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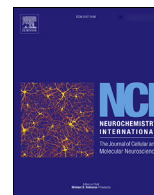

Expression and regulation of CYP17A1 and 3β-hydroxysteroid dehydrogenase in cells of the nervous system: Potential effects of vitamin D on brain steroidogenesis

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ABSTRACT

Steroids are reported to have diverse functions in the nervous system. Enzymatic production of steroid hormones has been reported in different cell types, including astrocytes and neurons. However, the information on some of the steroidogenic enzymes involved is insufficient in many respects. Contradictory results have been reported concerning the relative importance of different cell types in the nervous system for expression of CYP17A1 and 3β-hydroxysteroid dehydrogenase (3β-HSD). 3β-HSD is important in all basic steroidogenic pathways and CYP17A1 is required to form sex hormones. In the current investigation we studied the expression of these enzymes in cultured primary rat astrocytes, in neuron-enriched cells from rat cerebral cortex and in human neuroblastoma SH-SY5Y cells, a cell line often used as an *in vitro* model of neuronal function and differentiation. As part of this study we also examined potential effects on CYP17A1 and 3β-HSD by vitamin D, a compound previously shown to have regulatory effects in steroid hormone-producing cells outside the brain. The results of our study indicate that astrocytes are a major site for expression of 3β-HSD whereas expression of CYP17A1 is found in both astrocytes and neurons. The current data suggest that neurons, contrary to some previous reports, are not involved in 3β-HSD reactions. Previous studies have shown that vitamin D can influence gene expression and hormone production by steroidogenic enzymes in some cells. We found that vitamin D suppressed CYP17A1-mediated activity by 20% in SH-SY5Ycells and astrocytes. Suppression of CYP17A1 mRNA levels was considerably stronger, about 50% in SH-SY5Y cells and 75% in astrocytes. In astrocytes 3β-HSD was also suppressed by vitamin D, about 20% at the enzyme activity level and 60% at the mRNA level. These data suggest that vitamin D-mediated regulation of CYP17A1 and 3β-HSD, particularly on the transcriptional level, may play a role in the nervous system.

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1. Introduction

Endogenous steroids, such as estrogens and androgens, are reported to have diverse functions in the nervous system, including effects on neuronal survival, inflammation, proliferation, brain plasticity and modulation of synaptic transmission (Arnold and Beyer, 2009; Brann et al., 2007; Porcu et al., 2016). Several metabolic pathways for biosynthesis of different neurosteroids exist in cells of the central nervous system (CNS). However, data indicate that, in line with tissue-specific differences observed in other parts of the body, metabolism varies in different brain cell types (Cascio et al., 2000; Hojo et al., 2004; Manca et al., 2012; Zwain and Yen, 1999).

Cells of the CNS consist of neurons and glial cells. The most abundant cell types in the CNS are the astrocytes and the oligodendrocytes (Brann et al., 2007; Jessen, 2004). Oligodendrocytes are important in the formation of myelin. The enzymatic

Abbreviations: CNS, central nervous system; CYP, cytochrome P450; DHEA, dehydroepiandrosterone; 3β-HSD, 3β-hydroxysteroid dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TBP, TATA box binding protein; TLC, thin layer chromatography.

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production of steroid hormones have previously been reported in neurons, astrocytes and oligodendrocytes (Zwain and Yen, 1999). Synthesis of steroid hormones, using cholesterol as starting material, requires several enzymes (Fig. 1) (Benkert et al., 2015; Lundqvist et al., 2010). The first step of this synthesis forms pregnenolone, which is then metabolized into various types of hormones via different pathways. A critical enzyme in these metabolic pathways is 3β-hydroxysteroid dehydrogenase (3β-HSD), necessary for production of the 3-oxo-group present in most of the mature hormones or in their immediate precursors. Deficiency of 3β-HSD leads to disturbed levels of steroid hormones, in particular a decreased or absent production of glucocorticoids and mineralocorticoids which, if untreated, can lead to death in infancy (Labrie et al., 1992).

Certain of the steroidogenic enzymes, such as CYP19A1 (aromatase) which catalyzes aromatization of the steroid A-ring of androstenedione and testosterone to form estrogens, have been well characterized (Garcia-Ovejero et al., 2005). However, for some of the other steroidogenic enzymes information is insufficient in many respects. For instance, conflicting results are reported regarding which cell type in the nervous system is most important for the expression of CYP17A1 and 3β-HSD. CYP17A1 is required to form dehydroepiandrosterone (DHEA) as well as androstenedione, the immediate precursor to testosterone and estrone. Several studies have reported expression of CYP17A1 almost exclusively in neurons (Do Rego et al., 2007; Hojo et al., 2004; Manca et al., 2012). In contrast, another study reported expression of CYP17A1 mRNA and enzyme activity mainly in astrocytes (Zwain and Yen, 1999), whereas yet other authors report undetectable levels of CYP17A1 mRNA in astrocytes (Cascio et al., 2000), or cultures of glial cells (Mellon and Deschepper, 1993).

As described above 3β-HSD has an essential role in all pathways (Fig. 1). Astrocytes appear to be a major site of 3β-HSD expression (Sinchak et al., 2003). However, it has also been reported that this enzyme is expressed mainly in neurons in the rat brain (Schumacher et al., 2004).

In addition to the confusion on which cell type in the nervous system is most important for CYP17A1 and 3β-HSD expression, the knowledge of their regulation is limited, with studies focusing mainly on effects by estrogens (Micevych et al., 2007; Sorwell et al., 2012).

In the current investigation, we studied the expression of CYP17A1 and 3β-HSD in different cells of the nervous system. In addition, we examined potential effects on these enzymes by vitamin D, a compound previously shown to have regulatory effects on genes and enzymes in steroid hormone-producing cells outside the brain (Lundqvist et al., 2010; Merhi et al., 2014). To our knowledge there are very few studies on potential influence of vitamin D in regulation of the metabolism of neurosteroids (Yagishita et al., 2012). We find this of particular interest since levels of vitamin D have been linked to several diseases that affect the brain (Eyles et al., 2013).

2. Materials and methods

2.1. Materials

Human neuroblastoma SH-SY5Y cells (ATCC CRL-2266) were purchased from the American Type Culture Collection (Manassas, VA, USA). Materials for cell culturing were obtained from Thermo Fisher Scientific (Life Technologies). 1α,25-Dihydroxyvitamin D_3 (sc-202877) was purchased from Santa Cruz Biotechnology Inc. Radiolabeled [1,2,6,7-^{3}H(N)]-dehydroepiandrosterone (NET814), [1β-^{3}H(N)]-androst-4-ene-3,17-dione (NET469) and [1,2,6,7-^{3}H(N)]-progesterone (NET381) were obtained from Perkin Elmer. Retinoic acid and unlabeled steroid hormones were purchased from Sigma.

2.2. Animals

Animal studies were approved by the regional ethics committee for research on animals in Uppsala (Sweden) and carried out in accordance with the policy of the Society for Neuroscience. Rats (Sprague-Dawley) were obtained from Charles River, Germany. Primary rat astrocytes and neuron-enriched cells from rat cerebral cortex were prepared from brain tissue of these animals as described below.

2.3. Preparation of neuron-enriched cortical cell cultures from rat embryos

Primary cortical cell cultures from rat brain, containing neurons and glia at a ratio of approximately 60/40, were prepared from embryos of pregnant Sprague-Dawley rats, removed at embryonic day 17, as described by Nylander et al. (2016), Diwakarla et al. (2016). and Kindlundh-Högberg et al. (2010). The obtained cortical cells were cultured on poly-L-lysine coated plates for 2 weeks in Neurobasal media (which favors neuronal cell growth), supplemented with B-27 (2%), glutamine (600 μM) and antibiotics/antimycotics (1%), prior to experimentation. Media changes were performed twice a week. The amount of neurons in these cultures were determined by assay of microtubule-associated protein 2-positive cells (Nylander et al., 2016).

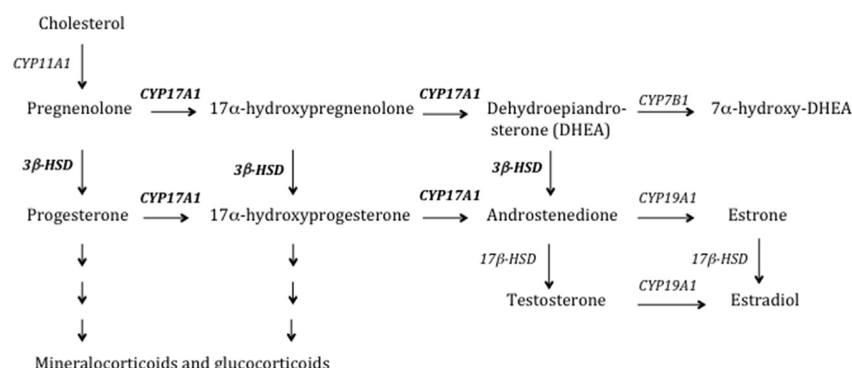

Fig. 1. Overview of enzymes in steroidogenesis.

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