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INFLUENCE OF MITOCHONDRIAL ENZYME DEFICIENCY ON ADULT NEUROGENESIS IN MOUSE MODELS OF NEURODEGENERATIVE DISEASES

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Abstract

Mitochondrial defects including reduction of a key mitochondrial tricarboxylic acid cycle enzyme α -ketoglutarate–dehydrogenase complex (KGDHC) are characteristic of many neurodegenerative diseases. KGDHC consists of α -ketoglutarate dehydrogenase, dihydrolipoyl succinyltransferase (*E2k*), and dihydrolipoamide dehydrogenase (*Dld*) subunits. We investigated whether *Dld* or *E2k* deficiency influences adult brain neurogenesis using immunohistochemistry for the immature neuron markers, doublecortin (Dcx) and polysialic acid–neural cell adhesion molecule, as well as a marker for proliferation, proliferating cell nuclear antigen (PCNA). Both *Dld*- and *E2k*-deficient mice showed reduced Dcx-positive neuroblasts in the subgranular zone (SGZ) of the hippocampal dentate gyrus compared with wild-type mice. In the *E2k* knockout mice, increased immunoreactivity for the lipid peroxidation marker, malondialdehyde occurred in the SGZ. These alterations did not occur in the subventricular zone (SVZ). PCNA staining revealed decreased proliferation in the SGZ of *E2k*-deficient mice. In a transgenic mouse model of Alzheimer's disease, Dcx-positive cells in the SGZ were also reduced compared with wild type, but *Dld* deficiency did not exacerbate the reduction. In the malonate lesion model of Huntington's disease, *Dld* deficiency did not alter the lesion-induced increase and migration of Dcx-positive cells from the SVZ into the ipsilateral striatum. Thus, the KGDHC subunit deficiencies associated with elevated lipid peroxidation selectively reduced the number of neuroblasts and proliferating cells in the hippocampal neurogenic zone. However, these mitochondrial defects neither exacerbated certain pathological conditions, such as amyloid precursor protein (APP) mutation-induced reduction of SGZ neuroblasts, nor inhibited malonate-induced migration of SVZ neuroblasts. Our findings support the view that mitochondrial dysfunction can influence the number of neural progenitor cells in the hippocampus of adult mice.

Keywords

mitochondrial enzyme; neurogenesis; neuroblasts; doublecortin; polysialic acid–neural cell adhesion molecule; lipid peroxidation; subgranular zone

Reduction of the mitochondrial enzyme α -ketoglutarate–dehydrogenase complex (KGDHC) is implicated in the pathogenesis of many neurodegenerative diseases including Alzheimer's and Huntington's disease (Gibson et al., 1988; Klivenyi et al., 2004). Mammalian KGDHC is composed of α -ketoglutarate dehydrogenase (*E1k* subunit, EC1.2.4.2), dihydrolipoyl succinyltransferase (*E2k* subunit, EC 2.3.1.61) and dihydrolipoylamide dehydrogenase (*Dld*) (encoded by *Dld* gene; *E3* subunit, EC 1.8.1.4). We have previously reported that in models of Huntington's and Parkinson's disease, *Dld*-deficient mice show increased vulnerability to the mitochondrial toxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), malonate and 3-nitropropionic acid (Klivenyi et al., 2004). These studies support the idea that mitochondrial defects may contribute to the pathogenesis of neurodegenerative diseases. The impact of mitochondrial KGDHC defects on the generation of neuronal precursors (neurogenesis) has not been explored. In the current study, we tested whether a deficiency of *Dld* or *E2k* influences adult neurogenesis in mice including mouse models of Alzheimer's and Huntington's disease.

In normal adult mammals, persistent neurogenesis occurs in the subgranular zone (SGZ) of the hippocampal dentate gyrus as well as the subventricular zone (SVZ) that lines the lateral ventricles in the forebrain. Impairment of neurogenesis has been associated with increased oxidative stress. For example, a previous study documented that oxidative stress reduces the number of hippocampal neural precursor cells both *in vitro* and *in vivo*, and that this reduction could be reversed with the antioxidant α -lipoic acid (Limoli et al., 2004). Oxidative stress can also inhibit the proliferative potential of neural precursor cells derived from rat hippocampus (Limoli et al., 2006). We previously reported that *Dld*-deficient mice have significantly increased levels of the lipid peroxidation marker malondialdehyde (MDA) in the striatum (Klivenyi et al., 2004) raising the possibility that neurogenesis may be altered in these mice.

The current investigation focuses on the impact of KGDHC subunit deficiencies on the numbers of neural progenitor cells in neurogenic zones of adult mouse brains in health and in disease models. The influence of KGDHC subunit deficiencies on differentiation of progenitor cells and integration of newborn neurons is beyond the scope of the present study. The term neurogenesis used in this report pertains mainly to the number of immature neurons in the neurogenic zones of adult mouse brain.

EXPERIMENTAL PROCEDURES

Animals

Heterozygous *Dld* knockout mice (*Dld*^{+/-}; C57BL/6) have been developed and characterized (Johnson et al., 1997; obtained from Dr. Mulchand S. Patel from the State University of Buffalo, Buffalo, NY, USA). Deficient in murine *Dld*, these mice have reduced brain KGDHC activity compared with controls. Mice deficient in the *E2k* subunit (*E2k*^{+/-}; C57BL/6 and 129SV/EV hybrid) were obtained from Lexicon Pharmaceuticals (The Woodlands, TX, USA). *E2k*^{+/-} (*n*=10; five male, five female) and *Dld*^{+/-} (*n*=6; three male, three female) mice and their respective wild-type littermate controls (*n*=10 for *E2k*; five male, five female; *n*=5 for *Dld*; three male, two female) were used in these studies. Both the *Dld*^{+/-} and *E2k*^{+/-} mice have no overt phenotype.

The Tg19959 mouse model of amyloid plaque formation similar to Alzheimer's disease was obtained from Dr. George A. Carlson, McLaughlin Research Institute, Great Falls, MT, USA. These mice express two amyloid precursor protein 695 (APP₆₉₅) mutations (KM670/671NL and V717F), under control of the PrP gene promoter. To test the impact of *Dld* deficiency on neurogenesis in Alzheimer transgenic mice, *Dld*^{+/-} mice were crossed with Tg19959 mice to generate Alzheimer transgenic mice with (*n*=5) or without (*n*=6) *Dld*.

All mice were maintained under constant temperature (70 °F), humidity (50%) and 12-h light/dark cycle with food and water available *ad libitum*. Mice were killed at 4 months as described below.

Malonate lesion

To determine the influence of KGDHC deficiency on the generation of doublecortin (Dcx) - positive immature neurons in a mouse model of Huntington's disease, we performed brain lesion experiments using the mitochondrial complex II inhibitor, malonate. The selective neuropathology in human Huntington's disease brain can be mimicked in laboratory animals by direct intrastriatal infusion of malonate. Thus, malonate lesioning in rodents has been widely used as a model of Huntington's disease (Beal et al., 1993). Four-month-old wild-type ($n=11$) and $E2k+/-$ ($n=10$) mice were anesthetized with isoflurane inhalation (1–3%). Mice received a unilateral stereotactically-guided injection of malonate (1.5 μ l in 1.0 μ l saline) into the left striatum using coordinates (anterior to bregma +0.5 mm; lateral +1.5 mm; ventral from dura –4 mm) ascertained from the mouse brain atlas of Paxinos and Franklin (2003). The needle was left in place for 2–5 min to prevent backflow of infusate up the needle track. Mice were allowed to recover, and on day 7 post-surgery, mice were killed.

Tissue preparation for histological analysis

Killing was performed under deep anesthesia with sodium pentobarbital (120 mg/kg i.p.) by transcardial perfusion with 0.9% saline, followed by 4% paraformaldehyde. The brains were removed, post-fixed in the same fixative, cryoprotected in 30% sucrose and sectioned coronally (50 μ m) using a cryostat. All animal procedures were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Cornell University. In addition, the investigators took all steps to minimize the number of animals used and their suffering in conducting these studies.

Immunohistochemistry

Dcx and polysialic acid-neural cell adhesion molecule (PSA-NCAM) served as markers of immature neurons. Dcx is a 43–53 kDa microtubule-associated protein required for normal neural migration, cortical layering and interneuron migration during development. It is widely expressed by migrating neuronal precursors of the developing CNS (Gleeson et al., 1998; Friocourt et al., 2007) but not by cells expressing antigens specific to glia or undifferentiated cells (Rao and Shetty, 2004). Although the Dcx-expressing population in the developing and adult brain contains multipotential precursors in addition to neuronal-lineage cells (Walker et al., 2007), Dcx has become a suitable and widely used immunohistological marker of migrating neuroblasts, and a means to perform relative quantitation of the level of neurogenesis under normal and pathological conditions (Couillard-Despres et al., 2005). Furthermore, the number of Dcx-positive cells directly correlates with cognitive function including spatial memory and discrimination learning in aged canine brain (Siwak-Tapp et al., 2007). PSA is a linear homopolymer of α 2-8-*N* acetylneuraminic acid whose major carrier in vertebrates is NCAM. PSA on NCAM plays a role in various forms of neural plasticity including adult neurogenesis (Bonfanti, 2006). Thus, the use of Dcx and PSA-NCAM immunostaining in studies of adult neurogenesis has become increasingly popular. For evaluating cell proliferation, immunostaining for proliferating cell nuclear antigen (PCNA) was used. Although the conventional technique for studying proliferation is by *in vivo* labeling with bromodeoxyuridine (BrdU), PCNA was used in this study avoiding the disadvantages of BrdU such as toxicity, requirement for careful and accurate control of dosage, and nonspecific labeling (Gould and Gross, 2002).

For histological demonstration of lipid peroxidation and generation of immature neurons in neurogenic zones of the brain, a modified avidin–biotin–peroxidase immunohistochemistry was used. Free-floating sections were pretreated with 3% H₂O₂ in 0.1 M sodium phosphate-buffered saline (PBS) for 30 min. The sections were incubated sequentially in (a) 1% bovine serum albumin (BSA) and 0.2% Triton X-100 in PBS for 30 min, (b) affinity-purified goat polyclonal antibody against a peptide mapping at the C-terminus of Dcx (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:200), mouse monoclonal antibody against PSA-NCAM (Chemicon, Temecula, CA, USA; 1:300), rabbit polyclonal antibody against PCNA (Santa Cruz Biotechnology; 1:1000), polyclonal rabbit anti-MDA (provided by Dr. Craig E. Thomas from Hoechst-Marion-Roussel; Hall et al., 1997; 1:1000), or polyclonal rabbit anti-glial fibrillary acidic protein (GFAP; DAKO, Carpinteria, CA, USA; 1:1000), (c) appropriate secondary antibody, either biotinylated anti-rabbit IgG, anti-goat IgG or anti-mouse IgM (Vector Laboratories, Burlingame, CA, USA) diluted at 1:200 in PBS/0.5% BSA for 18 h, and (d) avidin–biotin–peroxidase complex (Vector) with both reagents A and B diluted at 1:200 in PBS for 1 h. The immunoreaction product was visualized using 3,3'-diaminobenzidine tetrahydrochloride dihydrate (DAB, Vector).

Methodological specificity control was carried out by replacing the primary antibody with PBS/0.5% BSA. Since Dcx immunostaining was used predominantly, immunological specificity controls were carried out for Dcx antibody by incubating the tissue sections either in goat IgG or in antibody that was preincubated with the Dcx peptide (Santa Cruz Biotechnology).

Silver staining

To determine whether degeneration of immature neurons in the SGZ occurs before maturation, silver staining with the FD Neuro-silver kit (FD Neurotechnologies, Inc., Baltimore, MD, USA) was used. The principle of this staining technique is based on the findings that some components of degenerating neurons, such as lysosomes, axons, dendrites and terminals become particularly argyrophilic. As a positive control, sections from mice with 3-ni-tropropionic acid-induced striatal neurodegeneration from another study were stained simultaneously with the tissues from the current investigation.

Quantification

For quantitation of Dcx-positive immature neurons and PCNA-labeled cells, we used a stereological technique based upon unbiased principles of systematic uniformly random sampling. The optical fractionator method in the Stereo Investigator (v3.45) software program (Microbrightfield, Burlington, VT, USA) was used to obtain counts of Dcx- or PCNA-labeled cells in the SGZ. Slide labels were concealed before the analysis so the experimenter was blind to genotype until completion of the quantification. The same measure was taken for non-quantifiable analysis of MDA staining intensity. For Dcx analysis, only intensely labeled immature neurons with distinct nuclei were counted. For PCNA, only cells with robust nuclear immunoreactivity were considered positive. SGZ was defined as the region between the granule cell layer and the hilus of the dentate gyrus. All labeled cells in the SGZ were counted except those touching the granule cell layer on the border of the molecular layer. For the SGZ analysis, cell counts were made in four serial sections (250 μ m apart) per mouse, beginning rostrally from the level of bregma -1.7 mm through bregma -2.45 . The size of the x–y sampling grid was 140 μ m. The counting frame thickness was 14 μ m. In this study, the SVZ was defined as the layer of cells lining the lateral ventricle covering the entire dorsoventral distance. For the SVZ analysis, cell counts were made in four serial sections (250 μ m apart) per mouse beginning rostrally from the level of bregma 1.18 through bregma 0.43. The size of the x–y sampling grid was 140 μ m.

The counting frame thickness was 14 μm . The stereological cell counts represent the total number of cells in the SGZ or SVZ within the specified brain region analyzed.

Statistics

Data are expressed as mean $\pm$ standard error of the mean (S.E.M.). Statistical analysis of the data was performed using one-way analysis of variance followed by the Student-Newman-Keuls post test or unpaired Student's *t*-test, when appropriate, using the GraphPad Instat software (San Diego, CA, USA). A *P* value of <0.05 was considered statistically significant.

RESULTS

The specificity of Dcx immunostaining was verified by (a) performing competition experiments on adjacent brain sections from a wild-type control mouse, and (b) incubating the sections in goat IgG. While intense Dcx immunoreactivity occurred in neuroblasts of the SGZ as in published reports, incubation of adjacent sections with antibody that was preabsorbed with the Dcx peptide, or incubation with goat IgG in place of the Dcx antibody abolished the staining (data not shown).

We first sought to determine the influence of *E2k* deficiency on the number of immature neurons in the SGZ of 4 month-old mice. Dcx-immunoreactive neuroblasts occurred in the SGZ of both wild-type controls (Fig. 1A, A') and *E2k* $^{+/-}$ mice (Fig. 1B, B'). Stereological quantitation of the Dcx-positive cells revealed a striking difference in the number of immature neurons in *E2k* $^{+/-}$ mice relative to the wild-type controls (Fig. 1C). The number of Dcx-positive neuroblasts in the SGZ of *E2k* $^{+/-}$ mice (4234 ± 336) was significantly reduced compared with wild-type controls (7351 ± 588 ; $P<0.001$). Morphologically, Dcx-positive cells in the SGZ of control mice displayed complex arborization of dendrites toward the molecular layer of the dentate gyrus (Fig. 1A'). In *E2k* $^{+/-}$ mice, the Dcx-labeled cells exhibited a reduced dendritic arborization pattern (Fig. 1B').

Immunostaining using another immature neuron marker, PSA-NCAM revealed a similar pattern of staining as Dcx in the SGZ of controls (Fig. 2A, A') and *E2k* $^{+/-}$ mice (Fig. 2B, B'). For this reason, quantitative analysis and subsequent experiments were conducted using Dcx.

Dld deficiency also influenced the number of neuroblasts in the SGZ. Compared with wild-type controls (8166 ± 693 ; $P<0.01$; Fig. 3A, A', E), *Dld* $^{+/-}$ mice (Fig. 3B, B', E) showed significantly less Dcx-labeled cells (5410 ± 885). We next determined whether *Dld* deficiency influences the reduction of hippocampal neurogenesis in a mouse model of Alzheimer's disease. Previous studies have documented decreased hippocampal neurogenesis in Alzheimer transgenic mice (Donovan et al., 2006; Zhang et al., 2006). In the present study, we crossed the Tg19959 model of Alzheimer's disease with *Dld* deficient mice to generate Alzheimer mice with or without *Dld* deficiency. Consistent with other models of Alzheimer's disease, the Tg19959 mice showed a striking reduction in Dcx-immunoreactive cells in the SGZ (3274 ± 483 ; Fig. 3C, C', E) compared with wild-type controls ($P<0.001$). Interestingly, in *Dld*-deficient Tg19959 mice (Fig. 3D, D', E), the number of Dcx-labeled neuroblasts (3586 ± 419) did not differ significantly from Tg19959 mice without *Dld* deficiency ($P>0.05$). Thus, *Dld* deficiency neither exacerbated nor ameliorated the impairment of adult hippocampal neurogenesis in a mouse model of Alzheimer's disease.

To test whether *E2k* deficiency also influences proliferation in the SGZ, sections adjacent to those used for Dcx staining were processed for PCNA immunohistochemistry (wild type, Fig. 4A, A'; *E2k* $^{+/-}$, 4B, B') and stereological analysis (Fig. 4C). PCNA immunoreactivity occurred as irregular or oval shaped intensely stained nuclei (Fig. 4A, A'; 4B, B'). PCNA-

positive cells were distributed singly or clustered throughout the rostrocaudal extent of the SGZ. Quantification revealed that *E2k* deficiency significantly diminished the number of PCNA-labeled cells (1124 ± 77) compared with wild-type mice (1796 ± 128 ; $P < 0.001$).

To test whether the reduction of Dcx-labeled immature neurons in the SGZ of *E2k*-deficient mice is due to a shift of the phenotypic fate of progenitors toward the astrocytic lineage, GFAP immunostaining was performed. Fig. 5 shows that there was no significant difference between the number of GFAP-labeled astrocytes in wild-type controls (8274 ± 214 ; Fig. 5A, C) and that of *E2k*^{+/-} mice (8217 ± 386 ; $P > 0.05$; Fig. 5B, C).

We also investigated whether increased immature neuronal degeneration contributed to the reduction in the number and dendritic arborization of Dcx-positive neurons in *E2k*^{+/-} and *Dld*^{+/-} mice. Silver staining of SGZ sections from *E2k*^{+/-} (Fig. 6B), *Dld*^{+/-} (Fig. 6D) mice, and their respective controls (Fig. 6A, C) did not reveal any increased argyrophilic structures similar to those found in 3-nitropropionic acid-lesioned striatal sections (Fig. 6E) which are known to exhibit darkly stained argyrophilic neuron bodies and terminals.

Both the SGZ and SVZ were tested for *in situ* levels of lipid peroxidation. Using high performance liquid chromatography (HPLC), we previously showed that *Dld*-deficient mice have significantly increased levels of the lipid peroxidation marker, MDA in the striatum, as measured by amounts of thiobarbituric acid–MDA adducts (Klivenyi et al., 2004). In the current study, we localized oxidatively damaged cells within the hippocampal formation by immunohistochemistry using a polyclonal antibody to MDA-modified proteins. We employed this *in situ* technique to enable direct comparison of the dentate gyrus of control and *E2k*^{+/-} mice. Consistent with the HPLC results, we found elevated MDA immunoreactivity diffusely in the dentate gyrus including the SGZ of *E2k*^{+/-} (Fig. 7B) compared with wild-type controls (Fig. 7A). Intensely stained cells were also found in the SGZ layer of *E2k*^{+/-} mice (Fig. 7B inset). No MDA immunoreactivity occurred in the SVZ of either the *E2k*^{+/-} (Fig. 7D) or wild-type control (Fig. 7C) mice.

While neurogenesis in the SGZ was reduced in both the *E2k*^{+/-} and *Dld*^{+/-} mice, the generation of immature neurons in the SVZ did not appear to be affected by the mitochondrial defects in this study. Fig. 8 shows that the numbers of Dcx-positive immature neurons in the SVZ of *E2k*^{+/-} (7811 ± 460 ; Fig. 8B, C) and *Dld*^{+/-} (8429 ± 481 ; Fig. 8E, F) mice did not significantly differ from their respective wild-type controls (9103 ± 668 , wild type for *E2k*; 9114 ± 1143 , wild type for *Dld*; Fig. 8A, C, D, F; $P > 0.05$ for both groups).

Finally, we investigated the neurogenic response of the progenitor cells in the SVZ to neuron loss induced by intrastratial malonate, and tested the impact of *E2k* deficiency on this response. The close proximity of the SVZ to the adjacent striatum (caudate putamen) makes this model ideal for these experiments. Intrastratial malonate injections in rats and mice produce lesions that mimic the neuropathology of Huntington's disease (Beal et al., 1993; Greene et al., 1993). Seven days following malonate injection, a well-defined area of neuron loss occurred in the ipsilateral hemisphere of wild-type control mice (lesion volume = 1.3 ± 0.4 mm³). In the contralateral hemisphere of wild-type controls, robust Dcx immunoreactivity was confined to the SVZ lining the lateral ventricle, but virtually absent in the striatum (Fig. 9). In the ipsilateral side however, Dcx labeling occurred in the SVZ as well as in many cells scattered in the dorsomedial striatum, extending from the SVZ into the site of the lesion. Some of the malonate-generated Dcx-positive neuroblasts appeared fusiform with elongated processes while others had several processes extending in different directions.

To test whether *E2k* deficiency suppressed the malonate-induced increases in neurogenesis, we examined the lesioned striatum of *E2k*^{+/-} mice (lesion volume = 2.6 ± 0.5 mm³) in comparison with the lesioned hemisphere of wild-type controls. The pattern of Dcx staining

in the *E2k*^{+/-} mice was similar to that of controls (Fig. 9). Quantification of the immature neurons in the lesioned hemispheres outside the SVZ revealed that *E2k* deficiency did not impair the malonate-induced increases in neurogenesis. The number of Dcx-positive immature neurons in the ipsilateral striatum of wild-type mice (23.6±1 cells per section) did not differ significantly from that of *E2k*^{+/-} (23.8±1 cells per section). As in wild-type mice, malonate lesion also induced astrogliosis in *E2k*^{+/-} mice (data not shown). These studies demonstrate that malonate lesion enhanced the generation of immature neurons in the damaged striatum with or without *E2k* deficiency.

DISCUSSION

Our findings show that KGDHC subunit deficiencies selectively reduced the number of Dcx-labeled immature neurons as well as PCNA-immunoreactive proliferating cells in the hippocampal SGZ. This study is the first demonstration that mitochondrial enzyme defects can reduce the number of neural progenitor cells in adult mice.

KGDHC deficiency is associated with increased lipid peroxidation as evidenced by elevation of the lipid peroxidation marker MDA in brain of *Dld*^{+/-} mice as measured by HPLC (Klivenyi et al., 2004) and by immunohistochemistry in the *E2k*^{+/-} mice in the current study. In a rat model of chronic alcoholism, ethanol, which can damage tissues by lipid peroxidation (Montoliu et al., 1995; Mi et al., 2000), selectively impairs hippocampal neurogenesis (Herrera et al., 2003). This reduction of neurogenesis can be completely mitigated by the organo-selenium antioxidant ebselen, a drug that decreases oxidative stress through inhibition of peroxynitrite generation and lipid peroxidation (Briviba et al., 1996; Herrera et al., 2003). In the D-galactose model of neurotoxicity in mice, increased MDA levels are associated with reduced numbers and migration of new neurons in the SGZ (Zhang et al., 2005; Cui et al., 2006). Furthermore, *in vitro* studies show that lipid peroxidation is involved in the developmental impairment of neuronal progenitor cells by amyloid β 1–40 (Mazur-Kolecka et al., 2006). It is conceivable, therefore, that the reduced neurogenesis in the SGZ of *E2k*^{+/-} mice is due to the increased lipid peroxidation in the hippocampal dentate gyrus. This idea is supported by the absence of neurogenesis reduction in the SVZ of *Dld*^{+/-} or *E2k*^{+/-} where MDA was virtually undetectable by immunohistochemistry in the present study. In Alzheimer transgenic mice, the reduced neurogenesis could also be attributed in part to lipid peroxidation. A previous study showed that in the Tg2576 Alzheimer mouse model, lipid peroxidation in the hippocampus is increased compared with wild-type mice (Pratico et al., 2001). Whether the increased lipid peroxidation is a direct consequence of *Dld* or *E2k* deficiency is unclear. It is possible that the increased lipid peroxidation associated with KGDHC deficiency inhibited proliferation of neural progenitor cells, as evidenced by the reduction of PCNA-labeled cells, and also prevented the growth and maturation of neuroblasts by causing cytoskeletal alterations. Further studies are required to elucidate the exact mechanisms involved in the reduction of immature neurons in mice with KGDHC subunit deficiency. Nevertheless, the current findings are consistent with the view that mitochondrial defects and oxidative stress can inhibit adult neurogenesis.

While dendritic branches of Dcx-labeled neuroblasts extended radially toward the molecular layer of the dentate gyrus in the wild-type mice, dendritic branching was apparently reduced in the SGZ of *E2k*^{+/-} and *Dld*^{+/-} mice. Based on our silver staining results, this reduction did not result from degeneration. Argyrophilic profiles suggestive of degenerating neuroblast processes were not detected in mice lacking either *E2k* or *Dld*. Thus, the reduced arborization pattern most likely reflects inhibition of maturation of neuroblasts, possibly due to oxidative stress.

GFAP staining analysis revealed that the number of astrocytes in the SGZ was not elevated in mice lacking *E2k* compared with wild-type controls. This finding suggests that the reduction of neuroblasts in the *E2k*^{+/-} mice was not a consequence of a shift of progenitor cell differentiation toward the astrocytic lineage.

The hippocampal SGZ is a brain region that is evidently vulnerable to mitochondrial KGDHC deficiency. Our observations are consistent with the recent demonstration that thiamine deficiency simultaneously impairs hippocampal neurogenesis and induces cognitive dysfunction in mice (Zhao et al., 2008). KGDHC is a thiamine pyrophosphate-dependent enzyme of oxidative metabolism. It is well established that thiamine deficiency is characterized by reduced activity of KGDHC in brain (Gibson et al., 1984).

In several mammalian species such as rat and vole, adult hippocampal neurogenesis is modulated by both gender and endogenous levels of estradiol (Galea et al., 2006). However, a recent study documented that adult mice do not have gender differences in hippocampal proliferation or neurogenesis (Lagace et al., 2007). These results validate the use of male and female mice in adult neurogenesis studies. In the current experiments, male and female mice were pooled for each group.

Owing to the reduction of immature neurons in the *Dld*^{+/-} and AD transgenic mice, one would expect an exacerbation of the reduction in AD transgenic mice that lack *Dld*. However, the level of Dcx immunoreactivity did not differ significantly between AD transgenic mice with and without *Dld*. Compensatory phenomena most likely play a role in counteracting the mitochondrial enzyme deficit. Another possibility is that the pathological changes in *Dld* deficiency and amyloid precursor protein (APP) mutation are induced by the same factor such as lipid peroxidation, and after reaching a certain level, these changes do not go any further. These observations suggest that even in the presence of a mitochondrial KGDHC deficiency, AD transgenic mouse brains maintain a capacity for self-repair.

The results from our malonate lesion studies confirm the view that pathological stimuli can induce endogenous neurogenesis in regions such as the striatum where adult neurogenesis is non-existent under physiological conditions. Our findings agree with a previous study on rats lesioned with quinolinic acid, an excitatory amino acid agonist that has been extensively used as another animal model of Huntington's disease (Beal et al., 1986). Tattersfield and colleagues (2004) demonstrated that quinolinic acid-induced cell loss in rat striatum increases SVZ neurogenesis and migration of neuroblasts to the damaged areas. Here we present evidence that malonate lesions also led to the generation of Dcx-labeled cells migrating from the SVZ into the damaged striatum of wild-type control mice. Another possibility is that malonate enhanced neuronal precursor migration toward the lesioned area without change in new neuron generation. Surprisingly, mice with *E2k* deficiency also exhibited a similar response. It is possible that in the presence of an additional insult such as an excitotoxic lesion, the brain can overcome the compromised mitochondrial KGDHC function, and trigger compensatory mechanisms to sustain endogenous neurogenesis. This may explain why the same level of neurogenesis occurred in lesioned wild-type and *E2k*^{+/-} mice. More studies are necessary to understand the mechanism by assessing the rate of proliferation of neural progenitors and tracking their phenotypic fate into neurons or glia.

CONCLUSION

In summary, both *Dld* and *E2k* deficiencies selectively reduced the numbers of young neurons in the SGZ, but these mitochondrial defects do not exacerbate certain pathological conditions, such as APP mutation-induced reduction of SGZ neuroblasts or malonate

excitotoxicity-induced migration of SVZ neuroblasts. Our findings are consistent with the view that mitochondrial dysfunction can influence adult neurogenesis.

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Abbreviations

APP	amyloid precursor protein
BrdU	bromodeoxyuridine
BSA	bovine serum albumin
Dcx	doublecortin
Dld	dihydrolipoamide dehydrogenase
E2k	dihydrolipoyl succinyltransferase
GFAP	glial fibrillary acidic protein
HPLC	high performance liquid chromatography
KGDHC	α -ketoglutarate–dehydrogenase complex
MDA	malondialdehyde
PBS	phosphate-buffered saline
PCNA	proliferating cell nuclear antigen
PSA-NCAM	polysialic acid–neural cell adhesion molecule
SGZ	subgranular zone
SVZ	subventricular zone

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Fig. 1.

Dcx immunoreactivity in the SGZ of wild-type control (A, A') and *E2k*^{+/-} (B, B') mice showing a reduction of Dcx-labeled cells in the latter. High magnification photos depict reductions in the number and dendritic branching of Dcx-labeled neurons in the *E2k*^{+/-} mice (B') compared with wild-type control (A'). Stereological evaluation (C) revealed a significant loss of Dcx-positive cells in the SGZ of *E2k*^{+/-} mice compared with wild-type controls (*** $P < 0.001$). The stereological cell counts represent the total number of cells in the SGZ within the specified brain region included in the analysis (see Experimental Procedures).

Fig. 2. PSA-NCAM immunoreactivity in the SGZ of wild-type control (A, A') and *E2k+/-* (B' B') mice revealed a similar pattern of staining as Dcx.

Fig. 3.

Dcx immunoreactivity in the SGZ of wild-type control (A, A'), *Dld*^{+/-} (B, B'), an Alzheimer mouse model Tg19959 (C, C') and Tg19959 without *Dld* (D, D'). Stereological quantitation (E) shows a significant reduction of Dcx-positive cells in the *Dld*^{+/-} and Tg19959 mice (** $P < 0.01$, *** $P < 0.001$ vs. wild type). *Dld* deficiency did not affect the number of Dcx-positive cells in Tg19959.

Fig. 4. PCNA immunoreactivity in the SGZ of wild-type control (A, A') and *E2k*^{+/-} (B, B') mice showing a reduction of PCNA-labeled progenitor cells in the latter. High magnification photos (A', B') show labeled nuclei occurring singly or in clusters in the SGZ. Stereological evaluation (C) revealed a significant loss of PCNA-immunoreactive cells in the SGZ of *E2k*^{+/-} mice compared with wild-type controls (***) $P < 0.001$.

Fig. 5. GFAP immunoreactivity in representative sections through the dentate gyrus of wild-type control (A) and *E2k*^{+/-} (B) mice. Stereological counts of GFAP-positive astrocytes in the SGZ (C) revealed no statistically significant difference between the two groups ($P>0.05$).

Fig. 6. Silver staining of wild-type (A) and *E2k+/-* (B) mice, and wild-type (C) and *Dld+/-* (D) mice showing absence of degenerating immature neuron profiles in mice with mitochondrial enzyme defects. A positive control section through 3-nitropropionic acid-lesioned caudate putamen (E) displayed dense black silver grains in degenerating cell bodies and terminals.

Fig. 7. MDA immunoreactivity in the hippocampal dentate gyrus (A, B) and SVZ (C, D) of wild-type control (A, C) and *E2k+/-* (B, D) mice. Elevated MDA immunostaining was found in the dentate gyrus but not the SVZ of *E2k+/-* mice compared with wild-type control. Although staining was diffuse in the dentate gyrus, intense cellular localization of MDA staining occurred in the SGZ of *E2k+/-* mice (inset). CPu, caudate putamen.

Fig. 8. Dcx immunoreactivity in the SVZ of wild-type (A) and *E2k*^{+/-} (B) mice, and wild-type (D) and *Dld*^{+/-} (E) mice with high magnification photomicrographs of labeled neurons (insets). Quantification of Dcx-immunoreactive immature neurons revealed no significant reduction in either *E2k*^{+/-} (C) or *Dld*^{+/-} (F) mice.

Fig. 9.

Dcx immunoreactivity in the caudate putamen (CPu; striatum) and the SVZ lining the lateral ventricle (LV) of wild-type and *E2k*^{+/-} mice with unilateral malonate lesion. Dcx-positive cells migrating from the SVZ to the lesion site (arrows) occurred in both wild-type and *E2k*^{+/-} mice, and are shown at high magnification in the lower panel. Labeled cells are virtually absent in the intact caudate putamen. cc, Corpus callosum; MS, medial septal nucleus.