Changes in the glomerulosa cell phenotype during adrenal regeneration in rats

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Engeland, W. C., and B. K. Levay-Young. Changes in the glomerulosa cell phenotype during adrenal regeneration in rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1374–R1382, 1999.—In situ hybridization was used to examine cellular differentiation during rat adrenal regeneration, defining zona glomerulosa [cytochrome P-450 aldosterone synthase (P-450aldo) mRNA positive], zona fasciculata [cytochromeP-45011β-hydroxylase (P-45011β) mRNA positive], or zona intermedia [negative for both but 3β-hydroxysteroid dehydrogenase (3β-HSD) mRNA positive]. After unilateral adrenal enucleation with contralateral adrenalectomy (ULE/ULA), the expression of all mRNA was reduced at 2 days. From 5 to 10 days, P-45011β and 3β-HSD mRNA increased while P-450aldo remained low; at 20 days, all mRNA were increased. From 2 to 10 days, cells adjacent to the capsule showed intermedia cell differentiation; by 20 days, the subcapsular glomerulosa cells reappeared. This suggests that after enucleation the glomerulosa dedifferentiates to zona intermedia. The experiment was repeated in rats where the postenucleation ACTH rise was prevented. Rats underwent ULE with sham ULA (ULE/SULA) or ULE/SULA with ACTH treatment. Adrenals from ULE/SULA rats expressed increased P-450aldo mRNA at 10 days and reduced P-45011β mRNA and adrenal weight at 30 days. ACTH treatment reversed the pattern toward that seen in ULE/ULA. These findings show that the enucleation-induced dedifferentiation of the glomerulosa cell may result in part from elevated plasma ACTH and that prevention of dedifferentiation may result in impaired regeneration.

adrenal cortex; cytochrome P-450 aldosterone synthase; adrenocorticotropic hormone; adrenal enucleation

IN RESPONSE TO ENucleATION, the adrenal cortex regenerates from capsular and adherent glomerulosa cells to restore the outer zona glomerulosa and an inner zona fasciculata/reticularis (8). The glomerulosa cell has been implicated as the stem cell for regeneration (8), yet the absence of glomerulosa-type mitochondrial cristae (22, 33) and the reduction of aldosterone secretion compared with corticosterone secretion (1) suggest that dedifferentiation of glomerulosa cells may occur immediately after enucleation. The synthesis of aldosterone by glomerulosa cells is dependent on aldosterone synthase, which is the product of the CYP11B2 gene, whereas the synthesis of corticosterone by fasciculata and reticularis cells is dependent on 11β-hydroxylase, which is a product of the CYP11B1 gene (20, 25). The two genes are distinct and have been cloned (16, 35), and specific probes can distinguish cytochrome P-450 aldosterone synthase (P-450aldo) and cytochrome P-450 11β-hydroxylase (P-45011β) transcripts, permitting identification of glomerulosa and fasciculata/reticularis cells, respectively (16, 35).

In a preliminary study, in situ hybridization histochemistry was used to monitor steroidogenic cell phenotype during adrenal regeneration (6). After enucleation, the expression of P-450aldo and of P-45011β mRNA decreased as a consequence of the loss of adrenal tissue and remained low for 1 wk after enucleation. In this earlier study, using an oligonucleotide probe designed to detect both P-450aldo and P-45011β mRNA, an area between the zona glomerulosa and fasciculata was identified in intact adrenals that did not express either P-450aldo or P-45011β mRNA (6). A recent immunohistochemical study (19) has shown that these cells do not express either P-450aldo or P-45011β protein. This area was defined previously as a "sudanophil zone" because of its reduced lipid content (34) or a "zona intermedia" based on its position between the zona glomerulosa and fasciculata (3). Because the zona intermedia may provide progenitor cells for regeneration, in situ hybridization histochemistry was used to monitor changes in intermedia cells after adrenal enucleation over a longer time course.

Optimal regeneration is dependent on establishing a hypocorticotrophic cellular environment. Enucleation-induced regeneration is suppressed in rats administered corticosteroids (31) or in rats in which the paired adrenal remains in situ (13). To extend this study of normal regeneration, the pattern of steroidogenic gene expression was determined during impaired regeneration of enucleated adrenals in the presence of an intact adrenal.

METHODS

Male Sprague-Dawley rats (175–225 g body wt; Sasco Labs) were housed under a 12:12-h light-dark cycle (on 0700–1900 h) with food and water available ad libitum. Animals were allowed to acclimate to the housing facility and light cycle for at least 1 wk before experiments. Under pentobarbital sodium anesthesia (6–7 mg/100 g ip), rats underwent left adrenal enucleation and right unilateral adrenalectomy (ULE/ULA) or right sham ULA (ULE/SULA). Enucleation consisted of slitting the capsule and extruding the inner cortical and medullary tissue. Antibiotic (Ancef, 10 mg/kg) was administered, and animals were kept warm until fully ambulatory, when they were returned to the animal care facility.

Protocols. Two experiments were done to establish the time course of the response to enucleation. At 2, 5, 7, and 10 days (experiment 1) or 10, 20, and 30 days (experiment 2) after ULE/ULA, rats (n = 4/group) were killed by decapitation in

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the morning, and trunk blood and adrenals were collected. Two additional experiments assessed regenerative responses in the presence or absence of an intact adrenal. At 2, 5, and 10 days (experiment 3) or 30 days (experiment 4) after ULE/ULA or ULE/SULA, rats (n = 4/group) were killed, and trunk blood and adrenals were collected. In experiment 3, an additional group of ULE/SULA rats was treated daily with ACTH [1 U repository ACTH in gelatin per rat (Rhone-Poulenc, Collegeville, PA)] and killed at 10 days after enucleation. In experiment 4, an additional group of rats was included that underwent ULA only. Also, at the end of experiment 4, nonstress blood samples were collected by tail clip (10), a maximal dose of ACTH (100 ng rat ACTH; Peninsula Labs) was injected subcutaneously, and trunk blood was collected at 15 min after stimulation. Adrenals were removed, cleaned of fat and connective tissue, immersed in OCT compound, and frozen in cold isopentane.

Hormone analyses. Plasma ACTH was measured by RIA as described previously (14). The intra-assay and the interassay coefficients of variation (CV) on a pool value of 75 pg/ml were 7.6 and 13.3%, respectively. Plasma aldosterone was measured by RIA using a kit (Diagnost Product, Los Angeles, CA); the intra-assay and the interassay CV for aldosterone were 15.9 and 21.8%, respectively. The intra-assay and the interassay CV for cortisol were 7 and 13%, respectively. Plasma corticosterone was measured by RIA using a kit (ICN Biomedical, Costa Mesa, CA) as described previously (14). The intra-assay and the interassay CV for cortisol were 7.6 and 13.3%, respectively. Plasma aldosterone was measured by RIA using a kit (Diagnost Product, Los Angeles, CA); the intra-assay and the interassay CV for aldosterone were 15.9 and 21.8%, respectively.

In situ hybridization. Adrenals were frozen-sectioned (14 µm) and mounted onto ProbeOn slides (Fisher Scientific, Pittsburgh, PA). In situ hybridization histochemistry was done using a method (7) adapted from that of Dagerlind et al. (4). Slides were incubated at 42°C overnight with 5–10 ng of 35S-labeled probe per milliliter of hybridization solution. Sections were washed five times for 15 min each in 1× SSC at 54°C, rinsed, dehydrated, air-dried, and exposed to Biomax film (Kodak). Some sections were dipped in NTB-2 emulsion (Kodak) and exposed for 3–7 days. Dipped slides were developed, counterstained with bisbenzimide (30), and dehydrated. Oligonucleotide probes, purchased from Keystone Labs (Menlo Park, CA) or from Gibco (Grand Island, NY), were designed to detect cytochrome P-450 side-chain cleavage (P-450scc; nt 1368–1403 of rat P-450scc; Ref. 26), 3β-hydroxysteroid dehydrogenase (3β-HSD; nt 1294–1329 of type 1; Ref. 36), (P-450Ω1H; nt 608–643 of mouse CYP21 cDNA; Ref. 24); P-45011β (nt 814–849; Ref. 23), and P-450aldo (nt 918–953; Ref. 17) mRNA specifically. A “generic” P-45011β probe was designed to detect both P-450aldo and P-45011β mRNA (nt 154–189 of the P-450aldo and 50–85 of the P-45011β). For all probes, specificity was confirmed by demonstrating the absence of hybridization when using the corresponding sense probe and/or when excess (100×) unlabeled probe was added to the hybridization solution.

Sections from each group of adrenals and from all treatment groups within an experiment were hybridized in the same assay. Film exposure was chosen to ensure that the density of hybridization was below film saturation. Autoradiographs were scanned using a UMAX scanner calibrated to a density scale (Stouffer) using a Macintosh II computer and the public domain NIH Image program (W. Rasband, NIH) and available from the Internet by anonymous FTP from zippy.nih.gov. Image analysis provided information concerning both the mean density of hybridization (i.e., mean pixel value) and hybridization area (i.e., pixel number). Mean density values were corrected for background hybridization by subtracting mean density values obtained by scanning areas of adrenal sections that did not express steroidogenic enzyme mRNA (i.e., adrenal medulla in intact adrenals and fibrin clot in regenerating adrenals). Hybridization area was measured for each transcript after autothresholding. For each adrenal, the mean density and area of hybridization for three to five randomly chosen tissue sections were measured; means were calculated for each adrenal collected from a group of four rats. To control for differences in steroidogenic enzyme mRNA between adrenals that could result from sampling at different levels of the adrenal cortex, measurement...
ments for intact adrenals were made on sections containing the adrenal medulla. For regenerating adrenals where the adrenal medulla was removed, values averaged for each adrenal represented levels throughout the full extent of the gland. In all cases, adjacent sections were hybridized for P-450scc and P-450aldo mRNA.

Data analyses. The relative levels of P-450aldo, P-45011ß, and 3ß-HSD mRNA were compared between groups using ANOVA; individual means were compared using the Fisher’s least-squares differences test. Plasma hormone concentrations were subjected to logarithmic transformation before ANOVA to reduce variance. For all statistical analyses, P < 0.05 was required for statistical significance.

RESULTS

In intact adrenals, P-450sc mRNA was uniformly expressed throughout the adrenal cortex (Fig. 1A); the pattern of hybridization was similar for 3ß-HSD and P-45021OH mRNA (data not shown). As expected, the hybridization pattern for P-45011ß and P-450aldo mRNA was zone specific in that P-45011ß mRNA was restricted to the inner cortex (Fig. 1C), whereas P-450aldo mRNA was observed underlying the capsule (Fig. 1B). This pattern was observed using either the specific or generic P-45011ß (Fig. 1D). The generic P-45011ß probe, which detects both P-45011ß and P-450aldo transcripts, demarcated the zona intermedia as an area devoid of silver grains between the glomerulosa and fasciculata. Because transcripts representing earlier steps in the steroidogenic pathway (i.e., P-450scc, 3ß-HSD, and P-45021OH) were expressed in the zona intermedia, this area contains cells in which steroidogenesis occurs but is limited by the absence of P-45011ß and P-450aldo.

Steroidogenic enzyme gene expression after adrenal enucleation. Hybridization area and density of P-450aldo, P-45011ß, and 3ß-HSD mRNA were measured in regenerating adrenals collected at 2, 5, 7, and 10 days and at 10, 20, and 30 days after enucleation in two separate experiments. Hybridization area of P-450aldo and P-45011ß mRNA was viewed as an index of the presence of zona glomerulosa and fasciculata/reticularis cells, respectively. Hybridization area of 3ß-HSD mRNA reflected total steroidogenic tissue present in regenerating adrenals, because 3ß-HSD mRNA was expressed in all cortical zones (Fig. 1). As expected, in intact adrenals (time 0) hybridization area of P-450aldo was less than that of P-45011ß and 3ß-HSD (Fig. 2A; note that P-450aldo values are plotted at 5 times). In regenerating adrenals at 2 days postenucleation, hybridization area was reduced for all transcripts. At 5, 7, and 10 days postenucleation, labeling for P-45011ß and 3ß-HSD mRNA increased compared with 2 days but remained less than that of intact adrenals; in contrast, the labeling for P-450aldo mRNA did not increase from 2 to 10 days postenucleation (Fig. 2A). The pattern at 10 days was similar in experiment 2; hybridization area was reduced for all transcripts (Fig. 2B). However, at 20 and 30 days, regenerating adrenals showed labeling for P-45011ß and 3ß-HSD mRNA that was equivalent to that of intact adrenals, whereas the labeling for P-450aldo mRNA remained below that of intact adrenals (Fig. 2B). These data suggested that regeneration of the zona fasciculata/reticularis is complete by 20 days, whereas regeneration of the zona glomerulosa remains incomplete at 30 days. Hybridization density did not change for any transcript after enucleation (data not shown).

To determine the fate of zona intermedia cells after adrenal enucleation, emulsion autorads of adjacent sections hybridized with P-450scc, P-450aldo, 3ß-HSD, and specific P-45011ß probes were compared. Zona intermedia cells were defined as cells negative for P-450aldo and P-45011ß but positive for P-450scc and 3ß-HSD mRNA. In all samples examined, P-450scc (Figs. 3A and 4A) and 3ß-HSD (Figs. 3B and 4B) hybridization was seen extending out to the capsule; days 10 (Fig. 3) and 30 (Fig. 4) are shown as examples. At 2–10 days, cells adjacent to the capsule showed an absence of P-450aldo (Fig. 3B) and P-45011ß mRNA (Fig. 3D). By 20 and 30 days, cells near the capsule expressed P-450aldo mRNA (Fig. 4B), but not P-45011ß mRNA (Fig. 4D), reestablishing the preenucleation...
pattern (compare with Fig. 1). These results suggest that adrenal enucleation results in the loss of the glomerulosa cell phenotype concomitant with the appearance of the intermedia cell phenotype. The glomerulosa cell phenotype is restored in the late stages of regeneration (i.e., by 20 days).

Plasma hormonal responses to enucleation. Plasma ACTH was elevated at 2, 5, and 7 days after ULE/ULA compared with 10 days (experiment 1); plasma ACTH decreased between 10 and 20 days (experiment 2) but did not change between 20 and 30 days after enucleation (Table 1). Although small increases in plasma corticosterone were observed between 2 and 10 days after enucleation (experiment 1), plasma corticosterone did not vary from 10 to 30 days (experiment 2). Plasma aldosterone was at the limit of assay detection in all animals bearing regenerating adrenals (6 pg/ml). Animals bearing only an intact adrenal were not included.
in this experiment, precluding statistical testing. However, in parallel studies in which blood was collected from rats \((n = 4)\) under nonstress conditions at 14 days after ULA, plasma ACTH, corticosterone, and aldosterone values were \(34 \pm 5\) pg/ml, \(18 \pm 9\) ng/ml, and \(17 \pm 11\) pg/ml, respectively.

Steroidogenic enzyme gene expression after adrenal enucleation in the presence of an intact adrenal. Analysis similar to experiment 1 was repeated for P-45011\(\beta\) and P-450aldo mRNA in 2-, 5-, and 10-day enucleated adrenals regenerating in the absence (ULE/ULA) or in the presence (ULE/SULA) of an intact adrenal. There was no difference between enucleated adrenals from ULE/ULA and ULE/SULA at days 2 or 5, and the results were similar to experiment 1 for both time points (data not shown). However, enucleated adrenals in the presence of an intact adrenal (ULE/SULA) showed reduced P-45011\(\beta\) mRNA at 10 days compared with ULE/ULA (Fig. 5A). P-450aldo mRNA, on the other hand, was elevated at 10 days in ULE/SULA adrenals compared with ULE/ULA adrenals, and there was no difference between ULE/SULA and intact adrenals at 10 days (Fig. 5B). Treatment of ULE/SULA rats with ACTH did not significantly affect P-45011\(\beta\) mRNA (Fig. 5A) but reversed the elevated P-450aldo mRNA observed at 10 days (Fig. 5B). The data suggest that the presence of an intact adrenal impairs regeneration as reflected by reduced P-45011\(\beta\) mRNA and that reduced regeneration is associated with increased P-450aldo mRNA. In addition, treatment of ULE/SULA rats with ACTH decreases P-450aldo mRNA, suggesting that elevated plasma ACTH observed in ULE/ULA rats contributes to decreased expression of P-450aldo.

To confirm that the presence of an intact adrenal suppresses adrenal regeneration as reflected by adrenal mass and to extend the comparison to normal regeneration, treatment groups were compared at 30 days after enucleation. Adrenal weight and hybridization area for P-450sc and P-45011\(\beta\) were decreased in the regenerating adrenal from ULE/SULA rats compared with ULE/ULA or intact adrenals (Fig. 6, A–C). As in experiment 2 at day 30 (Fig. 2B), ULE/ULA adrenals were similar to intact adrenals in all of these parameters, that is, fully recovered. In contrast, the expression of P-450aldo mRNA was the same in the regenerating adrenals from ULE/ULA and ULE/SULA rats. As in experiment 2 at day 30 (Fig. 2B), P-450aldo was reduced in the enucleated adrenals compared with intact adrenals (Fig. 6D). These results show that the presence of an intact adrenal suppresses adrenal regeneration as reflected by adrenal weight and by expression of P-450sc and P-45011\(\beta\) mRNA. The presence of an intact adrenal does not impair recovery of P-450aldo mRNA. However, P-450aldo mRNA was not elevated at 30 days of impaired regeneration unlike 10 days after enucleation (experiment 3), suggesting that P-450aldo mRNA may reach a plateau as regeneration is suppressed.

Plasma hormonal response to enucleation in the presence of an intact adrenal. Plasma ACTH was elevated at 2 and 5 days after ULE/ULA or ULE/SULA compared with the respective 10-day value (Table 2). However, plasma ACTH was decreased in the ULE/SULA rats compared with the ULE/ULA rats at 2 and 5 days, but not at 10 days after enucleation. Plasma corticosterone also decreased in both treatment groups between 2 and 10 days. Differences in plasma corticosterone between ULE/ULA and ULE/SULA rats were observed only on day 2. Because day 2 samples were...
collected in the afternoon, circadian changes might have contributed to elevated plasma corticosterone in the ULE/SULA rats. Plasma ACTH and corticosterone values in ULE/SULA rats treated for 10 days with ACTH were 122 ± 11 pg/ml and 13 ± 6 ng/ml, respectively, at 10 days after enucleation. Plasma aldosterone was not assayed in this experiment, since the presence of an intact adrenal precluded use of plasma aldosterone as a meaningful reflection of P-450aldo expression in the regenerating adrenal.

To assess the long-term effect of the presence of an intact adrenal on enucleation-induced responses, both nonstress plasma corticosteroids and adrenal responses to a maximal dose of ACTH were measured at 30 days after enucleation. There was no difference in nonstress plasma ACTH or corticosterone between ULA rats (i.e., a single intact adrenal), ULE/ULA rats, or ULE/SULA rats (Table 3). As expected, plasma ACTH, corticosterone, and aldosterone values were increased at 15 min after injection. In response to ACTH injection, plasma corticosterone and aldosterone, but not plasma ACTH, were decreased in ULE/ULA rats compared with ULA and with ULE/SULA rats (Table 3).

**DISCUSSION**

The capacity of the adrenal cortex to restore its zonation (8) and secretory function (32) after enucleation has been thoroughly documented. Regeneration requires both proliferation to replace tissue mass and phenotypic differentiation to reestablish zonation (33). To characterize this process, oligonucleotide probes that specifically identify glomerulosa and fasciculata/reticularis cells were used with quantitative densitometry and emulsion autoradiography to delineate the restoration of adrenal zonation after enucleation.

Enucleation removes the adrenal medulla and the inner adrenal cortex, but not the glomerulosa and intermedia (data not shown). Surprisingly, the glomerulosa cell phenotype, as defined by the presence of P-450aldo mRNA, is downregulated after enucleation, and the glomerulosa cells assume an intermedia cell phenotype; the loss of P-450aldo explains reduced secretion of aldosterone in regenerating adrenals as reported by others (1) and confirmed in the present study. Restoration of the fasciculata precedes reestablishment of the glomerulosa; indeed, full recovery of P-450aldo mRNA had not occurred by 30 days of regeneration, whereas P-45011β mRNA had been restored by 20 days. In addition, when P-450aldo mRNA was increased in enucleated adrenals by the presence of an intact adrenal, recovery of P-450scc mRNA, P-45011β mRNA, and adrenal weight were suppressed. The suppression could be partially reversed by ACTH treatment, suggesting that elevated plasma ACTH resulting from the loss of steroid negative feedback

![Fig. 6. Comparison of adrenal P-45011β and P-450aldo mRNA expression at 30 days after adrenal enucleation in rats bearing a single intact adrenal (ULA; solid bar), a single regenerating adrenal (ULA/ULE; open bar), or one regenerating and one intact adrenal (ULE/SULA). Paired bars denoted as ULE-SULA represent values for paired regenerating (ULE; hatched bar) and intact (SULA; stippled bar) adrenals from ULE/SULA rats. Data represent means ± SE of 4 adrenals. Values with different superscripts are significantly different (P < 0.05) from other treatment groups. A: adrenal weight; B: P-450scc mRNA; C: P-45011β mRNA; D: P-450aldo mRNA.]

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<td>ULE/ULA</td>
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<td>ACTH, pg/ml</td>
<td>298 ± 50</td>
<td>136 ± 14*</td>
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<tr>
<td>Corticosterone, ng/ml</td>
<td>28 ± 6</td>
<td>176 ± 47*</td>
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Values are means ± SE of 4 rats per group. Plasma was collected in the afternoon on day 2 and in the morning on days 5 and 10. *P < 0.05 vs. ULE/ULA for a given time point. †P < 0.05 vs days 2 and 5 for a given treatment.
ADRENAL REGENERATION

Table 3. Comparison of plasma ACTH and corticosteroids before and after adrenal stimulation in rats bearing a single intact adrenal (ULA), a single regenerating adrenal (ULA/ULE), or one regenerating and one intact adrenal (ULE/SULA) for 30 days

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<td>Pre-ACTH</td>
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<td>Pre-ACTH</td>
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<tr>
<td>ACTH, pg/ml</td>
<td>89 ± 8</td>
<td>520 ± 102*</td>
<td>101 ± 8</td>
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<tr>
<td>Corticosterone, ng/ml</td>
<td>4 ± 1</td>
<td>364 ± 35**</td>
<td>11 ± 4</td>
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<tr>
<td>Aldosterone, pg/ml</td>
<td>NA</td>
<td>575 ± 74†</td>
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Values are means ± SE of 4 rats per group. Plasma was collected at 30 days after surgery in the morning by tail cut (pre-ACTH) and at 15 min after injection of a maximally stimulating dose of ACTH (100 ng/rat sc). NA, not assayed. *P < 0.05 vs. pre-ACTH for a given treatment. †P < 0.05 vs. ULE/ULA (post-ACTH).

Contributes to the suppression of the glomerulosa phenotype and the restoration of the fasciculata phenotype. These data support the contention that the reduction or loss of the glomerulosa cell phenotype contributes to enucleation-induced regeneration of the adrenal cortex.

Both systemic and local factors could contribute to the downregulation of P-450αldo expression after enucleation of the adrenal. As observed in the present study and shown by others (2, 12), enucleation results in increases in plasma ACTH. The presence of an intact adrenal has been shown previously to impair enucleation-induced regeneration (2, 12), presumably by secreting corticosterone that acts to suppress plasma ACTH. Because the loss of the glomerulosa cell phenotype occurs concomitantly with elevated plasma ACTH, steroidogenic enzyme gene expression was monitored in regenerating adrenals from rats in which steroid secretion was maintained by a contralateral intact adrenal. Results showed that regenerating adrenals collected from rats with a paired intact adrenal expressed increased P-450αldo mRNA and reduced P-450scc mRNA at 10 days. Because plasma ACTH was reduced in the presence of an intact adrenal, it is probable that the downregulation of P-450αldo resulted from elevated plasma ACTH. This possibility was tested by injecting ACTH to offset the inhibitory effects of corticosterone produced by the intact adrenal; results showed that ACTH treatment suppressed P-450αldo mRNA expression. These findings show that the enucleation-induced dedifferentiation of the glomerulosa cell may result in part from elevated plasma ACTH. These data are consistent with earlier work showing that stimulation of intact adrenals with ACTH decreases P-450αldo expression (11, 29). In addition to ACTH, local adrenal responses, including hemorrhage, edema, and infiltration by inflammatory cells (8), could affect P-450αldo mRNA expression. The vascular changes could deprive the remaining glomerulosa cells of nutrients required for survival, resulting in cell death; although cell death most likely occurs after enucleation, the expression of P-450scc and 3β-HSD mRNA in cells underlying the capsule supports their viability. However, changes in blood flow could produce local hypoxia. Because systemic hypoxia decreases P-450αldo expression (27), reduction in tissue oxygenation could contribute to enucleation-induced changes in adrenal cell phenotype. In addition, local inflammation may play a role by providing cytokines, like tumor necrosis factor and interleukin-1, that have been shown to inhibit aldosterone responses to ACTH and ANG II in vitro (21).

The physiological relevance of the dedifferentiation of glomerulosa cells to intermedia cells to the regenerative response is unknown. It is intriguing that enucleation results in the expansion of the zona intermedia, because others have suggested that intermedia cells represent stem cells for regeneration (18). However, in our initial studies of adrenal regeneration, proliferating cells as defined by expression of the Ki67 antigen appeared to be excluded from the zona intermedia (5). Although a more rigorous examination is required to establish the phenotype of proliferating cells throughout regeneration, it is clear that during the initial stages of regeneration, proliferation is not restricted to the zona intermedia. To establish the possible significance for the reduction of P-450αldo in the early stages of regeneration, it will be necessary to prevent the reduction and assess the effect on regeneration.

It is also possible that the reduction of P-450αldo per se is not a required element for regeneration, but instead is an epiphenomenon of decreased glomerulosa cell stimulation by ANG II, perhaps via downregulation of ANG II (AT) receptors. Neither changes in adrenal AT receptors nor responses of the renin-angiotensin system after enucleation have been reported to date. Adrenalectomy results in elevated ANG II (28), and in preliminary studies, ULA/ULE results in increases in plasma renin activity during the initial 3 days after enucleation (C. Wotus, A. I. Fraticelli, and W. C. Engeland, unpublished observations). Thus it is unlikely that circulating angiotensin contributes to the reduction of P-450αldo expression in regenerating adrenals, because ANG II upregulates the AT receptor and is a positive regulator of P-450αldo expression (11, 15, 35). More likely, because P-450αldo expression decreases after enucleation, factors like ACTH are required to offset the positive effect of AT receptor activation on P-450αldo expression.

Perspectives

The adrenal cortex demonstrates the uncommon capacity to regenerate after injury produced by transplantation or enucleation. This phenomenon was described early in this century (see Ref. 13) and subse-
quently has been characterized as a regenerative response that requires both cell proliferation and differentiation (22, 33). The novelty of the present study stems from the ability to define the phenotype of the cortical cell during restoration of zonation. The results implicate modulation of glomerulosa cell differentiation as a critical component of adrenal cortical regeneration. However, there are a host of unresolved issues concerning the regenerative process, including the nature of intermedia cell differentiation, the identity of proliferating cells in the regenerating cortex, the nature of the factors that regulate the loss of P-450aldo expression, and the mechanism by which glomerulosa cell differentiation is reestablished. Expression of factors linked to differentiation of adrenal glomerulosa/intermedia cells, such as Prep-1 (9), may play a role in these processes. It will be of interest to determine the cellular mechanisms by which systemic factors like ACTH and ANG II and local inflammatory factors regulate phenotypic expression and regeneration in the adrenal cortex.

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