Neurochemistry International 53 (2008) 118–125

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Lateralization of hippocampal nitric oxide mediator system in people with Alzheimer disease, multi-infarct dementia and schizophrenia

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ARTICLE INFO

Article history:
Received 25 April 2008
Received in revised form 25 June 2008
Accepted 26 June 2008
Available online 3 July 2008

Keywords:
Nitric oxide synthases
Hippocampal lateralization
Planum temporale
Dementias
Psychosis

ABSTRACT

There is evidence that brain lateralization underlying hemispheric specialization can be observed also at biochemical level. However, hemispheric differences in nitric oxide mediator system have not yet been evaluated. The hippocampus and planum temporale are highly asymmetrical regions but the degree of their laterality is altered in demented or psychotic people. In the study, L-glutamate/L-arginine/L-citrulline concentrations, nitric oxide synthase activities/expressions and nitrites/nitrates levels were estimated in *autoptic* hippocampi. Right/left laterality in endothelial synthase activity and in nitrites/nitrates was observed in controls. Lateral changes were estimated in patients with Alzheimer disease (a marked increase in activities of constitutive synthases and in expression of inducible enzyme in the left side) and schizophrenia (an increase in activities of all enzymes especially in the right side). Significant shifts from positive to negative correlations were found between laterality of some components of nitric oxide pathway and of planum temporale volumetry under pathological conditions. The hippocampal nitric oxide system appears to be globally right/left lateralized, especially *via* actions of highly asymmetrical endothelial synthase. The results suggest a specific involvement of all synthases in the development of selected diseases and show that lateral analyses are of sufficient sensitivity to reveal subtle links. The volumetric asymmetry of the planum temporale as a marker of handedness is not probably simply linked to brain laterality at biochemical level but reflects alterations due to pathological processes.

1. Introduction

Many studies have demonstrated lateralization of human brains at structural or functional level. Alterations in degree of lateralization can be observed in neurodegenerative disorders (e.g., Alzheimer disease (AD) and multi-infarct dementia (MID)) as well as in neurodevelopmental (e.g., schizophrenia (SCHIZ)) disorders. Research in this area often focuses on the hippocampus and planum temporale (PT), two of the most asymmetrical brain regions (for review, see Toga and Thompson, 2003). It is suggested that brain asymmetry underlies the functional specialization of hemispheres and that the structural/functional lateralization is also reflected at biochemical level. Although a direct relationship has not yet been established, right–left differences have been observed, e.g., in certain neurotransmitter or neuropeptide systems (Toga and Thompson, 2003; Ramírez et al., 2004). Laterality has not yet been evaluated for the nitric oxide (NO) mediator system. Although the left hemisphere is dominant for language in the majority of people (approximately 96% of right- and 73% of left-handers show left-hemisphere language localization, e.g., Knecht et al., 2000), the dominant hemisphere or handedness should be determined to correctly evaluate brain laterality. In human *autoptic* tissue, the relevant information is often not accessible, data however suggest that volumetric asymmetry of the PT could be used as a possible marker since the degree of its laterality is higher in right- than in left-handers (Toga and Thompson, 2003).

The NO molecule plays a key role in many physiological processes but is also involved in the development of various diseases including those mentioned above. In the brain, the balance between neuroprotective (anti-apoptotic) and neurotoxic (pro-apoptotic) NO effects appears to depend especially on its amount or oxidation. NO and L-citrulline are synthesized by NO synthase (NOS, EC 1.14.13.39) through a conversion process from

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doi:10.1016/j.neuint.2008.06.009

L-arginine. Two of the three NOS isoforms, the neuronal and the endothelial synthase (nNOS and eNOS), are constitutively expressed calcium-dependent enzymes, while the inducible synthase (iNOS) is expressed as a part of response to activation and is calcium-independent. An important role is attributed also to L-glutamate. This amino acid stimulates NO synthesis through nNOS *via* *N*-methyl-D-aspartate receptors and, inversely, the NO molecule enhances its release (Girault and Greengard, 1999). NO undergoes various reactions in biological fluids resulting in the formation of oxidized metabolites, nitrites or nitrates (for review, see Guix et al., 2005).

The NO molecule also plays an important role in pathogenesis of AD. As its production or oxidation probably increases at the early stages of the disease and decreases as neuronal cell loss progresses (e.g., Tohgi et al., 1998), experimental data often yield contradictory results. In comparison with age-matched controls, patients with AD exhibit decreased concentrations of L-glutamate in the neocortex (Lowe et al., 1990) or in CSF (Kuiper et al., 2000) but concentrations of L-arginine/L-citrulline in their CSF or plasma remain unchanged (Kuiper et al., 2000; Selley, 2003). Increased expression of nNOS was observed for instance in hippocampal neurons containing neurofibrillary tangles and in reactive astrocytes near amyloid plaques (for review, see Guix et al., 2005). On the other hand, some authors have reported reduced expression of hippocampal nNOS and no changes in corresponding mRNA levels (for review, see Molina et al., 1998). No significant alterations in nNOS activity were observed in brains of transgenic mice with pathological changes analogous to AD (Lahiri et al., 2003). Expression of eNOS/iNOS however seems to increase in astrocytes near plaques in AD patients as well as in transgenic mice (Lüth et al., 2001), while the expression of eNOS in endothelial cells with amyloid deposits was reported to be lower than in healthy vessels (de la Monte et al., 2000). Levels of nitrites/nitrates in CSF of patients with AD were described as normal or decreased (Molina et al., 1998) and attenuated as the dementia progressed (Tohgi et al., 1998).

MID is a type of vascular dementia characterized by multiple small cerebral infarcts associated with multiple brain embolism leading to ischemia. Although dysfunctions of vascular endothelium and of eNOS are generally hypothesized, data obtained directly from patients with MID are rare, in contrast to AD. It seems, e.g., that concentrations of nitrites/nitrates in CSF are significantly higher in the MID group when compared to controls and that the levels remain elevated throughout all stages of the disease (Tohgi et al., 1998). However, some authors have detected no alterations in nitrate CSF levels (Tarkowski et al., 2000). On the other hand, in a rat model of MID, nitrite/nitrate levels in the striatum were increased during 24–48 h following an experimentally induced ischemia and the results indicated an involvement of especially nNOS (Shirakura et al., 2005). Nevertheless, different animal models of global or focal cerebral ischemia indicate detrimental (especially nNOS/iNOS) or protective (eNOS) roles of all three enzymes (Guix et al., 2005).

SCHIZ is a heterogeneous mental disorder with unclear etiology; however, an impairment of many neurotransmitter (especially L-glutamate) or mediator systems (including that of NO) is suggested. Many experiments were performed directly on *autoptic* brain tissue of patients with SCHIZ and the results show possible region-dependent differences. In the prefrontal cortex for example, no changes in binding of (3*H*)L-NG-nitro-arginine (Dean et al., 1999) but decreased activities of constitutive nNOS/eNOS with no alterations in their expression (Xing et al., 2002) were reported. However, an increase in nNOS expression was found here, too (Baba et al., 2004). In the striatum, no changes in binding of (3*H*)L-NG-nitro-arginine were observed (Dean and Hussain, 2001) but some NOS-positive interneurons exhibited morphological abnormalities such as abnormal size and branching pattern, or they were markedly reduced in number (Lauer et al., 2005). In the cerebellum, there were no changes in binding of (3*H*)L-NG-nitro-arginine in molecular or granule cell layer (Doyle and Slater, 1995) but an increased number of NOS-positive neurons in cerebellar vermis (Karson et al., 1996) was reported. Finally, a reduction of NOS-positive neurons in the hypothalamus (Bernstein et al., 1998) and significantly increased levels of nitrites/nitrates in the nucleus caudatus (Yao et al., 2004) were observed. Neurodevelopmental models of SCHIZ in rats *via* prenatal stress or neonatal nonhandling suggest a decreased number of NOS-positive neurons in the adult hippocampus (Veenman et al., 1999). Moreover, neonatal destruction of the ventral hippocampus resulted in increased NOS immunostaning in the prefrontal and entorhinal cortex (Bernstein et al., 1999). In human subjects, changes such as lowered levels of nitrite/nitrates in CSF were reported (Ramirez et al., 2004). Outside the CNS, an increased activity of constitutive enzymes in platelets of drug naïve or drug treated patients was found (Das et al., 1995) and increased levels of asymmetric dimethylarginine, an endogenous competitive inhibitor of the synthases, as well as, decreased levels of nitrates were detected in plasma of drug naïve first episode patients (Das et al., 1996). The reduced nitrate levels were also observed in cultured skin fibroblasts (Das et al., 1998).

The aims of the study are as follows: (i) to estimate lateralization of hippocampal NO mediator system in nondemented nonpsychotic controls using estimations of corresponding amino acids, of all synthases and of nitrites/nitrates, (ii) to evaluate changes due to AD, MID and SCHIZ, and finally (iii) to compare the results with these of volumetric analysis of the PT.

2. Materials and methods

2.1. Tissue sampling

All experiments are in accordance with the declaration of Helsinki. The research was formally approved by the local ethics commission of the Prague Psychiatric Center/Prague, Czech Republic, and is in agreement with Laws 129/2003 and 130/2003. Human brain tissues of 50 patients were obtained at autopsy (*postmortem* delays were shorter than 24 h in all cases).

2.2. Histological analysis

Five brain areas from both hemispheres were dissected (three areas of neocortex: *gyrus frontalis medius*, *gyrus temporalis superior et medius*, and *lobulus parietalis inferior*; one of the hippocampus: *gyrus parahippocampalis*; one of the cerebellum: *lobulus semilunaris inferior*) and evaluated using silver staining technique in accordance with our previous study (Krištofiková et al., 1995). Samples were divided into five groups: controls (nondemented nonpsychotic patients with various types of tumors in the majority of cases, no marked histological changes indicative of AD pathology), AD (clinically diagnosed dementia, number of senile plaques and tangles in given areas of the cortex and in the hippocampus higher than would be expected for age, the criteria were consistent with those used in the classification of Mirra et al., 1991), MID (clinically diagnosed dementia, number of senile plaques and tangles corresponding to normal aging, vascular changes, neuropathologic lesions and gliosis present, other subtypes of vascular dementia were discarded), MIX (clinically diagnosed dementia, AD and MID simultaneously) and SCHIZ (clinically diagnosed psychosis, no marked histological changes indicative of AD pathology).

2.3. Biochemical analysis

The CA1 region of the rostral hippocampus was dissected out from both hemispheres. The tissue was divided into three exactly corresponding parts, wrapped up in aluminum and stored at −80 °C until assayed.

2.3.1. Concentrations of L-glutamate, L-arginine and L-citrulline

Amino acids were measured in extracts (methanol/acetic acid) taken from the outer parts of the CA1 region after derivatization with dansyl chloride (L-glutamate, see Simmaco et al., 1990) and with *o*-phthalaldehyde (L-arginine and L-citrulline, see Graser et al., 1985). The chromatographic system consisted of a Bio-Rad AS 100 autosampler with a 20-μl sample loop, an Agilent 1100 binary gradient pump and a