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Analysis of cholinergic markers, biogenic amines, and amino acids in the CNS of two APP overexpression mouse models

Debby Van Dam[a], Bart Marescau[a], Sebastiaan Engelborghs[a,b], Thomas Cremers[c], Jan Mulder[d], Matthias Staufenbiel[e], Peter Paul De Deyn[a,b,*]

[a]*Laboratory of Neurochemistry and Behaviour, Born-Bunge Institute, Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium*
[b]*Department of Neurology/Memory clinic, Middelheim Hospital, Lindendreef 1, B-2020 Antwerp, Belgium*
[c]*Department of Biomonitoring and Sensoring, University Center for Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands*
[d]*Department of Animal Physiology, Graduate School of Behavioural and Cognitive Neurosciences, University of Groningen, Kerklaan 30, NL-9750 AA Haren, The Netherlands*
[e]*Novartis Institutes of Biomedical Research Basel, CH-4002 Basel, Switzerland*

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Abstract

Two transgenic mouse models expressing mutated human amyloid precursor protein and previously found to display cognitive and behavioural alterations, reminiscent of Alzheimer patients' symptomatology, were scrutinised for putative brain region-specific changes in neurochemical parameters. Brains of NSE-hAPP$_{751}$m-57, APP23 and wild-type mice were microdissected to perform brain region-specific neurochemical analyses. Impairment of cholinergic transmission, the prominent neurochemical deficit in Alzheimer brain, was examined; acetylcholinesterase and choline acetyltransferase activity levels were determined as markers of the cholinergic system. Since Alzheimer neurodegeneration is not restricted to the cholinergic system, brain levels of biogenic amines and metabolites, and amino acidergic neurotransmitters and systemic amino acids were analysed as well.

Cholinergic dysfunction, reflected in reduced enzymatic activity in the basal forebrain nuclei, was restricted to the APP23 model, which also exhibited more outspoken and more widespread changes in other neurotransmitter systems. Significant changes in compounds of the noradrenergic and serotonergic system were observed, as well as alterations in levels of the inhibitory neurotransmitter glycine and systemic amino acids. These observations were clearly in occurrence with the more pronounced histopathological and behavioural phenotype of the APP23 model. As transgenic models often do not represent an end-stage of the disease, some discrepancies with results from post-mortem human Alzheimer brain analyses were apparent; in particular, no significant alterations in excitatory amino acid levels were detected. Our findings of brain region-specific alterations in compound levels indicate disturbed neurotransmission pathways, and greatly add to the validity of APP23 mice as a model for Alzheimer's disease. Transgenic mouse models may be employed as a tool to study early-stage neurochemical changes, which are often not accessible in Alzheimer brain.

Keywords: Transgenic mouse model; Alzheimer's disease; Amino acids; Biogenic amines; Cholinergic system; APP23

1. Introductory statement

Alzheimer's disease (AD) is a prevalent neurodegenerative disorder characterized by progressive cognitive decline, behavioural disturbances, changes in personality and impaired language skills, severely interfering with daily functioning. Patients exhibit a devastating loss of most cortical and subcortical functions, ultimately leading to death (Selkoe, 2001).

The cognitive and behavioural disturbances marking AD can – at least partially – be attributed to alterations in

* Corresponding author. Tel.: +32 3 820 26 20; fax: +32 3 820 26 18.
E-mail address: peter.dedeyn@ua.ac.be (P.P. De Deyn).

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multiple neurotransmitter systems. Although neurotransmitter alterations may not be the principal cause of AD, they are extensively studied as they represent important therapeutic targets. The first neurochemical alterations described in AD were situated in the cholinergic system (Davies and Maloney, 1976; Whitehouse et al., 1982). Degeneration of cholinergic neurons in the nucleus basalis of Meynert, situated in the basal forebrain and primarily projecting to the neocortex, occurs early in the course of the disease and leads to a marked decline in the activities of choline *O*-acetyltransferase (EC 2.3.1.6; ChAT) and acetylcholinesterase (EC 3.1.1.7; AChE) (Davies, 1979; Coyle et al., 1983; Perry et al., 1992). A correlation between cholinergic deficits and both cognitive symptomatology and the extent of neuropathological alterations in AD was reported (Perry et al., 1978; Martin et al., 1987; Dournaud et al., 1995; Bierer et al., 1995). Neurochemical alterations observed in AD brain, however, are not confined to the cholinergic system. Moreover, while the cholinergic involvement in cognitive AD symptomatology is incontestable, many data indicate that the neurochemical alterations underlying cognitive deterioration and related disturbances in cortical processing implicate more widespread neurodegeneration that cannot be entailed solely to the cholinergic system (for review see Dringenberg, 2000).

Many publications have reported neuronal loss and, inherently, alterations in the concentration of neurotransmitters and metabolites of the noradrenergic, adrenergic, dopaminergic and serotonergic system (Cross et al., 1983; Arai et al., 1984; Francis et al., 1985; Palmer et al., 1987b; Ebinger et al., 1987; Herregodts et al., 1989; Nazarali and Reynolds, 1992; for review see Engelborghs and De Deyn, 1997). Besides these alterations in monoaminergic neurotransmission, often contradictory, changes in the levels of neurotransmitter and systemic amino acids (AA) in CSF and brain of AD patients have been published (Tohgi et al., 1993; Young, 1987; for review see Engelborghs and De Deyn, 1997). Nevertheless, research clearly suggests a role for AA in the clinical manifestation and pathogenesis of AD. Excitotoxicity, referring to a process of neuronal death caused by excessive or prolonged activation of receptors for excitatory AA neurotransmitters, has been implicated in the aetiology or progression of human neurodegenerative diseases, including AD (for review see Doble, 1999).

Our group previously reported significant cognitive and behavioural disturbances, reminiscent of clinical symptomatology in AD, in two murine models expressing mutated human amyloid precursor protein (hAPP) (D'Hooge et al., 1996; Van Dam et al., 2003, Van Dam et al., in press). While no neurodegeneration or AD hallmarks have been reported in the NSE-hAPP$_{751}$m-57 model (Mucke et al., 1994), the APP23 model shows clear signs of neurodegeneration, and from the age of 6 months onwards, the first scarce plaques have been observed (Sturchler-Pierrat et al., 1997; Sturchler-Pierrat and Staufenbiel, 2000; Van Dam et al., 2003). The 3-month-old NSE-hAPP$_{751}$m-57 model displayed only mild changes in Morris water maze (MWM) learning, which were restricted to the acquisition phase of the test (D'Hooge et al., 1996). The APP23 model, on the other hand, exhibited severely impaired learning curves during the acquisition phase, as well as significantly impaired spatial accuracy during the MWM probe trial from the age of 3 months onwards (Van Dam et al., 2003; Van Dam et al., in press). Behavioural alterations, e.g. altered patterns of cage activity and decreased exploration levels, were also reported in the APP23 model (Van Dam et al., 2003; Van Dam et al., in press; Vloeberghs et al., 2004). Both transgenic strains can be considered valid models for AD, as they mimic several cognitive and behavioural and/or histopathological hallmarks of the human condition. Like all animal models developed up to date, both the NSE-hAPP$_{751}$m-57 and the APP23 model are partial models for the human condition. Nevertheless, they can be considered essential in further unravelling the nature, and spatial and temporal development of the complex molecular pathology underlying this condition, and potentially will allow the future evaluation of innovating therapies (De Deyn et al., 2000). Accordingly with histopathology, cognitive and behavioural alterations were more outspoken in the APP23 model, leading to the expectation that the APP23 model is a better neurochemical model for AD. An ensuing step in the validation of these models consisted of an extensive neurochemical evaluation of neurotransmitter and non-neurotransmitter compound levels in 14 different microdissected CNS regions. ChAT and AChE enzyme activity were examined as markers of the cholinergic system. High-pressure liquid chromatography with electrochemical detection (HPLC-ED) was used to measure levels of biogenic amines (BA) and certain metabolites. The brain concentration of a total of 19 AA, both systemic AA and neurotransmitters, was determined with an AA analyser using cation exchange chromatography. To our knowledge, this is the first study to report an elaborate neurochemical data set in transgenic mouse models of AD. Neurochemically validated animal models will enable the examination of early-stage neurochemical changes in AD, whereas neurochemical studies in patients too often reflect end-stage disease.

2. Experimental procedures

2.1. Mouse models

Transgenic mice and WT littermates were bred from previously described lines (Mucke et al., 1994; Sturchler-Pierrat et al., 1997), which had been backcrossed to a C57BL/6J background for at least six generations. Both models overexpress cDNA-based constructs of hAPP, 751 amino acids in length, driven by a neuron-specific murine promoter. The transgene in the APP23 model is seven-fold overexpressed and contains the Swedish double mutation (K670N/M671L) driven by the Thy-1 promoter (Sturchler-