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Neurosteroid biosynthetic pathways changes in prefrontal cortex in Alzheimer's disease

Sabina Luchetti [a,b,*], Koen Bossers [a], Saskia Van de Bilt [a], Vincent Agrapart [c], Rafael Ramirez Morales [c], Giovanni Vanni Frajese [c,d], Dick F. Swaab [a]

[a] *Netherlands Institute for Neuroscience (NIN), An Institute of the Royal Netherlands Academy of Arts and Sciences, Neuropsychiatric Disorders Lab, Meibergdreef 47, 1105 BA Amsterdam, The Netherlands*

[b] *Istituto Dermopatico Immacolata (IDI-IRCCS), Santa Lucia Foundation Scientific Institute, Rome, Italy*

[c] *Internal Medicine Department, Endocrinology Lab, University of Rome "Tor Vergata", Rome, Italy*

[d] *Faculty of Motor Sciences, University of Cassino, Cassino, Italy*

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Abstract

Expression of the genes for enzymes involved in neurosteroid biosynthesis was studied in human prefrontal cortex (PFC) in the course of Alzheimer's disease (AD) ($n = 49$). Quantitative RT-PCR (qPCR) revealed that mRNA levels of diazepam binding inhibitor (DBI), which is involved in the first step of steroidogenesis and in GABAergic transmission, were increased, as were mRNA levels for several neurosteroid biosynthetic enzymes. Aromatase, 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1) and aldo-keto reductase 1C2 (AKR1C2), were all increased in the late stages of AD. Several GABA-A subunits were significantly reduced in AD. Increased expression of aromatase in the PFC was confirmed by immunohistochemistry and was found to be localized predominantly in astrocytes. These data suggest a role for estrogens and allopregnanolone produced by astrocytes in the PFC in AD, possibly as part of a rescue program. The reduced gene expression of some synaptic and extra-synaptic GABA-A subunits may indicate a deficit of modulation of GABA-A receptors by neuroactive steroids, which may contribute to the neuropsychiatric characteristics of this disease.

Keywords: Neurosteroids; GABA-A receptors; Alzheimer's disease; Postmortem; Prefrontal cortex; Aromatase; Gene expression

1. Introduction

The sex steroids, i.e. estrogens, androgens and progesterone, when synthesized and metabolized in the central nervous system (CNS), are known as neurosteroids (Baulieu, 1998). In neural tissue, the enzymes involved in steroidogenesis are present both in glial cells and neurons (Do Rego et al., 2009; Mellon and Vaudry, 2001; Stoffel-Wagner, 2001). A scheme of the sex steroid biosynthesis pathway in the brain and the abbreviations used in the text are shown in Fig. 1. There is substantial evidence suggesting that sex steroids can mediate neuroprotection and influence neuronal survival, neuronal and glial differentiation and myelination in the CNS by regulating gene expression of neurotrophic factors and anti-inflammatory molecules (Behl, 2002; Bialek et al., 2004; Djebaili et al., 2005; Melcangi et al., 2008; Schumacher et al., 2007).

Progesterone, testosterone and estradiol have been shown to have neuroprotective and regenerative effects in in vitro models of neurodegeneration and in animal models of brain injury (Gouras et al., 2000; Schumacher et al., 2003; Vongher and Frye, 1999). On the other hand, some metabolites of pregnenolone, progesterone, testosterone and deoxycorticosterone (DOC) are also regarded as "neuroactive" because of their ability to modulate neurotransmitter activity. Among these, 3α5α-tetrahydro progesterone (3α5α-THP or allopregnanolone), androstanediol and 3α5α-tetrahydro DOC (3α5α-THDOC) are positive allosteric modulators of

* Corresponding author at: Meibergdreef 47, 1105 BA Amsterdam, The Netherlands. Tel.: +31 205665505; fax: +31 205666121.
E-mail address: s.luchetti@nin.knaw.nl (S. Luchetti).

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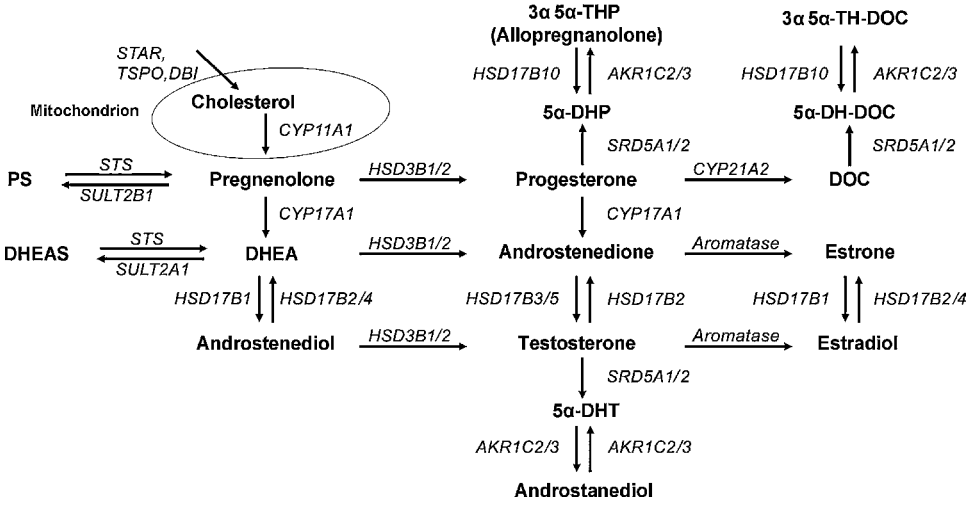

Fig. 1. Sex steroid biosynthetic pathway. Sex steroids as well as many of their metabolites have been shown to have pleiotropic actions in the CNS, all of which could contribute to neurodegenerative processes and repair (Schumacher et al., 2000). All the main steps of sex steroids synthesis are shown, including the initial transport of cholesterol into the mitochondria, mediated by the complex: STAR, TSPO and DBI. Numbers after the enzyme name indicate the isoform type. *Abbreviations*: AKR1C, aldo-keto reductase 1C; CYP11A1, cytochrome P450scc; CYP17A1, cytochrome P450c17A1; CYP21A2, cytochrome P450c21B; DBI, diazepam binding inhibitor; DOC, deoxycorticosterone; 5α-DH-DOC, 5α-dehydro-doxycorticosterone; DHEA, dehydroepiandrosterone; 5α-DHP, 5α-dehydro-progesterone; HSD3B, 3β-hydroxysteroid dehydrogenase; HSD17B, 17β-hydroxysteroid dehydrogenase; STAR, steroid acute regulator; SRD5A, 5α-reductase; SULT, sulphotransferase; STS, steroid-sulphatase; 3α5α-THDOC, 3α 5α-tetrahydro-deoxycorticosterone; 3α5α-THP, 3α5α-tetrahydroprogesterone; TSPO, 18 kDa translocator protein.

ionotropic γ-amino-butyric acid (GABA-A) receptors. In particular, allopregnanolone is considered the most potent allosteric modulator of GABA-A receptors, acting in a benzodiazepine (BDZ)-like manner (Belelli and Lambert, 2005).

The modulatory activity of neuroactive steroids on the GABA system is well-established (Belelli and Lambert, 2005). This involves interaction with post-synaptic GABA-A receptors, most commonly containing the α1 (GABRA1), β2 (GABRB2) and γ2 (GABRG2) subunits, and extra-synaptic GABA-A receptors commonly containing α4 (GABRA4), δ (GABRD) or ε (GABRE) subunits (Farrant and Nusser, 2005). Neuroactive steroids can also regulate the expression of GABA-A receptor subunit genes in vitro and in vivo (Biggio et al., 2001). As a consequence of these properties, allopregnanolone and the other neuroactive compounds modulate memory processes, anxiety, sleep processes, responses to stressful stimuli and seizure susceptibility and may influence cognitive and neuropsychiatric symptoms such as those seen in AD (Dubrovsky, 2005).

While a role for sex steroids in neuroprotection has been demonstrated in animal studies including AD models (Carroll et al., 2007; Ciriza et al., 2004; He et al., 2004), information is lacking about the neurosteroid biosynthetic pathway in the human CNS during neurodegenerative processes in AD, partly because of the difficulty in obtaining suitable human brain tissue. Decreased blood levels of sex steroids with aging have been associated with an increased risk of AD (Cholerton et al., 2002; Pike et al., 2006). Combined with evidence of reduced levels of steroids such as testosterone in human AD brain (Rosario et al., 2004), this raises the possibility that alterations in gene expression of the enzymes which synthesize neurosteroids may be involved in the pathology of AD, which may in turn result in reduced neuroprotective actions.

Furthermore, the evidence that the GABA system is relatively conserved in AD prefrontal cortex (PFC) compared to other neurostransmitter systems (Francis, 2003; Lowe et al., 1988; Reinikainen et al., 1988) suggests that this system represents an important target of the neurosteroids, especially in late stage AD when the neurodegenerative process is advanced.

The goal of the present study is to elucidate the gene expression of the enzymes involved in the synthesis of neurosteroids in the human PFC during the neuropathological progression of AD. Using quantitative RT-PCR (qPCR) we analyzed a list of 37 genes including the key biosynthetic enzymes, the steroid hormone receptors, and the GABA-A receptor subunits on which the neurosteroids exert their modulatory actions in the brain. Immunohistochemistry (IHC) experiments were also performed to confirm the main qPCR findings at the protein level.

2. Materials and methods

2.1. Subjects

Postmortem human brain tissue was obtained from The Netherlands Brain Bank, Netherlands Institute for Neuroscience, Amsterdam (NBB). Donors or their next of kin gave written informed consent to the NBB to allow the brain autopsy and to use the material and clinical information for research purposes.