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NUCLEUS BASOLATERALIS (NBL) OF MALE RATS NEONATALLY TREATED WITH PROGESTERONE

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The relative volume of cell nuclei (Vvn), cytoplasm (Vvc) and neuropil (Vvnp), as well as the numerical density (Nv) of NBL amygdalea (amy) neurons were investigated using stereological methods in control and treated male rats. Newborn male rats were treated on the 5th day of life with 1.25 mg of progesterone (P) and sacrificed with the control group on the 62nd day of life.

Stereological analysis showed a statistically significant decrease in the relative volume of neuropil (Vvnp) ($p<0.001$), while the numerical density (Nv) of NBL neurons of treated male rats increased compared to the Vvnp and Nv values of the controls.

Key words: Amigdalea, progesterone, hormones, NBL

INTRODUCTION

Many brain regions, particularly parts of the limbic system (such as the amy), have a great number of target neurons for steroid hormones. Sex steroids can induce significant changes in these regions, especially if they are applied neonatally, during the so called "critical" period of brain development. Beyer and Feder (1987) stated that steroid effects on the central nervous system (CNS) may be analyzed from two points of view : the place of their effects and the duration of their action. Primary sites of steroid hormone action are the neurons with classical intracellular receptors that receive information directly from the steroid molecule, and secondary, neurons which are activated indirectly, transsynaptically or by secretory processes originating in the classical, steroid sensitive neurons. In rats, steroid receptors may be detected a few days before birth (Attardi and Ohno, 1976; Kato, 1976; Mac Luskey, 1978, 1979; McEwen et al., 1978), and neonatally they are found in the cerebral cortex, hypothalamus, preoptical area and amygdalea (Attardi and Ohno, 1976; McEwen et al., 1978). Progesterone receptors (PR) have been confirmed, using biochemical methods, in different parts of the brain, including the cerebral cortex, hypothalamus, middle brain, preoptical area and amygdalea (Blaustein, 1978, 1980; Thorton, 1986).

MATERIALS AND METHODS

Ten male Wistar rats were treated on the 5th day of life with 1.25 mg of progesterone (P), and ten rats were used as controls. All animals were sacrificed on the 62nd day of life. Amygdalea were isolated and fixed in Bouin solution. After dehydration and paraffin embedding, serial sections (5 um thick) were stained with Herlant, Klüver Barera and alcal fuchsin.

Weibel's multipurpose test system (P:42) was used for the stereological analysis. Sampling was performed so that the first, middle and last sections of each particular amy were used, and the statistical significance of differences was tested using Student's t-test.

RESULTS

NBL belongs to the basolateral cell group of the amy. Two morphologically different — anterior and posterior — parts. were noticed in the NBL of the control and treated male rats. The anterior part of the NBL of controls was characterized by a less abundant population of neurons which from an oval structure, while the population of neurons of the posterior part of the NBL was richer, and covered a greater area of the basolateral part of amy. However,

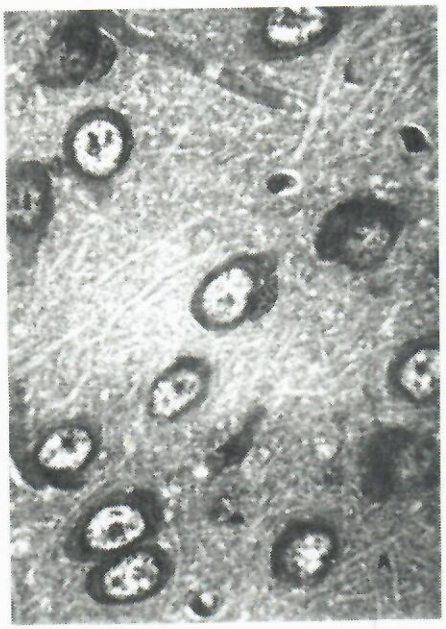

Figure 1. A. Nucleus basolateralis (NBL), male rats (controls) sacrificed on the 62th day of life (1080x)

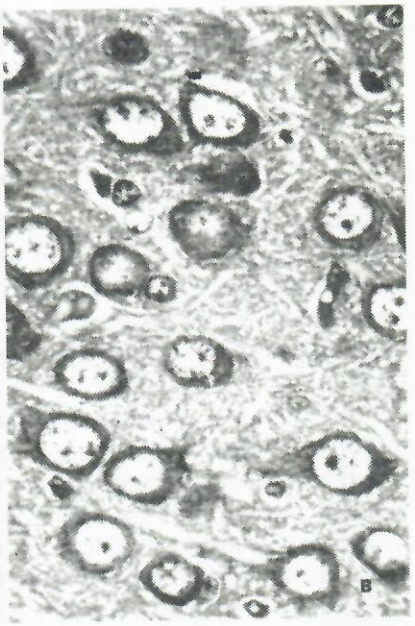

Figure 1. B. Nucleus basolateralis (NBL) of male rats, progesterone (P) treated on the 5th day of life, sacrificed on the 62th day of life (1080x)

the neuron population in the anterior part of treated animals was more numerous and denser compared to the population of neurons of the anterior part of the NBL in controls. The same observation was made for the population of neurons of the posterior part of the NBL. Dimensions of the cell nuclei of the neurons from both parts of the NBL of the treated group were smaller compared to the controls, and their shape was changed into irregularly round or oval, particularly in the anterior part.

In the interstital space of the NBL amy of the control animals could be seen the form of oligodenendroglia which are characteristic for the neuropil of "mature" neural tissue, so called "dark oligodendrocytes", while in the treated animals we observed the presence of oligodendroglia with large, light nuclei ("light oligodendrocytes"), characteristic for the interstitial space of "immature" neural tissue. (Figures 1 A and 1B).

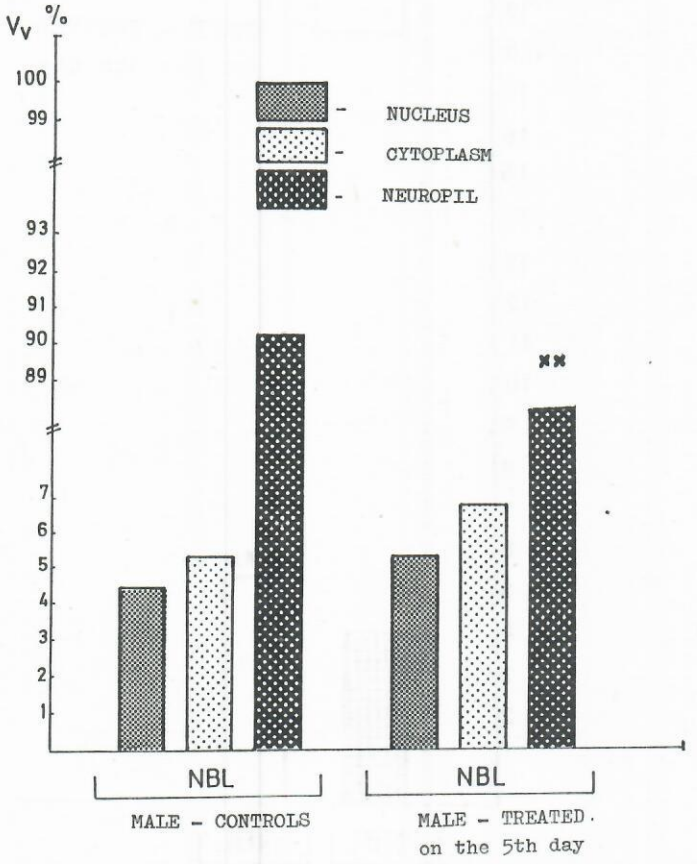

Figure 2. Volume density (Vv) of NBL male rats (controls) and rats treated with progesterone (P) on the 5th day of life, sacrificed on the 62th day of life.

Stereological results

Values for the Vvn, Vvc and Vvnp of the control male rats sacrificed on the 62nd day of the life were 0.045 (mm°) or 4.5%, 0.0538 (mm°) or 5.38% and 0.9014 (mm°) or 90.14% of the NBL investigated, respectively. The Nv of NBL neurons of the control group of male rats was $3.95 \times 10^4 (mm^{-3})$ and the average diameter of cell nuclei was 10.3 μm (Figure 2.).

The Vvn of NBL neurons of neonatally treated male rats was 0.0523 (mm°) or 5.23%; Vvc — 0.0676 (mm°) or 6.76%, and Vvnp was 0.88 (mm) or 88%, compared to the total number of examined neurons of the investigated nucleus. The Nv of NBL neurons of the treated male rats was $5.31 \times 10_4 (mm^{-3})$, and the average diameter of cell nuclei was 9.08 μm (Figure 3.).

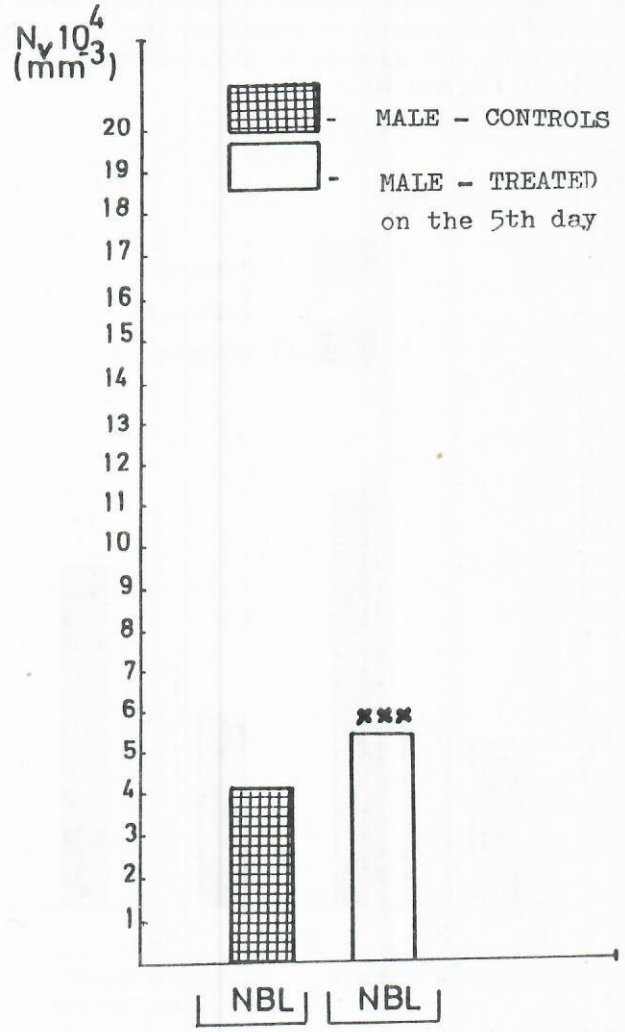

Figure 3. Numerical density (Nv) of NBL male rats (controls) and rats treated with progesterone (P) on the 5th day of life. sacrificed on 62th day of life.

DISCUSSION

Treatment with progesterone, or some other progesterone metabolites, inhibits electrical activity of the brain, as well as the neuronal branching of neonatal rats (Gonzales and Mariscal, 1982). During pregnancy progesterone is secreted in large quantities from the maternal ovaries (corpus luteum graviditas) and the fetal adrenal cortex, but progesterone levels significanly decrease during the perinatal period (Beyer and Feder, 1987). This decrease in progesterone concentration is very important concerning the great sensitivity of the neonatal brain to the inhibitory effects of this steriod (Holmes and Weber, 1987).

Dramatic changes in neural activity of the brain of males are expected immediately after birth as a result of the combined influence of endocrine factors from one side (increase in testosterone and estradiol, and decrease in progesterone level), and "catastrophic" changes resulting from the sudden transition from fetal to neonatal life, on the other side (Beyer and Feder, 1987).

Our results showed that (P) given on the 5th day of life, in this particulary sensitive period for brain development, caused changes which could be registered on the 62nd day of life, at the time of sacrificen. The increase in the Nv of NBL neurons of neonatally treated male rats (compared to the controls) was statistically significant ($p<0.001$), as well as the decrease in the Vvnp ($p<0.001$). According to our suggestions, (P), as a sexually nonspecific hormone given neonatally to male rats in this "critical" period of brain development, activates genetic processes which result in the biosynthesis of elements responsible for inhibition of dendro and axonogenesis, which are particularly intensive during the first postnatal days. Thus, Wakefeld and Lavin (1985), using Golgi morphometric analysis, found the greatest increase of NBL neurons (dendrites, dendritic branching and dendritic field radius) of neonatally treated kittens at 5-14 and 16-28 days of life. This increase in NBL neurons, compared to the values immediately after birth, was 75% at 1-4 days, and even 125% at 5-14 days of life.

According to our results, it may be suggested that the changes which occurred after the treatment with (P) are the result of interaction of this steriod with intracellular receptors in the NBL neurons. However, considering that in the last few years the ability of oligodendrocytes to synthesize pregnenolone from cholesterol has been established (Le Goascogne et al., 1987; Hu et al., 1987), as well as the estrogenic induction of progesterone receptors in tissue cultures of oligodendrocytes of neonatal brain (Jung-Testas et al., 1989; 1991), the question of the sites of action of progesterone (neurons, oligodendrocytes) has broadened, that is, stays open. Our results, contributing to the former view, established that the intercellular space of neonatally treated male rats is abundant in numerous large oligodendrocytes with light nuclei, which are characteristic for the neuropil of "immature" neural tissue.

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NUCLEUS BASOLATERALIS (NBL) MUŽJAKA PACOVA NEONATALNO TRETIRANIH PROGESTERONOM

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SADRŽAJ

Stereološkom analizom ispitivani su volumenska gustoća jedara (Vvn), citoplazme (Vvc) i međućelijskog prostora (Vvnp) neurona NBL, kao i numerička gustoća (Nv) ćelija neurona NBL amygdalea (amy) mujaka pacova tretiranih neonatalno, 5. dana života sa 1.25 mg progesterona (P) i njihovih kontrola, žrtvovanih 62. dana života.

Stereološkom analizom utvrđeno je statistički značajno smanjenje volumenske gustoće međućelijskog prostora (Vvnp) ($p<0,001$), uz statistički značajno povećanje numeričke gustoće (Nv) ćelija neurona NBL neonatalno tretiranih pacova u odnosu na vrednosti (Vvnp) i (Nv) neurona NBL pacova kontrola iste starosti.