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THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI OF FETAL AND NEONATAL OFFSPRING OF RATS TREATED WITH DEXAMETHASONE DURING GESTATION

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We have investigated the development and differentiation of the paraventricular (PVN) and supraoptic (SON) nuclei in 20-day-old male offspring (3-and 14-day-old pups) of rats treated with dexamethasone (Dx) (0.3 mg/kg b. w./day) from day 16-20 of gestation.

The analyses were made in serial frontal hypothalamic sections by measuring the maximum diameter (Dmax) and area of neurosecretory cell nuclei in five subgroups of PVN (anterior parvocellular and magnocellular, medial parvocellular and magnocellular and posterior magnocellular) at eight levels. Prolonged Dx-treatment of pregnant rats resulted in a significant decrease of Dmax and the area of PVN neurosecretory cell nuclei of their offspring at levels 3,4,5,6 and 7 where anterior and medial parvocellular subgroups of neurons were present or dominant. Morphometrical data were in accordance with the histological appearance of parvocellular neurons where nuclei were visibly diminished.

Dx-treatment of pregnant rats resulted in a significant decrease of Dmax and the area of SON neurosecretory cell nuclei of fetuses and 3-day-old rat pups.

Our findings about the differentiation of hypothalamic nuclei indicate that Dx-treatment of pregnant rats lowers the synthesizing activity of PVN and SON neurosecretory neurons of fetuses and neonatal offspring.

Key words: Hypothalamus, nucleus paraventricularis, nucleus supraopticus, fetal period, neonatal period, dexamethasone.

INTRODUCTION

Both the paraventricular (PVN) and the supraoptic (SON) nucleus of the rat hypothalamus differentiate between days 14 and 17 of fetal life. At the beginning of this period mitotic divisions of the neurosecretory cells are present in great number, but from day 17 of fetal life dividing cells are very scarce (Anderson, 1978). During ontogenesis of the rats, between days 15.5 and 17.5 of gestation, immunopositive CRH - neurons first appear in the PVN. The number of these

neurons increases to the end of the fetal period. Neurons of the PVN extend their axons to the median eminence (ME) and at fetal day 17.5 CRH-immunopositive neurosecretory fibers are seen in the zona externa. Neurosecretory neurons form a link between the nervous and the endocrine systems (Scharrer, 1967). Hypothalamic regulatory mechanisms start to control ACTH release between fetal days 16.5 and 17.5 (Daikoku et al., 1984). ACTH-cells in the pars distalis differentiate between days 16 and 17 of gestation (Dupouy and Magre, 1973; Sétáló and Nakane, 1976), but Nemeskéri and Halász (1989) discovered immunopositive ACTH-cells already at 14 days.

There are few data about the influence of hormones of the maternal hypothalamo-pituitary-adrenal axis (HPA) on the development of fetal hypothalamic nuclei.

In the present study we examined whether changes in the concentration of corticosteroids in pregnant rats Dx-treated from 16 to 20 day of gestation have some influence on the differentiation of the parvocellular and magnocellular divisions in the PVN and SON of fetuses and neonatal offspring.

MATERIAL AND METHODS

For these investigations we used Wistar strain pregnant rats, their male fetuses (20-day-old) and neonatal offspring (3-and 14-day-old). Three - month old rats were mated in the laboratory. The day when females were sperm-positive was considered as the first day of pregnancy. The sperm-positive test was done between 8.00 and 9.00 a. m. Pregnant rats were housed individually in conditions of controlled heating (22^0 C) and lighting (12 h light/dark cycle with the light on at 6.00 a. m.). Food and water were freely available. These females were divided into two groups. The experimental group consisted of five pregnant rats injected s. c. with Dx (Krka, Novo Mesto, Slovenia) (0.3 mg/kg b. w./day) during five days starting from day 16 of gestation. The control group included five females that received an equivalent volume of saline (0.3 ml/kg b.w./day) for five days beginning from the same day of gestation as the experimental group.

The dams and their fetuses (20-day-old) were sacrificed 24 h after the last injection under ether narcosis between 9.00 and 11.00 h a. m. on day 21 of gestation. The neonatal (3-and 14-day-old) offspring of both experimental and control dams, were sacrificed in the same way. The hypothalamus was quickly removed, fixed in Bouin's fluid and embedded in paraffin wax. Serial sections 5 mm thick were stained with Gabe-Azan. Differences in the structure of the PVN and SON cells were estimated through the size of neurosecretory cell nuclei. The size of these nuclei was measured using a Mop-Videoplan image analysis system, by determining the maximum diameter (Dmax) and the area. These analyses were made on micrographs of serial frontal hypothalamic sections, from the anterior to the caudal part. The cytoarchitectonic subdivision of PVN was determined using the classification according to Swanson and Kuypers (1980) and Armstrong et al. (1980). For analysis of five distinct subdivisions, three magnocellular and two parvocellular (anterior parvocellular - ap, anterior magnocellular -

am, medial parvocellular - mp, medial magnocellular - mm and posterior magnocellular - pm subdivision), morphometrical and histological analysis were performed on 50 cells at eight levels for each animal in the group. Parvocellular neurons in the complete PVN border were mixed with the magnocellular neurons. For this reason morphometrical data obtained for the parvocellular and magnocellular subdivisions are presented together at each of the eight levels, where the dominant subdivision on each level is underlined.

The SON was divided by the optic tract into the principal and retrochiasmatic part (Rhodes et al., 1981). We analyzed 50 cells in each SON.

Statistics. The results are expressed as means for five animals in each group ± S. E. Statistical comparison of the data was done by the Wilcoxon test.

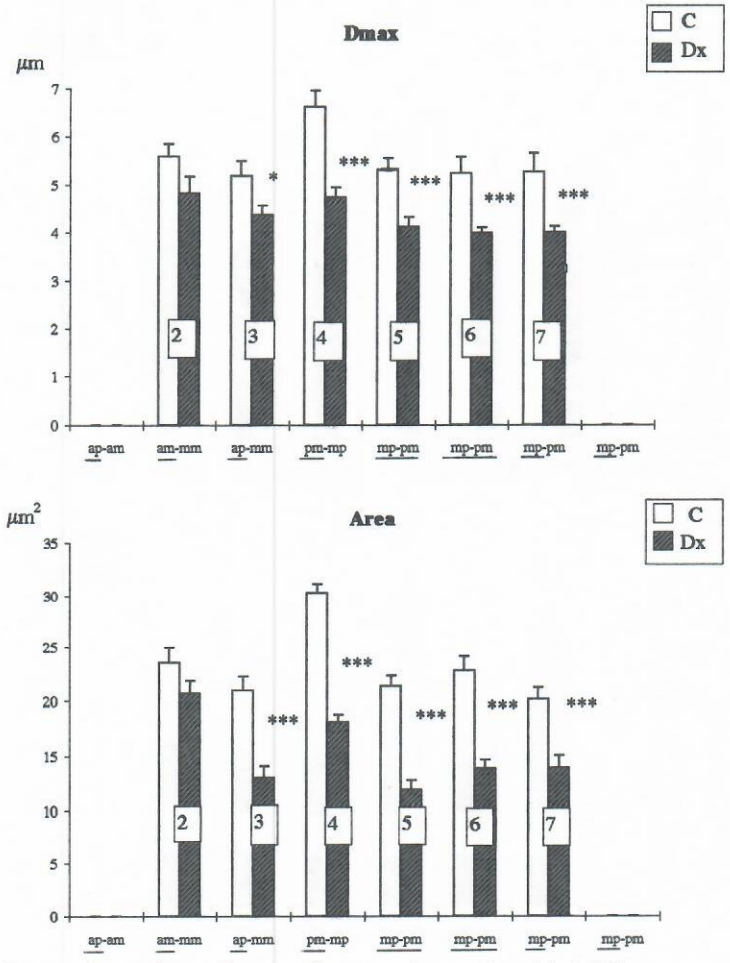

Figure 1. Changes in maximum diameter (Dmax) and area of nuclei of PVN neurons (subgroups: ap, am, mp, mm, and pm) of 20-day-old fetuses, of rats treated with Dx for five days, starting from day 16 of gestation. * $P < 0.05$; *** $P < 0.005$.

RESULTS

Morphometric analysis of PVN. Prolonged Dx-treatment of pregnant rats resulted in a significant decrease of Dmax and the area of neurosecretory PVN cell nuclei of 20-day-old fetuses at the five levels where parvocellular neurons were present (Dmax, level: 3-16%, 4 - 28%, 5 - 23%, 6 - 24% and 7 - 24%; Area, level: 3 - 38%, 4 - 41%, 5 - 44%, 6 - 39% and 7 - 31%). Significant differences were not seen at level 2, where only magnocellular neurons were present. The areas of neurosecretory neuron nuclei were markedly decreased on the levels where anterior parvocellular (ap) and medial parvocellular (mp) subgroups of neurons were present or were dominant in comparison with corresponding controls (Figure 1.).

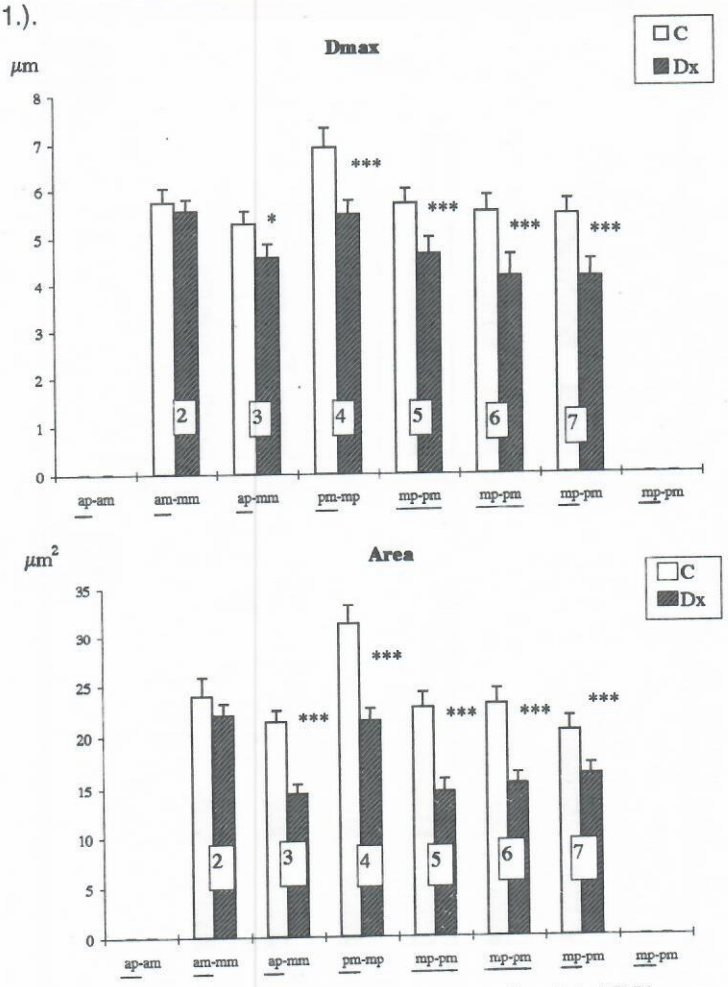

Figure 2. Changes in maximum diameter (Dmax) and area of nuclei of PVN neurons (subgroups: ap, am, mp, mm and pm) of 3-day-old offspring of rats treated with Dx during five days, starting from day 16 of gestation. *P <0.05; ***P< 0.005.

In the 3-day-old neonatal offspring of dams treated with Dx similar changes in Dmax and the area of the neurosecretory cell nuclei were seen as in the 20-day-old fetuses (Figure 2.). Dmax and area of the neurosecretory cell nuclei in the experimental group were significantly decreased at levels: 3 - 14% and 32%, 4 - 21% and 32%, 5 - 19% and 35%, 6 - 25% and 33%, 7 - 24% and 21%, respectively.

Morphometrical analysis of PVN neurosecretory cells of 14-day-old pups showed that the significant decrease of Dmax in fetuses and 3-day-old neonates was retained at levels 4 - 6 (19%, 14% and 14%, respectively) and of area at levels 3 - 6 (21%, 33%, 26% and 27%, respectively), where medial and posterior parvocellular subgroups of neurons were dominant (Figure 3.).

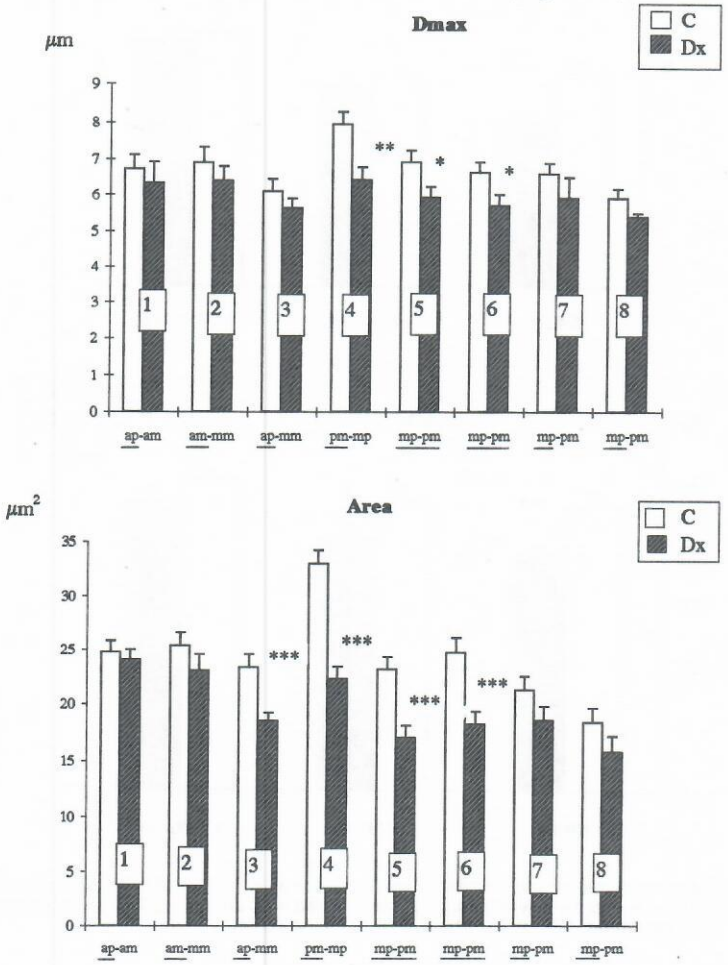

Figure 3. Changes in maximum diameter (Dmax) and area of nuclei of PVN neurons (subgroups:ap, am, mp, mm and pm) of 14-day-old offspring of rats treated with Dx during five days, starting from day 16 of gestation. *P< 0.05; **P < 0.01; ***P < 0.005.

Morphometric analysis of SON. The analyses of the morphometric parameters of SON neurosecretory cells of fetal and neonatal rats showed that Dx - treatment of female rats during gestation resulted in a decrease of neurosecretion neuron nuclei in all three examined periods. However, significant decreases were found only in fetuses (Dmax - 21% and area - 18%) and 3-day-old neonates (area - 14%) (Figure 4).

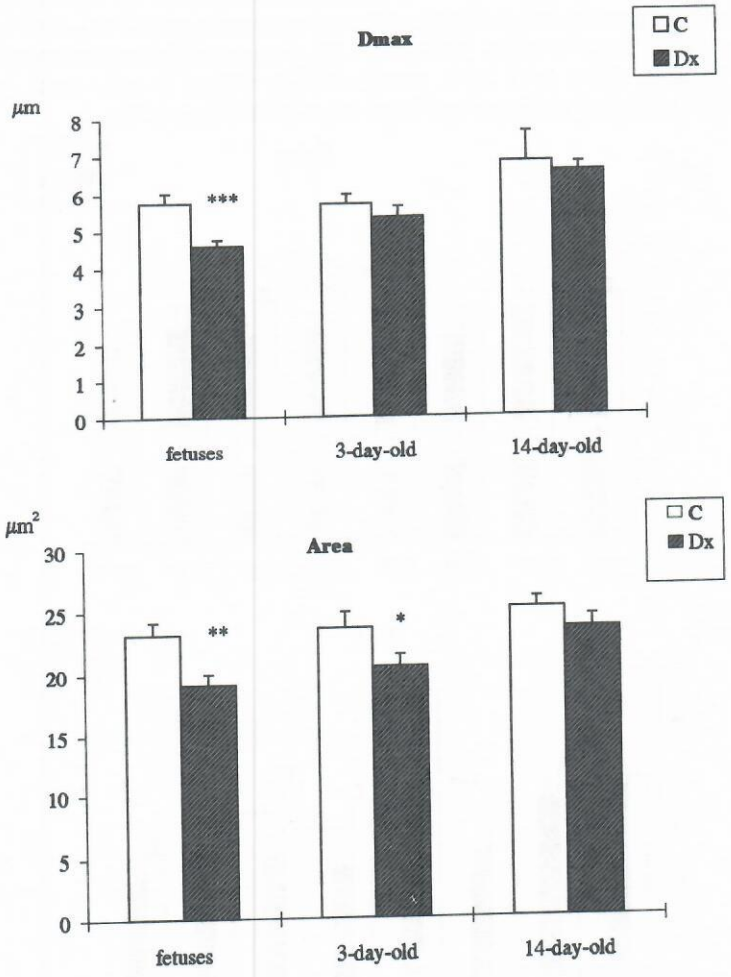

Figure 4. Changes in maximum diameter (Dmax) and area of nuclei of SON neurons of 20-day-old fetuses, 3- and 14-day-old pups of rats treated with Dx during five days, starting from day 16 of gestation. *P< 0.05; **P < 0.01; ***P < 0.005.

Histological analysis. Histological examination of the parvocellular and magnocellular subgroups of PVN in fetuses and neonatal offspring of Dx-treated

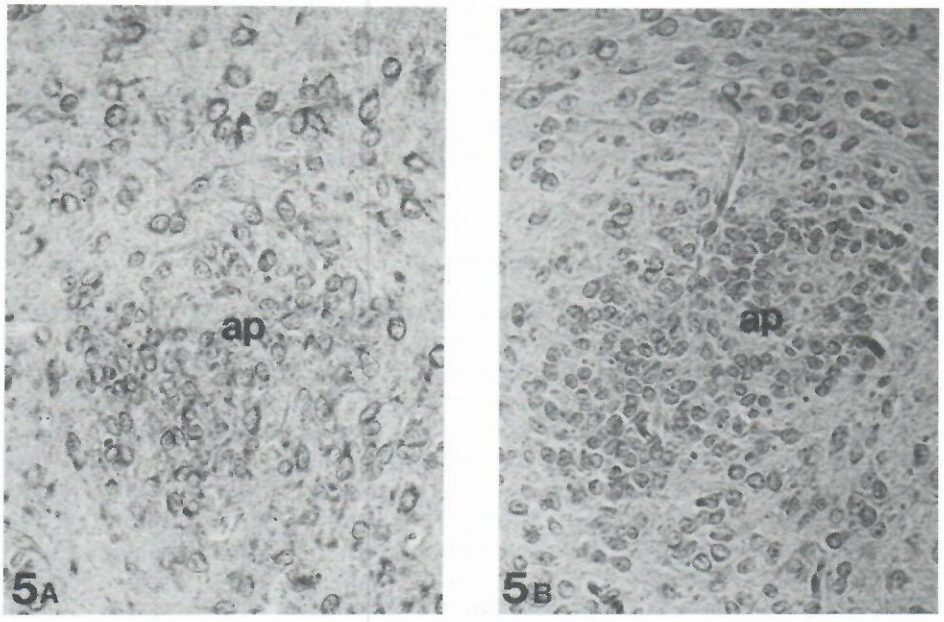

Figure 5. Neurosecretory neurons of anterior parvocellular subgroups (ap) of PVN (level 3) of 3-day-old offspring of controls (A) and rats treated with Dx during gestation (B), (640 x).

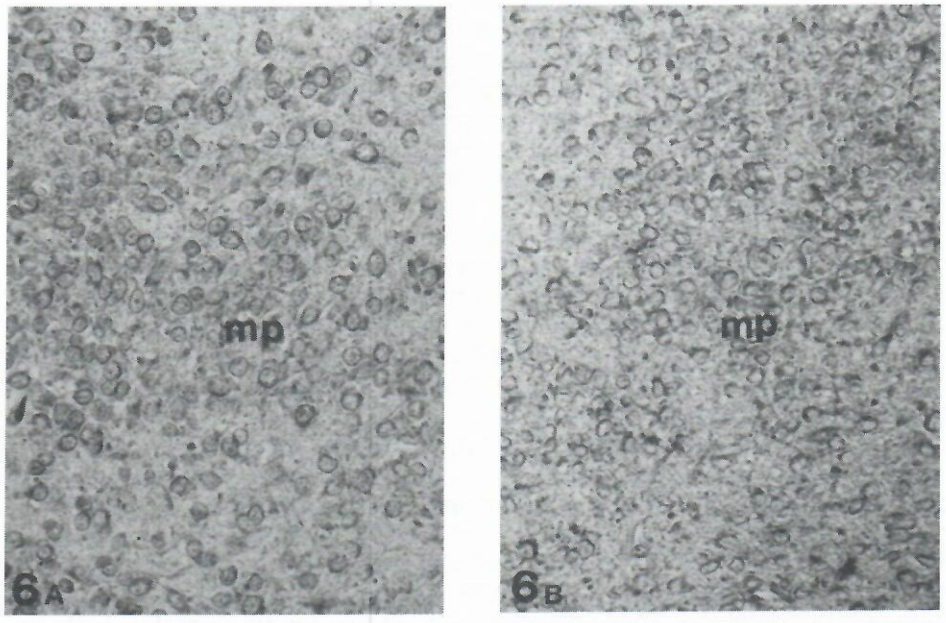

Figure 6. Neurosecretory neurons of medial parvocellular subgroups (mp) of PVN (level 4) of 3-day-old offspring of controls (A) and rats treated with Dx during gestation (B), (640 x).

pregnant rats revealed significant changes in the structure of neurosecretory neurons. The diameter and volume of the nuclei were decreased and their shape was altered (Figure 5 and 6).

Neurosecretory neurons from ap and am subgroups at level 1 and mp and pm at level 8, could not be morphometrically analyzed because of a decreased number in the experimental animals, which had a structure of cells consistent with a lower function.

DISCUSSION

Swanson and Kuypers (1980) have shown, on the basis of cytoarchitectonic analysis, that the PVN of the hypothalamus consists of eight distinct subdivisions, three magnocellular and five parvocellular. Immunocytochemical investigations indicate that CRH-immunoreactive neurons are present in the PVN of rats, in other hypothalamic nuclei (dorsomediolateral region and nucleus schiasmaticus) as well as in the extrahypothalamic regions (Daikoku et al., 1985). Prolonged treatment of rats with corticosterone influenced the CRH level only in the hypothalamic nuclei (Owens et al., 1990). Corticotropin-releasing hormone (CRH - 41) is synthesized in the parvocellular neurons of the PVN and released from nerve terminals to the hypophysial portal blood system (Antoni, 1986). CRH - 41 is the principal peptide that regulates the release of adrenocorticotropin (ACTH) from the ACTH-cells of the anterior pituitary (Gillies and Lowry, 1986).

Morphometrical analyses of PVN neurosecretory cell nuclei of fetuses and neonatal rats indicate that Dx given to pregnant rats causes significant changes of PVN neurosecretory cells of their offspring in all three examined periods (20-day-old fetuses and 3-and 14-day-old pups), while the changes found in the SON of fetuses and 3-day-old neonates had disappeared in 14-day-old pups. Significant decreases of Dmax and the area of PVN neurosecretory cell nuclei were found at the levels where parvocellular neurons are present or are dominant. These changes discovered in the fetal period were maintained till 3- and 14- days after birth.

Anterior parvocellular and magnocellular, medial parvocellular and posterior magnocellular subgroups of neurons localized at levels one and eight could not be morphometrically analyzed in the experimental animals because of an insufficient number of cells. On the basis of these data it may be supposed that Dx given to pregnant rats represses the proliferative activity of PVN neurosecretory cells, which is the most intensive, according to Anderson (1978), between 14 and 17 days of gestation.

Morphometrical data were in accordance with the histological appearance of parvocellular neurons in which the nuclei were visibly diminished and of changed shape. The results obtained indicate that prolonged treatment of pregnant rats with Dx caused significant changes in the structure and number of PVN neurosecretory cell of fetuses and neonatal offspring. The changes were markedly expressed in the parvocellular groups of neurons of fetuses and 3-day-old neonatal rats. These neurons are probably, for the most part, CRH-immunopositive cells.

CRH-immunopositive neurons of intact rats are numerous in the medial parvocellular subgroup of neurons, but in adrenalectomized rats they are found in the posterior magnocellular group of neurons also, near the OT-synthesising neurons (Burlet et al., 1983). After repeated stress (once daily immobilization of rats for 16 days) CRHmRNA, CRH and vasopressin (AVP) were increased in the parvocellular cell bodies despite elevated corticosterone levels (DeGoeij et al., 1992). On the other hand corticosterone implants in the PVN of rats prevented the rise in serum ACTH, corticosterone and CRH - 41 in the ME, following neural stimuli, possibly acting through type II hypothalamic receptors. Implants of corticosterone into the dorsal hippocampus had no effect on the function of the hypothalamo-pituitary-adrenal system (Kovacs et al., 1986: Feldman et al., 1992). Dx-treatment decreased the level of CRH in PVN and ME (Jessop et al., 1990) as well as in the pars neurointermedia (Suda et al., 1983).

Prolonged Dx-treatment of pregnant rats caused a decrease of Dmax and the area of SON neurosecretory cell nuclei of their offspring during the fetal and neonatal period, but this decrease was significant only in 20-day-old fetuses and 3-day-old neonatal rats. The evidence that CRH-41 peptide and CRHmRNA have been detected in the oxytocin - containing magnocellular neurons of the PVN and SON is in accordance with our results (Young et al., 1986; Lightman and Young, 1987).

Our findings about the differentiation of PVN and SON neurons indicate that Dx-treatment of pregnant rats lowers the synthetic activity of certain subgroups of parvocellular and magnocellular neurons of PVN and SON of fetal and neonatal offspring. These changes in the PVN lasted to 14 days after birth. Considering the atrophic changes in all three examined zones of the adrenal gland (Hristić et al., 1996) it is clear that Dx-treatment of the dams had a suppressive effect on the hypothalamo-pituitary-adrenal system of fetuses and their offspring.

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NUCLEUS PARAVENTRICULARIS I NUCLEUS SUPRAOPTICUS FETUSA I NEONATALNIH POTOMAKA PACOVA TRETIRANIH DEKSAMETAZONOM ZA VREME GESTACIJE

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SADRŽAJ

Ispitivali smo razvoj i direrenciranje nucleus paraventricularis-a (PVN) i nucleus supraopticus-a (SON) fetusa od 20 dana i neonatalnih pacova od 3 i 14

dana, potomaka pacova tretiranih deksametazonom (Dx) (0,3 mg/kg t.m./dan) od 16 - 20 dana gestacije.

Analiza je izvršena na serijskim frontalnim presecima hipotalamusa merenjem maksimalnog dijametra (Dmax) i površine preseka (area) jedara neurosekretnih ćelija u pet podgrupa PVN (anteriorne parvocelularne i magnocelularne, medialne parvocelularne i magnocelularne i posteriorne magnocelularne podgrupe) na osam nivoa. Dugotrajno tretiranje pacova u toku gestacije sa Dx prouzrokovalo je značajno smanjenje maksimalnog dijametra i površine preseka jedara neurosekretnih ćelija PVN potomaka, na trećem, četvrtom, petom, šestom i sedmom nivou, gde su prisutne ili preovlađuju anteriorna i medialna parvocelularna podgrupa neurona. Morfometrijski podaci su u skladu sa podacima dobijenim histološkom analizom. Jedra parvocelularnih neurona su vidno smanjena.

Tretiranje sa Dx gravidnih ženki utiče značajno na smanjenje maksimalnog dijametra i površine preseka jedara neurosekretnih ćelija SON fetusa i neonatalnih potomaka od 3 dana.

Podaci naših istraživanja o diferenciranju hipotalamičnih jedara ukazuju da tretiranje gravidnih ženki pacova sa Dx smanjuje sintetsku aktivnost neurosekretnih neurona PVN i SON fetusa i neonatalnih potomaka.