green in Fig. 1B, e) but not in dendrites in primary cultured hippocampal neurons.

We wanted to characterize further whether the endogenous GLP-2R functionally responded to GLP-2, its native ligand. The intact cell binding assay was used to test the GLP-2 binding curve. Primary hippocampal neurons showed a saturable and specific binding to the 125I-labeled human GLP-2 (Fig. 2a). By regression analysis, the specific binding at the top plateau, i.e., the maximal number of binding sites (Bmax) was 1.42 fmol/mg protein and 1.42 ± 0.33 fmol/mg protein and KD = 0.48 ± 0.05 nM.Similar to a high-affinity binding (0.57 nM) of 125I-Tyr34 human GLP-2 in COS cells stably transfected with rat GLP-2R cDNA, which has a maximal binding at 1.839 fmoles/mg protein (45). This saturation binding indicated that the affinity of human GLP-2 for mouse endogenous GLP-2R was comparable to that of overexpressed rat GLP-2R. However, the binding site density was lower in the intact primary neurons.

Next, we wanted to test whether primary neurons acutely responded to GLP-2 treatment. c-fos, an immediate early gene widely used as a functional marker of activated neurons (33), regulates expression of target genes that can influence neuronal excitability and survival in the hippocampus (68). Under basal condition, the c-Fos protein was expressed and localized exclusively to the nucleus in scattered neurons (Fig. 2b). Upon GLP-2 stimulation for 30 min, the c-Fos protein was revealed exclusively to the nucleus in scattered neurons (Fig. 2c), indicating c-Fos protein transient translocation from
Fig. 1. A: glucagon-like peptide-2 receptor (GLP-2R) mRNA expressed in the mouse brain. First, the spatial pattern for glp2r mRNA expression was revealed using a GLP-2R cRNA antisense probe by in situ hybridization in the mouse sagittal section. The positive signal for GLP-2R mRNA in blue was detected in the hippocampus marked in a square (c) and further magnified to cellular resolution (d). Second, levels of glp2r mRNA abundance was quantified by an automated image processing technique and painted in yellow, blue, and red, respectively, for the weak, moderate, and strong expression on a digital false-color map (e and f) representing the original expression pattern. [NB: images a (unpainted) and b (painted) were negative controls for in situ hybridization using a GLP-2R cRNA sense probe.] B: GLP-2R protein expressed in hippocampal neurons. The expression pattern for the GLP-2R protein was confirmed in confocal immunohistochemistry (a–c) or immunocytochemistry (d–f). GLP-2R protein was stained in green (b and e) and neuronal marker PGP9.5 in red (a and d). GLP-2R protein was expressed in the hippocampus (in yellow) and marked in a square (a) and localized on the plasma membrane of the neuronal body when further magnified in b. Furthermore, GLP-2R protein was expressed in hippocampal neurons cultured on DIV 5 (d–f). [NB: negative controls were performed without primary antibody against GLP-2R in the incubation (images not shown)].
the nucleus to the cytoplasm (and dendrites) or reverse. Although the c-fos gene can be rapidly induced within 5 min and 2 h (4, 32), c-Fos protein is unstable in the cytoplasm through ubiquitin- and/or proteosome-dependent degradation pathways. This spatiotemporal regulation of c-Fos may be mediated by the GLP-2R-activated cAMP pathway (53, 55), altering neuronal activity in the hippocampus.

GLP-2 enhanced Ba\(^{2+}\) currents predominately through L-type VGCC potentiation. Because Ca\(^{2+}\) influx can directly increase neuronal excitation through synaptic receptors and voltage-gated channels (37), we wanted to examine whether GLP-2 stimulation could modulate activity of VGCC in primary neurons. To minimize the confounding effects of Ca\(^{2+}\) on the kinetics of VGCC, Ba\(^{2+}\) currents (I\(_{Ba}\)) were recorded flowing through all subtypes of VGCC in primary neurons. Ba\(^{2+}\) currents were recorded through a voltage step protocol (from −90 to 40 mV). The I-V curve showed reverse potentials and peaks (at −40 mV) as typical values for Ba\(^{2+}\) currents (Fig. 3A, a and b). No shift was found in the voltage dependence of Ca\(^{2+}\) channel activation, indicating that I\(_{Ba}\) was increased throughout the voltage range. Moreover, the peak of the whole cell I\(_{Ba}\) was also achieved at 0 mV. GLP-2 potentiated the whole cell I\(_{Ba}\) in cultured hippocampal neurons (Fig. 3A, e, \(P < 0.01\)). The peak of I\(_{Ba}\) was 139 ± 13 pA for neurons under the basal condition, whereas it was 202 ± 10 pA for neurons in the presence of 20 nM GLP-2, indicating that GLP-2 enhanced VGCC activity by 45%. This effect was reversed after washout of GLP-2.

L-type VGCC are predominant VGCC in hippocampal neurons. We further characterized a GLP-2-mediated increase in the peak of Ba\(^{2+}\) inward currents flowing through L-type VGCC by isolating it electrophysiologically (at a holding potential of −40 mV shown in Fig. 3A, b, d, and f) (18, 60); and defined it pharmacologically using an L-type Ca\(^{2+}\) channel blocker, nifedipine (at 10 \(\mu\)M) (Fig. 4, a, c, and e). Approximately 80% of the peak I\(_{Ba}\) was attributed to GLP-2-mediated enhancement of L-type VGCC activation under the basal condition or under the stimulatory condition, indicating that the peak I\(_{Ba}\) (reached at 0 mV) evoked at the holding potential of −40 mV was flowing substantially through L-type VGCC. Importantly, −85% of peak I\(_{Ba}\) was inhibited pharmacologically in the presence of nifedipine at 10 \(\mu\)M, representing that recorded I\(_{Ba}\) was mainly through L-type channels. Moreover, the peak I\(_{Ba}\) was not increased in GLP-2-treated neurons pretreated with nifedipine. The average peak I\(_{Ba}\) was at −15 ± 10 pA for nifedipine-treated neurons and −17 ± 13 pA for nifedipine-preloaded, GLP-2-treated neurons. However, after a washout procedure, it increased again (−129 ± 13 pA, \(P < 0.01\)).
0.01) by 51% in the presence of GLP-2 compared with that of −85 ± 11 pA under the washout condition. These data suggest that GLP-2-enhanced whole cell $I_{\text{Ba}}$ was mediated mainly by potentiating L-type VGCC activity in primary neurons.

GLP-2-enhanced L-type $I_{\text{Ba}}$ in a PKA-dependent manner. In the present study, we focused on GLP-2-mediated L-type VGCC activity by recording Ba$^{2+}$ currents in primary neurons, removing the complication of Ca$^{2+}$-induced inactivation of L-type VGCC. Therefore, we further analyzed the activation kinetics from the G–V curve (Fig. 3B). The $V_{1/2}$ was at −1.06 ± 0.32 mV for the controlled neurons and at −4.11 ± 0.38 mV for the GLP-2-treated neurons. This was a shift ($P < 0.01$) toward a more hyperpolarized activation, indicating that an enhanced activation of L-type VGCC might be attributed to
GLP-2-increased inward $I_{Ba}$. Hyperpolarization shift in the $G$-$V$ curve reflects an improved coupling between gating charge movement and channel opening.

PKA is shown to enhance activation of L-type VGCC by phosphorylating the $\alpha_{1C}$ subunit (of Cav1.2) (20, 24). It has been demonstrated that GLP-2 increases intracellular cAMP accumulation and enhances PKA activity in HEK cells overexpressing GLP-2R. Thus, GLP-2-induced potentiation of L-type VGCC activation might be mediated through activating cAMP-dependent PKA signaling pathways. To assess this possibility, we tested whether GLP-2-mediated potentiation of L-type VGCC activity was attenuated in neurons pretreated with the membrane-permeable PKA inhibitor PKI14–22. We found that GLP-2 treatment did not increase the peak $I_{Ba}$ in neurons that were preloaded with PKI14–22 (at 1 $\mu$M) (Fig. 4, b, d, and f). There was no difference in the peak $I_{Ba}$ ($-64 \pm 8$ pA for PKI14–22-treated neurons and $-66 \pm 10$ pA for PKI14–22-preloaded, GLP-2-treated neurons). After a washout procedure, however, the peak $I_{Ba}$ was increased in the presence of GLP-2 as described above.

GLP-2-mediated stimulation in glucose uptake. To determine whether hippocampal neurons directly take up glucose, we first used 6-NBDG as a fluorescent, nonhydrolyzable glucose analog to trace glucose uptake and transport (31, 64). Neurons could directly take up and utilize glucose as energy sources in vitro (Fig. 5a), which is in agreement with recent reports (31, 52). Furthermore, we detected the protein expression of the facilitative glucose transporter (GLUT3) mainly responsible for neuronal glucose uptake, which may play a key role in synaptic energy supply and neurotransmitter synthesis (19). GLUT3 (labeled in green in Fig. 5b) was expressed not only in neurons (identified by a neuron-specific marker PGP9.5

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Fig. 4. GLP-2 increased L-type $I_{Ba}$ by activating PKA signaling pathway. Holding potential ($V_h$) at $-40$ mV was applied to trace $I_{Ba}$ flowing through L-types of VGCC by whole cell patch clamp. Representative $I$-$V$ curves are shown using the voltage step protocol from $-40$ to $40$ mV in a step of $10$ mV (a and b). NB: no shift was observed in voltage dependence of Ca$^{2+}$ channel activation. Peak $I_{Ba}$ were reached at 0 mV test potential (c–d). Averaged peak $I_{Ba}$ (reached at test voltage of 0 mV) are represented for the holding potential of $-40$ mV (e and f). Curves or bars in black, red, pink, green, and gray represent recording procedures for control, GLP-2 stimulation (20 nM), L-type VGCC blocker nifedipine (10 $\mu$M), or PKA inhibitor PKI (1 $\mu$M), GLP-2 + nifedipine or PKI, and washout, respectively. **$P < 0.01$. 

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in red), but also in glial cells. However, the abundance of GLUT3 protein did not increase in neurons after treatment with GLP-2 at 20 nM for 30 min (data not shown). These data are in agreement with reports that expression of the GLUT3 protein does not acutely increase within neurons treated with brain-derived neurotrophic factor (6, 51). However, we cannot rule out the possibility that acute GLP-2-stimulated glucose uptake might be attributed to subcellular translocation of GLUT3.

Moreover, we quantified whether GLP-2 modulates glucose uptake in primary neurons by the [3H]2-DG uptake assay. 2-DG is a glucose analog that is transported specifically by ATP-independent, facilitative glucose transporters, but not by ATP-dependent Na+/glucose cotransporters. In the presence of cytochalasin B (50 μM), a competitive inhibitor of glucose transport through facilitative glucose transporters, 2-DG uptake was at 3.5%. Thus 96.5% of 2-DG uptake was cytochalasin B-sensitive, mainly through facilitative GLUT-mediated uptake. GLP-2 stimulated glucose uptake in a dose-dependent manner by GLP-2 treatment for 30 min and maximized at 20 nM. Experiments were repeated independently 3 times. *P < 0.05 and **P < 0.01.
promote neuronal survival. We wanted to examine whether GLP-2-mediated stimulation of 2-DG uptake was mediated partially through the activation of cAMP-dependent PKA. Although primary neurons were preloaded with PKI_{14-21} (1 μM) 30 min prior to GLP-2 treatment, GLP-2-mediated stimulation in 2-DG uptake was abolished completely by PKI_{14-21} (Fig. 6a). To examine whether GLP-2-stimulated uptake of glucose was associated with the potentiation of L-type VGCC activity, hippocampal neurons were treated with GLP-2 in the presence of nifedipine (10 μM). GLP-2-mediated stimulation on 2-DG uptake was completely blocked by nifedipine (Fig. 6b). Moreover, the basal uptake of glucose was suppressed by the PKA inhibitor but not by the L-type VGCC blocker. (Note that the abundance of GLUT3 protein was not increased in neurons treated with GLP-2 at 20 nM for 30-min. Thus, the abundance of GLUT3 protein might not be attributed to GLP-2-acutely mediated stimulation of glucose uptake.) Together, these data indicate that GLP-mediated stimulation of glucose uptake might involve the potentiation of L-type VGCC activity.

DISCUSSION

In the present study, we characterized functionally the primary culture model of mouse hippocampal neurons that expressed endogenous GLP-2R, and we showed that GLP-2 increased Ba^{2+} currents through potentiated activation of L-type Ca^{2+} channels by using the whole cell voltage clamp. This potentiation was mediated by activating the cAMP-dependent PKA signaling pathway. Moreover, GLP-2 stimulated glucose uptake by primary neurons, and this stimulation was not only dependent upon activation of PKA, but also associated with potentiation of L-type Ca^{2+} channels. We speculate that GLP-2-mediated potentiation of L-type Ca^{2+} channel activity may play an important role in Ca^{2+}-triggered neurotransmitter release and hormone secretion from GLP-2R-positive neurons and endocrine cells, respectively. In addition, GLP-2-mediated potentiation of L-type VGCC activity would induce long-term potentiation in the hippocampal pyramidal neurons, which may play a critical role in coupling cellular signal transduction to learning and memory (61) or neuroprotection (39).

Characterization of endogenous GLP-2R in primary neurons. GLP-2R is expressed in endocrine cells, neurons, and myofibroblasts. GLP-2R function and signaling have been studied mainly in cell lines overexpressing the receptor, and functional expression of endogenous GLP-2R has not been characterized in primary cells. We found that GLP-2R was expressed not only in primary hippocampal neurons but was also specifically and functionally responsible for GLP-2-mediated stimulation. We were the first to characterize binding properties of endogenous GLP-2R and showed a saturated curve with the binding affinity (K_D) similar to that in GLP-2R-overexpressed membrane preparation. Moreover, subcellular translocalization of the early gene c-fos might reflect an acute GLP-2-mediated action. Therefore, this characterization of endogenous GLP-2R would validate the usefulness of working with primary cultures of hippocampal neurons in further studies of GLP-2 function and signaling.

GLP-2 potentiates activation of L-type VGCC. One of the major findings in the present study is that GLP-2 increased the peak I_{Ca}\_L by ~50% (48). Moreover, L-type Ca^{2+} channels are predominantly and functionally expressed in the somatodendritic compartment of hippocampal neurons cultured for ~5 days (48). L-type Ca^{2+} channels are functionally present in CA1 pyramidal neurons, contributing to spinal Ca^{2+} influx (29). L-type currents in neurons are evoked within ~40 to 0 mV (14). Barium currents were recorded at a holding potential of ~40 mV in voltage step protocols in the present study. 3) GLP-2-mediated potentiation was completely blocked by the L-type Ca^{2+} channel blocker...
nifedipine, providing evidence for GLP-2-mediated increases in i_{Ba} flowing through L-type VGCC in hippocampal neurons. L-type Ca^{2+} channels (including Ca_{1.2} and Ca_{1.3} in endocrine cells and neurons) serve as key transducers of plasma membrane potential changes into local intracellular Ca^{2+} transients. This L-type Ca^{2+} current initiates distinct signaling pathways in regulation of hormone secretion, neurotransmitter release, synaptic plasticity, and gene transcription (9, 13, 14, 27). For example, potentiation of L-type VGCC may increase Ca^{2+} influx into hippocampal neurons, which is associated with late-phase, long-term potentiation underlying learning and memory (28, 44).

GLP-2-mediated potentiation of L-type VGCC activity through activating PKA signaling. GLP-2 increased i_{Ba} flowing through L-type VGCC as a result of enhanced activation of those channels indicated by a significant leftward shift in the G-V curve. Furthermore, GLP-2-mediated potentiation of L-type VGCC activity was blocked by a PKA inhibitor, suggesting that GLP-2-mediated potentiation was dependent on activation of the PKA signaling. GLP-2 increases intracellular cAMP production by activating GLP-2R-coupled G_{i} protein. The cAMP-dependent PKA regulates a wide array of cellular functions, including modulating the activity of the L-type VGCC in hippocampal neurons (20, 21, 29). L-type VGCC is composed minimally of pore-forming α_{1}-subunits along with accessory β- and α_{2}β-subunits. L-type Ca^{2+} channel (Ca_{1.2}) is associated with the G protein-coupled receptor in hippocampal neurons (20). Importantly, the activity of Ca_{1.2} can be enhanced in hippocampal neurons by β-adrenergic ligands, which is mediated by PKA-dependent phosphorylation of the α_{1}-subunit (at Ser^{1058}) (20, 21, 24, 26, 29, 47). Thus, we speculate that GLP-2 modulates α_{1}-subunit phosphorylation and facilitates L-type VGCC activation, causing a left shift in the conductance-voltage relationship and potentiation peak i_{Ba} at hyperpolarized membrane potentials. However, we cannot exclude the possibility that suppression of the inactivation process of the L-type Ca^{2+} channel is involved in the GLP-2-mediated increase in Ba^{2+} currents.

Glucose uptake in hippocampal neurons. In the present study, we have shown that glucose can be taken up directly by primary neurons, as indicated by the intracellular accumulation of 6-NBDG. In addition, the GLUT3 protein was highly expressed in primary neurons transporting glucose across the plasma membrane. It has been hypothesized that neurons obtain their energy source from glial lactate, especially during neuronal activation (56). However, this concept is challenged by a recent model that indicates that the neuron is the primary site of glucose uptake and utilization under both steady-state and stimulated conditions (56). Neuronal uptake of glucose is mediated by facilitative glucose transporters such as GLUT3 (19, 41). Insulin promotes GLUT3 translocation to the plasma membrane, enabling neurons to respond to energy demand induced by increased neuronal activity (59). However, glucose transport (via GLUT3) is largely independent of insulin in the CNS (42). In neurons, phosphorylated CREB (cAMP regulatory element-binding) can bind the GLUT3 gene, trans-activating its expression (49). However, we could not find any increase in the abundance of GLUT3 protein or subcellular translocation of GLUT3 protein in hippocampal neurons treated with GLP-2 for 30 min. We cannot exclude the involvement of other isoforms (e.g., GLUT1, -4, and -8) in GLP-2-mediated stimulation of glucose uptake. We speculate that insulin-stimulated glucose uptake in the present study might be attributed to insulin-responsive GLUT4 and -8 that are also expressed in hippocampal neurons (1, 5, 10, 50, 54). The novel role of GLP-2-stimulated glucose uptake in neurons might be important, since cellular glucose uptake is predominantly insulin independent in the brain. Further studies are warranted to confirm which isoforms of facilitative glucose transporters in neurons are specifically regulated by GLP-2.

Another major finding in the present study is that GLP-2-mediated stimulation of glucose uptake was involved with the potentiation of L-type VGCC activation in primary neurons. Previous studies suggest that Ca^{2+} influx through L-type VGCC is involved in insulin-stimulated glucose transport in skeletal muscle (66). Thus, we hypothesized that GLP-2-mediated enhancement of Ca^{2+} channel activity would alter glucose uptake in neurons. Interestingly, GLP-2-mediated stimulation of glucose uptake was completely blocked in the presence of nifedipine, an L-type Ca^{2+} channel inhibitor, suggesting that this metabolic action is mediated partially by enhanced L-type Ca^{2+} channel activity. Recent studies show that increased influx of Ca^{2+} across the plasma membrane is associated with insulin-mediated increase in glucose uptake (34, 35), possibly through facilitating docking, fusion, and/or insertion of GLUT4 in the membrane via Ca^{2+} sensor (such as synaptotagmin I) in the neurons (34, 35, 62) or through modulating calcium-calmodulin-dependent kinase II signaling (30). However, nifedipine did not affect the basal level of 2-DG uptake. This is in agreement with studies that a solitary decrease in Ca^{2+} influx is not enough to alter basal glucose uptake (12, 35, 62).

The novel function of GLP-2-mediated potentiation of L-type Ca^{2+} channel activity might be physiologically relevant to its neuroendocrine modulation of neurotransmitter release and hormone secretion. As mentioned previously, it is assumed that GLP-2 executes cellular actions and metabolic effects mainly through secondary mediators such as neurotransmitters (e.g., nitric oxide, serotonin, and vasoactive intestinal peptide) and hormones (e.g., glucagon, IGF-I, and KGF). GLP-2-mediated enhancement of L-type Ca^{2+} channel activity would increase cellular Ca^{2+} influx, resulting in Ca^{2+}-triggered release of neurotransmitters and secretion of hormones. Moreover, GLP-2-mediated potentiation of L-type Ca^{2+} channel activity would induce long-term potentiation, which is highly relevant to key functions (e.g., learning and memory) of the hippocampus. Finally, GLP-2-stimulated uptake of glucose might be equally relevant to energetic demand for neuronal activities and regulation of glucose metabolism in the brain. This regulatory process might be physiologically important, because uptake and utilization of glucose in the brain is insulin independent. However, further studies are warranted to define physiological significance of GLP-2-mediated neuronal action and signaling by using neuron-specific GLP-2R deficiency mouse models.

In summary, we showed that GLP-2-mediated potentiation of L-type Ca^{2+} channel activity was mediated through the activation of PKA. GLP-2-mediated stimulation of glucose uptake was not only dependent upon activation of PKA, but also coupled with potentiated activation of L-type Ca^{2+} channels. GLP-2-potentiated activation of L-type Ca^{2+} channels may be physiologically relevant to neurotransmitter release, synaptic plasticity, and energetic demand for memory forma-
tion in the hippocampus. However, further studies are warranted to establish whether GLP-2-potentiated activation of L-type Ca²⁺ channels initiates Ca²⁺-triggered neurotransmitter release and hormone secretion from GLP-2R-positive neurons and endocrine cells and to define whether GLP-2R activation modulates phosphorylation of α₁-subunits of L-type VGCC in those cells.

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DISCLOSURES

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