

Short communication

Localization of 20 α -hydroxysteroid dehydrogenase mRNA in mouse brain by in situ hybridization

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Abstract

The enzyme 20 α -hydroxysteroid dehydrogenase (20 α -HSD) catalyzes the conversion of progesterone into its inactive form, 20 α -hydroxyprogesterone. We studied the expression of 20 α -HSD mRNA in mouse brain by in situ hybridization. 20 α -HSD mRNA was exclusively found in neurons in cortex and hippocampus. In the cortex, the labelled cells were concentrated in the external granular layer, the external pyramidal layer and the inner granular layer. In the hippocampus, the labelling was mostly located over pyramidal cells of the CA1 layer. These results suggest that progesterone can be inactivated by 20 α -HSD in some specific brain areas.

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It is well documented that nerve cells can de novo synthesize progesterone (for a review, see Ref. [8]) which can be converted successfully into 5 α -dihydroprogesterone and 3 α , 5 α tetrahydroprogesterone (allopregnanolone) by the combined action of 5 α -reductase and 3 α -hydroxysteroid dehydrogenase (3 α -HSD) [5]. Conversion of progesterone into 20 α -reduced metabolites by the brain of several species, including male and female rats, has also been reported, indicating some brain 20 α -hydroxysteroid dehydrogenase (20 α -HSD) activity [5]. So far, the expression and distribution of 20 α -HSD in the brain has not been reported. Very recently, we have localized by in situ hybridization 20 α -HSD mRNA in a variety of tissues in male and female mice. In the adrenal cortex, liver, kidney and skin, the expression was higher in female than in male animals [10]. In order to evaluate the expression and precise localization of 20 α -HSD mRNA in the brain, we have proceeded to in situ hybridization

studies using a radiolabelled 20 α -HSD cRNA probe in male and female adult mouse brains.

Three adult male (26–30 g) and female (24–27 g) C57BL6 mice were used. The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study was performed in accordance with the CCAC Guide for Care and Use of Experimental Animals. The animals were all perfused with 50 ml 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The perfused females were on diestrus day 1 because, at this stage, the plasma levels of estradiol are low and relatively stable [12]. The stages of the estrous cycle were determined by examination of vaginal smears using Papanicolaou staining as described [12]. The brains and three ovaries were excised and postfixed in the same fixative for 24 h at 4 °C. They were placed in 15% sucrose in 0.1 M phosphate buffer before being quickly frozen in isopentane chilled in liquid nitrogen.

Coronal sections (10 μ m thick) were serially cut at –20 °C through the entire brain. Each second section was mounted onto gelatin- and poly-L-lysine-coated slides.

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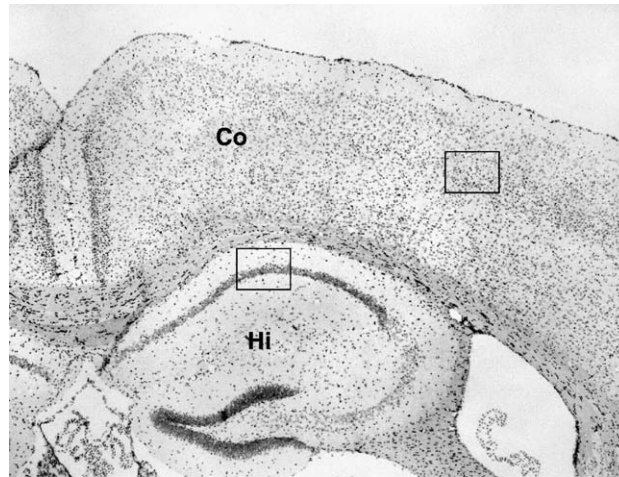


Fig. 1. Micrograph showing the dorsal area of the mouse brain. The circumscribed areas in the cortex (Co) and CA1 layer of the hippocampus (Hi) correspond to the areas shown at high magnification in Figs. 2 and 3. Magnification: $\times 60$.

The vector used for the production of cRNA probe was constructed by insertion into a pBSKSII+ vector (Stratagene, La Jolla, CA) of a cDNA fragment of 252 bp

of mouse 20 α -HSD (Genebank accession number AB059565). The cDNA fragment located at position 36–288 downstream from the ATG start codon was obtained

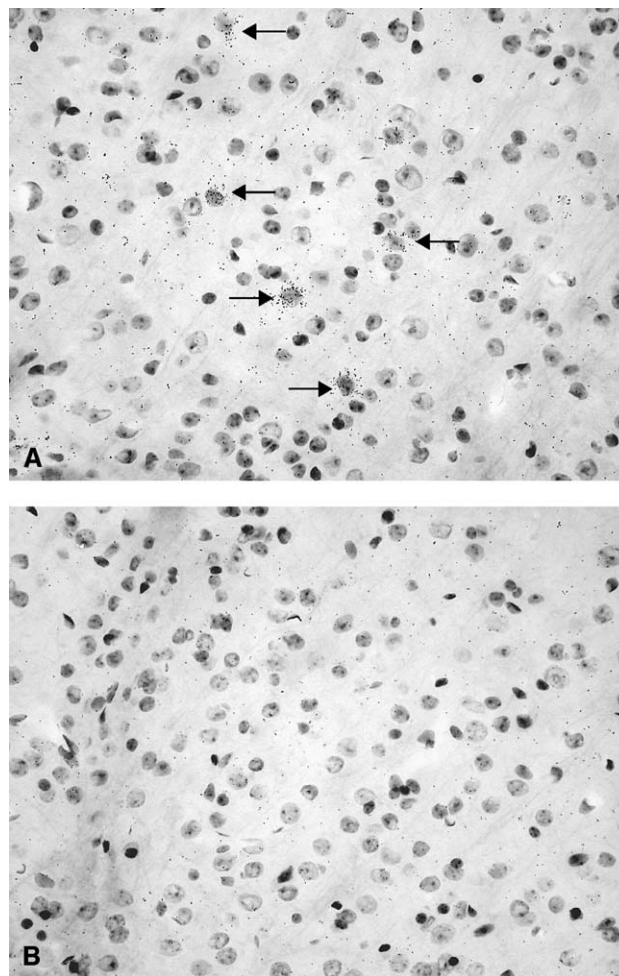


Fig. 2. (A) Micrographs showing the localization of 20 α -HSD mRNA in the external granular layer of the parietal cortex in a female mouse. A few neurons are labelled (\rightarrow). (B) Consecutive section hybridized with the sense probe. Only diffuse labelling can be detected. Magnification: $\times 600$. Exposure time: 45 days.

by amplification using polymerase chain reaction. In situ hybridization with the antisense and sense ^{35}S -labelled cRNA probes was performed as previously described [2,10]. After hybridization, the sections were dehydrated and coated with liquid photographic emulsion (Kodak-NTB2; diluted 1:1 with water). After 10–45 days of exposure, the sections were processed and counterstained with haematoxylin.

Ovaries were used as a positive control for 20 α -HSD mRNA localization. As previously reported [10], strong labelling was observed in corpora lutea (not shown). No autoradiographic reaction could be obtained following hybridization with the labelled sense probe. In the brain, radiolabelling was almost exclusively observed in the cerebral cortex and hippocampus. A neuron was considered as specifically labelled when the number of grains exceeded five times the background level. Labelled neurons were observed in the frontal, parietal, temporal and visual cortex. They were mostly concentrated in the external granular layer, the external pyramidal cell layer and the inner granular layer (Figs. 1 and 2A). The

molecular layer was generally devoid of any specific reaction. The localization of labelled neurons was the same in both sexes. In the hippocampus, the labelling was mostly detected over pyramidal cells of the CA1 layer. Occasionally labelled neurons were detected in the oriens layer dorsal to the CA1 layer (Figs. 1 and 3A). In adjacent sections which had been hybridized with the sense-labelled cRNA probe, no cell labelling could be detected (Figs. 2B and 3B).

Using in situ hybridization, we have then demonstrated for the first time that 20 α -HSD mRNA is expressed in specific areas of the male and female mouse brain. These findings suggest that neurons in cortical and hippocampal areas can convert progesterone to its inactive form, 20 α -hydroxyprogesterone. It is noteworthy that the brain regions expressing 20 α -HSD mRNA have been shown to highly express 3 β -HSD, the enzyme which converts pregnenolone to progesterone [1,3,8]. Recently, Ibanez et al. [4], using in situ hybridization to detect 3 β -HSD mRNA, have reported a laminar pattern of labelling in the rat cerebral cortex, without identifying the layers exhibiting the hybridization signal. In

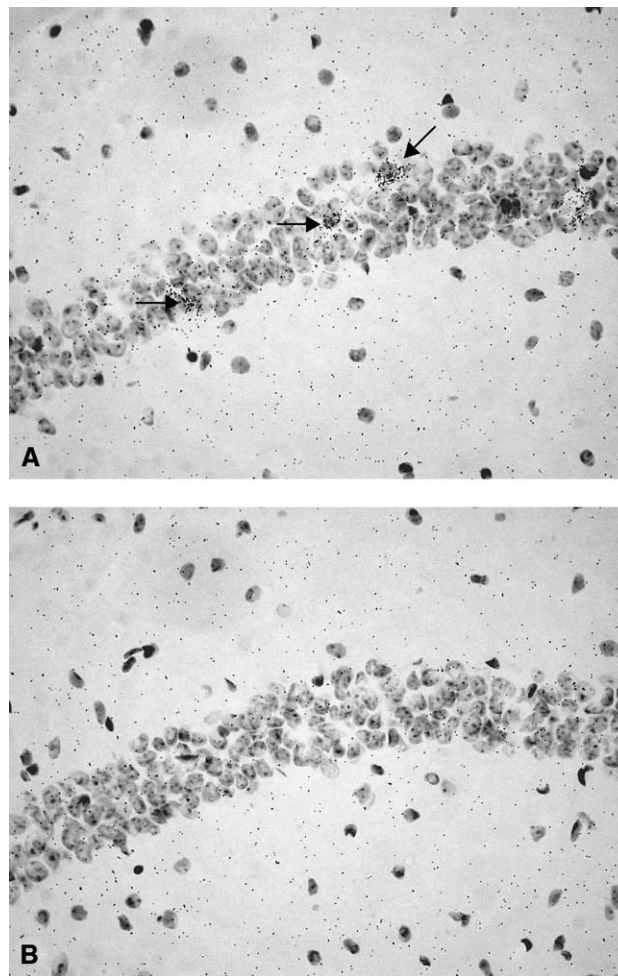


Fig. 3. (A) Micrographs illustrating the localization of 20 α -HSD mRNA in the CA1 layer of the hippocampus. A few pyramidal neurons (\rightarrow) exhibit labelling. (B) Consecutive section hybridized with the sense probe. Only diffuse labelling can be observed. Magnification: $\times 600$. Exposure time: 45 days.

the hippocampus, they also reported that pyramidal cells of CA1, CA2 and CA3 layers were all expressing 3 β -HSD mRNA, the highest labelling being found in the CA1 layer. These results might indicate that, in some brain areas, the same nerve cells might express both 3 β -HSD and 20 α -HSD. The expression of both enzymes in the same cells has been observed in peripheral organs, such as corpus luteum of the ovary, Leydig cells of the testis, adrenal cortex, hepatocytes, mammary gland (epithelial cells) and skin (sebocytes) [7,9,10,13,14]. Interestingly, progesterone receptors have been localized in neurons of the external granular layer, external pyramidal layer, inner granular layer and inner pyramidal layer of the cortex as well as pyramidal cells of the CA1 layer of the hippocampus which also express 20 α -HSD mRNA [6]. 20 α -HSD might therefore regulate the availability of locally produced progesterone for progesterone receptors, and thus control the influence of progesterone on neuronal activity. So far, there is no evidence that 20 α -hydroxyprogesterone might act as a neuroactive steroid [8,11].

In summary, we report for the very first time that 20 α -HSD mRNA is expressed in cortex and hippocampus in both male and female mouse brain. The enzyme might be involved in regulation of progesterone availability at the cellular level in cortical and hippocampal areas.

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