Review

Suppression of brain aging and neurodegenerative disorders by dietary restriction and environmental enrichment: molecular mechanisms

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Abstract

Dietary restriction (reduced calorie intake with nutritional maintenance) can extend lifespan and may increase the resistance of the nervous system to age-related diseases including neurodegenerative disorders. An environment enriched in intellectual and physical activities can also allay many of the adverse effects of aging on the brain. The mechanisms underlying the beneficial effects of dietary restriction and environmental enrichment on the brain involve stimulation of the expression of neurotrophic factors and ‘stress proteins’. The neurotrophic factors and stress proteins induced by dietary restriction may protect neurons by suppressing oxynradical production, stabilizing cellular calcium homeostasis and inhibiting a form of programmed cell death called apoptosis. Interestingly, dietary restriction and environmental enrichment also increase numbers of newly-generated neural cells in the adult brain suggesting that these behavioral modifications can increase the brain’s capacity for plasticity and self-repair. A better understanding of the cellular and molecular mechanisms...
underlying these effects of diet and behavior on the brain is leading to novel therapeutic agents that mimic their beneficial effects. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Alzheimer’s disease; Calories; Heat shock protein; Oxidative stress; Mitochondria; Neurotrophic factor; Parkinson’s disease; Stroke

1. Introduction

The lifespans (both mean and maximum lifespan) of many different species of animals can be increased by up to 50% simply by reducing their calorie intake with maintenance of micronutrient intake (dietary restriction). This has been shown to be the case in many organisms commonly used in biomedical research including the yeast S. cerevisiae (Ashrafi et al., 2000; Lin et al., 2000), the roundworm C. elegans (Sze et al., 2000) and mice and rats (Weindruch and Sohal, 1997). Ongoing studies suggest the same will be true in monkeys (Lane et al., 1999). In mammals, dietary restriction (DR) reduces the development of age-related cancers (Raffoul et al., 1999), cardiovascular disease (Maeda et al., 1985) and deficits in immune function (Spaulding et al., 1997). Clinical and epidemiological studies of humans are entirely consistent with beneficial effects of DR. Overeating increases the risk of many age-related diseases in humans including cardiovascular disease, diabetes and cancers (Lebovitz, 1999; Levi et al., 1999; Brochu et al., 2000). Accordingly, a decrease in calorie intake can reduce risk of the same diseases. The vast majority of research on the effects of calorie intake on aging and disease have focussed on organ systems other than the brain. Although benefits of DR on the cardiovascular, immune and endocrine systems have been demonstrated, its effects on the nervous system are only now being studied. Emerging data suggest that a similar scenario may apply to neurodegenerative disorders including Alzheimer’s disease (AD), Parkinson’s disease (PD) and stroke (Logroscino et al., 1996; Bruce-Keller et al., 1999; Duan and Mattson, 1999; Yu and Mattson, 1999).

In the present article we define environmental enrichment (EE) as a sustained increase in daily cognitive, sensorimotor and/or physical activity. This definition is broader than the definition usually used by neuroscientists who focus on increased sensory stimulation as the paradigm of EE (Mohammed et al., 1993). It should be noted that, from the standpoint of the nervous system, it is difficult to separate the effects of physical exercise on energy metabolism from effects on activity of the nervous system. In mammals from rodents to man, physical exercise can reduce the incidence of age-related diseases, particularly cardiovascular disease and cancer (Duncan et al., 1997; Schell and Myers, 1997). However, compared to the dramatic extension of maximum lifespan by DR, exercise has very little effect on maximum lifespan in rodents (Goodrick et al., 1983). The effects of mental and physical activity on the aging nervous system are not well understood, but recent findings described below suggest that EE can counteract adverse effects of aging on the brain. The purpose of the present article is to review the evidence concerning the
effects of caloric intake, and physical and mental exercise, on the brain, with particular emphasis on age-related neurodegenerative disorders.

2. Evidence that dietary restriction can protect neurons and promote neuroplasticity

Studies of rats and mice maintained on DR suggest that a decreased calorie intake can slow age-related molecular changes in the brain including increases in levels of glial fibrillary acidic protein and oxidative damage to proteins and DNA (Dubey et al., 1996; Finch and Morgan, 1997). Analyses of relative expression levels of thousands of genes in the brains of young rats, and old rats that had been fed either ad libitum or reduced calorie diets, have revealed quite striking changes in gene expression in brain cell during aging, and have shown that DR can suppress those changes (Table 1). Age-related changes in the expression of genes that encode proteins involved in innate immune responses, oxidative stress and energy metabolism are counteracted by DR (Lee et al., 2000a). This retardation of aging at the molecular level may underlie retardation of brain aging at the functional level. As evidence, DR can attenuate age-related deficits in learning and memory ability and motor function in rodents (Ingram et al., 1987; Stewart et al., 1989). A

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Examples of the effects of dietary restriction on changes in gene expression in the brain during aginga</th>
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<tbody>
<tr>
<td><strong>Gene</strong></td>
<td><strong>Usual diet</strong></td>
</tr>
<tr>
<td><strong>Energy-related</strong></td>
<td></td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>Decreased expression</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>Decreased expression</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase</td>
<td>Increased expression</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>Increased expression</td>
</tr>
<tr>
<td><strong>Stress-related</strong></td>
<td></td>
</tr>
<tr>
<td>HSP-70</td>
<td>No change or decrease</td>
</tr>
<tr>
<td>GRP-78</td>
<td>No change or decrease</td>
</tr>
<tr>
<td>Gadd153</td>
<td>Increased expression</td>
</tr>
<tr>
<td>Proteosome z subunit</td>
<td>Decreased expression</td>
</tr>
<tr>
<td><strong>Inflammation-related</strong></td>
<td></td>
</tr>
<tr>
<td>GFAP</td>
<td>Increased expression</td>
</tr>
<tr>
<td>Complement C1q</td>
<td>Increased expression</td>
</tr>
<tr>
<td>Complement C4</td>
<td>Increased expression</td>
</tr>
<tr>
<td><strong>Plasticity-related</strong></td>
<td></td>
</tr>
<tr>
<td>NMDA receptor NR1</td>
<td>Decreased expression</td>
</tr>
<tr>
<td>BDNF</td>
<td>Decreased expression</td>
</tr>
<tr>
<td>TrkB</td>
<td>Decreased expression</td>
</tr>
<tr>
<td>α-synuclein</td>
<td>Decreased expression</td>
</tr>
</tbody>
</table>

a Taken from data in references (Aspnes et al., 1997; Finch and Morgan, 1997; Croll et al., 1998; Nicoletti et al., 1998; Duan and Mattson, 1999; Yu and Mattson, 1999; Eckles-Smith et al., 2000; Lee et al., 2000a,b; Duan et al., 2001a,b).
parallel may be found in human populations where the risk of developing Alzheimer’s disease (AD), Parkinson’s disease (PD) and stroke may be lower in individuals with a low calorie intake (Logroscino et al., 1996; Grant, 1997; Mayeux et al., 1999).

Because life expectancy is increasing, more and more persons will suffer from age-related neurodegenerative conditions with AD, PD and stroke being the most prevalent. Each of these disorders is characterized by selective dysfunction and degeneration of specific populations of neurons in the brain (Fig. 1). Degeneration and death of neurons in brain regions involved in learning and memory processes, such as the hippocampus and cerebral cortex, occurs in AD patients (Mattson, 1997; Ray et al., 1998). In PD, degeneration of dopaminergic neurons in the substantia nigra occurs and results in motor dysfunction (Jenner and Olanow, 1998; Schulz and Dichgans, 1999). When a stroke (occlusion or rupture of a cerebral blood vessel) occurs neurons in the brain tissue supplied by that vessel degenerate (Dirnagl et al., 1999). In each of the aforementioned neurodegenerative conditions neurons may die by a stereotyped biochemical cellular cascade of events called apoptosis (Mattson, 2000). The negative impact of age-related neurodegenerative disorders is emphasized by the fact that more dollars are required to care for
of patients with AD, PD and stroke than are spent on care for patients with cardiovascular disease and cancer.

The identification of specific genetic and environmental factors that may initiate the neurodegenerative process in AD, PD and stroke has led to the development of useful animal models of these disorders (Table 2). In the case of AD the models include transgenic mice overexpressing mutant forms of amyloid precursor protein which are known to cause early-onset inherited AD (Games et al., 1995; Hsiao et al., 1986), transgenic and knockin mice expressing mutant forms of presenilin-1 linked to inherited forms of AD (Duff et al., 1996; Guo et al., 1999), and administration of amyloid β-peptide and/or excitotoxins into the brains of rats and mice (Geula et al., 1998; Bruce-Keller et al., 1999). Models of PD include administration of the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to adult monkeys and mice which results in selective loss of substantia nigra dopaminergic neurons and associated motor dysfunction (Duan et al., 1999), and α-synuclein mutant mice which exhibit degeneration of dopaminergic neurons and a behavioral phenotype that mimicks several features of PD (Masliah et al., 2000). Stroke models include transient or permanent occlusion of the middle cerebral artery in rats and mice (Dirnagl et al., 1999; Yu and Mattson, 1999). We have investigated the impact of DR on the neurodegenerative process in several of these animal models.

In many of our studies we employed an every other day feeding DR regimen; this DR protocol results in an approximately 30% reduction in calorie intake over time and extends the lifespans of rats and mice by 30–40%. Maintenance of rats on the alternate day DR feeding regimen for 2–4 months results in resistance of hippocampal neurons to kainate-induced degeneration, a model of epileptic seizure-induced brain damage and memory loss (Bruce-Keller et al., 1999). The reduced damage to hippocampal neurons is correlated with a striking preservation of learning and memory in a water maze spatial learning task (Fig. 2A). In order to determine whether DR might counteract the pathogenic actions of mutations in

Table 2
Genetic and environmental factors that increase risk for age-related neurodegenerative disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Genetic factors</th>
<th>Environmental factors</th>
</tr>
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<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>Mutations in APP, presenilins variations in ApoE, α2-M</td>
<td>Head trauma, calorie intake, education</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>Mutations in α-synuclein, parkin</td>
<td>Toxins, head trauma, calorie intake</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>Mutation of huntingtin</td>
<td>Toxins?</td>
</tr>
<tr>
<td>ALS</td>
<td>Mutation of Cu/Zn-SOD</td>
<td>Toxins?</td>
</tr>
<tr>
<td>Stroke</td>
<td>Mutations in Notch-3, LDL receptor variations in ApoE</td>
<td>Calorie intake, smoking, lack of exercise</td>
</tr>
</tbody>
</table>

*α2-M, α2-macroglobulin; ALS, amyotrophic lateral sclerosis; ApoE, apolipoprotein E; Cu/Zn-SOD, Cu/Zn- superoxide dismutase.
Fig. 2. DR enhances learning and memory and increases resistance of neurons to degeneration in experimental models of Alzheimer’s disease. (A) Rats were maintained for 3 months on a DR feeding regimen or were fed ad libitum (AL). The excitotoxin kainate was then injected into the dorsal hippocampus bilaterally, and 24 h later the spatial memory ability of the rats was measured using the Morris water maze method. Goal latency data showing that kainate caused severe impairment in the AL group, but not in the DR group. (B) Graph showing relative levels of the lipid peroxidation product 4-hydroxynonenal in the hippocampus of wild-type mice and presenilin-1 mutant knockin mice 12 h after kainate administration. The mice had been maintained for 3 months on either DR or AL diets. Note the increased level of 4-hydroxynonenal in the AL-fed PS-1 mutant mouse compared to the AL-fed wild-type mouse, and the marked suppression of 4-hydroxynonenal levels in wild-type and PS-1 mutant mice that had been maintained on the DR feeding regimen.
presenilin-1 and the amyloid precursor protein (APP), we maintained presenilin-1 mutant knockin mice and APP mutant transgenic mice on the alternate day feeding regimen. We had previously shown that presenilin-1 mutations increase the vulnerability of hippocampal and cortical neurons to excitotoxicity and apoptosis by a mechanism involving enhanced calcium release from the endoplasmic reticulum (Guo et al., 1999). Presenilin-1 mutant knockin mice that had been maintained on DR for 3 months exhibited increased resistance of hippocampal CA1 and CA3 neurons to excitotoxic injury compared to mice fed ad libitum (Zhu et al., 1999). Levels of oxidative stress in the hippocampus following kainate administration were lower the DR mice compared to mice fed ad libitum, indicating that suppression of oxidative stress may be one mechanism underlying the neuroprotective effect of DR (Fig. 2B). Thus, the neurodegeneration-promoting effect of a mutation that causes AD can be counteracted by a reduced calorie diet.

APP mutant mice exhibit progressive age-dependent deposition of Aβ in their brains which is most prominent in the cerebral cortex and hippocampus. In an initial test of the hypothesis that DR would suppress Aβ deposition in APP mutant mice, we obtained a quite surprising result. When APP mutant mice were placed on an alternate day feeding schedule, they died within 2–3 weeks (Pedersen et al., 1999). The APP mutant mice became severely hypoglycemic during the days they were without food, and apparently succumbed to the hypoglycemia. Further analyses revealed marked abnormalities in regulation of the stress-responsive hypothalamic-pituitary-adrenal axis in the APP mutant mice characterized by abnormal glucocorticoid and blood glucose regulation in response to restraint stress, for example (Pedersen et al., 1999). More recent findings suggest that there are abnormalities in glucocorticoid feedback mechanisms in association with Aβ accumulation in the cerebral cortex, hippocampus and hypothalamus of the APP mutant mice that may contribute to their inability to ‘cope’ with stress (Pedersen et al., 2000). Interestingly, when the alternate day feeding regimen is begun in APP mutant mice that are less than 3 months of age they survive. Our preliminary data suggest that under the latter conditions, deposition of Aβ in the brain is decreased.

DR also has a beneficial effect in experimental models of the movement disorders. For example, the vulnerability of midbrain dopaminergic neurons to MPTP toxicity is decreased in mice maintained on DR (Duan and Mattson, 1999). More dopaminergic neurons survive exposure to MPTP, and deficits in motor function are markedly decreased in DR rats (Fig. 3). Selective degeneration of neurons in the striatum of patients with Huntington’s disease (HD) is responsible for their inability to control body movements properly. An animal model of HD involves administration of the succinate dehydrogenase inhibitor (mitochondrial toxin) 3-nitropropionic acid (3NP) to rats and mice. Maintenance of rats on a DR regimen for several months prior to administration of 3NP results in increased resistance of striatal neurons to 3NP and improved motor function (Bruce-Keller et al., 1999).

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by progressive degeneration of spinal cord motor neurons resulting in progressive paralysis. A small percentage of cases of ALS result from mutations in the gene encoding the antioxidant enzyme Cu/Zn-superoxide dismutase. Transgenic mice expressing mu-
Fig. 3. (Continued)
tant Cu/Zn-SOD exhibit progressive motor neuron degeneration and a clinical phenotype remarkably similar to ALS patients (Pedersen and Mattson, 1999). We maintained Cu/Zn-SOD mutant mice on a DR regimen to determine the impact of a reduced calorie diet on age of disease onset and disease progression. In contrast to the beneficial effects of DR in the PD and HD models, Cu/Zn-SOD mutant mice do not benefit from DR (Pedersen and Mattson, 1999). DR did not delay disease onset and, once mice became symptomatic, disease progression was actually accelerated. These findings are important because they show that DR cannot overcome the pathogenic action of the Cu/Zn-SOD mutation suggesting that the neurodegenerative cascade in this mouse model is fundamentally different than that in the AD, PD and HD models. However, an alternative interpretation is that not all populations of neurons benefit equally from DR. From the clinical perspective these findings are also of interest because it is well known that increased energy intake has a beneficial effect on disease progression in ALS patients.

We have also determined whether DR can modify outcome in a rat stroke model in which the middle cerebral artery is transiently occluded resulting in damage to the cerebral cortex and striatum supplied by that artery, and associated motor dysfunction. Rats that had been maintained on DR for several months exhibited reduced brain damage and improved behavioral outcome following transient occlusion of the middle cerebral artery (Yu and Mattson, 1999). DR is also effective in reducing focal ischemic brain damage in mice (unpublished data). The experimental findings just described directly demonstrate profound neuroprotective effects of DR in animal models relevant to the pathogenesis of AD, PD, HD and stroke. The striking neuroprotective effects of DR in these experimental models suggest that DR may prove beneficial in reducing the incidence and/or severity of many different human neurodegenerative disorders. The following epidemiological data support the latter possibility.

Studies of world populations and well-characterized cohorts in urban settings suggest that individuals with a low calorie intake may have reduced risk for AD and PD. Analyses of different populations throughout the world reveals a strong relation between per capita food consumption and risk for AD (Grant, 1997). For example, persons in China and Japan have relatively low calorie intakes (1600–2000 calories per day) as compared to persons in the United States and Western Europe (2500–3000 calories per day). The incidence of AD in China and Japan is approximately half that in the United States and Western Europe, although it

Fig. 3. Dietary restriction increases resistance of dopaminergic neurons to degeneration and improves motor performance in an experimental model of Parkinson’s disease. (A) Micrographs showing dopamine-producing neurons (stained with an antibody against tyrosine hydroxylase) in the substantia nigra 7 days after administration of the Parkinsonian toxin MPTP in mice that had been maintained for 3 months on either DR or ad-libitum feeding regimens. (B) The motor performance of mice was measured using rotary rod apparatus in mice that had been maintained for 3 months on either DR or ad-libitum feeding regimens. Mice were administered MPTP and their motor function was assessed at the indicated time points. Note that DR mice were able to maintain themselves on the rotary rod for a longer time period compared to the ad-libitum fed control mice.
should be recognized that per capita food consumption is a very poor measure of energy intake. It may also be the case that AD is underdiagnosed in countries such as China, and therefore the kinds of relationships established in such cross-cultural comparisons are not conclusive. More compelling evidence that DR can protect against neurodegenerative disorders comes from studies of a large cohort of people living in New York City. Individuals with the lowest daily calorie intakes had the lowest risk for AD (Mayeux et al., 1999) and PD (Logroscino et al., 1996). In both of these studies nutrient intake was assessed using a semi-quantitative food-frequency questionnaire which included 61 foods, use of vitamin and mineral supplements, types of breakfast cereals consumed, type of fats used for frying and baking, use of sugar and salt, and alcohol; food had a fixed portion size, and nutrient content of each portion was estimated from USDA food composition data. Subjects were asked how often the consumed each food, and were asked to report their usual dietary pattern over the last year. Possible disease-related changes in diet of the subjects were controlled for by recording dietary habits in the last 10 years. Finally, the epidemiological data suggesting that overeating is a major risk factor for stroke is quite compelling (Bronner et al., 1995). Experimental data suggest that a high calorie intake can worsen outcome following stroke (Yu and Mattson, 1999), consistent with the possibility that a low calorie diet will improve outcome following stroke in humans.

3. Evidence that environmental enrichment can protect neurons and promote neuroplasticity

Data from epidemiological studies have documented an inverse relationship between educational attainment and risk for AD such that more educated persons are at reduced risk (Evans et al., 1997). A study of a population of nuns further suggested that those nuns with the best linguistic abilities were at reduced risk for AD (Snowdon et al., 1996). One interpretation of these data is that intellectual activity is neuroprotective. Animal studies are consistent with this interpretation. The early work of Greenough and coworkers showed that rats raised in enriched environments exhibit increased complexity of dendritic arbors and synapses in the hippocampus and cerebellum, suggesting an increased functional reserve (Black et al., 1989; Kleim et al., 1997). Synaptic density decreases in hippocampus during aging in rats, and maintenance of rats in an enriched environment can prevent such age-related synaptic loss (Saito et al., 1994). In addition, when rodents are raised in an enriched environment in which they have many objects to play with, neurogenesis is enhanced and learning and memory ability improved (Kempermann et al., 1997; Nilsson et al., 1999; Young et al., 1999). EE may also increase resistance of neurons to injury and promote recovery after injury. For example, functional deficits caused by bilateral lesions of the frontal cortex were ameliorated in rats maintained in an enriched environment, and this was correlated with reduced structural damage (Kolb and Gibb, 1991). In addition to enhancing synaptic connectivity and increasing resistance of neurons to injury, EE can improve
outcome after a stroke in rats (Johansson, 1996), suggesting an attenuation of the delayed neurodegenerative process or enhanced recovery of function of surviving neurons. Similarly, postinjury EE improves learning and memory performance in a water maze test after lesion of cholinergic basal forebrain neurons in rats (van Rijzingen et al., 1997).

Interestingly, physical activity (which can reduce body weight) may also counteract the adverse effects of aging and disease on the brain. Thus, mice allowed access to a running wheel exhibit increased neurogenesis and improved learning and memory compared to ‘couch potato’ mice (van Praag et al., 1999). Further data supporting a beneficial effect of exercise on the brain comes from epidemiological studies in humans which show that regular vigorous physical activity can reduce risk for ischemic stroke (Lee et al., 1999a), although it has not been established that prior physical activity can improve outcome after a stroke, this would seem plausible. Physical activity can also benefit the brain after injury. For example, exercise after brain injury improved functional outcome in rats, and the improved outcome was associated with enhanced structural plasticity in the motor cortex (Jones et al., 1999).

4. Cellular and molecular mechanisms underlying the beneficial effects of dietary restriction and environmental enrichment on the brain

How does DR increase resistance of neurons to neurodegenerative disorders? In order to answer this question it is necessary to understand the biochemical cascades that occur in neurons that result in their dysfunction and death in aging and neurodegenerative disorders. Although the genetic and environmental factors that initiate the neurodegenerative process may differ among diseases, considerable evidence suggests that a common set of alterations ensues that ultimately kills the neuron. Three major alterations involved in the neurodegenerative process are increased oxidative stress, perturbed cellular ion homeostasis and impaired energy metabolism (Mattson, 2000). Increased oxidative stress results in damage to proteins, lipids and nucleic acids, impaired regulation of cellular ion homeostasis results in aberrant increases in intracellular calcium levels, and impaired energy metabolism can result from and contribute to mitochondrial dysfunction. These alterations render neurons vulnerable to apoptosis, a form of cell death that involves a cascade of molecular interactions mediated by proteins such as Par-4, and Bcl-2 family members and caspases (Mattson, 2000). In AD the neurodegenerative cascade can be initiated by the aging process, in combination with a specific genetic predisposition or environmental factors (e.g. head trauma or a high calorie diet). In each case there is increased production and extracellular deposition of a neurotoxic proteolytic peptide product of APP called amyloid β-peptide (Aβ). Aβ promotes neuronal apoptosis and excitotoxicity by a mechanism involving membrane lipid peroxidation and impairment of ion-motive ATPases and glucose and glutamate transporters (Mattson, 1997), PD may be caused primarily by environmental factors, although a very small percentage of inherited cases have been linked
to mutations in a synaptic protein called α-synuclein. The neurodegenerative process may be triggered in dopaminergic neurons by factors that induce oxidative stress such as iron and dopamine metabolites. Oxidative stress and perturbed calcium regulation are also intimately involved in the neurodegenerative cascades that occur in HD and stroke. Because DR increases neuronal resistance to dysfunction and death in each of the just-mentioned disorders, it seems likely that the mechanism underlying this neuroprotective effect of DR somehow impinges upon the shared features of the neurodegenerative cascades, namely, oxidative stress and perturbed calcium homeostasis.

What accounts for the ability of DR to increase resistance of neurons to a broad array of insults? We have begun to address this important question by measuring levels of expression of proteins known to confer resistance of neurons to many different insults. Two major classes of genes affected by DR include those encoding cellular stress proteins and neurotrophic factors. Measurements of levels of several different stress proteins and neurotrophic factors were made in brain tissues from rats maintained for 3 months on a DR feeding regimen in comparison with control rats fed ad libitum. Levels of heat-shock protein-70 (HSP-70) and glucose-regulated protein-78 (GRP-78) were increased in cortical, striatal and hippocampal neurons of DR rats compared to rats fed ad libitum (Duan and Mattson, 1999; Yu and Mattson, 1999; Guo et al., 2000). Levels of heat-shock protein-60 were unchanged. Previous studies in this and other laboratories had provided evidence that HSP-70 and GRP-78 can protect neurons against excitotoxic and oxidative injury (Lowenstein et al., 1991; Yu et al., 1999), suggesting that they contribute to the neuroprotective effect of DR. These findings further suggested that DR induces a mild stress response in neurons, presumably because of a decreased energy (glucose) availability to the neurons. Such a 'preconditioning' mechanism of action of DR is supported by studies showing that metabolic stress induced by administration of 2-deoxy-D-glucose (a non-metabolizable analog of glucose) to animals fed ad libitum also increases resistance of neurons to injury. For example, rats given 2-deoxy-D-glucose exhibit reduced damage to hippocampal neurons and improved learning and memory ability following kainate administration (Lee et al., 1999b), and reduced damage to cortical and striatal neurons and improved behavioral outcome following transient occlusion of the middle cerebral artery (Yu and Mattson, 1999). In addition, mice pretreated with 2-deoxy-D-glucose, exhibit decreased damage to dopaminergic neurons in the substantia nigra and marked attenuation of motor deficits (Duan and Mattson, 1999). 2-deoxy-D-glucose treatment resulted in increased levels of HSP-70 and GRP-78 in each brain region examined (Duan and Mattson, 1999; Yu and Mattson, 1999). In cultured neurons 2-deoxy-D-glucose pretreatment suppresses oxidative stress, preserves mitochondrial function, stabilizes calcium homeostasis and attenuates neuronal death following exposure to excitotoxic, metabolic and oxidative insults (Duan and Mattson, 1999; Lee et al., 1999b; Yu and Mattson, 1999; Yu et al., 1999; Guo et al., 2000).

Considerable evidence suggests that the neurodegenerative process begins when alterations occur in synaptic terminals that result in synaptic dysfunction and activation of apoptotic and excitotoxic cascades (Mattson et al., 1998). We have
found that cortical synaptosomes prepared from rats maintained on DR exhibit increased resistance to oxidative and metabolic insults, as indicated by relative preservation of glucose and glutamate transport and mitochondrial function (Guo et al., 2000). Synaptosomes from rats given 2-DG are also more resistant to various insults (Guo and Mattson, 2000). The content of HSP-70 and GRP-78 in the synaptosomes is increased suggesting that levels of neuroprotective proteins increase locally in synaptic compartments in response to DR. Thus, DR bolsters the ability of synapses to cope with the oxidative and metabolic stress associated with aging.

An intriguing aspect of the effects of DR on the brain was revealed in recent studies showing that levels of several neurotrophic factors, the most notable of which is brain-derived neurotrophic factor (BDNF), are increased in brain cells of rats and mice maintained on a DR feeding regimen (Lee et al., 2000b; Duan et al., 2001a,b). Studies performed in our laboratories during the past 12 years have documented the neuroprotective activities of several different neurotrophic factors in experimental models of neurodegenerative disorders (Mattson, 1996; Guo and Mattson, 1999). In general, the neurotrophic factors protect neurons by inducing an increase in the levels of proteins that suppress oxidative stress (antioxidant enzymes and Bcl-2) and stabilize cellular calcium homeostasis (calcium-binding proteins and glutamate receptor subunits). BDNF and other neurotrophic factors have also been shown to exert beneficial effects on synaptic plasticity and may thereby facilitate learning and memory (Jankowsky and Patterson, 1999). BDNF may play a particularly prominent role in the neuroprotective effect of DR because infusion of a BDNF blocking antibody into the lateral ventricles of rats maintained on DR significantly attenuates the protective effect of DR (Fig. 4) (Duan et al., 2001a,b).

Work performed during the past decade has established that the adult brain contains populations of cells that are capable of dividing and then differentiating into neurons or glial cells, a process called neurogenesis. In rodents and primates such neural stem cells are most abundant in the subventricular zone and the dentate gyrus of the hippocampus (Momma et al., 2000). The presence of stem cells in the adult brain suggests that they may provide a reserve of neural cells that can be used to replace cells that die as the result of various injuries and diseases. Indeed, the proliferation of neural stem cells can be stimulated by ischemic and excitotoxic brain injuries (Parent et al., 1997; Liu et al., 1998). Interestingly, an ‘enriched’ environment and physical exercise can also enhance neurogenesis (Young et al., 1999; van Praag et al., 1999) suggesting that the stem cells can respond not only to injury, but also to increased functional demands upon neural circuits. We have found that DR can modulate numbers of newly generated neural cells in the brains of rats (Lee et al., 2000b). Rats that had been maintained on ad libitum and DR (alternate day feeding) regimens for 3 months were given five daily injections of the DNA precursor bromodeoxyuridine (BrdU). Rats were killed either 1 day or 3 weeks after the last BrdU injection and numbers of newly generated cells in the dentate gyrus were quantified by unbiased stereological methods. There was no difference in numbers of newly generated cells at the 1 day time point indicating that DR did not affect the proliferation rate of the neural stem cells. However,
significantly more BrdU-positive cells remained at the 3 week time point in the DR rats compared to the ad libitum-fed rats suggesting that DR promotes survival of newly generated neural cells (Fig. 5). Although not yet established, it is conceivable that BDNF plays a role in the enhanced survival of newly-generated neural stem cells in the dentate gyrus of rats maintained on DR because BDNF is known to have a similar effect on neural stem cells (Young et al., 1999).

Molecular genetic approaches to elucidating mechanisms of aging have been successfully applied to studies of the invertebrates *C. elegans* (roundworm) and *D. melanogaster* (fruit fly). Lifespan of these organisms can be affected by mutations in two major regulatory systems, the insulin signaling pathway and antioxidant systems. Interestingly, recent studies of *C. elegans* have shown that insulin-like signaling in neurons (via a receptor tyrosine kinase called DAF-2 and a PI3 kinase called AGE-1) can impose a limit on lifespan and, accordingly, mutations in these genes can increase lifespan (Wolkow et al., 2000). Moreover, mutations that cause defects in sensory perception can extend lifespan in *C. elegans* and suggest that the mechanism may involve the insulin signaling pathway (Apfeld and Kenyon, 1999). The lifespan of *D. melanogaster* can be significantly increased by overexpressing the antioxidant enzyme Cu/Zn-SOD in motor neurons (Parkes et al., 1998), providing further evidence for an important role for the nervous system in regulation of lifespan.
What are the mechanisms whereby environmental enrichment enhances neuronal plasticity and resistance to injury? Increased plasticity might result from changes in neurotransmitter signaling. As evidence, rats raised in an enriched environment exhibit decreased ability to upregulate AMPA (a glutamate receptor agonist) binding in response to calcium in hippocampal neurons, without a change in levels of AMPA receptor subunit mRNA or protein levels (Gagne et al., 1998). Levels of several different neurotrophic factors are increased in the brains of animals maintained in enriched environments compared to control animals maintained in impoverished environments. For example, levels of BDNF and NGF are increased in cerebral cortex, hippocampus, basal forebrain and hindbrain of rats maintained in an enriched environment (Ickes et al., 2000). The latter study also showed that levels of both the high and low affinity NGF receptors are increased in the basal forebrain of rats maintained in the enriched environment. Levels of BDNF are increased in the hippocampus of rats given access to a running wheel (Russo-Neustadt et al., 1999), suggesting a role for BDNF in the beneficial effects of exercise on brain function and plasticity. Collectively, the available data suggest that DR and EE exert similar beneficial effects on neurons in the brain, and share a mechanism involving increased neurotrophic factor production (Fig. 5).

5. Calorie restriction mimetics: a substitute for a weak will?

The implementation of DR and EE for the general population is a daunting task because many individuals do not have the motivation or commitment to incorporate these behavioral changes into their daily routines. Moreover, the plague of overeating now facing our industrialized countries is largely the result of a severe addiction to food which has proven very difficult to overcome. Indeed, only a very
small percentage of overweight people are able to bring their body weight into the healthy range and then keep it there. For these reasons we have begun a series of studies aimed at identifying and characterizing compounds that limit glucose availability at the cellular level and may thereby mimick the beneficial effects of DR. As described above, many of the beneficial effects of DR are the result of either a pre-conditioning effect and/or a decreased production of reactive oxygen species due to decreased glucose availability or utilization. Our first ‘hit’ in the DR mimetic program is 2-deoxy-D-glucose (2-DG).

When rats or mice are given 2-DG via daily intraperitoneal injections (100–200 mg/kg body weight), neurons in their brains are more resistant to injury. The neuroprotective effect of 2-DG is evident in experimental models relevant to the pathogenesis of an array of disorders including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease and stroke (Duan and Mattson, 1999; Lee et al., 1999b; Yu and Mattson, 1999). Hippocampal neurons in rats given 2-DG were more resistant to damage induced by the amensetic excitotoxin kainic acid, and this increased resistance was reflected in a preserved learning and memory ability (Lee et al., 1999b). Damage to dopaminergic neurons caused by the Parkinsonian toxins MPTP and rotenone was significantly decreased in mice given 2-DG, and this neuroprotection was correlated with an amelioration of motor dysfunction (Duan and Mattson, 1999); similar beneficial effects of 2-DG were obtained in a model of Huntington’s disease (author’s unpublished data). Transient occlusion of the middle cerebral artery in rats results in focal ischemic damage to neurons in the cerebral cortex and striatum supplied by that blood vessel. Rats given 2-DG exhibit a greatly reduced amount of cortical and striatal damage and improved behavioral outcome in this stroke model (Yu and Mattson, 1999). More recently we have found that 2-DG can be incorporated into the food of mice and rats and, at an amount that has no obvious side effects (approximately 1 mg 2-DG per kcal of energy intake) can decrease neuronal damage and improve behavioral outcome in the different models of neurodegenerative disorders just described (manuscripts submitted and in preparation).

The mechanism whereby 2-DG supplementation increases resistance of neurons to injury appears to be similar to that of DR. Thus, levels of the stress proteins HSP-70 and GRP-78 are increased in neurons of rats and mice given 2-DG (Logroscino et al., 1996; Duan and Mattson, 1999; Lee et al., 1999b). Moreover, when synaptosomes were isolated from the cerebral cortex of rats given 2-DG, they exhibited increased resistance to dysfunction caused by oxidative stress and amyloid β-peptide (Guo and Mattson, 2000). The ability of the synapses to maintain glucose and glutamate transport, and to maintain mitochondrial function, were greatly improved in rats given 2-DG. Interestingly, levels of HSP-70 and GRP-78 were increased in synaptosomes from rats given 2-DG, suggesting that 2-DG protects the synaptic terminals by increasing levels of proteins that stabilize proteins and maintain organellar function. The latter findings in animals given 2-DG are similar to findings in studies of synaptosomes from animals maintained on DR (Guo et al., 2000). The ability of 2-DG to protect synaptic
Fig. 6. The effects of 2-deoxy-D-glucose, a prototypical calorie restriction mimetic dietary supplement, on body weight and food intake in mice. Mice were maintained on the usual diet or the same diet supplemented with 0.4% 2-DG (w/w). Body weights and food intake were recorded weekly. Note that the mice fed the diet containing 2-DG ate more for several months after initiation of the diet and exhibited a slightly greater body weight during that time period.
terminals against insults relevant to the pathogenesis of age-related neurodegenerative disorders suggest that dietary supplementation with 2-DG may prove effective in reducing risk for neurodegenerative disorders.

Might dietary supplementation with 2-DG also retard the aging process and extend lifespan? While the jury is still out on this important issue, preliminary data look promising. Rats maintained for 6 months on a diet supplemented with 2-DG exhibit changes in biomarkers of aging similar to those observed in rats maintained on DR (Lane et al., 1998). These changes include a decreased body temperature, decreased insulin levels and a modest decrease in blood glucose levels. Animals fed a 2-DG-supplemented diet increase their food intake for several weeks after initiation of the diet, and then normalize to the level of food intake of animals in the control group (Fig. 6). Interestingly, dietary supplementation with 2-DG causes no decrease in body weight in mice (Fig. 6), which contrasts with the decreased body weight of mice maintained on DR. A study is currently underway to determine whether lifelong dietary supplementation with 2-DG will extend lifespan in rats, and the question should be answered within the next 18 months. The search for DR mimetic compounds that can be incorporated into the diet is expanding. The agents being examined impact at various points in the pathways that control energy metabolism in cells, and preliminary data suggest that several other compounds can mimic the neuroprotective effects of DR and 2-DG.

6. Conclusions

In many different experimental animal models DR increases resistance of neurons to the kinds of adverse conditions believed to promote the neurodegenerative process. Findings from animal studies are supported by epidemiological data, and together strongly suggest that reduced calorie intake increases resistance of the nervous system to disease. DR may exert its beneficial effects by inducing a mild ‘stress response’ which results in the expression of genes that encode proteins such as neurotrophic factors and heat-shock proteins that serve to suppress oxyradical production and stabilize cellular calcium homeostasis. EE appears to activate a similar neuroprotective mechanism, likely as the result of the mild stress associated with increased activity in neuronal circuits. When extrapolated to humans, the data obtained from animal studies suggest that DR (in the range of 1800–2200 calories per day) and EE (daily engagement in intellectual activities and exercise) may dramatically reduce the incidence and severity of age-related disorders of the nervous system including AD, PD and stroke. For individuals that are unable to maintain the strict dietary and behavioral regimentation required for successful implementation of DR and EE, dietary supplementation with DR mimetic compounds such as 2-DG may prove effective, although this remains to be established in humans.
References


