Exendin-4 Improves Glycemic Control, Ameliorates Brain and Pancreatic Pathologies and Extends Survival in a Mouse Model of Huntington’s Disease

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ABSTRACT

Objective: The aim of this study was to find an effective treatment for the genetic form of diabetes that is present in some Huntington’s disease (HD) patients and in HD mouse models. HD is a neurodegenerative disorder caused by a polyglutamine expansion within the huntingtin protein. HD patients exhibit neuronal dysfunction/degeneration, chorea and progressive weight loss. Additionally, they suffer from abnormalities in energy metabolism affecting both the brain and periphery. Similarly to HD patients, mice expressing the mutated human huntingtin protein also exhibit neurodegenerative changes, motor dysfunction, perturbed energy metabolism, and elevated blood glucose levels.

Research Design and Methods: HD mice were treated with an FDA-approved anti-diabetic glucagon-like peptide 1 receptor agonist, exendin-4, to test if euglycemia could be achieved, if pancreatic dysfunction could be alleviated and if the mice showed any neurological benefit. Blood glucose and insulin levels and various appetite hormone concentrations were measured during the study. Additionally, motor performance and life-span were quantified and mutant huntingtin aggregates were measured in both the pancreas and brain.

Results: Exendin-4 treatment ameliorated abnormalities in peripheral glucose regulation and suppressed cellular pathology in both brain and pancreas in a mouse model of HD. The treatment also improved motor function and extended the survival time of the HD mice. These clinical improvements were correlated with reduced accumulation of mutant huntingtin protein aggregates in both islet and brain cells.

Conclusions: Targeting both peripheral and neuronal deficits, exendin-4 is an attractive agent for therapeutic intervention in HD patients suffering from diabetes.
Huntington’s disease (HD) is an inherited neurodegenerative disorder typified by involuntary body movements, as well as psychiatric and cognitive abnormalities. The incidence of HD is approximately 5-10 cases per 100,000 worldwide making it one of the most common inherited neurodegenerative disorders (1). The genetic defect underlying HD involves expansion of CAG trinucleotide repeats in exon 1 of the HD gene, resulting in polyglutamine expansions in the huntingtin (htt) protein (2). Polyglutamine expansion in htt leads to its abnormal processing and deleterious intracellular aggregation. The number of polyglutamine repeats in htt is inversely correlated with the age of onset, with 70-100 repeats leading to juvenile onset (1). The wild-type htt protein is thought to be a scaffolding protein involved in multiple processes including vesicle movement and cell metabolism. Mutant htt forms abnormal intracellular aggregates in degenerating neurons in the striatum and cerebral cortex (3).

Despite being considered primarily a neurological disorder, HD patients also exhibit peripheral symptoms, including progressive weight loss, appetite dysfunction and poor glycemic control (4). HD patients suffer from an unusual combination of a hypermetabolic state due in part to continuous body movements (5), and despite this, glucose metabolism is paradoxically impaired in both brain and periphery (6,7). Dietary supplementation with creatine has been shown to reduce brain damage and delay the onset of motor dysfunction in huntingtin mutant mice (8), which suggests a potential benefit of increasing brain energy availability in HD. High levels of mutant htt have been documented in peripheral tissues including muscle and gonads (9), and in the pancreatic islet cells of the R6/2 HD mice, which exhibit decreased β-cell mass and impaired insulin release capacity (10, 11). However, the ‘diabetic-like’ condition in the HD mice is not improved by treatment with hypoglycemic agents such as insulin or metformin (12, 13). Disruption of glycemic homeostasis is likely to affect nutrient availability to neurons and could alter neuronal function and contribute to neurodegeneration and motor deficits in HD. The emerging view of HD as a ‘body-wide’ disorder supports the increasing evidence that the maintenance of a healthy nervous system is tightly linked with peripheral metabolic health (14). Therefore, treatment of both the peripheral and central pathophysiologies of HD could form the basis of a more effective HD therapeutic strategy.

Glucagon-like peptide-1 (GLP-1), a hormone secreted by intestinal enteroendocrine L cells in response to food ingestion, and the natural ligand of the GLP-1 receptor, acts on multiple target tissues to enhance energy metabolism; it stimulates the production and release of insulin from beta cells in islets of Langerhans in the pancreas, and increases insulin sensitivity by multiple mechanisms (15, 16). These actions however appear to be highly complicated and context-specific as several studies have not demonstrated a link between GLP-1 activity and increases in insulin sensitivity (17). With respect to the anti-diabetic actions of GLP-1 have been shown to improve glucose regulation in human subjects (18) and a long-acting GLP-1 receptor agonist, exendin-4 (Ex-4), is now a treatment for type 2 diabetes (19). In addition to peripheral actions, GLP-1 and Ex-4 have been shown to act on neurons in the brain. GLP-1 receptors are widely expressed in neurons throughout the brain (20), and Ex-4 readily crosses the blood brain barrier (21). GLP-1 and Ex-4 have been shown to exert neuroprotective actions in experimental models of excitotoxic brain injury (22) and peripheral neuropathy (23). The anti-diabetic and direct neuroprotective activities of Ex-4 suggested to us its potential to ameliorate both
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the central and peripheral abnormalities in HD. We therefore determined whether daily administration of Ex-4 could attenuate disease progression and alleviate metabolic abnormalities in the N171-82Q mouse model of HD. Our findings show that Ex-4 treatment suppresses the development of mutant huntingtin inclusions in the pancreas and brain, ameliorates metabolic defects and motor dysfunction, and extends the survival of HD mice.

RESEARCH DESIGN AND METHODS

Animals and drug administration. Forty male B6C3-Tg(HD82Gln)81Dbo/J (common name N171-82Q) mice (24) and 43 age-matched wild-type mice (WT) were used in this study. Transgenic mice were identified by PCR analysis of tail DNA. All procedures using these HD mice and their wild-type littermates were approved by the institutional Animal Care and Use Committee (ACUC) of the National Institute on Aging. The N171-82Q mice express 82 CAG repeats and display many HD-like symptoms including huntingtin aggregate formation in and degeneration of striatal and cortical neurons, motor impairment, progressive weight loss, and significantly elevated plasma glucose levels (24). All mice were group housed on a 12 hr light/12 hr dark cycle, and had ad libitum access to food and water. Either Exendin-4 (Bachem, Torrance, CA) or saline (PBS, Sigma, St. Louis, MO) was administered by a once-daily subcutaneous injection (300 µl of a 0.1 µM solution of Ex-4). This study was repeated in multiple cohorts of the N171-82Q mice.

Body weight and glucose measurements. Body weight (g) and blood glucose levels (mg/dl) were measured weekly. Glucose levels were measured in blood collected from the tail vein blood using a Bayer Glucometer Elite XL blood glucose meter.

In vivo insulin sensitivity test. An insulin tolerance test was performed on a cohort of mice (6-8 animals per group) to investigate insulin sensitivity. Human, rapid-acting insulin (Novo Nordisk) was diluted to 0.1 units/ml in isotonic NaCl 0.05% bovine serum albumin (Sigma). The mice were injected subcutaneously in the neck region with 1mU/g bodyweight of insulin and blood glucose levels were measured at various time points (t = 0, 15, 30, 45, 60, 90, 120), from the tail vein using a Bayer Glucometer Elite XL blood glucose meter.

Motor performance assessment. Motor coordination was tested using an accelerating rotarod (Med. Associates Inc, Georgia, VT). Mice were trained to use the rotarod apparatus during a 2-minute habituation trial (4 rpm) on the day prior to the first day of testing. On test days the rotarod apparatus was gradually accelerated from 4-40 rpm over 5 minutes. The latency to fall was measured and averaged over two trials per test day. Testing was started the week prior to initial treatment and continued bi-weekly throughout the course of the study.

Tissue collection. Mice were euthanized by isofluorene overdose inhalation and decapitation. Brains were removed and the cerebral cortex was isolated by dissection. Additionally, the pancreas was removed from each animal and blood was collected for analyses of energy metabolism hormones. Blood was centrifuged at 3000 rpm for 30 minutes at 4°C and plasma was aspirated off. The pancreata were fixed in 4% formalin for 48 hours and stored in PBS until processing. The pancreatic tissue was processed and embedded in paraffin wax. Pancreatic sections were cut at 5 µm thickness using a microtome and subsequently the sections were adhered to poly-L-lysine coated microscope slides (Fisher, Springfield, NJ).

Insulin and glucagon immunohistochemistry. Pancreatic sections were immunostained according to a previously described protocol (25). Briefly, tissue was incubated with the primary insulin antibody (guinea pig anti-swine insulin,
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DakoCytomation, Carpinteria, CA; 1:300) or glucagon antibody (guinea pig anti-glucagon, Millipore, Billerica, MA; 1:500) for 2 hours at room temperature, then incubated with secondary antibody (Alexa Fluor 488 goat anti-guinea pig, 1:200 or Alexa Fluor 568 goat anti-guinea pig, 1:1000, Invitrogen, Carlsbad, CA) for 1 hour at room temperature. Sections were imaged with an Olympus Fluoview IX70 confocal microscope (Olympus America Inc, Center Valley, PA).

Pancreatic islet image analysis. Quantification of immunohistochemistry images was performed in Matlab (Mathworks) using novel software in conjunction with the image processing toolbox. Intensity readings of each image ranged from 0 to 256, with 256 being the greatest pixel density and hence the highest staining intensity. The region of interest (ROI) was drawn around each islet after background subtraction. The pixels within the bounds of the ROI and above the set threshold of 8 were selected, from which actual islet area was calculated. The normalized variance of the ROI was used to calculate an artificial ellipse from which the major and minor axis was determined. The major axis is the longest diameter that can be drawn in the ellipse and the minor axis is the shortest diameter, both giving an accurate approximation for the range of the actual islet diameter. Islet morphometry and sizing analyses were performed in an unbiased, random fashion.

Adipokine and hormone measurements. These were measured by ELISA and RIA methods according to the kit manufacturers’ instructions: adiponectin (ELISA, Linco Research, St. Charles, MO), leptin (ELISA, Chrystal Chem Inc, Downers Grove, IL), insulin (ELISA, Crystal Chem Inc, Downers Grove, IL), and ghrelin (RIA, Phoenix Pharmaceuticals, Belmont, CA). Homeostatic model of insulin resistance values (HOMA) were calculated from glucose and insulin values using the HOMA2 software available from the Oxford Centre for Diabetes, Endocrinology, and Metabolism Diabetes Trials Unit (www.dtu.ox.ac.uk).

Western immunoblotting. Cortex samples were homogenized and sonicated in a Nonidet P-40-based lysis buffer, as described previously (26). Subsequently, the samples were centrifuged at 14,000 rpm for 15 minutes and the supernatant was removed for protein analysis. Samples were diluted 1:1 with Laemmli buffer and resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred onto a PVDF membrane for immunoblotting. Membranes were blocked by soaking in methanol and allowed to air dry, and were then incubated with primary antibody diluted in a 4% bovine serum albumin, 50 mM Tris-HCl, pH 7.0, 0.05% Tween 20, and 0.05% Nonidet P-40 blocking solution for 1 hour at room temperature (S830 anti-HD exon 1 transgene protein, goat polyclonal, 1:3000; anti-Hsp-70 mouse monoclonal, (Stressgen, Ann Arbor, MI), 1:1000; anti-actin, mouse monoclonal (Sigma, St. Louis, MO), 1:3000. Membranes were probed with secondary alkaline phosphatase antibody diluted in 4% BSA/TBS-T solution for 1 hour (anti-goat or anti-mouse IgG, Sigma, 1:3000), then developed with ECF Substrate (GE Healthcare). A Typhoon 9410 Variable Mode Imager (Amersham Biosciences) was used for signal detection and band intensities were calculated using ImageQuant software (Molecular Dynamics).

Mutant huntingtin immunohistochemistry. Pancreatic sections were immunostained as previously described (27). S830, a goat polyclonal antibody to the HD exon 1 transgene protein, was used at a dilution of 1:2000. A biotinylated secondary rabbit anti-goat antibody was used at 1:500 (Vector Laboratories, Burlingame, CA). Slices were complexed with avidin-biotin using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and developed using a Dako
Liquid DAB Substrate Chromogen System (Dako Cytomation Corporation, Carpinteria, CA). Images were acquired using an Olympus Fluoview IX70 confocal microscope and Nikon Coolpix P5000 digital camera.

**Statistical analyses.** The data represent the means ± SEM. Differences between mean values for variables within individual experiments were compared statistically by student’s t-test and ANOVA. Comparisons were performed by using Graphpad Prism (GraphPad Software, San Diego, CA) and Excel. p<0.05 was considered statistically significant.

**RESULTS**

**Exendin-4 treatment induces euglycemia in HD mice.** Pre-symptomatic male N171-82Q HD (which express cDNA encoding an N-terminal fragment (171 amino acids) of huntingtin with 82 glutamines) mice and age-matched male wild-type (WT) littermates were injected daily with either Ex-4 or saline (Control). Body weight and non-fasting plasma glucose levels were measured weekly and motor coordination was measured bi-weekly (Fig. 1A). Daily Ex-4 treatment in the WT mice resulted in a small reduction in resting glucose levels, compared to the saline treated control WT mice (Fig. 2A). The HD mice exhibited significantly elevated plasma glucose levels compared to the WT mice (Fig. 2B), which corroborates with previous reports of elevated peripheral blood glucose levels in HD mouse models (10, 13). Daily treatment with Ex-4 resulted in a significant and progressive reduction in glucose levels in the HD mice (p < 0.05; Fig. 2B). Mean blood glucose levels across the whole study period were significantly reduced in the WT Ex-4-treated mice (p<0.05, Fig. 2C) and to a much greater extent in the Ex-4 treated HD mice (p<0.001, Fig. 2D). We attempted to achieve euglycemia in the HD mice by administering daily or twice daily injections of long-acting insulin (Glargine), however we were unable to affect plasma glucose levels in the HD mice, even with a high dose (up to 8 IU/kg) of insulin, and animals died more quickly than non-treated HD mice (data not shown). The insensitivity of the N171-82Q mice to insulin is similar to that reported for R6/2 mice, another HD mouse model, treated with insulin or metformin (12, 13).

Severe and progressive weight loss is a common symptom of HD that also occurs in many mouse models of HD (8, 10). The control HD mice lost a significant amount of body weight throughout the study (Fig. 2F, H) and their weight was significantly lower than the control WT mice (p < 0.001). Ex-4 treatment exacerbated this weight loss and both the HD and WT Ex-4 treated mice weighed significantly less than their saline-treated control counterparts (p < 0.001, p < 0.01; Fig. 2E-H). This reduction in body weight is in accord with previous reports which have shown that Ex-4 treatment leads to progressive weight loss in human subjects (28).

**Energy- and appetite-regulating hormones are altered by Exendin-4 treatment.** An alteration in energy homeostasis has been reported in HD patients and in several of the HD mouse models (29, 30). A disruption in energy balance could contribute to some of the HD symptomology, including extreme weight loss and alterations in appetite. We measured plasma levels of the main energy-regulating hormones in the HD and WT mice to determine whether Ex-4 had caused any alterations in these hormones. Ex-4 treatment induced a significant reduction in plasma insulin levels in the WT mice (p<0.05) and a small reduction in plasma insulin levels in the HD mice (Fig. 3A,B). Ex-4 is a proven and effective treatment for type 2 diabetes, as it not only protects pancreatic β-cell function, but it also significantly increases insulin sensitivity (31, 32). Ex-4 treatment increased insulin sensitivity by approximately 50% in the HD mice (Fig 3b insert), as judged by the
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homeostatic model of insulin resistance (HOMA). As the HOMA index was created to measure classical diabetic states and not the idiosyncratic diabetic pathophysiology in HD mice, we also employed an insulin tolerance test in a separate cohort of animals to assess insulin resistance. This demonstrated that Ex-4 improved insulin-stimulated glucose uptake in both HD and WT animals (Supplementary Figure 1).

The extreme and uncontrolled weight loss in HD patients and mice has been linked to alterations in hypothalamic function (29) including perturbations of the gastric hormone ghrelin and the adipocyte hormone leptin (30). Compared to WT animals the HD mice had significantly lower leptin, ghrelin and adiponectin levels (Fig. 3C-H). In both WT and HD mice, Ex-4 treatment significantly lowered plasma leptin levels (Fig. 3C,D, WT p<0.001; HD p<0.05). Plasma ghrelin levels were reduced in the Ex-4 treated WT mice compared to the control WT mice (Fig. 3E), and this effect has also been observed in human subjects (33). The control HD mice had significantly lower ghrelin levels compared to the WT mice, and Ex-4 treatment unexpectedly restored ghrelin levels in the HD mice to that of WT mice (Fig. 3F). This suggests that Ex-4 treatment might ameliorate some of the appetite-related dysfunctions in HD patients. Ex-4 treatment did not affect plasma adiponectin levels in HD or WT mice (Fig. 3 G, H), although adiponectin levels were significantly lower in the HD mice compared to the WT mice (p<0.001). Decreased adiponectin levels are a universal finding in insulin resistant states (34).

Exendin-4 improves pancreatic islet structure in HD mice. In the N171-82Q mice and in other HD mouse models there is a build-up of mhtt aggregates within the islets of Langerhans, which may contribute to their ‘diabetic-like’ condition (11). HD mice showed significant alterations in islet structure, evidenced by greatly reduced numbers of insulin immunoreactive beta cells compared to their WT control counterparts (Fig. 4A, B). Ex-4 treatment restored numbers of β-cells in the HD mice to levels close to those of WT mice (Fig. 4C, D, and Table 1). The alterations in islet size and structure were also confirmed by glucagon staining. In control and Ex-4-treated WT mice, the glucagon-expressing α-cells were situated on the periphery of the islets (Fig. 4E, G). In HD mice some of the α-cells were displaced into the center of the islets (Fig. 4F), causing abnormal islet structure. Ex-4 treatment largely restored the α-cell topography of islets in the HD mice (Fig. 4H).

Motor coordination is improved in Exendin-4 treated HD mice. Motor coordination was measured on a bi-weekly basis using an accelerating rotarod (Fig. 5 A, B). As HD patients and HD mice become symptomatic, they exhibit impaired motor coordination (8, 35). The HD control mice spent significantly less time on the rotarod than the WT control mice, which indicates that motor function was significantly impaired in the HD control mice. Ex-4 treatment significantly enhanced the ability to stay on the rotarod for both WT (p<0.05) and HD (p<0.05) mice. Generally, the WT and HD mice that were treated with Ex-4 showed increased levels of general activity in their home cages (data not shown). We propose that the improved performance of the WT Ex-4 treated mice on the rotarod could be due to their increased activity levels. In humans, nausea is often observed with Ex-4 treatment, however in our paradigm it is unlikely that this was in effect as this would likely have negatively affected the animals’ rotarod performance.

Ex-4-treated HD mice showed superior motor control compared to the HD control animals, and treatment with Ex-4 recovered HD rotarod performance times to a similar proficiency as the WT control mice (Fig. 5A). As HD symptoms progressed in the N171-82Q mice, the control and Ex-4-treated HD mice performed progressively worse on each rotarod
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test. However, Ex-4 treatment significantly attenuated this decline in rotarod performance in the HD mice, which indicates that Ex-4 can ameliorate motor symptoms in HD mice (Fig. 5B). Comparing the differences in performance (percent change from baseline) from week to week of treatment, we demonstrated that as a ratio of Ex-4:control animals that there was a consistent superiority of the Ex-4 treated HD animals compared to saline-treated HD animals. Hence both saline and Ex-4 treated animals declined in performance over time but the Ex-4-treated animals each week had a better performance than control animals., i.e. greater positive change to baseline at week 4 and then lesser decreases from baseline at weeks 6 and 8 (Fig. 5B and C).

**Mutant huntingtin aggregates are reduced with Exendin-4 treatment.** Inclusions of aggregated huntingtin protein in cortical, striatal and hippocampal neurons are present in the HD mice, as well as in HD patients. In the N171-82Q mice, intranuclear inclusions and neuritic aggregates (all immunoreactive with an antibody to the N-terminus (amino acids 1-17) of huntingtin) have been shown to be present in multiple populations of neurons (24). We determined whether Ex-4 treatment had any efficacy at reducing the number of mhtt aggregates in both the brain (cortex) and pancreas (islets of Langerhans). Ex-4 treatment caused a reduction in the amount of mhtt aggregates in the cortex of the HD mice (Fig. 6A, p<0.01). Additionally, there was a small, but non-significant, increase in heat-shock protein-70 (Hsp-70) levels in the cortex of both the WT and HD Ex-4-treated mice compared to vehicle-treated mice. In the pancreas, Ex-4 treatment caused a significant reduction (Fig. 6B; p<0.001) in the number of mhtt aggregates in the islets of Langerhans. The HD control mice showed a large number of mhtt aggregates in the pancreatic islets. Typically, the HD control mice had two types of islets; very small islets containing a large number of mhtt aggregates and somewhat larger irregular-shaped islets containing a large number of mhtt aggregates. The Ex-4-treated HD mice on the other hand demonstrated that only the smaller islets contained a low number of aggregates and larger islets had few or no aggregates.

**Exendin-4 treatment extends the survival of HD mice.** Any effective therapeutic agent for the treatment of HD symptomology would ideally delay the onset of symptoms (i.e. motor dysfunction) and extend life span. In addition to ameliorating motor dysfunction (Fig. 5), Ex-4 treatment resulted in a highly significant increase in the survival of the HD mice compared to vehicle-treated control HD mice (Fig. 6C). The onset of mortality in HD mice treated with Ex-4 was significantly delayed compared to the control HD mice and the mean life span was significantly increased from 140 days to 165 days (p < 0.01; Fig. 6C). This life span-extending effect of Ex-4 of nearly one month represents an 18% increase over the life span of vehicle-treated control HD mice.

**DISCUSSION**

We have shown that Ex-4, a potent long-acting agonist of GLP-1 used to treat type 2 diabetes, significantly improved glycemic control and pancreatic cellular architecture, attenuated motor performance decline, reduced mhtt aggregation in the brain and pancreas and significantly increased life span in the N171-82Q mouse model of HD. This is the first study to report that euglycemia can be achieved and maintained and that pancreatic islets can be preserved in a mouse model of HD by daily administration of a widely-used drug. The beneficial effects of Ex-4 on abnormalities of energy metabolism (glycemic control) and brain pathology (mhtt-associated degeneration and motor dysfunction) are likely due to a combination of peripheral and central actions of Ex-4. The global effects of Ex-4 are clearly beneficial to
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the animal even with the presence of reduced body mass, thus the treatment is still effective even with this unwanted side-effect. In human patients, cognoscence of this action may be offset by an increase in input calories in their diet, which may be actually facilitated by Ex-4 treatment as in the animals it reduced leptin levels and increased ghrelin levels. A simultaneous reduction in satiety (lower leptin) and increase in hunger (increased ghrelin) would help foster elevated caloric intake in the HD patients.

The high basal glucose levels in HD mice may be associated with accumulation of mhtt and therefore disruption of function in pancreatic β-cells, reductions in numbers of β-cells and perturbed α-cell topography. Others have reported the presence of mhtt aggregates in pancreatic cells associated with perturbed structure and endocrine function of the pancreas in HD mouse models (11). We found that Ex-4, based on the morphological findings, obviously ameliorates mhtt-related β-cell pathologies suggesting that the beneficial effects of Ex-4 on the pancreas are, at least in part, responsible for its ability to restore glycemic control in the HD mice. Approximately 20% of HD patients are reported to have diabetes (10, 36). This incidence may prove to be higher if all HD patients were tested. Insulin treatment (up to 8IU/kg daily or twice daily) of long-acting insulin, a dose that typically induces lethal hypoglycaemia in normal mice, did not control blood glucose in the HD mice, illustrating a severe insulin resistance, and actually appeared to shorten the life-span of the HD mice.

Ex-4 had profound effects on insulin resistance, as demonstrated by the marked improvement in the HOMA index. Plasma glucose levels of the Ex-4 treated HD mice were significantly lower than those of the non-treated HD animals, for the same prevailing plasma insulin levels, clearly indicating improved insulin action. We propose that this is due to direct effects of Ex-4 on the brain as a result of reducing mutant htt aggregates, and we suggest that the therapeutic locus of improvement may be at the level of the hypothalamus. Evidence indicates that while insulin is not a major regulator of glucose use by the brain (37), the brain is clearly not insulin insensitive. In normal rats, blockade of insulin receptor signaling in the hypothalamus by phosphoinositide 3-kinase (PI3-K) inhibitors leads to hepatic insulin resistance and its inevitable consequence of increased hepatic glucose production (38, 39). Centrally acting insulin may regulate glucose metabolism via neuronal systems that are partially independent of one another (39). Defective insulin receptor substrate (IRS)/PI3K signaling is implicated in insulin resistance in peripheral tissues (40) and presumably may be causing neuronal/hypothalamic insulin resistance in HD patients. In addition to insulin control of PI3-K activity in the hypothalamus, the satiety controlling hormone leptin also activates PI3-K in the hypothalamus (41). Therefore defective insulin/PI3-K signaling in hypothalamus would be compounded by the presence of low leptin levels, as we found to be the case in the HD mice. Intriguing data also points to centrally-acting CPT-1, the mitochondrial protein that regulates the rate of fatty acid oxidation, being a regulator of peripheral insulin sensitivity. Its inhibition centrally led to increased hepatic insulin sensitivity that was functionally blocked by vagotomy (42). Lipid sensing by the brain appears to regulate hepatic glucose metabolism via activation of vagal afferent fibers. We therefore suggest that in HD severe peripheral insulin resistance may develop via insulin resistance in the hypothalamus. This may also lead to upregulation of CPT-1 and increased fatty acid oxidation, further decreasing signaling via the vagus to the liver. The defects related to glucose metabolism in the HD mice were not corrected by exogenous insulin (and presumably also could not be corrected by
endogenous insulin action), suggesting that mutant htt aggregates are directly impacting the insulin receptor down-stream signaling pathways. As Ex-4 lessened aggregates in both the brain and the pancreas in our HD mice, we hypothesize that this is the primary mechanism underlying the ability to markedly improve insulin action. Taken together, such a mechanism further reinforces the potential impact of considering hormonal periphery-central interactions with respect to therapeutics for neurodegenerative disorders.

Neither the normal function of huntingtin nor the mechanism whereby the polyglutamine expansions result in selective loss of striatal neurons is fully understood, although impaired energy metabolism (43), excitotoxicity (44) and oxidative stress (45) have all been implicated. Mutant htt may cause neuronal dysfunction and death by inducing oxidative stress, impairing energy metabolism, inhibiting neurotrophic factor expression and triggering apoptosis (46). Deficits in striatal and cortical glucose metabolism have been shown to precede the appearance of symptoms in HD patients (47), and mhtt impairs neuronal energy metabolism in cultured neurons and transgenic mice (48, 49). The ability of Ex-4 to suppress mhtt accumulation in brain cells and improve motor performance in HD mice indicates that Ex-4 counteracts the adverse effects of mhtt on neurons at a relatively early stage in the neurodegenerative process. Previous studies have shown that Ex-4 crosses the blood-brain barrier (21) and that small fragments of Ex-4 have been shown in one study to mediate neuronal protection against excitotoxic and metabolic insults in culture and in vivo (22). Our findings suggest that Ex-4 can protect neurons against the pathogenic actions of mhtt, thereby delaying disease onset and extending the survival of the HD mice.

Our understanding of neurodegenerative brain disorders has evolved to a point at which brain health and somatic health are intimately associated in the disease process. The contribution of peripheral and glycemic health is critical to the maintenance of a healthy brain (14). In searching for an effective therapeutic strategy for HD, previous research has focused only on the detrimental effects of aberrant mutant htt processing in brain cells, while largely overlooking the fact that peripheral pathophysiology (such as altered glucose control, disrupted energy expenditure and severe uncontrolled weight loss) could contribute to HD symptomology and exacerbate neuronal dysfunction and disease progression. Our findings show that Ex-4, an agent that targets both the central (i.e. neuronal dysfunction) and peripheral (abnormal energy and glucose regulation, appetite dysregulation) pathological processes in HD, is a therapeutic agent in HD mice. When taken together with the increasing evidence that GLP-1 and Ex-4 can protect neurons against a range of insults (22) and can stimulate neurogenesis (50), our findings suggest that Ex-4 might also prove efficacious in other complex neurodegenerative disorders that involve metabolic disturbances, including Alzheimer’s and Parkinson’s diseases. Regardless of all else, our data suggests that Ex-4, now marketed as exenatide, should be seriously considered for treating metabolic deficits in HD patients.

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REFERENCES

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Table 1. Pancreatic islet size analysis of wild-type and N171-82Q HD mice treated with either saline or Exendin-4.

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<th>Wild-type</th>
<th>N171-82Q HD</th>
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<tr>
<td></td>
<td>Control</td>
<td>Ex-4</td>
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<tr>
<td>Islet Area (μm²)</td>
<td>15101 ± 2546</td>
<td>12606 ± 2652</td>
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<tr>
<td>Major Axis (μm)</td>
<td>211.5 ± 19.0</td>
<td>185.3 ± 21.5</td>
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<tr>
<td>Minor Axis (μm)</td>
<td>154.1 ± 13.5</td>
<td>132.7 ± 13.6</td>
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**p<0.01; ***p<0.001.
Figure 1. Experimental design. (A). Experimental timeline of the study. Male N171-82Q and age-matched WT mice were injected daily with either Exendin-4 (Ex-4) or saline (control). Body weight and glucose measurements were recorded weekly and rotarod performance was assessed bi-weekly. Upon euthanization, cortex, pancreas and plasma were collected for further analyses. (B). Ex-4 is an agonist of the glucagon-like peptide 1 (GLP-1) receptor. Several amino acids differ between GLP-1 and Ex-4 sequences, most importantly in the N-terminal region where the substitution of alanine to glycine renders Ex-4 resistant to proteolysis by dipeptidyl peptidase-IV (DPP-IV). While GLP-1 has a half-life of less than two minutes in circulation, Ex-4 has biological activity for approximately 6 hours. This ensures that Ex-4 has potent and long-acting effects on both the periphery (pancreas) and the brain. (C). Proposed mechanisms of action of Ex-4 in peripheral tissues and the brain. Ex-4 promotes pancreatic β cell growth and insulin production and secretion, and increases insulin sensitivity of muscle and liver cells. Ex-4 crosses the blood-brain barrier and acts on neurons in the brain to promote their survival and support their high energy demands.
Figure 2. Exendin-4 normalizes plasma glucose levels in HD mice. Weekly (A, B) and average (C, D) glucose levels of saline- and Ex-4-treated wild type (WT) and HD mice are shown throughout the treatment period. Ex-4 treatment significantly reduced plasma glucose levels in HD mice from the first week of treatment and euglycemia was maintained throughout the course of the study period. Ex-4 also significantly reduced plasma glucose levels in WT mice. Weekly (E, F) and average (G, H) body weight measurements for saline- and Ex-4-treated WT and HD mice throughout the study. Ex-4 treatment caused a significant reduction in body weight in both WT and HD mice. Values are means ± SEM, n = 18-24 animals per group. *p<0.05; **p<0.01; ***p<0.001.
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Figure 3. Modification of plasma levels of energy-regulating hormones by Ex-4 treatment in wild-type and HD mice. Plasma concentrations of insulin (A, B), leptin (C, D), ghrelin (E, F), and adiponectin (G, H) were measured in WT and HD mice treated with either saline (control) or Ex-4. Ex-4 treatment significantly reduced plasma insulin levels in WT mice, compared to control WT animals (p<0.05). In HD mice, Ex-4 treatment did not significantly alter circulating levels of insulin, compared to HD control animals. Thus, despite having lower circulating levels of insulin, the Ex-4-treated WT and HD mice exhibit improved insulin sensitivity, as demonstrated by the reduction in plasma glucose levels (A, B). Using plasma insulin and glucose measurements, HOMA (homeostasis model of insulin resistance) values were calculated (B inset). Ex-4 treatment significantly reduced leptin levels in both WT (p<0.001) and HD (p<0.05) mice, consistent with the decrease in the body weight in the Ex-4-treated mice (C, D). There were no significant effects of Ex-4 on plasma ghrelin levels in WT and HD mice, although there were trends towards decreased levels in the WT mice and increased levels in the HD mice (E, F). There were no significant alterations in the plasma adiponectin levels with Ex-4 treatment for both the WT and HD mice, and HD mice had significantly lower plasma adiponectin levels compared to WT mice (p<0.01) (G, H). Values are means ± SEM, n = 18-24 animals per group. *p<0.05; ***p<0.001.
Figure 4. Treatment with Ex-4 improves pancreatic islet physiology in HD mice. Immunostaining of pancreatic tissue for the β-cell derived hormone insulin and corresponding phase contrast images. Saline treated HD mice had small, significantly diminished islets compared to saline treated WT mice (A, B). Treatment with Ex-4 restored islet size in HD mice, but did not significantly affect islet size in WT mice (C, D). Immunostaining for the α-cell derived hormone glucagon confirmed these improvements in islet physiology with Ex-4 treatment. In non-diabetic mice, glucagon-positive cells are typically arranged in a “halo” around the edge of the islet (E, G). In the HD mice, the islet structure was altered and α-cells were displaced into the center of the islet (F); this α-cell abnormality was improved with Ex-4 treatment in HD mice (H). Values are means ± SEM, n = 6-8 animals per group.
Figure 5. Exendin-4 treatment improves motor coordination in HD mice. Motor performance was measured bi-weekly using an accelerating rotarod apparatus. Mice were placed on the rotarod, which accelerated from 4-40 rpm over a 5-minute test period, and latency to fall was recorded. Average time spent on the rotarod during the course of treatment for each study group is shown (A). Treatment with Ex-4 significantly increased the rotarod times of HD mice (p<0.05). Ex-4 treatment also increased the rotarod latencies of WT mice (p<0.01). Analysis of percent change in rotarod performance from baseline confirmed that the performance of the Ex-4 treated HD mice showed a much slower rate of motor control decline than the saline treated HD mice (B). Hence at each time trial Ex-4 treated animals showed a greater time on the rotarod compared to saline-treated. (C) Demonstrates the relative week to week differences (modulus) in rotarod performance (percent change from baseline) between Ex-4 and control treated HD animals expressed as a ratio. Hence an increase in the ratio denotes a relative increase in maintenance of rotarod performance in the Ex-4 treated animals compared to control. Values are means ± SEM, n = 19-24 animals per group.
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Figure 6. Exendin-4 reduces mutant huntingtin aggregates in both the cortex and islets of Langerhans and significantly increases lifespan of HD mice. Western immunoblot analysis with S830, an antibody against the HD exon 1 transgene protein, showed that the amount of mutant huntingtin (mhtt) aggregation was significantly reduced in cortical tissue of Ex-4 treated HD mice, compared to saline-treated controls (p<0.01, A, left). Heat shock protein-70 (Hsp-70) is a molecular chaperone whose expression is increased in times of cellular stress, and it acts to prevent protein misfolding and aggregation in response to environmental insults or disease. Western immunobloting showed that cortical levels of Hsp-70 were slightly elevated with Ex-4 treatment in both HD and WT mice (A, right). Immunostaining of pancreatic tissue with S830 antibody showed that Ex-4 treatment caused a significant decrease in the number of mhtt aggregates in the pancreatic islets of Langerhans. There was a significant decrease in the average number of mutant huntingtin aggregates per islet (p<0.001) and an increase in the number of islets containing no mhtt aggregates in the Ex-4 treated HD mice (B, bar charts). Representative images of both HD control and HD Ex-4 treated islets are shown. The HD control mice generally had 2 types of islets, some very small and others large and irregularly shaped, and both types containing large numbers of mhtt aggregates. The small islets in Ex-4-treated HD mice had a small number of mhtt aggregates, while large islets had a more regular structure and little or no mhtt aggregates (B). Ex-4 treatment caused a significant increase in the survival of HD mice (p<0.01). Ex-4-treated HD mice lived an average of 25.3 days longer than saline-treated controls, an 18% increase in lifespan (C). Values are means ± SEM, n = 21-24 animals per group. **p<0.01; ***p<0.001.