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Oxidative stress modulates Sir2α in rat hippocampus and cerebral cortex

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Abstract

Silent information regulator 2 (Sir2) helps survival and longevity in lower organisms during challenging situations. We investigated the possibility that Sir2α could be involved with brain plasticity under challenging situations. A diet high in saturated fat and sucrose, which has been shown in rodents to reduce synaptic plasticity and cognition, decreased Sir2α levels in the hippocampus and cerebral cortex, in proportion to an increase in protein oxidation. Vitamin E supplementation normalized, in the hippocampus and cerebral cortex, Sir2α levels that had been reduced by the high-fat diet. Neither the high-fat diet nor vitamin E supplementation affected cerebellar Sir2α. Vitamin E reduced, in the hippocampus, the oxidized nucleic acids that were increased by the high-fat diet. Western blot analysis showed higher contents of Sir2α in the hippocampus and cerebellum than in the cerebral cortex. Sir2α immunostaining was predominantly localized in the mossy fibre system and the dentate gyrus granule layer of the hippocampal formation. The high-fat diet decreased Sir2α immunostaining while vitamin E supplementation reversed these effects. Given that oxidative stress is a subproduct of dysfunctional energy homeostasis, we measured AMP-activated protein kinase (AMPK) to have an indication of the energy status of cells. Hippocampal levels of total and phosphorylated AMPK were reduced after high fat consumption and levels were normalized by vitamin E treatment. The present results show that oxidative stress and energy homeostasis associated with the consumption of the high-fat diet are critical for the regulation of Sir2α, with important implications for mechanisms of neural repair.

Introduction

The silent information regulator 2 (Sir2) has surfaced as an important modulator of genomic stability and cellular homeostasis. The function of Sir2 in cellular metabolic homeostasis (Lin *et al*., 2000, 2004; Anderson *et al*., 2003; Rogina & Helfand, 2004) has been shown to be significant for promoting longevity in yeasts, nematodes and flies. Seven mammalian Sir2 homologs have been identified, named SIRTs 1–7 (Cheng *et al*., 2003), in which SIRT1, also known as Sir2α, is the closest homolog of yeast Sir2 (Hisahara *et al*., 2005). Recent studies indicate that the actions of Sir2α on cellular homeostasis can be extended to the maintenance of proper brain function. Deletion of the Sir2α gene in mice causes developmental deficits in the brain (Cheng *et al*., 2003; Sakamoto *et al*., 2004). In the adult brain, Sir2α can protect neurons against challenging conditions (Araki *et al*., 2004; Bordone & Guarente, 2005; Parker *et al*., 2005; Sinclair, 2005). For example, activation of Sir2α reduces degeneration of severed axons (Araki *et al*., 2004) and suppresses neuronal death caused by toxic insults (Parker *et al*., 2005; Sinclair, 2005). Cells normally have specific mechanisms for repairing sustained DNA damage, and dysfunction in these mechanisms may increase the vulnerability of the brain to insults (Cardozo-Pelaez *et al*., 2000; Hamilton *et al*., 2001), and lead to neurological deficits (Nunomura *et al*., 1999; Liu *et al*., 2001, 2003). It has been shown that Sir2 increases the ability of cells to repair incurred DNA damage (Guarente, 2000; Kirkwood & Austad, 2000; Tran *et al*., 2002; Lombard *et al*., 2005). Everything seems to indicate that Sir2α may confer neuronal resistance against insults and help to maintain homeostatic mechanisms important for optimal brain function.

The capacity of dietary factors to affect plasticity and function of neural circuits is becoming well established, yet the molecular mechanisms involved with these effects require further elucidation. Dietary components such as a diet high in saturated fat have been shown to decrease brain function and cognitive abilities in rodents and humans (Molteni *et al*., 2004; Wu *et al*., 2004). Consumption of a diet high in saturated fat is associated with cumulative oxidative stress, which represents a major threat for normal maintenance of synaptic plasticity and cognitive function. Antioxidant dietary supplementation compensates for the deleterious effects of the high-fat diet (Molteni *et al*., 2002, 2004; Wu *et al*., 2003, 2004, 2006), portraying oxidative stress as a critical condition under which dietary factors can affect synaptic plasticity and cognitive function. Given the critical roles of Sir2α on the regulation of antioxidant gene expression (Brunet *et al*., 2004; Daitoku *et al*., 2004), here we have explored the possibility that Sir2α can preserve neuronal health against insults encountered in daily living such as those related to the consumption of poor diets.

Given the involvement of Sir2α on the regulation of cellular energy homeostasis and the critical role of this process in maintaining brain plasticity, we have evaluated the role of energy homeostasis in our paradigm. Indeed, oxidative stress is a subproduct of dysfunctional energy homeostasis. Adenosine monophosphate (AMP)-activated protein kinase (AMPK), a highly conserved serine–threonine kinase,

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acts as a critical sensor of cellular energy status in all eukaryotic cells. AMPK activation plays an important role in maintaining energy homeostasis (Gadalla *et al*., 2004; Rafaeloff-Phail *et al*., 2004; Shaw *et al*., 2004; Lee *et al*., 2005). The fact that the dysfunction of AMPK can increase vulnerability to stress (Shaw *et al*., 2004) has led us to examine this system in our study.

Materials and methods

Experimental designs and tissue preparation

Sprague–Dawley rats (Charles River Laboratories, Inc., Wilmington, MA, USA) weighing between 200 and 240 g were maintained in environmentally controlled rooms (22–24 °C) with a 12-h light–dark cycle. After acclimatization for 1 week on standard rat chow, four sets of rats were randomly assigned, one set each to a high-fat diet or regular diet, with or without 500 IU/kg vitamin E, for 2 months. The diets, fed *ad libitum*, were provided in powder (Purina Mills Inc, TestDiet Inc., Richmond, IN, USA) in a large bowl and contained a standard vitamin and mineral mix with all essential nutrients, as described previously (Molteni *et al*., 2002; Wu *et al*., 2003). Regular diet is low in saturated fat (13% of energy from fat). High-fat diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of corn oil; 39% energy). At the end of 2 months' feeding the rats were killed by decapitation; the fresh tissues including hippocampus were dissected, frozen in dry ice and stored at −70 °C until use for biochemical analyses ($n = 8$ within each group). For immunohistochemistry ($n = 4$ within each group), the rats were deeply anaesthetized (Nembutal, 75 mg/kg) and then transcardially perfused with 400 mL 4% paraformaldehyde and 100 mL 20% sucrose. The fixed brains were then removed and stored at −70 °C until use. All experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Western blot

The tissues from hippocampus, cerebral cortex (caudal) and cerebellum were homogenized in a lysis buffer containing NaCl, 137 mM; Tris–HCl pH 8.0, 20 mM; polyglycol ether (nonionic) surfactant NP40, 1%; glycerol, 10%; PMSF, 1 mM; aprotinin, 10 μg/mL; benzethonium chloride, 0.1 mM; and sodium vanadate, 0.5 mM. The homogenates were then centrifuged, the supernatants were collected and total protein concentration was determined according to the MicroBCA procedure (Pierce, Rockford, IL, USA), using bovine serum albumin as standard. Sir2α levels were analysed using Western blot. Briefly, protein samples were separated by electrophoresis on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. Nonspecific binding sites were blocked in Tris-buffered saline (TBS) overnight at 4 °C, with 2% bovine serum albumin and 0.1% Tween-20. Membranes were rinsed for 10 min in buffer (0.1% Tween-20 in TBS) and then incubated with antiactin (1 : 2000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by antigoat IgG horseradish peroxidase conjugate (Santa Cruz Biotechnology), or with anti-Sir2α (1 : 1000; Upstate, Chicago, IL, USA) followed by antirabbit IgG horseradish peroxidase conjugate (Santa Cruz Biotechnology). After rinsing with buffer, the immunocomplexes were visualized with chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using NIH Image software. Actin was used as an internal control for Western blot such that data were standardized according to actin values.

Sir2α immunohistochemistry

Serial coronal sections (25 μm) were cut on a cryostat, mounted on gelatin-coated slides and processed for immunohistochemistry, as previously described (Gomez-Pinilla *et al*., 2001). A 1 : 1000 dilution was used for the rabbit polyclonal anti-Sir2α. This antibody can recognize mammalian Sir2α, a homolog of yeast Sir2, and has been used to determine Sir2α expression in mammalian cells. For example, this antibody has been shown to specifically detect Sir2α in rat cardiac myocytes (Alcendor *et al*., 2004). Immunohistochemistry controls were performed with omission of the primary antibody. The results of immunohistochemistry controls were negative as no staining was observed in cell structures.

Detection of oxidized nucleosides

The sections were prepared as described above and incubated with monoclonal anti8-hydroxy-2′-deoxyguanosine/8-hydroxyguanosine (8OHdG/8OHG; 1 : 2000; QED Bioscience, San Diego, CA, USA) and visualized by using standard immunohistochemical methods (Gomez-Pinilla *et al*., 2001).

Measurement of oxidized proteins

The oxidized protein levels in rat hippocampus were measured by Western blot analysis of dinitrophenylhydrazine (DNPH)-derived carbonyl groups on oxidized proteins using an Oxyblot kit (Intergen, Purchase, NY, USA). Briefly, the protein sample (10 μg) was reacted with 1 × DNPH for 15 min, followed by neutralization with a solution containing glycerol and β-mercaptoethanol. These samples were electrophoresed on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. After blocking, membranes were incubated overnight with a rabbit anti-DNPH antibody (1 : 150) at 4 °C, followed by incubation in goat antirabbit (1 : 300) for 1 h at room temperature. After rinsing with buffer, the immunocomplexes were visualized with chemiluminescence using the ECL kit (Amersham) according to the manufacturer's instructions. Because of the large number of samples (32; four groups with eight animals per group), we ran separate gels with the same experimental controls included in each gel. Experimental values were standardized according to the internal control values. The entire band was integrated in each group and analysed using NIH image software.

Statistical analysis

Actin was employed as internal standard for Western blot. For Western blot, the values were expressed as a percentage of control and presented in bar graphs as mean + SEM. The data were analysed using ANOVA followed by Fisher's protected least significance *post hoc* test. Statistical differences were considered significant at $P < 0.05$.

Results

Sir2α expression

In this study, we applied a well-characterized polyclonal anti-Sir2α antibody to detect Sir2α expression in rat brain. This antibody recognizes mammalian Sir2α, a homolog of yeast Sir2, and has been used to determine Sir2α expression in mammalian cells. For example, this antibody has been shown to specifically detect Sir2α in rat cardiac myocytes (Alcendor *et al*., 2004). Further, this antibody was used

successfully to measure Sir2α proteins in mammalian cells (Daitoku *et al*., 2004). Accordingly, our Western blot also showed that this antibody recognized rat Sir2α.

Our results showed that the Sir2α level in the hippocampus of rats fed the high-fat diet was significantly lower (81% of control, $P < 0.05$; Fig. 1A) than that of rats fed regular diet. Our previous study showed that consumption of a high-fat diet increases the levels of oxidized proteins and nuclear acids in the hippocampus (Wu *et al*., 2004), suggesting the potential role of oxidative stress in mediating the effects of diet on neuronal health and function. Accordingly, in the present study the high-fat diet was supplemented with vitamin E, resulting in normal hippocampal levels of Sir2α (98% of control, $P > 0.05$; Fig. 1A). In addition, vitamin E supplementation significantly increased Sir2α levels in animals that consumed a regular diet (138% of control, $P < 0.05$; Fig. 1A).

To determine relative expression of Sir2α across brain regions, we measured levels of Sir2α in selected brain regions such as hippocampus, cerebral cortex and cerebellum. The results showed that Sir2α in the hippocampus did not differ from that in the cerebellum, but it was significantly higher than that in the cerebral cortex (100% vs. 79%, $P < 0.05$; Fig. 1D). We also performed Western blot to measure Sir2α in these regions under all experimental conditions. The results showed that Sir2α in the cerebral cortex was significantly reduced by the high-fat diet (80% of control, $P < 0.05$; Fig. 1B); this reduction was reversed by vitamin E supplementation. Vitamin E did not affect the Sir2α levels in the cerebral cortex of animals fed regular diet (Fig. 1B). Neither vitamin E nor the high-fat diet changed Sir2α levels in cerebellum (Fig. 1C).

Sir2α staining was predominantly distributed in the mossy fibre system and in the granule layer of the dentate gyrus in the hippocampal formation (Fig. 2A). Sir2α immunostaining was diffuse in other brain regions such as cerebral cortex (Fig. 2A). Rats fed a high-fat diet showed a qualitative reduction in Sir2α immunoreactivity in the hippocampus, whereas vitamin E supplementation counteracted this reduction (Fig. 2B). Vitamin E supplementation appeared to increase hippocampal Sir2α expression in rats fed regular diet (Fig. 2B).

Oxidized protein levels

Protein oxidation was assessed in the hippocampus by using Western blot analysis of DNPH-derived carbonyl groups. Representative examples of Oxyblot are shown in Fig. 3A. High-fat diet consumption increased hippocampal levels of oxidized protein (150% of control, $P < 0.01$; Fig. 3B) compared to regular diet, whereas vitamin E supplementation dramatically reduced the oxidized protein levels (72% of control, $P < 0.05$; Fig. 3B). Vitamin E supplementation also decreased oxidized protein levels in rats fed regular diet (79% of control, $P < 0.05$; Fig. 3B). In addition, the oxidized protein levels were negatively correlated with the levels of Sir2α in the hippocampus of rats fed the high-fat diet ($r = –0.88$, $P < 0.05$).

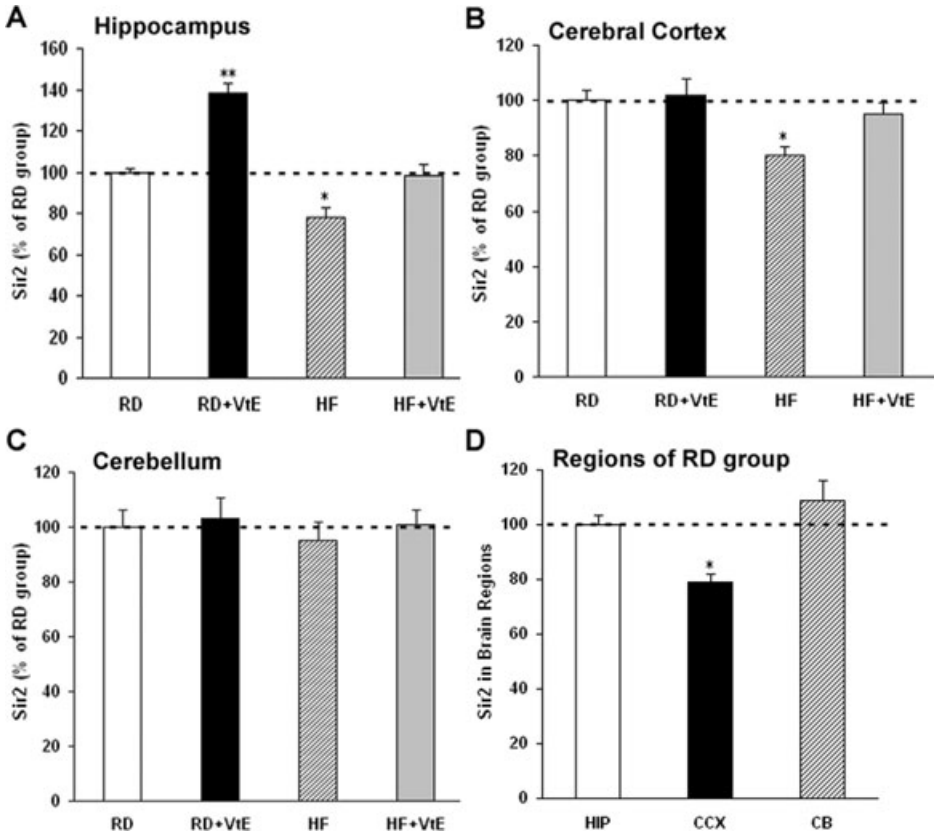

FIG. 1. Expression of Sir2α in rat brain revealed by Western blot analysis. (A) Sir2α levels in the hippocampus of rats fed the high-fat diet (HF) was significantly lower than that of rats fed regular diet (RD), whereas supplementation with vitamin E (VtE) normalized hippocampal levels of Sir2α. In addition, vitamin E supplementation significantly increased Sir2α levels in animals fed regular diet. (B) Sir2α levels in the cerebral cortex (caudal) were significantly reduced by the high-fat diet; the reduction was reversed by vitamin E, but vitamin E did not affect Sir2α in animals fed regular diet. (C) Sir2α in the cerebellum was not changed by either vitamin E or by the high-fat diet. (D) Sir2α levels in different brain regions of the regular diet group. Sir2α in hippocampus (HIP) was higher than that in caudal cerebral cortex (CCX) but not significantly different from that in cerebellum (CB). The values are expressed as a percentage of the RD group (mean + SEM). *$P < 0.05$; **$P < 0.01$.

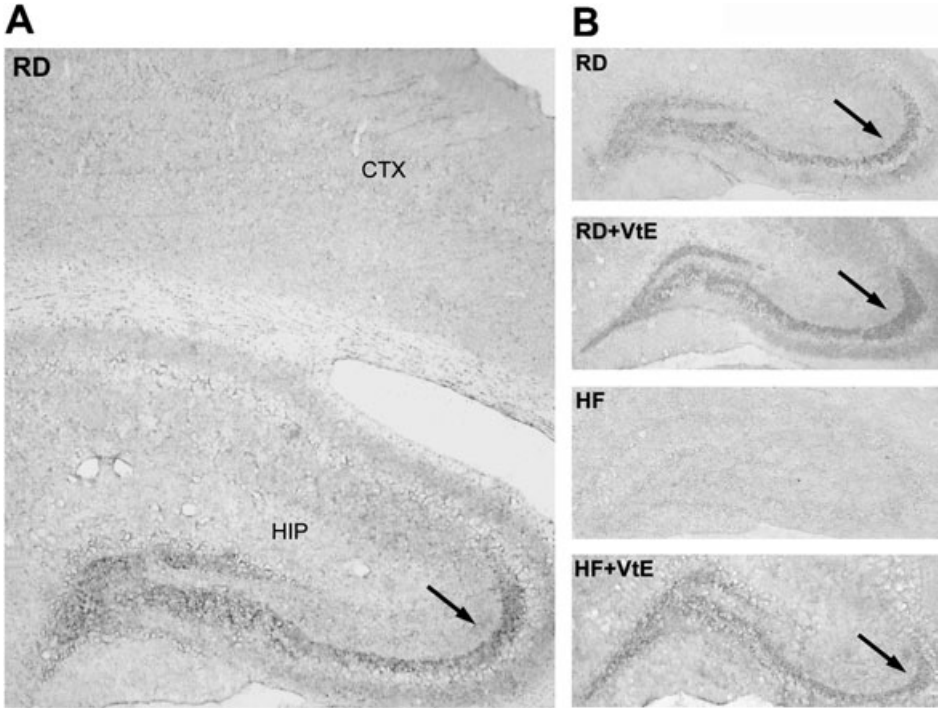

FIG. 2. Sir2α distribution revealed by immunohistochemical analysis. (A) Sir2α immunostaining was predominantly distributed in the mossy fibre system, and in the granule layer of the dentate gyrus of the hippocampal formation (arrows). The immunostaining for Sir2α in cortex was very diffuse relative to Sir2α immunoreactivity in the hippocampus. (B) High fat diet consumption resulted in a qualitative reduction in Sir2α immunoreactivity that was attenuated by vitamin E supplementation. In addition, vitamin E supplementation increased Sir2α expression in rats fed regular diet. RD, regular diet; HF, high-fat diet; VtE, vitamin E; HIP, hippocampus; CTX, cerebral cortex.

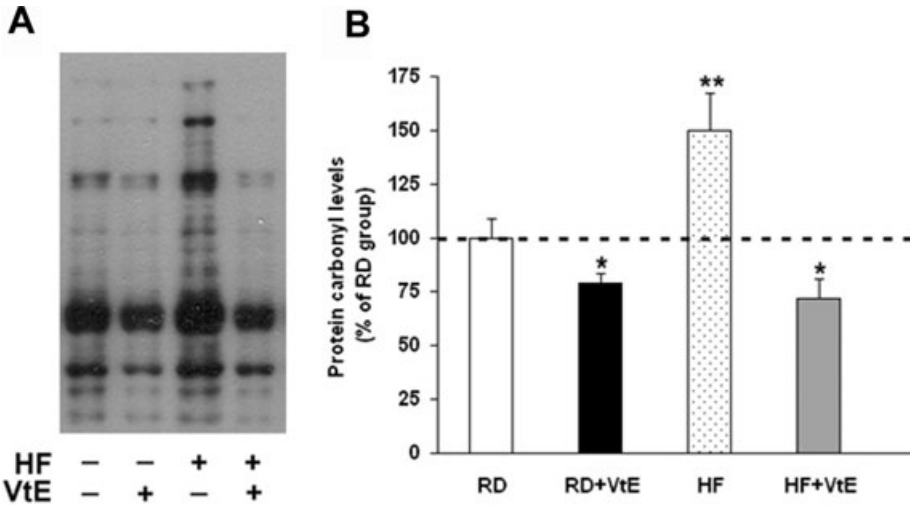

FIG. 3. Measurement of oxidized protein levels in rat hippocampus by Western blot analysis of DNPH-derived carbonyl groups on oxidized proteins. (A) Representative examples of Oxyblot. (B) HF consumption increased oxidized protein levels compared to the RD group, whereas vitamin E supplementation reduced the oxidized protein levels (72%). Vitamin E supplementation decreased oxidized protein levels (79%) in rats fed regular diet. The values are expressed as a percentage of the RD group (mean + SEM). *$P < 0.05$, **$P < 0.01$. RD, regular diet; HF, high-fat diet; VtE, vitamin E.

Oxidized nucleic acids

Oxidative damage to nucleic acids was assessed using immunohistochemistry with an antibody against 8OHdG/8OHG that detects oxidized nucleic acids. Oxidized nucleic acid labeling appeared markedly increased in all the neuronal cell body layers of the hippocampus after consumption of a high-fat diet (Fig. 4), whereas dietary supplementation with vitamin E appeared to dramatically reduce the labeling (Fig. 4). In addition, vitamin E supplementation appeared to reduce 8OHdG/8OHG staining in rats fed regular diet (Fig. 4).

AMPK and phosphorylated AMPK (p-AMPK) levels

Given the association of Sir2α with energy metabolism and the newly discovered roles of AMPK in maintaining energy balance (Gadalla

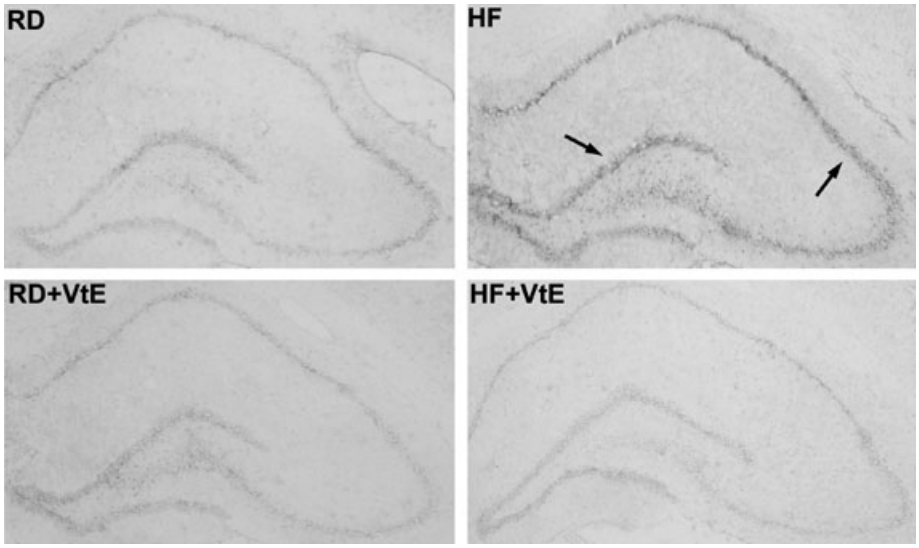

FIG. 4. Detection of oxidized nucleic acids in rat hippocampus by immunohistochemistry using an 8OHdG/8OHG antibody. The staining intensity was markedly increased in HF-fed rats (arrows), but decreased in rats fed HF supplemented with VtE. In addition, vitamin E supplementation reduced 8OHdG/8OHG staining in rats fed regular diet. RD, regular diet; HF, high-fat diet; VtE, vitamin E.

et al., 2004; Rafaeloff-Phail *et al*., 2004; Shaw *et al*., 2004; Lee *et al*., 2005), we have evaluated the role of AMPK in our paradigm. We measured levels of total AMPK and pAMPK using Western blot analysis in the same tissue used for Sir2α measurements. Our results showed reduced AMPK levels in the hippocampus of rats fed the high-fat diet (77% of control, $P < 0.05$; Fig. 5A) compared to animals fed regular diet. Vitamin E supplementation normalized levels of Sir2α in high-fat diet-fed rats (103% of control, $P > 0.05$; Fig. 5A). Furthermore, vitamin E supplementation normalized the levels of p-AMPK (92% of control, $P > 0.05$; Fig. 5B) which had been decreased by the effects of the high-fat diet (74% of control, $P < 0.05$; Fig. 5B). Vitamin E supplementation increased AMPK levels (127% of control, $P < 0.05$; Fig. 5A) and p-AMPK levels (120% of control, $P < 0.05$; Fig. 5B) in rats fed regular diet. In addition, in cerebral cortex (Fig. 5C and D) and cerebellum (Fig. 5E and F), neither high-fat diet nor vitamin E affected the AMPK or p-AMPK levels.

We performed a correlation analysis to evaluate the possible association between Sir2α and AMPK levels in the hippocampus. There was a significant positive correlation between Sir2α and AMPK ($r = 0.87$, $P < 0.05$) which was disrupted by high-fat feeding ($r = –0.30$, $P > 0.05$). Vitamin E supplementation restored the correlation ($r = 0.84$, $P < 0.05$). The correlation analysis also revealed a significant positive correlation between Sir2α and p-AMPK ($r = 0.91$, $P < 0.05$), which was disrupted by high-fat diet consumption ($r = –0.19$, $P > 0.05$) and restored by vitamin E supplementation ($r = 0.82$, $P < 0.05$).

Discussion

Recent research emphasizes the critical implications of diet and other lifestyle factors for the maintenance of neural function and plasticity. A diet rich in saturated fat has been identified as a predictor of reduced capacity of neurons to withstand insult (Wu *et al*., 2003). In contrast, Sir2α has been implicated in cellular mechanisms that enable organisms to cope with adverse conditions. Even more recently, it has been found that this protective action of Sir2α can extend to the brain (Araki *et al*., 2004; Bordone & Guarente, 2005; Parker *et al*., 2005; Sinclair, 2005). The present results show that the pervasive action of the high-fat diet on brain plasticity may involve a reduction in hippocampal levels of Sir2α. Our results also indicate that elevated oxidative stress and disrupted energy homeostasis are critical factors for the reduced expression of Sir2α in the hippocampus after consumption of the high-fat diet.

Sir2α and oxidative stress

Cells normally have buffering mechanisms acting to defend them against damage induced by free radicals. Cell function and viability are compromised when reactive oxygen species (ROS) production exceeds the buffering capacity of the cell. Our results indicate that elevated levels of oxidative stress associated with the high-fat diet have a negative impact on Sir2α expression. Specifically, these results showed that levels of Sir2α were reduced proportionally to the increase in protein oxidation, and that vitamin E treatment normalized levels of Sir2α. These findings suggest a possible role for Sir2α on the mechanisms by which the high-fat diet impacts brain homeostasis and neuronal resilience to insults. It is notable that high-fat diet affects Sir2α in both hippocampus and cerebral cortex, but not in cerebellum, suggesting a regional effect of the high-fat diet on the brain. As discussed below, Sir2α may also indirectly contribute to reduced oxidative stress. According to this, lowered levels of Sir2α associated with the high fat would diminish the buffering capacity of the brain against oxidative stress, thus compromising neuronal resilience to insults. Indeed, Sir2α has been shown to provide protection to neurons against the action of various insults (Araki *et al*., 2004; Bordone & Guarente, 2005; Parker *et al*., 2005; Sinclair, 2005).

The mechanisms by which oxidative stress would affect Sir2α expression are not well understood. It is known that Sir2 activation is linked to elements of the cellular respiratory chain (Nemoto *et al*., 2005; Rodgers *et al*., 2005). Free radicals are highly reactive molecules generated predominantly during cellular respiration and normal metabolism. Sir2 is a nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase (Imai *et al*., 2000; Landry *et al*., 2000; Vaziri *et al*., 2001; Blander & Guarente, 2004), suggesting an

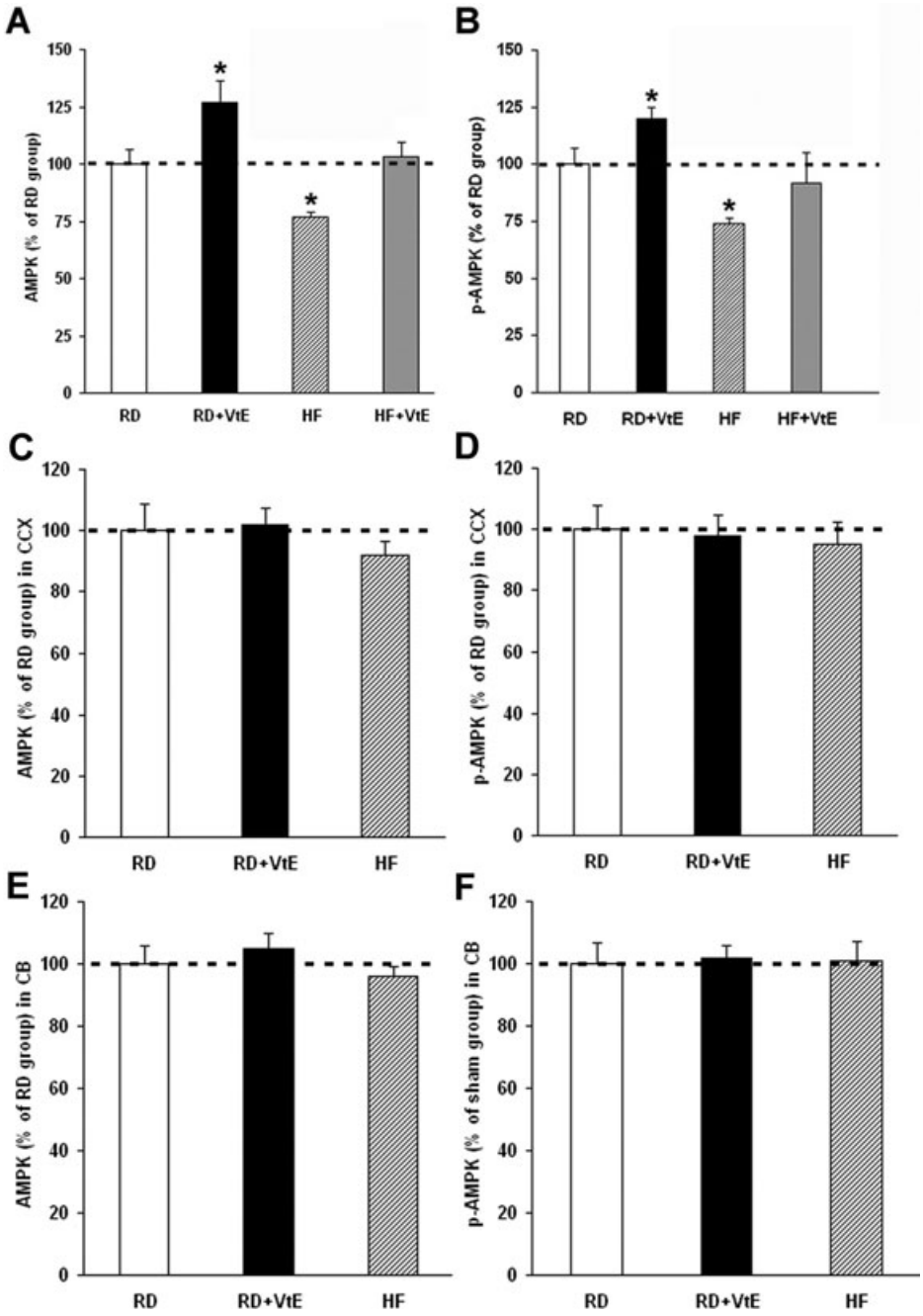

FIG. 5. Expression of (A) AMPK and (B) p-AMPK in rat hippocampus revealed by Western blot analysis. Both AMPK and p-AMPK were significantly reduced by high-fat diet consumption, whereas supplementation with vitamin E significantly increased levels of both. Vitamin E supplementation increased (A) AMPK and (B) p-AMPK expression in rats fed regular diet. In cerebral cortex (caudal; C and D) and cerebellum (E and F), neither high-fat diet nor vitamin E changed AMPK or p-AMPK levels. The values are expressed as a percentage of the RD group (mean + SEM). $*P < 0.05$. RD, regular diet; HF, high-fat diet; VtE, vitamin E; HIP, hippocampus; CCX, cerebral cortex (caudal); CB, cerebellum.

important role for NAD in the regulation of Sir2. It has been shown that reduced caloric intake elevates Sir2 expression, probably by reducing glucose metabolism and subsequently increasing the ratio of NAD to its reduced version NADH (Lin *et al*., 2004). It was found that increasing NAD biosynthesis by elevated nicotinamide mononucleotide adenylyltransferase 1 can activate Sir2α and this seems to mediate the protective action of Sir2α after axonal damage (Araki *et al*., 2004). Sir2 can increase the expression of manganese superoxide dismutase, a potent antioxidant enzyme, which in turn can increase the cellular ability to detoxify reactive oxygen species (Kops *et al*., 2002; Nemoto & Finkel, 2002; Brunet *et al*., 2004; Daitoku *et al*., 2004).

It has been known that mitochondria play an important role in energy metabolism and production of ROS. There is evidence indicating that SIRT3, which is located in mitochondria, can reduce ROS production and increase respiration rates in adipose tissue (Anekonda & Reddy, 2006). Mitochondrial DNA is particularly susceptible to oxidative damage, and it has been suggested that mitochondrial deficits caused by oxidative damage are a major

contributor to cellular, tissue and organismal ageing (Mecocci *et al*., 1993; Shigenaga *et al*., 1994). Our results show increased oxidative damage to nucleic acids in animals fed a high-fat diet and that this damage was reversed by antioxidant treatment. Thus, whether mitochondrial DNA was involved in the oxidative damage arising from high-fat diet consumption, and whether both Sir2α (SIRT1) and SIRT3 have protective actions, need to be further investigated.

Our results showed that the high-fat diet reduced levels of AMPK and that vitamin E supplementation restored levels of AMPK in hippocampus. However, neither high-fat diet nor vitamin E affected AMPK or p-AMPK in cerebral cortex and cerebellum, suggesting some levels of specificity for the action of the diet on AMPK. It is known that AMPK serves as an energy sensor (Gadalla *et al*., 2004; Rafaeloff-Phail *et al*., 2004; Shaw *et al*., 2004; Lee *et al*., 2005), such that reduced levels of AMPK in our results may be a product of the high caloric content of the high-fat diet that can lead to oxidative stress (Mattson *et al*., 2003).

Our data showed a significant positive correlation between levels of Sir2α, AMPK and p-AMPK in animals fed the regular diet, which may reflect a close association between Sir2α and energy status during homeostatic conditions. Interestingly, those correlations disappeared in high-fat-fed animals, which may be the result of disrupted homeostasis associated with this diet's elevated oxidative stress. It is known that impaired energy homeostasis is related to dysfunction in the AMPK system, and that this condition makes the brain more vulnerable to stress (Culmsee *et al*., 2001). New studies have supported a mechanistic association between Sir2 and AMPK, as they show that NAD, a critical substrate for Sir2 function, is activated by AMPK in a dose-dependent manner (Rafaeloff-Phail *et al*., 2004).

Sir2α distribution in hippocampal formation and cerebral cortex

Immunohistochemical analysis showed that Sir2α was predominantly localized in the hippocampal formation, a brain region critical for learning and memory and supporting cognitive abilities (Sugaya *et al*., 1996; Clayton *et al*., 2002; Steffenach *et al*., 2002; Drapeau *et al*., 2003; Kelly *et al*., 2003). Sir2α labeling was more prominent along the mossy fibre system and in the granule layer of the dentate gyrus in the hippocampal formation. The pattern of Sir2α distribution is markedly similar to that of brain-derived neurotrophic factor (BDNF), a critical factor for synaptic plasticity associated with spatial memory (Wu *et al*., 2003, 2004). Further studies are required to evaluate a possible association between Sir2α and BDNF. The mossy fiber system has been well implicated in the hippocampal circuits that conform memory formation, suggesting a supporting role of Sir2α in events associated with synaptic plasticity and learning and memory. However, the Sir2α distribution is very diffuse in cerebral cortex (Fig. 2A). As far as we know, our report is the first to demonstrate the cellular location of Sir2α in the brain. It has been recently reported that calorie restriction increased Sir2α protein levels in rat brain tissue (Cohen *et al*., 2004), but there was no description of brain areas affected.

Implications of Sir2α function for neural repair

The hippocampus is a brain region important for processing and storage of information vital for the survival of the species. Accordingly, the potential role of Sir2α in protecting hippocampal cells from the effects of poor diets has special relevance for biological adaptation to environmental challenges. Sir2α up-regulation may reduce nucleic acid oxidation and contribute to enhancing DNA repair mechanisms in hippocampal neurons. Given the actions of Sir2 on gene stability and DNA repair (Guarente, 2000; Kirkwood & Austad, 2000; Tran *et al*., 2002; Lombard *et al*., 2005), our findings support the possibility that hippocampal neurons can protect themselves against oxidative DNA damage by increasing Sir2α. Sir2 was originally described for its role in promoting longevity in invertebrates under challenging situations. In turn, the current results suggest that Sir2α may maintain homeostasis and protect the brain against incurred oxidative damage. Given that normal neuronal function requires proper regulation of energy metabolism to balance oxidative stress, it is critical to further elucidate the role of Sir2α in energy homeostasis to better understand mechanisms of neuronal repair.

Acknowledgements

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Abbreviations

8OHdG/8OHG, 8-hydroxy-2′-deoxyguanosine/8-hydroxyguanosine; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; DNPH, dinitrophenylhydrazine; NAD, nicotinamide adenine dinucleotide; p-AMPK, phosphorylated AMPK; ROS, reactive oxygen species; Sir2, silent information regulator 2; SIRTs, mammalian Sir2 homologs.

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