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Regional brain and sex differences in the plasma progesterone concentration of sheep

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Abstract

Progesterone concentration was measured in specific brain regions of the ram and anestrous ewe to provide reference values for future studies to investigate if local CNS lesions resulting from neurodegenerative diseases of sheep are associated with progesterone loss. Using radioimmunoassay, plasma progesterone was recorded throughout all brain regions assayed. No significant differences were found between the ewe and ram for any brain regions. There were however, significant differences between the different regions of an individual brain ($P < 0.05$). Plasma progesterone concentration for the highest to the lowest value recorded was as follows: 2.93 ± 0.85 and 2.77 ± 0.51 ng/g in the frontal cortex; 2.33 ± 0.67 and 2.25 ± 0.48 ng/g in the parietal cortex; 1.32 ± 0.36 and 1.29 ± 0.35 ng/g in the temporal cortex; 1.25 ± 0.32 and 1.25 ± 0.31 ng/g in the occipital cortex; 1.24 ± 0.30 and 1.23 ± 0.31 ng/g in the corpus callosum; 1.16 ± 0.30 and 1.21 ± 0.38 ng/g in the cerebellum; 1.09 ± 0.30 and 1.12 ± 0.39 ng/g in the medulla oblongata of the ewe and ram, respectively. Plasma progesterone concentration in the ewe (0.28 ± 0.06 ng/ml) was significantly higher than that in the ram (0.10 ± 0.03 ng/ml) ($P < 0.001$). Furthermore, plasma progesterone concentration in all sheep was several times lower than that of any regions of the brain. The results indicate that the sheep brain accumulates progesterone in significant levels, which may be independent of the circulating progesterone. The brain progesterone concentration in CNS regions assayed was similar for the ram and anestrous ewe. Neurodegenerative processes in visna, border disease and enzootic ataxia should be questioned in further studies if they are associated with local progesterone loss.

Keywords: Central nervous system; Ewe; Plasma; Progesterone; Ram

1. Introduction

Progesterone is among the steroids that can be synthesized by the nervous system from cholesterol de novo, independent of the gonads and adrenal glands (Robel et al., 1987; Jung-Testas et al., 1989). Although glial cells are primarily responsible for the synthesis of neurosteroids in the central and peripheral nervous system, neurosteroidogenesis in the neurons has also been recognized (Usui et al., 1995; Ukena et al., 1998; Zwain and Yen, 1999). In addition to its de novo synthesis, circulating progesterone can cross the blood–brain barrier and accumulate within the brain of several mammalian species (Lanthier and Patwardhan, 1986; Robel et al., 1986, 1987), avian species (Tsutsui and Yamazaki, 1995; Usui et al., 1995) and amphibians (*Cynops pyrrhogaster*) (Inai et al., 2003). Pregnenolone, the immediate precursor of progesterone, can also cross the blood-brain barrier and accumulate within the brain tissues. Subsequently,

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it can be utilized in progesterone synthesis in the brain (Schumacher et al., 2004).

Progesterone either produced de novo or originating from circulation may regulate the transcription of important genes, binding to intracellular receptors in the glial cells and neurons (McEwen, 1991). Acting on ion-gated channel receptors, including $GABA_A$ and glycin, progesterone also modules interneuronal communication and neuronal excitability (Majewska, 1992). By way of such mechanisms, progesterone mediates several brain functions and activities, including sexual drive, sexual behaviour, and adaptation to stress (Morin, 1977; Gonzalez Deniselle et al., 2002; Schumacher et al., 2004). A study by Frye et al. (1998) suggested that progesterone and 3α,5α-tetrahydroxy-progesterone enhances the sexual motivation, receptivity, and proceptivity in female rats. Observing a high expression of progesterone in the diencephalon during the breeding season and in the preoptic region during the brooding period, Clark et al. (1999) suggested that progesterone regulates the reproductive and parenting behaviours in the male ring dove (*Streptopelia risoria*), while progesterone has also been reported to regulate fetal and maternal behaviour in pregnant ewes (Crenshaw et al., 1966; Crossley et al., 1997).

Research during the past decade has indicated that progesterone is involved in the establishment of the cerebral circuit during the neonatal life—promoting neuronal and glial growth and synaptic junctions of the neurons (Tsutsui et al., 2000). Progesterone also prolongs the survival of the neurons and arranges the synaptic junctions and growth of the neurites in the adult nervous system (Garcia-Segura et al., 1999). By causing an increase in the expression of myelin proteins in the oligodendrocytes and Schwann cells, progesterone exerts pro-myelinating effects in the central and peripheral nervous system (Koenig et al., 1995; Chan et al., 1998; Schumacher et al., 2004). Neuroptective effects of progesterone have been demonstrated in traumatic brain injury, spinal cord injuries, multiple sclerosis, etc. (Drew and Chavis, 2000; Gonzalez Deniselle et al., 2002; Shear et al., 2002). It is not known if progesterone offers therapeutic possibilities for the prevention and treatment of neurodegenerative diseases of sheep such as visna, border disease, and enzootic ataxia, which are associated with hypomyelination and demyelination (Jubb and Huxtable, 1993). The beneficial effects of progesterone may be due to local progesterone loss in such diseases. So for instance, the cerebellar progesterone concentration is lower in Alzheimer's patients and dogs with canine distemper (Weill-Engerer et al., 2002; Yarim et al., 2006).

The objective of the present study was to determine progesterone concentrations in specific CNS regions and the plasma of the ram and anestrous ewe. The data generated through this study could provide reference values for future studies to investigate if local CNS lesions resulting from neurodegenerative diseases of sheep such as visna, border disease and enzootic ataxia are associated with progesterone loss.

2. Materials and methods

2.1. Animals and tissue samples

One to two-year-old healthy ewes ($n = 28$) and rams ($n = 25$) of the Akkaraman breed were utilized in the study. All sheep used in the study, being brought to the local slaughterhouse. This study was carried out during the postpartum anestrous period (in summer) of the ewe and post-mortem evaluation of the ovaries was conducted to confirm anestrous and the absence of pathologies. Blood samples (5 ml) were collected from the jugular vein in vacutainer tubes containing heparin prior to slaughter. Plasma was aspirated following centrifugation at $1550 \times g$ (4 °C) for 10 min and the plasma frozen at −20 °C, until assayed. Immediately after slaughter, the brain was removed from the skull, separated from their meninges, rinsed in a cold saline solution, and dried on tissue paper. Following dissection, samples of the cortex (frontal, parietal, temporal, occipital), corpus callosum, cerebellum, and medulla oblongata were collected.

2.2. Extraction of progesterone from central nervous system

Tissue extraction of progesterone was performed according to the method described by Lacroix et al. (1987). Briefly, samples of a known wet weight (1 g) were homogenized in a phosphate gelatine buffer (PGB) (2 ml/g, w/w), using an Ultra-Turrax homogenizer (4 °C). After 30 min of equilibration and agitation at room temperature, the homogenate was extracted in 10 ml cyclohexane–ethyl acetate (50:50), by vortexing for 1 min. The dried extract was vortexed in 2 ml of PGB and the mixture kept at 4 °C overnight, before centrifugation. The residue was then separated from the aqueous phase by centrifugation and supernatant extracted twice using the same procedure.

2.3. Radioimmunoassay of tissue and plasma progesterone

The dried extract of each specific brain region was dissolved in 2 ml of PGB, using a progesterone (RIA) kit