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Proliferation and distribution of adrenocortical cells in the gland of ACTH- or dexamethasone-treated rats

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Summary. The effects of prolonged (7-day) ACTH and dexamethasone administrations on rat adrenocortical-cell turnover have been investigated by combined stereological and metaphase-arrest techniques. ACTH was found to increase the number of parenchymal cells in each adrenal zone; however, ACTH altered the cell distribution in the cortex, lowering their percentage in the zona glomerulosa (ZG) and zona fasciculata (ZF) and enhancing it in the zona reticularis (ZR). The cell birth-rate was markedly raised by ACTH exclusively in ZG and ZF. Dexamethasone notably decreased the number of ZF and ZR cells, without altering that of ZG cells. Moreover, dexamethasone increased the percentage of parenchymal cells in ZG and ZF, and lowered it in ZR. In the adrenal cortices of dexamethasone-administered animals, metaphases were virtually absent. These data indicate that ACTH increases the cell birth-rate in ZG and possibly ZF, and enhances the centripetal migration of newly-formed cells and their accumulation in ZR. Dexamethasone inhibits both proliferation of adrenocortical cells in the outer cortical layers and their centripetal migration into ZR. Moreover, it appears to cause parenchymal-cell loss in the inner adrenocortical layers.

Key words: Adrenocortical-cell turnover, ACTH, Dexamethasone, Rat

Introduction

Cell turnover in the adrenal cortex has been the subject of extensive studies which have largely confirmed the theory of cell migration first proposed by Gottschau (1883). The existence of a centripetal migration of cells from the zona glomerulosa (ZG) to the zona reticularis (ZR), throughout the zona fasciculata (ZF), has been demonstrated mainly by autoradiography with ^{3}H-thymidine, and numerous investigations based on this technique indicate that the outer part of the cortex may be considered as the progenitor compartment in adrenocortical-cell renewal (Ford and Young, 1963; Reiter and Pizzarello, 1966; Wrigth, 1971a; Wright et al., 1973; Wrigth and Voncina, 1977; Belloni et al., 1978; Bertholet, 1980; Malendowicz and Jachimowicz, 1982; Zajicek et al., 1986). Detailed kinetic studies also gave quantitative data on cell proliferation and migration within the adrenal cortex of prepubertal or adult rats (Wright and Voncina, 1977; Zajicek et al., 1986). However, to our knowledge, literature is not available which attempt to correlate the rates of proliferation and migration with the changes in the number of parenchymal cells either in particular adrenocortical zones or in the entire gland. It therefore seemed worthwhile to investigate, by combined stereologic and metaphase-arrest techniques, the proliferation and distribution of adrenocortical cells in the gland of ACTH- or dexamethasone-treated rats.

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Materials and methods

Experimental protocol

Female Wistar rats, weighing 80-90 g, were used. They were maintained under standardized conditions of light (14h on/10h off) and temperature (22 ± 2 °C), and fed laboratory pellets with free access to tap water.

At the beginning of the experiment, 6 rats were decapitated and served as a control. The remaining animals were divided into three groups, which were subcutaneously injected for 7 days with daily doses of 0.1 mg ACTH (Synacthen Depot, Ciba), 0.1 mg dexamethasone phosphate (Decadron, Merck) or 0.1 ml of solvent (0.9% NaCl). 24 h after the last injection, the animals were given (at 9.00 a.m.) 0.1 mg vincristine (Gedeon-Richter, Budapest) intraperitoneum, and subsequently decapitated after 0, 60, 120 and 240 min (5 rats per each time-point). Adrenals were promptly

removed, freed of adherent adipose tissue and weighed. The glands were then fixed for 24 h in Bouin's solution and embedded in paraffin.

Stereology

In addition to control rats, 6 animals from each experimental group were randomly chosen. Adrenals were serially sectioned at 5-6 μm and stained with hematoxylin-eosin. Stereologic studies were made by differential point counting (Weibel, 1979), as detailed by Malendowicz (1987). The following parameters were evaluated: volumes of adrenocortical zones and their parenchymal cells; and number of adrenocortical cells in each zone and in the entire cortex.

Methapase-arrest count

The metaphase index was estimated on medulla-containing sections, by counting 5000 parenchymal cells in each zone. In addition, the number of metaphases per medulla-containing section was evaluated according to Michat and Nouet (1975). For each rat 8-10 sections were analyzed.

Statistical treatment of results

From stereologic data, means and S.E. were calculated for each group, and results were compared with control group by Student's t-test. In the case of metaphase-arrest technique, the rate of accumulation of arrested metaphases was plotted against the time, and the equation of the regression line was calculated by the least square method. The slope of the regression line gives the estimate of the birth rate, mitotic rate or rate of entry in mitosis. Moreover, the correlation coefficient was computed among the number of metaphases per adrenal section and metaphase index in ZG and ZF. All calculations were done with an IBM Personal Computer.

Results

Adrenal glands of saline-injected rats were heavier than those of control animals, and this increase depended on the rise in the volume of the ZR and in the number of ZG and ZR cells (Table 1).

As expected, ACTH treatment resulted in significant increases in the adrenal weight, in the volume of all adrenocortical zones and their cells, and in the number of parenchymal cells in each zone (as well as in the entire cortex). Compared with the control group, adrenal cortex of ACTH-treated rats contained a lower percentage of parenchymal cells in ZG and ZF and a markedly higher percentage in ZR (Table 1). On the contrary, dexamethasone administration caused a significant decrease in the adrenal weight, in the volume of all adrenal zones and their cells, and in the number of parenchymal cells in ZF and ZR (and in the entire cortex). ZG and ZF comprised higher and ZR lower percentages of parenchymal cells than in control rats (Table 1).

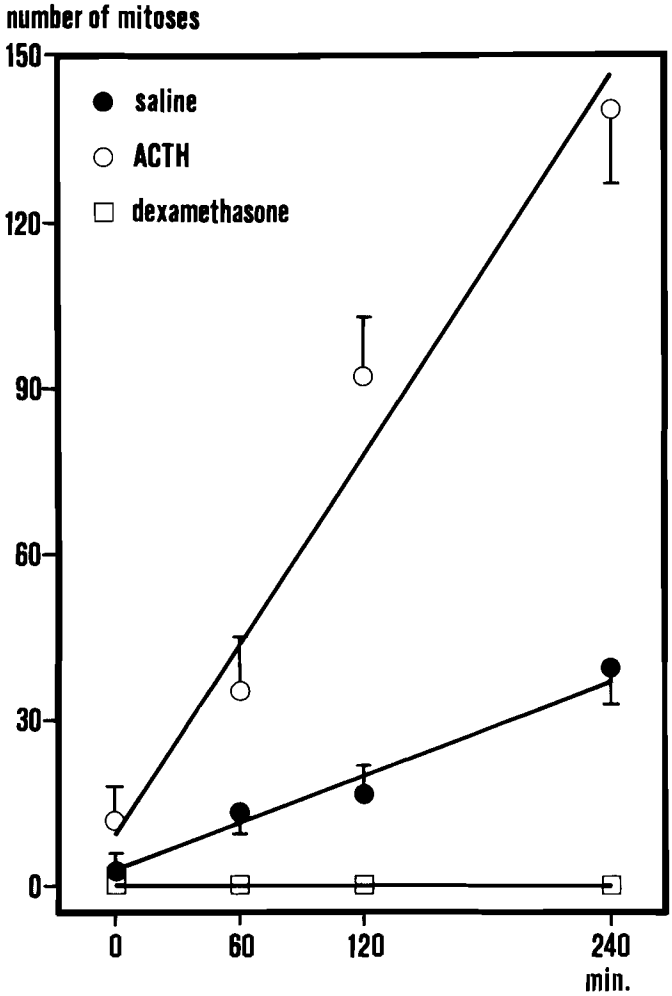

Fig. 1. Metaphase index in the zona glomerulosa of saline-, ACTH- and dexamethasone-injected rats. Equations of regression lines are: saline, $y = 0.44 + 0.013x$; ACTH, $y = 1.55 + 0.094x$. Standard errors are indicated.

Metaphase-arrest technique with vincristine gave linear increase in the number of metaphases with time. Metaphase index notably increased in ZG and ZF of ACTH-treated rats, while in dexamethasone-administered animals, metaphases were virtually absent (Figs. 1, 2). Similar results were obtained if metaphase counting was done per adrenal section (Fig. 3). There was a close correlation between metaphase count per adrenal section and metaphase index of ZG and ZF cells, with values of 0.958 and 0.943, respectively (obviously, only data from saline- and ACTH-injected animals were considered).

The birth rate of adrenocortical cells was calculated from both stereologic and metaphase-arrest technique data (Table 2). As may be seen, the increase in the total number of cells calculated from stereologic data cannot be explained by parallel changes in the metaphase index. Stereology gave markedly higher increase in the total number of parenchymal cells in saline- and ACTH-injected rats when compared with data calculated from metaphase index. On the other hand, stereology indicated a notable cell loss in dexamethasone-treated

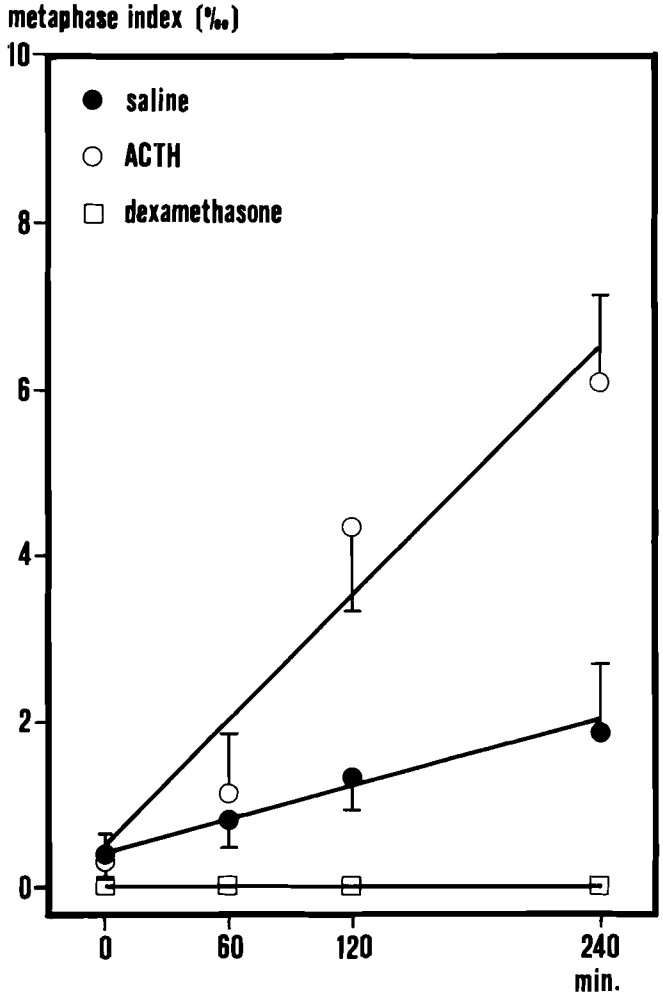

Fig. 2. Metaphase index in the zona fasciculata of saline-, ACTH- and dexamethasone-injected rats. Equations of regression lines are: saline, $y = 0.35 + 0.07x$; ACTH, $y = 0.10 + 0.026x$. Standard errors are indicated.

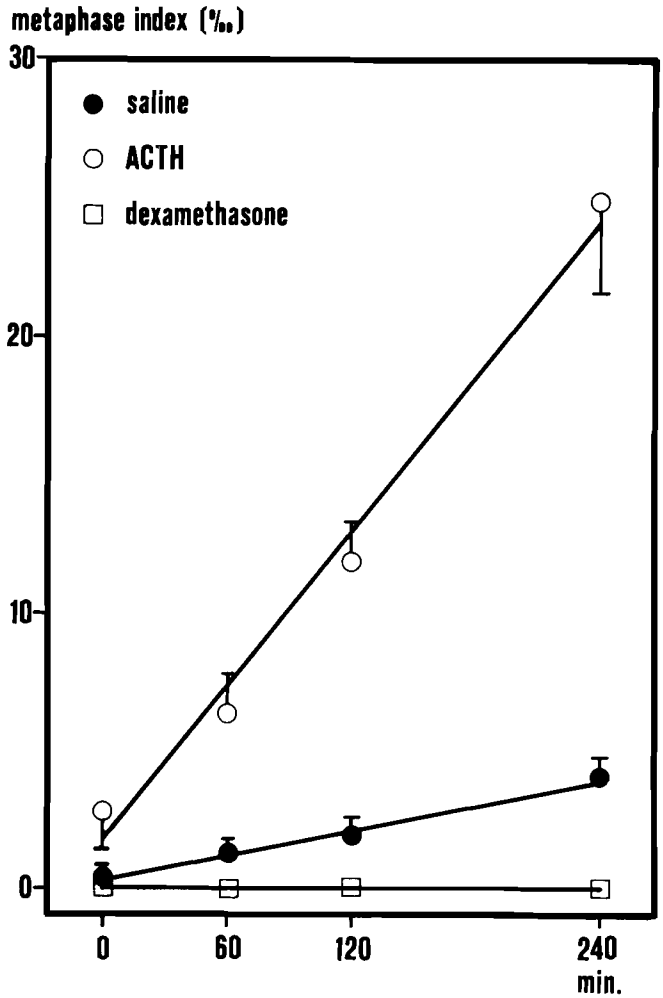

Fig. 3. Number of vincristine-arrested metaphases per medulla-containing section of the adrenal gland of saline-, ACTH- and dexamethasone-injected rats. Equations of regression lines are: saline, $y = 3.46 + 0.143x$; ACTH, $y = 0.78 + 0.612x$. Standard errors are indicated.

animals. In saline-injected rats, the increases in the quantity of ZG and ZF cells calculated from metaphase index were respectively lower and higher than those computed from stereology. The opposite was true in ACTH-treated rats. Mitoses were virtually absent in dexamethasone-administered animals. Likewise, mitoses were not observed in the ZR of saline-injected and ACTH-treated rats, while in both groups of animals stereology revealed a notable increase in the number of cells in that zone.

Discussion

Control of adrenocortical growth by ACTH in vivo is well documented and there is general agreement that ACTH primarily causes cell hypertrophy followed by hyperplasia (Dallman, 1984, 1985; Nussdorfer, 1986). We have presently shown that ACTH also causes changes in the distribution of parenchymal cells in the cortex, lowering their percentage in ZG and ZF and enhancing it in ZR, with overall increase in the total number of adrenocortical cells. This finding is consistent with earlier reports (Belloni et al., 1978; Malendowicz, 1986). On the contrary, dexamethasone, a drug inhibiting POMC synthesis and release (Khalid et al., 1982), results in a notable atrophy of parenchymal cells in all zones and significantly diminishes their number, especially as far as inner adrenocortical layers are concerned. Dexamethasone also alters the distribution of parenchymal cells in the cortex, with higher percentage in ZG and ZF and a lower one in ZR.

Metaphase arrest technique with vincristine allowed us to calculate cell birth-rate in adrenal cortex and, to our knowledge, this is the first attempt to correlate the proliferative activity of adrenocortical cells with the changes in their number as evaluated by stereology. However, to our surprise, marked differences were found in the cell birth-rates calculated from stereological data and mitotic index. In general, stereology gave notably higher values in saline-injected and ACTH-

Table 1. Adrenal weight and stereologic parameters of the adrenal cortex of control and treated rats.

	Control rats	Saline-injected rats	ACTH-injected rats	Dexamethasone-injected rats
Adrenal weight (mg)	22.5 ± 1.0	25.4 ± 0.4$^+$	77.0 ± 6.0*	15.4 ± 0.6*
Volume of zones (mm^3)				
ZG	1.624 ± 0.074	1.827 ± 0.030	2.837 ± 0.259*	1.733 ± 0.071
ZF	12.130 ± 0.628	12.613 ± 0.278	37.369 ± 2.794*	7.589 ± 0.305*
ZR	6.497 ± 0.306	8.336 ± 0.511$^+$	31.108 ± 3.060*	3.928 ± 0.207*
Volume of cells (μm^3)				
ZG	938 ± 11	889 ± 14$^+$	1055 ± 23*	893 ± 7*
ZF	1961 ± 36	1999 ± 68	2532 ± 69*	1370 ± 16*
ZR	716 ± 7	726 ± 22	929 ± 20*	666 ± 7*
Number of cells ($\times 10^3$)				
ZG	1710.7 ± 76.6	2024.3 ± 55.6*	2659.0 ± 229.6*	1923.5 ± 82.2
ZF	5528.5 ± 278.8	5739.6 ± 215.4	12587.5 ± 991.8*	4748.9 ± 189.6$^+$
ZR	7312.8 ± 329.3	9350.2 ± 740.9$^+$	26682.1 ± 2632.1*	4735.2 ± 232.4*
total	14552.1 ± 659.5	17114.1 ± 684.3$^+$	41928.6 ± 3553.2*	11407.6 ± 462.4*
Distribution of cells in the zones (%)				
ZG	11.76 ± 0.10	11.86 ± 0.26	6.42 ± 0.50*	16.87 ± 0.25*
ZF	37.97 ± 0.55	33.82 ± 1.88	30.29 ± 1.51*	41.67 ± 0.73*
ZR	50.27 ± 0.64	54.32 ± 2.03	63.29 ± 1.96*	41.46 ± 0.94*

Results are group means ± S.E. $^+$, $P < 0.05$ and *, $P < 0.01$ versus control group (n = 6).

Table 2. Birth-rate of adrenocortical cells in saline–, ACTH– or dexamethasone-injected rats, as calculated from stereology and metaphase-arrest technique.

		Saline-injected rats	ACTH-injected rats	Dexamethasone-injected rats
Data from stereology				
($\times 10^3$/day)	ZG	44.8	135.5	30.4
	ZF	30.1	1008.4	–111.4
	ZR	291.1	2767.0	–368.2
	total	366.0	3910.9	–449.2
Data from metaphase-index				
($\times 10^3$/day)	ZG	32.4	205.3	0
	ZF	55.7	204.3	0
	ZR	0	0	0
	total	88.1	409.6	0

Stereology-derived values were obtained by subtracting means from control group; those from metaphase-arrest technique were calculated from equations of regression lines shown in Figs. 1, 2.

treated rats, and lower ones in dexamethasone-administered animals. These differences, which are most marked in ZR, may depend on several factors:

(i) It may be that stereology overestimates the number of cells per unit volume of the cortex. In the present study, this parameter was calculated by the formula of Weibel and Gómez (Weibel, 1979); however, we obtained similar results by employing the De Hoff-Rhines method (Weibel, 1979) (data not shown). (ii) Metaphase-arrest technique could underestimate the cell birth-rate, possibly due to the incomplete vincristine-induced arrest of dividing cells in metaphase or to the escape of some cells from metaphase. However, the present study shows that vincristine method gives a linear increase with time in the metaphase index, as well as in the number of mitoses per adrenal section. Moreover, vincristine-evoked metaphase arrest may last even 12 h (Wright and Alison, 1984). (iii) Other factors which should be taken into account are the well-known rhythms of proliferative activity of adrenocortical cells (Michat and Nouet, 1975; Kirillov and Kurilenko, 1977; Ueberberg, 1982). Such natural rhythms may conceivably be altered by the high doses of ACTH and dexamethasone administered in the present study. In the case of ACTH, another possibility cannot be ruled out. It is known that ACTH synchronizes the proliferation of adrenocortical cells in rats and that bursts of mitoses appear periodically (Nussdorfer, 1986). Therefore it is possible that a one time-point sampling study, such as that applied in the present investigation, has missed such

a burst of ACTH-induced proliferation, thus leading to an underestimation of the birth rate of parenchymal cells. Experiments aimed at elucidating this problem are in progress.

Dexamethasone is known to inhibit proliferative activity of adrenocortical cells, and a direct action of this drug on the adrenal cortex has been suggested (Wright, 1971b; Wright et al., 1974). This inhibition of proliferative activity is also connected with a marked loss of parenchymal cells in the adrenocortical gland. In the adrenals of prepubertal rats, apoptotic cell-loss has been reported to be confined to the inner cortical layers (Wyllie et al., 1973a, b). Our findings indicate that ZG of dexamethasone-treated rats contains about the same number of cells as those of control animals, while in the remaining zones (especially ZR) cell loss occurs. Moreover, dexamethasone-treated glands, if compared with the control ones, contain a higher percentage of parenchymal cells in ZG and a lower one in ZR. This would indicate that dexamethasone inhibits both proliferation of adrenocortical cells and their centripetal migration. In ACTH-treated rats, centripetal migration is easily demonstrated by the marked increase in the number of ZR cells coupled with a rise in the birth-rate only in ZG and ZF.

Before concluding, we wish to stress that the present study also demonstrates the close correlation between the mitotic (metaphase) index of adrenocortical cells and the number of mitoses per medulla-containing section of the gland. Therefore, this last parameter may be used for evaluating the proliferative activity of adrenal cortex.

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