Zone-specific cell proliferation during compensatory adrenal growth in rats

W. C. Engeland, W. B. Ennen, A. Elayaperumal, D. A. Durand, and B. K. Levay-Young
Departments of Surgery and Neuroscience, University of Minnesota, Minneapolis, Minnesota

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Engeland, W. C., W. B. Ennen, A. Elayaperumal, D. A. Durand, and B. K. Levay-Young. Zone-specific cell proliferation during compensatory adrenal growth in rats. Am J Physiol Endocrinol Metab 288: E298–E306, 2005.—Compensatory adrenal growth after unilateral adrenalectomy (ULA) leads to adrenocortical hyperplasia. Because zonal growth contributions are not clear, we characterized the phenotype of cortical cells that proliferate using immunofluorescence histochemistry and zone-specific cell counting. Rats underwent ULA, sham adrenalectomy (sham), or no surgery and were killed at 2 or 5 days. Adrenals were weighed and sections immunostained for Ki67 (proliferation), cytochrome P-450 aldosterone synthase (P450aldo, glomerulosa), and cytochrome P-450 11β-hydroxylase (P45011B, fasciculata). Unbiased stereology was used to count proliferating glomerulosa and fasciculata cells. Adrenal weight increased after ULA compared with sham and no surgery at both time points, and there was no difference between sham and no surgery. However, either ULA or sham increased Ki67-positive cells in the outer fasciculata at both time points compared with no surgery. Outer fasciculata-restricted proliferation is thus associated with adrenal weight gain in ULA but not sham. Experiment repetition using proliferating cell nuclear antigen and bromodeoxyuridine showed similar results. After ULA, adrenal DNA, RNA, and protein increased at both time points, whereas after sham, only adrenal DNA increased at 2 days. Compensatory growth thus results from hyperplasia and hypertrophy, whereas sham induces only a transient adrenal hyperplasia. Dexamethasone pretreatment prevented the increase in adrenal weight after ULA and blocked Ki67 labeling in the outer fasciculata but not zona glomerulosa in all groups. These results clearly show that the outer fasciculata is the primary adrenal zone responsible for compensatory growth, responding to steroid-suppressible stress signals that alone are ineffective in increasing adrenal mass.

Address for reprint requests and other correspondence: W. C. Engeland, Box 120 UMHC, 516 Delaware St. SE, Minneapolis, MN 55455 (E-mail: engel002@umn.edu).

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processes responsible for changes in adrenal weight. The finding that the pattern of cell proliferation observed during compensatory adrenal growth was similar to that observed in rats undergoing sham adrenalectomy led to additional experiments assessing proliferative responses after suppression of pituitary-adrenal hormonal responses to surgery by dexamethasone.

**METHODS**

**Materials**

The following supplies and chemicals were purchased: Vectashield and Antigen Unmasking Solution from Vector Laboratories (Burlingame, CA); Nacetyl from Pfizer (New York, NY); pentobarbital sodium (Nembutal) from Abbott Laboratories (North Chicago, IL); bicinchoninic acid (BCA) protein assay kit from Pierce (Rockford, IL); dexamethasone phosphate from American Pharmaceutical Partners (Los Angeles, CA); corticosterone RIA kits from MP Biomedical (Irvine, CA); 125I-labeled ACTH from Diasorin (Stillwater, MN); anti-bromodeoxyuridine (BrdU) antibody from Becton-Dickinson (San Jose, CA); PicoGreen dsDNA quantitation reagent from Molecular Probes (Eugene, OR); RNAlater from Ambion (Austin, TX); mouse anti-PCNA antibody from Zymed (South San Francisco, CA); Cy5-labeled donkey anti-rabbit IgG, AlexaFluor488-labeled goat anti-mouse IgG, F(ab')2 fragment donkey anti-mouse IgG, and Cy3-labeled donkey anti-mouse IgG from Jackson ImmunoResearch Laboratories (West Grove, PA); and mouse anti-Ki67 antigen from DakoCytomation (Carpinteria, CA).

**Animals**

Male Sprague-Dawley rats (175–225 g body wt, Charles River Laboratories) were housed two per cage in a light-controlled facility (lights on from 0600 to 1800). Food and water were provided ad libitum. Animals were allowed to accimate to the housing facility and light cycle for at least 1 wk before experiments. All procedures were approved by the University of Minnesota Animal Care and Use Committee.

**Adrenal Surgery**

Rats were anesthetized (pentobarbital sodium, 6–7 mg/100 g ip) and underwent right unilateral adrenalectomy (ULA) or sham surgery in which the adrenal was exposed but not manipulated (sham). Skin incisions were closed with wound clips, antibiotic (Nacetyl, 2 mg/kg im) was administered, and animals were kept warm until fully ambulatory.

**Experimental Protocols**

**Experiment 1.** To determine the relationship between adrenal weight and zone-specific cell proliferation during compensatory adrenal growth, rats (n = 5–6/group) were decapitated in the morning at 2 or 5 days after ULA or sham surgery. An additional control group was included at each time point consisting of rats that remained in the animal facility and had no surgery. Left adrenals were cleaned of connective tissue and fat, weighed, and frozen for triple-label immunohistochemistry.

**Experiment 2.** To establish that Ki67 labeling was accurately reflecting cell proliferation, additional rats (n = 5–6/group) were decapitated in the morning at 2 days after ULA, sham surgery, or no surgery. Left adrenals were bisected, and the fragments were weighed and processed for adrenal protein, RNA, and DNA.

**Experiment 5.** To determine adrenal growth after suppression of pituitary-adrenal hormonal responses to surgery, rats were pretreated with dexamethasone phosphate (400 μg sc) or saline (100 μl sc) 2 h before adrenal surgery. Rats were decapitated in the morning at 2 days after ULA, sham surgery, or no surgery; left adrenals were weighed and frozen for immunohistochemistry. To assess the effectiveness of dexamethasone to block stress-induced plasma ACTH and corticosterone, additional rats (n = 6/group) were sampled at 30 min after surgery; blood was collected for RIA of ACTH and corticosterone as described previously (41).

**Immunofluorescence Histochemistry**

**Triple labeling.** Staining for Ki67, P450aldo, and P45011B was performed by a triple-labeling procedure adapted from a previous double-labeling method (44). Frozen adrenals were sectioned (30 μm), postfixed in Zamboni’s solution, and incubated overnight with a mouse anti-Ki67 primary, followed by a donkey anti-mouse Cy3 secondary and a blocking antibody [F(ab')2 donkey anti-mouse, Jackson Laboratories]. Primary antibodies (Ab) directed against P450aldo (rabbit Ab) and P45011B (mouse Ab), generously supplied by C. Gomez-Sanchez of the University of Mississippi Medical Center, were then applied. After overnight incubation, sections were incubated with secondary antibodies (goat anti-mouse Alexa488 and donkey anti-rabbit Cy5) for 1 h, rinsed, and coverslipped in aqueous mounting media (Vectashield).

PCNA labeling and BrdU labeling. For the detection of PCNA, paraformaldehyde (4%)–fixed sections were immersed in hot Antigen Unmasking Solution and incubated overnight with mouse anti-PCNA primary. Sections were then incubated with secondary antibody (donkey anti-mouse Cy3) and coverslipped in Vectashield. For the detection of BrdU, Zamboni-fixed sections were denatured in 4 N HCl, neutralized in 0.1 M sodium tetraborate, and incubated overnight with mouse anti-BrdU primary. Sections were then incubated with secondary antibody (goat anti-mouse Alexa488) and coverslipped in Vectashield.

**Photomicroscopy and Unbiased Stereology**

Optical images were collected with a monochrome charge-coupled device camera, captured with a Scion LG-3 frame grabber, and processed on a Macintosh computer using NIH Image software. Triple-labeled images were pseudocolored and overlapped using Adobe Photoshop.

Cell counting was done with the use of unbiased stereology as described by Howard and Reed (23). Cells labeled for Ki67 and P450aldo or for Ki67 and P45011B were counted as proliferating glomerulosa cells or fasciculata cells, respectively. Outer fasciculata cells were classified as P45011B-positive cells adjacent to the zona intermedia; the zona intermedia expresses neither P450aldo nor P45011B. Inner fasciculata cells were classified as P45011B-positive cells adjacent to the medulla. Sampling areas within each zone were randomly selected. Labeled cells within an area circumscribed by a two-dimensional box (e.g., 40.1 × 400.5 μm and 133.5 × 133.5 μm for glomerulosa and fasciculata, respectively) were counted throughout the depth of the section. With the use of these templates, a sampling area in the glomerulosa extended inward from the adrenal capsule and included 5–10 cell layers or ~1,000 cells; a sampling area in the fasciculata included 15–20 cell layers or ~750 cells extending inward from the zona intermedia (outer fasciculata) or extending outward from the medulla (inner fasciculata). Approximately 200 Ki67-labeled cells from eight distinct sampling areas and four different sections were counted for each adrenal, and numerical density (labeled cells per volume) was determined.

Cells stained for PCNA and BrdU were counted in a similar manner as for Ki67. Tissue processing for PCNA and BrdU staining...
precluded labeling for steroidogenic enzymes. Cell counts were made in the outer cortex with the border of the sampling area adjacent to the capsule. Cells in the zona glomerulosa and outer zona fasciculata were included in the outer cortex counts.

**DNA and RNA Measurement**

Adrenals were bisected; adrenal halves were weighed and either frozen on dry ice (protein) or stored in RNALater. For adrenal protein, tissue was homogenized in SDS sample buffer, and protein was determined by BCA assay. For RNA and DNA, adrenal tissue was homogenized in guanidine buffer (8), and DNA was determined by PicoGreen assay. RNA was extracted from guanidine and quantified by absorbance at 260 nm. Adrenal DNA, RNA, and protein were corrected by fragment weight to determine amount per adrenal.

**Plasma Hormone Measurement**

Plasma corticosterone was measured by RIA with a commercially available kit. The intra-assay and interassay coefficients of variation (CVs) for corticosterone were 7 and 13%, respectively. Plasma ACTH was measured via RIA as described previously (25), using $^{125}$I-ACTH as tracer. The intra-assay and interassay CVs for ACTH were 7.6 and 13.3%, respectively.

**Statistics**

Data are shown as mean values ± SE. Statistical significance was determined by ANOVA, with repeated measures when appropriate. When necessary, homogeneity of variance was obtained by logarithmic transformation followed by ANOVA. Differences between groups were determined via a Newman-Keuls post hoc analysis; differences were considered significantly different when the test yielded $P < 0.05$.

**RESULTS**

**Experiment 1**

Adrenal weight was increased in the ULA group compared with sham and no surgery groups at 2 and 5 days, but there was no difference between sham and no surgery groups at either time point (Fig. 1). Triple-label immunostaining showed a high density of Ki67(+) cells in outer fasciculata in sham surgery and ULA adrenals compared with no surgery adrenals (Fig. 2). In response to ULA or sham surgery, Ki67(+) cells in the outer fasciculata increased at 2 days compared with no surgery; at 5 days, Ki67(+) cells in the outer fasciculata decreased from day 2 levels but remained elevated compared with no surgery rats (Fig. 3). In addition, there was a small increase in Ki67(+) in the inner fasciculata at 2 days, but not 5 days, after ULA or sham surgery.

![Fig. 1. Adrenal weight at 2 and 5 days after unilateral adrenalectomy (ULA), sham surgery, and no surgery. Values are means ± SE; n = 5–6/group. *P < 0.05 vs. no surgery and sham surgery.](#)

![Fig. 2. Representative sections from rat adrenals collected at 2 days after no surgery (A), sham surgery (B), and ULA (C), immunostained for cytochrome P-450 aldosterone synthase (P450aldo; red), cytochrome P-450 11β-hydroxylase (P45011B; green), and Ki67 (blue). CP, capsule; ZG, zona glomerulosa; ZI, zona intermedia; ZF, zona fasciculata.](#)
Experiment 2

Adrenal weight was increased in ULA rats compared with sham and no surgery rats at 2 days (Table 1). Although phenotypic markers could not be used to determine precise zonation, immunostaining for PCNA showed a high density of proliferating cells in the cortex underlying the capsule in sham surgery and ULA adrenals compared with no surgery adrenals (Fig. 4). The number of PCNA(+) cells was increased in ULA and sham surgery adrenals compared with no surgery adrenals at 2 days (Table 1). In sections from the same adrenals, immunostaining for BrdU showed parallel changes in proliferation; the number of BrdU(+) cells in the outer adrenal cortex increased in ULA and sham surgery compared with no surgery groups (Table 1). The results from PCNA and BrdU labeling confirm the Ki67 data.

Experiments 3 and 4

At 2 and 5 days, adrenal weight, RNA, and protein were increased in ULA rats compared with sham and no surgery rats. At 2 days, adrenal DNA was increased in ULA and sham surgery compared with no surgery rats; by 5 days, adrenal DNA was increased in ULA compared with both sham and no surgery rats (Fig. 5). The protein-to-DNA and RNA-to-DNA ratios increased in the ULA group at 2 days.

Experiment 5

Treatment with dexamethasone 2 h before surgery suppressed stress-induced changes in ACTH and corticosterone. After either ULA or sham surgery, plasma ACTH and corticosterone in dexamethasone-treated rats were decreased at 30 min after surgery compared with saline-treated rats (Table 2). Treatment with dexamethasone 2 h before surgery prevented the increase in adrenal weight at 2 days postsurgery normally produced by ULA (Table 3). In rats treated with saline, Ki67 labeling was prominent in the outer fasciculata in sham surgery and ULA rats; after dexamethasone, Ki67 labeling was reduced in this adrenal zone in all groups (Fig. 6). In saline-pretreated rats, the density of Ki67(+) cells in the outer fasciculata increased in response to ULA or sham surgery compared with no surgery; there were no changes in proliferation between groups in the glomerulosa or inner fasciculata (Fig. 7). However, after dexamethasone pretreatment, the density of Ki67(+) cells in the outer fasciculata was reduced in all groups, and there were no surgery- or ULA-mediated differences. Cell proliferation was not affected by dexamethasone in the zona glomerulosa, but Ki67(+) cells were increased in the ULA group after dexamethasone compared with the sham surgery, but not the no surgery, group (Fig. 7).

DISCUSSION

The present study was designed to determine unambiguously which adrenal cortical zones proliferate during compensatory adrenal growth. With the use of triple-label immunostaining to identify the phenotype of the cortical cell, results showed that cell proliferation after unilateral adrenalectomy occurs primarily in the outer fasciculata. In addition, increases in cell proliferation were observed after sham surgery with a distribution and magnitude similar to that observed after unilateral adrenalectomy. However, despite a small and transient increase in adrenal DNA at 2 days after sham surgery, the proliferative response observed at 2 and 5 days was not reflected by increases in adrenal weight. In contrast, unilateral adrenalectomy results in increases in adrenal DNA, RNA, and protein.

Table 1. Comparison of adrenal wet wt, PCNA, and BrdU cell counts at 2 days after no surgery, sham surgery, or ULA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Wt, g</th>
<th>Adrenal Wt, mg/100 g body wt</th>
<th>No. of PCNA(+) Cells, $\times 10^3$/mm$^2$</th>
<th>No. of BrdU(+) Cells, $\times 10^3$/mm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No surgery</td>
<td>267±7</td>
<td>7.7±0.3</td>
<td>16.52±1.09</td>
<td>9.54±0.99</td>
</tr>
<tr>
<td>Sham surgery</td>
<td>244±10</td>
<td>8.7±0.1</td>
<td>25.09±1.05*</td>
<td>14.35±1.20*</td>
</tr>
<tr>
<td>ULA</td>
<td>248±6</td>
<td>11.4±0.5†</td>
<td>32.69±4.18*</td>
<td>20.51±3.97*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. PCNA, proliferating cell nuclear antigen; BrdU, bromodeoxyuridine; ULA, unilateral adrenalectomy. *P < 0.05 vs. no surgery. †P < 0.05 vs. sham surgery.
that are associated with sustained increases in adrenal weight. These data suggest that stress-related increases in cell proliferation in the outer fasciculata contribute to the initial increases in compensatory growth, but that surgical stress does not induce a proliferative response that alone is sufficient to alter adrenal weight. The finding that dexamethasone prevents fasciculata cell proliferation during compensatory growth and after surgical stress supports previous work suggesting the involvement of POMC-derived peptides in adrenal growth.

After unilateral adrenalectomy, growth of the remaining adrenal occurs to compensate for the loss of adrenal mass. As shown previously (9, 11), compensatory adrenal growth is characterized by a rapid increase in DNA content. Because cell hyperplasia is a major component of the response, it is important to determine the cortical cells that proliferate. Although standard histological approaches can distinguish glomerulosa cells underlying the adrenal capsule from inner cortical cells, the outer cortex is composed of three adjacent cell zones: the glomerulosa and outer fasciculata separated by the intermedia. Accurate identification of cells in these zones requires the use of specific phenotypic markers (33, 44). In addition, a concise quantitative assessment of proliferation as a function of cell phenotype required a triple-labeling approach. Our laboratory published a brief report using Ki67 as a proliferation marker with phenotypic labeling of an adjacent adrenal section (21). However, the current approach consisting of labeling in a single section allows accurate identification of proliferating glomerulosa and fasciculata cells; in addition, the use of unbiased stereology offers a quantitative assessment of proliferating cell density. The results show that, in control rats undergoing no surgery, cell proliferation is comparable in the glomerulosa and outer fasciculata. However, at 2 and 5 days after unilateral adrenalectomy, the predominant response is an increase in cell proliferation restricted to the outer fasciculata. The finding that glomerulosa cells are not involved in the proliferative response appears inconsistent with previous work showing that unilateral adrenalectomy increases the rate of cell proliferation in capsule-glomerulosa preparations in vitro (2). However, because outer fasciculata cells are known contaminants of these preparations (22), it is possible that fasciculata cells are responsible, at least in part, for the observed response. In addition, experiments were not done to compare glomerulosa and outer fasciculata cell preparations to determine relative differences in proliferation rates. If this comparison was not made, on the basis of the Ki67 data reported in the current study, it is likely that a preparation enriched with outer fasciculata cells would have a greater proliferation rate than that observed in a glomerulosa cell preparation.

To characterize compensatory adrenal growth, comparisons were made between rats undergoing unilateral adrenalectomy and those undergoing sham surgery. This comparison is critical to control for the effects of anesthesia and surgical stress on the hypothalamic-pituitary-adrenal axis (11, 35), factors that could influence adrenal growth. Another control group consisting of rats undergoing no surgery was included after a pilot experiment suggested that Ki67 labeling was increased in the outer fasciculata in rats after sham surgery. The experiments clearly show that sham surgery results in increases in Ki67 labeling in the adrenal cortex that are indistinguishable from those observed after unilateral adrenalectomy. To insure that Ki67 labeling accurately reflected cell proliferation, PCNA and BrdU labeling was used; the data verified the Ki67 data showing that both sham surgery and unilateral adrenalectomy increased cell proliferation compared with unoperated control rats. Although cell proliferation increases after sham surgery, there is no parallel increase in adrenal weight. To determine
whether other indexes of adrenal growth were stimulated by sham surgery, adrenal DNA, RNA, and protein contents were measured. In response to sham surgery, adrenal DNA increased at 2 days but not at 5 days after surgery, and there were no changes in adrenal RNA or protein. After unilateral adrenalectomy, increases in adrenal DNA, RNA, and protein occurred at 2 and 5 days. These data are consistent with previous work showing that both adrenal hyperplasia and hypertrophy occur after unilateral adrenalectomy (9, 11). Because sham surgery induces a transient adrenal hyperplasia, it is likely that the stress associated with adrenal surgery contributes to the proliferative response during the early stages of compensatory adrenal growth. The hyperplasia resulting from sham surgery is insufficient to evoke a change in adrenal weight. Although increases in adrenal weight are considered a hallmark of the stress response, repeated exposure to stress (18, 39) or chronic stress (20) may be required to elicit this response. Additional experiments are required to determine whether repeated stimulation evokes incremental changes in adrenal DNA or RNA that ultimately would result in increases in adrenal weight.

The finding that the pattern of cell proliferation observed during compensatory adrenal growth was similar to that observed in rats undergoing sham adrenalectomy led to experiments assessing whether the stress of surgery mediated the response by increasing pituitary-adrenal activity. Rats were pretreated with dexamethasone immediately before surgery to block the pituitary response; dexamethasone treatment was effective in preventing stress-induced increases in plasma ACTH and corticosterone. Increases in cell proliferation in the outer fasciculata after unilateral adrenalectomy or sham surgery were completely prevented by dexamethasone. These data strongly suggest that pituitary activation is required for stress-induced adrenal hyperplasia. In addition to affecting cell proliferation, dexamethasone blocked increases in adrenal weight after unilateral adrenalectomy. This observation was unexpected in light of previous data showing that compensatory

Table 2. Effect of dexamethasone pretreatment on plasma ACTH and corticosterone at 30 min after ULA or sham surgery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma ACTH, pg/ml</th>
<th>Plasma Corticosterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>529±98</td>
<td>622.2±31.8</td>
</tr>
<tr>
<td>Sham surgery</td>
<td>784±227</td>
<td>355.9±31.1†</td>
</tr>
<tr>
<td>ULA</td>
<td>59±3*</td>
<td>36.9±6.7*</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham surgery</td>
<td>54±2*</td>
<td>24.3±3.8*</td>
</tr>
<tr>
<td>ULA</td>
<td>59±3*</td>
<td>36.9±6.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. *P < 0.05 vs. saline. †P < 0.05 vs. sham surgery.

Table 3. Effect of dexamethasone pretreatment on adrenal wet wt at 2 days after ULA, sham surgery, or no surgery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Wt, g</th>
<th>Adrenal Wt, mg/100 g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>251±3</td>
<td>7.9±0.2</td>
</tr>
<tr>
<td>Sham surgery</td>
<td>246±6</td>
<td>9.0±0.6</td>
</tr>
<tr>
<td>ULA</td>
<td>245±4</td>
<td>10.8±0.5†</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No surgery</td>
<td>237±4</td>
<td>7.5±0.2</td>
</tr>
<tr>
<td>Sham surgery</td>
<td>236±5</td>
<td>7.5±0.3</td>
</tr>
<tr>
<td>ULA</td>
<td>240±5</td>
<td>8.2±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. *P < 0.05 vs. no surgery. †P < 0.05 vs. sham surgery.
adrenal growth occurs after dexamethasone treatment (9, 14, 19). Our inability to observe compensatory growth might be due to procedural differences from earlier studies, including the dose or regimen of dexamethasone treatment. In the present study, a single injection of dexamethasone at a dose of 2 mg/kg body wt was administered 2 h before surgery. Dexamethasone given 2 h before surgery at a dose of 0.25 mg/kg body wt or given as multiple doses beginning 18 h before surgery and continuing after surgery (9, 14, 19) failed to prevent compensatory growth. In addition, compensatory adrenal growth reflected by adrenal weight and mitotic index occurred in rats treated with dexamethasone for 14 days before surgery (32). However, other investigators have shown that dexamethasone blocks the growth response when given before (27) or after surgery (35). Dexamethasone suppresses pituitary activity and produces adrenal atrophy, resulting in relatively small absolute changes in adrenal weight after unilateral adrenalectomy. The present study confirms the observation that compensatory adrenal growth can be prevented by dexamethasone. However, it is possible that differences in experimental protocols in combination with biological variability determine whether compensatory growth is observed after steroid treatment.

Dexamethasone treatment also reduced proliferation in the outer fasciculata in rats that underwent no surgery. This observation supports previous work supporting a role for ACTH in the maintenance of adrenal cortical mass under nonstress conditions. For example, circadian increases in fasciculata cell proliferation that are preceded by increases in plasma ACTH can be blocked by dexamethasone (34), and adrenal atrophy produced by chronic dexamethasone treatment can be prevented (28, 40) or reversed (29) by ACTH replacement. In contrast to its suppressive effect in the outer fasciculata, dexamethasone treatment unmasked a proliferative response to unilateral adrenalectomy in glomerulosa cells. This observation differs from previous results showing that dexamethasone treatment decreases glomerulosa cell proliferation in rats (34, 45). There are two possible explanations for this apparent inconsistency. First, there is a biphasic response of adrenocor-

Fig. 6. Representative sections immunostained for P450aldo (red), P45011β (green), and Ki67 (blue) from adrenals collected at 2 days after rats were treated with saline (A) or dexamethasone (B), followed by ULA. Horizontal bar = 100 µm.

Fig. 7. Adrenal zone-specific cell proliferation estimated by the density of Ki67(+) cells at 2 days after treatment with dexamethasone in rats undergoing no surgery, sham surgery, and ULA. Values are means ± SE; n = 5–6/group. *P < 0.05 vs. saline. +P < 0.05 vs. no surgery. x P < 0.05 vs. sham surgery.
tical cells to a single injection of dexamethasone; suppression of proliferation in the zona glomerulosa occurs during the initial 12–24 h (34, 45), followed by an increase in proliferation occurring from 48 to 72 h (45). In the present study, with assessment of proliferation at 48 h, the suppressive effect of dexamethasone on glomerulosa cells may have been missed. Second, previous studies have not examined glomerulosa cell proliferation after dexamethasone in rats undergoing compensatory adrenal growth. The unique finding that proliferation in the zona glomerulosa occurs under these conditions suggests that the removal of ACTH and other POMC-derived peptides or a direct adrenal effect of dexamethasone affects the mitogenic response of glomerulosa cells to unilateral adrenalec-
tomy. Because there are numerous mitogens specific to glo-
erulosa cells, including angiotensin (31), endothelin (30), and adrenomedullin (1), it is possible that dexamethasone treatment results in receptor upregulation for one or more of these factors. Additional experiments are required to address this possibility.

There is abundant evidence supporting the hypothesis that compensatory adrenal growth is effected through a reflex that involves both afferent and efferent neural limbs (11–13, 42). Although the cellular mechanism for adrenal growth has not been identified, the proliferative process is thought to occur through neural activation, with local production of mitogen.

Basic fibroblast growth factor (bFGF) is a potent adrenal growth factor (17) concentrated in the glomerulosa and outer fasciculata in rat adrenal cortex (2). Because bFGF receptor internalization is altered in compensatory growth (3), bFGF may play a role in the growth response. However, bFGF receptors are not localized in the outer fasciculata, the primary site for cell proliferation (3), and experiments have not been done to selectively block bFGF activity to determine the effect on compensatory growth. Other studies have identified an adrenal secretory protease (AsP) with activity to produce NH2-terminal POMC peptides that could act locally as adrenal mitogens (5). The finding that AsP increases during compensatory growth (5) supports an earlier hypothesis that neural activation of an adrenal proteolytic enzyme is the efferent mechanism of the neural reflex (27). A role for ACTH and other pituitary-derived POMC peptides in compensatory growth has been somewhat discounted, since hypophysectomy does not prevent the growth response (9, 14, 26). However, hypophysectomy, like dexamethasone treatment, reduces the absolute magnitude of the compensatory growth response (9, 14, 19, 27, 32, 35), suggesting that either ACTH or NH2-terminal POMC-derived peptides could contribute to compensatory growth. Although ACTH treatment increases adrenal weight, the cellular mechanism differs from that of compensatory growth; ACTH stimulates increases in adrenal RNA followed by increases in DNA (16, 24), whereas compensatory growth induces rapid increases in DNA (Fig. 5; Refs. 9, 11). Selective neutralization of ACTH with specific antibodies does not affect compensatory adrenal growth (27, 36), and treatment with ACTH inhibits the growth response (9). In contrast, selective removal of NH2-terminal POMC peptides decreases compensatory growth (27). Taken together, these data are consistent with the hypothesis that adrenal nerves act via locally produced mitogens like NH2-terminal POMC peptides to mediate compensatory adrenal growth. Because the primary site of cell proliferation during compensatory growth is the outer fasciculata, identifying receptors in this area to locally produced adrenal mitogens will be an important step in defining the extracellular signaling pathways involved.

ACKNOWLEDGMENTS

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GRANTS

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