Impaired Regulation of Brain Mitochondria by Extramitochondrial Ca\textsuperscript{2+} in Transgenic Huntington Disease Rats

Received for publication, November 21, 2007, and in revised form, July 3, 2008. Published, JBC Papers in Press, July 3, 2008, DOI 10.1074/jbc.M709555200

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Huntington disease (HD) \textsuperscript{2} is characterized by polyglutamine expansions of huntingtin (htt), but the underlying pathomechanisms have remained unclear. We studied brain mitochondria of transgenic HD rats with 51 glutamine repeats (htt\textsubscript{51Q}), modeling the adult form of HD. Ca\textsubscript{2+}\textsubscript{\textsc{free}} up to 2 \( \mu \)M activated state 3 respiration of wild type mitochondria with glutamate/malate or pyruvate/malate as substrates. Ca\textsubscript{2+}\textsubscript{\textsc{free}} above 2 \( \mu \)M inhibited respiration via cyclosporin A-dependent permeability transition (PT). Ruthenium red, an inhibitor of the mitochondrial Ca\textsubscript{2+} uniporter, did not affect the Ca\textsubscript{2+}-dependent activation of respiration but reduced Ca\textsubscript{2+}-induced inhibition. Thus, Ca\textsubscript{2+} activation was mediated exclusively by extramitochondrial Ca\textsubscript{2+}, whereas inhibition was promoted also by intramitochondrial Ca\textsubscript{2+}. In contrast, htt\textsubscript{51Q} mitochondria showed a deficient state 3 respiration, a lower sensitivity to Ca\textsubscript{2+} activation, and a higher susceptibility to Ca\textsubscript{2+}-dependent inhibition. Furthermore htt\textsubscript{51Q} mitochondria exhibited a diminished membrane potential stability in response to Ca\textsubscript{2+}, lower capacities and rates of Ca\textsubscript{2+} accumulation, and a decreased Ca\textsubscript{2+} threshold for PT in a substrate-independent but cyclosporin A-sensitive manner. Compared with wild type, Ca\textsubscript{2+}-induced inhibition of respiration of htt\textsubscript{51Q} mitochondria was less sensitive to ruthenium red, indicating the involvement of extramitochondrial Ca\textsubscript{2+}. In conclusion, we demonstrate a novel mechanism of mitochondrial regulation by extramitochondrial Ca\textsubscript{2+}. We suggest that specific regulatory Ca\textsubscript{2+} binding sites on the mitochondrial surface, e.g. the glutamate/aspartate carrier (aralar), mediate this regulation. Interactions between htt\textsubscript{51Q} and distinct targets such as aralar and/or the PT pore may underlie mitochondrial dysregulation leading to energetic depression, cell death, and tissue atrophy in HD.

Huntington disease (HD) \textsuperscript{2} is a progressive neurodegenerative disorder caused by a CAG repeat expansion in the coding region of the huntingtin (htt) gene resulting in an expanded polyglutamine stretch in the htt protein (htt\textsubscript{expQ}) \textsuperscript{(1,2)}. The CAG repeat length of htt\textsubscript{expQ} correlates inversely with the time point of disease onset \textsuperscript{(3)}. Unmodified htt and even htt\textsubscript{expQ} in HD are abundantly expressed in most tissues \textsuperscript{(2)}, but neither the biological function of htt nor the mechanism of cytotoxic action of htt\textsubscript{expQ} has been understood \textsuperscript{(4)}.

Several lines of evidence suggest that the cellular energy metabolism is impaired in HD (for reviews, see Refs. 5 and 6) possibly because of the mitochondriotoxic effects of htt\textsubscript{expQ} \textsuperscript{(4–11)}. Indeed decreased Ca\textsubscript{2+} accumulation capacities of mitochondria from brain of YAC\textsubscript{22Q} mice \textsuperscript{(7)}, from skeletal muscle of htt\textsubscript{140Q} R6/2 mice \textsuperscript{(8)}, from liver of htt\textsubscript{111Q} mice \textsuperscript{(9)}, from HD patient’s lymphocytes \textsuperscript{(7)}, and from htt\textsubscript{111Q} striatal progenitor cells \textsuperscript{(10)} have been reported. Furthermore an impaired mitochondrial function and a Ca\textsubscript{2+} dyshomeostasis were detected in PC12 cells after transfection with htt\textsubscript{expQ} plasmids \textsuperscript{(11)}. In contrast, increased Ca\textsubscript{2+} loading capacities were observed in brain mitochondria from several HD mice lines \textsuperscript{(12,13)}.

Recently we have presented the first experimental evidence for an impaired oxidative phosphorylation in isolated HD mitochondria from skeletal muscle of R6/2 mice after their exposure to elevated Ca\textsubscript{2+} levels \textsuperscript{(8)}. Similarly in situ measurements of mitochondrial respiration revealed a declined oxidative phos-
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phorylation in HD150Q striatal cells but only after N-methyl-D-aspartate receptor-induced Ca2+ stress (13). A Ca2+-induced decrease of respiration was also identified in mitochondria isolated from htt111Q striatal cells (10). In addition, we could recognize a compromised mitochondrial function in fibroblasts from a HD patient with htt43Q (14). Taken together, all these data imply a disturbed interaction between HD mitochondria and cytosolic Ca2+ ([Ca2+], but the underlying targets and pathomechanisms have remained uncertain.

Elevated [Ca2+], mitochondrial Ca2+ uptake via the Ca2+-uniporter, and subsequent intramitochondrial Ca2+ accumulation (15) activate mitochondrial respiration due to stimulation of mitochondrial dehydrogenases (16). Elevated intramitochondrial Ca2+ levels also induce permeability transition (PT) if distinct [Ca2+] thresholds are exceeded (17). In addition, a reduced mitochondrial membrane potential (ΔΨ) and a decreased redox pressure, defined by the ratio of NADH/NAD+, further reinforce the tendency for undergoing mitochondrial PT (17).

Recently activation of the mitochondrial malate/aspartate shuttle was observed at low, i.e. submicromolar, [Ca2+] levels. This effect was caused by a Ca2+-dependent stimulation of the glutamate/aspartate translocator (18, 19). This carrier, termed aralar in brain mitochondria, was shown to also be involved in the transport of reducing hydrogen into mitochondria via a reconstituted malate/aspartate shuttle (18).

Considering this background, it was the first goal of this study to answer the question whether or not httexpQ is mitochondrialotoxic and if so to identify the underlying pathomechanism. For this purpose, we investigated the mitochondrial function in a newly generated transgenic HD rat strain with 51 glutamine repeats (htt11Q) (20). In contrast to httQ150 R6/2 mice, a model of the juvenile form of HD (21), the htt51Q rat strain exhibits specifically an adult-related onset of the neurological HD phenotype (20).

Because HD-specific changes in mitochondrial function may be related to alterations in the intracellular Ca2+ homeostasis (7–11, 22, 23), the second goal of this study was to evaluate the influence of extramitochondrial Ca2+ on the function of wild type (WT) and htt51Q mitochondria. We studied the effects of extramitochondrial Ca2+ on mitochondrial Ca2+ accumulation and oxidative phosphorylation in mitochondria isolated from striatum and brain. We found that extramitochondrial Ca2+ in the submicromolar range was able to activate mitochondrial glutamate uptake, probably via binding on the high affinity Ca2+ binding sites of the aspartate/glutamate translocator (alaral) that are exposed into the mitochondrial intermembrane space. Htt51Q mitochondria showed a deficient state 3 respiration, a lower sensitivity to Ca2+ activation, and a higher susceptibility to Ca2+-dependent inhibition. Furthermore htt51Q mitochondria exhibited a diminished membrane potential stability in response to Ca2+, lower capacities and rates of Ca2+ accumulation, and a decreased Ca2+ threshold for PT in a substrate-independent but CsA-sensitive manner. Compared with WT, Ca2+-induced inhibition of respiration of htt51Q mitochondria was less sensitive to ruthenium red (RR), indicating the involvement of extramitochondrial Ca2+. In summary, our data suggest that mitochondrialotoxic actions of htt51Q might be realized by affecting the regulatory Ca2+ binding sites of mitochondrial carrier proteins like aralar and the PT pore, finally leading to energetic depression (6, 24), mitochondrial cell death, and atrophy of affected tissues (6).

EXPERIMENTAL PROCEDURES

Animals—Transgenic animals were obtained from the central animal facility of the University of Tübingen, Tübingen, Germany. HD rats expressed 727 amino acids of the htt51Q gene corresponding to 22% of the full-length gene. Tail tips were removed from all rats at the age of 3 weeks, and genotypes were determined by Southern blot analysis. We used male and female rats aged 21–27 months and compared them with age-matched littermate WT rats from the same source. For some studies, normal adult Wistar WU rats were also used (Charles River Laboratories). All research and animal care procedures were performed according to European guidelines.

Isolation of Mitochondria—Mitochondria were prepared either from striatum or from the remaining part of the brain (total brain minus striatum) using a slightly modified protocol according to Kudin et al. (25), avoiding bovine serum albumin (BSA) during the preparation and investigation of mitochondria. In brief, animals were anesthetized with CO2 and killed by decapitation. Brain tissue was immediately transferred into the ice-cold isolation medium (IM) consisting of 225 mM mannitol, 75 mM sucrose, 20 mM MOPS, 1 mM EGTA, and 0.5 mM dithiothreitol, pH 7.4. Then we minced the tissue, added 10 mM of nargase medium (IM + 0.05% (w/v) nargase)/1 g of tissue, and homogenized the mixture with a glass/glass homogenizer. Then the homogenate was diluted 1:4 with nargase-free IM and centrifuged at 2,000 × g for 4 min. After centrifugation, the supernatant was passed through a cheesecloth and centrifuged at 12,000 × g for 9 min. To permeabilize the synaptosomes, the resulting pellet was suspended in 10 mM of ice-cold digitonin medium (IM + 0.02% digitonin), transferred to a small glass homogenizer, and manually homogenized 8–10 times to obtain a homogenous suspension. Finally the suspension was centrifuged at 12,000 × g for 11 min, and the resulting pellet was suspended in 400 μl of IM/g of tissue.

Respirometry—Mitochondrial respiration was measured with a Clark-type oxygen electrode by means of high resolution respirometry using an OROBOROS Oxygen-2k (Oroboros, Innsbruck, Austria) at 30 °C. Respiration of mitochondria (0.06 mg of protein/ml) was investigated in EGTA medium (120 mM mannitol, 40 mM MOPS, 5 mM KH2PO4, 60 mM KCl, 5 mM MgCl2, and 0.1 mM EGTA, pH 7.4). Extramitochondrial concentrations of free Ca2+ (Ca2+free) were adjusted either by up to six sequential Ca2+- additions (each of 20 μM) or by one single addition of 50 μM Ca2+ into the medium. Ca2+free under each condition was verified by Fura-2 measurements as described below.

Ca2+ Accumulation Measurements—Ca2+ accumulation into isolated mitochondria (0.25 mg of protein/ml) was monitored fluorimetrically in medium A (120 mM mannitol, 40 mM MOPS, 5 mM KH2PO4, and 60 mM KCl, pH 7.4) containing 0.5 μM Calcium Green-5N (Invitrogen). Measurements were performed in stirred and thermostated (30 °C) cuvettes using a Cary Eclipse fluorimeter (Varian Deutschland GmbH) as
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RESULTS

Stimulation of Glutamate-dependent Respiration by Extramitochondrial Ca$^{2+}$—To investigate the Ca$^{2+}$ dependence of oxidative phosphorylation, a model system allowing the stepwise increase of Ca$^{2+}$ from nm to $\mu$M concentration ranges was developed. We used an incubation medium containing 100 $\mu$M EGTA to keep Ca$^{2+}$ negligibly low (EGTA medium). Fig. 1A shows a respirogram of brain mitochondria isolated from normal rats using 10 mM glutamate and 2 mM malate as substrates. After addition of 2.5 mM ADP, an unusually low glutamate-dependent respiration rate (state 3$_{\text{glu/mal}}$) was obtained. To clarify whether such a modest respiration resulted from an insufficient complex I-related metabolism, we measured the complex II-dependent respiration by applying the specific complex I inhibitor rotenone and succinate as complex II-specific substrate (state 3$_{\text{suc}}$ respiration). As shown in Fig. 1A, succinate caused a normal state 3$_{\text{suc}}$ respiration that clearly exceeded the state 3$_{\text{glu/mal}}$ respiration. This finding suggests an inadequate complex I-mediated respiration. However, if the same experiment was performed in the presence of 1.35 mM Ca$^{2+}$ (Fig. 1B), the state 3$_{\text{glu/mal}}$ respiration was more than doubled compared with that in Ca$^{2+}$-free conditions (Fig. 1, A and B). Thus, the increase in Ca$^{2+}$ induced a normalization of the mitochondrial complex I-dependent respiration in normal brain mitochondria.

In a second approach, state 3$_{\text{glu/mal}}$ respiration was titrated by sequential Ca$^{2+}$ additions (Fig. 1C). Again state 3$_{\text{glu/mal}}$ was very low under Ca$^{2+}$-free conditions but increased stepwise until Ca$^{2+}$ reached 2 $\mu$M. At this Ca$^{2+}$ concentration, the maximum state 3$_{\text{glu/mal}}$ respiration was observed, whereas further Ca$^{2+}$ additions provoked an inhibitory effect. When Ca$^{2+}$-induced respiration rates were normalized against the corresponding respiration rate under Ca$^{2+}$-free conditions and plotted versus Ca$^{2+}$ (Fig. 1F), a 2-fold increase of state 3$_{\text{glu/mal}}$ respiration by 2 $\mu$M Ca$^{2+}$ was revealed. To further ascertain whether the pronounced Ca$^{2+}$ stimulation of state 3$_{\text{glu/mal}}$ respiration is a characteristic property of the glutamate metabolism, we investigated the substrate dependence of this effect in more detail. In particular, we considered pyruvate/malate as an alternative substrate of complex I-dependent respiration. We found that state 3$_{\text{pyr/mal}}$ respiration was also significantly activated (16 $\pm$ 3%) but to a much lesser extent than state 3$_{\text{glu/mal}}$ (87 $\pm$ 8%; Fig. 1, E and F). Additional experiments revealed that the activation of state 3$_{\text{pyr/mal}}$ is not caused by extramitochondrial Ca$^{2+}$ but is a result of large time requirement for complete

![FIGURE 1. Stimulation of glutamate-dependent respiration in normal brain mitochondria by extramitochondrial Ca$^{2+}$.](image)

*Measurements of Ca$^{2+}$ in EGTA Medium—Ca$^{2+}$ in the EGTA medium was measured fluorimetrically with Fura-2 (10 $\mu$M) as described previously (28, 29). Because the EGTA medium contained 5 mM Mg$^{2+}$ that competitively binds Fura-2 (29), the dissociation constant ($K_d$) of the Ca$^{2+}$-Fura-2 complex was measured experimentally under these conditions and found to be 0.3 $\mu$M, which was similar to that found in a previous study (28).

$\Delta$Ψ—Mitochondrial $\Delta$Ψ was monitored fluorimetrically by the release of safranine (30). Fluorescence was measured at 495 nm excitation and 586 nm emission using a Cary Eclipse fluorimeter (Varian) with 10 $\mu$M safranine in stirred and thermostated cuvettes (30 $^\circ$C). Measurements were performed in medium A using mitochondria adjusted to 0.25 mg of protein/mL.

Protein Determination—Mitochondrial protein concentrations were determined by the biocinchoninic acid assay (31). BSA was used as standard.

Statistical Analysis—All results are presented as mean $\pm$ S.D. or mean $\pm$ S.E. as indicated. Statistical significance was analyzed by paired and unpaired two-tailed t test or Bonferroni’s test.

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* C. Tanne and F. N. Gellerich, unpublished observation.
TABLE 1

| Condition | WT | Htt51Q |
|-----------|----|--------|
|           | Without RR | With RR | Without RR | With RR |
| Glutamate/malate | 121 ± 4 (16) | 122 ± 3 (16) | 83 ± 8* (16) | 82 ± 5* (13) |
| Ca²⁺ stimulation | +60 ± 6 (16) | +52 ± 5 (16) | 31 ± 6* (16) | +30 ± 6* (13) |
| Ca²⁺ inhibition | -47 ± 7 (16) | -8 ± 5* (16) | -63 ± 11 (16) | -28 ± 11* (13) |
| Pyruvate/malate | 184 ± 10 (6) | 174 ± 10 (6) | 122 ± 7* (4) | 119 ± 6* (4) |
| Ca²⁺ stimulation | +26 ± 34 (6) | +34 ± 13 (6) | +4 ± 4 (4) | +9 ± 4 (4) |
| Ca²⁺ inhibition | -73 ± 9 (6) | -39 ± 10* (6) | -81 ± 3 (4) | -56 ± 3* (4) |
| Succinate/rotenone | 178 ± 9 (10) | 181 ± 8 (10) | 156 ± 6* (6) | 151 ± 5* (6) |
| Ca²⁺ inhibition | -34 ± 10 (10) | -10 ± 8* (10) | -62 ± 3 (10) | -45 ± 8* (10) |
| RCI (without Ca²⁺) | 13.3 ± 0 (16) | 13.9 ± 1.1 (16) | 9.5 ± 0.9* (16) | 10.7 ± 1.4* (13) |
| Glutamate/malate | 18.7 ± 1.9 (6) | 18.9 ± 1.7 (6) | 11.5 ± 0.9* (4) | 10.8 ± 1.0* (4) |
| Pyruvate/malate | 5.2 ± 0.2 (10) | 5.2 ± 0.3 (10) | 5.0 ± 0.5 (6) | 4.8 ± 0.4 (6) |

*p < 0.01 compared with WT mitochondria.
*p < 0.002 compared with data without RR.

activation of pyruvate dehydrogenase. State 3Succ respiration remained unaffected by Ca²⁺free (Fig. 1D). Thus, glutamate/malate-dependent respiration could be identified as the most sensitive target of Ca²⁺free. The Ca²⁺free concentration required for half-maximum activation of state 3glu/mal respiration was 0.26 ± 0.02 µM. However, this value was much lower than the Km calculated for mitochondrial Ca²⁺ accumulation via the Ca²⁺ uniporter under similar conditions (2.5 ± 0.2 µM). This finding suggests that Ca²⁺-dependent activation of state 3Succ respiration cannot be mediated by the mitochondrial Ca²⁺ uniporter. If this assumption is valid, state 3Succ respiration should also be activated by Ca²⁺free in the presence of RR, an inhibitor of the Ca²⁺ uniporter. Indeed 250 nM RR, a dose able to block mitochondrial Ca²⁺ uptake through the Ca²⁺ uniporter completely under the conditions used here (not shown), did not prevent the Ca²⁺free-dependent state 3Succ activation (Fig. 1F). Thus, mechanisms underlying the stimulation of glutamate/malate-dependent respiration do not require intramitochondrial Ca²⁺ accumulation and therefore must be initiated outside the Ca²⁺-impermeable mitochondrial inner membrane.

Glutamate is taken up by mitochondria either by aralar, leading to its subsequent transamination by aspartate aminotransferase (inhibitable by aminoxyacetic acid (AOA)), or via the glutamate/OH carrier followed by its desamination by glutamate dehydrogenase (19, 32–34). However, the activity of the glutamate/OH carrier is low in most organs except liver and kidney (34). If so, Ca²⁺ activation of state 3Succ respiration should be inhibited by AOA. Indeed the Ca²⁺-dependent state 3Succ activation was significantly suppressed by 2 mM AOA (55 ± 5%), confirming the involvement of the aralar/transaminase pathway in the Ca²⁺ activation. In addition, we found that AOA (250 nM) did not affect the mitochondrial respiration with pyruvate/malate as substrates (not shown). It is therefore likely that activation of state 3Succ respiration by extramitochondrial Ca²⁺free is mediated by an enhanced glutamate transport into the mitochondrial matrix via aralar.

However, the functional relationship between Ca²⁺free and state 3Succ respiration rate exhibited a biphasic nature. At 6.3 µM or higher Ca²⁺free, a marked decrease in state 3Succ respiration was monitored (Fig. 1, C and F). Because this change was significantly attenuated by 1 µM CsA (data not shown), it was most likely caused by partial opening of the PT pore due to an intramitochondrial Ca²⁺ overload. This conclusion was further validated by the following finding. In contrast to the Ca²⁺-dependent activation of state 3Succ respiration, Ca²⁺-dependent inhibition of state 3Succ respiration at high Ca²⁺free was diminished by 2 µM RR (Fig. 1F). We therefore suppose that an intramitochondrial Ca²⁺ overload via the Ca²⁺ uniporter induces PT in a CsA-sensitive and thus cyclophilin D-dependent manner. Accordingly a significant inhibition of respiration by elevated Ca²⁺free was also found with pyruvate/malate and to a lesser extent with succinate (Fig. 1, D, E, and F).

**Impaired Oxidative Phosphorylation of Brain Mitochondria from htt51Q Rats**—In the next series of experiments, htt51Q and WT brain mitochondria were compared in EGTA medium in the presence of various substrates. State 3 respiration was adjusted by 2.5 mM ADP, whereas Ca²⁺free was kept low initially. As shown in Table 1, state 3Succ (−31%) and state 3Pyr (−32%) respiration of htt51Q mitochondria was lowered significantly compared with WT. Comitant reductions (between −20 and −29%) were also observed in the respiratory control indices (RCIs; calculated as the ratio of state 3/state 2 respiration) with NADH-linked complex I substrates. In contrast, complex II-dependent state 3Succ respiration of htt51Q mito-
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In general, RR did not influence the Ca$^{2+}$-dependent activation of state 3$_{\text{glycine/malate}}$ and state 3$_{\text{pyruvate/malate}}$ respiration (Fig. 2, B, D, F, and G, and Table 1). However, we could identify significant RR effects on the Ca$^{2+}$-induced inhibition of state 3 respiration regardless of whether htt$_{51Q}$ or WT mitochondria were studied and independently of the substrates used (Fig. 2, B, D, F, G, and H, and Table 1). Interestingly, the extent of RR effects on state 3 respiration was larger in WT mitochondria (Fig. 2, F and G, and Table 1), indicating that extramitochondrial Ca$^{2+}$ increased the tendency for PT pore opening in htt$_{51Q}$ mitochondria. Furthermore, RR shifted the maximum state 3 respiration rates with glutamate/malate or pyruvate/malate to higher Ca$_{\text{free}}$ levels exclusively in WT mitochondria (Fig. 2, E–G). In contrast, RR-dependent maximum state 3 rates of htt$_{51Q}$ mitochondria were moved toward lower Ca$_{\text{free}}$ levels as for pyruvate/malate or remained unchanged as for glutamate/malate (Fig. 2, E–G).

We also addressed the issue of whether complex II-specific state 3 respiration, realized by the simultaneous application of the FADH-generating substrate succinate and the specific complex I inhibitor rotenone, would be affected by Ca$_{\text{free}}$ RR, and/or htt$_{51Q}$. Under Ca$^{2+}$-free conditions, the state 3$_{\text{suc/rot}}$ respiration of WT and htt$_{51Q}$ mitochondria was comparable to or even higher than the corresponding complex I-linked respiration with glutamate/malate or pyruvate/malate (Figs. 1D and 2H and Table 1). Furthermore, Ca$_{\text{free}}$ did not exert any activation of state 3$_{\text{suc/rot}}$ respiration either in WT or in htt$_{51Q}$ mitochondria. Nevertheless, there was a moderate and Ca$^{2+}$-dependent inhibition of state 3$_{\text{suc/rot}}$ respiration if Ca$_{\text{free}}$ was increased into the range of 6.3–32 µM (Figs. 1D and 2H). Although slightly more pronounced in htt$_{51Q}$ mitochondria than in WT, this effect was in general smaller compared with complex I substrates (Table 1). In line with the results obtained with complex I-specific substrates, RR did not alter the basal state 3$_{\text{suc/rot}}$ respiration under Ca$^{2+}$-free conditions but reduced significantly the Ca$^{2+}$-dependent inhibition of respiration at high Ca$_{\text{free}}$ levels. Notably, this effect was exclusively observed in WT mitochondria but not in htt$_{51Q}$ mitochondria (Fig. 2H and Table 1).

For a more detailed evaluation of substrate dependences of htt$_{51Q}$-specific impairments of mitochondrial function, relative respiration efficiencies were calculated as the decrease of state 3 respiration in relation to corresponding WT values (Fig. 2, E–H). In general, these calculations were based on experimental data obtained in the absence of RR. For both complex I-de-
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In the next step, we addressed the question of which parts of oxidative phosphorylation, i.e. ΔΨ generation (respiration) and/or ΔΨ utilization (ADP phosphorylation and Ca2+-accumulation), contribute to htt51Q-specific changes of brain mitochondria. Respiration and ADP phosphorylation were experimentally separated by the uncoupler FCCP, which was added to mitochondria after the induction of state 3 respiration by 100 μM ADP. However, state 3 glutamate/malate respiration was not affected by FCCP either in htt51Q or in WT brain mitochondria (not shown). Similar results were obtained with pyruvate/malate and succinate as substrates (not shown). We therefore concluded that ADP phosphorylation was not compromised in htt51Q mitochondria. Rather htt51Q-dependent changes of mitochondrial function might be caused by impairments within the ΔΨ generating metabolism and/or by altered PT.

Decreased Stability of the Inner Membrane Potential ΔΨ of htt51Q Mitochondria—To study the underlying mechanism(s) of htt51Q-dependent changes in the ΔΨ generating metabolism of oxidative phosphorylation, we asked whether htt51Q is able to affect the stability of ΔΨ. Therefore, the influence of Ca2+-on ΔΨ was investigated in EGTA-free medium using the ΔΨ-sensitive fluorescence indicator safranine and different substrates (30). As shown in Fig. 3, A and B, glutamate/malate-consuming WT and corresponding htt51Q brain mitochondria accumulated safranine to a similar extent, indicating comparable membrane potentials in both types of mitochondria. After the first addition of 10 μM Ca2+ and subsequent mitochondrial Ca2+ uptake, WT brain mitochondria released 42 ± 15% of their total safranine (Fig. 3, A and C). The corresponding fluorescence increase visualized a Ca2+-dependent decrease of ΔΨ. The following Ca2+ additions (each 10 μM Ca2+) induced a further but stepwisely reduced depolarization. Finally FCCP (70 nM) was applied to abolish the residual electrochemical proton gradient of the inner mitochondrial membrane and thus to abrogate ΔΨ.

In contrast to WT mitochondria, htt51Q mitochondria released 72 ± 14% of their total safranine already after the first Ca2+ addition (Fig. 3, B and C). Similar results were obtained with pyruvate/malate or succinate as complex I- and complex II-specific substrates, respectively (not shown). These findings revealed a significantly higher sensitivity of ΔΨ of htt51Q mitochondria against destabilizing effects of extramitochondrial Ca2+ than WT mitochondria.

Impaired Ca2+ Accumulation of htt51Q Mitochondria—Because the mitochondrial Ca2+ uniporter is driven by ΔΨ of the inner mitochondrial membrane, the htt51Q-promoted Ca2+ sensitivity of ΔΨ may have an impact on the kinetics of mitochondrial Ca2+ uptake and accumulation. To prove this assumption, isolated brain mitochondria were kept in medium A containing the fluorescence indicator Calcium Green to monitor extramitochondrial Ca2+ and either 10 mM glutamate and 2 mM malate, 10 mM pyruvate and 2 mM malate, or 10 mM succinate and 2 mM rotenone (Figs. 4 and 5). Mitochondrial Ca2+ accumulation rates were calculated as the first derivative of fluorescence intensity versus time. Ca2+ added with the first addition (10 μM) was taken up by htt51Q mitochondria to a large extent of their total safranine to a similar extent, indicating comparable membrane potentials in both types of mitochondria. After the first addition of 10 μM Ca2+ and subsequent mitochondrial Ca2+ uptake, WT brain mitochondria released 42 ± 15% of their total safranine (Fig. 3, A and C). The corresponding fluorescence increase visualized a Ca2+-dependent decrease of ΔΨ. The following Ca2+ additions (each 10 μM Ca2+) induced a further but stepwisely reduced depolarization. Finally FCCP (70 nM) was applied to abolish the residual electrochemical proton gradient of the inner mitochondrial membrane and thus to abrogate ΔΨ.

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FIGURE 5. CsA counteracts the impaired Ca\(^{2+}\) accumulation of htt\(_{110}\) brain mitochondria in a substrate-independent manner. htt\(_{110}\) (A, B, E, F, G, and H) and WT (C and D) mitochondria (0.25 mg of mitochondrial protein/ml) were suspended in medium B including 10 \(\mu\)M ADP with 10 mM glutamate and 2 mM malate (A–D), 10 mM pyruvate and 2 mM malate (E and F), or 10 mM succinate and 1.5 \(\mu\)M rotenone (G). Measurements were performed in parallel without (A, C, E, and G) or in the presence of 2 \(\mu\)M CsA (B, D, F, and H). Calcium Green was used to monitor extramitochondrial Ca\(^{2+}\) levels in response to sequential Ca\(^{2+}\) additions (10 \(\mu\)M each). Calcium Green fluorescence (upper recordings) in arbitrary units (A.U.) and its first derivative, corresponding to the rate of mitochondrial Ca\(^{2+}\) uptake (lower recordings), are presented. H, htt\(_{110}\)-dependent Ca\(^{2+}\) accumulation indices (see Fig. 3 for explanation) in the presence of 2 \(\mu\)M CsA were normalized against the corresponding values obtained without CsA using the substrates glutamate/malate (GM; \(n = 5\)), pyruvate/malate (PM; \(n = 7\)), and succinate/rotenone (SR; \(n = 4\)). *, \(p < 0.05\) compared with CsA-free conditions.

extent. As seen in Fig. 4A, this process was reflected by a fast fluorescence increase due to the binding of added Ca\(^{2+}\) to extramitochondrial Calcium Green followed by a slower fluorescence decrease almost to levels seen before Ca\(^{2+}\) addition. The latter fluorescence decrease displayed mitochondrial Ca\(^{2+}\) accumulation. In contrast, the second Ca\(^{2+}\) addition (10 \(\mu\)M) was only partially accumulated by htt\(_{110}\) mitochondria (25% instead of 100%). Already after the third Ca\(^{2+}\) addition, htt\(_{110}\) mitochondria started to release Ca\(^{2+}\) into the medium possibly via PT pore opening. This scenario remained unchanged if pyruvate/malate (Fig. 5E) or succinate/rotenone (Fig. 5G) instead of glutamate/malate were used. However, when htt\(_{110}\) mitochondria were compared with WT mitochondria, two major differences became evident. (i) In response to sequential Ca\(^{2+}\) additions (each 10 \(\mu\)M), Ca\(^{2+}\) was completely accumulated by WT mitochondria until the fifth addition (Fig. 4B), meaning that WT mitochondria are characterized by a significantly higher Ca\(^{2+}\) accumulation capacity than htt\(_{110}\) mitochondria. (ii) The velocity of mitochondrial Ca\(^{2+}\) uptake, illustrated by the slope of the decline of repetitive Calcium Green spikes (Fig. 4, A–D, lower traces) was significantly higher in WT than in htt\(_{110}\) mitochondria.

A further significant alteration was identified by comparing mitochondria from brain and striatum (Fig. 4). Striatal WT mitochondria revealed about a 3 times lower Ca\(^{2+}\) accumulation rate than those from the rest of the brain (Fig. 4, compare B and D, lower traces). On the other hand, Ca\(^{2+}\) accumulation rates were similarly reduced in htt\(_{110}\) mitochondria regardless of whether they were derived from brain or striatum (Fig. 4, compare A and C, lower traces). We also analyzed mitochondrial Ca\(^{2+}\) accumulation indices that were calculated as the product of Ca\(^{2+}\) accumulation rate and corresponding completeness of mitochondrial Ca\(^{2+}\) uptake. The completeness of mitochondrial Ca\(^{2+}\) uptake was defined as the ratio of remaining Calcium Green signal after mitochondrial Ca\(^{2+}\) uptake (Fig. 4A, b)/maximum Calcium Green signal after the previous Ca\(^{2+}\) addition (Fig. 4A, a). As illustrated in Fig. 4E, Ca\(^{2+}\) accumulation indices were significantly different between htt\(_{110}\) and WT mitochondria. Furthermore Ca\(^{2+}\) thresholds linked to PT pore opening and mitochondrial Ca\(^{2+}\) efflux (Fig. 4, A–D) were markedly decreased in htt\(_{110}\) mitochondria compared with WT (Fig. 4F). Reduced Ca\(^{2+}\) thresholds and Ca\(^{2+}\) accumulation rates of htt\(_{110}\) mitochondria were also observed with pyruvate/malate (Fig. 5, E and F) and with succinate/rotenone (Fig. 5G).

CsA Normalizes the Impaired Ca\(^{2+}\) Accumulation of htt\(_{110}\) Brain Mitochondria In Vitro—If Ca\(^{2+}\)-induced functional impairments of htt\(_{110}\) mitochondria (Fig. 4) were related to the opening of the PT pore, CsA should diminish this effect. To verify this, the influence of CsA (2 \(\mu\)M) on the kinetics of mitochondrial Ca\(^{2+}\) accumulation was monitored with Calcium Green (Fig. 5). The inhibiting effect of CsA on the PT in isolated brain mitochondria is known to depend on the composition of the incubation medium (35–37), i.e., for maximum effects of CsA, the presence of ADP and Mg\(^{2+}\) is required. Thus, CsA effects on htt\(_{110}\) mitochondria were studied in Mg\(^{2+}\) and ADP-containing medium B. Again htt\(_{110}\) mitochondria showed their typical features as a low Ca\(^{2+}\) uptake rate, a reduced capacity of Ca\(^{2+}\) accumulation, and an early progression to PT (Fig. 5, A, E, and G). However, all these changes were diminished by 2 \(\mu\)M CsA regardless of whether glutamate/malate, pyruvate/malate, or succinate/rotenone were consumed (Fig. 5, A, B, E, F, G, and H). Consequently Ca\(^{2+}\) accumulation curves became like those of WT mitochondria (Fig. 5C). Furthermore CsA caused a significant increase in the Ca\(^{2+}\) accumulation rates of htt\(_{110}\) mitochondria (not shown). As illustrated in Fig. 5H, CsA increased the Ca\(^{2+}\) accumulation indices (see definition above) following the first three Ca\(^{2+}\) additions to htt\(_{110}\) mitochondria by about 96–158%. In line with this finding, there was a only a low CsA effect (18 ± 12%) on Ca\(^{2+}\) accumulation rates of WT mitochondria.
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exposed to glutamate/malate (Fig. 5, C and D), pyruvate/malate, or succinate rotenone (not shown).

DISCUSSION

Activation of Oxidative Phosphorylation by Extramitochondrial Ca\(^{2+}\): a Novel Mechanism of Regulation—For the first time, this study reveals that state 3\(_{\text{glu/mal}}\) respiration of brain mitochondria is strongly and exclusively activated by extramitochondrial Ca\(^{2+}\) in the nanomolar to low micromolar concentration range. This finding might reflect an important mechanism for the regulation of oxidative phosphorylation by Ca\(^{2+}\). To explain this type of activation, the presence of specific regulatory Ca\(^{2+}\) binding sites as metabolic sensors of extramitochondrial Ca\(^{2+}\) in the outer compartment of mitochondria must be considered. Aralar, the cerebral isoform of the Ca\(^{2+}\)-dependent aspartate/glutamate carrier, serves as an important component of the mitochondrial malate/aspartate shuttle. As recently reported, aralar contains a Ca\(^{2+}\)-binding site that is localized in the intermembrane space and regulates the activity of the aspartate/glutamate carrier in a Ca\(^{2+}\)-dependent manner (18, 19). Two basic findings of our study support an involvement of aralar in the regulation of oxidative phosphorylation by extramitochondrial Ca\(^{2+}\). (i) This carrier is activated by low Ca\(^{2+}\) concentrations (S\(_{0.5} = 0.3 \mu M\) Ca\(^{2+}\)_free\(\rightleftharpoons\) (18). Such Ca\(^{2+}\) levels are very close to those required for the Ca\(^{2+}\) activation of state 3\(_{\text{glu/mal}}\) identified under our experimental conditions. (ii) Ca\(^{2+}\)-stimulated state 3\(_{\text{glu/mal}}\) respiration was suppressed by AOA, an inhibitor of the mitochondrial aspartate aminotransferase, which metabolizes glutamate after its transport into the mitochondrial matrix via aralar. Consistently Ca\(^{2+}\)-stimulated state 3\(_{\text{glu/mal}}\) respiration was not affected by RR, an inhibitor of mitochondrial uptake by the Ca\(^{2+}\) uniporter.

In line with our results, Pardo et al. (18) have demonstrated an activation of the malate/aspartate shuttle by Ca\(^{2+}\) via an increased transport of reducing hydrogen into brain mitochondria. Furthermore Palmieri et al. (19) could detect a Ca\(^{2+}\)-activation of the glutamate/aspartate carrier because of increased rates of mitochondrial glutamate decarboxylation in the human cell line HEK-293T. An example for the importance of aralar has been also shown in patients with Mohr-Tranebjaerg syndrome, which is characterized by a decreased NADH shuttle activity due to mutations in the TIMM8a protein complex. TIMM8a is responsible for the import of the aspartate/glutamate carrier protein into mitochondria (38).

In contrast to the Ca\(^{2+}\)-dependent activation of state 3\(_{\text{glu/mal}}\) and state 3\(_{\text{pyr/mal}}\) respiration by nanomolar and low micromolar Ca\(^{2+}\)_free levels, mitochondrial respiration was rather inhibited if Ca\(^{2+}\)_free was increased to \(>2.0 \mu M\) (Fig. 1). Because of its pronounced sensitivity to RR, this Ca\(^{2+}\)-dependent inhibition of state 3 respiration was most likely caused by the intramitochondrial accumulation of Ca\(^{2+}\) following its uptake via the Ca\(^{2+}\) uniporter. Because mitochondrial inhibition was also attenuated by CsA, it became evident that PT pore opening, induced by intramitochondrial Ca\(^{2+}\) accumulation, must be involved in the suppression of respiration.

Association of HD with Impaired Regulation of Oxidative Phosphorylation by Extramitochondrial Ca\(^{2+}\)—This study identified a deficient oxidative phosphorylation in the brain of htt\(_{51Q}\) rats (Fig. 2 and Table 1). Compared with WT, mitochondria of htt\(_{51Q}\) donors were characterized by a 30–34% lower state 3 respiration prior to extramitochondrial Ca\(^{2+}\) additions regardless of whether complex I- or complex II-dependent substrates were used (Table 1 and Fig. 2, E, G, and H). In general, htt\(_{51Q}\) mitochondria were less prone to activation but more susceptible to inhibition by Ca\(^{2+}\) and unable to keep ΔΨ stable during the Ca\(^{2+}\) influx. The state 3\(_{\text{glu/mal}}\) of WT mitochondria was stable against elevations of Ca\(^{2+}\)_free concentrations up to 2 \(\mu M\), whereas the htt\(_{51Q}\) mitochondria were stable up to 0.9 \(\mu M\) Ca\(^{2+}\)_free only. In contrast, the maximum state 3\(_{\text{pyr/mal}}\) at 0.9 \(\mu M\) Ca\(^{2+}\)_free remained unaffected by htt\(_{51Q}\). This finding reflects a stronger effect of extramitochondrial Ca\(^{2+}\) on the glutamate/malate respiration than on pyruvate/malate respiration of htt\(_{51Q}\) mitochondria.

Because the uncoupled respiration (induced by FCCP) was similar to the state 3 respiration of htt\(_{51Q}\) and WT mitochondria, a defect of ADP phosphorylation itself can be excluded to underlie mitochondrial effects of htt\(_{51Q}\) (39). Thus, not the system utilizing the proton motive force but rather the machinery generating this force must be deficient in htt\(_{51Q}\) mitochondria. This kind of insufficiency was clearly substrate-specific because the complex I-dependent respiration of glutamate/malate or pyruvate/malate was more affected than the complex II-specific consumption of succinate (Fig. 2 and Table 1). This finding fits well with our recent study revealing a decreased state 3/1 ratio of skeletal muscle mitochondria from HD R6/2 mice compared with WT animals (8).

The larger relative deficiency of HD mitochondria utilizing complex I-dependent substrates (Fig. 2, E–H) could be caused by the following mechanism. At low Ca\(^{2+}\)_free activation of state 3 respiration by extramitochondrial Ca\(^{2+}\) might be hindered because of interactions of htt\(_{51Q}\) with regulatory Ca\(^{2+}\)-binding sites of mitochondrial substrate carriers such as aralar. At high Ca\(^{2+}\)_free levels and intramitochondrial Ca\(^{2+}\) accumulation, inhibition of complex I-dependent respiration may be at least partially caused by the release of NADH and NAD\(^{+}\) from the matrix space due to PT pore opening. The latter effect was seen by an increase of respiration after NADH addition to brain mitochondria exposed to inhibiting Ca\(^{2+}\) levels (not shown). A similar effect of NADH was demonstrated recently in HD mitochondria from striatal neurons (10). In contrast, under the same conditions of high Ca\(^{2+}\)_free, the succinate respiration was not dramatically affected likely because the coenzyme FAD is covalently bound to the succinate dehydrogenase and therefore cannot leave the mitochondrial matrix during PT. Direct measurements of PT of htt\(_{51Q}\) mitochondria with Calcium Green revealed significantly reduced Ca\(^{2+}\) thresholds and decreased Ca\(^{2+}\) accumulation rates for complex I- and complex II-dependent substrates (Figs. 4 and 5). Previously we identified decreased Ca\(^{2+}\) thresholds for PT in skeletal muscle mitochondria of htt\(_{40Q}\) R6/2 mice with both succinate/rotenone and pyruvate/malate as substrates (8). These data implicate an impaired regulation of mitochondrial PT possibly because of an interaction of htt\(_{51Q}\) with the PT pore.

HD-specific impairments of the Ca\(^{2+}\)-dependent regulation of mitochondrial function are in line with several previous reports (7–11, 14). For example, instability of ΔΨ and lower
Ca\textsuperscript{2+} accumulation capacities of HD mitochondria have been identified in lymphocytes of HD patients (7); in brain, liver, and muscle of transgenic mice (7–9); and in spiny striatal neurons containing htt\textsubscript{exp}, (10). Swelling of liver mitochondria of htt\textsubscript{111Q} knock-in mice was shown to be more sensitive to Ca\textsuperscript{2+} additions compared with WT mitochondria (9). However, consensus in this issue is lacking because other investigators have either not observed an increased Ca\textsuperscript{2+} sensitivity of PT (12) or observed an enlarged Ca\textsuperscript{2+} loading capacity (13) of mitochondria in HD mice models. Most probably, methodological differences underlie these conflicting results. For instance, studies by Brustovetsky et al. (12) and Oliveira et al. (13) were performed using non-synaptosomal mitochondria isolated and assessed in the presence of BSA. Notably Panov et al. (40) found that the defective Ca\textsuperscript{2+} handling of brain mitochondria can only be observed consistently if these organelles are isolated in the absence of BSA. Presumably BSA displaces htt\textsubscript{expQ} from its binding places in mitochondria. Therefore, we generally avoided the exposure of mitochondria to BSA during isolation and experiments.

A further difference compared with previous studies concerns the isolation of brain mitochondria. Brustovetsky et al. (12) and Oliveira et al. (13) used mitochondria purified with a Percoll gradient that systematically excludes synaptosomal mitochondria. In contrast, we utilized non-synaptosomal and synaptosomal mitochondria according to the method described by Kunz and coworkers (25). This aspect is important because it considers the greater susceptibility of synaptosomal mitochondria to PT because of their higher content of cyclophilin D compared with non-synaptosomal mitochondria (41). Thus, isolated non-synaptosomal HD mitochondria could be more resistant to Ca\textsuperscript{2+} challenges than a more physiological mixture of both fractions.

Up to now, the nature of htt\textsubscript{expQ}-caused cytotoxicity is unclear. Because binding of htt and htt\textsubscript{expQ} at the outer mitochondrial membrane has been detected (7, 9, 11, 42), specific interactions with mitochondrial target proteins should be taken into account. Aralar may represent one of the target candidates that is affected by htt\textsubscript{51Q} via its regulatory Ca\textsuperscript{2+} binding site. However, other proteins exposing their regulatory binding sites to extramitochondrial Ca\textsuperscript{2+} probably also play a role in htt\textsubscript{51Q}-induced dysregulation of mitochondria. For example, Bernardi et al. (43) have described an external Ca\textsuperscript{2+} binding site of the PT pore. Based on this, it has been postulated that cytosolic Ca\textsuperscript{2+} decreases the opening probability of the pore by interfering with this site (43). In contrast, Kowaltowski and Castilho (44) postulate a stimulation of PT by extramitochondrial Ca\textsuperscript{2+}. Our finding that the inhibition of mitochondrial Ca\textsuperscript{2+} uptake by RR diminished the inhibition of state 3 respiration by Ca\textsuperscript{2+} levels above 2.0 \textmu M largely in WT but only to a minor extent in htt\textsubscript{51Q} mitochondria (Fig. 2 and Table 1) supports the hypothesis of Bernardi et al. (43). Indeed we provide the first evidence for an interaction of htt\textsubscript{51Q} with a regulatory Ca\textsuperscript{2+} binding site of the PT pore by showing that htt\textsubscript{51Q} effects are opposite on aralar and PT. Aralar is insufficiently activated followed by a limited mitochondrial substrate supply and thus a decreased activation of respiration by extramitochondrial Ca\textsuperscript{2+} concentrations \leq 2 \textmu M. In contrast, htt\textsubscript{51Q} sensitizes the PT pore to extramitochondrial Ca\textsuperscript{2+} levels > 1 \textmu M, leading to pore opening and decreased respiration rates even in the presence of RR. It is therefore conceivable that htt\textsubscript{51Q} interacts with the Ca\textsuperscript{2+} binding site of the PT pore, thereby blocking a protective effect of extramitochondrial Ca\textsuperscript{2+} against PT. This effect could explain the increased susceptibility of htt\textsubscript{51Q} mitochondria to PT and their compromised Ca\textsuperscript{2+} retention capacity.

Besides aralar and the PT pore, further potential target proteins of htt\textsubscript{expQ} deserve attention, e.g. a subfamily of Ca\textsuperscript{2+}-binding mitochondrial carriers (45–47). These carriers are isoenzymes of the ATP-Mg/P\textsubscript{i} transporter responsible for the net influx or efflux of adenine nucleotides into or from mitochondria (48). Their Ca\textsuperscript{2+} binding moieties in the N terminus may serve as sensors of cytosolic Ca\textsuperscript{2+}. Notably the mitochondrial Ca\textsuperscript{2+} uniporter itself provides a regulatory Ca\textsuperscript{2+} binding site at the outer side of mitochondrial inner membrane (15, 49). Interactions of htt\textsubscript{expQ} with this site could decrease the Ca\textsuperscript{2+} accumulation of HD mitochondria. Finally the porin pore of the mitochondrial outer membrane, termed the voltage-dependent anion channel (VDAC), may also be regulated by cytosolic Ca\textsuperscript{2+}. (50). Htt\textsubscript{51Q}-dependent changes of mitochondrial regulation may uncover important pathophysiological consequences. For instance, decreased Ca\textsuperscript{2+} thresholds of htt\textsubscript{expQ} mitochondria for undergoing PT might be responsible for accelerated mitochondrial death, in particular under conditions of elevated cytosolic Ca\textsuperscript{2+} concentrations seen in HD (6). Moreover impairments of HD mitochondria may limit the cellular function also at physiological Ca\textsuperscript{2+} levels.

The protective CsA effects on the kinetics of Ca\textsuperscript{2+} accumulation might contribute to a normalization of the Ca\textsuperscript{2+} homeostasis in affected HD tissues. These assumption fits with recent studies showing that CsA is able to increase and normalize the mitochondrial Ca\textsuperscript{2+} uptake threshold of HD mitochondria from clonal striatal cells (10).

As a novel finding, we demonstrated that protective CsA effects occur not only under conditions of Ca\textsuperscript{2+} overload but also within physiological concentration ranges of extramitochondrial Ca\textsuperscript{2+} in htt\textsubscript{51Q} mitochondria. This may have important, disease-specific consequences in vivo when an altered Ca\textsuperscript{2+} homeostasis affects the energy metabolism and vice versa.

It is widely accepted that HD attacks preferably the striatum, whereas other brain regions are less affected. We have addressed this issue by analyzing the function of mitochondria from striatum and the remaining brain regions within most of the protocols used. We found that the mechanisms by which htt\textsubscript{51Q} modifies mitochondrial function might not differ between these two sources of mitochondria.

In summary, our data provide several lines of evidence for htt\textsubscript{51Q}-induced pathomechanisms underlying the initiation and progression of HD. Ca\textsuperscript{2+}-dependent impairments of mitochondrial oxidative phosphorylation due to a limited substrate supply and/or altered PT are suggested as major causes for the increased vulnerability of HD mitochondria against Ca\textsuperscript{2+} stress. The protective effect of CsA may provide a basis for the development of new therapeutic modalities.
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Acknowledgments—We thank Doreen Jercebeck, Veronica Wöllner, and Ellen Fröhlich for technical assistance. We thank Prof. Dieter Brdiczka for helpful comments on the manuscript and Dr. Ulrike Bandemer-Greulich for statistical calculations.

REFERENCES

1. Ambrose, C. M., Duyao, M. P., Barnes, G., Bates, G. P., Lin, C. S., Srinidhi, J., Baxendale, S., Hummerich, H., Lehrach, H., and Altherr, M. (1994) 

2. Li, S. H. L., Schilling, G., Young, W. S., III, Li, X. J., Margolis, R. L., Stine, O. C., Wagster, M. V., Abbott, M. H., Franz, M. L., Ranen, N. G., Folstein, S. E., Hedeen, J. C., and Ross, C. A. (1993) Neuron 11, 985–993

3. Langbehn, D. R., Brinkman, R. R., Falush, D., Paulsen, J. S., and Hayden, M. R. (2004) Clin. Genet. 65, 276–277

4. Harjes, P., and Wanker, E. E. (2003) Trends Biochem. Sci. 28, 425–433

5. Browne, S. E., and Beal, M. F. (2005)

6. Seppet, E., Gizatullina, Z., Trumbeckaite, S., Zierz, S., Striggow, F., and Gellerich, F. N. (2003)

7. Gizatullina, Z., Lindenberg, K. S., Harjes, P., Chen, Y., Kosinski, C. M., Landwehrmeyer, B. G., Ludolph, A. C., Striggow, F., Zierz, S., and Gellerich, F. N. (2004) Ann. Neurol. 59, 407–411

8. Gizatullina, Z. Z., Lindenberg, K. S., Harjes, P., Chen, Y., Kosinski, C. M., Landwehrmeyer, B. G., Ludolph, A. C., Striggow, F., Zierz, S., and Gellerich, F. N. (2004)

9. Choo, Y. S., Johnson, G. V., MacDonald, M., Detloff, P. J., and Lesort, M. (2004) Hum. Mol. Genet. 13, 1407–1420

10. Milakovic, T., Quintanilla, R. A., and Johnson, G. V. (2006) J. Biol. Chem. 281, 34785–34795

11. Rockabrand, E., Slepkova, J., Pantalone, A., Nukala, V. N., Kazantsev, A., Marsh, J. L., Sullivan, P. G., Steffan, J. S., Sensi, S. L., and Thompson, L. M. (2007) Hum. Mol. Genet. 16, 61–77

12. Brustovetsky, N., LaFrance, R., Purl, K. J., Brustovetsky, T., Keene, C. D., Vieira-Saecker, A. M., Paul, M., Jones, L., Lindenberg, K. S., Landwehrmeyer, B., Bauer, I., Vaarmann, A, Rathke-Hartlieb, S., Schulz, J. B., Grasshoff, U., Bauer, I., Freisinger, P., and Sperl, W. (2007) Arch. Biochem. Biophys. 462, 44–52

13. Novgorodov, S. A., Gudz, T. I., Brierley, G. P., and Pfeiffer, D. R. (1994) Arch. Biochem. Biophys. 311, 219–228

14. Roesch, K., Hynds, P. J., Varga, R., Tranenjaerg, L., and Koehler, C. M. (2004) Hum. Mol. Genet. 13, 2101–2111

15. Mayr, J. A., Merkel, O., Kohlwein, S. D., Gebhardt, B. R., Bohles, H., Fotschl, U., Koch, J., Jaksch, M., Lochmuller, H., Horvath, R., Freisinger, P., and Sperl, W. (2007) J. Biol. Chem. 282, 478–484

16. Panov, A. V., Andreeva, L., and Gellerich, F. N. (2004) J. Neurochem. 93, 1361–1370

17. Oliveira, J. M., Jekabsons, M. B., Chen, S., Lin, A., Rego, A. C., Goncalves, J., Ellerby, L. M., and Nichols, D. G. (2007) J. Neurochem. 101, 241–249

18. Kosinski, C. M., Schlangen, C., Gellerich, F. N., Gizatullina, Z., Deschauer, M., Schiefer, J., Young, A. B., Landwehrmeyer, G. B., Toyka, K. V., Selhausen, B., and Lindenberg, K. S. (2007) Mov. Disord. 22, 637–640

19. Litsky, M. L., and Pfeiffer, D. R. (1997) Biochemistry 36, 7071–7080

20. Hansford, R. G., and Zorzio, D. (1998) Mol. Cell. Biochem. 184, 359–369

21. Bernardi, P. (1992) J. Biol. Chem. 267, 8834–8839

22. Pardo, B., Contreras, L., Ferrero, A., and Sperl, W. (2007) Cell Calcium 42, 4127–4135

23. Ng, K. K., Sullivan, P. G., and Geddes, J. W. (2007) J. Neurosci. 27, 7469–7475

24. Petrasch-Parwez, E., Nguyen, H. P., Lobbecke-Schumacher, M., Habbes, H. W., Wieczorek, S., Riess, O., Andres, K. H., Dermietzel, R., and von Horsten, S. (2007) J. Comp. Neurol. 501, 716–730

25. Bernardi, P., Veronese, P., and Petronilli, V. (1993) J. Biol. Chem. 268, 1005–1010

26. Kowaltowski, A. J., and Castilho, R. F. (1997) Biochem. Biophys. Res. Commun. 232, 17347–17358