Neuronal and Glial Coexpression of Argininosuccinate Synthetase and Inducible Nitric Oxide Synthase in Alzheimer Disease

MICHAEL T. HENCKA, MD, HEINRICH WIESINGER, PHD, LUCIA DUMITRESCU-OZIMEK, PHD, PETER RIEDERER, MD, DOUGLAS L. FEINSTEIN, PHD, AND THOMAS KLOCKGETHER, MD

Abstract. The enzyme argininosuccinate synthetase (ASS) is the rate limiting enzyme in the metabolic pathway leading from L-citrulline to L-arginine, the physiological substrate of all isoforms of nitric oxide synthases (NOS). ASS and inducible NOS (iNOS) expression in neurons and glia was investigated by immunohistochemistry in brains of Alzheimer disease (AD) patients and nondemented, age-matched controls. In 3 areas examined (hippocampus, frontal, and entorhinal cortex), a marked increase in neuronal ASS and iNOS expression was observed in AD brains. GFAP-positive astrocytes expressing ASS were not increased in AD brains versus controls, whereas the number of iNOS expressing GFAP-positive astrocytes was significantly higher in AD brains. Density measurements revealed that ASS expression levels were significantly higher in glial cells of AD brains. Colocalization of ASS and iNOS immunoreactivity was detectable in neurons and glia. Occasionally, both ASS- and iNOS expression was detectable in CD 68-positive activated microglia cells in close proximity to senile plaques. These results suggest that neurons and astrocytes express ASS in human brain constitutively, whereas neuronal and glial ASS expression increases parallel to iNOS expression in AD. Because an adequate supply of L-arginine is indispensable for prolonged NO generation, coinduction of ASS enables cells to sustain NO generation during AD by replenishing necessary supply of L-arginine.

Key Words: Alzheimer disease; Argininosuccinate synthetase; Human brain; L-arginine; L-citrulline; Neuroinflammation; Nitric oxide synthase.

INTRODUCTION

Alzheimer disease (AD) is a chronic neurodegenerative disorder clinically characterized by impairment of cognitive function, memory loss, and finally severe dementia. Neuropathologically, the disease is characterized by extensive formation of neurofibrillary tangles (NFT), senile neuritic plaques, and progressive neuronal cell death. An inflammatory response by reactive glia cells surrounding the respective brain lesions may participate in impairment of neuronal function and eventually neuronal cell death (1–3).

Despite increasing evidence that inflammatory processes cause oxidative damage and modification of proteins and nucleic acids in AD (4), neither the source nor the underlying mechanism are completely understood. However, the inflammation-dependent release of nitric oxide (NO) and subsequent formation of peroxynitrite by reaction with superoxide may contribute to the oxidative stress in AD. Both the expression of the inducible nitric oxide synthase (iNOS) in neurons and astrocytes (5, 6) as well as increased protein nitration around lesion sites, indicative of NO-mediated oxidative stress, have been described in AD (7, 8). This finding has direct implications for the understanding of AD pathomechanisms since iNOS-derived NO plays a role in a variety of physiological and pathological processes in brain, acting either as a signaling molecule or as part of a cytotoxic host defense mechanism (9, 10). However, when released in excess, iNOS-derived NO can be detrimental to the host. Thus, NO released by immunostimulated astrocytes or microglia causes neuronal death in various models of neuronal-glial coculture (11–13), and we recently described that iNOS expression in neuronal PC12 cells and in primary cerebellar granule neurons caused neuronal apoptotic cell death (14, 15). Hence, the prolonged production of NO can lead to concentrations sufficient to induce cell damage and death.

In addition to iNOS expression, sustained NO generation is dependent on an adequate supply of L-arginine, the only known substrate of NO synthases. L-arginine supply may be secured by uptake from the extracellular space or by recycling from the NOS coproduct L-citrulline through the combined action of the urea cycle enzymes, argininosuccinate synthetase (ASS, the rate limiting step) and argininosuccinate lyase. ASS activity has been detected in primary glial cells (16). Under proinflammatory conditions ASS expression is upregulated in glioma cells as well as in mixed glial and pure astroglial cultures, and a functional role in the recycling of L-citrulline for the generation of NO has been demonstrated (17, 18). Based on our findings that ASS is upregulated by immunostimulation in vivo (19), we hypothesized that ASS may be coexpressed with iNOS in AD. We report here that ASS,
as well as iNOS, is increased in AD brains versus age-matched control brains, thus providing a sustained source of substrate for iNOS activity.

MATERIALS AND METHODS

Tissue

Neocortex, hippocampal, and entorhinal brain tissue was obtained from 10 clinically and pathologically confirmed cases of nondominantly inherited Alzheimer disease (NDAD) with an age range of 70–90 yr and a duration of disease from 6–18 yr. All NDAD patients met Consortium to Establish a Registry for AD (CERAD) pathological criteria (20) and were also diagnosed as Braak and Braak Alzheimer stage VI (21). Control brains were obtained from 6 age-matched, nondemented individuals without clinical history or pathological signs of AD (age range: 74–89 yr). Postmortem intervals before fixation were between 2 and 8 hours (h) in both groups. Medication of patients in both groups included antidepressants and cardiovascular drugs. After fixation, tissue was dehydrated through ascending ethanol and embedded in paraffin.

Processing of Brain Tissue and Immunohistochemistry

Serial coronal sections of paraffin-embedded human brain were cut 8-μm thick using a Leitz microtome and mounted on poly-L-lysine-coated slides. The following antibodies were used in the respective dilution: 1) Antiserum directed against the peptide sequence 196–222 of mouse liver ASS (1:250) (17). 2) mAB MCA 363, raised against glial fibrillary acidic protein GFAP (1:200, Serotec, Darmstadt, Germany). 3) mAB N32020 directed against iNOS (1:100, Transduction Laboratories, KY). 4) mAB CAT0876 raised against human CD68 (1:100, DAKO, Hamburg, Germany). 5) pAB CAT44–344 raised against human β-amyloid 1–42 (1:1,000, QCB, Hopkinton, MA). For detection, slides were immersed in 10 mM citrate buffer (pH 6.0) and heated in a microwave oven, 4 cycles of 5 minutes (min) each, to unmask antigen sites. Thereafter slides were removed and cooled for 20 min at room temperature before washing in PBS. Endogenous peroxidase activity was inhibited by rinsing slides in 0.1% hydrogen peroxide for 10 min. Following washing in PBS (pH 7.4), nonspecific binding was prevented by incubating sections in blocking solution containing 10% normal goat serum in PBS for 1 h at room temperature. After washing in PBS, sections were incubated overnight or for 24 h at 4°C with antiserum (1). Thereafter, the primary antibody was removed and sections were washed intensively with PBS followed by incubation with a biotinylated anti-rabbit IgG (1:200, Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Immunohistochemical localization was performed using an avidin-biotinylated peroxidase complex method (Vectastain ABC-Kit, Vector Laboratories) with 3,3′-diaminobenzidine (DAB, Sigma, Germany) as peroxidase substrate. For costaining with a second antibody, slides were washed twice in PBS and blocked with 10% normal horse serum. After incubation with the primary antibody (2, 4, or 5) for 30 min and 1 h, respectively, slides were washed in PBS and incubated with a biotinylated horse anti-mouse IgG. Immunohistochemical localization was detected as described above using DABROblue as substrate (DACROblue substrate kit, Vector Laboratories). In some cases, immunostained sections were counterstained with hemalum Mayer, dehydrated, and cleared with xylene before mounting in DePeX (Fluka, Buchs, Switzerland). Negative controls included utilization of nonspecific IgG instead of the primary antibodies and preabsorption of primary antibodies with the respective cognate peptides. No labeling was observed in either condition.

Quantification and Statistical Analysis

We compared 5 AD patients and 5 age-matched controls. In each brain, we independently studied glial and neuronal expression of the proteins indicated below. For glial expression, 30 sections of each region (hippocampus, entorhinal, or frontal cortex) were colabeled for ASS/GFAP, iNOS/GFAP, ASS/CD68, or iNOS/CD68. Cells that were positive for the 2 antigens were counted. Because the number of CD68-positive cells expressing iNOS or ASS was low compared to GFAP-positive cells, we presented together the quantitation of CD68 and GFAP-containing cells as glial.

To evaluate neuronal immunoreactivity, 15 sections of each region were double-stained for iNOS/NSE or ASS/NSE. In addition, fifteen 4-μm serial sections were stained for NSE, iNOS, or ASS.

In each section 20 randomly chosen fields were counted. To further analyze immunoreactive cells and to compare the levels of ASS and iNOS expression in AD and control brains, density measurements were performed using a digital camera (SONY, Model DXC-9100P; SONY, Köln, Germany) connected to a PC system with LUCIA software (LUCIA 32G, version 4.11; Laboratory Imaging, Düsseldorf, Germany) and given as original data (O.D.). Statistical analysis was performed by ANOVA and post hoc Tukey test, a p value of less than 0.05 was considered to be significant.

RESULTS

β-Amyloid_{1–42} Staining

Costaining with GFAP and CD 68: Costaining for β-amyloid_{1–42} and either GFAP or CD68 was performed to correlate the occurrence of activated glial cells with AD neuropathology. GFAP-positive cells surrounding β-amyloid_{1–42}-Positive senile plaques were observed in the hippocampus, entorhinal, and frontal cortex (Fig. 1A). In addition to activated astrocytes, CD68-positive activated microglia cells were found within senile plaques in the same areas (Fig. 1B).

Costaining for β-Amyloid_{1–42} and ASS and iNOS: Costaining for β-amyloid_{1–42} and ASS and iNOS was investigated to demonstrate that expression of NO pathway enzymes occurs in close proximity to senile plaque pathology. iNOS and ASS expressing neurons (Fig. 1C, D) and astrocytes (Fig. 1E, F) were found around senile plaques. However, iNOS- and ASS-positive astrocytes were not restricted to the near environment of senile plaques, but rather showed widespread distribution in the hippocampus, entorhinal, and frontal cortex of AD brains.

ASS Expression

ASS in Neurons: In the 3 brain areas investigated (hippocampus, frontal, and entorhinal cortex), a basal ASS
Fig. 1. Double-immunolabeling with β-amyloid1-24, GFAP, CD68, iNOS and ASS. Immunostaining of β-amyloid1-24 showed deposition in senile plaques (A, brown) and surrounding GFAP-positive astrocytes (A, dark blue). B: Double-staining for β-amyloid1-24 (B, brown) and CD68-positive microglia cells (B, dark blue). C and D: iNOS-positive neurons (C, brown) and ASS expressing neurons (D, brown) nearby β-amyloid1-24-stained senile plaques (C, D, dark blue). E and F: iNOS (E, brown) and ASS (F, brown) immunopositive astrocytes close to senile plaques (E, F, dark blue). Scale bar = 50 μm.

expression was detected in neurons of age-matched controls (Fig. 2A, C, E). The number of ASS-positive neurons was almost identical in all brain regions of control brains (Fig. 6A). Density measurements revealed that the level of neuronal ASS expression was comparable in the frontal cortex and hippocampus, whereas the entorhinal cortex showed a higher basal level of ASS expression (Fig. 6B). In contrast, neuronal ASS expression was markedly increased in the respective areas of AD brains (Fig. 2B, D, F). Both the total number of immunopositive neurons as well as the expression level increased in all AD brains compared to controls. The most pronounced increase of ASS-positive cells was detected in the frontal cortex showing a 3-fold increase, whereas the number of
Fig. 2. Neuronal ASS expression in AD. Sections from AD brains or age-matched control brains were stained for the presence of ASS. A, C, E: Sections from age-matched control brains; B, D, F: sections from AD brains. A, B: Hippocampus. C, D: Frontal cortex. E, F: Entorhinal cortex. Scale bar = 100 μm.
ASS-positive cells increased by 2.1-fold and 2.3-fold in the hippocampus and entorhinal cortex, respectively (Fig. 6A). Expression levels of neuronal ASS were significantly higher in the frontal cortex and hippocampus of AD brains (Fig. 6B). However, no specific distribution pattern of ASS expression was detectable within the brain regions investigated.

ASS in Glia: Morphological considerations and co-staining with antibodies directed against the astroglial marker protein glial fibrillary acidic protein (GFAP) revealed that glial ASS expression was found predominantly in astrocytes (Fig. 3A, B, D). In contrast to neuronal ASS expression, the number of ASS-positive astrocytes was not elevated in AD brains compared to controls, and was comparable in the hippocampus, entorhinal, and frontal cortex of all brains investigated (Fig. 6A). However, in AD brains, expression levels of astrogial ASS increased significantly in all brain regions (Fig. 6B). In AD, ASS immunoreactivity was rarely detectable in round or oval activated CD 68-positive microglia cells in close proximity to microvessels (Fig. 3C) or surrounding senile plaques in the frontal lobe and entorhinal cortex. Occasionally, ASS-positive astrocytes were observed in the adjacent white matter.

iNOS Expression

iNOS in Neurons: In brain regions of age matched controls, neuronal iNOS expression was rarely detectable (Fig. 6A). As in neuronal ASS, the number of iNOS-positive neurons was significantly higher in AD brains, revealing an increase of 16.5-fold in the frontal cortex,
4.5-fold in the hippocampus, and 4.3-fold in the entorhinal cortex, respectively (Fig. 6A). Compared to controls, the number of iNOS-positive neurons and the level of expression were significantly increased in the frontal and entorhinal cortex (Fig. 6B). However, this phenomenon was not observed in the hippocampus where the neuronal expression levels were not significantly different between AD and control brains (Fig. 6B).

iNOS in Glia: Glial iNOS expression was detected in all brain regions investigated (Fig. 4 A–C). Characteristic morphology and co-staining with GFAP identified the vast majority of iNOS-positive glial cells as astrocytes (Fig. 4C). In contrast to ASS expression, the number of iNOS-positive glial cells was significantly higher in AD brains compared to controls. Thus, an increase of 1.5-fold, 3.9-fold, and 2.0-fold was detected in the frontal cortex, the entorhinal cortex, and hippocampus, respectively (Fig. 6A). In addition, in AD brains an increased level of glial iNOS expression was found in all brain regions investigated without a prevalence for a specific region (Fig. 6B). Interestingly, iNOS expressing astrocytes were not restricted to the gray matter but were also observed in the adjacent white matter areas. Furthermore, co-staining of iNOS with the human microglia marker CD68 double-labeled a number of cells having a round to oval morphological appearance and immunopositive processes, indicative of microglia cells in different states of activation; these were predominantly in the frontal lobe in close proximity to brain vessels or senile plaques (Fig. 4D).

ASS and iNOS Coexpression

To investigate whether ASS and iNOS are coexpressed by identical cells in AD brains, sequential parallel sections of 8 μM were stained for both proteins. All brain regions investigated showed a greater number of cells

**Fig. 4.** Glial iNOS expression in AD. Glial iNOS expression was exclusively detected in AD brains: iNOS expressing astrocytes within the hippocampus indicated by arrows and in an enlarged inlay (A) and entorhinal cortex (B). C: Double-labeling for iNOS (brown) and glial fibrillary acidic protein (GFAP, dark blue). D: Double-labeling for human microglia epitope CD68 (dark blue) and iNOS (brown) surrounding a senile plaque. Scale bars: A, B = 50 μm; C, D = 25 μm.
with positive ASS immunoreactivity compared to the number of iNOS positive cells. However, costaining was observed in neurons of the hippocampus, entorhinal, and frontal cortex (Fig. 5). In glial cells the arborization of astrocytes and microglia cells made it difficult to judge from parallel sections whether the same cells (Fig. 5F) expressed both proteins. Nevertheless, ASS and iNOS-positive immunoreactivity was either detected in cells with identical locations to morphological landmarks or in close proximity.

**DISCUSSION**

In this report we demonstrate that expression of the enzyme ASS is increased in brains of AD patients versus age-matched controls. Basal ASS immunoreactivity was detected in both neurons and glial cells in brain regions of controls (hippocampus, entorhinal cortex, and frontal cortex). The number of ASS-positive neurons was markedly increased in AD brains compared to controls. In addition, a significant higher level of ASS expression was found in the hippocampus and frontal cortex of AD brains. In contrast, no increase in the number of ASS-positive glial cells was observed in AD. Nevertheless, the level of glial ASS expression was significantly higher in AD brains than in controls. This finding suggests that in AD, glial ASS expression is increased while neuronal ASS expression is upregulated and induced de novo. In the same samples, iNOS immunoreactivity was detected in neurons, astrocytes, and CD68 microglial cells. In AD brains, a pronounced increase in the number and expression level of iNOS was detected in all 3 regions, but was only rarely apparent in sections of control brains. Finally, using double-labeling methods in serial sections we identified ASS and iNOS colocalized in neurons, and possibly to a lesser extent in glial cells. These results confirm previous reports of iNOS expression in AD brains (5, 6) and demonstrate for the first time that the enzyme ASS, whose activity is necessary to replenish levels of the iNOS substrate L-arginine, is also induced in AD. These observations raise the possibility that the concomitant induction of ASS together with iNOS allows for prolonged production of NO during AD, which can contribute to neuronal damage and death.

Expression of iNOS in neurofibrillary tangle (NFT)-containing neurons of brain tissue from AD patients has been described before (5). iNOS-derived NO may react with superoxide to form peroxynitrite when both molecules are generated in close proximity. In accordance with this hypothesis, peroxynitrite-mediated nitration of tyrosine residues has been found in regions of AD pathology (7, 8, 22). In fact, glycated proteins and herein glycated tau in NFTs are a potential source of superoxide generation (23, 24). Glycated proteins as well as β-amyloid peptides are potent inducers of NOS in cultured glial cells (25, 26), and thus may give the impression of permanent generation of NO in areas of Alzheimer pathology. However, the concentration of essential cofactors or of the substrate L-arginine may become limiting for long-lasting NO production in brain cells and compensatory mechanisms may exist. Indeed, cultured murine astroglial cells upregulate L-arginine transport and the expression of ASS in parallel to iNOS in vitro (18, 27) and in vivo ASS is upregulated in a rat model of brain inflammation (19). Furthermore, it has been shown that biosynthesis of NO requires tetrahydrobiopterin is induced in glial cells by immunostimulation (28, 29). Therefore, evidence for modulation of any of these pathways auxiliary to the biosynthesis of NO in human CNS has been lacking so far.

Our finding that ASS is upregulated and colocalized to iNOS in neurons and glial cells in AD is the first evidence for modulation of this enzyme in a human neurological disorder. In contrast to glial cells, evidence concerning the constitutive expression of ASS in neurons throughout the rat brain in the absence of immunostimulation has been presented before (30, 31). Distribution and size of ASS-positive cells pointed to localization of the enzyme mainly in interneurons (30).

In the present study, basal ASS expression was detected in neurons and glial cells throughout the hippocampus, entorhinal, and frontal cortex of age-matched, nondemented controls. In AD brains, staining intensity and the number of neurons stained for ASS strongly increased in all areas investigated, whereas glial cells did not increase in number but showed a higher level of expression in AD brains. In control brains, iNOS expression was rarely detectable in both neurons and glial cells. This finding is in line with previous reports detecting low levels of iNOS expression in astrocytes and neurons in normal aging brain (32). However, other reports had failed to detect any iNOS expression in normal aging brain (5, 33). In all brains of patients suffering from AD, glial iNOS and ASS were strongly expressed and mainly colocalized to the astrocytic marker GFAP. In addition to astrocytic glia, reactive microglia cells surrounding senile plaques and tangle-bearing neurons have been described earlier in AD (34). In the present study, control brains only rarely showed CD68-positive microglia close to brain vessels and neither iNOS nor ASS expression was detectable in these cells. In contrast, iNOS and ASS coexpression was found in a minority of CD68-positive microglia (in different states of activation as judged by morphological means) that were located in close proximity to either microvessels or senile plaques in AD brains. Microglia cells expressing both ASS and iNOS are a potent source for sustained NO release in AD.
Fig. 5. Coexpression of iNOS and ASS in neurons and glial cells. Coexpression of iNOS and ASS was detected in astrocytes and neurons of all brain regions examined: parallel sections of AD frontal cortex was stained for ASS (A) and for iNOS (B). C: ASS-positive pyramidal neurons in AD hippocampus and coexpression of iNOS in the parallel section (D). ASS- (E) and iNOS-positive (F) astrocytes in parallel sections of AD entorhinal cortex. Asterisk indicates marker brain vessels in the respective sections. Scale bars: A, B = 20 μm; C, D = 100 μm; E, F = 50 μm.
Fig. 6. Quantification and statistical analysis of iNOS and ASS in neurons and glial cells. A: The number of iNOS and ASS expressing cells was quantified in glial cells and neurons of all brain regions examined: frontal cortex, entorhinal cortex, and hippocampus. B: The level of iNOS and ASS expression was determined by density measurements of immunopositive cells in the respective regions. Statistical analysis was performed using ANOVA with a Tukey post hoc test. A p value less than 0.05 was considered significant (*, p < 0.05; **, p < 0.01; ***, p < 0.001).

Since iNOS and ASS were predominantly upregulated in neurons, it seems likely that the neuronal loss and gliosis in AD may lead to a lower number of iNOS- and ASS-positive cells. Taking into account that iNOS-mediated, long-term generation of NO also induces neuronal cell death (14), it seems likely that the actual staining underestimates the total number of iNOS and ASS expressing neurons. Moreover, iNOS and ASS are transiently expressed and therefore neurons that survived the consequences of transient expression may revert to being immunonegative for both enzymes.

A number of possible inducers may account for the induction or increased expression of iNOS and ASS in AD. In vitro, parallel induction and upregulation of both
enzymes by the pro-inflammatory cytokine interferon-γ in primary astrocytes has been shown (18). In addition, interleukin-1β (IL-1β), another potent iNOS inducing cytokine, has been implicated in the pathogenesis of AD, and an enhanced immunoreactivity for this cytokine was found in glial cells of AD brains (35). β-amyloid, a key protein that accumulates and forms senile plaques in the course of AD, induced iNOS in astrocytes and microglia in vitro (26). In the present study, activated astrocytes and microglia cells were observed in close proximity to β-amyloid-positive plaques, linking the deposition of β-amyloid with the occurrence of proinflammatory changes in AD brains. Furthermore, iNOS- and ASS-positive neurons and glial cells were found near senile plaque pathology, supporting the aforementioned in vitro data. In astrocytes, amyloid beta-mediated iNOS induction is potentiated by several cytokines (36). The finding that iNOS and ASS expression in astrocytes is also found in adjacent white matter regions raises the possibility that despite the fact that cortical pathology, and especially β-amyloid deposition, may be the major trigger for proinflammatory changes, cytokines and other inflammatory mediators are not necessarily restricted to their site of generation and thus may lead to a more widespread iNOS and ASS induction.

Furthermore, S-100 beta, which has also been found to be elevated in AD, caused NO-dependent neuronal cell death by induction of iNOS in astrocytes (37). Although in vitro data are lacking, it seems likely that either one of these iNOS inducers also accounts for the upregulation of ASS, or that the later reaction is driven by secondary mediators in response to the above-mentioned stimulants. Future experiments will investigate the possibility of induction and regulation of neuronal and astrocytic ASS expression in vitro.

The recycling of the coproduct of NOS (L-citrulline) to the substrate of this enzyme (L-arginine) may be a prerequisite for NOergic neurotransmission (38–40) and may play an important role in the regulation of cerebral vascular tone (41) under physiological conditions. However, upregulation of ASS in response to a pathological stimulus will result in an adequate supply of L-arginine for long-lasting NO generation because ASS catalyzes the rate limiting step in recycling the L-citrulline to L-arginine. Although the histochemical evidence obtained in the present study has to be supported by functional data, the results of this study suggest that both glial cells and neurons have to be taken into account as arginine-regenerating cells.

Our finding fits into a model integrating generation of NO and L-citrulline by iNOS, recycling L-citrulline to L-arginine by ASS, subsequent long-term generation of NO, reaction of NO with superoxide forming peroxynitrite, and finally, oxidation damage of proteins vital to cell survival. Because a short exposure to oxidative stress might be compensated by antioxidants such as glutathione (42), upregulation of ASS securing L-arginine supply and thereby long-term NO generation is an important finding. It should be noted that long-lasting NO generation may lead to cell death via alternative pathways. We recently found that iNOS-derived NO causes apoptotic cell death in neuronally differentiated PC 12 cells and in primary neurons involving activation of caspase 3 and induction of DNA fragmentation. Interestingly, this cell death was potentiated in both models by exogenous addition of L-arginine (14), suggesting that substrate availability regulates cell damage and death.

Although coexpression of ASS and iNOS has been detected in astrocytes and neurons, upregulation of ASS seems to be more prominent compared to iNOS expression. Previously, a partly differential localization has been reported for ASS and constitutive NOS in vivo (31, 43) and for ASS and iNOS in vitro (18). This raises the possibility that generation and consumption of arginine does not necessarily occur in the same cell and that neuronal-neuronal or neuronal-glial/endothelial cell trafficking of the respective amino acids may occur. However, this ability appears to be negligible under normal physiological conditions, but might be substantially increased under conditions mimicking an inflammation of the brain (19) or in AD (present study). Further studies have to determine whether upregulation of ASS concomitant with iNOS induction is a phenomenon restricted to AD or is a generalized mechanism occurring in other neurodegenerative disorders.

REFERENCES

2. Floyd RA. Neuroinflammatory processes are important in neurodegenerative diseases: An hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development. Free Radic Biol Med 1999;26:1346–55


