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Compensatory Adrenal Growth and Steroidogenesis after Unilateral Adrenalectomy

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Abstract. To examine the effect of unilateral adrenalectomy on compensatory growth and steroidogenesis in the remaining adrenal gland, we determined the tissue concentrations of protein, DNA and corticosterone in the remaining adrenal gland and the circulating corticosterone and ACTH concentrations at 1, 3, 5, 7 and 14 days after unilateral adrenalectomy in male rats. The remaining adrenal weight and total protein content increased steadily over the 14 days after the operation, while the plasma ACTH increased transiently on the 1st day. The adrenal total DNA level was not significantly changed after unilateral adrenalectomy, whereas the protein/DNA ratio was significantly increased by the 14th day. These findings suggest that compensatory growth is not induced by an increase in ACTH and that hypertrophy may occur rather than hyperplasia in the remaining gland. The serum corticosterone levels and corticosterone concentration in the remaining gland were significantly increased by the 3rd day, when the plasma ACTH levels returned to normal. The aldosterone/corticosterone ratio in the remaining gland did not change during the experiment. These results indicate that steroidogenesis stimulating factors other than ACTH may be present and stimulate rather the early stages than the late stages of steroidogenesis under conditions of unilateral adrenalectomy.

Key words: Compensatory adrenal growth, Unilateral adrenalectomy, Steroidogenesis.

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IT IS KNOWN that, following unilateral adrenalectomy, compensatory adrenal growth occurs in the remaining gland. Such compensatory growth was previously believed to result from an increase in ACTH secretion due to temporarily low glucocorticoid production [1]. However, it has been reported that the plasma ACTH levels and plasma corticosterone levels in unilateral adrenalectomized rats remained unchanged as compared to those of sham-operated rats except in the early period after the operation [2]. Dallman [3] indicated that the adrenal wet weight, DNA, protein and cell number increased in the early stages (12–24 h) after unilateral adrenalectomy. Nevertheless, what stimulates the growth in the remaining gland and whether hyperplasia or hypertrophy occurs is not yet completely established, and the adrenal steroid concentration in the remaining gland was not determined. To evaluate the effects of unilateral adrenalectomy on steroidogenesis and compensatory growth in the remaining gland, we measured the concentrations of corticosterone and ACTH in blood, and tissue contents of DNA, protein and steroids in the remaining adrenal gland at 1, 3, 5, 7 and 14 days after unilateral adrenalectomy in male rats.

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Materials and Methods

Eight-week-old male Wistar rats, weighing 250–300 g, were used. Four rats per cage were housed for 2 weeks in an artificially lighted room with an automatically controlled light cycle (lights on from 0600–1800 h) and a room temperature of 24°C. Rat chow and water were made available *ad libitum*. The rats were anesthetized with pentobarbital sodium in an intraperitoneal injection of 40 mg/kg body weight. The right adrenal gland was removed through a dorsal midline skin incision. Groups of rats were killed by decapitation in the morning (0900–1000 h) before the operation (day 0) and on the 1st, 3rd, 5th, 7th and 14th days after the operation. Blood was collected in ice-cooled tubes. Following confirmation of no subadrenal gland, the adrenal gland was cleaned of fat and connective tissue, and then weighed.

The whole adrenal gland was homogenized in 2 m*l* of 33% ethanol containing 0.55 pmol 19-nortestosterone (19-NT) as an internal standard. The homogenate was washed with 5 m*l* hexane, and the washed material was centrifuged after the addition of 5 m*l* dichloromethane and 0.25 m*l* of 1 N NaOH. The supernatant was aspirated and discarded. The residue was washed with 1 m*l* of 0.01 N NaOH and 1 m*l* H_2O. After washing, the extracted solution was dried under N_2 gas. Extraction of steroids from the plasma was performed by a similar method to that for the adrenal gland. The serum steroid levels and steroid contents of the adrenal gland were measured by high performance liquid chromatography (HPLC) [4–8]. Corticosterone and aldosterone were clearly separated by their single peaks and retention times (corticosterone; 10.6 min, aldosterone 5.4 min) of HPLC. The plasma ACTH assay was performed with a ^{125}I-labelled ligand. Sensitivity of the assay was 7 pg/m*l*. The intra-assay coefficient of variation was 10%. Samples from each experiment was assayed in a single run to avoid interassay variation. There was 0.2–0.5% cross-reactivity with α-melanocyte-stimulating hormone but no cross-reactivity with ACTH (25–39) [9]. The DNA in the remaining adrenal gland was estimated by the diphenylamine method [10], and protein content was measured by Lowry's method [11].

All values are shown as the means and the standard error (SE). One way analysis of variance was employed for statistical analysis.

Results

As shown in Fig. 1, the remaining adrenal weight was significantly increased at the 1st, 5th, 7th and 14th days after the operation ($P<0.05$). The protein in the remaining adrenal gland was also significantly increased at the 7th and 14th days as compared to before the operation ($P<0.05$). The adrenal DNA was not significantly different before and after the operation ($P>0.05$). As shown in Fig. 2, no significant change in the

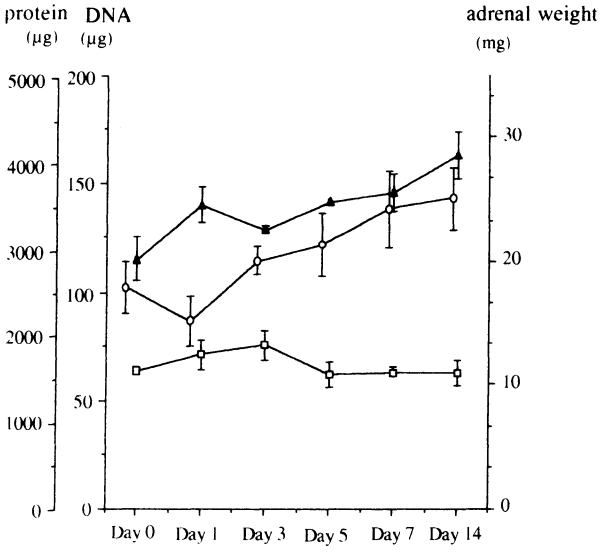

Fig. 1. Changes in compensatory adrenal growth after unilateral adrenalectomy. ▲, adrenal weight; ○, adrenal total protein content in the remaining adrenal gland; □, adrenal total DNA content in the adenal gland. Day 0, before the operation. The data are the mean ± SEM (n=5).

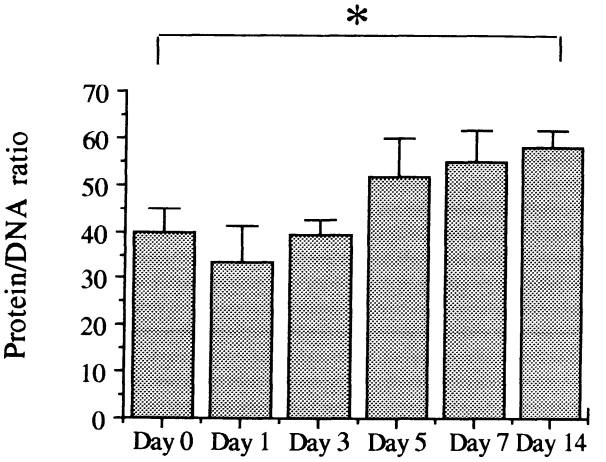

Fig. 2. Changes in the protein/DNA ratio in the remaining adrenal gland. The data are the mean ± SEM (n=5). *, $P<0.05$, significant difference compared to Day 0 (the day before the operation).

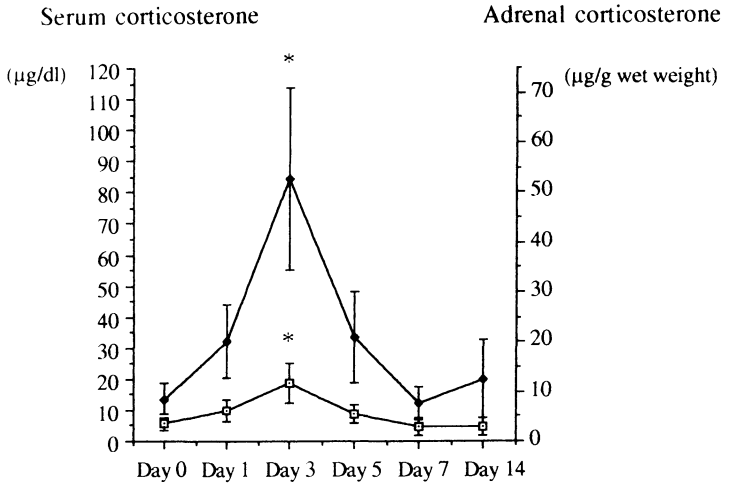

Fig. 3. Changes in serum corticosterone levels and corticosterone content in the remaining adrenal gland. □, serum corticosterone level; ◆, corticosterone content in the adrenal gland. The data are the mean ± SEM (n=5). *, $P<0.05$, significant difference compared to Day 0 (the day before the operation).

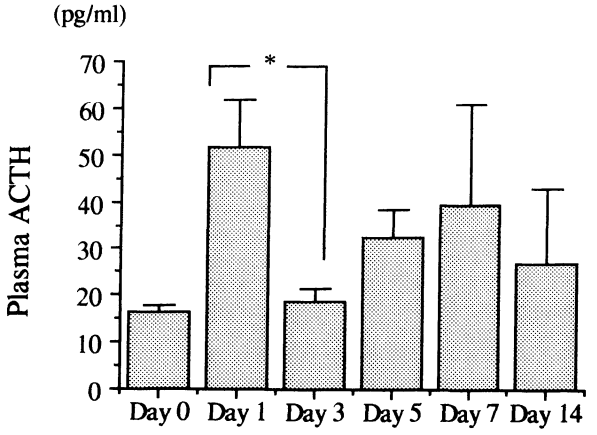

Fig. 4. Changes in plasma ACTH levels after unilateral adrenalectomy. The data are the mean ± SEM (n=5). *, $P<0.05$, significant difference.

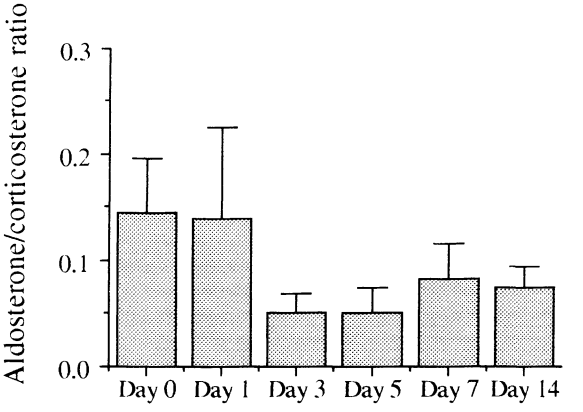

Fig. 5. Changes in the aldosterone/corticosterone ratio in the remaining adrenal gland after unilateral adrenalectomy. The data are the mean ± SEM (n=5).

protein/DNA ratio was observed for 7 days after the operation, however the ratio revealed a significant increase at the 14th day ($P<0.05$). The protein/adrenal weight ratio did not change significantly at the 14th day as compared to before the operation (126.4±10.5 *vs.* 126.3±8.6, not significant), while the DNA/adrenal weight ratio was significantly decreased at the 14th day as compared to before the operation (2.18±0.15 *vs.* 3.25±0.24, $P<0.05$).

The serum and adrenal corticosterone levels in the unilateral adrenalectomized rats are shown in Fig. 3. The serum corticosterone level exhibited a significant increase ($P<0.05$) at the 3rd day after the operation. The corticosterone concentration in the remaining gland was much higher values ($P<0.05$) on the 3rd day than before the operation. As shown in Fig. 4, circulating ACTH was quite high ($P<0.05$) 1 day after unilateral adrenalectomy, but returned to the control level after 3 days. The alterations in the aldosterone/corticosterone ratio in the remaining adrenal gland are shown in Fig. 5. There was virtually no change in the unilateral adrenalectomized rats.

Discussion

Unilateral adrenalectomy has been shown to induce compensatory growth of the remaining adrenal gland. The generally accepted hypothesis in the 1940s was that after removal of one adrenal, the total glucocorticoid secretion rate decreased, the low circulating glucocorticoid level then caused an increase in ACTH secretion, and the increased ACTH mediated compensatory adrenal growth [1]. However, Miahle *et al.* [12] reported that circulating ACTH and corticosterone were not altered by the removal of one adrenal. Engeland *et al.* [2] found that the plasma ACTH was significantly increased in relation to that of sham-operated rats only at 2 h after the operation and not before or after this time.

Furthermore, it has been reported that adrenal compensatory growth after unilateral adrenalectomy occurs even when endogenous ACTH is suppressed with dexamethasone or with dexamethasone plus exogenous ACTH [13]. Adrenal compensatory growth also occurs after unilateral adrenalectomy and hypophysectomy without exogenous ACTH. These findings indicate that compensatory adrenal growth following unilateral adrenalectomy is not mediated by ACTH.

Lowry *et al.* [14] suggested that neurally mediated proteolytic cleavage of the circulating inactive mitogenic precursor pro-γ-MSH at the adrenal gland is the major mechanism of rapid compensatory growth from 24 h after unilateral adrenalectomy. However, it is not known if pro-γ-MSH acts for 2 weeks.

Several reports has been indicated that adrenal hypertrophy shows increased RNA and protein, while adrenal hyperplasia shows increased DNA and cell number [15–20]. Bransome [15] reported that RNA was increased in the canine adrenal cortex following ACTH administration in an *in vivo* study. The gain in size and weight of the adrenal gland after the ACTH administration was not accompanied by any significant change in DNA, suggesting that cell hypertrophy rather than cell proliferation occurred in response to ACTH. While the increase in adrenal DNA and RNA following unilateral adrenalectomy was much less than that in the ACTH-treated animal, the cells showed proliferation rather than hypertrophy. Other investigators have demonstrated that the total DNA content in the remaining gland after unilateral adrenalectomy is virtually unchanged [21]. In the present series of experiments, the remaining adrenal weight and total protein content increased steadily over a period of 14 days after the operation, while the plasma ACTH increased transiently only on the 1st day. The adrenal total DNA content did not change significantly during the experiments. The protein/DNA ratio was significantly increased at the 14th day. These observations indicate that the compensatory growth may not be induced by an increase in ACTH and that hypertrophy rather than hyperplasia may occur in the remaining adrenal gland.

Pellegrino *et al.* [21] reported that the corticosterone secretion from the remaining gland collected by cannulation of the renal vein was considerably increased at the 7th day after unilateral adrenalectomy. However, at the 12th, 15th and 30th days, average secretion decreased to near the control value. In the present experiments, plasma corticosterone was increased at the 3rd day and returned to the control level by the 7th day. The change in plasma corticosterone parallel to that in adrenal corticosterone indicates that the increased corticosterone level reflected its production and secretion in the remaining adrenal gland. The production of corticosterone may be controlled by agents other than ACTH, since the corticosterone concentration in the remaining gland had increased significantly by the 3rd day, when the plasma ACTH level returned to the control level.

Our experiments revealed that the aldosterone/corticosterone ratio in the remaining gland did not alter during the 14 days. Then, steroid stimulating factors may act on rather the early stages of steroidogenesis than the conversion of corticosterone to aldosterone, or may enhance the effect of ACTH or another physiological growth factors.

In conclusion, our data indicate that adrenal growth-stimulating factors other than ACTH may be present and may induce hypertrophy rather than hyperplasia in the remaining gland, and that steroidogenesis stimulating factors other than ACTH may also be present and stimulate rather the early stages than the late step of steroidogenesis under conditions of unilateral adrenalectomy.

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