Protease-Resistant Glucose-Dependent Insulinotropic Polypeptide Agonists Facilitate Hippocampal LTP and Reverse the Impairment of LTP Induced by Beta-Amyloid

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Gault VA, Hölscher C. Protease-resistant glucose-dependent insulinotropic polypeptide agonists facilitate hippocampal LTP and reverse the impairment of LTP induced by beta-amyloid. J Neurophysiol 99: 1590–1595, 2008. First published January 30, 2008; doi:10.1152/jn.01161.2007. Type 2 diabetes has been identified as a risk factor for Alzheimer’s disease (AD). Insulin signaling is often impaired in AD, contributing to the neurodegeneration observed in AD patients. One potential strategy to overcome this impairment is to normalize insulin signaling in the brain. In the present study, we have examined the effects of an enzyme-resistant analogue of glucose-dependent insulinotropic polypeptide (GIP), N-AcGIP, on synaptic plasticity. N-AcGIP is a stable, long-acting peptide hormone that regulates glucose homeostasis and insulin release. We tested the effects of native GIP and the agonist N-AcGIP on synaptic plasticity [long-term potentiation (LTP)] in the hippocampus [15 nmol, administered intracerebroventricularly (icv)] and report for the first time that both peptides have enhancing effects on LTP. In contrast, the antagonist of GIP, Pro(3)GIP (15 nmol icv), reduced LTP. Injection of beta-amyloid(25–35) (100 nmol), a peptide that aggregates in brains of AD patients, also impaired LTP. The injection of N-AcGIP (15 nmol icv) 30 min prior to injection of amyloid(25–35) (100 nmol icv) fully reversed the impairment of LTP induced by beta-amyloid. The results demonstrate for the first time that GIP (particularly enzyme-resistant forms) not only directly modulates neurotransmitter release and LTP formation, but also protects synapses from the detrimental effects of beta-amyloid fragments on LTP formation. The use of enzyme-resistant analogues of GIP show great promise as a potential novel treatment for preventing neurodegenerative processes in AD and other related disorders.

INTRODUCTION

Type 2 diabetes has recently been identified as a risk factor for developing Alzheimer’s disease (AD) (Biessels and Kappelle 2005; Li and Hölscher 2007). For example, an analysis of the Mayo clinic patient database showed a clear correlation between AD and type 2 diabetes. About 85% of AD patients also had diabetes or impaired fasting glucose levels (Janson et al. 2004). A different study found that the occurrence of AD is doubled in diabetic subjects (Ott et al. 1999). Type 2 diabetes is often associated with the desensitization of insulin receptors, thereby compromising insulin signaling and cell metabolism. Desensitization of insulin receptors has been observed in the brains of AD patients (Biessels et al. 2006; Carro and Torres-Aleman 2004) and neuronal glucose and energy metabolism are impaired in early stages of AD (Steen et al. 2005). In addition, it was found that late-onset sporadic AD disease changes in the brain are similar to those caused by type 2 diabetes (Hoyer 2004). Recently, it has been shown that beta-amyloid fragments bind to insulin receptors in the brain and cause a strong reduction of receptor numbers on dendrites (Zhao et al. 2008). This could be part of the underlying basis for the link between AD and type 2 diabetes.

The role of insulin receptors in the brain has been under investigation for many decades. It has been found that diabetes is associated with cognitive deficits and that insulin given to diabetic or nondiabetic people improves attention and short-term memory (Hoyer 2004; Stockhorst et al. 2004; Strachan 2005). People with diabetes have reduced global memory, attention, abstract reasoning, and visual motor task performance (Trudeau et al. 2004). In animal models of diabetes, memory impairments have been observed in learning spatial tasks and, furthermore, treatment with insulin was shown to reverse these impairments. Long-term potentiation of synaptic transmission (LTP) in the hippocampus of diabetic animals was found to be impaired and, in one study, 12 wk after streptozotocin treatment to induce diabetes, N-methyl-D-aspartate–dependent LTP in areas CA3 and CA1 was impaired (Gispen and Biessels 2000; Trudeau et al. 2004). Studies in our laboratory have demonstrated that beta-amyloid, the peptide that aggregates to form plaques in the brain of AD patients, also affects the induction of LTP in the hippocampus. Low amounts of different fragments of amyloid injected intracerebroventricularly (icv) can impair or block LTP in the hippocampus in vivo in a fast and immediate time course (Freir et al. 2001; Gengler et al. 2007). This impairment also affects the ability to learn spatial tasks and is reversible (Hölscher et al. 2007).

It is therefore of interest to investigate in further detail the role of insulin-signaling on synaptic plasticity and whether insulin-signaling can alter the impairment of LTP induced by beta-amyloid fragments. Since insulin affects blood glucose concentrations, which on its own can have powerful effects on neuronal activity, we did not attempt to directly activate the insulin receptor. Instead, we chose to use a novel strategy examining the effects of the insulinotropic hormone, glucose-dependent insulinotropic polypeptide (GIP), and an enzyme-resistant analogue on synaptic plasticity. GIP is a 42-amino acid peptide hormone whose major physiological role is targeting pancreatic islets to enhance insulin secretion and help...
reduce postprandial hyperglycemia (Gault et al. 2003). In addition to its actions in modulating insulin release, GIP has been shown to promote pancreatic beta-cell growth, differentiation, proliferation, and survival (Gault et al. 2003). Furthermore, GIP elicits several physiologically important glucose-lowering actions through a variety of extrapancreatic mechanisms (Irwin et al. 2006). Taken together, these beneficial actions highlight GIP as an attractive therapeutic approach for type 2 diabetes. However, GIP is rapidly degraded in the circulation by the enzyme dipeptidylpeptidase-IV (DPP-IV) and, as such, stable, enzyme-resistant forms have recently been developed (Irwin et al. 2006).

Interestingly, GIP receptors are expressed in the brain, including the hippocampus, and are found on neurons and on neuronal progenitor cells in the hippocampus (Nyberg et al. 2005, 2007). We have previously shown that GIP’s sister incretin hormone, glucagon-like peptide-1 (GLP-1), has acute and prominent enhancing effects on LTP and can reverse the effects of beta-amyloid fragments on LTP in the hippocampus (unpublished data). Since GIP has properties very similar to those of GLP-1, we decided to test the effects of GIP and a stable analogue on LTP induction in the hippocampus. The effects of a novel GIP antagonist Pro(3)GIP on LTP was also tested (Gault et al. 2005). Furthermore, effects of N-AcGIP on impairment of LTP by the beta-amyloid fragment (25–35) were examined.

METHODS

Surgery and LTP induction protocols

Male Wistar rats (Harlan, UK) weighing 220–280 g were anesthetized with urethane (ethyl carbamate, 1.8 g/kg, administered intraperi-

FIG. 1. A: glucose-dependent insulinotropic polypeptide (GIP) was injected [15 nmol in 5 μl, administered intracerebroventricularly (icv)] to test the effects on long-term potentiation (LTP) induced by a weak stimulation protocol. An ANOVA showed a difference between the GIP group and control ($P < 0.01$). Interaction between factors was not significant. All groups $n = 6$. Averaged excitatory postsynaptic potentials (EPSPs) are shown recorded 5 min pretetanus and 1 h posttetanus. Calibration bars are 10 ms horizontal, 1 mV vertical. B: the novel stable GIP agonist N-AcGIP (15 nmol in 5 μl icv) enhanced LTP induced by a weak stimulation protocol. An ANOVA showed a difference between the N-AcGIP group and control ($P < 0.001$). Averaged EPSPs are shown recorded 5 min before high-frequency stimulation (pre-HFS) and 1 h post-HFS. Calibration bars are 10 ms horizontal, 1 mV vertical. All groups $n = 6$. 

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Peptides

Beta-amyloid(25–35) and other peptides used in this study were synthesized on an Applied BioSystems automated peptide synthesizer (Model 432A) using standard solid-phase Fmoc (i.e., 9-fluorenylmethoxycarbonyl) protocols. Peptides were judged pure by reversed-phase high-performance liquid chromatography on a Waters Millennium 2010 chromatography system and peptides were subsequently characterized using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as described previously (Gengler et al. 2007; Hölscher et al. 2007). Peptides were stored in dry form and dissolved in double-distilled water before the experiments; 5 μl of peptides solution was injected icv.

Statistics

Each group consisted of six animals. Data were analyzed using either a two-way repeated-measures ANOVA or a three-level two-way repeated-measures ANOVA with post hoc tests to discriminate between groups (PRISM, GraphPad Software, San Diego, CA). Data were analyzed in the ANOVA ranged from post-HFS onward until the end of recording because no difference was expected pre-HFS. All data points post-HFS were included.

RESULTS

Effects of GIP and N-AcGIP on LTP

The native hormone GIP was injected (15 nmol in 5 μl icv) to test the effects on LTP using a weak stimulation protocol [control group, 142 ± 8.3% (SE) of baseline values; GIP, 179 ± 11.2% (SE)]. A two-level two-way repeated-measures ANOVA showed a difference between the GIP group and control [df(1,119); F = 14.1; P < 0.01] and over time [df(1,119); F = 1.7; P < 0.01]. Interaction between factors was not significant. All groups n = 6 (see Fig. 1A).

The novel stable GIP agonist N-AcGIP was tested (15 nmol in 5 μl icv) using a weak stimulation protocol. A two-level two-way repeated-measures ANOVA showed a difference between the N-AcGIP group LTP [133 ± 19.2% (SE)] and control [df(1,110); F = 17.5; P < 0.001; 180 ± 14.1% (SE)] and over time [df(1,119); F = 1.9; P < 0.005]. Interaction between factors was not significant (see Fig. 1B). All groups n = 6.

Effects of the antagonist Pro(3)GIP on LTP

As a control for this study Pro(3)GIP (Gault et al. 2002), a GIP antagonist, was tested (15 nmol in 5 μl icv) using a strong stimulation protocol. A two-level two-way repeated-measures ANOVA showed a difference between the Pro(3)GIP group and control [df(1,10); F = 21; P < 0.001] and over time.
[df(1,119); F = 1.96; P < 0.005]. Interaction between factors was not significant (see Fig. 2). All groups n = 6.

Effects of N-AcGIP on the impairment of LTP by beta-amyloid(25–35)

When injecting either 100 or 10 nmol beta-amyloid(25–35) icv, a significant difference on LTP induced by a strong stimulation protocol was found compared with a control group that was injected with an inactive scrambled peptide sequence version of beta-amyloid(25–35) [control: 158 ± 10.7% (SE); 10 nmol: 129 ± 7.8% (SE); 100 nmol: 108 ± 9.4% (SE); see Fig. 3; 10 nmol data not shown in the graph]. A three-level two-way repeated-measures ANOVA found an overall difference between groups [df(1,119); F = 6.2, P < 0.001] and time [df(2,119); F = 1.9; P < 0.01]. Interaction between factors was not significant. A two-level two-way repeated-measures ANOVA showed a difference between the N-AcGIP group and control [df(1,119); F = 16.1; P < 0.005] and over time [df(1,119); F = 1.5; P < 0.001]. Interaction between factors was not significant. A two-level two-way repeated-measures ANOVA showed a difference between the 10 nmol group and control [df(1,119); F = 9.1; P < 0.01] and over time [df(1,119); F = 1.38; P < 0.005]. Interaction between factors was not significant. There was no difference between the 10 and the 100 nmol groups (n = 6 per group).

When injecting N-AcGIP (15 nmol in 5 μl icv) 30 min before beta-amyloid(25–35) (100 nmol in 5 μl icv), the impairing effect of beta-amyloid on LTP was completely reversed [drug combination: 147 ± 11.3% (SE) vs. control: 158 ± 10.7% (SE)]. A three-level two-way repeated-measures ANOVA found an overall difference between groups [df(1,119); F = 10.3, P < 0.001] and over time [df(1,119); F = 4.1; P < 0.001]. A two-level two-way repeated-measures ANOVA showed a difference between the beta-amyloid(25–35) group and control [df(1,119); F = 21; P < 0.001] and over time [df(1,119); F = 2.2; P < 0.001]. A two-level two-way repeated-measures ANOVA showed a difference between the N-AcGIP group and beta-amyloid(25–35) combination group and control [df(1,119); F = 18.5; P < 0.001] and over time [df(1,119); F = 1.6; P < 0.01]. No difference was found between the control group and the drug combination group. All groups n = 6 (see Fig. 3).

Drug effects on LTP induction

To investigate whether the drugs increase LTP of fEPSPs in absolute terms or increase the likelihood of inducing LTP (but not increasing LTP in absolute terms), the average LTP results for each animal were drawn up in a scatterplot. The plot shows that the effect of the GIP ligands has a general effect on each group, increasing or decreasing the amount of overall LTP for all animals, rather than increasing the probability of inducing LTP in a rat (Fig. 4).

DISCUSSION

The results show for the first time that GIP and the stable analogue N-AcGIP have direct and acute modulating effects on LTP in a rat (Fig. 4).
synaptic transmission and can enhance the induction of LTP. The novel stable antagonist Pro(3)GIP has detrimental effects on LTP. The fact that the GIP receptor agonists enhance LTP and the GIP antagonist reduces LTP suggests that the effects of these peptides are due to the activation or the block of neuronal GIP receptors. The enhancing effect of LTP triggered by the activation of GIP receptors could be the result of modulation of vesicle release at the presynaptic site. Similar to modulating the release of insulin under hyperglycemic conditions, GIP receptor activation would only modulate transmitter release, which explains the lack of effects on basic transmission while enhancing LTP after HFS. We know that GIP receptors on beta-cells in the pancreas modulate insulin release via a mechanism that involves closure of K⁺ channels, depolarization of the cell membrane that activates voltage-dependent calcium channels (VDCCs), and the increase of cyclic adenosine monophosphate (cAMP) levels. The subsequent influx of Ca²⁺ then activates Ca²⁺-sensitive enzymes such as PLA₂, phospholipase C (PLC), adenylyl cyclase that forms cAMP, and protein kinase A (PKA), and activates the mechanisms of vesicle exocytosis to release insulin into the extracellular space (Green et al. 2004; Irwin et al. 2005, 2006; Leech and Hølscher 1997; Suzuki et al. 1997). The same biochemical mechanisms that control the release of neurotransmitters into the synaptic cleft via vesicles are found in neurons (Okamoto et al. 1994; Wheeler et al. 1994; Winder and Conn 1993). Indeed, it has been shown that in neuronal cell cultures, the incretin GLP-1 (that part of the hormone family to which GIP belongs) also modulates glutamate-induced Ca²⁺ influx. This effect is due to altered VDCC activity. Ca²⁺ influx induced by K⁺ conductance is also altered. Furthermore, GLP-1 induces cAMP formation, activates PKA, mitogen-activated protein kinases, and more (Gilman et al. 2003). In addition, insulin receptors have been identified at the presynaptic site (Zhao et al. 2008) and they also modulate the release of neurotransmitter in neurons and affect the induction of LTP in the hippocampus (Gispen and Biessels 2000; Li and Hølscher 2007). We therefore postulate that the mechanism by which GIP increases insulin release in the pancreas is similar to the effects on LTP and synaptic transmission observed in the present study in the brain. However, other mechanisms of action are feasible and our theory will have to be tested by further experiments.

Beta-amyloid fragments also have been shown to affect synaptic transmission. Beta-amyloid has detrimental effects on LTP (Freir et al. 2001). The underlying mechanism of this impairment includes the change of K⁺-channel activity (Jalonen et al. 1997; Pannaccione et al. 2004), reduction of VDCC activity, and Ca²⁺ influx (Abe and Kimura 1996; Freir and Herron 2003), which in turn affect Ca²⁺-sensitive enzyme activity (Wang et al. 2004) and reduce vesicle release (Arias et al. 1995; Harris et al. 1996). Interestingly, the release of beta-amyloid is affected and reduced by GLP-1 (Perry et al. 2003).

The results of the present study also show that the facilitating effects of GIP on synaptic plasticity can prevent the detrimental effects that beta-amyloid(25–35) fragments have on LTP. The fact that GIP has to be applied ≥30 min before beta-amyloid makes it unlikely that both compounds act at the same binding sites on neurons. Instead, it appears that the activation of GIP receptors triggers mechanisms that prime synapses for increased LTP and prevent or counteract the effects that beta-amyloid has on synaptic plasticity by altering VDCC and other ion channel activity. A possible mechanism would be that GIP activates cAMP levels in neurons in a similar way that it activates in beta cells (Green et al. 2004). The cAMP increase can then enhance vesicle release and make synaptic activity less dependent on VDCC activity, which is affected by beta-amyloid (Freir and Herron 2003). Since the chronically increased activation of Ca²⁺ channels leads to neurotoxic processes such as the increased production of free radicals (Hølscher 1998, 2005), the observation that GIP receptor activation prevents the effects of beta-amyloid holds the great promise that the early degenerative effects of beta-amyloid can be reduced, and the downstream processes that lead to neurodegeneration can be prevented. In addition, the growth factor–like effects that GIP has on neurons by increasing stem cell proliferation and neuronal regeneration could help prevent or reduce long-term damage induced by beta-amyloid activity and plaque-induced gliosis (Perry and Greig 2005; Perry et al. 2003). These properties of GIP suggest that the treatment of AD patients with stable GIP agonists could be an effective prophylactic treatment of Alzheimer’s disease. However, further research is required to identify the underlying biochemical processes of the acute and chronic GIP effects in the CNS.

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GRANTS

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REFERENCES

Increased immunohistochemical


