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# Choroid epithelial cells: source cerebrospinal fluid progesterone in sheep?

SIYAMI KARAHAN, GUL FATMA YARIM*, MURAT YARIM**

Department of Basic Sciences, Faculty of Veterinary Medicine, Kirikkale University 71450, Yahsihan, Kirikkale, Turkey
*Department of Biochemistry, Faculty of Veterinary Medicine, University of Ondokuzmayis, 55139, Kurupelit, Samsun, Turkey
**Department of Pathology, Faculty of Veterinary Medicine, University of Ondokuzmayis, 55139, Kurupelit, Samsun, Turkey

Karahan S., Yarim G. F., Yarim M.

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Summary

**The present study was conducted to immunolocalize 3$\beta$-hydroxysteroid dehydrogenase (3$\beta$-HSD), an enzyme metabolizing pregnenolone to progesterone in the choroid plexus of the lateral ventricle in sheep, as well as to measure progesterone concentration in cerebrospinal fluid (CSF) and plasma using radioimmunoassay (RIA). Akkaraman breed rams (n = 16) and ewes (n = 16) were utilized in the study. 3$\beta$-HSD was immunolocalized in choroid epithelial cells of the choroid plexus with an apparent cytoplasmic immunoreactivity. Progesterone was detected in CSF with no significant differences between the ewes (0.76 ± 0.14 ng/mL) and rams (0.74 ± 0.13 ng/mL) ($p > 0.05$). However, the plasma progesterone concentration in the ewes (0.27 ± 0.04 ng/mL) was significantly higher than that of the rams (0.11 ± 0.02 ng/mL) ($p < 0.001$). Consequently, CSF in sheep contains progesterone in significant levels. As evidenced by 3$\beta$-HSD immunoreactivity, choroid epithelial cells may be a site of progesterone synthesis in sheep.**

**Keywords: 3$\beta$-hydroxysteroid dehydrogenase, cerebrospinal fluid, progesterone, sheep**

Progesterone in circulation can cross the blood-brain barrier and accumulates in various brain regions and cerebrospinal fluid (CSF) of several mammalian species (11, 13, 15, 17). Progesterone is also synthesized *de novo* from cholesterol by other sites including nervous system, independent of gonads and adrenal glands. Such steroids are called „neurosteroids" (2, 4, 21). Cholesterol taken up by cells is converted to pregnenolone by cytochrome P450 side-chain cleavage enzyme (P450scc) in the inner membrane of mitochondria. Pregnenolone is then transferred from mitochondria to microsomal compartment where it is converted to progesterone by the $\Delta$5-3$\beta$-hydroxysteroid dehydrogenase isomerase (3$\beta$-HSD). The enzymes 5$\alpha$-reductase and 5$\beta$-reductase reduce progesterone further into the 5$\alpha$-dihydroprogesterone and 5$\beta$-dihydroprogesterone, respectively (3, 8, 9). In the nervous system, glial cells are primarily responsible for synthesis of neurosteroids; however, neurons have also been recognized as a site of steroidogenesis (1, 2, 22, 26).

Neurosteroids including progesterone have important roles in the central nervous system (CNS), mediating several brain functions and activities such as sexual drive, behaviour, and adaptation to stress (5, 14, 16). A study by Frye at al. (1998) suggested that progesterone and 3$\alpha$,5$\alpha$-tetrahydroxy-progesterone enhance sexual motivation, receptivity, and proceptivity in female rats (7). Progesterone regulates foetal and maternal behaviours in pregnant ewes (5).

As reported from different species including human and rodents, neurosteroids including progesterone are present CSF in various concentrations (15, 23). Plasma progesterone is implicated as the origin of progesterone in CSF (20, 23); however, local production of neurosteroids by other sites independent of gonads is well recognized (2, 21). Importantly, data on sheep CSF progesterone as well as its local sources were scare. Thus, the objectives of the present study were to investigate presence of immunoreactivity for 3$\beta$-HSD, the key enzyme metabolizing pregnelone to progesterone (8), in the choroid plexus as well as to measure progesterone in CSF and plasma in the ewe and ram.

## Material and methods

**Animals and tissue, blood, and CSF samples.** 1-2 years old healthy ewes (n = 16) and rams (n = 16) of Akkaraman breed were utilized in the study. All sheep used in the study were those brought to the Kirikkale slaughterhouse. This study was carried out in postpartum period of the ewe. Blood samples were collected from the jugular vein in sterile heparin-containing tubes. Plasma was isolated by centrifugation at 1550 × g at 4°C for 10 min. Samples of CSF were

obtained using a 20 gauge catheter from the subarachnoid cavity during slaughter. Plasma and CSF samples were stored at –20°C and –80°C, respectively until assayed. Samples were thawed at 21°C just prior to assay. The choroid plexus of the lateral ventricle was collected and processed for immunohistochemistry.

**Radioimmunoassay (RIA) for progesterone.** Progesterone was measured using a commercially available progesterone RIA kit for ovine (Immunotech SA, Marseille Cedex, France) in plasma and CSF according to the manufacturer's instruction. All determinations were made in duplicate. The recovery percentages obtained were between 95% and 122%. The intra- and inter-assay coefficients of variation were 5.4% and 9.1%, respectively.

**Immunohistochemistry.** Cryostat sections of fresh frozen tissues were cut at 5 µm and mounted onto organosilane (3-aminopropyl triethoxysilane)-coated glass slides and immediately fixated for 10 minutes in ice-cold acetone. Frozen sections were prepared using a commercial universal LSAB2 horseradish peroxidase (HRP) kit (DAKO, Carpinteria, CA). The frozen sections were immediately air-dried, using a hair dryer (cold air) to avoid autolysis of cells, and then kept at –20°C. Endogenous peroxidase activity was quenched by in 3% hydrogen peroxidase ($H_2O_2$) in absolute methanol for 5 min. Sections were rinsed with Tris-buffer (pH 7.4) three times for 10 min. between the consecutive steps of the test. Non-specific bindings were blocked with 5% normal goat serum for 5 min. and 1% bovine serum albumin (BSA) in PBS containing Triton X-100 (0.3%) for 30 min. at room temperature. Sections were then incubated with the primary antiserum (rabbit anti-mouse adrenal/gonadal 3$\beta$-HSD which cross-reacts with ovine tissue (24) at a dilution of 1 : 512 overnight at 4°C. Next day, sections were treated with the anti-rabbit secondary antibody in PBS for 10 min. at room temperature and with the streptavidin-peroxidase enzyme for 10 min. at room temperature. Sections were incubated with 3-amino-9-ethylcarbazole (AEC) chromogen (DAKO, Carpentaria, CA) for 5-10 min. and counterstained very lightly with Mayer's haematoxylin. Finally, sections were mounted in aqueous mounting medium (Shandon, Pittsburgh, PA). The ram prostate was processed as positive control. The primary and secondary antibodies were omitted in separate slides to control the assay.

Statistical Analysis. Student's *t* test was used to determine whether significant differences exist between the ewes and rams for progesterone concentrations in CSF and plasma. A p value of $< 0.05$ was considered significant. All data are expressed as mean ± standard deviation.

## Results and discussion

Progesterone was measured in significant levels in plasma and CSF samples assayed (tab. 1). There were no significant differences for CSF progesterone concentration between the ewe (0.76 ± 0.14 ng/mL) and ram (0.74 ± 0.13 ng/mL) ($p > 0.05$). However, the plasma progesterone concentration in the ewe (0.27 ± 0.04 ng/mL) was significantly higher than that of the ram (0.11 ± 0.02 ng/mL) ($p < 0.001$). CSF progesterone concentration in both ewes and rams significantly higher compared to that of plasma ($p < 0.001$).

**Tab. 1. Plasma and cerebrospinal fluid (CSF) progesterone concentration (ng/mL) in ewes and rams**

| | Ewes | Rams |
|---|---|---|
| Plasma | 0.27 ± 0.04 ng/mL | 0.11 ± 0.02 ng/mL |
| CSF | 0.76 ± 0.14 ng/mL | 0.74 ± 0.13 ng/mL |

Explanation: * – $p < 0.001$

3$\beta$-hydroxysteroid dehydrogenase immunoreactivity was detected in choroid epithelial cells of the choroid plexus with an apparent cytoplasmic staining (fig. 1).

Progesterone is present and locally synthesized *de novo* in the nervous system and, in turn, involves many functions including certain behaviours, adaptation to stress, and neuroprotection (19, 21). Progesterone concentration in CNS regions is even higher than plasma progesterone levels (10, 12, 25). As found by the present study the sheep CSF contains a significant amount of progesterone content, higher than the circulating progesterone level.

Due to their lipophilic features, neurosteroids including progesterone can cross the blood-brain barrier; however, active transporters such as P-glycoproteins may regulate and limit steroids passage through the blood-brain barrier (18). In ovariectomised ewes, administration of progesterone into the carotid artery resulted in much lower progesterone concentration in CSF compared to plasma (20). Due to limitation of

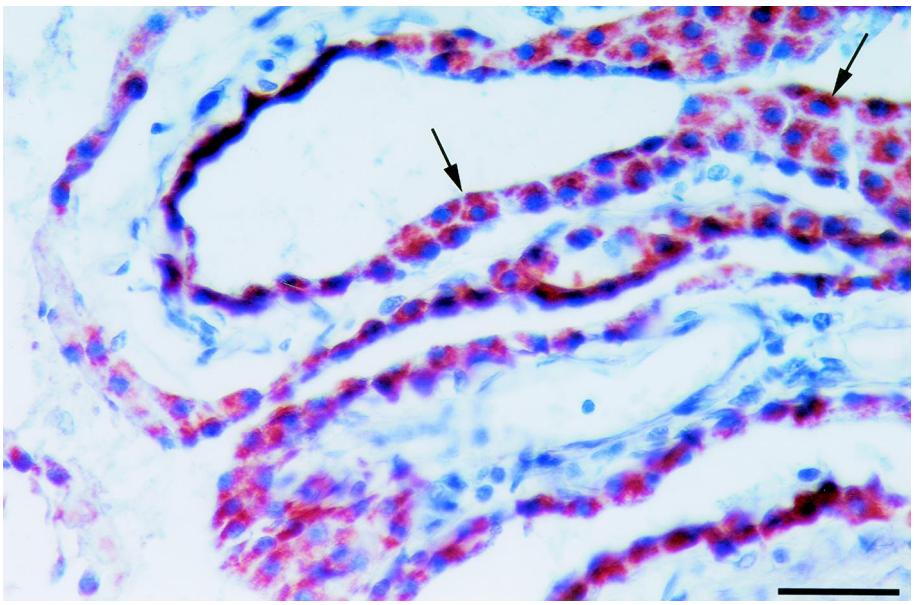

**Fig. 1. 3$\beta$-hydroxysteroid dehydrogenase immunoreactivity in the sheep choroid plexus. The choroid epithelial cells (arrows) of the choroid plexus are intensely immunoreactive with an apparent cytoplasmic staining. Bar = 30 µm**

plasma progesterone passage to CSF, progesterone in CSF most likely originates and is synthesized from local sources. In this sense, 3$\beta$-HSD immunoreactive choroid epithelial cells of the choroid plexus seems a viable candidate for local progesterone synthesis. The enzyme 3$\beta$-HSD is responsible for conversion of pregnelone to progesterone (8).

Unlike plasma progesterone, CSF progesterone concentration is not significantly different between ewes and rams as found in the present study, which suggests that the influence of circulating progesterone on CSF progesterone is limited at the most. Some physiological conditions such as pregnancy and postmenopausal state in women and photoperiod changes in sheep and pathological conditions such as Parkinson's disease have been reported to change CSF progesterone concentrations (6, 20). In this respect, further studies should be conducted to determine the influence of physiological and pathological conditions such as parturition, stress, central nervous systems disorders such as enzootic ataxia, scrapie on CSF progesterone concentration should be investigated.

In conclusion, sheep CSF contains significant levels of progesterone. Importantly, choroid epithelial cells of the choroid plexus are 3$\beta$-HSD immunoreactive, which suggests that choroid epithelial cells are the possible sites of progesterone synthesis.

## References


1. *Akwa Y., Young J., Kabbadj J. K., Sancho M. J., Zucman D., Vourch C., Jung-Testas I., Hu Z. Y., Le Goascogne C., Jo D. H., Corpéchot C., Simon P., Baulieu E. E., Robel P.*: Neurosteroids: biosynthesis, metabolism and function of pregnenolone and dehydroepiandrosterone in the brain. J. Steroid Biochem. Mol. Biol. 1991, 40, 71-81.
2. *Baulieu E. E., Robel P., Schumacher M.*: Neurosteroids: beginning of the story. Int. Rev. Neurobiol. 2001, 46, 1-32.
3. *Celotti F., Melcangi R. C., Martini L.*: The 5 alpha-reductase in the brain: molecular aspects and relation to brain function. Front. Neuroendocrinol. 1992, 13, 163-215.
4. *Compagnone N. A., Mellon S. H.*: Neurosteroids: biosynthesis and function of these novel neuromodulators. Front. Neuroendocrinol. 2000, 21, 1-56.
5. *Crossley K. J., Nicol M. B., Hirst J. J., Walker D. W., Thorburn G. D.*: Suppression of arousal by progesterone in fetal sheep. Reprod. Fertil. Dev. 1997, 8, 767-773.
6. *di Michele F., Longone P., Romeo E., Lucchetti S., Brusa L., Pierantozzi M., Bassi A., Bernardi G., Stanzione P.*: Decreased plasma and cerebrospinal fluid content of neuroactive steroids in Parkinson's disease. Neurol. Sci. 2003, 24, 172-173.
7. *Frye C. A., Scalise T. J., Bayon L. E.*: Finasteride blocks the reduction in ictal activity produced by exogenous estrous cyclicity. J. Neuroendocrinol. 1998, 10, 291-296.
8. *Jung-Testas I., Hu Z. Y., Baulieu E. E., Robel P.*: Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. Endocrinology 1989, 125, 2083-2091.
9. *Kabbadj K., el-Etr M., Baulieu E. E., Robel P.*: Pregnenolone metabolism in rodent embryonic neurons and astrocytes. Glia 1993, 7, 170-175.
10. *Lacroix C., Fiet J., Benais J. P., Gueux B., Bonete R., Villette J. M., Gourmel B., Dreux C.*: Simultaneous radioimmunoassay of progesterone, androst-4-enedione, pregnenolone, dehydroepiandrosterone and 17-hydroxyprogesterone in specific regions of human brain. J. Steroid Biochem. 1987, 28, 317-325.
11. *Lanthier A., Patwardhan V. V.*: Sex steroids and 5-en-3 beta-hydroxysteroids in specific regions of the human brain and cranial nerves. J. Steroid Biochem. 1986, 25, 445-449.
12. *Le Goascogne C., Eychenne B., Tonon M. C., Lachapelle F., Baumann N., Robel P.*: Neurosteroid progesterone is up-regulated in the brain of jimpy and shiverer mice. Glia 2000, 1, 14-24.
13. *Mathur C., Prasad V. V., Raju V. S., Welch M., Lieberman S.*: Steroids and their conjugates in the mammalian brain. Proc. Natl. Acad. Sci. 1993, 90, 85-88.
14. *McEwen B. S.*: Stress and hippocampal plasticity. Annu. Rev. Neurosci. 1999, 22, 105-122.
15. *Molnar G., Kassai-Bazsa Z.*: Gonadotropin, ACTH, prolactin, sexual steroid and cortisol levels in postmenopausal women's cerebrospinal fluid (CSF). Arch Gerontol Geriatr. 1997, 24, 269-280.
16. *Morin L. P.*: Progesterone: inhibition of rodent sexual behavior. Physiol Behav. 1977, 4, 701-715.
17. *Robel P., Bourreau E., Corpechot C., Dang D. C., Halberg F., Clarke C., Haug M., Schlegel M. L., Synguelakis M., Vourch C.*: Neuro-steroids: 3 beta-hydroxy-delta 5-derivatives in rat and monkey brain. J. Steroid Biochem. 1987, 27, 649-655.
18. *Schinkel A. H., Wagenaar E., van Deemter L., Mol C. A., Borst P.*: Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin. J. Clin. Invest. 1995, 96, 1698-1705.
19. *Schumacher M., Guennoun R., Robert F., Carelli C., Gago N., Ghoumari A., Gonzalez Deniselle M. C., Gonzalez S. L., Ibanez C., Labombarda F., Coirinib H., Baulieu E. E., De Nicola A. F.*: Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. Growth, Hormone IGF Res. 2004, 14, 18-33.
20. *Thiery J. C., Robel P., Canepa S., Delaleu B., Gayrard V., Picard-Hagen N., Malpaux B.*: Passage of progesterone into the brain changes with photoperiod in the ewe. Eur. J. Neurosci. 2003, 8, 895-901.
21. *Tsutsui K., Ukena K., Usui M., Sakamoto H., Takase M.*: Novel brain function: biosynthesis and actions of neurosteroids in neurons. Neurosci. Res. 2000, 36, 261-273.
22. *Ukena K., Usui M., Kohchi C., Tsutsui K.*: Cytochrome P450 side-chain cleavage enzyme in the cerebellar Purkinje neuron and its neonatal change in rats. Endocrinology 1998, 139, 137-147.
23. *van den Berg M. P., Verhoef J. C., Romeijn S. G., Merkus F. W.*: Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats. Eur. J. Pharm. Biopharm. 2004, 58, 131-135.
24. *Yarim M., Karahan S., Kabakci N., Atmaca T.*: Immunolocalization of 3beta-hydroxystreoid dehyrogenase in normal and hyperplastic ovine prostates. Domest. Anim. Endocrinol. 2006, 30, 17-27.
25. *Yarim G. F., Karahan S.*: Regional brain and sex differences in the plasma progesterone concentration of sheep. Small Anim. Res. 2006 (in press).
26. *Zwain I. H., Yen S. S. C.*: Neurosteroidogenesis in astrocytes, oligodendrocytes and neurons of cerebral cortex of rat brain. Endocrinology 1999, 140, 3843-3852.


**Authors address: Dr. Karahan Siyami DVM, PhD, Department of Basic Sciences, Faculty of Veterinary Medicine, Kirikkale University, 71450, Yahsihan, Kirikkale, Turkey; e-mail: siyamikarahan@yahoo.com**