Glucose-dependent insulinotropic polypeptide receptor knockout mice are impaired in learning, synaptic plasticity, and neurogenesis

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Faivre E, Gault VA, Thorens B, Hölscher C. Glucose-dependent insulinotropic polypeptide receptor knockout mice are impaired in learning, synaptic plasticity, and neurogenesis. J Neurophysiol 105: 1574–1580, 2011. First published January 27, 2011; doi:10.1152/jn.00866.2010.—Glucose-dependent insulinotropic polypeptide (GIP) is a key incretin hormone, released from intestine after a meal, producing a glucose-dependent insulin secretion. The GIP receptor (GIPR) is expressed on pyramidal neurons in the cortex and hippocampus, and GIP is synthesized in a subset of neurons in the brain. However, the role of the GIPR in neuronal signaling is not clear. In this study, we used a mouse strain with GIPR gene deletion (GIPR KO) to elucidate the role of the GIPR in neuronal communication and brain function. Compared with C57BL/6 control mice, GIPR KO mice displayed higher locomotor activity in an open-field task. Impairment of recognition and spatial learning and memory of GIPR KO mice were found in the object recognition task and a spatial water maze task, respectively. In an object location task, no impairment was found. GIPR KO mice also showed impaired synaptic plasticity in paired-pulse facilitation and a block of long-term potentiation in area CA1 of the hippocampus. Moreover, a large decrease in the number of neuronal progenitor cells was found in the dentate gyrus of transgenic mice, although the numbers of young neurons was not changed. Together the results suggest that GIP receptors play an important role in cognition, neurotransmission, and cell proliferation.

Alzheimer; incretin; insulin; long-term potentiation; memory

GLUCOSE-DEPENDENT INSULINOTROPIC PEPTIDE (GIP) is a 42-amino acid peptide that belongs to the secretin-glucagon family of gastrointestinal regulatory peptides. GIP is synthesized in and secreted from the endocrine K-cells in the intestinal epithelium (Baggio and Drucker 2007; Drucker et al. 2006). Recently, GIP and also GIP receptor (GIPR) expression has been reported in the large pyramidal neurons in the cortex and the hippocampus (Nyberg et al. 2005, 2007). Furthermore, GIP promotes growth, differentiation, proliferation, and survival of β-cells (Irwin et al. 2006; Trumper et al. 2001) and neuronal progenitor cells (Nyberg et al. 2005).

Although GIP and GIPRs have been detected in the brain, few studies have looked at the potential roles that GIP could play in the central nervous system (CNS). The GIPR is a member of the family of seven-transmembrane domain G protein-coupled receptors (GPCR) (Yip et al. 2000). GIP mRNA and GIPR mRNA are expressed in several regions, including the hippocampus, cerebellum, and olfactory system (Nyberg et al. 2007; Usdin et al. 1993). Activation of GIPR signaling is coupled to increase in cAMP and intracellular Ca2+ levels, as well as activation of phosphatidylinositol 3-kinase (PI-3K), protein kinase A (PKA), protein kinase B (PKB), mitogen-activated protein kinase (MAPK), and phospholipase A2 (Lu et al. 1993; Volz et al. 1995). Although very little is known about the role of GIP in the brain, GIP and its receptor were found to be involved in memory, emotions, and vegetative functions (Usdin et al. 1993). GIP-overexpressing transgenic mice show better sensorimotor coordination (in rotarod and open-field tasks) and better memory performance (in Y-maze tasks) compared with control mice (Ding et al. 2006). Moreover, physical exercise has been shown to lead to an increase in GIPR expression in the dentate gyrus of aged rats, accompanied by enhanced spatial memory in the water maze (Figueiredo et al. 2010). In contrast, active immunization with GIP vaccine led to a significant change in brain function and behavior in rats (Tian et al. 2010).

GIP and GIPR activation have clear effects on synaptic plasticity and neuroprotection (Hölscher and Li 2010). Activation of GIPRs by native GIP or protease-resistant analogs, such as N-acetyl-GIP, enhanced synaptic plasticity and protected synapses from the detrimental effects of β-amyloid fragments on long-term potentiation (LTP) formation (Gault and Hölscher 2008). Coadministration of GIP and Aβ(1–40) abolishes the severe impairments of spatial learning and memory induced by intracerebroventricularly infusion of Aβ(1–40) during the water maze task (Figueiredo et al. 2010). Furthermore, GIP has been shown to promote axonal regeneration after sciatic nerve injury. GIPR-deficient mice were associated with impaired spontaneous nerve regeneration compared with the normal mice (Buhren et al. 2009).

GIP also activates the proliferation of neuronal progenitor cells and therefore may contribute to neurogenesis. Chronic intracerebroventricular infusion of GIP increased the rate of progenitor cell proliferation in the hippocampus of adult rats. In contrast, GIPR knockout (KO) mice displayed a marked decrease in cell proliferation in the dentate gyrus (DG) (Nyberg et al. 2005, 2007). An increase of apoptosis was also found in the hippocampus of rats immunized against GIP (Tian et al. 2010).

The aim of this study was to identify the role of GIP and its receptor in cognitive processes, neuronal transmission, and neurogenesis in the GIPR KO mouse strain.

MATERIALS AND METHODS

Animals. GIPR KO mouse breeding pairs were kindly provided by Prof. Bernard Thorens (Lausanne, Switzerland) and bred in the Ulster University animal unit. The background and generation of GIPR-deficient mice used in this study has been previously described (Preitner et al. 2004). Age-matched wild-type (WT) mice with the same C57BL/6 genetic background were used as controls (Harlan
Animals were maintained on a 12:12-h light-dark cycle (lights on at 0800, off at 2000), in a temperature-controlled room (21.5 ± 1°C). Animals were individually caged and received food and water ad libitum. After ~2 wk of acclimatization, the animals were handled daily for at least 2 wk before the study. All experiments were licensed according to United Kingdom Home Office regulations (UK Animals Scientific Procedures Act 1986) and European Union laws.

Open-field task. Mice received a session of 5 min in the empty open field (58-cm diameter, 31-cm wall height) constructed of aluminum with painted gray walls and a gray floor. A 60-W lamp placed 2 m directly above the arena dimly illuminated the open field. Motor activity was recorded as total path, number of lines crossed, and speed. The number of rearing events (forepaws elevated from the floor) was analyzed as an index of exploratory behavior. The anxiety level was assessed as the percentage of time spent in the center vs. periphery of the arena and the number of grooming events.

Object recognition task. Twenty-four hours after the open-field assessment, each mouse was subject to a 10-min acquisition trial, during which they were placed in the open field in presence of two identical objects A (cube or ball) situated at 15 cm from the arena wall (acquisition task). On completion of 10 min of exploration, the mouse was returned to its cage for a 3-h delay. After the 3-h retention interval, the mice were placed back into the box and exposed to the familiar object A and to a novel object B for a further 10 min (test task). The objects were placed in the same locations as the previous ones. The position of the novel object was randomized to avoid preferences not based on novelty. The total time spent exploring each of the two objects (when the animal’s snout was directly toward the object at a distance ≤2 cm) was recorded. A recognition index defined as the amount of time exploring the familiar object (TA) or the novel object (TB) divided by the total time spent exploring both objects and multiplied by 100 was used to measure recognition memory: [TA or TB/(TA + TB)] × 100. In the acquisition and retention trials, if the exploration time was <30 s and <15 s, respectively, the mice were excluded from the trial.

Object location task. The apparatus and procedures were the same as in the object recognition task. The objects were blue cubes (1.8 cm wide) and black and red bottle tops (3.2-cm diameter; 2.8-cm wall height). After a 5-min session of habituation in the open field, the animals were subjected to a 10-min acquisition trial with two identical objects. After a delay of 3 h, the mice received a second trial (test trial) identical to the first trial, except that one of the objects was placed in the new location.

Morris water maze task. Animals were trained in a pool with a diameter of 120 cm and wall height of 40 cm, filled with water kept at 25°C to avoid hypothermia. A small escape platform (10 × 6.5 cm, 21.5 cm high) was placed in the center of one quadrant and was hidden 1 cm beneath the water surface. The room contained a number of fixed visual cues on the walls. The acquisition trial phase consisted of six training days with four trials per day with a 15-min intertrial interval. Four points equally spaced along the circumference of the pool (north, east, south, west) served as the starting position, which was randomized across the four trials per day. If an animal did not reach the platform within 90 s, it was guided to the platform, where it had to remain for 30 s before being returned to its home cage. The escape latencies and speed were recorded using water maze analysis software (Bio Signal, Brooklyn, NY). One day after the acquisition task was finished, a probe trial was performed to assess the spatial memory (24-h delay). The platform was removed from the maze, and animals were allowed to swim freely for 90 s. Spatial acuity was expressed as a percentage of time spent in each of four quadrants of the pool. The number of times the mice crossed the learned escape platform location as well as the time spent was assessed.

The reversal task commenced on the next day and lasted 3 days. It was conducted in a manner identical to acquisition training, except that the escape platform was moved to the middle of a new quadrant. One day after the acquisition task was finished, a probe trial was performed as described previously.

The visual task was conducted with the escape platform lifted 1 cm above water level and shifted to a new quadrant. A flag (10 wide × 17 cm high) was inserted on the top of the escape platform. Each mouse received four trials with 15-min intertrial to reach the visible platform.

Surgery and LTP recording in the hippocampus. At 10 mo of age, mice were anesthetized with urethane (ethyl carbamate) for the duration of the experiment. The skull was exposed and three holes 0.8 mm in diameter were drilled. Electrodes (tungsten with Teflon coating; Bilaney, Kent, UK) were implanted in the following coordinates: 1.5 mm posterior and 1.0 mm lateral to the midline for the recording electrode, and 2.0 mm posterior to bregma and 1.5 mm lateral to the midline for the stimulating electrode. The earth electrode location was at 3.0 mm posterior to bregma and 2.5 mm lateral to the midline, contralateral to the electrode sites. The electrodes were slowly lowered through the cortex and the upper layers of the hippocampus and into the CA1 region (~1.2 mm) until the appearance of a negative deflecting excitation postsynaptic potential (EPSP) that had a latency of about 10 ms. Recordings of field excitatory postsynaptic potentials (fEPSPs) were made from the stratum radiatum in the CA1 region of the right hippocampal hemisphere in response to stimulation of the Schaffer collateral/commissural pathway. fEPSPs were recorded on a computerized stimulating and recording unit (PowerLab, ADInstruments) in which the trigger threshold was adjustable. The triggered unit activated a constant current stimulus isolation unit (Neurolog, Welwyn Garden City, UK). The data acquisition system was triggered simultaneously to record all events. Sampling speed was at 20 kHz for recordings of fEPSPs. The high-frequency stimulation protocol for inducing LTP consisted of 3 trains of 200 stimuli, intertrain interval of 1 s, and interstimulus intervals of 5 ms (200 Hz). Stimulation intensity was 70% of the maximal fEPSP, as analyzed by establishing an input-output correlation. LTP was measured as a percentage of baseline fEPSP slope recorded over a 15-min period before application of high-frequency stimulation. This value was taken as 100% of the EPSP slope, and all recorded values were normalized to this baseline value.

Paired-pulse facilitation (PPF) was measured to analyze presynaptic functions and interneuron activity. Two stimuli were given at 60% of maximal fEPSP response. The interval between two stimuli was changed from 25 ms to 50, 80, 120, 160, and 200 ms to analyze PPF in relation to time. The PPF induced at short interstimulus intervals is considered to be triggered by presynaptic transmitter release-facilitating processes (Chen et al. 1996), whereas later PPF is considered to be linked to GABA_A and GABA_B interneuronal synaptic transmission (Schulz et al. 1995; Tsai et al. 2008). The size of the fEPSP response was measured by analyzing the change from baseline to the lowest point of the fEPSP curve. Data were normalized by taking the first fEPSP value as 100% and comparing the second fEPSP with it.

Immunohistochemistry. Mice were injected with bromodeoxyuridine (BrdU; 50 mg/kg body wt; Sigma-Aldrich, Poole, UK) intraperitoneally and killed 24 h later. Animals were perfused transcardially with 30 ml of ice-cold PBS and 30 ml of ice-cold 4% paraformaldehyde to postfix the brain.Brains were transferred into fresh 30% sucrose solution to cryoprotect tissue and cut at 40 µm on a cryostat. Sections were chosen according to stereotological rules, with the first section taken at random and every 5th section afterwards. Between 7 and 10 sections were analyzed per brain. Dilutions of primary antibody used were as follows: monoclonal mouse BrdU (1:200; Sigma catalog no. 2531), polyclonal goat DCX (double-cortin; 1:400; Santa Cruz Biotechnology catalog no. sc-8066). For visualization, the Vectastain Elite ABC kit was used. Quantification of BrdU (a marker of cell proliferation) and DCX (a marker of immature neurons) cells was performed by counting the number of positive cells in the subgranular zone of the dentate gyrus (Axio Scope 1; Carl Zeiss, Stuttgart, Germany).
Fig. 1. Measurement of spontaneous behavior of control and glucose-dependent insulinotropic polypeptide receptor (GIPR) knockout (KO) mice across a period of 5 min in the open field: path length (A), number of lines crossed (B), number of rears (C), speed (D), the ratio of time spent in the center of the arena to that in the periphery (E), and anxiety levels measured as number of grooming events (F). Data are means ± SE of 12 control mice and 13 GIPR KO mice. **P < 0.01 (Student’s t-test).

Statistics. Statistical analyses were performed using Prism (GraphPad Software) with the level of probability set at 95%. Value are means ± SE. Data were analyzed using two-way repeated-measures ANOVA followed by the Bonferroni post hoc comparison, or Student’s t-tests.

RESULTS

Open-field assessment. Distance travelled and numbers of lines crossed were higher for GIPR KO mice than control mice (Student’s t-test, P < 0.01, Fig. 1, A and B). Exploratory activity as shown in number of rears was not significantly different (Fig. 1C). Travel speed was higher on average in the GIPR KO mice (P < 0.01, Fig. 1D). The ratio of time spent in the center of the arena to the periphery was not different between groups (Fig. 1E). No difference was found in number of grooming events (Fig. 1F).

Object recognition task. During the acquisition task, where two identical objects were shown to the two groups, no difference between exploration of familiar objects was found (Student’s t-test, P > 0.05, Fig. 2A). During the test, one familiar and one novel object were shown to the two groups 3 h later. Control mice spent the largest time exploring the novel object of overall exploration time (Student’s t-test, P < 0.01, Fig. 2B). GIPR KO mice did not discriminate between the novel and the familiar object (P > 0.05).

Object location task. Both GIPR KO mice and control mice spent more time exploring the object in the new location during the test trial (Student’s t-test, P < 0.01, and P < 0.001, respectively, Fig. 3), suggesting location memory is intact.

Morris water maze task. During the acquisition phase, GIPR KO mice showed a deficit in the learning of the location of the escape platform per day compared with the control (2-way ANOVA, P < 0.0001; post hoc test, P < 0.05 for day 5 and P < 0.01 for day 6, Fig. 4A). No difference in swim speed was found between groups (Fig. 4B). During the probe (recall) trial, control mice spent more time in the target quadrant than GIPR KO mice (2-way ANOVA, P < 0.0001; post hoc test, P < 0.0001, Fig. 4C). GIPR KO mice spent equal amounts of time in each quadrant and did not recall the location of the platform.

Reversal task. During the reversal training, GIPR KO mice took longer to reach the escape platform than control group (2-way ANOVA, P < 0.0001); however, both groups improved over time (P < 0.0001, Fig. 5A). No difference in speed was found between groups (Fig. 5B). In the reversal probe trial, both groups spent more time in the target quadrant than in the other quadrants (time spent > 40%; 2-way ANOVA, P < 0.0001). However, the control group spent significantly more time crossing the exact previous location of the platform than GIPR KO mice (Student’s t-test, P < 0.05, Fig. 5C). No difference in escape latency was found between groups in visual acuity during the visible platform control task (Fig. 5D).

Synaptic plasticity. High-frequency stimulation induced robust LTP in the control mice, whereas the induction of LTP in the area CA1 of the hippocampus of GIPR KO mice was impaired. A clear difference between groups was found (2-way ANOVA, P < 0.0001), but not over time (2-way ANOVA, P > 0.05, Fig. 6A). GIPR KO mice had impaired paired-pulse
facilitation compared with control mice at 25 and 50 ms (2-way ANOVA, \( P < 0.0001 \); post hoc test, \( P < 0.05 \), Fig. 6B).

**Immunostaining.** The DG of GIPR KO mice contained less than one-half the number of BrdU-positive cells observed in control mice (Student’s \( t \)-test, \( P < 0.0001 \), Fig. 7C). However, no difference in the number of DCX-positive young neurons was found between groups (Fig. 7F).

**DISCUSSION**

In the present investigation, we investigated the role of GIP receptors in different physiological processes in the brain. The results demonstrate that GIPR KO mice have impairments in cognitive performance, synaptic plasticity, and neurogenesis.

Expression of the GIP gene and GIPR is widespread throughout the CNS (Usdin et al. 1993), and GIP and GIPRs are expressed on pyramidal neurons in the cortex and the hippocampus, indicating that GIP plays a central role in neuronal communication in these areas. However, the precise role of these receptors in normal neuronal function is not clear. GIPR KO mice showed an increase in locomotor activity during the open-field task that may be related to increased speed. No difference was found in exploratory level and anxiety-like behavior. GIP receptor downregulation as a consequence of high GIP circulating levels has resulted in the same changes in spontaneous behavior (Ding et al. 2006). Rodents show an innate preference for novelty translated by a bigger exploration of novel over familiar objects (Dere et al. 2007), and memory formation for familiar objects as well as the detection and encoding of a novel object memory could be assessed by the object recognition task (Ennaceur 2009). In this task, both groups spent equal time exploring the familiar objects, indicating that general exploratory behavior, motivation, and motor activity were not affected by the gene deletion. During the test task, control mice spent more time exploring the novel object, indicating that object recognition memory was intact. However, GIPR KO mice failed to discriminate between the novel and familiar objects. Absence of the GIPR clearly affects performance in this memory task, indicating that this type of memory is dependent on GIP signaling between neurons. The same memory impairment was also found in a mouse strain that lacked gene expression of the glucagon-like polypeptide-1 receptor (GLP-1R), another incretin hormone receptor (Abbas et al. 2009). This suggests that formation of this memory is dependent on unimpaired signaling of several incretins, which appear to have independent functions in neuronal signaling, since the deletion of one type of receptor cannot be compensated by signaling of the other incretin type. Interestingly, when GIPR KO animals were tested in the object location task, no difference was found between groups. This suggests that the lack of GIP affects specific types of learning while leaving others unimpaired. It also demonstrates the specific memory impairment that is not based on nonspecific impairments such as reduced motor activity or exploration.

The Morris water maze is used to assess hippocampus-dependent spatial learning and memory in rodents (D’Hooge and De Deyn 2001). In this task, GIPR KO mice took longer to locate the escape platform. This deficit was not due to reduced swim speed, since transgenic mice swim as fast as control mice. GIPR KO mice also failed to remember the location of the hidden platform in the recall test. In the reversal task, the platform was moved to another location. This task requires a relearning of the location and a repression of the previous memory. This task is often harder to learn by animals that are impaired in learning (Hölscher and Schmidt 1994). During reversal training, GIPR KO mice were clearly impaired. Although both groups spent more time in the target quadrant during the reversal probe trial, control mice spent more time in the correct zone (where the platform was previously located), indicating that these mice may have a more accurate memory...
for the platform location than GIPR KO mice. GIP-overexpressing mice previously demonstrated improved performance in the Y-maze (Ding et al. 2006), and our results in the GIPR KO strain underscore the importance of GIP signaling in memory formation. Moreover, increased GIP receptor expression in the hippocampus after chronic running exercise led to better spatial memory performance in the Morris water maze task (Figueiredo et al. 2010).

Fig. 5. Performance of GIPR KO mice in the reversal Morris water task. Escape latency (A) and (B) speed for the control and GIPR KO mice across 3 days during reversal training. *P < 0.0001 (2-way ANOVA). C: time spent crossing the platform previously located. *P < 0.05 (Student’s t-test). D: difference of escape latency during the visible platform control task. Data are means ± SE of 12-control mice and 13 GIPR KO mice.

Fig. 6. In vivo recording in the hippocampus of control and GIPR KO mice. A: recording of field excitatory postsynaptic potentials (fEPSPs) from the stratum radiatum in response to stimulation of the Schaffer collateral/commissural pathway. P < 0.0001 (2-way repeated-measures ANOVA). B: paired-pulse facilitation (PPF) in area CA1. *P < 0.05 (post hoc test). Data are means ± SE of 6 mice per group. Sample fEPSPs are shown 10 min before and 30 min after high-frequency stimulation (HFS). Calibration bars: 10 ms horizontally, 1 mV vertically.
In the electrophysiological study, LTP was completely abolished in GIPR KO mice, whereas control group showed stable and long-lasting LTP in area CA1 of the hippocampus. This effect may be due to a decrease in synapse vesicle release, since an impairment in paired-pulse facilitation at short inter-stimulus intervals in GIPR KO mice is an indication that vesicle release was affected (Chen et al. 1996). We have previously shown that activation of GIPRs in the hippocampus by the GIP agonist N-acetyl-GIP enhanced the induction of LTP, whereas the GIP antagonist (Pro³)GIP reduced LTP (Gault et al. 2003; Gault and Hölscher 2008). These results support the concept that GIPR modulates transmitter release and is involved in synaptic plasticity induction. We know that activation of GIPRs on β-cells in the pancreas leads to enhanced insulin release in high-glucose conditions (Creutzfeldt 2001; Meier et al. 2002). This release of insulin vesicles involves cAMP increase, PKA activation, K⁺ channel modulation, and membrane depolarization, a signaling mechanism similar to the release mechanism that controls neurotransmitter vesicle release at the synapse (Okamoto et al. 1994; Pang and Sudhof 2010; Winder and Conn 1993). We therefore postulate that GIPRs modulate synaptic vesicle release in a subpopulation of neurons that play a role in forming memories in the cortex and hippocampus.

It has been reported previously that GIP induces progenitor cell proliferation (Nyberg et al. 2005). In the current study we observed that GIPR KO mice had a large reduction in the number of BrdU-positive cells in the DG compared with control mice. This area contains neuronal progenitor cells that can differentiate to neurons in the granule cell layer (Encinas et al. 2006), and 5-bromo-2-deoxyuridine (BrdU) is a marker of dividing cells (Kang et al. 2006). Others have also reported that GIP increased progenitor cell proliferation both in vivo and in vitro (Nyberg et al. 2005; Tian et al. 2010). GIP also promotes axonal growth after nerve crush (Buhren et al. 2009), indicating that GIP has growth factor-like properties that can be of use in neuroprotection and neuroregeneration. We also tested the number of immature neurons as identified by DCX, a cell marker for immature neurons (Li et al. 2010), to test whether the lack of progenitor cells in the GIPR KO mice leads to a reduction in young neurons. Interestingly, the numbers in control and GIPR KO mice were identical. These findings suggest that GIPR may act as a growth factor and play a specific regulatory role in progenitor cell proliferation but not in neuronal cell differentiation. Levels of neuronal cell differentiation agents such as nerve growth factor appear to be normal in these mice, and neurogenesis is not directly affected.

In conclusion, lack of GIPR affects memory formation, synaptic plasticity, and progenitor cell proliferation. These findings clearly demonstrate that the GIPR plays an important role in neuronal communication and brain function. Although the GLP-1 receptor plays similar roles in the brain (Abbas et al. 2009; Gault et al. 2010; Hölscher and Li 2010), GIP must play a specific role that is uniquely different from GLP-1, since GLP-1 signaling is still intact in the GIPR KO mice but cannot compensate for the lack of GIPR expression. The beneficial actions of GIP suggest that the use of long-lasting analogs may be an attractive therapeutic approach for the treatment of neurodegenerative diseases such as Alzheimer's disease, in which memory and synaptic plasticity is impaired (Holscher 2010).


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