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Regional distribution of 5α-reductase type 2 in the adult rat brain: An immunohistochemical analysis

M. Paola Castelli [a,b,c,*], Alberto Casti [a], Angelo Casu [a], Roberto Frau [a,c], Marco Bortolato [c,e], Saturnino Spiga [d], Maria Grazia Ennas [a,c]

[a] *Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology and Division of Cytomorphology, University of Cagliari, Cagliari, Italy*
[b] *Center of Excellence for the Neurobiology of Addictions, University of Cagliari, Cagliari, Italy*
[c] *Tourette Syndrome Center, University of Cagliari, Cagliari, Italy*
[d] *Department of Life and Environmental Sciences, University of Cagliari, Italy*
[e] *Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA*

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Summary The enzyme 5α-reductase (5αR) catalyzes the conversion of testosterone and other Δ^4-3-ketosteroids into their 5α-reduced metabolites. Of the five members of the 5αR family, the type 2 enzyme (5αR2) plays a key role in androgen metabolism, and is abundantly distributed in the urogenital system. Although 5αR2 has been reported to be highly expressed in the brain during early developmental stages, little is currently known on its anatomical and cellular distribution in the adult brain. Thus, the present study was designed to determine the detailed localization of 5αR2 in the adult rat brain, using a highly specific polyclonal antibody against this isoform. Parasagittal and coronal sections revealed 5αR2 immunoreactivity throughout most brain regions,

Abbreviations: Acb, accumbens nucleus; AcC, accumbens nucleus core; AcSh, accumbens nucleus shell; AOL, anterior olfactory nucleus lat; AON, anterior olfactory nucleus; Arc, arcuate hypothalamic nucleus; BLA, basolateral amygdaloid nucleus ant; Cb, cerebellum; CA1—CA3, fields of Ammon's horn; Cg, cingulate cortex; CLi, caudal linear nucleus raphe; cp, cerebral peduncle; CPu, caudate putamen; DEn, dorsal endopiriform nucleus; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; DR, dorsal Raphe nucleus; Ent, entorhinal cortex; Fr, frontal cortex; f, fornix; GP, globus pallidum; Gr, granular layer; HA, anterior hypothalamus; Hi, hippocampus; HDB, nucleus of horizontal limb of diagonal band; Hyp, hypothalamus; IC, inferior colliculus; LC, locus coeruleus; LaDL, lateral amygdaloid nucleus dorsolateral; La, lateral amygdaloid nucleus; LD, lateral dorsal thalamic nuclei; LV, lateral ventricle; LPL, lateral posterolateral thalamic nucleus; M2, motor cortex area 2; Me5, mesencephalic trigeminal nucleus; MePV, medial amygdaloid nucleus posteroventral; MD, medial dorsal thalamic nuclei; MG, medial geniculate nucleus; MHb, medial habenular nucleus; Mi, mitral cell layer of olfactory bulb; MnR, median raphe nucleus; Mo, molecular layer; O, stratum oriens; OCC, occipital cortex; opt, optic tract; Par1, parietal cortex area 1; Pc, Purkinje cells; Pir, piriform cortex; Pn, pontine nuclei; PnC, pontine reticular nucleus caudal; PMCo, posteromedial cortical amygdaloid nucleus; PoThal, posterior thalamic nucleus; Py, pyramidal cell layer; R, stratum radiatum; Rt, reticular thalamic nucleus; S, subiculum; SuG, superficial gray layer superior colliculus; SN, substantia nigra; SNC, substantia nigra compacta; SNR, substantia nigra reticular; STh, subthalamic nucleus; TT, tenia tecta; Th, thalamus; Tu, olfactory tuberclue; VL, ventral lateral thalamic nucleus; VMH, ventromedial hypothalamus nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; VTA, ventral tegmental area; 3V, 3rd ventricle; 4V, 4th ventricle.

* Corresponding author at: Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, Cittadella Universitaria, SS 554, km. 4,500, I-09042 Monserrato, Cagliari, Italy. Tel.: +39 070 6754065; fax: +39 070 6754320.
E-mail address: castelli@unica.it (M.P. Castelli).

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with strong immunolabeling in the layers III and VI of the prefrontal and somatosensory cortex, olfactory bulb, thalamic nuclei, CA3 field of hippocampus, basolateral amygdala and Purkinje cell layer of cerebellum. Lower 5αR2 levels were detected in the hypothalamus and midbrain. Moreover, double labeling fluorescence with confocal laser scanning microscopy (CLSM) revealed that 5αR2 is localized in neurons, but not in glial cells. Specifically, the enzyme was documented in the pyramidal neurons of the cortex by CLSM analysis of simultaneous Golgi-Cox and immunofluorescent staining. Finally, low levels of 5αR2 expression were identified in GABAergic cells across the cortex, hippocampus and striatum. These findings show that, in the adult brain, 5αR2 is distributed in critical regions for behavioral regulation, suggesting that the functional role of this isoform is present throughout the entire lifespan of the individual.

1. Introduction

Steroid 5α-reductases (5αRs) are a family of enzymes catalyzing the saturation of the 4,5 double bond of the A ring of several Δ^4-3-ketosteroid substrates, including progesterone, glucocorticoids, mineralocorticoids and androgens (see Russell and Wilson, 1994; Paba et al., 2011). Of the five known 5αRs, only the types 1 (5αR1) and 2 (5αR2) are believed to be physiologically involved in steroidogenesis. Although these two isoenzymes share common genetic components (Langlois et al., 2010), similar size and catalytic activities (see Paba et al., 2011), their differences in substrate affinity and anatomical distribution suggest that they exert distinct physiological functions. In particular, 5αR2 is posited to convert testosterone into its metabolite 5α-androstan-17β-ol-3-one (dihydrotestosterone; DHT), the most potent androgen hormone, which stimulates the acquisition of the majority of secondary sexual traits in men (Breedlove, 1992).

In the central nervous system (CNS), 5αR catalyzes the main rate-limiting reaction for the synthesis of neurosteroids such as allopregnanolone (AP), a derivative of progesterone that regulates stress and anxiety responses by acting as a potent allosteric modulator of the γ-aminobutyric acid A ($GABA_A$) receptor (Barbaccia et al., 2001; Girdler and Klatzkin, 2007). In addition to AP, other 5α-reduced neurosteroids have been associated with important functions in the brain; for example, DHT and its metabolite 5α-androstan-3α,17β-diol (3α-diol), have been shown to play cardinal roles in the regulation of emotion and cognition, stimulation of myelination as well as development of sexually dimorphic areas in the central nervous system (Valencia et al., 1992; Goldstein and Sengelaub, 1994; Beyer and Hutchinson, 1997; Frye et al., 2001; Melcangi et al., 2003; Sato et al., 2004; Edinger and Frye, 2005).

Previous research has shown that numerous brain regions produce DHT from testosterone, suggesting the presence of 5αR2 in their neural tissues. Nevertheless, while several studies have shown that 5αR1 is abundantly expressed in the CNS throughout all developmental stages (Poletti et al., 1998), the brain distribution of 5αR2 was originally considered essentially limited to late fetal and early postnatal periods (Poletti et al., 1998). In contrast with this finding, subsequent studies have documented the presence of 5αR2 in brain regions of adult rodents and humans, albeit at lower levels than 5αR1 (Normington and Russell, 1992; Lephart, 1993; Torres and Ortega, 2003, 2006; Kimoto et al., 2010; Bortolato et al., 2011). In humans, whereas 5αR1 immunoreactivity is present in both neurons and glia, 5αR2 distribution has been found only in pyramidal cells, but not in small neurons and glial cells, pointing to cell-specific patterns in the expression of this enzyme throughout the brain (Eicheler et al., 1994; Aumüller et al., 1996).

Recently, the whole localization of the 5αR2 transcript in the adult mouse brain was reported in the Allen Brain Atlas, showing that the molecule is indeed present in most brain regions, and particularly expressed in the olfactory lobe, neocortex, hippocampus and cerebellum (http://mouse.brain-map.org/gene/show/60858). In spite of these results, the complete anatomical and cellular distribution of 5αR2 protein in the brain remains elusive.

Here we report the detailed localization of 5αR2 in the brain of the adult rat, as detected by immunohistochemical analyses performed with a highly specific anti-5αR2 polyclonal antibody. In addition, the distribution of this enzyme in neurons, glia and GABAergic cells were carried by double-labeling immunostaining, analyzed by CLSM. Finally, we visualized the presence of 5αR2 in cortical pyramidal neurons by means of the simultaneous Golgi-Cox and immunofluorescence staining.

2. Methods

2.1. Animals

Male Sprague—Dawley rats (220—250 g; Charles River, Como, Italy) were used in all experiments. Animals were housed in groups of four at a temperature of 24 °C and with 60% humidity under a 12-h light/dark cycle (lights on from 0700 to 1900 h). All experimental procedures were conducted between 0900 h and 1300 h, with methods aimed at minimizing environmental stress, in view of its impact on brain 5αR2 expression (Sánchez et al., 2009; Bortolato et al., 2011). Experiments were carried out in accordance with the guidelines of the European Communities Directive of 24 November 1986 (86/609/EEC) and the Italian Legislation (D.P.R. 116/92).

2.2. Brain tissue preparation

Rats were deeply anaesthetized with Equithesin (0.97 g pentobarbital, 2.1 g magnesium sulphate, 4.25 g chloral hydrate, 42.8 mL propylene glycol, 11.5 mL ethanol 90%, 5 mL kg^{-1}, intraperitoneal) and transcardially perfused with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). Brains were rapidly removed and post-fixed in the same fixative for 6 h. After repeated washing in 0.1 M PBS, brains were cryoprotected in