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Retinoic acid normalizes nuclear receptor mediated hypo-expression of proteins involved in β-amyloid deposits in the cerebral cortex of vitamin A deprived rats

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Recent data have revealed that disruption of vitamin A signaling observed in Alzheimer's disease (AD) leads to a deposition of β-amyloid (Aβ). The aim of this study was to precise the role of vitamin A and its nuclear receptors (RAR) in the processes leading to the Aβ deposits. Thus, the effect of vitamin A depletion and subsequent administration of retinoic acid (RA, the active metabolite of vitamin A) on the expression of RARβ, and of proteins involved in amyloidogenic pathway, e.g., amyloid precursor protein (APP), β-secretase enzyme (BACE), and APP carboxy-terminal fragment (APP-CTF) was examined in the whole brain, hippocampus, striatum, and cerebral cortex of rats. Rats fed a vitamin A-deprived diet for 13 weeks exhibited decreased amount of RARβ, APP695, BACE, and of APP-CTF in the whole brain and in the cerebral cortex. Administration of RA is able to restore all expression. The results suggest that fine regulation of vitamin A mediated gene expression seems fundamental for the regulation of APP processing.

Keywords: Vitamin A deficiency; Retinoic acid nuclear receptor (RAR); Amyloid precursor protein (APP); β-Site APP-cleaving enzyme (BACE); APP carboxy-terminal fragment (CTF); Rat cerebral cortex

Introduction

Retinoic acid (RA), the active metabolite of vitamin A, mediates its effect by inducing or repressing transcription of genes through binding to specific nuclear receptors which are transcription factors: RAR (whose ligands are the *all-trans* RA and *9-cis* RA isomers) and RXR (whose ligand is the *9-cis* RA isomer) (Marill et al., 2003). It is well-known that retinoids, and particularly retinoic acid, play a critical role in a variety of essential life processes, including reproduction, embryonic development, and modulation of the growth and differentiation of a wide variety of mammalian cell types (Bonet et al., 2003).

Presently, the role of retinoids in the adult central nervous system is less conspicuous than their role in development and has only recently attracted scientific attention. Some data suggest that fine regulation of retinoid mediated gene expression seems fundamentally important for optimal brain functioning such as LTP, synaptic plasticity, learning, and memory (Enderlin et al., 1997; Chiang et al., 1998; Etchamendy et al., 2001, 2003; Misner et al., 2001; Cocco et al., 2002). Similarly, changes at the retinoid level appear capable of producing alterations in neuronal target proteins and consequently may affect physiological maintenance processes in the mature brain (Malik et al., 2000; Lane and Bailey, 2005). Among the many genes whose expression is regulated by RA, there are those coding for their own nuclear (Yamagata et al., 1993) receptors and those coding for neuron-specific proteins involved in many activities in the mature brain, e.g., nerve growth factor (Fiorentini et al., 2002), *N*-methyl-D-aspartate receptor (Beczkowska et al., 1996), dopamin receptor 2 (Farooqui, 1994), choline acetyltransferase (Kobayashi et al., 1994), neurogranin (Iñiguez et al., 1994).

Recently, data from a number of studies have argued for the involvement of retinoid signaling in the etiology of Alzheimer's disease (AD) (Goodman and Pardee, 2003; Corcoran et al., 2004). This disease is characterized by the presence of two major pathologic lesions, neuritic plaques, and neurofibrillary tangles. Neuritic plaques are composed of extracellular fibrillar deposits of β-amyloid peptide. The involvement of APP, an integral membrane glycoprotein, in the mechanisms of these deposits is well documented (Neve et al., 1990; Sinha, 2002). The APP gene undergoes alternative splicing to yield three major mRNA transcripts, APP770, APP751, and APP695 with two minor transcripts,

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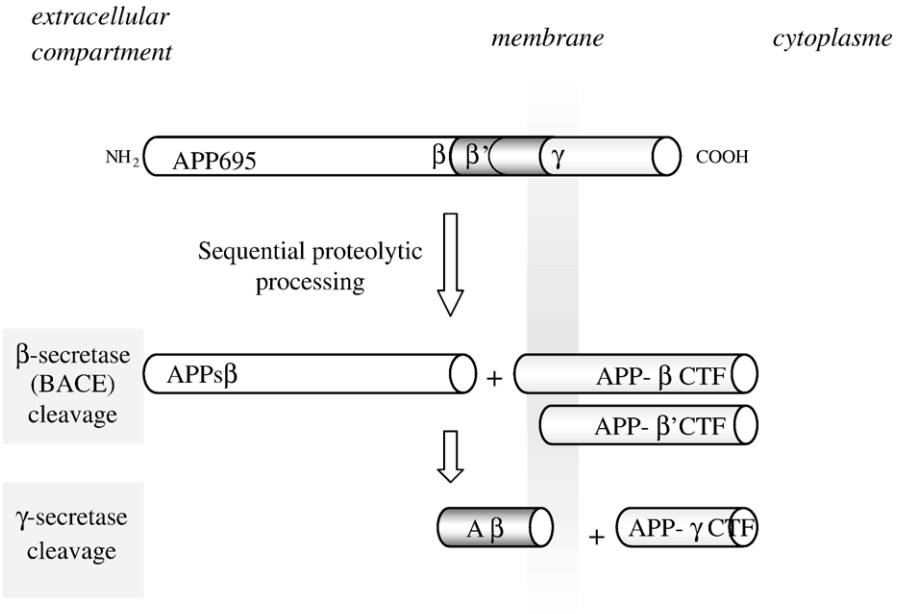

Fig. 1. Schematic representation of the APP amyloidogenic pathway. The β-secretase (BACE) cleaves APP695 leading to the secreted form of APP (APPsβ) and to the carboxy-terminal fragments (APP-βCTF and APP-β′CTF). The latter are cleaved by the γ-secretase complex, which generates a carboxy-terminal fragment (γCTF) and the peptide Aβ.

APP563, and APP714 (Tanzi et al., 1988). The APP770 and APP751 isoforms are protected by the presence of the Kunitz protease inhibitor (KPI) domain, whereas the APP695 isoform, lacking this KPI domain, undergoes amyloidogenic pathway-related sequential proteolytic processing by β- and γ-secretases, generating the β-amyloid peptide (Aβ peptide) (Leblanc et al., 1996; Simons et al., 1996; Gao and Pimplikar, 2001) (Fig. 1). The more amyloidogenic fragment $A\beta_{1-42}$ aggregates and forms the nidus of plaque leading to cell death (Dugué et al., 2003). The recently identified enzyme BACE is the major β-secretase responsible for generation of Aβ peptides by neurons (Cai et al., 2001; Bodendorf et al., 2002). This enzyme leads to the cleavage of the APP ectodomain at the N-terminus region, producing membrane-bound C-terminal fragments (APP-βCTF and APP-β′CTF), the direct precursors of Aβ (Evin et al., 2003). APP-CTF is considered to be a potential early marker for the biological diagnosis of AD (Sergeant et al., 2002). Interestingly, several studies have shown that expression of some of AD-related genes are under control of RA in the brain, e.g., β-site APP-cleaving enzyme (BACE) (Satoh and Kuroda, 2000), amyloid precursor protein (APP) (Hung et al., 1992; Yang et al., 1998; Murray and Ihwe, 2003), or α-synuclein, a non-Aβ component of amyloid plaques (Satoh and Kuroda, 2001). Moreover, recent studies have revealed a decrease in serum retinol in AD patients (Bourdel-Marchasson et al., 2001; Rinaldi et al., 2003). Some authors have reported that (i) hypo-functioning of retinoid signaling pathway is a key factor in development of AD (Goodman and Pardee, 2003), and (ii) disruption of retinoid signaling causes deposition of β-amyloid in the adult brain (Corcoran et al., 2004). Finally, vitamin A or retinoid seems to impair and destabilize preformed Aβ aggregates and consequently seems to protect against plaque formation, probably via its nuclear receptors (Ono et al., 2004; Sahin et al., 2005). Such effects of vitamin A are of primary importance in that attenuating Aβ mediated neuro-degeneration is, today, of major consideration in a potential treatment for AD.

Thus, in the present study, the role of vitamin A, and its receptors, in the processes leading to amyloid deposits was studied in vivo. Therefore, using a model of postnatal vitamin A deprivation, the effect of a vitamin A-free diet, with or without administration of RA, on the amount of mRNA of RA nuclear receptor (RARβ) and on three target genes involved in amyloid deposits (APP695 and APP770-751 and the β-secretase BACE) in the whole brain and in hippocampus, striatum, and cortex of adult rat was evaluated. We have choice to favor, in this study, the quantification of RARβ mRNA since this isoform (i) is the major receptor in the adult brain (Maret et al., 2005), (ii) seems up-regulated by its own ligand in several organs including brain (Kato et al., 1992; Yamagata et al., 1993; Alfos et al., 2001), and (iii) is implicated in optimal adult brain functioning (Chiang et al., 1998; Enderlin et al., 1997; Etchamendy et al., 2001, 2003; Husson et al. 2004).

The levels of mRNA were measured using a real-time polymerase chain reaction (PCR). Moreover, using a Western blot analysis, the amount of APP carboxy-terminal fragment (APP-

Fig. 2. Western blot analysis of APP-CTF in 13-week vitamin A deprived rats treated with RA or not. (A) Example of Western blot analysis of tubulin and APP-CTF in the cortex of control rats, vitamin-A-deficient rats (VAD rats) and VAD rats treated by RA (VAD + RA rats). The five bands for the APP-CTF correspond to (A) phosphorylated form of APP-βCTF (APP-βCTF^{p}); (B) APP-βCTF; (C) phosphorylated form of APP-β′CTF (APP-β′CTF^{p}); (D) phosphorylated form of APP-αCTF (APP-αCTF^{p}) and APP-β′CTF; (E) APP-αCTF. (B) Abundance of phosphorylated APP-βCTF (APP-βCTF^{p}), APP-βCTF, phosphorylated APP-β′CTF (APP-β′CTF^{p}), phosphorylated APP-αCTF + APP-β′CTF (APP-αCTF^{p}/APP-β′CTF) and APP-αCTF in the hippocampus, striatum, and cerebral cortex of 13-week vitamin A deprived rats treated with RA or not. Data represent mean values of measures performed on five animals ($n = 5$), with the standard error represented by vertical bars. *Mean value was significantly different from control rats ($P < 0.05$). □ Depleted. ■ Depleted + RA.