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Reduction in asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in the cerebrospinal fluid during aging and in patients with Alzheimer's disease

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Abstract

To investigate the significance of nitric oxide (NO) -mediated neuron death in aging and Alzheimer's disease (AD), the concentration of asymmetrical dimethylarginine (ADMA), an endogenous NO synthase inhibitor, in the cerebrospinal fluid was determined in neurologically normal controls and patients with AD. The ADMA concentration significantly decreased with age, whereas the arginine concentration was unaltered. In patients with AD, the ADMA concentration was significantly decreased, compared with controls of a similar age (−48%, $P = 0.0001$), and it significantly decreased with decreasing cognitive functions ($r_s = 0.58$, $P < 0.05$), whereas the arginine concentration did not change. These findings suggest that ADMA may play an important role in regulating NO synthesis in brain aging and AD.

Keywords: Nitric oxide; Asymmetrical dimethylarginine; Aging; Alzheimer's disease; Cerebrospinal fluid

Oxidative stress has been implicated in brain aging and the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative disorders [5,15,17]. Superoxide reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), a powerful oxidant which may directly oxidize proteins, lipids and DNA through a nitronium-like intermediate, with resultant carbonyl formation from cleavage of side chains and peptide bonds [2]. $ONOO^-$ also reacts directly with Cu/Zn superoxide dismutase (SOD) via a nitronium ion (NO_2^+) intermediate that nitrates tyrosine amino acids of proteins to form the stable compound 3-nitrotyrosine. NO synthase (NOS) has been localized in discrete neuronal populations in the human central nervous system. The increases in NO and 3-nitrotyrosine productions have been recently reported in various neurodegenerative disorders including AD [3,9,20,21,22] and in normal aging [22].

The methylated arginine analogue asymmetrical dimethylarginine (ADMA) is a substance that occurs naturally in the brain [11], although the distribution of ADMA in human brain tissues and in human cerebrospinal fluid (CSF) has not been determined in detail. NO synthesis can be inhibited in vitro and in vivo by guanidino-substituted arginine analogues such as ADMA, which is also present in human plasma and urine [23]. This raises the possibility that guanidino-substituted arginine analogues may regulate NOS activity in the brain and thus modulate the NO metabolism, which may play a role in brain aging and the pathogenesis of AD. Nevertheless, whether ADMA is present in the CSF and, if present, whether its concentration changes in brain aging and AD patients have not yet been reported. In our previous study, the 3-nitrotyrosine concentrations in the CSF significantly increased both with advancing age and in patients with AD compared with controls of similar age [22]. The aim of the present study was to determine alterations in ADMA concentration in the CSF during normal aging and in patients with AD.

Subjects were 15 controls (age, 65.1 ± 7.8 years (mean ± SD); eight males and seven females) and 14 patients with AD (age, 68.9 ± 4.2 years; six males and eight females). Controls were neurologically normal patients who had undergone minor surgery. Diagnosis of AD was made according to the DSM-IV [1] and NINDS [14] criteria and Hachinski's ischemic score [8]. Cognitive function was assessed based on Mini-Mental-State Examination (MMSE) scores [7]. In patients with AD, duration of the illness was 4.0 ± 2.7 years, and the average mean MMSE score was 12.2 ± 3.9. Informed consent was obtained from all the patients.

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CSF was obtained between 09:00 and 10:00 h after overnight bed-rest and before breakfast. The CSF samples drawn from the patients were rapidly frozen and stored at −80°C until assayed. CSF samples were passed through a 10,000 NMWL ultrafilter (UFC3 LGC00, Japan Millipore Ltd., Tokyo), and concentrations of free ADMA and arginine were determined according to the method described by Donzanti and Yamamoto [6], with some modifications. The working derivatizing solution was prepared by diluting 54 mg *o*-phthalaldehyde (OPA) in 1 ml methanol with 10 μl of β-mercaptoethanol (BME) and 9 ml of 0.10 M sodium borate (pH 9.5). Precolumn amino acid derivatization was accomplished by mixing 75 μl of a CSF sample with 20 μl of the working OPA/BME reagent for exactly 2 min prior to injection onto the analytical column. The mobile phase consisted of 0.10 M NaH_2PO_4 and 25% methanol, and was adjusted to pH 6.75 with NaOH. ADMA and arginine were separated by injection of 30-μl volumes of the reaction mixture onto an NBS C_{18} reversed-phase column (150 × 4.6 mm) (MC Medical, Tokyo, Japan). For sample analysis, we used a coulometric electrochemical detector (Coulochem II Model 5200: ESA Inc., Bedford, MA). The electrode potentials were maintained at 0.45 V for the guard cell, 0.25 V for detector I, and 0.4 V for detector II. The detection limits for ADMA and arginine were both 0.001 μM. The standards for ADMA and arginine were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Statistical analysis was performed using a non-parametric Mann–Whitney's test or Spearman's rank correlation coefficient (r_s). The significance level was designated as $P < 0.05$.

In controls, the ADMA concentration ranged from 0.014 to 0.040 μM, and the arginine concentration ranged from 30.6 to 55.1 μM (Fig. 1a). Within these control ranges, the ADMA concentration significantly decreased with advancing age ($r_s = -0.56$, $P < 0.05$). In contrast, the arginine concentration did not significantly change with age ($r_s = 0.19$, $P = 0.48$).

The arginine concentration in patients with AD did not significantly differ from that of age-matched controls (44.1 ± 8.0 vs. 39.3 ± 5.2 μM, respectively). However, ADMA concentration was significantly lower in the AD patients than in age-matched controls (0.014 ± 0.006 vs. 0.027 ± 0.008 μM, respectively) ($P = 0.0001$) (Fig. 1b). The ADMA concentration did not correlate with duration of illness ($r_s = -0.11$, $P = 0.69$), but did show a significant positive correlation with MMSE scores ($r_s = 0.58$, $P < 0.05$) (Fig. 1c).

The present results demonstrated that ADMA was detected in the CSF and that the levels of ADMA in the CSF significantly decreased with age and in patients with AD (−48%, compared to controls). Methylated arginine analogues such as ADMA can inhibit NOS and may play an important role in regulating signal transduction through the NO system. It has been reported that ADMA (but not symmetrical dimethylarginine) accumulation [23] acts as an endogenous inhibitor of NOS in vitro and in vivo, and this suggests that a similar mechanism for controlling NO synthesis may exist in the brain and could be related to the pathogenesis of AD. Transmethylation is catalyzed by *S*-adenosyl-L-methionine (SAM) [19]. Previous studies reported reduced SAM levels in the CSF [4] and brains [16] of AD patients. Such reductions in SAM levels in the CSF and brains of AD patients may cause a reduction in ADMA concentrations in the CSF and brain. In our previous study, 3-nitrotyrosine concentrations in CSF increased with advancing age [22]. This increase in 3-nitrotyrosine with age may result from increased NOS activity [13,18] due to a decrease in ADMA.

The present significant reduction in ADMA in patients with AD is consistent with studies reporting increases in NADPH diaphorase- and 3-nitrotyrosine-positive neurons in AD brains [3,20,21], implicating NO and $ONOO^-$ in the pathogenesis of AD. The fact that NOS-containing neurons are relatively spared in AD [10] and the activity

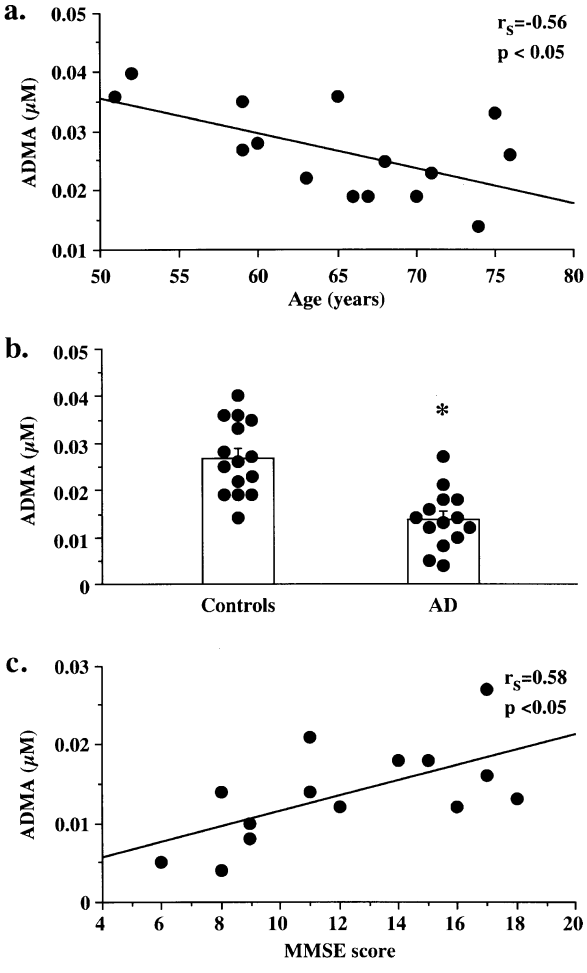

Fig. 1. (a) Concentrations of ADMA as a function of age in control subjects. (b) Concentrations of ADMA (Scatterogram and mean ± SEM) in AD patients and controls. *$P = 0.0001$ compared to controls (Mann–Whitney's test). (c) Concentrations of ADMA in AD patients as a function of MMSE score.

of Cu/Zn SOD (which reacts with $ONOO^-$ to nitrate tyrosine residues) is unaltered in AD brains [12] suggests that the increase in 3-nitrotyrosine is due to increased production of NO and oxidation of NO to $ONOO^-$ in AD. Whereas 3-nitrotyrosine concentrations in the CSF of AD patients significantly increased with disease progression in our previous study [22], ADMA decreased significantly with increasing severity of AD in the present study. Both results suggest that NOS activity increases with disease progression, resulting in increased production of NO, $ONOO^-$ and 3-nitrotyrosine. Whether the findings in the present results reflect changes in brain tissues remains to be investigated.

In conclusion, ADMA levels in the CSF decrease significantly with age and are also significantly lower in patients with AD than in controls. These findings suggest that ADMA may play an important role in regulating NO synthesis in brain aging and the pathogenesis of AD.

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