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Research Report

Changes in the expression and subcellular localization of RARα in the rat hippocampus during postnatal development

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ABSTRACT

Retinoic acid receptors (RARs) are reported to mediate the effects of retinoid acid and participate in the maintenance of normal hippocampal function during embryonic and postnatal stages. RARα is the only one that has been reported to be continuously expressed among RARs in the CA1–CA3 areas of the hippocampus, at both the mRNA and the protein level. Here, we show the expression and subcellular localization of RARα in granule and pyramidal cells in various regions of the hippocampus during postnatal development of rats. We discovered that the expression level of RARα in postnatal hippocampal tissue gradually decreased over time with increasing developmental maturity of the nervous system. Moreover, the subcellular localization of RARα expression showed a phenomenon of intracellular translocation during the postnatal development period. This new discovery is inconsistent with a traditional viewpoint according to which RARα, as a nuclear transcription factor, is mainly expressed inside nucleus. This phenomenon suggests that RARα may have different actions during each stage of hippocampal development.

1. Introduction

Vitamin A and its derivatives have been found to be closely related to the central nervous system (CNS) development and function. Insufficient or excessive dietary vitamin A can cause characteristic congenital malformations of the CNS, including hydrocephalus, spina bifida, anophthalmia, and microphthalmia (Maden, 2002; McCaffery et al., 2003). More recently, evidence has emerged that vitamin A is required for several aspects of adult brain function, especially for learning and memory (Mey and McCaffery, 2004; Mey, 2004). Rodents fed with vitamin A deficient diet result in more errors than controls in the radial maze spatial learning task. Replenishing dietary vitamin A reverses these deficits in rats and attenuates them in mice (Cocco et al., 2002). Whether vitamin A affects brain development or adjusts mature brain function, it acts mainly through its active metabolic products, all-*trans*-retinoic acid (atRA) and 9-*cis*-retinoic acid (Jacobs et al., 2006;

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Abbreviation: RAR, retinoic acid receptor; atRA, all-*trans*-retinoid acid; CNS, central nervous system; RA, retinoic acid; LTP, long-term potentiation; LTD, long-term depression; RXR, retinoic X receptor; DG, dentate gyrus; P, postnatal day; CLSM, confocal laser-scanning microscope; AMPAR, the α-amino-3-hydroxyl-5-methyl-4-isoxazole propionate receptors

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Maden, 2002). Members of the retinoic acid (RA) signaling pathway, such as transporters, metabolic enzymes, and receptors, have all been detected in embryonic and mature brain tissue. Mature brain tissue is one of the organs shown high concentrations of retinoic acid, especially in the hippocampus, where the RA level is higher than anywhere else in the brain (Werner and Deluca, 2002). RA is also essential for cellular growth and differentiation in the development and adult animals (Maden, 2007). RA has been shown to induce undifferentiated cells to differentiate into neurons and glia (Maden, 2001). This has been observed consistently using various strains of embryonic carcinoma cells and stem cells from mice, rats, and humans (Maden, 2001). Additionally, RA is required for the maintenance of neurites, and plays an important role in the survival of developed neurites, such as those found in the adult CNS (Maden, 2007). Supplementing atRA can partially recover learning and memory defects caused by vitamin A deficiency in adult rats, and ingesting atRA can also improve learning and memory decline in aged rats (Etchamendy et al., 2001; Etchamendy et al., 2003). Adding atRA into the hippocampus slices of vitamin A deficient rat can rescue reduced long-term potentiation (LTP) levels and long-term depression (LTD) in area CA1 of the hippocampus (Misner et al., 2001).

Recently, many studies have suggested that the reason why RA has such functions is that it can regulate the expression of multiple functional genes. During embryonic and early postnatal brain development, RA regulates genes controlling neuronal differentiation, neurite outgrowth, and pattering of the anteroposterior axis of the neural tube (Maden, 2002; McCaffery et al., 2003). In the mature brain, RA can control genes associated with synaptic plasticity, which is thought to be the neurophysiologic basis of learning and memory (Etchamendy et al., 2001). Moreover, there have also been reports that RA regulates genes of neurogenesis and differentiation of neurons in the mature hippocampus (Lane and Bailey, 2005). The RA signaling pathway is important for RA to carry out its physiological functions. Two members of the intercellular receptor superfamily, RARs and retinoic X receptors (RXRs), bind to RA ligands with high affinity and specificity (Allenby et al., 1993). The RARs and RXRs act as heterodimers to recognize consensus sequences known as RA-response elements in the control elements of RA-responsive genes (Chambon, 1994; Kastner et al., 1997; Maden, 2002; Mangelsdorf et al., 1990). RARs bind to all-*trans*-RA and 9-*cis*-RA with approximately equal affinity, whereas RXRs bind only to 9-*cis*-RA (Chambon, 1996). Current studies have mainly focused on the expressions of these important RA receptors in embryonic neurodevelopment and mature brain function (Maden, 2007). However, there are fewer studies on the role of RA in postnatal development, which is a critical period for the development of brain functions. As known, human newborns have lower synaptic density than adults. However, by several months after birth, synapse formation in the infant cerebrum exceeds the level in adults. At age 4, synaptic density has already reached its peak in all areas of the brain, and exceeds the level in adults by 50%. Around puberty, pruning processes cause the number of synapses to decrease, a pattern which continues into adulthood (Huttenlocher and Dabholkar, 1997). In the hippocampus, synaptic loops that are closely associated with learning and memory are also initially established and mature during this stage of development (Huttenlocher and Dabholkar, 1997).

The hippocampus is part of the limbic system, a region of phylogenetically and architectonically primitive cortex. A crucial role of the hippocampus lies in the generation of episodic, declarative and spatial learning and memory, which are all important parts of brain function (McCaffery et al., 2006). atRA has important effects on synaptic plasticity and neurogenesis in the adult hippocampus (McCaffery et al., 2006). There are three known subtypes of each RA receptor subfamily, classified as -α, -β, and -γ, which display specific expression patterns throughout development and function in cell growth and differentiation (Fitzgerald et al., 1997; Lohnes, 1999). Among RARs, RARα is the only one that has been reported to be continuously expressed in area CA1–CA3 of the hippocampus. RARα expression in the hippocampal dentate gyrus (DG) is also seen in rodents (Krezel et al., 1999; Zetterstrom et al., 1999). In the present study, we investigated the expression and subcellular localization of RARα in nerve cells in various areas of the hippocampus during postnatal development in rats. It was found that the expression level of RARα in postnatal hippocampal tissue gradually decreased with increasing maturity of the nervous system. Moreover, the subcellular localization of RARα showed a phenomenon of intracellular translocation during the postnatal development period. This new discovery is inconsistent with a traditional viewpoint, which holds that RARα, as a nuclear transcription factor, is mainly expressed in the nucleus. This phenomenon indicates that RARα may have a different function during each development stage of the hippocampus.

2. Results

2.1. *Changes in expression level of RARα mRNA during hippocampal development*

RARα mRNA expression was detected at P1, P28, and P56 ($n=6$, $F=21.44$, $P=0.000$, one-way ANOVA). As shown in Fig. 1, RARα

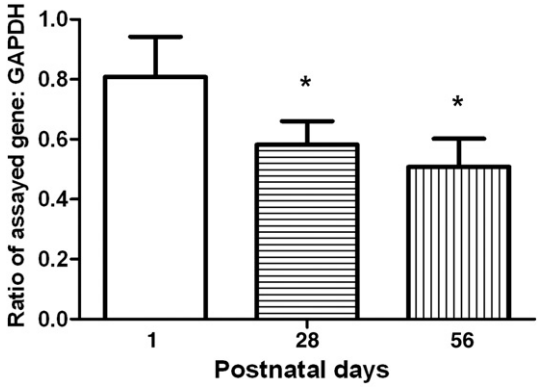

Fig. 1 – Expression of RARα mRNA in the hippocampus during postnatal development (P1, P28, and P56). Values are mean ± SEM, $n=6$, $F=21.44$, $P=0.000$, one-way ANOVA. RARα mRNA at P28 and P56 was decreased compared to P1. *Different from P1, $P<0.05$.