

STEREOLOGICAL ANALYSIS OF THE ADRENAL GLAND CORTEX IN THE MALE RATS TREATED WITH HYDROCORTISONE

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ABSTRACT

In this work, the reaction of the adrenal cortex of the male rats to treatment with different hydrocortisone doses applied during the juvenile stage of development was studied using stereological methods. Animals were divided into 6 groups. Groups 1, 2, 3 and 5 were treated for four weeks with 2, 4, 8 or 32 mg hydrocortisone (Hy)/kg b.w./day, respectively. Animals from the group 4 were injected with decreasing Hy doses (16, 8, 4 and 2 mg Hy/kg b.w./day), changing the dose every week. All Hy-treated groups were compared with the corresponding control animals. Animals were sacrificed 24 h after the last Hy injection. The results obtained by stereological analysis showed differences in the adrenal cortex reaction to Hy application which were dependent on whether physiological (2 and 4 mg) or pharmacological (8; 16, 8, 4 and 2; 32 mg) doses of this glucocorticoid hormone were used. Physiological Hy doses led to a significant increase of the absolute volume of the adrenal cortex, as well as that of zona fasciculata volume and total number of the cells within this zone in comparison with the controls. At the same time, the application of pharmacological doses of Hy resulted in a conspicuous decrease of the same parameters comparing to the controls.

KEY WORDS: * Adrenal gland cortex * hydrocortisone * male rats *
* stereology *

INTRODUCTION

It was shown previously that hydrocortisone treatment of the male rats during neonatal, early and late juvenile period of development results in a significant reduction of the adrenal cortex width which was especially pronounced in zona fasciculata (Hristić et al., 1986, 1987, 1988). Similar changes were observed in female rats treated with hydrocortisone during the juvenile stage of development (Milošević and Kalafatić, 1990). Kahri et al. (1979) studied changes in the adrenal cortex upon hydrocortisone application from a biochemical aspect

and found that this glucocorticoid hormone acts inhibiting both biosynthesis and secretion of 18-hydroxy-deoxycorticosterone in the culture of fetal adrenocortical cells. It was also shown that dexamethasone inhibits biosynthesis and secretion of aldosterone (Nussdorfer et al., 1986) and corticosterone (Maz-zocchi et al., 1987) in adult males rats

In this work we have examined the reaction of adrenal cortex and its individual zones to different hydrocortisone doses applied during the juvenile period of development to the male rats using stereological and histological methods.

MATERIAL AND METHODS

Treatment of the animals. Thirty, 15 day old male rats of Wistar strain were i.p. injected with hydrocortisone during four weeks, Saturdays and Sundays being the rest period. Animals were divided into five experimental groups: Groups 1 (2 mg Hy/kg b.w./day) and 2 (4 mg Hy/kg b.w./day) received physiological doses of hydrocortisone. Animals of groups 3 (8 mg Hy/kg b.w./day) and 5 (32 mg Hy/kg b.w./day) were treated with a pharmacological doses of hydrocortisone. Animals of group 4 were treated with 16, 8, 4 and 2 mg Hy/kg b.w./day, the dose being changed every week. Control group consisted of animals of corresponding age and was injected with 0.1 ml of physiological saline according to the same schedule.

Light microscopy. All animals were sacrificed 24 h after the last injection. Left adrenal glands were excised, fixed in Bouin's solution, embedded in paraffin and serially cut into 5 μ m thick sections which were stained with hematoxylin-eosin and examined under a light microscope (Opton).

Morphometry.

Stage 1. Zonation of the adrenal gland. In order to evaluate the volume densities of the three adrenocortical zones every 10th section of the gland was analyzed using a magnification of 125 x and the multipurpose test system M (Weibel, 1979).

The absolute volume of the glands was calculated on the basis of their weights assuming that 1.039 g/cm³ represents an average specific gravity of the adrenal (Swinyard, 1938).

Stage 2. Size and number of adrenocortical cells. For each adrenal gland one of the section with the largest area was chosen and 50 test-areas of ZF were counted in it at a magnification of 1000 x and with the multipurpose test system M₄ (Weibel, 1979). On the basis of the previous karyometric studies (Malendowich, 1974), the shape coefficient β which relates N_v to N_a and V_v and depends on the axial ratio of the estimated nuclei, was assumed to be 1.382 for the ZF. The number of nuclei of adrenocortical cells per mm³ was calculated according to Weibel and Gomez (1962). Since rat adrenocortical cells are mononuclear, the numerical density of the nuclei corresponds to the number of cells per mm³. Differences between the groups were evaluated by the Student t-test (Snedecor, 1966).

RESULTS

Effects of hydrocortisone treatment on body weight, absolute and relative weight of the rat adrenal gland. The results summarized in Table 1 show that hydrocortisone treatment of the

Table 1. The effect of hydrocortisone on body weight, absolute and relative weight of the adrenal gland in the male rats

Groups	Hy dose/kg b.w./day (mg)	Body weight (g)	Absolute weight of the adrenal gland (mg)	Relative weight of the adrenal gland (mg)
Control	0	160.0 ± 10.0	24.8 ± 1.9	15.5 ± 1.4
1	2	162.5 ± 5.0*	27.8 ± 1.9	17.1 ± 2.3
2	4	125.0 ± 8.4*	42.2 ± 16.5 ^o	34.2 ± 14.6 ^{oo}
3	8	77.0 ± 9.7*	20.5 ± 6.8*	26.5 ± 7.6 ^{oo}
4	16, 8, 4, 2	80.8 ± 20.6*	17.3 ± 1.3*	23.2 ± 8.8
5	32	55.0 ± 19.1	8.8 ± 1.2*	18.6 ± 9.9

The results are MEANS ± S.D.; *p<0.001; ^op<0.025; ^op<0.05

male rats led to a significant decrease of body weight with the exception of animals from the group 1 treated with the lowest Hy dose (2 mg Hy/kg b.w. /day), in comparison with the controls. Absolute weight of the adrenal gland was

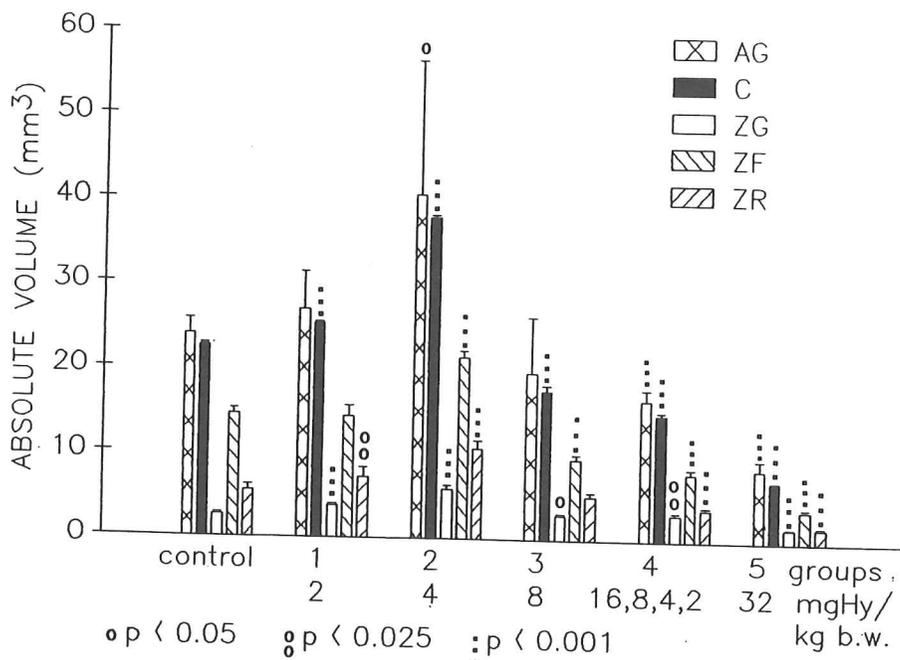


FIG. 1 Absolute volume of: adrenal gland (AG), adrenal cortex (C), zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) after treatment with hydrocortisone in 5 experimental groups and controls. The results are MEANS ± S.D.

significantly increased in group 2 (4mg Hy/kg b.w./day) and decreased in groups 4 (16,8,4 and 2 mg Hy/kg b.w./day) and 5 (8 mg Hy/kg b.w./day) comparing to the corresponding controls. Relative weight of the adrenals was increased in all hydrocortisone-treated groups, but this increase was statistically significant only in groups 2 and 3.

Morphometric and histological picture of the adrenal cortex. Changes in the absolute volume of the adrenal gland are graphically presented in Fig. 1.

Hydrocortisone application led to a significant increase of the absolute volume of the adrenal gland in the group 2, while in the groups 4 and 5 a significant reduction of this value was recorded. Absolute volume of the adrenal cortex was found to be very significantly increased ($p < 0.001$) in the groups 1 and 2, while treatment with 8 mg Hy/kg b.w./day (group 3), 32 mg Hy/kg b.w./day (group 5) and with decreasing Hy doses (16,8,4, and 2 mg Hy/kg b.w./day; group 4) resulted in a significant decrease of this value comparing to the corresponding controls. Relative volume of the adrenal cortex (Fig. 2) was not significantly changed upon hydrocortisone treatment in the groups 1 and 2 of the animals, while in the other three experimental groups a significant decrease of this value was recorded comparing with the controls.

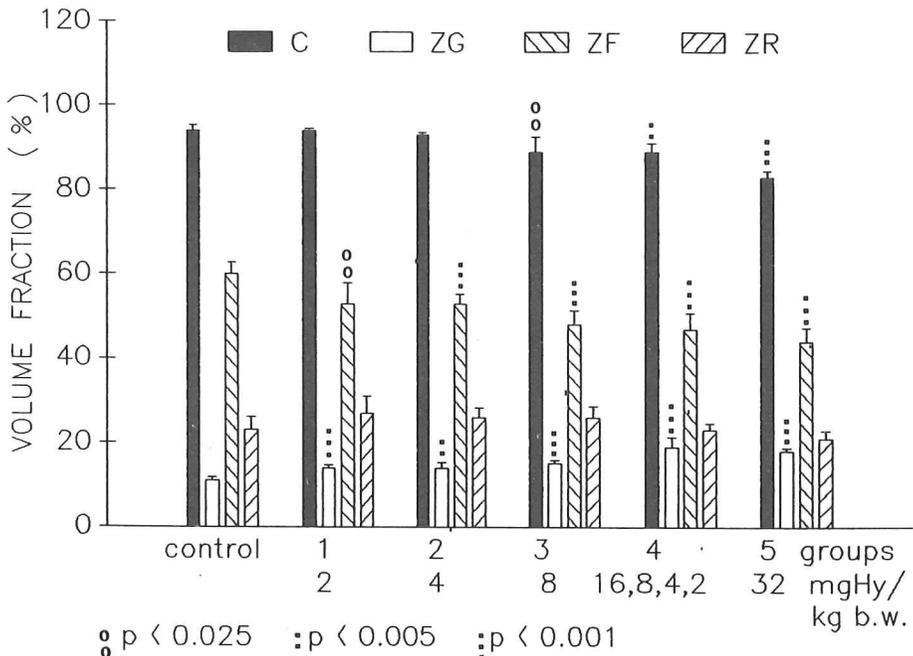


FIG. 2 Relative volume of the adrenal cortex (C), zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) in the adrenal glands of the male rats after treatment with hydrocortisone in 5 experimental groups and controls. The results are MEANS \pm S.D.

In all groups of the animals all zones of the adrenal cortex :zona glomerulosa, fasciculata,reticularis and zona intermedia, were clearly visible.

Zona glomerulosa. Cell population of this zone is very dense. Round and oval nuclei have clearly distinguished nucleoli. Poor cell vacuolization is expressive in this zone. Vacuolisation of the cells was weaker in the animals treated with hydrocortisone than in the corresponding controls. Absolute volume of this zone (Fig.1) was significantly increased in the groups 1 and 2 and decreased in the groups 3, 4 and 5 in relation to the corresponding controls. Relative volume of this cortical zone (Fig.2) was significantly increased in all hydrocortisone-treated groups of animals.

Zona intermedia. This zone consisted from two to three cell layers and it was sporadically interrupted. No hydrocortisone-related changes in the histological structure of the cells within this zone were observed.

Zona fasciculata. In intact rats, this zone is made of polygonal cells arranged in the form of bands with clearly visible vacuolization. No significant changes in histological structure of these cells have been observed in the rats treated with physiological hydrocortisone doses (2 and 4 mg/kg b.w./day). Absolute volume of this zone was significantly increased in the group 2 in relation to the control (Fig.1). Relative volume of this zone was significantly reduced in both groups comparing to the controls (Fig.2).

Table 2. Stereological parameters in the zona fasciculata of the male rats adrenal gland upon treatment with different hydrocortisone doses.

Groups	Hy dose/kg b.w./day (mg)	Absolute volume (μm^3)	
		Cells	Nuclei
Control	0	1249 \pm 34.1	120 \pm 3.3
1	2	1006 \pm 25.2 ^{***}	110 \pm 9.1 ^o
2	4	1128 \pm 39.3 ^{***}	109 \pm 3.1 ^{***}
3	8	1035 \pm 19.5 ^{***}	115 \pm 2.2 ^{oo}
4	16,8,4,2	1032 \pm 6.4 ^{***}	114 \pm 0.7 ^{***}
5	32	749 \pm 26.8 ^{***}	86 \pm 1.9 ^{***}

Groups	Hy dose/kg b.w./day (mg)	Number of cells per mm ³ of total (1×10^6)	
		Cells	Nuclei
Control	0	753 \pm 20.4	10.922 \pm 0.3
1	2	862 \pm 18.8 ^{***}	12.323 \pm 0.3 ^{***}
2	4	821 \pm 23.5 ^{***}	17.580 \pm 0.5 ^{***}
3	8	869 \pm 7.2 ^{***}	8.259 \pm 0.2 ^{***}
4	16,8,4,2	872 \pm 5.6 ^{***}	6.890 \pm 0.1 ^{***}
5	32	1170 \pm 25.7 ^{***}	4.446 \pm 0.1 ^{***}

The results are MEANS \pm S.D.; ^{***}p<0.001, ^{**}p<0.005, ^op<0.025, ^{oo}p<0.05

Absolute volume of the cells and nuclei in the groups 1 and 2 was significantly decreased comparing to the controls. Number of cells expressed per mm^3 , as well as total number of cells were significantly increased in comparison with the controls (Table 2.).

Histological picture of the adrenal gland of the animals treated with pharmacological hydrocortisone doses (8 or 32 mg Hy/kg b.w. / day) was altered comparing to the controls. Cell vacuolization was less expressive. In both these groups an increased number of mitosis was observed, which was especially pronounced in the group receiving 8 mg Hy/kg b.w./day. Absolute and relative volume of this zone was significantly reduced in the groups 3 and 5 (8 and 32 mg Hy/kg b.w./day, respectively) in comparison with the controls.

Absolute volume of the nuclei was decreased in all groups, especially in groups 3 and 5. Number of cells expressed per mm^3 was significantly increased. Total number of the cells was significantly increased in group 1 and 2, but reduced in groups 3, 4 and 5 comparing to the controls (Table 2.).

Histological picture in the group 4 of the rats which were treated with gradually decreasing hydrocortisone doses (16, 8, 4 and 2 mg Hy/kg b.w. / day) was similar to that of the controls. Mitoses were seen, but they were less frequent than observed in the animals from the groups 3 and 5. Absolute and relative volume of zona fasciculata were significantly reduced in comparison with the controls (Fig.1 and 2).

Absolute volume of both the cells and the nuclei was also significantly decreased related to the controls. Number of cells expressed per mm^3 was increased in comparison with the controls, while total number of cells was reduced (Table 2.).

Zona reticularis. This zone of hydrocortisone-treated rats contained quite a number of cells with dark cytoplasm. In all animals receiving this glucocorticoid hormone islets of cells similar to pseudonodules were observed in higher number than in the controls. Absolute volume of this zone (Fig.1) was significantly increased in the groups 1 and 2 comparing to the controls. In the other three groups of animals receiving this hormone, a certain decrease of absolute volume was seen, but it was statistically significant only in the groups 4 and 5 in relation to the control.

Statistically significant differences were not found in relative volume of zona reticularis (Fig.2.).

DISCUSSION

Our results presented in this work demonstrate the difference in the response of the adrenal gland to treatment with hydrocortisone during the juvenile period of development which are dependent on whether physiological or pharmacological doses of this glucocorticoid hormone were applied.

Physiological doses of hydrocortisone (2 or 4 mg Hy/kg b.w./day) continuously applied during four weeks to the male rats led to insignificant changes in histological structure of the adrenal gland cortex. However, absolute volume of the adrenal gland, especially in the animals treated with 4 mg Hy/kg b.w./day was found to be increased. Similar changes have been observed in the female rats treated with the corresponding

hydrocortisone doses during the juvenile period of development (Milošević and Kalafatić, 1990). Determination of the absolute volume of individual adrenal cortex zones revealed an increase of zona glomerulosa, zona fasciculata (only in group 2) and zona reticularis in the male rats receiving physiological hydrocortisone doses. Relative volume for individual adrenal cortex zones show an increase of zona glomerulosa and decrease of zona fasciculata. Stereological measurements of cellular and nuclear volume of the zona fasciculata revealed a decrease of the volume of both the cells and the nuclei, but increased in number of cells per mm^3 and total number of cells in both groups of animals. Our earlier data showed an increased extent of (^3H)-thymidine incorporation into the cells of the all adrenal cortical zones of the male rats treated with physiological hydrocortisone doses suggesting a hydrocortisone-related stimulation of cell division (Hristić et al., 1986, 1988). These our results seem to suggest that the treatment of 15-day old rats four weeks with Hy, that Saturday and Sunday being the rest period, directly stimulates adrenocortical cell division without any remarkable influence on secretion of trophic hormones. Volume of the cortex and individual cortex zones was increased due to number of cells but not of their volume.

Pharmacological hydrocortisone doses applied to the male rats during the juvenile period of life for four weeks led to prominent changes in the adrenal gland as compared to the animals receiving physiological doses of this hormone, as well as to the corresponding controls. Reduction in the adrenal cortex resulted from a remarkable decrease of the zona fasciculata. The numerous mitoses together with poor vacuolisation of the zona glomerulosa and fasciculata cells has been observed. Further analyses revealed a remarkable decrease of both cellular and nuclear volumes, thus resulting in the increase of their number per mm^3 in the zona fasciculata. However, total number of cells within this zone was decreased, meaning that even such a high cell proliferation could not compensate for the decrease of the zona fasciculata and the total cortex provoked by pharmacological hydrocortisone doses. Dexamethasone notably decreased the number of ZF and ZR cells without altering that of ZG cells (Stachowiak et al., 1990). A reduction of the adrenal cortex was earlier observed in the adult male rats treated with a single pharmacological dose of hydrocortisone during neonatal and juvenile period of development (Hristić et al., 1986, 1987). Nussdorfer et al. (1986) demonstrated a significant decrease of plasma aldosterone level upon dexamethasone treatment, and Mazzocchi et al. (1987) found similar effect of dexamethasone on plasma corticosterone level. Treatment of adult male rats with dexamethasone (10 $\mu\text{g}/\text{kg}$ b.w.) during seven consecutive days led to a significant reduction of the zona glomerulosa volume, as well as of both cellular and nuclear volumes, but total number of the cells within this zone was found to be unaltered (Mazzocchi et al., 1987). Prolonged dexamethasone and captopril infusion, though not provoking evidential structural qualitative changes, induced a morphometrically demonstrable striking atrophy of zona glomerulosa cells (Rebuffat et al., 1986). It seems that pharmacological hydrocortisone doses applied throughout our work lead to the atrophic changes of

the adrenal gland and to a strong proliferation of the cells in zona glomeruloza and zona fasciculata. Higher doses seem to cause growth inhibition of the gland, possibly due to inhibition of ACTH secretion and inhibition of growth hormone secretion, as suggested by the small weight of the animals in the effectively exposed groups.

During our studies presented in this work special attention has been paid to the effects of decreasing hydrocortisone doses (16, 8, 4, and 2 mg Hy/kg b.w./day) applied for four weeks to the male rats during juvenile period of life. Histological picture of the adrenal gland and the number of mitoses in these animals resembled that of the control rats, but the other changes observed correspond more to those of high hydrocortisone doses (decrease of the weight and absolute volume of the adrenal gland, reduction of the zona fasciculata volume, as well as cellular and nuclear volume and the decrease in the total number of nuclei within this zone).

This data together with the results reported here suggest that decreasing hydrocortisone doses lead to less expressive changes in the structure and function of the adrenal gland than high doses of this hormone and thus they seem more suitable for therapeutical purpose. Also low doses of hydrocortisone do not affect the endocrinological balance remarkably even hyperplasia; they are recommended over large doses. Higher doses, on the other hand, do and they should not be used too freely.

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