Increased energy metabolism rescues glia-induced pathology in a *Drosophila* model of Huntington’s disease

Marie-Thérèse Besson1,†, Pascale Dupont1,†, Yih-Woei C. Fridell2 and Jean-Charles Liévens1,*

1Centre de Recherche en Neurobiologie-Neurophysiologie de Marseille, CNRS UMR 6231, Université d’Aix-Marseille II et III, Institut Jean-Roche, 13344 Marseille Cedex 15, France and 2Department of Allied Health Sciences, University of Connecticut, 358 Mansfield Road, Storrs, CT 06269, USA

Received April 2, 2010; Revised and Accepted June 10, 2010

Huntington’s disease (HD) is a polyglutamine (polyQ) disease caused by an expanded CAG tract within the coding region of Huntingtin protein. Mutant Huntingtin (mHtt) is ubiquitously expressed, abundantly in neurons but also significantly in glial cells. Neuron-intrinsic mechanism and alterations in glia-to-neuron communication both contribute to the neuronal dysfunction and death in HD pathology. However, it remains to be determined the role of glial cells in HD pathogenesis. In recent years, development of *Drosophila* models facilitated the dissection of the cellular and molecular events in polyQ-related diseases. By using genetic approaches in *Drosophila*, we manipulated the expression levels of mitochondrial uncoupling proteins (UCPs) that regulate production of both ATP and reactive oxygen species in mitochondria. We discovered that enhanced levels of UCPs alleviated the HD phenotype when mHtt was selectively expressed in glia, including defects in locomotor behavior and early death of *Drosophila*. In contrast, UCPs failed to prevent the HD toxicity in neurons. Increased oxidative stress defense was found to rescue neuron but not glia-induced pathology. Evidence is now emerging that UCPs are fundamental to adapt the energy metabolism in order to meet the metabolic demand. Thus, we propose that UCPs are glioprotective by rescuing energy-dependent functions in glia that are challenged by mHtt. In support of this, increasing glucose entry in glia was found to alleviate glia-induced pathology. Altogether, our data emphasize the importance of energy metabolism in the glial alterations in HD and may lead to a new therapeutic avenue.

INTRODUCTION

Huntington’s disease (HD) is an autosomal dominant late-onset neurodegenerative disorder. HD is caused by an expanded CAG repeat in the first exon of the *IT15* gene, resulting in increased length of a polyglutamine (polyQ) stretch in the N-terminus of Huntingtin protein. HD pathology is observed when patients carry more than 36 CAG repeats and symptoms include progressive dyskinesia, chorea, cognitive decline and psychiatric disturbances. Despite ubiquitous expression of mutant Huntingtin (mHtt) in neurons throughout the brain, only specific neuronal subpopulations degenerate mostly in the striatum and, to a lesser extent in the cerebral cortex. However, HD should not simply be considered as only pathology of neuronal loss since evidence shows that neuronal functioning impairments appear to be the primary cause for the early symptoms in HD patients.

Major insights into the mechanisms leading to neuronal dysfunction and death have arisen from transgenic models. Among the intrinsic neuronal mechanisms, mHtt alters a large variety of pathways including cellular trafficking, synaptic transmission, transcription regulation, proteosomal function and mitochondrial metabolism. Recently, it was assumed that non-cell autonomous pathways also participate in neuronal dysfunction and death in HD. For instance, glial cells express Huntingtin (1,2) and the expression of HD mutation specifically in glia induces locomotor defects and shortened lifespan in fly or mouse models (3–5). However, the mechanisms by which mHtt-expressing glia may challenge neuronal functioning remains poorly defined. So far, we and

†To whom correspondence should be addressed. Tel: +33 491698880; Fax: +33 491698977; Email: jean-charles.lievens@univmed.fr

†M.T.B. and P.D. contributed equally to this work.

© The Author 2010. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org
others have demonstrated that glutamate removal from the synaptic cleft by astroglial cells is impaired in the mouse model and HD patient brains (6–9). This is due to direct effect of mHtt on glial functioning because such a defective glutamate uptake is also found in transgenic flies or mouse expressing mHtt solely in glial cells (5,10). It remains to be determined whether and how other crucial glial functions can contribute to HD pathogenesis.

In the brain, the main site of ATP synthesis and expenditure is the neuron mostly to support synaptic activity. Glial cells, while taking up half or more of the glucose consumed by the brain, use a very small part of energy and supply neurons with energy intermediate substrates (11,12). Energetic defects are a profound hallmark of HD (reviewed in 13,14). The presence of mHtt disrupts the mitochondria functioning and thereby alters the oxidative production of ATP. In particular, impaired activities of the complexes II/III and IV of the respiratory chain have been found in post-mortem samples of HD patients (15,16). Data on the respiratory chain in transgenic animals are more conflicting and they differ between models and the disease stage (13,14). In contrast, dramatic reductions in the activity and expression of the tricarboxylic acid cycle (TCA) enzyme aconitase have been reported in both HD patients (17,18) and mouse models (19,20). Pyruvate dehydrogenase, the key enzyme linking the glycolysis metabolic pathway to the TCA, was also shown to be altered in HD brain (21,22). It is now widely recognized that the majority of reactive oxygen species (ROS) are generated by mitochondria as a byproduct of the respiratory chain. Besides energetic defects, there is evidence that direct interaction of mHtt with mitochondria triggers ROS overproduction and thereby results in oxidative stress in HD brain (23).

The fruitfly Drosophila melanogaster is a powerful model organism to identify pathways involved in human brain diseases. Using the UAS-GAL4 bipartite system (24), mHtt can be selectively expressed either in neurons or independently in glia. We recently designed an original sensitive model in Drosophila to dissect out mechanisms underlying the mHtt-mediated glial pathology (3). In particular, we found that flies expressing mHtt in glia suffered from altered locomotor performance and abnormal susceptibility to mechanical stress. Under mechanical shock, flies exhibited paralysis and convulsions, a phenotype that was previously correlated with HD brain (23). This view is further supported by our data showing that increasing glucose utilization rescues the glial phenotype in HD flies.

RESULTS

Drosophila UCP5 rescues glial pathology in HD flies

Whereas sequence homology analysis revealed four putative UCPs in Drosophila: DmUCP4a, DmUCP4b, DmUCP4c and DmUCP5 (28), to date only DmUCP5 has been functionally characterized and reported to have uncoupling activity in Drosophila (29). We here generated transgenic flies to overexpress the full-length DmUCP5 cDNA selectively in specific cell subtypes by using the UAS-GAL4 bipartite system. Two independently transformed lines were used in parallel: DmUCP5.2 and DmUCP5.3. They were crossed to the actin-GAL4 driver to induce transgene expression in all cell types. Semi-quantitative RT–PCR analysis showed that levels of DmUCP5 transcript were dramatically increased in flies actin-GAL4; UAS-DmUCP5.2 and actin-GAL4; UAS-DmUCP5.3 as compared with control flies actin-GAL4 (Fig. 1).

We first examined the effects of DmUCP5 on mHtt-induced glial pathology. For that purpose, Httex1p Q93 was exclusively expressed in all glial cells by using the repo-GAL4 driver. Climbing ability of flies was evaluated at 12 days post-eclosion when Httex1p Q93-expressing flies exhibit strong locomotor defects (3). Figure 2A shows the percent of flies that reached the top or remained at the bottom of the column 30 s after startle. Whereas all control flies containing the repo-GAL4 driver alone were able to reach the top of the column within 30 s (≏90% of flies), only 26% of flies expressing Httex1p Q93 in glia reached the top of the column and ≏46% remained at the bottom. DmUCP5 had a significant beneficial effect on the climbing performance of Drosophila expressing mHtt in glia. When DmUCP5 was overexpressed, 52% of flies succeeded to reach the top of the column while ≏22% remained at the bottom. Similar results were observed with both DmUCP5.2 and DmUCP5.3 lines. An intriguing phenotype when mHtt is expressed in Drosophila glia is that flies progressively displayed temporary paralysis/convulsion after a mechanical stress (3). Such a bang-sensitive phenotype reflects the inability of flies to perform the escape reflex in response to mechanical stress. At 12 days post-eclosion, ≏40% of flies expressing Httex1p Q93 under the control of the repo-GAL4 driver were no longer able to perform any motor activity but were able to startle when tapped at the bottom of the column. Similar results were obtained under the control of the actin-GAL4 driver (Fig. 2B). These results are consistent with the data showing that DmUCP5 rescues locomotor defects in HD flies.
driver were bang-sensitive (Fig. 2B). DmUCP5 significantly ameliorated the bang-sensitivity since only 23% of flies co-expressing DmUCP5 and Httex1pQ93 in glia showed a paralytic/convulsive behavior after mechanical stress. The lifespan of flies expressing mHtt in glia was also improved in the presence of DmUCP5 (Fig. 2C). Whereas 70% of adult flies expressing Httex1p Q93 alone were dead at 16 days, 78% of flies co-expressing DmUCP5 and Httex1p Q93 remained alive at the same age. Life expectancy of flies co-expressing DmUCP5 together with Httex1p Q93 in glia was increased by 23–25% as compared with flies expressing Httex1p Q93 alone. As a control for potentially non-specific suppression effects, green fluorescent protein (GFP) was co-expressed with Httex1p Q93 in glial cells using the UAS-GFP-S65T transgenic line. We found no significant changes in the locomotor defects and premature death of Drosophila expressing mHtt in glia (Table 1 and Supplementary Material, S1). No change in the Drosophila lifespan was observed when DmUCP5 was expressed alone under the control of the pan-glial repo-GAL4 driver (Supplementary Material, S2).

Glia-mediated alterations were also described when mHtt expression was driven by the deAAT1-GAL4 driver (3). deAAT1 is the only glutamate transporter expressed in glia subset that lies at the periphery of the brain and sends cytoplasmic projections in the central neuropil where synaptic contacts occur (30). Whereas their role has not been fully elucidated, one particular function of deAAT1-positive glia is to remove and recycle extracellular glutamate. Again, we found that DmUCP5 rescued the early lethality of flies expressing Httex1p Q93 in deAAT1-positive glia (Fig. 3A). The presence of DmUCP5 increased by 20% the life expectancy of flies expressing Httex1p Q93 under the regulation of the glial driver repo-GAL4. We also study the effects of DmUCP5 loss of function on the glial phenotype. For that, we used BmcpBG02446 flies carrying a P-element inserted into the translational start codon of the DmUCP5 gene. Those flies were previously reported to show impaired expression of the DmUCP5 transcript (31). We found that the presence of one mutant allele of DmUCP5 did not modify the early lethality of flies expressing Httex1p Q93 under the control of the glial driver.
flies. Flies expressing Httex1p Q93 in neurons did not survive beyond 28 days post-eclosion (Fig. 4B). We found that co-expression of DmUCP5 failed to ameliorate both climbing performances and the survival time of flies that expressed Httex1p Q93 in neurons (Fig. 4A and B). No change in the Drosophila lifespan was observed when DmUCP5 was expressed alone in neurons (Supplementary Material, S2).

Neuronal expression of mHtt is also known to induce neuronal loss in restricted cerebral structure in Drosophila such as the mushroom bodies (3,32), a central brain structure involved in locomotion, learning and memory (33). To visualize the mushroom bodies, we used flies expressing GFP under control of the mushroom body-specific OK107 driver (line OK107-GFP) as previously described (32). Figure 4C shows the loss of the mushroom body γ-lobes, whereas the α- and β-lobes remained intact in 12-day-old flies expressing Httex1p Q93 in mushroom bodies. Quantitative analysis (Fig. 4D) indicated that in the Httex1p Q93-expressing flies, the fluorescence intensity in the γ-lobes fell to only 18% of the control flies. Flies co-expressing DmUCP5 and Httex1p Q93 displayed a similar loss of the γ-lobes as compared with flies expressing mHtt alone (Fig. 4C and D). In contrast, we previously reported that overexpression of the HSP70 chaperone, a potent suppressor of mHtt toxicity, was able to abolish the γ-lobe loss in similar conditions (3). Therefore, although DmUCP5 restores neuron–glia interaction when co-expressed with mHtt in glia, DmUCP5 did not protect against direct effects of mHtt on neuronal functioning and survival.

**Human UCP2 alleviates glial pathology in HD flies**

To attest further that UCPs prevent glial HD pathology, we also assessed the effects of human uncoupling protein UCP2. We therefore used the transgenic flies UAS-hUCP2 that were previously generated (34). We first investigated whether or not the presence of hUCP2 modifies the phenotype of flies when mHtt was expressed under the control of the pan-glial repo-GAL4 driver. The climbing ability of HD flies was significantly improved by hUCP2 as late as 12 days of adult age (Fig. 5A). Whereas >60% of flies expressing Httex1p Q93 alone in glia remained at the bottom of the column within 30 s, only 32% of flies co-expressing hUCP2 with Httex1p Q93 did not climb. The presence of hUCP2 strongly reduced the rate of bang-sensitive flies (Fig. 5B). When Httex1p Q93 alone was expressed in glia, 46% of flies exhibited a temporary paralysis after mechanical stress. In contrast, only 10% of flies co-expressing hUCP2 and Httex1p Q93 in glia displayed bang-sensitivity. Whereas almost all flies expressing Httex1p Q93 alone did not survive beyond 20 days, 75% of flies co-expressing hUCP2 and Httex1p Q93 remained alive at 20 days (Fig. 5C). Life expectancy of flies co-expressing hUCP2 together with Httex1p Q93 in glia was increased by 30% as compared with flies expressing Httex1p Q93 alone. Similar improvement in survival was also found for flies co-expressing mHtt and UCP2 in deEAAT1-positive glia (Fig. 5D). The presence of hUCP2 increased by 21% the life expectancy of flies expressing Httex1p Q93 under the control of the glial driver deEAAT1-GAL4. Therefore, these data provide strong evidence that UCPs protect against glia-induced toxicity of mHtt.

### Table 1. Genetic modifiers for the glia-induced pathology in flies

<table>
<thead>
<tr>
<th>Transgenes</th>
<th>Life expectancy</th>
<th>Locomotor performance</th>
<th>Bang-sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DmUCP5.2 or DmUCP5.3</td>
<td>Increased</td>
<td>Ameliorated</td>
<td>Decreased</td>
</tr>
<tr>
<td>BMCpBG02446</td>
<td>No change</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>hUCP2</td>
<td>Increased</td>
<td>Ameliorated</td>
<td>Decreased</td>
</tr>
<tr>
<td>MnSOD7; OAT-Catalase5</td>
<td>No change</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>DmGlut1I</td>
<td>Increased</td>
<td>Ameliorated</td>
<td>n.d.</td>
</tr>
<tr>
<td>GFP-S65T</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
</tbody>
</table>

n.d., not determined.

**Drosophila UCP5 fails to rescue neuronal pathology in HD flies**

Next we tested whether DmUCP5 may have a protective effect when co-expressed with mHtt in neurons. Neuronal expression of transgenes was then driven in all neurons by the elav-GAL4 driver. As previously described (3), flies expressing mHtt in neurons displayed reduced climbing ability (Fig. 4A). Under this condition, only 59% of flies succeeded to reach the top of the column within 30 s versus 90% in the case of control flies. Flies expressing Httex1p Q93 in neurons did not
Superoxide dismutase and catalase do not rescue glia-induced pathology in HD flies

It has been proposed that UCPs lead to cell protection by controlling ROS generation (34–36). Superoxide anion (O$_2^-$) radical is the first source of ROS that is generated as a by-product of the mitochondrial respiratory chain. Superoxide dismutase (SOD) and catalase act in tandem to eliminate ROS by converting O$_2^-$ radicals first to hydrogen peroxide (H$_2$O$_2$) and then into inactive metabolites (H$_2$O + O$_2$), respectively. To determine whether the beneficial effects of UCPs in our conditions result from a reduced oxidative stress, we evaluated whether simultaneous increase in Mn-SOD and catalase levels may be protective against HD toxicity in glia. Transgenic flies bearing one additional copy of the *Drosophila* Mn-SOD gene, together with the *Drosophila* catalase coding sequence containing the mitochondrial targeting sequence of the OAT (ornithine aminotransferase) gene, were previously described.
to resist to oxidative stress (37). We here showed that the presence of both Mn-SOD and catalase in the mitochondrial matrix did not prevent the early death of flies expressing Httex1p Q93 in glia under the control of the pan-glial driver repo-GAL4. In contrast, increasing expression of MnSOD and catalase together into the mitochondria strongly improves the survival of flies expressing mHtt under the control of the neuronal driver elav-GAL4 (Fig. 6A). In this case, the lifespan of flies was significantly increased by 64% versus flies expressing mHtt alone. Therefore, whereas oxidative stress is likely a key event that leads to mHtt toxicity in neurons, it seems that other crucial pathways are involved in the mHtt-induced alterations in glial cells.

**Drosophila glucose transporter rescues glial pathology in HD flies**

UCPs are also reported to adapt the way energy is supplied in cells in order to meet the metabolic demand. One possible mechanism is that UCPs may enhance glycolysis (25). To
Neuron–glia interactions are crucial to ensure optimal brain function and accumulation of mHtt compromises crosstalk between the two cell types (2,3,5,10). Interestingly, whereas both neuronal and glial expression of mHtt triggers decreased climbing performance, only flies expressing mHtt in glia suffered from bang-sensitivity following a mechanical stress. Thus, the bang-sensitive phenotype likely reflects the disruption of glial-specific functions by expanded polyQ proteins. The exact mechanism that triggers the bang-sensitive behavior in Drosophila is not well understood. So far, bang-sensitivity has been related to depletion in ATP levels and impairment of the Na+/K+ ATPase pump (39,40). Interestingly, two crucial functions of glia are to supply neurons with energy metabolites and to maintain ionic composition of the extracellular environment.

We here report that DmUCP5 rescues the bang-sensitive phenotype and the climbing performance when co-expressed with mHtt in glia. Moreover, DmUCP5 significantly prolongs the survival of flies expressing mHtt in glia. Similarly, the exogenous expression of hUCP2 confers protection against mHtt toxicity in glia. In contrast, DmUCP5 fails to alleviate the deleterious phenotype when co-expressed with mHtt in neurons. Thus, we provide the first evidence that UCPs mediate differential effects on the glial- and neuronal-specific physiology.

Numerous studies have raised the possibility that UCPs could confer neuroprotection in models of brain injury or neurodegenerative diseases. Evidence come mainly from transgenic mice containing a copy of human UCP2 and UCP3 genes cloned under the regulation of their endogenous promoters, respectively (41). Accordingly to the regional distribution of UCPs, those mice show a moderate overexpression of UCP2 into the brain (42). UCP2/UCP3 transgenic mice showed a lower MPTP-induced neurodegeneration and reproduce a syndrome clinically similar to Parkinson's disease in animal models. Whereas UCP2 knock-out mice display an enhanced sensitivity to MPTP exposure, UCP2/UCP3 transgenic mice showed a lower MPTP-induced loss of dopaminergic neurons in the substantia nigra (45). Similarly, selective overexpression of UCP2 in catecholaminergic neurons by using the tyrosine hydroxylase promoter protects nigral neurons from acute MPTP toxicity (46). Stroke, ischemia and acute MPTP treatment appear to lead to neuronal death by a common pathway: they all alter mitochondrial metabolism and increase ROS release. Accordingly, in the aforementioned studies, the neuroprotective effect of UCP2 determine whether or not increased entry of glucose in glial cells may have beneficial effects on mHtt-induced glial alterations, we generated transgenic flies to overexpress the full-length Drosophila homologue of glucose transporters, DmGluT1 (38), in glia by using the UAS-GAL4 system. Over-expression of DmGluT1 significantly improved locomotor behavior and survival of flies when co-expressed with mHtt in glia under the regulation of the repo-GAL4 driver. Whereas only 17% of 12-day-old flies expressing Httex1p Q93 in glia reached the top of the column, 58% of flies co-expressing Httex1p Q93 and DmGluT1 succeeded to reach the top of the column (Fig. 7A). More importantly, whereas almost all flies expressing mHtt alone died before 20 days of age, Drosophila co-expressing mHtt and DmGluT1 survived to as late as 32 days (Fig. 7B). Life expectancy of flies co-expressing Httex1p Q93 and DmGluT1 in glia was significantly increased by 32% as compared with flies expressing mHtt alone. No change in the Drosophila lifespan was observed when DmGluT1 was expressed alone in glia (Supplementary Material, S3). Thus, we showed that increasing glucose uptake in glial cells is a suppressor of mHtt toxicity in glia.

**DISCUSSION**

As summarized in Table 1, the main outcome of the present study is that manipulating energy metabolism alleviates the glial-induced pathology in a Drosophila model of HD. Neuron–glia interactions are crucial to ensure optimal brain function and accumulation of mHtt compromises crosstalk between the two cell types (2,3,5,10). Interestingly, whereas both neuronal and glial expression of mHtt triggers decreased climbing performance, only flies expressing mHtt in glia suffered from bang-sensitivity following a mechanical stress. Thus, the bang-sensitive phenotype likely reflects the disruption of glial-specific functions by expanded polyQ proteins. The exact mechanism that triggers the bang-sensitive behavior in Drosophila is not well understood. So far, bang-sensitivity has been related to depletion in ATP levels and impairment of the Na+/K+ ATPase pump (39,40). Interestingly, two crucial functions of glia are to supply neurons with energy metabolites and to maintain ionic composition of the extracellular environment.

We here report that DmUCP5 rescues the bang-sensitive phenotype and the climbing performance when co-expressed with mHtt in glia. Moreover, DmUCP5 significantly prolongs the survival of flies expressing mHtt in glia. Similarly, the exogenous expression of hUCP2 confers protection against mHtt toxicity in glia. In contrast, DmUCP5 fails to alleviate the deleterious phenotype when co-expressed with mHtt in neurons. Thus, we provide the first evidence that UCPs mediate differential effects on the glial- and neuronal-specific physiology.

Numerous studies have raised the possibility that UCPs could confer neuroprotection in models of brain injury or neurodegenerative diseases. Evidence come mainly from transgenic mice containing a copy of human UCP2 and UCP3 genes cloned under the regulation of their endogenous promoters, respectively (41). Accordingly to the regional distribution of UCPs, those mice show a moderate overexpression of UCP2 into the brain (42). UCP2/UCP3 transgenic mice exhibit a reduction in cell death occurring after experimental induction of epileptic seizures (43) or following ischemia and traumatic brain injury (36,44). Acute treatment with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) is currently used to induce dopaminergic neuron degeneration and reproduce a syndrome clinically similar to Parkinson’s disease in animal models. Whereas UCP2 knock-out mice display an enhanced sensitivity to MPTP exposure, UCP2/UCP3 transgenic mice showed a lower MPTP-induced loss of dopaminergic neurons in the substantia nigra (45). Similarly, selective overexpression of UCP2 in catecholaminergic neurons by using the tyrosine hydroxylase promoter protects nigral neurons from acute MPTP toxicity (46). Stroke, ischemia and acute MPTP treatment appear to lead to neuronal death by a common pathway: they all alter mitochondrial metabolism and increase ROS release. Accordingly, in the aforementioned studies, the neuroprotective effect of UCP2...
was correlated with the reduction of oxidative stress in these models (36,43,45,46). Since mHtt is known to interact with mitochondria and lead to increased ROS production (23), it is tempting to propose that UCPs are glioprotective by reducing oxidative stress in glia. In the present study, we evaluated the potential benefit of increasing ROS defense when mHtt is selectively expressed in Drosophila glia or neurons. Whereas increasing MnSOD and catalase levels rescued the early death of flies expressing mHtt in neurons, no improvement was found when mHtt was present selectively in glia. Thus, we further confirm that ROS overproduction is a crucial pathway by which mHtt leads to neuronal dysfunction and/or death in HD. However, despite the impact of UCPs on ROS production, this may not be sufficient to counteract the mHtt-induced alterations in neurons. Our data also reveal that oxidative stress is likely not the primary cause of HD glial pathogenesis and the beneficial effects of UCPs on glial cells are likely not due to the UCP-mediated proton leak and the subsequent reduction of ROS.

In recent years, an increasing amount of data indicate that UCPs may not only act as uncoupling agents. They may be fundamental for metabolic sensing and adaptive energetic metabolism in order to meet the energy demand. For instance, Pecqueur et al. (47) reported that UCP2 knock-out fibroblasts display enhanced proliferation associated with a higher pyruvate oxidation rate and a reduced fatty acid oxidation in mitochondria. On the contrary, UCP2 overexpression decreases the glucose-dependent proliferation of CHO cells (47). This is consistent with a ‘metabolic hypothesis’ whereby the role of UCP2 would be to promote oxidation of fatty acids rather than that of glucose-derived pyruvate (25). Data also suggest that UCPs may physically conduct free fatty acid anions and thereby, actively participate to the fatty acid cycling (48,49). Finally, since UCPs shunt the oxidation of pyruvate in mitochondria, this may give rise to increased glycolysis followed by lactate production: a process known as the Warburg effects (50). In respect to this, overexpression of UCP3 and UCP4 in muscle and neuronal cultured cells, respectively, was found to stimulate glucose transport and shifting the way of ATP production from mitochondrial production to glycolysis (51–53). In the brain, glycolysis is predominantly an astrocytic metabolic process, whereas oxidation is primarily neuronal (11,54). Therefore, we propose that UCP overexpression in the presence of mHtt would ameliorate the glial-induced alterations by enhancing glycolysis. In support of this hypothesis, overexpression of the Drosophila glucose transporter DmGluT1 ameliorated the locomotor deficiency and the lifespan of flies expressing mHtt in glia. A role for glucose metabolism as a modulator for mHtt toxicity was previously suggested on HD cell models as cell death was significantly reduced by glucose transporter overexpression (55,56).

---

**Figure 7.** DmGluT1 rescues locomotor deficiency and improves survival of flies expressing Htt(ex) Q93 in glia. (A) Climbing performances of 12-day-old flies expressing, Htt(ex) Q93 alone or Htt(ex) Q93 plus DmGluT1 (line DmGluT1.1) under the control of the glial driver repo-GAL4. The proportions of flies that climbed to the top of the column or that remained at the bottom were determined after 30 s. Statistical significance was assessed by Student’s t-test (**P < 0.01; ***P < 0.001). (B) Survival rates of flies expressing Htt(ex) Q93 alone, or together with DmGluT1 (line DmGluT1.1) under the control of the glial driver repo-GAL4. Cumulative survival curves were generated by the Kaplan–Meier method, and statistical significance was determined by the logrank test (**P < 0.001).
of glucose is beneficial against mHtt-induced glial dysfunction. The exact steps whereby enhanced glucose metabolism rescues glia-induced alterations in HD flies remains to be clarified. As a possible mechanism, raised intracellular glucose levels may induce autophagy via mTOR signaling (55).

In conclusion, we propose that defects in energetic metabolism are involved in mHtt-induced glial alterations and that increasing glucose metabolism may be beneficial to rescue abnormal glia-to-neuron communication in HD. Further work is required to fully understand the importance of energy metabolism in the abnormal neuron–glia crosstalk in HD pathogenesis.

MATERIALS AND METHODS

Drosophila transgenic lines

The DmUCP5 cDNA was cloned into the pUAST transformation vector as described previously (31). The DmGluT1 clone (Flybase clone LD20062) was digested with EcoR1 and Xho1 restriction enzymes. The excised 2.2 kb fragment was purified, then inserted into the pUAST plasmid. All transgenic flies were generated in a w1118 background. Germ-line transformation for DmGluT1 and DmUCP5 was performed by BestGene (BestGene Inc., CA, USA). In the present study, we used two independent DmUCP5 lines (DmUCP5.2 and DmUCP5.3) and one DmGluT1 line (DmGluT1.1) carrying the respective transgene on the second chromosome.

Fly stocks and culture

Flies were reared on a standard agar/cornmeal/yeast diet. The following fly stocks were used: deEAAT1-GAL4 (30), repo-GAL4 (generous gift from A. Giangrande), UAS-Httex1p Q93 (57), UAS-hUCP2 (34), Mn-SOD7; OAT-Catalase5 (37) and OK107-GFP (32). All control experiments were carried out in the absence of the respective transgene on the second chromosome.

Reverse transcription polymerase chain reaction

Total RNA was extracted from 15 heads of 48 h post-eclosion flies using Trizol (Invitrogen). Reverse transcription was performed with Improm-II Reverse Transcriptase (Promega). The DmUCP5 and Actin 5C primers were added together in the same PCR tube. Cycling conditions were 26 cycles: 30 s at 92°C, 30 s at 60°C and 1 min at 72°C. The DmUCP5 primers (5′-AAGGTCAGAAAATCGACCAATCG and 5′-AGCTCA CACGAGGCGCTAATG) and Actin 5C primers (5′-CGAC AACGGCTCTGGCATGT and 5′-GCAATCAGACTGCGACCAATCG) amplify a 450 and 1092 bp cDNA fragment, respectively. Control experiments were carried out in the absence of reverse transcriptase. PCR products were separated on 1% agarose gels and visualized by Sybr Green (Invitrogen) staining.

Lifespan analysis

Lethality analysis was assessed on 80–120 flies of each strain divided into five to eight vials (10–20 flies per vial). For each desired genotype, flies were reared at 25°C. Cumulative survival curves were generated by the Kaplan–Meier method and statistical analysis was performed by the logrank test (GraphPad Prism software).

Climbing assay

Climbing ability was assessed with a negative geotaxis assay as described previously (3). Eight female flies were anesthetized with CO2 and placed in a plastic column (length: 18 cm × diameter: 1.3 cm). After 30 min recovery, columns were disposed vertically and flies were gently tapped to the bottom of the column. Flies having reached the top and remaining at the bottom of the column were counted after 30 s and four to six trials were performed at 1 min intervals in each experiment. The data are presented as the number of flies at the top (ntop) or at the bottom (ntop) and are expressed as percentage ± SEM of the total number of flies (ntotal). Statistical significance was assessed by using Student’s t-test.

Bang-sensitive assay

Testing for bang-sensitive behavior was performed on 12-day-old flies (>80 flies) of each strain divided into more than seven trials (10–15 flies/trial). Flies were separated in independent vials at least 12 h before testing. Individual flies were mechanically stimulated by manual banging (three times). The data are presented as the proportion of flies that displayed the bang-sensitive phenotype and are expressed as percentage ± SEM of the total number of flies (noverall). Statistical significance was assessed by using Student’s t-test.

Fluorescence detection of mushroom bodies

Mushroom bodies were visualized by using the OK107-GFP fly line as described previously (32). Adult Drosophila brains were dissected in phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, rinsed in PBS and mounted in Mowiol (Calbiochem, La Jolla, CA, USA). Confocal image acquisition was performed on a Leica (Wetzlar, Germany) TSC SP2 laser scanning microscope. Images of GFP-stained mushroom bodies were obtained using the 488 nm band of an argon laser for excitation. Fluorescence images were collected automatically as frame-by-frame sequential series, each image being produced from an average of three frame scans. For quantitative analysis, microscopic examination was performed using an AX10 Zeiss Apotome. Quantification of the signal was done with AxiosVision (Zeiss) and Image J software. The specific signal was obtained after subtracting the background signal and then was expressed as percent of the mean of control flies. Data from at least five flies per condition were averaged and were presented as mean ± SEM. Statistical analysis was performed using a Student’s t-test.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.
ACKNOWLEDGEMENTS

We thank Leslie M. Thompson and J. Lawrence Marsh (UC Irvine) for the UAS-Hitex1p Q93 flies, Serge Birman for the deAAAT1-GAL4 driver flies, Rajind Sohal (Dallas) for the Mn-SOD7; OAT-Catalase5 flies and the Bloomington Drosophila Stock Center for transgenic flies. The authors are grateful to C. Faivre-Sarrailh for support and critical reading of the manuscript.

Conflict of Interest statement. None declared.

FUNDING

This work was supported by a generous grant from the CHDI Foundation, Inc.

REFERENCES

34. Fridelli, Y.W., Sanchez-Blanco, A., Silvia, B.A. and Helfand, S.L. (2005) Targeted expression of the human uncoupling protein 2 (hUCP2) to adult flies and the Bloomington Drosophila Stock Center for transgenic flies. The authors are grateful to C. Faivre-Sarrailh for support and critical reading of the manuscript.

Human Molecular Genetics, 2010, Vol. 19, No. 17 3381