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Insulin and IGF-1 regularize energy metabolites in neural cells expressing full-length mutant huntingtin

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder linked to the expression of mutant huntingtin. Bioenergetic dysfunction has been described to contribute to HD pathogenesis. Thus, treatment paradigms aimed to ameliorate energy deficits appear to be suitable candidates in HD. In previous studies, we observed protective effects of insulin growth factor-1 (IGF-1) in YAC128 and R6/2 mice, two HD mouse models, whereas IGF-1 and/or insulin halted mitochondrial-driven oxidative stress in mutant striatal cells and mitochondrial dysfunction in HD human lymphoblasts. Here, we analyzed the effect of IGF-1 *versus* insulin on energy metabolic parameters using striatal cells derived from HD knock-in mice and primary cortical cultures from YAC128 mice. *STHdh*$^{Q111/Q111}$ cells exhibited decreased ATP/ADP ratio and increased phosphocreatine levels. Moreover, pyruvate levels were increased in mutant cells, most probably in consequence of a decrease in pyruvate dehydrogenase (PDH) protein expression and increased PDH phosphorylation, reflecting its inactivation. Insulin and IGF-1 treatment significantly decreased phosphocreatine levels, whereas IGF-1 only decreased pyruvate levels in mutant cells. In a different scenario, primary cortical cultures derived from YAC128 mice also displayed energetic abnormalities. We observed a decrease in both ATP/ADP and phosphocreatine levels, which were prevented following exposure to insulin or IGF-1. Furthermore, decreased lactate levels in YAC128 cultures occurred concomitantly with a decline in lactate dehydrogenase activity, which was ameliorated with both insulin and IGF-1. These data demonstrate differential HD-associated metabolic dysfunction in striatal cell lines and primary cortical cultures, both of which being alleviated by insulin and IGF-1.

1. Introduction

Huntington's disease (HD) in an autosomal dominant neurodegenerative disorder caused by an abnormal expansion of CAG triplets in the *HTT* gene, encoding for mutant huntingtin (mHTT in humans, or mHtt in mice). HD clinical symptoms include involuntary movements, dementia, dramatic weight loss, and eventually death. Neuropathologically, HD is characterized by selective dysfunction and death of GABAergic projection medium spiny neurons in the striatum. Moreover, the degree of striatal atrophy correlates with the degeneration of cerebral cortex during the latest stages (Gil and Rego, 2008). Although striatal death underlies many symptoms in advanced stages of the disease (Vonsattel and DiFiglia, 1998), early deficits, which seem to occur years before the evident movement disorder, are more likely associated with neuronal and synaptic dysfunction in the cortex (e.g. Rosas et al., 2005). An expansion of more than 39 CAG repeats in the *HTT* gene underlies several mechanisms of neurodegeneration, such as oxidative stress, mitochondrial dysfunction, and deficits in energy metabolism (Naia et al., 2011). In particular, mHTT impairs mitochondrial respiration and ATP production (Milakovic and Johnson, 2005; Seong et al., 2005; Silva et al., 2013). Elevated levels of lactate were also detected in the striatum and cerebral cortex of HD patients (Koroshetz et al., 1997; Jenkins et al., 1998), as well as decreased ATP and phosphocreatine (PCr) levels following glycolysis inhibition in HD cybrids, exhibiting bioenergetically dysfunctional mitochondria (Ferreira et al., 2011). Additionally, a diversity of metabolic enzymes are altered in HD, namely, pyruvate dehydrogenase (PDH) (Perluigi et al., 2005; Ferreira et al., 2011), pyruvate

Abbreviations: ADP, adenosine diphosphate; Akt, protein kinase B; AMP, adenosine monophosphate; ATL, alanine aminotransferase; ATP, adenosine triphosphate; Cr, creatine; DG, 2-deoxy-D-glucose; FBS, fetal bovine serum; GLUC, glucose; IGF-1, insulin-like growth factor 1; IR/IGF-1R, insulin/IGF-1 receptor; LDH, lactate dehydrogenase; HD, Huntington's disease; mHTT, mutant huntingtin; PC, pyruvate carboxylase; PCr, phosphocreatine; PDH, pyruvate dehydrogenase; PI-3K, phosphoinositide 3-kinase.

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carboxylase (PC) (Butterworth, 1986; Lee et al., 2013), glucose-6-phosphate dehydrogenase (Ferreira et al., 2011), aconitase (Tabrizi et al., 2000), and aspartate aminotransferase (Perluigi et al., 2005).

During the last few years, our group analyzed the effects of insulin and insulin-like growth factor 1 (IGF-1) in HD, from peripheral to neural cells and also in *in vivo* models. The insulin/IGF-1 signaling has been studied for many years in several neurodegenerative disorders linked to toxic protein aggregation, but it has generated conflicting results. While some studies demonstrated that IGF-1 modulates the clearance of brain aggregation-prone proteins, such as amyloid-β peptide (Carro et al., 2002) or mHTT (Humbert et al., 2002), other authors claimed that reduced insulin/IGF-1 signaling regulate disaggregation of amyloid-β peptide, ataxin-3, and other polyglutamine proteins to promote cell survival (Morley et al., 2002; Cohen et al., 2006; Kapperler et al., 2008; Teixeira-Castro et al., 2011). Indeed, IGF-1 signaling was described to be deleterious in the regulation of lifespan in nematodes (Kenyon et al., 1993) and mouse models (Holzenberger et al., 2003); however, the ablation of IGF-1 or its receptor promoted brain growth retardation (D'Ercole et al., 2002), indicating that IGF-1 may stimulate neuronal development. A more recent study found no measurable effect between the inhibition of insulin/IGF-1 pathway and the decreased mHTT aggregation (Jakubik et al., 2014). Even so, IGF-1 plasma levels were shown to be increased in YAC128 mice (expressing human full-length mHTT), correlating with increased body weight (Pouladi et al., 2010), and high IGF-1 levels were associated with cognitive decline in HD patients (Saleh et al., 2010).

Counterweighting the previous data, recent findings in our group showed that peripheral administration of IGF-1 prevented metabolic abnormalities in a hemizygous R6/2 mouse model of HD, such as impaired glucose tolerance and age-related decrease in body weight by enriching blood insulin and IGF-1 levels (Duarte et al., 2011). Indeed, both insulin and IGF-1 may activate insulin/IGF-1 receptor (IR/IGF-1R), stimulating PI-3K/Akt signaling pathways, through direct phosphorylation of HTT by Akt at serine 421 (Humbert et al., 2002), which appears to promote mitochondrial function and modulate the expression of proteins involved in glucose metabolism and anti-apoptotic mechanisms (Duarte et al., 2008; Naia et al., 2015). The activation of the same pathway also counteracted the increase in lactate/pyruvate ratio in both YAC128 mice (Lopes et al., 2014) and HD human lymphoblasts (Naia et al., 2015). In addition, we showed that the activation of IGF-1/insulin signaling pathways in striatal cells expressing 111 glutamines precludes mitochondrial generation of reactive oxygen species and mitochondrial dysfunction, largely reducing apoptotic and senescent cells induced by mHtt expression (Ribeiro et al., 2014). Nevertheless, it remained unknown whether the improvement in mitochondrial function would also be linked to an improvement in cell bioenergetics. Therefore, we hypothesized that insulin or IGF-1 treatment could ameliorate metabolic function in the context of HD. Here, we show that both insulin and IGF-1 can rescue energy deficits in YAC128 primary cortical cells by ameliorating ATP, PCr, and lactate levels, the later involving modified lactate dehydrogenase (LDH) activity. Furthermore, IGF-1 alleviates anomalous pyruvate levels in homozygous ST*Hdh*$^{Q111/Q111}$ striatal cells derived from HD knock-in mice.

2. Materials and methods

2.1. Materials

Fetal bovine serum (FBS), B27 supplement and penicillin/streptomycin were from Gibco (Paisley, Scotland, UK). Insulin from porcine pancreas, DMEM medium, trypsin-type IX-S from porcine pancreas, IGF-1, glucose-6-phosphate, deoxy-D-glucose (DG), L-glutamine, poly-L-lysine, fatty acid free bovine serum albumin (BSA), trypan blue (0.4%), resazurin, adenine dinucleotide phosphate hydrate (NADP), nicotinamide adenine dinucleotide hydrate (NAD), ADP, glucose 6 phosphate dehydrogenase (G6P-DH), hexokinase, creatine kinase, cytosine β-D-arabinofuranoside, CoASH, and thiamine pyrophosphate (TPP) from Sigma Aldrich (St. Louis, MO, USA). Bio-Rad protein assay was from Bio-Rad (Hemel Hempstead, UK). Lactate, pyruvate, and phospho-PDH in-cell ELISA kit from Abcam (Cambridge, UK). All other reagents were of analytical grade.

2.2. Primary culture of cortical cells and treatments

Heterozygous YAC128 (containing the full-length human *HTT* gene with 128 CAG repeats) mice embryos with 16–17 days and age-matched WT mice (as controls) (FVB/N strain) embryos were used. YAC128 mice were previously described by Slow et al. (2003). These animals were obtained from our local colony (CNC/Faculty of Medicine Animal Facility, University of Coimbra) with breeding couples provided by Dr. Michael Hayden (University of British Columbia, Vancouver, Canada). Animals were kept under controlled light and humidity conditions, being sacrificed by cervical displacement and decapitation (EU guideline 86/609/EEC); the studies were performed according to the Helsinki Declaration and Guide for the Care and Use of Laboratory Animals (NIH, USA). Cortical cells were isolated using a previously described procedure (Duarte et al., 2008), with some minor modifications. Briefly, mouse brains were dissected out and the cortex was digested with 0.6 mg/mL trypsin for 5 min, at 37 °C in Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution containing (in mM): 137 NaCl, 5.36 KCl, 0.44 KH_2PO_4, 0.34 $Na_2HPO_4.2H_2O$, 4.16 $NaHCO_3$, 5 glucose, 1 sodium pyruvate and 10 HEPES, at pH 7.2. Cells were then plated at a density of 9×10^4 cells/cm^2 in poly-L-lysine (0.1 mg/mL) coated 96- or 12-well plates, according to the experimental procedure. Cells were cultured for 13 days *in vitro* (DIV) in 95% air and 5% CO_2, in serum-free neurobasal medium supplemented with 2% B27, 0.5 mM glutamine, and 0.12 mg/mL gentamicin. The glial cell marker GFAP was used to confirm the presence of astrocytes. According to this characterization, the percentage of neurons in our primary culture was about 60%, indicating the presence of a co-culture of neuronal and non-neuronal cells.

Treatment consisted in 1 nM insulin or IGF-1, added to cortical cultures 48 h before collection. For hyperglycemia induction, neurobasal medium was supplemented with 6 mM D-glucose (BASAL) and 56 mM D-glucose (+GLUC), 24 h before collection. For hypoglycemia conditions, neurobasal medium was replaced by Krebs medium containing the following (in mM): 132 NaCl, 4 KCl, 1.2 NA_2HPO_4, 1.4 $MgCl_2$, 1 $CaCl_2$ supplemented with 6 mM D-glucose (BASAL), or in the absence of glucose plus 2 mM deoxy-D-glucose (−GLUC + DG) 20 min before the experiment.

2.3. Cell lines culture and treatments

Striatal cells derived from mice expressing normal HTT (STHdh$^{Q7/Q7}$ or wild-type cells; clone 2aA5) or homozygous knock-in mice expressing mHTT with 111 glutamines (ST*Hdh*$^{Q111/Q111}$ or mutant cells; clone 109-1 A) were used. The cell lines were kindly donated by Dr. Marcy E. MacDonald (Department of Neurology, Massachusetts General Hospital, Boston, USA). The cells were maintained as described previously (Trettel et al., 2000). Striatal cells were plated on poly-L-lysine coated glass coverslips, multiwell chambers, or flasks at a density of 0.06×10^6 cells/cm^2 48 h before the experiments in order to allow the desired confluence. Twenty-four hours before the experiment, cells were incubated with insulin (0.1 nM) or IGF-1 (1 nM).

2.4. Measurement of intracellular adenine nucleotides, phosphocreatine, and pyruvate levels

Cells were washed with ice-cold PBS and centrifuged at 145×*g*, for 5 min (4 °C). Extracts were performed with 0.6 M perchloric acid supplemented with 25 mM EDTA-Na^+ and then centrifuged at 20,800×*g* for 2 min at 4 °C to remove cell debris. The resulting pellet was solubilized with 1 M NaOH and further analyzed for total protein content by