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Berry Fruit Supplementation and the Aging Brain

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The onset of age-related neurodegenerative diseases such as Alzheimer's or Parkinson's disease, superimposed on a declining nervous system, could exacerbate the motor and cognitive behavioral deficits that normally occur in senescence. In cases of severe deficits in memory or motor function, hospitalization and/or custodial care would be a likely outcome. This means that unless some way is found to reduce these age-related decrements in neuronal function, health-care costs will continue to rise exponentially. Thus, it is extremely important to explore methods to retard or reverse age-related neuronal deficits, as well as their subsequent behavioral manifestations, to increase healthy aging. In this regard, consumption of diets rich in antioxidants and anti-inflammatory polyphenolics, such as those found in fruits and vegetables, may lower the risk of developing age-related neurodegenerative diseases. Research suggests that the polyphenolic compounds found in berry fruits, such as blueberries and strawberries, may exert their beneficial effects either through their ability to lower oxidative stress and inflammation or directly by altering the signaling involved in neuronal communication, calcium buffering ability, neuroprotective stress shock proteins, plasticity, and stress signaling pathways. These interventions, in turn, may exert protection against age-related deficits in cognitive and motor function. The purpose of this paper is to discuss the benefits of these interventions in rodent models and to describe the putative molecular mechanisms involved in their benefits.

KEYWORDS: Berries; supplementation; brain; aging; cognition; antioxidants; anti-inflammatories; polyphenols; signaling

INTRODUCTION

There are numerous motor (*1, 2*) and cognitive (*3*) behavioral deficits that occur during aging, even in the absence of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. The alterations in motor function include decreases in balance, muscle strength, and coordination (*1*), whereas memory deficits are seen on cognitive tasks that require the use of spatial learning and memory (*4, 5*). These decrements have been reported in numerous studies in animals (*3–5*) and humans (*6, 7*). Age-related deficits in motor performance are thought to be the result of alterations in the striatal dopamine (DA) system (*8*) or in the cerebellum (*9, 10*), with both structures showing significant changes with age. Alterations in cognition appear to occur primarily in secondary memory systems and are reflected in the storage of newly acquired information (*8, 11*). It is thought that the hippocampus mediates allocentric spatial navigation (i.e., place learning), the dorsomedial striatum mediates egocentric spatial orientation (i.e., response and cue learning), and the prefrontal cortex is critical to acquiring the rules that govern performance in procedural knowledge (*12–15*).

Although the mechanisms involved in the behavioral deficits during aging remain to be discerned, it is clear that oxidative stress (OS) (*16*) and inflammation (*17, 18*) are involved. Increased susceptibility to the long-term effects of OS and inflammatory insults are thought to be contributing factors to the decrements in cognitive and/or motor performance seen in aging and other neurodegenerative diseases. Deficits in brain functions due to OS may be due, in part, to a decline in the endogenous antioxidant defense mechanisms (*19–24*) and the vulnerability of the brain to the deleterious effects of oxidative damage (*25*). Research also indicates that not only is the central nervous system (CNS) particularly vulnerable to OS, but this vulnerability increases during aging (see refs *26* and *27* for reviews) and may also enhance central vulnerability to inflammation (*26, 28*). With age, there are increases in inflammatory mediators (e.g., cytokines) (*29–31*), as well as increased mobilization and infiltration of peripheral inflammatory cells, which have been shown to produce deficits in behavior similar to those observed during aging (*17*). Furthermore, age-related changes in brain vulnerability to OS and inflammation may be the result of membrane changes and differential receptor sensitivity (*32*).

REDUCING THE VULNERABILITY IN AGING

Therefore, the key to reducing the incidence of age-related deficits in behavior might be to alter the neuronal environment such that neuroinflammation and oxidative stress, and the vulnerability to them, are reduced. Although there are numerous studies suggesting that various antioxidant supplements can be

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effective in antagonizing the behavioral deficits seen in aging (see ref *33* for a review), research from our laboratory and several others suggests that the combinations of antioxidant/anti-inflammatory polyphenolics found in fruits and vegetables may show efficacy in aging. Plants, including food plants (fruits and vegetables), synthesize a vast array of secondary chemical compounds that, although not involved in their primary metabolism, are important in serving a variety of ecological functions that enhance the plant's survivability. Interestingly, these antioxidant/anti-inflammatory compounds may be responsible for the multitude of beneficial effects that have been reported for fruits and vegetables on an array of health-related bioactivities. Polyphenolics are one class of phytochemicals of which over 4000 different structures have been identified (*34*), and these occur ubiquitously in foods of plant origin (e.g., fruits, vegetables, nuts, seeds, grains, tea, and wine). Examples of polyphenolic families include phenolic acids, stilbenes, coumarins, tannins, and flavonoids. Polyphenolic compounds have been recognized to possess many biological properties including antioxidant, antiallergic, anti-inflammatory, antiviral, antiproliferative, antitumorigenic, antianxiety, and anticarcinogenic (*35–37*). However, past interest in these compounds, with respect to their health-promoting benefits, has primarily been focused on examining their roles in protection against the incidence and mortality rates of cancer and ischemic heart disease, by reducing risk factors associated with these diseases (*36*). Consequently, there are few studies that have investigated their role with regard to the CNS, such as their effects on brain function and behavior. This paper will review studies which have shown that dietary supplementation with fruits, vegetables, or nuts can prevent or reverse decrements in brain and behavioral aging.

BENEFICIAL EFFECTS OF POLYPHENOLICS ON BEHAVIORAL AND NEURONAL DEFICITS IN AGING

Fruits and vegetables are known to contain numerous phytochemicals, and until recently, their beneficial health effects on brain function had not been scientifically studied. Although initially it was assumed that the vitamin component of fruits and vegetables was the primary source of dietary antioxidants, it is now well established that the phytochemical components also contribute substantially to the overall dietary antioxidant intake (*38*). Therefore, recently there has been an increasing interest in the beneficial effects of polyphenolic nutritional antioxidants on combating the deleterious effects of oxidative stress and inflammation in aging and age-related neurodegenerative diseases (*39–43*). Furthermore, epidemiological studies have shown that nutritional antioxidants may forestall the onset of dementia (*44*) and may provide protection against stroke (reviewed in refs *45* and *46*). The question arises, however, as to which of the nutritional antioxidants may be the most effective. It appears that some of the most beneficial effects can be derived from the large class of polyphenols known as flavonoids. A subset of the flavonoids known as anthocyanins are particularly abundant in brightly colored fruits such as berry fruits and Concord grapes and grape seeds. Anthocyanins are responsible for the colors in the fruits, and they have been shown to have potent antioxidant/anti-inflammatory activities (*47–49*), as well as to inhibit lipid peroxidation and the inflammatory mediators cyclooxygenase (COX)-1 and -2 (*50, 51*).

Fruit and vegetable extracts that have high levels of flavonoids also display high total antioxidant activity, as assessed via the oxygen radical absorbance capacity assay (ORAC) (*52*); these include spinach and strawberries (*53, 54*) and blueberries (*55*). Fruits and vegetables have antioxidant properties, and increases in oxidative stress combined with declines in antioxidant defense mechanisms have been postulated as causative factors in age-related decrements in behavior. Therefore, our laboratory, together with colleagues at the University of Colorado, decided to study the effects of fruit and vegetable supplementation for their ability to forestall or reverse age-related changes in cognitive and motor performance (*56–60*).

In our first study, Fischer 344 (F344) rats underwent long-term feeding from adulthood (6 months) to middle age (15 months) with a control diet (AIN-93) or diets supplemented with vitamin E (500 IU/kg of diet) or with extracts of strawberry or spinach that contained identical antioxidant content (based upon millimoles of Trolox equivalents) to determine if the feeding would prevent age-related decrements in motor and cognitive behavior as well as brain function (*59*). A number of different parameters known to be sensitive to oxidative stress were prevented by the antioxidant diets including (1) receptor sensitivity, as measured by oxotremorine-enhanced dopamine (DA) release in isolated striatal slices and cerebellar Purkinje cell activity; (2) calcium buffering capacity, that is, the ability of striatal synaptosomes to extrude calcium following depolarization, deficits of which ultimately result in reduced cellular signaling and eventually cell death; (3) changes in signal transduction assessed by carbachol-stimulated GTPase coupling/uncoupling in striatal membranes; and (4) cognition (spatial learning and memory) as measured by Morris water maze (MWM) performance (*59*). Spinach-fed rats demonstrated the greatest retardation of age-effects on all parameters except GTPase activity, for which strawberry had the greatest effect; strawberry and vitamin E showed significant but equal protection against these age-induced deficits on the other parameters.

Our subsequent experiments (*56–58*) found that dietary supplementations (for 8 weeks) with spinach, strawberry, or blueberry extracts in a control diet (AIN-93) were also effective in reversing age-related deficits in brain and behavioral function in aged (19 months) F344 rats. Whereas all of the supplemented diets showed positive effects on cognitive behavior, rats on the blueberry diet showed the greatest increases in motor performance, carbachol-stimulated GTPase activity, and oxotremorine-enhanced DA release (*58*). Additionally, the blueberry-fed group showed no decrements in calcium recovery following exposure to an oxidative stressor (*58*). Interestingly, blueberries were added to this study because they were found to have the highest antioxidant capacity (ORAC) of all fruits and vegetables tested (*55*). Yet, even though these diets were supplemented on the basis of equal antioxidant activity, as determined by the ORAC assay (*53*), they were not equally effective in preventing/reversing age-related changes. Therefore, it seems that antioxidant activity alone was not predictive in assessing the potency of these compounds against certain disorders affected by aging. In fact, oxidative stress markers (as measured by DCF fluorescence and glutathione levels in the brain) were only modestly reduced by the diets (*58*), suggesting that these fruit and vegetable polyphenolics possess a multiplicity of actions, aside from antioxidative, and that differences in the polyphenolic composition of these extracts could account for the positive effects observed.

A more recent study has suggested that, in addition to Morris water maze performance, cognitive declines in object recognition were effectively reversed by blueberry supplementation (*61*). Furthermore, the beneficial effects of blueberries were seen even when superimposed on an already well-balanced, healthy rodent diet ("chow"), which was more representative of a balanced human diet (*60*).

In the case of motor performance, research has suggested that blueberry supplementation improved performance of aged animals on tests of motor function that assessed balance and coordination (e.g., rod walking and the accelerating rotarod), whereas none of the other supplemented groups (e.g., spinach) differed from control on these tasks (*58*). However, in subsequent experiments, we have shown that the cognitive/neuronal variables are sensitive to a greater number of fruits than those seen with respect to motor behavior. Thus far, only blueberries, cranberries (*62*), Concord grape juice (*63*), and strawberries (*64*) have been effective in reversing the motor behavioral deficits. This result may be due to the brain region selectivity of the polyphenolic compounds from the various fruits and vegetables. Evidence for this hypothesis was seen in a recent study in which young rats (3 months of age) were given a control diet or one supplemented with either blueberry or strawberry extracts (2% for 8 weeks) and then exposed to whole-body irradiation with 1.5 Gy of 1 GeV/n high-energy ^{56}Fe particles (*65*). We had previously shown that these irradiations produce deficits in cognitive and motor behavior similar to those seen with respect to aging. In this study, strawberry or blueberry supplementations protected rats from radiation-induced deficits. Interestingly, the strawberry diet offered better protection against spatial deficits in the maze, because strawberry-fed animals were better able to retain place information (a hippocampally mediated behavior) on probe trials (when the platform was removed from maze) compared to controls. The blueberry diet, on the other hand, seemed to improve reversal learning (i.e., when the platform was moved from one quadrant to another in the Morris water maze), a behavior more dependent on intact striatal function. Therefore, it appears that the polyphenolic compounds in these fruits might be acting in different brain regions to produce their beneficial effects (*65*). However, it also seems that the beneficial effects of these supplementations may involve more than decreased sensitivity to oxidative stress or inflammation.

POSSIBLE MECHANISMS IN THE BENEFICIAL EFFECTS OF POLYPHENOLICS

Subsequent findings from our group and others have suggested that in addition to having antioxidant and anti-inflammatory effects, other possible mechanisms for the berry fruit's positive effects include the following: direct effects on signaling to enhance neuronal communication (*66*), the ability to buffer against excess calcium (*67*), enhancement of neuroprotective stress shock proteins (*68*), and reduction of stress signals such as nuclear factor kappa B (NF-κB) (*61*). Additionally, the anthocyanins contained in blueberries have been shown to enter the brain, and their concentrations were correlated with cognitive performance (*69*). Measures of hippocampal plasticity, including neurogenesis, were also enhanced in rats on a blueberry-enriched diet (*70*). Additionally, extracellular signal regulated kinase (ERK) activation and insulin-like growth factor-1 (IGF-1) were increased in the supplemented animals, and IGF-1 receptor levels positively correlated with improvements in spatial memory (*70*). Furthermore, the potential anti-inflammatory actions of polyphenolics are supported by a number of studies that have shown them to be able to antagonize arachidonic acid transport (*71*), suppress the 5-lipoxygenase pathway (*72*), and thus reduce inflammatory responses (*73, 74*). Polyphenolics have also been shown to regulate signal transduction processes/transcription factors involved in the regulation of inflammatory genes (*75*).

In general, the antioxidant effects of flavonoids also appear to involve transcriptional up-regulation of antioxidant enzymes related to glutathione synthesis and/or glutathione. The enzymes for glutathione (*76, 77*) or heme-oxygenase (*78*) synthesis are dependent on ERK 1/2, and gene expression of inducible nitric oxide synthase (iNOS) activity is regulated through ERK (*76*). Additionally, there is evidence that a possible direct link exists between the antioxidant activity of flavonoids and their putative mitogen-activated protein (MAP) kinase altering activity. Such direct effects would greatly expand the potential antioxidant/anti-inflammatory properties of the flavonoids to include direct mediation of cell signaling in a variety of functions. As examples, delphinidin inhibits endothelial cell proliferation and cell cycle progression by ERK 1/2 activation (*79*). Additional research indicates that phytochemicals can regulate MAP kinase and other signaling pathways at the level of transcription (*80*). Given the numerous studies showing the involvement of ERK in diverse forms of memory (*81–84*), these findings suggest that the putative signal-modifying properties of flavonoids may prove to be invaluable in altering the neuronal and behavioral effects of aging.

The importance of the signaling properties of the blueberries to functional measures was shown in a study from our laboratory in which APP/PS1 transgenic mice, given blueberry supplementation beginning at 4 months of age and continued until they were 12 months of age, exhibited greater Y-maze performance, as measured by increased alternation behavior, compared to the nonsupplemented transgenic animals and behavior similar to that seen in nontransgenic mice (*66*). Interestingly, no differences were seen between the supplemented and nonsupplemented APP/PS1 mice in the number of plaques, even though behavioral declines were prevented in the blueberry-supplemented animals (*66*). Further analyses of the data indicated that the blueberry-supplemented APP/PS1 mice exhibited enhancement of GTPase activity and greater levels of hippocampal ERK, as well as striatal and hippocampal protein kinase Cα (PKC), than those seen in the transgenic mice maintained on the control diet (*66*). Two of the most important functions of PKC may be the regulation of synaptic plasticity and modulation of short- to long-term memory. Studies have shown that PKC activity is important in memory formation, particularly spatial memory (see ref *85* for a review) and that treatment with PKC inhibitors impairs memory formation (*86*). Another study conducted in our laboratory the following year, this time in aged animals, confirmed that blueberries increased ERK and that these increases in ERK were positively correlated with increased neurogenesis (*70*). Therefore, it appears from these studies and others that at least part of the beneficial effects of the berry fruit supplementation on behavior in the aged animals may involve enhancements of cell signaling associated with learning and memory.

CONCLUSIONS

Therefore, it is evident that oxidative stress and inflammation are major sources contributing to the deleterious effects of aging and the development of age-related neurodegenerative diseases and that the numerous natural antioxidants/anti-inflammatories found in plant food matrices, such as fruits and vegetables, possess neuroprotective, as well as cardioprotective and chemoprotective, properties (*87–93*). Moreover, it appears that berry fruit, such as blueberries and strawberries, may exert their effects directly through alterations in cell signaling to improve or increase neuronal communication, calcium buffering ability, neuroprotective stress shock proteins, plasticity, and stress signaling pathways. These alterations, and others that are being studied, may be mediating the enhancements in cognitive and motor behavioral performance in berry fruit-supplemented

senescent animals. Thus, nutritional interventions containing polyphenolics, such as berry fruits, may prove to be a valuable asset in strengthening the brain against the ravages of time as they could retard or prevent the development of age-related neurodegenerative diseases. Early nutritional interventions may even prevent or delay the onset of diseases such as Alzheimer's disease, because they can reduce oxidative stress and inflammation superimposed upon a stress-vulnerable aging brain.

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