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The Influence of Prolonged Dexamethasone Treatment of Pregnant Rats on the Perinatal Development of the Adrenal Gland of Their Offspring

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ABSTRACT The effects of prolonged dexamethasone (Dx) administration to pregnant rats on the struture and function of the adrenal glands of fetal and neonatal offspring have been investigated by combined stereological and ultrastructural methods, as well as by metaphase index determination. Pregnant rats were injected subcutaneoisly with Dx (0.3 mg/kg body weight/day) during 5 days, starting from day 16 of gestation. The dams and their fetuses were killed 24 hr after the last injection. The neonatal offspring were killed in the same way on the 3rd and 14th day of life. Because in fetal and 3-day-old neonatal rats zona reticularis (ZR) was poorly defined and could not be clearly seen as a separate zone, zona fasciculata (ZF) and ZR were analyzed as one, inner zone (IZ). In 14-day-old rats ZF and ZR were analyzed separately. Proliferative activity of adrenocortical cells was estimated following the application of Vincristine sulphate.

Dx treatment of pregnant rats induced a marked decrease of fetal adrenal gland volume and the volumes of zona glomerulosa + capsula (ZG + C) and IZ as the consequence of atrophic changes in the gland and reduction of the average volume and total number of adrenocortical cells. Similar morphometric changes were found in 3- and 14-day-old pups. However, in 3-day-old animals the number of cortical cells in the ZG was increased, whereas on the 14th postnatal day cortical cell number remained decreased only in the ZF. The multinuclear giant cells, numerous lymphocytes, and the resorption zones, present in the adrenal cortex of fetuses and 3-day-old pups of both experimental and control dams, were not seen in 14-day-old offspring.

These results demonstrate that prolonged treatment of pregnant rats with Dx in the period when intensive differentiation of the fetal hypothalamo-hypophyseal system takes place inhibits proliferative activity of adrenocortical cells and evokes considerable atrophic changes in the adrenal glands of offspring from 20 days gestation to 14 days after birth. The histological appearance of the adrenal cortex and the ultrastructure of adrenocortical cells suggest that cortical cell function was inhibited, too. *J. Exp. Zool. 279:54–61, 1997.*

It is well known that numerous factors can affect the differentiation and secretory capacity of the fetal adrenal gland as well as adrenal development and function from the neonatal period to sexual maturity (Pepe and Albrecht, '90). Among these factors of substantial great importance is the status of those maternal hormones that influence the fetal hypothalamo-hypophyseal-adrenal system. Hence, it has been reported that the loss of maternal corticosteroids evoked by adrenalectomy stimulates fetal pituitary and adrenal glands (Milković et al., '73; Hristić et al., '78), which results in elevated plasma corticosterone levels at birth (Thoman et al., '70; Milkivić et al., '76). Removal of the maternal adrenals on day 16 of gestation inhibits adrenal growth both in fetal and neonatal offspring, but morphometric parameters point to stimulated secretory activity of the adrenocortical cells (Hristić et al., '94). Ultrastructural analysis of adrenocortical cells of newborn rats also shows signs of stimulated activity in response to adrenalectomy of the mothers (Nickerson et al., '78), whereas treatment of 21-day-old fetuses with ACTH increases adrenal steroidogenesis (Klepac and Milković, '75).

On the other hand, ACTH produced by tumor cells in pregnant rats, by stimulating maternal

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corticosteroidogenesis, inhibits the function of fetal adrenal glands (Milković and Domac, '73). Maternal treatment with prednisolone decreases fetal adrenal weight and increases adrenocortical apoptosis (Wyllie et al., '73b). Recently, we showed that administration of a single dose of dexamethasone (Dx) to pregnant rats on day 16 of gestation leads to marked atrophic changes and suppression of the function of the fetal adrenal glands, which are partially maintained up to the 14th day of postnatal life (Hristić et al., '95).

In the present study the effects of repeated Dx application to pregnant rats on fetal and neonatal adrenal gland morphometric and ultrastructural characteristics as well as the proliferative activity of cortical cells were examined. The single dose of Dx (1.5 mg/kg body weight [BW]) used in our previous work (Hristić et al., '95) was divided into five daily doses (0.3 mg/kg BW) to examine whether the offspring adrenal gland response differed from that induced by maternal treatment with the same quantity of Dx administered at once.

MATERIALS AND METHODS

Treatment of animals

Twenty female and 20 male, 3-mo-old Wistar strain rats were mated in the laboratory. The day when females were sperm positive was considered as the first day of pregnancy. Pregnant rats were housed individually in conditions of controlled heating (22°C) and lighting (12:12-hr light-dark cycle with 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 10 animals injected subcutaneously with Dx (0.3 mg·kg BW^{-1}·day^{-1}) during 5 days, starting from day 16 of gestation. The control group included 10 females that received an equivalent volume of saline (0.3 ml·kg^{-1}·day) during 5 days beginning from the same day of gestation as the experimental group.

The dams and their fetuses were killed 24 hr after the last injection by decapitation under ether narcosis between 9:00 and 11:00 A.M. on day 21 of gestation. The neonatal animals, offspring of both experimental and control dams, were killed in the same way on day 4 or 15 of life. Fetal and neonatal males will be referred to as 20-day-old fetuses and 3- or 14- day-old neonatal rats.

Light microscopic morphometry

The left adrenal glands from five animals per group were removed quickly, weighed and fixed for 24 hr in Bouin´s solution, embedded in paraffin, and serially sectioned at 5 μm. Sections were stained with hematoxylin and eosin.

Level I. Volume and zonation of the adrenal gland

Stereological measurements were performed by a simple counting method (Weibel, '79). Sections were analyzed with the aid of the Weibel multipurpose lattice M_{42} (42 points, 21 lines) inserted into the ocular of the microscope. To evaluate total volume of the adrenal gland and volumetric densities of the adrenocortical zones, every fifth section of the gland was measured at a magnification of ×400. In fetal and 3-day-old rat adrenals, zona reticularis (ZR) was poorly defined and could not be clearly seen as a separate zone. For this reason zona fasciculata (ZF) and ZR were analyzed as one, inner zone (IZ). In 14-day-old rat adrenals, three cortical zones (zona glomerulosa [ZG], ZF, and ZR) were analyzed.

Level II. Size and number of adrenocortical cells

Fifty test areas of each zone (ZG, IZ, ZF, and ZR) from the equatorial sections were counted at ×1,000 magnification by the test system M_{42} (Weibel, '79). Numerical density of adrenocortical cell nuclei, and thus of the cells, was determined according to Weibel and Gomez (Weibel, '79). The shape coefficient β depends on the axial ratio of estimated nuclear profiles. It was assumed to be 1.382 for the cell nuclei of all cortical zones (Malendowicz, '87). Mean volumes of the cells and nuclei were calculated from the numerical density values and number of the cells.

Electron microscopy

The right adrenal glands were removed quickly and 1-mm^3 slices were fixed in 3% glutaraldehyde buffered to 7.2 with 0.1 M phosphate. Tissue was postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (7.2), dehydrated in a graded series of acetone, and embedded in araldite. Semithin and thin sections were cut with glass knives on an LKB Ultramicrotome III and stained with methylene blue or uranyl acetate and lead citrate, respectively.

Metaphase index

For determination metaphase index of the adrenocortical cells five animals from each group received a single intraperitoneal injection of Vincristine sulphate (1 mg/kg BW) (Oncovin, Lek, Ljubljana, Slovenia with Eli Lilli-Co, Indianapo-

lis, IN), 3 hr before killing. Vincristin sulphate was given to the fetuses after laparotomy of their dams under light ether narcosis.

The metaphase-arrested adrenocortical cells were counted in each cortical zone from three equatorial sections of the gland stained with hematoxylin and eosin at a magnification of ×400. The metaphase index was expressed as the number of metaphases per 1,000 adrenocortical cells.

Number of multinuclear giant cells

The multinuclear giant cells in the adrenal gland were counted in each fifth section of the gland at a magnification of ×400. The area of gland in which these cells were counted was determined stereologically by using the M_{42} test-system. The number of multinuclear giant cells was then normalized and expressed per square millimeter of adrenal gland in fetuses and 3-day-old rats and per square millimeter of adrenal cortex in 14-day-old rats.

Statistical analysis

Data were expressed as means for five animals per group ± SEM. Statistical comparison of the data was made by the Wilcoxon test.

RESULTS

Body weights of 20-day-old fetuses of dams treated with Dx (0.3 mg·kg BW^{-1}·day^{-1}) for five consecutive days starting from day 16 of gestation were significantly decreased in comparison with the corresponding control fetuses (38%) (Table 1). The significant reduction of body weight was maintained up to day 3 (17%) (Table 1) and day 14 (27%) (Table 2) of neonatal life.

The absolute and relative adrenal gland weights of fetuses of dams treated with Dx were markedly decreased (67 and 46%, respectively) in comparison with control fetuses (Table 1). The absolute adrenal gland weights of 3-day-old (Table 1) and 14-day-old (Table 2) neonatal rats of dams treated with Dx were also decreased (36 and 23%, respectively).

Morphometric parameters

In the 20-day-old fetuses of dams treated with Dx stereological analyses (Table 1) also showed a marked decrease in the adrenal gland volume (60%). This was due to a reduction in the volumes of ZG + C and IZ (53 and 63%, respectively). The average volume of the cortical cells in ZG and the cortical cells and nuclei in IZ were decreased (ZG: 26%; IZ: 30 and 22%, respectively). Dx treatment of pregnant rats led to a decrease in the total number of fetal cortical cells in both zones (ZG: 44%; IZ: 46%) in comparison with control animals.

In 3-day-old pups of Dx-treated dams (Table 1) the volumes of the adrenal gland, ZG + C and IZ were decreased compared with corresponding controls (32, 23, and 35%, respectively). The average volumes of the cortical cells and nuclei in both zones were markedly reduced (ZG: 49 and 26%; IZ: 31 and 27%, respectively). However, the total number of parenchymal cells in ZG was signifi-

TABLE 1. Morphometric parameters of the adrenal cortex in fetuses and 3-day-old rats of dams treated with dexamethasone (Dx) or saline (controls) five consecutive days from day 16 of gestation[1]

	Fetuses		3-day-old rats	
	Controls	Dx-injected dams	Controls	Dx-injected dams
Body weight (g)	6.44 ± 0.13	4.02 ± 0.11***	8.62 ± 0.22	7.18 ± 0.17***
Adrenal weight (mg)	1.80 ± 0.10	0.60 ± 0.04***	1.06 ± 0.07	0.68 ± 0.07**
(mg/100 g BW)	27.96 ± 1.53	15.03 ± 1.43***	12.35 ± 0.98	9.50 ± 1.00
Adrenal volume (mm^3)	1.071 ± 0.30	0.424 ± 0.026***	0.778 ± 0.038	0.532 ± 0.030***
Volume (mm^3)				
ZG + C	0.245 ± 0.015	0.016 ± 0.007***	0.196 ± 0.018	0.151 ± 0.008*
IZ	0.824 ± 0.017	0.306 ± 0.019***	0.581 ± 0.024	0.380 ± 0.028***
Volume of cell (μm^3)				
ZG	1,657 ± 125	1,223 ± 28*	1,743 ± 107	884 ± 50***
IZ	2,590 ± 50	1,811 ± 23***	2,252 ± 158	1,564 ± 78***
Volume of nucleus (μm^3)				
ZG	220 ± 20	189 ± 14	252 ± 17	186 ± 12*
IZ	278 ± 9	217 ± 9***	250 ± 28	182 ± 12*
Number of cells ($\times 10^3$)				
ZG	76.04 ± 10.18	42.70 ± 2.7***	65.30 ± 7.03	92.80 ± 5.27*
IZ	214.55 ± 19.79	115.40 ± 5.7***	254.50 ± 19.69	208.30 ± 12.20*

[1]Results are given as means for five animals ± SEM. Statistical significance according to the Wilcoxon test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$. Zona glomerulosa + capsula (ZG + C), inner zone (IZ).

TABLE 2. Morphometric parameters of the adrenal cortex in 14-day-old pups of dams treated with dexamethasone (Dx) or saline (controls) five consecutive days from day 16 of gestation[1]

	14-day-old rats	
	Controls	Dx-injected dams
Body weight (g)	32.18 ± 1.58	23.50 ± 0.40**
Adrenal weight (mg)	2.34 ± 0.22	1.81 ± 0.11*
(mg/100 g BW)	7.34 ± 0.80	7.68 ± 0.44
Adrenal weight (mm^3)	2.036 ± 0.122	1.201 ± 0.058**
Cortex volume (mm^3)	1.835 ± 0.104	1.117 ± 0.058**
Volume (mm^3)		
ZG + C	0.310 ± 0.013	0.258 ± 0.017*
ZF	1.291 ± 0.078	0.638 ± 0.059**
ZR	0.235 ± 0.025	0.209 ± 0.011
Volume of cell (μm^3)		
ZG	1,658 ± 95	1,021 ± 61**
ZF	1,760 ± 87	1,433 ± 33**
ZR	1,575 ± 102	1,327 ± 38**
Volume of nucleus (μm^3)		
ZG	313 ± 15	191 ± 9**
ZF	299 ± 16	186 ± 8**
ZR	267 ± 16	184 ± 6**
Number of cells ($\times 10^3$)		
ZG	146.12 ± 10.20	153.04 ± 15.50
ZF	720.50 ± 60.00	330.36 ± 30.60**
ZR	111.70 ± 8.03	129.16 ± 8.50

[1]Results are given as means for five animals ± SEM. Statistical significance according to the Wilcoxon test: *$P < 0.05$; **$P < 0.005$. Zona glomerulosa + capsula (ZG + C), zona fasciculata (ZF), zona reticularis (ZR).

cantly increased (42%), whereas in IZ it was decreased (18%).

In 14-day-old neonatal rats of dams treated with Dx, similar changes in morphometric parameters of the adrenal glands (Table 2) were seen as in 3-day-old experimental neonatal rats. The volumes of adrenal gland, cortex, ZG, and ZF were considerably reduced (41, 39, 17, and 51%, respectively). The average volumes of the cortical cells and their nuclei in all three examined zones were significantly decreased (ZG: 38 and 38%; ZF: 19 and 38%; ZR: 16 and 31%, respectively). However, the cell number was only changed in ZF, where it was considerably decreased (54%).

The metaphase index of cortical cells

Determination of metaphase index (Table 3) demonstrated that Dx treatment of dams during gestation dramatically decreased proliferative activity of ZG and IZ cortical cells of fetuses (3.5 times and 3 times, respectively) and 3-day-old pups (36 and 46%, respectively). However, no significant differences in metaphase indices of cortical cells between 14-day-old offspring of Dx-treated and saline-injected dams were found.

When the metaphase indices of ZG cortical cells were compared between fetal and neonatal periods, a marked difference was observed in control animals (Table 3). Thus, the number of metaphase-arrested cells in the ZG declined from day 20 of fetal life to day 3 of postnatal life (3 times, $P < 0.005$) and then remained unaltered up to 14th postnatal day. These changes were not found in offspring of Dx-treated dams.

Histological analysis

Histological analysis of fetal adrenal gland of Dx-treated dams revealed the presence of a great number of lipid droplets in ZG cortical cells. In 3- and 14-day-old neonatal rats the accumulation of lipid droplets in some groups of ZG cells was also observed (Fig. 1a). In these postnatal periods groups of numerous light cells were found in ZG (Fig. 1b).

In the inner cortical zone of fetuses and 3-day-old rats of control dams, resorption zones with necrotic adrenocortical cells and numerous lymphocytes between parenchymal cells were observed. Frequent multinuclear giant cells were seen in the vicinity of resorption zones. In the adrenal gland of fetuses and 3-day-old pups of Dx-treated dams, necrotic changes were more expressed and parenchymal cells in various stages of degeneration were abundant, especially near the central part of the gland where ZR begins to differentiate (Figs. 2a, b and 3a, b). Apoptotic bodies were found in the IZ, too. The number of multinuclear giant cells in the adrenal glands of fetuses and 3-day-old rats from experimental dams was markedly lower than in corresponding controls ($P < 0.01$ and $P < 0.01$, respectively). It was 10.31 ± 1.77 in control and

TABLE 3. Methaphase index of cortical cells in 20-day-old fetuses and 3- and 4-day-old offspring of dams treated with Dx five consecutive days from day 16 of gestation[1]

Coritcal zones	Controls	Dx-injected dams
	Fetuses	
ZG	46.44 ± 10.09	13.37 ± 4.71**
IZ	4.01 ± 1.04	1.27 ± 0.50**
	3-day-old rats	
ZG	14.64 ± 2.02	9.44 ± 2.87*
IZ	3.34 ± 0.95	1.80 ± 0.35*
	14-day-old rats	
ZG	14.63 ± 2.53	14.04 ± 4.71
ZF	1.77 ± 0.48	2.32 ± 0.66
ZR	0.59 ± 0.18	0.42 ± 0.17

[1]Results are given as means for five animials ± SEM. Statistical significance according to the Wilcoxon test: *$P < 0.01$; **$P < 0.005$. Zona glomeruiosa (ZG), inner zone (IZ), zona fasciculata (ZF), zona reticularis (ZR).

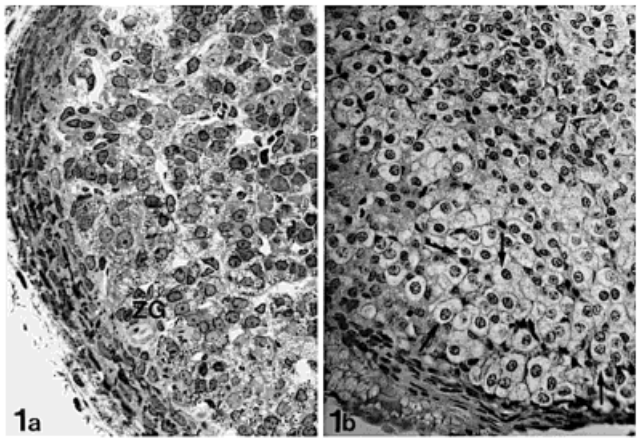

Fig. 1. Light micrographs of the adrenal gland of 3-day-old rats of Dx-treated dams. (**a**) Great number of lipid droplets in the cortical cells of zona glomerulosa (ZG). The volumes of these cortical cells and their nuclei are markedly reduced (×800). (**b**) The group of the numerous light cells (arrows) in ZG (×640).

2.78 ± 0.57 in fetuses from Dx-treated dams. The corresponding values for 3-day-old neonatal rats were 4.89 ± 1.67 and 1.50 ± 0.83. On day 14 of postnatal life the multinuclear giant cells, lymphocytes and the resorption zones were absent both in control and experimental rats. However, multinuclear giant cells were observed as necrotic cells between the medulla and ZR. The atrophic changes in the adrenal cortex were suppressed in these animals but still present mostly in ZF.

DISCUSSION

The results of this study demonstrate that prolonged administration of Dx to pregnant rats over 5 days, starting from day 16 of gestation, resulted in significant atrophic changes in the adrenal cortex and a decrease in the body weight of fetal and neonatal offspring.

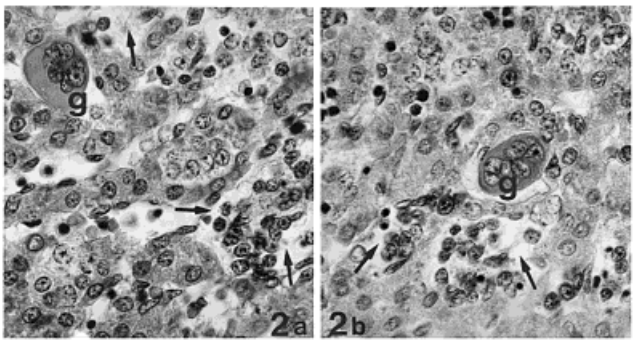

Fig. 2. In the inner cortical zone of (**a**) 20-day-old fetus and (**b**) 3-day-old rat of Dx-treated dams multinuclear giant cells (g) are localized in the vicinity of necrotic adrenocortical cells and resorption zones (arrows) (×880).

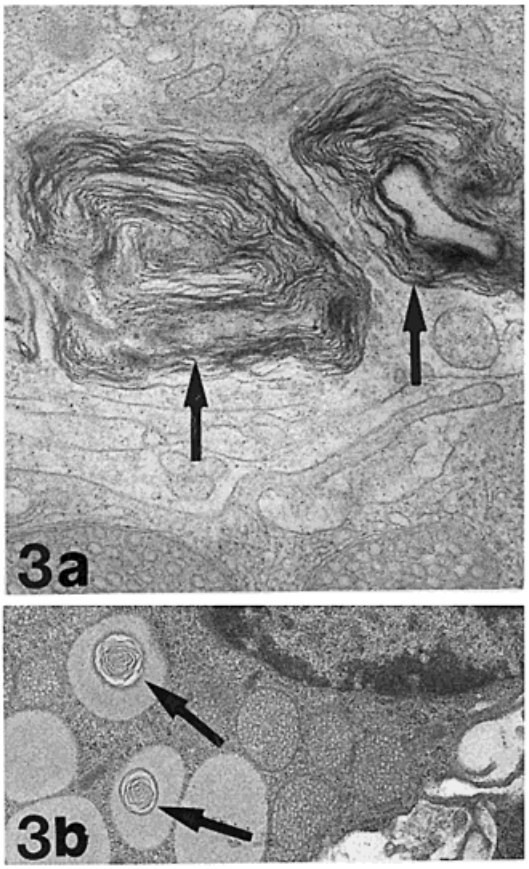

Fig. 3. Electron micrograph of the inner cortical zone of 3-day-old rats of Dx-treated dams. (**a**) The numerous myelin figures formed between cortical cells (×24,000). (**b**) The part of the adrenocortical cell with myelin figures localized in some lipid droplets (arrows) (×15,200).

The reduction in body weight evident in 20-day-old fetuses persisted until the 14th day of postnatal life. A similar influence of Dx treatment of pregnant dams on the body weight of offspring was found in our previous work (Hristić et al., '95) and by other investigators (Rotenberg andGewolb, '93). The effect of glucocorticoids seems to be complex one, because both stimulatory and inhibitory effects on body growth were reported. Glucocorticoids were shown to stimulate the expression of growth hormone in the fetal hypophysis (Nogami and Tachibana, '93). On the other hand, the clearly established inhibitory influence of these steroids on growth could involve stimulation of catabolic processes and somatostatin secretion (Calogero et al., '90; Wehrenberg et al., '90).

To our knowledge there are no data in the literature on the effects exerted by glucocorticoid treatment of pregnant rats on the morphometric characteristics of the adrenal gland in their off-

spring. The stereological analysis performed here revealed that, in the fetuses the volume of whole adrenal gland, ZG + C and IZ were significantly decreased as a result of the decreases both in parenchymal cell size and number. Similar atrophic changes were found in neonatal rats, although in 3-day-old pups the number of cortical cells in the ZG was increased, whereas in 14-day-old animal the cortical cell number was still reduced only in the ZF.

Determination of the metaphase index showed that in fetuses and 3-day-old pups from Dx-treated dams the proliferative activity of adrenocortical cells was significantly lower than in corresponding controls. Atrophic changes in the adrenal cortex and the diminution of cortical cell mitotic activity most probably are the consequences of strong inhibition from fetal ACTH secretion. The fetal hypophysis is essential for normal differentiation of the adrenal cortex (Wyllie et al., '73a; Laureno and Tseng, '76). In the rat, the synthesis of ACTH begins between the 16th and 17th day of fetal life when the first ACTH-immunoreactive cells become visible in the adenohypophysis (Dupouy and Magre, '73; Sétaló and Nakane, '76). Recent data indicate that hypophyseal ACTH production begins on day 14 of fetal life (Nemeskéri and Halász, '89; Stoeckel et al., '93). Dx administered to pregnant rats crosses the placental barrier, enters the fetal circulation (Zarrow et al., '70), and inhibits fetal ACTH secretion. It has been shown to inhibit transcription of the POMC gene and to decrease cAMP accumulation evoked by CRH (King and Baertschi, '90).

The deprivation of ACTH also stimulates apoptosis in the adrenal cortex both in neonatal (Wyllie et al., '73b) and adult rats (Wyllie et al., '73a; Bursch et al., ´92). This process is most prominent in the inner cortical zones, particularly in the ZF. Our finding that the number of parenchymal cells was invariably reduced in the ZF from the 20th day of fetal life to the 14th postnatal day suggests that apoptosis took place. The absence of apoptotic cells loss in the ZG might be responsible for the enhanced number of cortical cells in this zone of 3-day-old experimental rats in spite of decreased mitotic activity in these cells.

A considerable reduction in adrenocortical cell volume accompanied by decreased nuclear volume in the experimental offspring indicates functional inhibition. Although the plasma corticosteroid concentrations were not determined, the histological appearance of cortical cells also confirms that their activity was inhibited. In the ZG cells of fetuses and 3-day-old neonatal rats, accumulation of lipid droplets, as a sign of inhibited steroidogenesis, was detected (Nussdorfer, '86). Also, the unpublished data of the plasma ACTH concentration revealed a significant decrease in all examined periods (fetuses: 235.60 ± 42.24 vs. 144.34 ± 38.95, $P < 0.005$; 3-day-old neonatal rats: 227.99 ± 47.85 vs. 147.63 ± 54.79, $P < 0.025$; 14-day-old neonatal rats: 196.22 ± 51.72 vs. 67.79 ± 14.18, $P < 0.001$). The results of Mazzocchi et al. (1986) demonstrated that the decrease of cortical cells and nuclei volume in zona fasciculata of Dx-treated rats are followed by a decrease of the plasma corticosterone concentrations.

The significance of fetal ACTH for the adrenal development and function was demonstrated in fetal sheep (Boshier et al., '81; Robinson et al., '83) in which hypophysectomy markedly reduced adrenal weight and plasma cortisol concentrations, the effect being reversed with the infusion of ACTH (Coulter et al., '92).

The groups of numerous light cells in ZG, observed in the postnatal period, probably represent precursors, which give rise to the zona fasciculata cells where atrophic changes were most expressed after Dx treatment of the dams.

According to one hypothesis the adrenocortical cells differentiate from blasts localized in the zona capsularis (Jayne, '53; Lombardo and Cortesini, '88) or from cells of the zona glomerulosa that remained after enucleation and/or transplantation (Greep and Deane, '49; Taki and Nickerson, '85). The proliferating cells after enucleation were mainly light cells (Seki at al., '69). The cells with intense proliferative activity were localized in zona intermedia from where thay migrate to ZG or ZF (Nussdorfer, '86).

Dx inhibits proliferation of parenchymal cells in the outer cortical layer of young rats (80–90 g) and their centripetal migration into ZR (Stachowiak et al., '90).

It is of interest that, in the control adrenal glands of fetuses and 3-day-old rats, numerous multinuclear giant cells and lymphocytes were present among the cortical cells. Degenerative changes in the adrenal cortex of neonatal rats were also described by others (Wyllie et al., '73b; Nussdorfer, '86; Hristić et al., '95). The presence of multinuclear giant cells, macrophages, lymphocytes, as well as the resorption zones with necrotic cells in control fetal and 3-day-old neonatal adrenals is probably related to the significant decrease in adrenal weight in the first postnatal days in rats (Josimovich et al., '54; Manojlović et al., '95).

If the effects of Dx administration to pregnant rats were compared between pups whose mothers received a single dose of steroid (Hristić et al., '95) and those whose mothers received the same quantity of Dx but divided into five daily doses, it could be seen that prolonged treatment with smaller doses was more potent and had a longer effect. In fetuses and 3-day-old pups prolonged treatment of the dams affected both the size and number of offspring adrenocortical cells, whereas a single dose of Dx given to pregnant rats decreased only the number of cortical cells in the ZG and IZ. In 14-day-old pups whose dams were submitted to 5-day Dx treatment, the volumes of adrenal glands, ZG + C and ZF were still significantly decreased, whereas in animals of the same age from dams treated with a single dose of Dx only the volume of ZR was reduced.

The main conclusion that could be drawn from the results of this and our previous study is that administration of Dx to pregnant rats in the period of gestation when intensive differentiation of the fetal hypothalamo-pituitary-adrenal system occurs consistently induces atrophic changes in the adrenal glands and a reduction in body weight of the offspring at the fetal and neonatal stages. The magnitude and persistence of these changes depend more on the duration of Dx treatment than on the total Dx dose applied.

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