Journal of Neuroscience Research 63:395–401 (2001)

Role of Spermine in Amyloid β-Peptide-Associated Free Radical-Induced Neurotoxicity

Servet M. Yatin,[1] Mustafa Yatin,[2,3] Sridhar Varadarajan,[1] Kenneth B. Ain,[2,3] and D. Allan Butterfield[1,4]★

[1]Department of Chemistry and Center of Membrane Sciences, University of Kentucky, Lexington
[2]Thyroid Cancer Research Laboratory, Medical Service, Veterans Affairs Medical Center, Lexington, Kentucky
[3]Endocrinology and Molecular Medicine, University of Kentucky, Lexington
[4]Sanders-Brown Center on Aging, University of Kentucky, Lexington

The polyamines, relatively low-molecular-weight aliphatic compounds, are the main inducers of eukaryotic cell growth and proliferation. Although polyamine requirements for cell growth are well defined, their role is still enigmatic. We have previously reported that amyloid β-peptide (Aβ), the main constituent of senile plaques in Alzheimer's disease (AD) brain, is toxic to neurons through a free radical-dependent oxidative stress mechanism and that Aβ(1–42), the principal form of Aβ in AD brain, causes an increase in polyamine metabolism manifested by up-regulated polyamine uptake and increased ornithine decarboxylase (ODC) activity. Both effects were prevented by the free radical scavenger vitamin E. Spermine has been reported to function directly as a free radical scavenger. In the current study, we aimed to address whether up-regulation of polyamine metabolism is a defense against, or a result of, Aβ-induced oxidative stress by investigating the capability of spermine to quench Aβ-associated free radicals in solution and to assert a protective function of spermine in neuronal culture against Aβ. Pretreatment of cultured neurons with spermine prior to Aβ exposure failed to prevent Aβ-induced cell death. Indeed, Aβ plus spermine added to cultured neurons was even more neurotoxic than either agent alone. Additionally, inhibition of the polyamine synthesis by difluoromethylornithine (DFMO) did not protect cells from Aβ-induced free radical toxicity, and stimulation of the synthesis of putrescine and spermine by the aminopropyltransferase inhibitor S-adenosyl-1,8-diamino-thiooctane (AdoDATO), rather, further enhanced Aβ-induced toxicity. Although spermine is capable of scavenging free radicals generated by Aβ in solution as measured by electron paramagnetic resonance (EPR) spectroscopy, the up-regulated transport of exogenously added spermine together with Aβ may lead to overaccumulation of a cellular spermine pool, with resulting enhanced neurotoxicity. J. Neurosci. Res. 63:395–401, 2001.
© 2001 Wiley-Liss, Inc.

Key words: Alzheimer's disease; electron paramagnetic resonance; ornithine decarboxylase; oxidation; polyamine

The polyamines putrescine, spermidine, and spermine are a family of relatively low-molecular-weight biologic amines that are protonated under physiological conditions and are essential constituents of all mammalian cells. Although the pathways of polyamine biosynthesis have been well characterized and are a tightly controlled process (see Fig. 1), the role of polyamines in cellular growth is not fully known. Polyamines influence cellular processes at all stages from gene transcription to protein synthesis, stabilize nucleic acid conformations, and are central to regulation of cell growth and differentiation (Marton and Pegg, 1995). The composition and levels of polyamine pools are extremely vulnerable to the minute changes in chemical and physical conditions of the cellular environment.

Amyloid β-peptide (Aβ) is a 42- or 43-amino-acid-long peptide that plays a central role in the pathogenesis of Alzheimer's disease (AD; Selkoe, 1989). It has been reported by us and others that Aβ(1–42) and its 11-amino-acid subset, Aβ(25–35), are toxic to neurons through a free radical-dependent oxidative stress mechanism (Hensley et al., 1994; Behl et al., 1994; Harris et al., 1995; Butterfield, 1997; Mark et al., 1997; Subramaniam et al., 1998; Yatin et al., 1998, 1999a–c, 2000) and are responsible for up-regulated polyamine metabolism, that is, increased polyamine uptake and elevated ornithine decarboxylase (ODC) activity (Yatin et al., 1999b). Oxygen free radicals, particularly hydroxyl radical, were also re-

Contract grant sponsor: NIH; Contract grant number AG-10836; Contract grant number: AG-05119; Contract grant sponsor: NCI; Contract grant number: CA58935; Contract grant sponsor: VA; Contract grant number: 596-003. Servet M. Yatin is now at NERPRC/Neurochemistry, Harvard Medical School, Southborough, MA 01772-9102.

★Correspondence to: Prof. D. Allan Butterfield, Department of Chemistry, Center of Membrane Sciences and Sanders-Brown Center on Aging, 121 Chemistry-Physics Building, University of Kentucky, Lexington, KY 40506-0055. E-mail: dabcns@pop.uky.edu

Received 25 September 2000; Revised 21 November 2000; Accepted 22 November 2000

© 2001 Wiley-Liss, Inc.

ported to trigger induction of ODC activity, and the consequent increase of polyamine synthesis has been reported to have profound effects on neurogenesis and neurodifferentiation in the developing brain (Saito et al., 1997). The mechanism for increased endogenous polyamine levels in response to Aβ-induced oxidative stress is unknown, and insight into this mechanism is confounded by conflicting reports that these compounds may cooperate in the recovery process of oxidatively damaged neurons or that they may play a direct role in neurodegenerative processes (Slotkin and Bartolome, 1986; Gilad and Gilad, 1991; Gilad et al., 1993; Farbiszewski et al., 1996; Sparapani et al., 1997). The blockade of polyamine biosynthesis at various pathways was reported to be neuroprotective against neuronal cell damage (Dempsey et al., 1988; Dogan et al., 1999a,b). In contrast, pretreatment with polyamines was also reported as a rescuing strategy to prevent cells from dying after the infliction of various types of neurotrauma (i.e., mechanical injuries, neurotoxic insults, and ischemia; Farbiszewski, 1995; Gilad and Gilad, 1999). It is also reported that polyamines may act as substrates in an oxidative pathway that activates programmed cell death (Coffino and Poznanski, 1991), and, in a conflicting study, polyamines were reported to prevent apoptotic cell death (Harada and Sugimoto, 1997). Like all aliphatic amines, the polyamines spermine, spermidine, and putrescine are physical quenchers of most reactive oxygen species (ROS; Khan et al., 1992a,b; Matkowics et al., 1993); however, the unregulated accumulation of polyamines in injured neurons may overcome this protective effect and make polyamines toxic agents for the cells.

Among the three polyamines, spermine, having the largest number of amine groups, is believed to be particularly important in terms of regulation of gene expression, stabilization of chromatin, and prevention of endonuclease-mediated DNA fragmentation (Muscari et al., 1995). It was reported that spermine functions directly as a free radical scavenger (Ha et al., 1998), and it was hypothesized that the antioxidative effect of spermine may be due to metal chelation and/or to prevention of superoxide generation from stimulated neutrophils (Lovaas, 1995). In the current study, given the divergent reports of spermine as an antioxidant or prooxidant (Kauppila, 1992; Lovaas, 1995; Ha et al., 1998; Dogan et al., 1999a,b; Gilad and Gilad, 1999), we investigated the capacity of spermine to neutralize the effect of Aβ(1–42)-generated free radicals. Further, we studied the extent of the neuroprotective effect of spermine in oxidatively stressed rat embryonic hippocampal neuronal cultures by manipulation of intracellular polyamine levels, employing difluoromethylornithine (DFMO) and S-adenosyl-1,8-diamino-thiooctane (AdoDATO; Fig. 1). Exogenously introduced spermine was also tested as a neuroprotective agent.

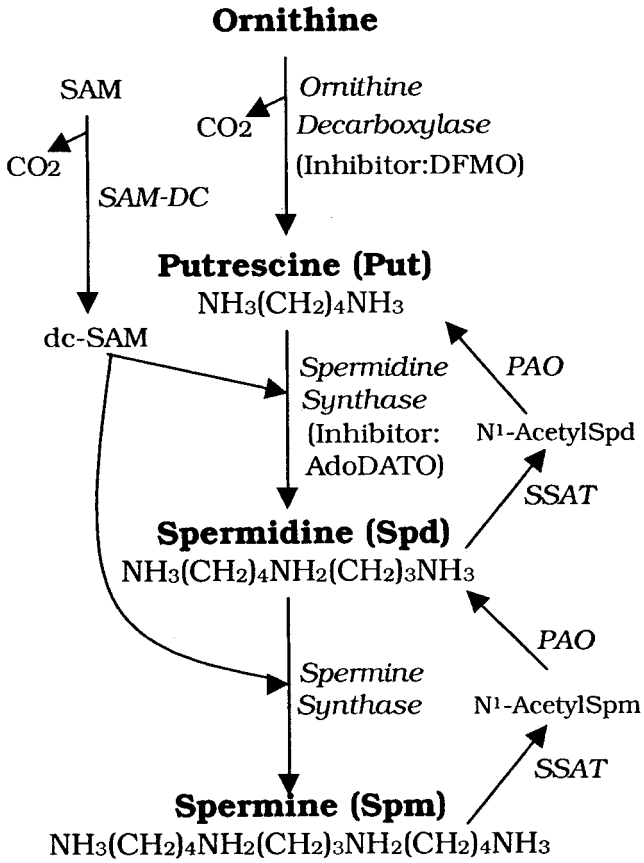

Fig. 1. Polyamine biosynthesis/interconversion pathway. The enzymes, substrates, and inhibitors involved are ornithine decarboxylase (ODC), the rate-limiting enzyme in the polyamine biosynthesis, catalyzes the conversion of ornithine to putrescine; spermidine synthase catalyzes the conversion of putrescine to spermidine; spermine synthase catalyzes the conversion of spermidine to spermine; spermidine/spermine N^1-acetyltransferase (SSAT) catalyzes the conversion of spermine and spermidine into N^1-acetylated forms, that are substrates for polyamine oxidase (PAO); PAO catalyzes the conversion of N^1acetylspermine and N^1acetylspermidine into spermidine and spermine subsequently; s-adenosylmethionine decarboxylase (SAM-dc) catalyzes the conversion of s-adenosylmethionine into its decarboxylated form, which is the substrate for spermidine and spermine synthase. Difluoromethylornithine (DFMO) is the specific inhibitor of ODC, and S-adenosyl-1-8-diamino-thiooctane (AdoDATO) is the inhibitor of spermidine synthase.

MATERIALS AND METHODS

Drugs and Aβ Treatment

Synthetic Aβ(1–42) (AnaSpec, San Jose, CA; lots 5811 and 7266) was dissolved in double-distilled water immediately before use at a concentration of 1 mg/ml. This stock solution was added to cultures to produce a final concentration of 10 μM. Twenty-four hour pretreatment with 5.0 μM spermine, 1.0 μM AdoDATO, or 2.0 mM DFMO was performed in growth medium; concentrations were chosen from the literature (Pegg et al., 1982, 1995; Holm et al., 1989; Yatin et al., 1999d,e). In selected experiments, spermine-treated cells were cultured with the addition of 1.0 mM aminoguanidine to verify the absence of interference from serum amine oxidases possibly present in the fetal bovine serum, B27, and N2 (Life Technologies, Bethesda, MD).

Spermine (tetrachloride salt) was obtained from Sigma (St. Louis, MO). DFMO was a generous gift of the Merrel Research

Center (Hoechst-Marion Roussel Pharmaceuticals, Cincinnati, OH). AdoDATO was designed, synthesized, and generously supplied by James K. Coward (Professor of Medicinal Chemistry, University of Michigan). All other chemicals used were of highest purity.

Cell Culture

Neuronal cultures were prepared from Sprague-Dawley (SD) 18 day rat fetuses as described previously (Yatin et al., 1998, 1999a,b) and were grown in B27- or N2-containing neurobasal medium (Gibco, Grand Island, NY). Cultures, maintained at 37°C in a 5% CO^2/95% room air-humidified incubator, were used for the experiments between 9 and 11 days. In B27/neurobasal medium, glial growth is reported to be less than 0.5% of the nearly pure neuronal population (Brewer et al., 1993).

Assessment of Neuronal Survival

Neuronal survival was evaluated by two different assays, the MTT tetrazolium colorimetric assay and trypan blue exclusion, as previously described (Yatin et al., 1998, 1999b).

EPR Spectroscopy and Spin Trapping

Ultrapure N-tert-butyl-α-phenylnitrone (PBN) was dissolved in PBS buffer (150 mM NaCl, 5 mM sodium phosphate buffer, pH 7.4) over chelex-100 beads and containing the iron chelator deferoxamine. Aβ(1–42) was dissolved in the PBN solution and divided into two aliquots. A solution of 1.0 mM spermine, resulting in a 500 μM final spermine concentration, was added to one of the aliquots. The final peptide concentration in each of the samples was 500 μM. The above-described samples and a control solution containing only PBN and deferoxamine mesylate were incubated at 37°C for 48 hr, and EPR spectra were recorded. EPR parameters were as follows: microwave power = 20 mW, modulation frequency = 100 kHz, modulation amplitude = 0.3 G. The PBN used was repeatedly recrystallized and resublimed and was shown to be pure by HPLC and NMR analyses and by the absence of an EPR spectrum even after addition of Fe^{3+} (Varadarajan et al., 1999).

Cellular Polyamine Levels

Cellular polyamine contents were determined as described previously (Morgan, 1998; Yatin et al., 1999b). Briefly, cells were washed with ice-cold phosphate-buffered saline solution, lysed, harvested in 500 μl of 0.2 M $HClO_4$, and disrupted for 20 min by ultrasonification. Sonicates were left at 4°C overnight, then centrifuged at 40,000*g* for 20 min. One hundred microliters of saturated Na_2CO_3 and 500 μl of dansyl chloride (10 mg/ml acetone) were added to each 400 μl supernatant. This was mixed vigorously for 30 sec, wrapped in foil, and incubated for 10 min at 70°C. The dansylated polyamines were loaded onto Bond Elute Columns (Sep-Pak Cartridges; Waters Corp., Milford, MA) preconditioned with methanol-water washes. Dansylated polyamine mixes were eluted from each column with 750 μl methanol to separate unreacted polyamine and dansyl chloride substrates. Aliquots (50 μl) were loaded on an Ultrasphere C18 Nova-Pak HPLC column (Waters Corp.) and the dansylated polyamines eluted with a linear gradient of 10% acetonitrile:methanol:water (5:3:2) to acetonitrile:methanol (10:40) and quantitated with a Shimadzu model RF-535 fluorescence detector (Shimadzu Scientific Instruments, Inc., Columbia, MD), providing a 1.0 pmol detection limit for dansylated polyamines. The polyamine contents were normalized in terms of protein content as measured by the Pierce BCA protein assay (BCA; Pierce Chemical Co., Rockford, IL).

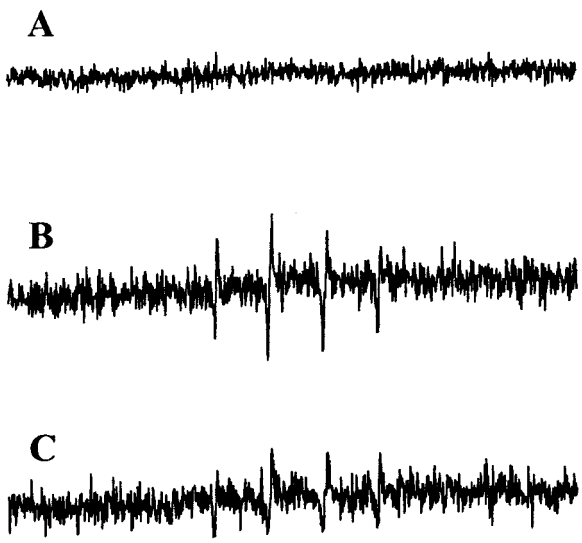

Fig. 2. Electron paramagnetic resonance (EPR) spectra of free radical spin adducts with spin trap PBN (50 mM) after 48 hr of incubation at 37°C. **A:** PBN/PBS control after 48 hr of incubation at 37°C showing no EPR signal. **B:** Aβ(1–42) (500 μM) in chelexed PBN/PBS solution after 48 hr of incubation at 37°C. **C:** Aβ(1–42) (500 μM) plus spermine (1.0 mM) in chelexed PBN/PBS solution after 48 hr of incubation at 37°C.

Statistical Analysis

The statistical significance of differences between treated cells vs. control and Aβ-treated cells vs. Aβ + spermine or Aβ + DFMO, or Aβ + AdoDATO were assessed by single factor analysis of variance (ANOVA) followed by Dunnett's test (Sigma Plot 3.0; Jandel Scientific, San Rafael, CA).

RESULTS

In spin trapping, a transient free radical reacts with a nonparamagnetic nitrone (PBN) to form a paramagnetic spin adduct that can be detected by EPR. The presence of EPR signal is prima facia evidence that a free radical was present (Butterfield, 1997; Butterfield et al., 1999), and a change in the intensity of the signal is evidence of a change in the scavenged free radical content (Ha et al., 1998; Yatin et al., 1998; Butterfield et al., 1999). Consistent with previous studies in our laboratory and that of others (Yatin et al., 1999a,b; Huang et al., 1999; Varadarajan et al., 2000), Figure 2B shows the presence of the free radicals in Aβ(1–42) solution; however, 500 μM spermine containing Aβ(1–42) solution shows a decrease in the signal intensity (Fig. 2C), suggesting that spermine may have a free radical scavenging property, although not all Aβ(1–42)-associated free radicals are quenched. The spin trap

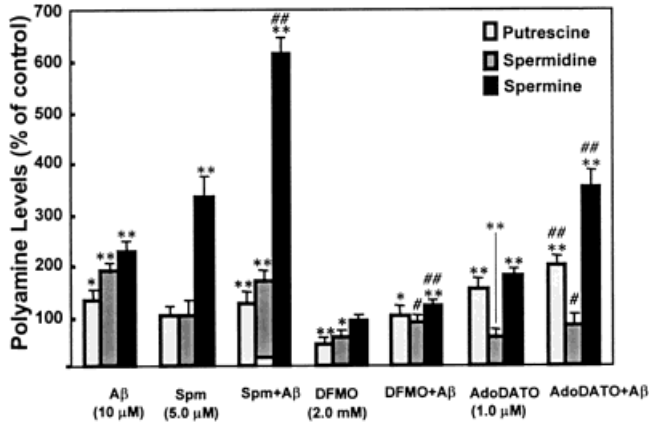

Fig. 3. Percentage variation of intracellular polyamine levels upon treatment with 10 μM Aβ(1–42), 5.0 μM spermine, 2.0 mM DFMO, 1.0 μM AdoDATO, and combination of these agents with Aβ-peptide. Aβ(1–42) itself increases polyamine levels and is able to increase further the already elevated polyamine levels, as in spermine + Aβ and AdoDATO + Aβ. Statistical comparisons were made using ANOVA followed by Dunnett's test. Error bars represent SEM values. Significant differences for treated cells vs. control $^{\star}P < 0.01$, $^{\star\star}P < 0.005$; and significant differences for Aβ alone vs. Aβ + spermine or Aβ + DFMO or Aβ + AdoDATO $^{\#}P < 0.01$, $^{\#\#}P < 0.005$ (n = 2; each n represents four replicates).

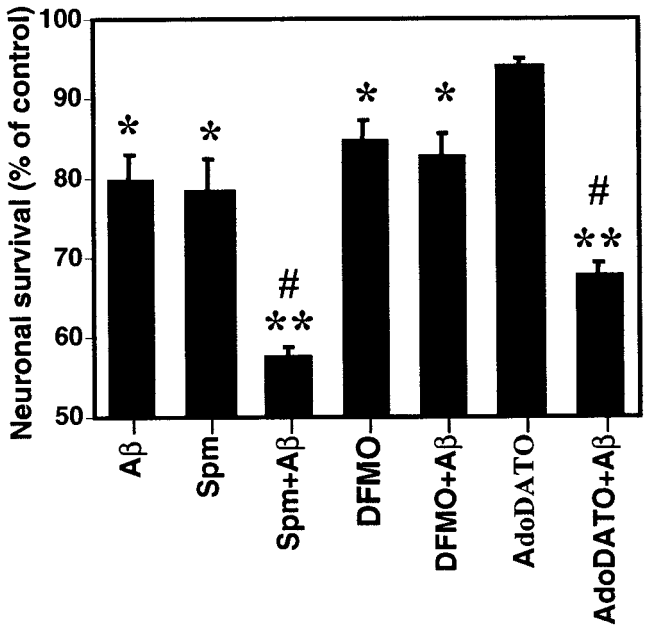

Fig. 4. Percentage variation of neuronal survival upon treatment with 10 μM Aβ(1–42), 5.0 M spermine, 2.0 mM DFMO, 1.0 μM AdoDATO, and combination of these agents with Aβ peptide. Statistical comparisons were made using ANOVA followed by Dunnett's test. Error bars represent SEM values. Significant differences from control $^{\star}P < 0.01$, $^{\star\star}P < 0.0001$; and significant differences from spermine vs. AdoDATO alone $^{\#}P < 0.005$ (n = 3; each n is three or four replicates).

PBN itself is EPR silent, that is, does not give any detectable spectra (Fig. 2A).

We have previously shown that polyamine transport in cultured hippocampal cells is an active and temperature-dependent process (Yatin et al., 1999b), confirming a similar observation in a different cell type (Aziz et al., 1998; Yatin et al., 1999e). Figure 3 shows that Aβ(1–42) increased the levels of all three polyamines but mostly spermine ($P < 0.01$, control vs. putrescine; and $P < 0.005$, control vs. spermidine or spermine). Exogenous addition of spermine increased only the spermine level ($P < 0.005$ vs. control). Interestingly, Aβ(1–42) plus spermine elevated spermine levels more than either agent alone did ($P < 0.005$, Aβ vs. Aβ + spermine). DFMO administration decreased the putrescine and spermidine levels as expected ($P < 0.005$, control vs. putrescine; $P < 0.01$, control vs. spermidine) but did not affect the spermine level; however, Aβ(1–42), even in the presence of DFMO, an inhibitor of the rate-limiting step in polyamine synthesis, was able to increase the putrescine and spermine levels. AdoDATO, a specific inhibitor of spermidine synthase, decreased the spermidine level by 50%, but putrescine and spermine levels increased ($P < 0.005$ vs. control). Similarly, in AdoDATO- plus Aβ(1–42)-treated culture, levels of putrescine and spermine were much higher than the polyamine levels following treatment by AdoDATO alone ($P < 0.01$, not assigned in Fig. 3). These results suggest that 1) intracellular polyamine levels can be modulated by exogenous addition of these polyamines as well as enzymatic manipulation and 2) Aβ(1–42) is capable of further increasing the already modulated polyamine levels.

Figure 4 shows that Aβ(1–42) decreased cell survival to 80% ($P < 0.01$ vs. control); however, pretreatment of cells with 5.0 μM spermine prior to Aβ peptide addition caused an additive effect in terms of neurotoxicity (58% cell survival; $P < 0.005$ vs. control) rather than a rescuing effect of free radical-induced cell death. Similarly, pretreatment with AdoDATO, itself not toxic to cells, further increased Aβ toxicity (68% cell survival; $P < 0.005$) than Aβ(1–42) alone. Aβ(1–42) toxicity did not change in the presence of DFMO (Fig. 4).

Polyamines can be metabolized by serum oxidases to yield toxic aldehyde derivatives from primary amine moieties (Seiler et al., 1981). Thus the oxidation of exogenously added spermine could lead to degradation products that interfere with the direct neurotoxicity of internalized spermine. This seems unlikely because pretreatment of the cells with 1.0 mM aminoguanidine, an inhibitor of amine oxidase activity, failed to influence the cytotoxic effect of spermine (data not shown).

DISCUSSION

AdoDATO is a specific inhibitor of spermidine synthase, an enzyme responsible for de novo production of spermidine from the precursor polyamine putrescine through the addition of an aminopropyl group of decarboxylated S-adenosylmethionine (dc-SAM; Tang et al., 1980; Pegg et al., 1995). Treatment of hippocampal neuronal cells with AdoDATO caused a marked decrease in

spermidine level together with accumulation of putrescine and spermine, confirming the same results with a different cell type (Pegg et al., 1982; Holm et al., 1989). The increase in spermine level can be explained by the accumulation of the aminopropyl donor dc-SAM (Pegg et al., 1995). This accumulation was caused by an increase in S-adenosylmethionine decarboxylase (SAM-DC) activity resulting from the absence of repression by spermidine, because spermidine synthesis is blocked by AdoDATO (data not shown). Thus, in the presence of AdoDATO, the use of the available dc-SAM by spermine aminopropyltransferase is favored, excess dc-SAM is available, and the spermidine, which is formed previously, is efficiently converted to spermine (Pegg et al., 1995). The results of this study suggest that the cellular accumulation of spermine through the control of de novo synthesis shows a nonprotective effect against the toxicity of Aβ(1–42) similar to that of exogenously added spermine. The unregulated uptake of added spermine in Aβ-treated cells caused an overaccumulation of spermine to toxic levels and significantly enhanced cell death. DFMO is a well-known specific inhibitor of ODC activity and usually causes a significant decrease in the amount of the simplest polyamine, putrescine, and a slight depletion in spermidine but has no effect on, or causes a slight increase in (Marton and Pegg, 1995), spermine levels. Pretreatment of neurons with DFMO prior to Aβ addition, did not change the spermine level and had no effect on cell survival.

Spermine acted as a free radical scavenger in solution, albeit not one that totally scavenged the Aβ(1–42)-associated free radicals (Fig. 2C). The efforts to increase the cellular amounts of spermine, either through manipulation of enzyme activities responsible for synthesis of individual polyamines by specific enzyme inhibitors or through exogenous addition, did not reveal any protective effect following accumulation of this particular polyamine. Importantly, the presence of spermine did not increase the production of Aβ(1–42)-associated free radicals. Thus, the potentiation of Aβ(1–42)-induced neurotoxicity by spermine is not due to this polyamine facilitating further generation of Aβ(1–42)-associated free radicals.

Polyamines such as spermine have multiple effects in the central nervous system and were shown to be neuromodulators and regulators of the activity of N-methyl-D-aspartate (NMDA) receptor (Rock and MacDonald, 1995). Spermine-induced neurotoxicity in in vitro and in vivo studies suggests an involvement of NMDA and non-NMDA glutamate receptors (Otusaki et al., 1995). It was reported that the exposure of chick cortical neurons to spermine significantly decreased the neuronal survival, and coapplication of dizocilpine, an NMDA-associated channel antagonist, completely inhibited the toxic effect of spermine (Fahey et al., 1993). In contrast, it was also reported that spermine at 1 mM concentration, much higher than the physiological concentration of free spermine, could be neuroprotective because it blocks the NMDA receptor and voltage-activated Ca^{2+} channels (Ferchmin et al., 2000). In a recent study, administration of NMDA antagonist MK-801 and/or free radical scavenger vitamin E and C complex was shown to have a neuroprotective effect in Aβ(1–42)-injected rats (Harkany et al., 1999). Our results, in agreement with some of the above-mentioned studies, suggest that Aβ(1–42) can increase spermine levels, activating the NMDA receptors, which then causes Ca^{2+} influx, leading to more free radical generation (Lafon-Cazal et al., 1993), followed by a cascade of events.

The increase in polyamine biosynthesis and transport is conceivably a result of an impairment in polyamine biosynthesis regulation, possibly oxidation of key enzymes in polyamine biosynthesis and transport. For example, change in ODC antizyme (the key regulatory enzyme in the polyamine biosynthesis pathway) activity and amount were observed under osmotic stress (Mitchell et al., 1998). Recently, ROS were reported to cause excretion of polyamines from cells through induced activity of spermidine/spermine N^1-acetyltransferase in human cancer cells, a process that was speculated to be a protective response to oxidative stress in mammalian cells by facilitating removal of polyamines (in the form of N^1 acetylated spermine and spermidine) from the cell to prevent their toxic accumulation (Chopra and Wallace, 1998). The acetylation of polyamines at primary nitrogens makes them substrates for polyamine oxidase and allows the interconversion pathway (see Fig. 1). Relative to the corresponding polyamine, the acetylated derivatives of spermidine and spermine are more easily excreted from the cell and cannot be readily transported back to the cell (Persson et al., 1996). A specific inhibitor of polyamine oxidase, MDL 72527, was reported to show a protective effect by decreasing putrescine levels in brain edema and ischemic injury, which are associated with elevated putrescine levels (Dogan et al., 1999a,b). These reports suggest that the polyamine degradation is a preferred response to disturbance in physical conditions of the cellular environment. Although cells may try to avoid overloading of polyamines by stimulating catabolic pathways and degradation of polyamines, the simultaneous up-regulation of the polyamine uptake in response to Aβ(1–42)-induced oxidative stress may be large enough to internalize the already excreted acetylpolyamines, and the steady-state influx may exceed the efflux.

Is Aβ-induced spermine accumulation protective or harmful to neurons? Our results support the latter possibility. Aβ is shown to oxidize numerous neuronal proteins (Aksenov et al., 1997; Butterfield, 1997; Yatin et al., 1998, 1999b,c; Butterfield et al., 1999). In previous studies polyamine biosynthesis inhibitors were reported as neuroprotective agents (Kindy et al., 1994; Baskaya et al., 1996). These agents may protect the neurons from overaccumulation of polyamines to toxic levels by inhibiting the specific enzymatic pathways. However, in the presence of Aβ even these polyamine biosynthesis enzymes may become oxidized and lose function, similar to the effects of Aβ on other enzymes and proteins.

Our results imply that up-regulation of polyamine metabolism due to Aβ(1–42)-associated free radicals is a result of oxidative stress. We hypothesize that other polyamine regulatory enzymes, such as ODC-antizyme, are damaged by oxidative insults and incapable of regulating polyamine uptake and synthesis, resulting in accumulation of intracellular polyamines to toxic levels. These results may have importance in understanding the mechanism of Aβ toxicity in AD brain. Further studies on the oxidation of polyamine regulatory enzymes after Aβ(1–42) addition and of polyamine levels in AD brain are underway in our laboratory.

REFERENCES

Aksenov MY, Aksenova MV, Carney JM, Butterfield DA. 1997. Oxidative modification of glutamine synthetase by amyloid β-peptide. Free Rad Res 27:267–281.

Aziz SM, Yatin M, Worthen DR, Lipke DW, Crooks PA. 1998. A novel technique for visualizing the intracellular localization and distribution of transported polyamines in cultured pulmonary artery muscle cells. J Pharmacol Biomed Anal 2:307–320.

Baskaya MK, Rao AM, Puckett L, Prasad RM, Dempsey RJ. 1996. Effect of difluoromethylornithine treatment on regional ornithine decarboxylase activity and edema formation after experimental brain injury. J Neurotrauma 13:85–92.

Behl C, Davis JB, Lesley R, Schubert D. 1994. Hydrogen peroxide mediates amyloid β-protein toxicity. Cell 77:817–827.

Brewer GJ, Torricelli JR, Evege EK, Price PJ. 1993. Optimized survival of hippocampal neurons in B27-supplemented neurobasal, a new serum-free medium combination. J Neurosci Res 35:567–576.

Butterfield DA. 1997. β-Amyloid associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. Chem Res Toxicol 10:495–506.

Butterfield DA, Yatin SM, Varadarajan S, Koppal T. 1999. Amyloid β-peptide-induced oxidative pathways of cell death. Methods Enzymol 309:746–768.

Chopra S, Wallace HM. 1998. Induction of spermidine/spermine N^1-acetyltransferase in human cancer cells in response to increased production of reactive oxygen species. Biochem Pharmacol 55:1119–1123.

Coffino P, Poznanski A. 1991. Killer polyamines? J Cell Biochem 45:54–58.

Dempsey RJ, Roy MW, Cowen DE, Combs DJ. 1988. Polyamine inhibition preserves somatosensory evoked potential activity after transient cerebral ischemia. Neurol Res 10:141–144.

Dogan A, Rao AM, Baskaya MK, Hatcher J, Temiz C, Rao VL, Dempsey RJ. 1999a. Contribution of polyamine oxidase to brain injury after trauma. J Neurosurg 90:1078–1082.

Dogan A, Rao AM, Hatcher J, Rao VL, Baskaya MK, Dempsey RJ. 1999b. Effects of MDL 72527, a specific inhibitor of polyamine oxidase, on brain edema, ischemic injury volume, and tissue polyamine levels in rats after temporary middle cerebral artery occlusion. J Neurochem 72:765–770.

Fahey JM, Pritchard GA, Miller LG. 1993. Polyamine neurotoxicity is antagonized in cultured chick cortical neurons. Neurosci Lett 161:109–112.

Farbiszewski R, Bielawski K, Bielawska A, Sobaniec W. 1995. Spermine protects in vivo the antioxidant enzymes in transiently hypoperfused rat brain. Acta Neurobiol Exp 55:253–258.

Farbiszewski R, Bielawski A, Szymanska M, Skrzydlewska E. 1996. Spermine partially normalizes in vivo antioxidant defense potential in certain brain regions in transiently hypoperfused rat brain. Neurochem Res 21:1497–1503.

Ferchmin PA, Perez D, Biello M. 2000. Spermine is neuroprotective against anoxia and N-methyl-D-aspartate in hippocampal slices. Brain Res 859:273–279.

Gilad GM, Gilad VH. 1991. Polyamines can protect against ischemia-induced nerve cell death in gerbil forebrain. Exp Neurol 111:349–355.

Gilad GM, Gilad VH. 1999. Novel polyamine derivatives as neuroprotective agents. J Pharmacol Exp Ther 291:39–43.

Gilad GM, Gilad VH, Wyatt RJ. 1993. Accumulation of exogenous polyamines in gerbil brain after ischemia. Mol Chem Neuropathol 18: 197–210.

Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero RA. 1998. The natural polyamine spermine functions directly as a free radical scavenger. Proc Natl Acad Sci USA 95:11140–11145.

Harada J, Sugimoto M. 1997. Polyamines prevent apoptotic cell death in cultured cerebellar granule neurons. Brain Res 753:251–259.

Harkany T, Mulder J, Sasvari M, Abraham I, Konya C, Zarandi M, Penke B, Luiten PG, Nyakas C. 1999. N-methyl-D-aspartate receptor antagonist MK-801 and radical scavengers protect cholinergic nucleus basalis neurons against β-amyloid neurotoxicity. Neurobiol Dis 6:109–121.

Harris ME, Carney JM, Cole PS, Hensley K, Howard BJ, Martin L, Bummer P, Wang Y, Pedigo NW Jr, Butterfield DA. 1995. Aβ peptide-derived, oxygen-dependent free radicals inhibit glutamate uptake in cultured astrocytes: implications for Alzheimer's disease. NeuroReport 6:1875–1879.

Hensley K, Carney JM, Mattson MP, Aksenova M, Harris M, Wu JF, Floyd RA, Butterfield DA. 1994. A model for Aβ aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer's disease. Proc Natl Acad Sci USA 91:3270–3274.

Holm I, Persson L, Pegg AE, Hebby O. 1989. Effects of S-adenosyl-1,8-diamino-3-thio-octane and S-methyl-5′-methylthioadenosine on polyamine synthesis in Ehrich asscites-tumor cells. Biochem J 261:205–210.

Kaupppila T. 1992. Polyamines enhance recovery after sciatic nerve trauma in the rat. Brain Res 575:299–303.

Khan AU, Mei YH, Wilson T. 1992a. A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen. Biophysics 98:11426–11427.

Khan AU, Mascio PD, Marisa HG, Wilson T. 1992b. Spermine and spermidine protection of plasmid DNA against single-strand breaks induced by singlet oxygen. Proc Natl Acad Sci USA 89:11428–11430.

Kindy MS, Hu Y, Dempsey RJ. 1994. Blockade of ornithine decarboxylase enzyme protects against ischemic brain damage. J Cereb Blood Flow Metab 14:1040–1045.

Lafon-Cazal M, Pietri S, Culcasi M, Bockaert J. 1993. NMDA-dependent superoxide production and neurotoxicity. Nature 364:535–537.

Lovaas E. 1995. Hypothesis: spermine may be an important epidermal antioxidant. Med Hypoth 45:59–67.

Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP. 1997. A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by Aβ-peptide. J Neurochem 68:255–264.

Marton LJ, Pegg AE. 1995. Polyamines as targets for therapeutic intervention. Annu Rev Pharmacol Toxicol 35:55–91.

Matkowics B, Keckskemeti V, Varga IS, Novak Z, Kertesz Z. 1993. Antioxidant properties of di- and polyamines. Comp Biochem Physiol 104B:475–479.

Mitchell JL, Judd GG, Leyser A, Choe CY. 1998. Osmotic stress induces variation in cellular levels of ornithine decarboxylase-antizyme. Biochem J 329:453–459.

Morgan DM. 1998. Polyamine protocols. In: Walker JM, editor. Methods in molecular biology, vol 79. Totowa, NJ: Humana Press. p 1–183.

Muscari C, Guarnieri C, Stefanelli C, Giaccari A, Caldarera CM. 1995. Protective effect of spermine on DNA exposed to oxidative stress. Mol Cell Biochem 144:125–129.

Otusaki M, Davidson M, Goodenough S, Wilce PA, Tase C, Matsumoto I. 1995. In vivo pharmacological study of spermine-induced neurotoxicity. Neurosci Lett 196:81–84.

Pegg AE, Tang KC, Coward JK. 1982. Effects of S-adenosyl-1-,8-diamino-3-thiooctane on polyamine metabolism. Biochemistry 21:5082–5089.

Pegg AE, Poulin R, Coward JK. 1995. Use of aminopropyltransferase inhibitors and of nonmetabolizable analogs to study polyamine regulation and function. Int J Biochem Cell Biol 27:425–442.

Persson L, Svensson F, Wallstrom EL. 1996. Regulation of polyamine metabolism. In: Nishioka K, editor. Polyamines in cancer: basic mechanisms and clinical approaches. Austin, TX: R.G. Landes Company. p 18–43.

Rock DM, MacDonald RL. 1995. Polyamine regulation of N-methyl-D-aspartate receptor channels. Annu Rev Pharmacol Toxicol 35:463–482.

Saito K, Packianathan S, Longo LD. 1997. Free radical-induced elevation of ornithine decarboxylase activity in developing rat brain slices. Brain Res 763:232–238.

Selkoe DJ. 1989. The deposition of amyloid proteins in the aging mammalian brain: implications for Alzheimer's disease. Ann Med 21:73–76.

Seiler N, Bolkenius FN, Rennert OM. 1981. Interconversion catabolism and and the elimination of polyamines. Med Biol 59:334–346.

Slotkin TA, Bartolome J. 1986. Role of ornithine decarboxylase and the polyamines in nervous system development: a review. Brain Res Bull 17:307–320.

Sparapani M, Dall'Olio R, Gandolfi O, Ciani E, Contestabile A. 1997. Neurotoxicity of polyamines and pharmacological neuroprotection in cultures of rat cerebellar granule cells. Exp Neurol 148:157–166.

Subramaniam R, Koppal T, Green M, Yatin S, Jordan B, Drake J, Butterfield DA. 1998. The free radical antioxidant vitamin E protects cortical synaptosomal membranes from Aβ(25–35) toxicity but not from hydroxynonenal toxicity: relevance to the free radical hypothesis of Alzheimer's disease. Neurochem Res 23:1403–1410.

Tang KC, Pegg AE, Coward JK. 1980. Specific and potent inhibition of spermidine synthase by the transition-state analog, S-adenosyl-3-thio-1-8-diaminooctane. Biochem Biophys Res Commun 96:1371–1377.

Varadarajan S, Yatin S, Kanski J, Jahanshahi F, Butterfield DA. 1999. Methionine residue 35 is important in amyloid β-peptide-associated free radical oxidative stress. Brain Res Bull 50:133–141.

Varadarajan S, Yatin S, Aksenova M, Butterfield DA. 2000. Review: Alzheimer's amyloid β-peptide associated free radical oxidative stress and neurotoxicity. J Struct Biol 130:184–208.

Yatin SM, Aksenova MV, Aksenov MY, Markesbery WR, Aulick T, Butterfield DA. 1998. Temporal relations among amyloid β-peptide-induced free-radical oxidative stress, neuronal toxicity, and neuronal defensive responses. J Mol Neurosci 11:183–197.

Yatin SM, Aksenov MY, Butterfield DA. 1999a. The antioxidant, vitamin E, modulates amyloid-peptide induced creatine kinase activity inhibition and increased protein oxidation: implications for the free radical hypothesis of Alzheimer's disease. Neurochem Res 24:427–435.

Yatin SM, Yatin M, Aulick T, Ain KB, Butterfield DA. 1999b. Alzheimer's amyloid β-peptide associated free radicals increase rat embryonic neuronal polyamine uptake and ornithine decarboxylase activity: protective effect of vitamin E. Neurosci Lett 263:17–20.

Yatin SM, Varadarajan S, Link CD, Butterfield DA. 1999c. In vitro and in vivo oxidative stress associated with Alzheimer's amyloid β-peptide (1–42). Neurobiol Aging 20:325–330.

Yatin M, Venkataraman GM, Marcinek R, Ain KB. 1999d. Polyamine biosynthesis and transport inhibition in a human anaplastic thyroid carcinoma cell line in vitro and as xenograft tumors. Thyroid 9:805–814.

Yatin M, Venkataraman GM, Marcinek R, Ain KB. 1999e. Demethylation treatment restores Na^+/I^- symporter gene expression in de-differentiated thyroid carcinoma. Paper presented at the American Thyroid Association 72nd Annual Meeting, Palm Beach, FL.

Yatin SM, Varadarajan S, Butterfield DA. 2000. Vitamin E prevents Alzheimer's amyloid β-peptide (1–42)-induced protein oxidation and reactive oxygen species formation. J Alzheimers Dis 2:123–131.