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CHANGES IN MYELINISATION OF NEURONS IN DIFFERENT BRAIN REGIONS IN PROGESTERONE-TREATED RATS

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The influence of progesterone on myelin of the brain in adult male Wistar rats was investigated by labelling the myelin of neurons in 5 μm thick brain sections with Nile blue stain. The following nuclei were analysed: hypothalamic nucleus arcuatus (ARC) and nucleus paraventricularis (NPV), claustrum (CL), nuclei of the corticomedial part of amygdala: nucleus medialis (NM), nucleus corticalis (NCO) and nucleus centralis (NCE) and in the basolateral part of amygdala, nucleus basolateralis (NBL), nucleus basomedialis (NBM) and nucleus lateralis posterior (NLP).

In control male rats sacrificed at 62 days of age a great number of neurons labelled with Nile blue for myelin were detected by stereological analysis.They were observed in; ARC and NPV, in the corticomedial amygdaloid nuclei (NM, NCE, NCO) as well as in the basolateral nuclei (NBL, NBM and NLP). In CL there was a smaller number of neurons with labelled myelin than in the other investigated regions.

In comparison to the controls, the number of neurons labelled with Nile blue for myelin in progesterone treated male rats was significantly reduced in ARC of hypothalamus and in NCO of amygdala. A significant increase was observed in NPV of hypothalamus, and in NM, NCE, NBL and NBM of amygdala. On the other hand, in CL the number of neurons labelled with Nile blue for myelin was not changed.

Key words: progesterone, neurones, hypothalamus, gyrus dentatus, hippocampus, claustrum, amygdala, Nile blue, myelin

INTRODUCTION

Sex steroid hormone receptors are thought to mediate the actions of their respective hormones by functioning as ligand activated nuclear transcription factors that alter the expression of specific sets of hormone responsive genes (Simerley and Young, 1991). Circulating sex steroid hormones influence the activity and development of hormone-sensitive neural circuitry in the brain through interactions with specific receptors that are found in discrete populations of neurons (Simerley and Young, 1991, Siburg *et al.*,1991, Hines *et al.*,1992). During the "critical period" of development estrogens (E) exert organizational (permanent) effects on gonadotropin secretion and sexual behaviour (Siburg *et al.*,1991, Gonzales *et*

al.,1990). Vito and Fox (1982) showed the presence of estrogen receptors (ER) already 7 days before birth, immediately followed by an approximately 5-fold increase prior to birth, while there was little increase at the time of birth. Differentiation of sexual behaviour in mammals is largely determined by the steroidal environment during transient "critical periods" of perinatal age (Baum, 1979). Cherry *et al.*, (1990) investigated the influence of perinatal gonadal steroid exposure on the development of morphological sex differences in the preoptic anterior hypothalamic area, as well as of masculine and feminine sexual behaviours in a carnivore. They concluded that formation of the preoptic area of anterior hypothalamus (PO/AH) is probably regulated by the aromatase dependent conversion of testosterone (T) into E.

The effects of E in the brain are often inhibited by progesterone (P), which has been shown to down regulate the concentration of ER in some circumstances (Blaustein *et al.*,1989, Blaustein and Olster ,1989).

Medroxyprogesterone acetate (MPA), a synthetic progestin accumulates predominantly in progestin target neurons (Michael *et al.*, 1991). However, progestins can act both as anti-androgens and as anti-estrogens (Gurpide, 1983). Such actions of MPA could also block the uptake of T or its 5 α - reduced metabolite 5 α dihydrotestosterone and they interfere with the uptake of aromatized metabolites in to the brain (Michael *et al.*, 1991).

In neonatally P treated male rats we observed long term effects on the number of neurons containing myelin labelled with Nile blue. This effect was different in different regions of the rat brain.

Some P is synthesized within both the central and the peripheral nervous systems, where it regulates neurotransmission and the important glial function of myelin. Therefore, P can be designated a "neurosteroid". Steroids act not only on the brain, but also on peripheral nerves, which offer many advantages in studies on the biological significance of locally produced neurosteroids: their remarkable plasticity and regenerative capacity and their relatively simple structure. Progesterone also increases the number of myelinated axons when added at a low concentration to cocultures of Schwann cells and sensory neurons (Baulieu, 1997, Baulieu *et al.*,1996, Baulieu *et al.*,1997, Xinghua *et al.*, 1998, DeVries *et al.*,1998, Rupprecht,2003).

MATERIAL AND METHODS

Animals. Male Wistar rats were kept under standard laboratory conditions (food and water ad libitum, temperature 22$\pm$1^{o}C, standard illumination).

Treatment. Ten male Wistar rats were treated with 1,25 mg P on the 5th day of life and an other ten male rats were used as controls. All animals were sacrificed on the 62nd day of life by ether anesthesia.

Histological procedure. Tissue blocks containing amygdala, hypothalamus and claustrum were isolated and fixed in 10% formaline. After dehydration and paraffin embedding, serial frontal (5 μm thick) sections were stained with Nile blue as a marker for myelin. Nile blue (a basic oxazine dye) has been used as a lipochrome since the beginning of the 20th century in lipid histochemistry. Commer-

cially available Nile blue contains small quantities of oxasone, named Nile red, which is oil-soluble and stains nonacid lipid pink. Hydrophobic unsaturated lipids, such as triglycerides, cholesterol esters and unsaturated fatty acids, are stained by the oxazone component of the dye, whereas the hydrophilic phosphoglycerides and sulphatides are stained blue by oxazine (Miklossy and VanderLoos, 1991).

Stereological analysis. The sections were observed on a light microscope (NU2 Carl Zeiss, Germany). Stereological analysis: Weibel's multipurpose test system (P:42) was used for stereological analysis and the number of neurons labelled with Nile blue was determined (N_A x10^2) per mm^2. The first, intermediate and last section of each particular hypothalamic or amygdala nucleus were examined.

RESULTS

Control rats. The number of neurons labelled with Nile blue, as a marker for myelin, was 298 per mm^2 in hypothalmus ARC, and 374 per mm^2 in NPV. In the investigated corticomedial part of amygdaloid nuclei the number per mm^2 was: 307 in NM, 200 in NCO and 173 in NCE. In the basolateral group of nuclei, the number of Nile blue labelled neurons per mm^2 was: in 163 NBL, 316 in NBM and 167 in NLP. There were 115 Nile blue labelled neurons per mm^2 in claustrum.

Treated rats. In P treated male rats we observed more intensely labeled perycarions, nuclei and dendrites. The Nile blue of neurons had more clearly visible spines than in corresponding controls. In treated male rats the number of neurons labelled with Nile blue per mm^2 was 219 in ARC, and 468 in NPV. In the corticomedial group of amygdaloid nuclei, the number of neurons labeled with Nile blue per mm^2 was: 310 in NM, 121 in NCO and 511 in NCE per mm^2. In the basolateral group of nuclei, the number of Nile blue neurons labelled per mm^2 was: 435 in NBL, 367 in NBM and 391 in NLP. In CL there were 277 neurons labelled with Nile blue per mm^2. Differences in the number of neurons per mm^2 labelled with Nile blue for myelin were found between P treated and control male rats, as well as between the different brain regions.

Figure 1. Neurons of the NCE of male rats: control (A) and progesterone treated (B), Nile blue labelled, x 1024 Neurons of the NBL of male rats: control (C) and progesterone treated (D), Nile blue labelled, x 1024

DISCUSSION

The formation of the alpha-reduced metabolites of T and P is a very active process in the brain, since the enzyme alpha-reductase (alpha-R) is present in al-

most all central nervous system structures. A particularly elevated alpha-R activity has been shown in myelin sheets (Polleti *et al.*,1997). The results of the present study reveal a wide distribution of P target cells in 62 day old male rats. These cells were found in various amygdaloid and hypothalamus nuclei and the number was changed differently under the influence of P on myelin. synthesis. Myelin sheets originate from distinct places on the oligodendrocyte (OLG) plasma membrane and, as opposed to the latter, myelin containing membranes are relatively enriched in glycosphingolipids and cholesterol. The OLG plasma membrane can be considered to exist in polarized cells; the myelin sheet is reminiscent of an apical membrane domain, and the OLG plasma membrane resembles the basolateral membrane (De Vries *et al.*, 1998). Experiments performed on the optic nerve, a myelinated structure very rich in alpha-R activity clearly indicated the presence of a specific type 1 enzyme immunoreactivity in the myelin sheets of the axon (Polleti *et al.*, 1997).

In the hypothalamus both ARC and ventromedial nuclei contain high densities of cells that express P receptors (Warembourg 1978). Moreover cells have been identified in these nuclei in the guinea pig that appear that contain E and P receptor immunoreactivities (Blaustein and Brown, 1984, Blaustein *et al.*, 1989, Warembourg *et al.*,1989, Genazzani *et al.*, 2000). Progesterone appears to decrease E binding in the uterus (Okulicz *et al.*,1981ab), but conflicting results have been reported for the brain (Barrachough *et al.*, 1986, Blaustein and Brown 1984) and no significant changes have been observed in neuron ER immunostaining in the arcuate and ventromedial pars ventrolateralis after P treatment.

Our results showed that, in male rats neonatally treated with P the number of Nile blue neurons labeled for myelin is significantly decreased in hypothalamic ARC and only slightly increased in NPV. In NM of amygdala P did not influence the number of neurons labelled for myelin. After P treatment the number of neurons labeled for myelin in amygdaloid NCO was decreased, while in ARC and in NCE of amygdala their number was significantly increased. These results for labelled myelin clearly indicate different reactivity of different nuclei from the same, corticomedial part of amygdala. This is in agreement with the findings (Don Carlos *et al.*, 1989) that E had no effect on the levels of P receptors in medial amygdala and that there was no sex difference in the preoptic area (Thorton *et al.*, 1989). In the basolateral part of amygdala in control rats the greatest number of neurons labelled for myelin was in NBL and NBM.

In treated male rats the effect of P caused significant changes in the neurons in NBL and in NLP. On the other hand, in controls the number of neurons labelled for myelin was the smallest there. Chen and Tu (1992), suggested sex differences in E and androgen binding activities in certain brain areas of intact hamsters. While the levels of nuclear receptors correlated well with the biological responses, the presence of cryptozoic steroid receptors has been regarded as a prediction index for the response of the tissue to the hormonal environment.

Our results suggest that the P is stronger action of progesterone on myelin in amygdaloid nuclei. Also, our results indicate that the NBL amygdaloid nuclei in controls have smaller numbers of neurons labelled for myelin than those in P treated male rats. Lozance *et al.* (1993) showed that P given at a particularly sensi-

tive period of brain development, on (5th day of life), caused changes, which could be registered at 62 days of age. Therefore they suggested that P, as a sexually nonspecific hormone, activates genetic processes which result in the biosynthesis of elements responsible for inhibition of dendro and axonogenesis. Chen and Tu (1992) found the highest level of E receptors in the medial preoptic area and the lowest in amygdala of the prestrus hamster.

In male rats neonatally treated with P the number of neurons labelled for myelin was significantly increased in basolateral group nuclei which in controls had small numbers. Sibug *et al.* (1991) suggested that the plasticity of ER systems in the rodent brain is not only dependent on development but also on the hormonal status of the animal and environmental sensory stimuli. The effect of estrogens exhibits regional specificity. For example, in developing rats the hypothalamus is sensitive to E induction of P receptors. That is confirmed by our results for synthesis of myelin.

Also, we conclude that in male rats, neonatally treated with P the number of neurons labelled with Nile blue for myelin increases significantly differently in different brain regions.

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PROMENE MIJELINIZACIJE NEURONA U RAZLIČITIM REGIONIMA MOZGA PACOVA TRETIRANIH PROGESTERONOM

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SADRŽAJ

Istraživan je uticaj progesterona na mijelinizaciju u mozgu pacova Wistar soja obeležavanjem mijelina bojom Nil plavo na histološkim presecima debljine 5 μm. Analizirane su sledeće grupe nukleusa: hipotalamični nucleus arcuatus (ARC) i nucleus paraventricularis (NPV), claustrum (CL), nukleusi kortikomedijalnog dela amigdale: nucleus medialis (NM), nucleus corticalis (NCO) i nucleus centralis (NCE) i u bazolateralnom delu amigdale: nucleus basolateralis (NBL), nucleus basomedialis (NBM) i nucleus lateralis posterior (NLP). Broj neurona progesteronom tretiranih mužjaka mijelinom obeleženih sa Nil plavo značajno je smanjen u ARC hipotalamusa i NCO amigdale u odnosu na kontrolne vrednosti. Značajno povećanje obeleženosti zapaženo je u NPV hipotalamusa, NM, NC, NBL i NMB amigdale. S druge strane, u CL broj obeleženih neurona nije se značajno promenio nakon tretmana.

Poštovani autori i čitaoci,

Izvinjavamo se što je zbog tehničke greške izostavljena 372. stranica časopisa "Acta veterinaria" broj 5-6/2003. godine, koju Vam dostavljamo u prilogu.

Redakcija

Respectable authors and readers,

We are very sorry, that page, number 372, of journal "Acta veterinaria" 5-6/2003. was leave out, because of tehnical mistake.

We deliver it in supplement.

Editorial