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L-Glutamate, L-arginine and L-citrulline levels in cerebrospinal fluid of Parkinson's disease, multiple system atrophy, and Alzheimer's disease patients

Short Communication

M. A. Kuiper[1], **T. Teerlink**[2], **J. J. Visser**[2,3], **P. L. M. Bergmans**[1], **P. Scheltens**[1], and **E. Ch. Wolters**[1]

Departments of [1]Neurology,
[2]Clinical Chemistry, and
[3]Surgery, Free University Hospital, Amsterdam, The Netherlands

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Summary. Alterations in neuronal nitric oxide (NO) production may play a role in the pathophysiology of Parkinson's disease (PD) Alzheimer's disease (AD), and multiple system atrophy (MSA). The biosynthesis of NO is dependent on the availability of L-arginine, the substrate for NO-synthase (NOS), and on L-glutamate, which stimulates NO synthesis via the NMDA receptor. In this process L-citrulline is formed. We measured the levels of these amino acids in cerebrospinal fluid (CSF) of 108 PD patients, 12 AD patients, 15 MSA patients and 21 healthy subjects. A slight but statistically significant elevation of CSF L-citrulline was found in MSA patients, while CSF L-glutamate was found to be significantly decreased in AD patients. We found no significant changes in L-arginine levels. Although the relation between the CSF levels of these amino acids and neuronal NO production is still unclear, our findings suggest that AD is associated with a decrease in NO synthesis.

Keywords: L-arginine, L-glutamate, L-citrulline, nitric oxide, nitrate, Parkinson's disease, Alzheimer's disease, multiple system atrophy, cerebrospinal fluid, biopterin.

Introduction

Nitric oxide (NO) has gained much interest in recent years as its role in vasodilatation (Moncada et al., 1988), macrophage cytotoxicity (Hibbs et al., 1987a; Boje and Arora, 1992) and neurotransmission (Bredt et al., 1990; Snyder and Bredt, 1991), has been established. NO is believed to play an

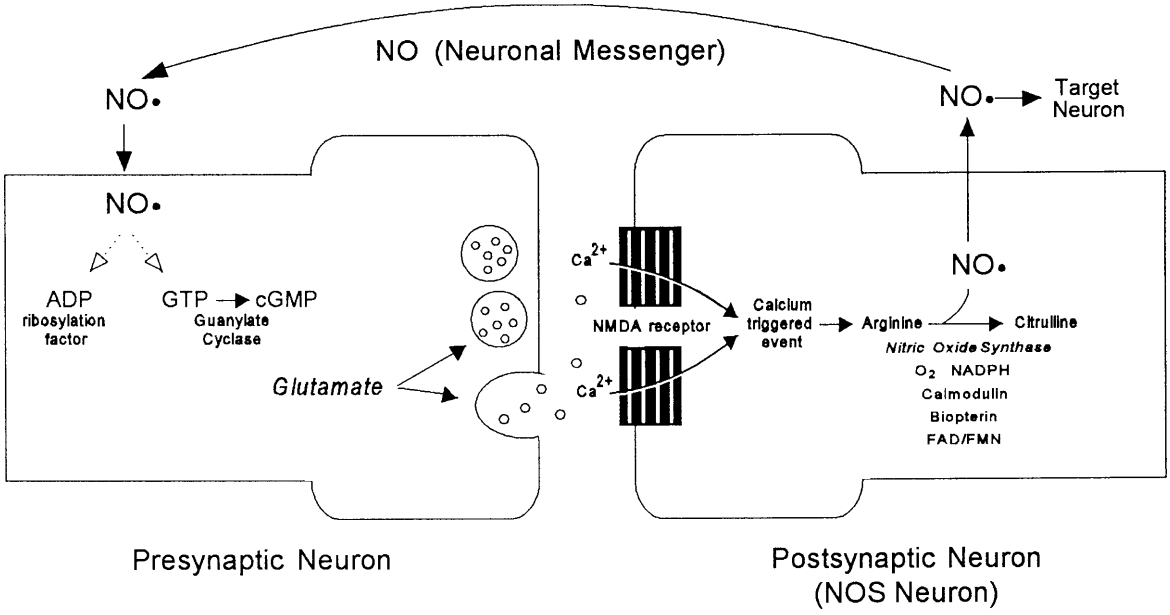

Fig. 1. The synthesis of NO. This figure depicts the events leading to NO production. Glutamate, with its agonistic effect on the N-Methyl-D-Aspartate (NMDA) receptor, induces Ca^{2+} influx. Ca^{2+}, together with calmodulin activates nitric oxide synthase (NOS). In presence of biopterin, NADPH, O_2, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), arginine is converted by NOS to citrulline and NO. NO can freely diffuse outside the neuron, and have its effect on adjacent cells. NO, being a free radical may act as a neurotoxin, or NO may act as a neurotransmitter. It activates guanylate cyclase to form cGMP or adenosine diphosphate (ADP) to mediate neurotransmitter release (after Dawson et al., 1992)

important role in memory function as a retrograde transmitter in long-term potentiation (Dawson et al., 1992).

NO is produced from the oxidation of the amino-acid L-arginine to L-citrulline by the enzyme NO synthase (NOS) (Hibbs et al., 1987b). Two forms of NOS are known: a constitutive, calcium-dependent enzyme, which is found in endothelial cells and specific neurons, and an inducible enzyme, which is expressed in macrophages in reaction to inflammatory stimuli (Moncada et al., 1991). NO production in NOS containing neurons is stimulated via the N-Methyl-D-Aspartate (NMDA) receptor by L-glutamate (see Fig. 1). NO is a very labile free radical with a half-life of only a few seconds. It is rapidly oxidized by tissue oxygen to the stable end products nitrate and nitrite. In the circulation, nitrite is almost totally converted to nitrate by hemoglobin.

As Alzheimer's disease (AD) and Parkinson's disease (PD) are reported to be associated with increased microglial activity (Rogers et al., 1988; McGeer et al., 1988), an increase in NO production via the inducible NOS could be expected. On the other hand, the amount of NO produced is strongly correlated with levels of tetrahydrobiopterin (BH4), which is a co-enzyme of NOS (Hevel and Marletta, 1992). In AD (Barford et al., 1984) and PD brain (Nagatsu et al., 1981) a decrease of BH4 has been established. This decrease

of BH4 may induce a diminished NOS activity and thus lead to a decrease in NO production in these diseases. A diminished production of NO may lead to deterioration of neuronal function, whereas at high levels, NO may have a neurotoxic effect. At suboptimal concentrations of BH4 or L-arginine however, NOS catalyzes the formation of reactive oxygen species, which are even more neurotoxic than NO itself (Heinzel et al., 1992).

We reported a decrease in CSF nitrate content in AD, PD, and MSA patients (Kuiper et al., 1994). In previous research we also confirmed the reported decrease of BH4 in CSF of these patients (Kuiper et al., 1993). Apart from a diminished BH4 content in AD and PD brain, changes in L-glutamate or L-arginine may contribute to the observed decrease in CSF nitrate. To further investigate the role of NO in neurodegenerative disorders as PD, AD, and MSA we determined CSF L-glutamate, L-arginine and L-citrulline content.

Materials and methods

Patients

We included in our study 89 PD patients without dementia, 19 PD patients with dementia (PDD), 15 MSA patients, 12 AD patients and 21 control subjects (C). The diagnosis PD was made according to criteria used by the Parkinson's Disease Society Brain Bank of London, U.K. (Hughes et al., 1992). Dementia in PD was diagnosed by applying the DSM-III-R criteria with additional neuropsychological assessment. Most PDD patients lacked typical cortical features. The clinical diagnosis of "probable" AD was made according to the NINCDS-ADRDA criteria (McKhann et al., 1984). MSA was diagnosed according to criteria reported by Quinn (1989). After IBZM-SPECT scanning, we included three patients with progressive supranuclear palsy (PSP), as all MSA and PSP patients showed a marked loss of dopamine D2 receptors in the striatum (Schwarz et al., 1992; Van Royen et al., 1993). Controls were mainly patients with lower back pain and a negative myelography, viz. no objective neurological disorder. Informed consent was obtained from all participating subjects.

Methods

All patients underwent lumbar puncture between 09.00 and 10.00 a.m. After the first 3 ml, which was used for cell counts, protein and glucose, 20 ml CSF was obtained and pooled. CSF was stored at $-70°C$ until assayed.

CSF amino acids were determined by high-performance liquid chromatography (HPLC) after deproteinization of samples with sulfosalicylic acid. The method is based on pre-column derivatization of primary amino acids with ortho-phthalaldehyde reagent containing 3-mercaptopropionic acid, separation of the derivatives by reversed-phase chromatography, and quantification by fluorescence detection. The derivatization step was fully automated. Complete separation was achieved within 12 minutes, using a C18 column containing 3 μm particles. Total analysis time, including derivatization, chromatography, and reequilibration of the column amounted to 17 minutes. Average within-run precision was 3.0% (range 1.9–6.4%). Between-run precision averaged 3.5% (range 2.1–7.2%). For further details see Teerlink et al. (1994).

Statistics

Statistical analysis comprised the Mann-Whitney U test, Spearman Rank correlation, Chi Square test and ANCOVA. The level of significance was set at $p < 0.05$. In case of multiple comparisons Bonferroni correction was used.

Table 1. CSF levels of L-arginine, L-citrulline and L-glutamate in Parkinson's disease, Alzheimer's disease and MSA patients

	N	Age ± SD	Arg ± SD	Cit ± SD	Glu ± SD
C	21	65.8 ± 11.9	21.3 ± 3.6	2.2 ± 0.6	1.9 ± 0.5
PD	89	66.0 ± 11.8	23.0 ± 3.6	2.6 ± 0.8	1.6 ± 0.7
PDD	19	75.3 ± 7.5*	23.5 ± 4.5	3.0 ± 1.3	1.7 ± 0.6
MSA	15	66.9 ± 10.4	23.6 ± 3.9	2.9 ± 0.9*	2.0 ± 0.9
AD	12	64.5 ± 7.8	22.2 ± 2.5	2.1 ± 0.7	1.5 ± 0.4*

N number of patients, *C* Controls, *PD* Parkinson's disease, *PDD* Parkinson's disease with dementia, *AD* Alzheimer's disease, *MSA* multiple system atrophy, *Arg* L-arginine, *Cit* L-citrulline, *Glu* L-glutamate. CSF L-arginine, L-citrulline and L-glutamate levels (mean ± SD) are given in μmol/l. *$p < 0.05$ compared with controls

The groupmeans were tested using Mann-Whitney U comparisons, correlations were tested using Spearman Rank correlation, nominal variables were tested with the Chi Square test and ANCOVA was used to analyse group differences with correction for age.

Each dataset is corrected for multiple comparisons using the Bonferroni.

All statistics were performed using SPSS-PC™.

Results

In all subjects normal cell counts, protein, IgG-index, glucose and a normal albumin CSF/serum ratio, which reflects the integrity of the blood-brain barrier, were found, with the exception of two AD patients who showed a slightly elevated albumin CSF/serum ratio. L-citrulline was found to be significantly increased in CSF of MSA patients compared to controls. L-Glutamate was significantly decreased in CSF of AD patients compared to controls. We found a relation between glutamate levels and age in the AD group ($r = 0.66$, $p = 0.02$), but not in the other patientgroups, nor in the controlgroup. The increase of L-arginine in PD patients compared with controls was statistically significant after Bonferroni correction, but no longer after correction for age and sex using ANCOVA. The PDD patients were significantly older than the control patients. The results are summarized in Table 1.

Discussion

We reported a decrease in CSF nitrate content in AD, PD, and MSA patients (Kuiper et al., 1994), which may indicate a decrease in NO production in the CNS of these patients. We hypothesized that an altered aminoacid metabolism in these patients might be underlying a decreased NO metabolism. Lakke et al. (1986), found a significant increase in L-arginine in CSF of PD patients. We could however not confirm this result.

We found a slight but statistically significant increase in CSF L-citrulline in MSA patients. This may indicate an increase in NOS activity and thus an increase in NO production, but this contradicts our earlier study (Kuiper et al., 1994). An increase in cerebrospinal fluid nitrite levels in PD patients was however reported by Qureshi et al. (1995), suggesting an increased NO pro-

duction in PD brain. Molina et al. (1996) found no change in cerebrospinal fluid nitrate levels in PD and both studies found a normal L-arginine concentration in PD.

The exact cause and the significance of the observed increases in CSF L-citrulline needs further investigation since L-citrulline and L-arginine are metabolized in several other pathways.

The reduction of CSF L-glutamate in AD patients confirms the results of Lowe et al. (1990), who found a decrease of glutamate in ante-mortem brain tissue of AD patients and may partly explain the impaired memory function in these patients (McEntee and Crook, 1993). Our results are also in agreement with the study of Toghi et al. (1992). This latter group not only found glutamate significantly reduced in CSF AD patients, but also of PD patients, a result we could not confirm (Toghi et al., 1991). Mally et al. (1997) also established a significant decrease in CSF glutamate in PD patients, while the level of glutamine was increased (and other aminoacids showed no significant changes). Jiménez-Jiménez et al. (1996) found no change in CSF glutamate of PD patients compared with controls. Pomara et al. (1992) found CSF glutamate to be significantly increased in AD patients. Methodological problems, which may underly and explain the different results in L-glutamate levels in CSF, were reported by Ferrarese et al. (1993). They suggested a method to inactivate CSF enzymes to get stable glutamate levels. As we already had collected our CSF samples, we could not take their considerations into account. It may be speculated that a decrease of intracerebral L-glutamate may lead to a diminished NO production and thus to a decrease of CSF nitrate in these patients. The well-established decrease of biopterin in PD and AD may also contribute to the observed decrease of CSF nitrate, since biopterin is a NOS co-enzyme.

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Authors' address: E. Ch. Wolters, Department of Neurology, Free University Hospital, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands, e-mail: e.wolters@azvu.nl