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DIFFERENTIAL EFFECTS OF ADRENOCORTICOTROPIC HORMONE ON STEROID HYDROXYLASE ACTIVITIES IN THE INNER AND OUTER ZONES OF THE GUINEA PIG ADRENAL CORTEX

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Summary—We have studied the effects of ACTH treatment on steroid hydroxylase activities in the inner (zona reticularis) and outer (zona fasciculata plus zona glomerulosa) zones of the guinea pig adrenal cortex. Animals received 5 or 10 U of ACTH daily for 6 days and enzyme activities were then assessed in isolated microsomal or mitochondrial preparations. In control animals, microsomal cytochrome *P*-450 concentrations were greater in the inner than outer zone, but mitochondrial *P*-450 levels were similar in the two zones. Microsomal 17α-hydroxylase and mitochondrial 11β-hydroxylase activities were greater in the outer than inner zone, but microsomal 21-hydroxylase activity was greater in the inner zone. ACTH treatment decreased cytochrome *P*-450 concentrations in inner but not outer zone microsomes; mitochondrial *P*-450 levels were unaffected in both zones. ACTH caused a dose-dependent increase in inner zone 17α-hydroxylase activity and decrease in 21-hydroxylase activity without affecting the activity of either enzyme in outer zone microsomes. ACTH also decreased 11β-hydroxylase activity in outer but not inner zone mitochondrial preparations. The net effect of ACTH treatment was to diminish the differences in steroid metabolism between the two zones. The results indicate that the effects of ACTH on steroid hydroxylase activities are both zone- and enzyme-dependent, suggesting the existence of multiple and independent regulatory mechanisms.

INTRODUCTION

There are three distinct anatomical zones which comprise the mammalian adrenal cortex, the outermost zona glomerulosa (ZG), intermediate zona fasciculata (ZF), and innermost zona reticularis (ZR) [see 1, 2]. Different functional roles in steroidogenesis have been proposed for each of these zones. It is well established that the ZG is solely responsible for aldosterone production and, therefore, involved in mineralocorticoid function [1, 2]. However, the relative importance of the ZF and ZR with respect to glucocorticoid and androgen production has yet to be fully resolved. In addition, it is not clear if the ZF and ZR share common regulatory mechanisms or are controlled independently of one another [1–4].

Among the problems associated with attempts to establish the functional differences between the ZF and ZR has been the difficulty of separating the two zones in adrenals from laboratory animals. However, the ZR of the guinea pig adrenal cortex is chromatically distinct from the rest of the gland, allowing it to be dissected apart from the ZF and ZG [5–7]. Studies with these inner (ZR) and outer (ZF plus ZG) zone preparations indicate that the guinea pig ZF produces far more cortisol than the ZR, and only ZF cells respond to ACTH with a substantial increase in cortisol secretion [6–9]. Limited investigations with human adrenal tissue have resulted in similar conclusions [1, 2, 10].

Several laboratories have been pursuing the mechanism(s) responsible for the differences in cortisol secretion by the inner and outer zones of the guinea pig adrenal cortex. Studies have focused on various regulatory aspects of adrenal steroidogenesis including cellular cAMP production, lipoprotein binding, cholesterol synthesis, and steroidogenic enzyme activities [11–18]. Zonal differences in the activities of several steroid hydroxylases have been reported [11, 12], with the net effect of the differences favoring cortisol synthesis to a far

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greater extent in outer than inner zone cells. The mechanisms responsible for these enzymatic differences between the two zones have not been resolved and little is known about the regulation of enzyme activities in each zone [19–21]. The studies described in this report were done to pursue the latter by comparing the effects of ACTH treatment on steroidogenic enzyme activities in the inner and outer zones of the guinea pig adrenal cortex.

EXPERIMENTAL

Male English Short-Hair guinea pigs weighing approx. 800–1000 g were obtained from Camm Research Institute (Wayne, NJ). Animals were maintained under standardized conditions of light (6 am–6 pm) and temperature (22°C) and received food and water *ad libitum*. Guinea pigs were allowed at least 7 days to become acclimated to the housing conditions prior to initiation of experiments. ACTH (Cortrophin-Zinc, Organon, West Orange, NJ) was administered by s.c. injection at doses of 5 or 10 USP U/kg body wt daily for 7 days. Controls received injections of saline only.

Animals were killed by decapitation between 8 and 9 am on the day after the last administration of ACTH. Adrenal glands were quickly removed and placed in cold 0.25 M sucrose containing 0.05 M Tris–HCl (pH 7.4) on ice. After being trimmed free of fat and connective tissue and weighed, adrenals were bisected longitudinally and the dark-brown inner zone, consisting primarily of ZR, was dissected away from the tan outer zone, consisting of the ZF and ZG, as previously described by Martin and Black [5]. Tissues were then homogenized in sucrose–Tris buffer, and washed mitochondrial and microsomal fractions were obtained by differential centrifugation as described previously [11, 12].

Mitochondrial 11β-hydroxylase activity was assayed as the rate of conversion of 11-deoxycortisol to cortisol as described previously [12]. Cortisol was measured fluorometrically [22]. Microsomal 21-hydroxylase activity was determined as the rate of conversion of 17α-hydroxyprogesterone to 11-deoxycortisol, and 17α-hydroxylase activity as the rate of conversion of progesterone to 17α-hydroxyprogesterone plus 11-deoxycortisol. Incubation conditions and HPLC analyses of metabolites were previously described in detail [11].

Microsomal benzo[*a*]pyrene hydroxylation was determined by the fluorometric method of Nebert and Gelboin [23]. Quinine sulfate was calibrated against authentic 3-OH-benzo-[*a*]pyrene and routinely used as the fluorescence standard. For all enzyme assays, conditions were established to ensure linearity of product formation with respect to protein concentrations and incubation times. Cytochrome *P*-450 was measured as the dithionite-reduced CO complex as described by Omura and Sato [24]. Microsomal and mitochondrial protein concentrations were determined by the method of Lowry *et al.* [25]. All data are expressed as means ± SE and were analyzed by the Newman–Keuls multiple range test for significant differences between group means.

RESULTS

Adrenal gland weights were increased by ACTH treatment, the higher dose causing an approx. 70% increase in adrenal mass (Table 1). ACTH treatment had no effect on adrenal mitochondrial protein or cytochrome *P*-450 levels in the inner or outer zone (Table 1). However, as

Table 1. Effects of ACTH treatment on cytochrome *P*-450 and protein concentrations in adrenal inner and outer zone mitochondrial and microsomal preparations[a]

	Control	ACTH (5 U/day)	ACTH (10 U/day)
Adrenal weight (mg)	357 ± 39	489 ± 51	608 ± 56[c]
Protein (mg/g adrenal)			
Inner microsomes	56.0 ± 3.7	45.9 ± 2.8[c]	41.4 ± 2.2[c]
Outer microsomes	31.2 ± 4.0[b]	33.6 ± 2.9[b]	31.8 ± 3.7
Inner mitochondria	31.3 ± 2.1	29.1 ± 2.6	28.5 ± 2.6
Outer mitochondria	32.4 ± 3.6	31.8 ± 3.6	29.7 ± 2.5
Cytochrome *P*-450 (nmol/mg prot)			
Inner microsomes	1.92 ± 0.21	1.49 ± 0.20[c]	1.33 ± 0.18[c]
Outer microsomes	1.16 ± 0.14[b]	1.21 ± 1.19	1.10 ± 0.13
Inner mitochondria	0.58 ± 0.17	0.63 ± 0.06	0.55 ± 0.06
Outer mitochondria	0.67 ± 0.08	0.62 ± 0.08	0.71 ± 0.07

[a]Values are expressed as means ± SE of 5–7 animals per group.
[b]$P < 0.05$ (vs corresponding inner zone value).
[c]$P < 0.05$ (vs corresponding control value).

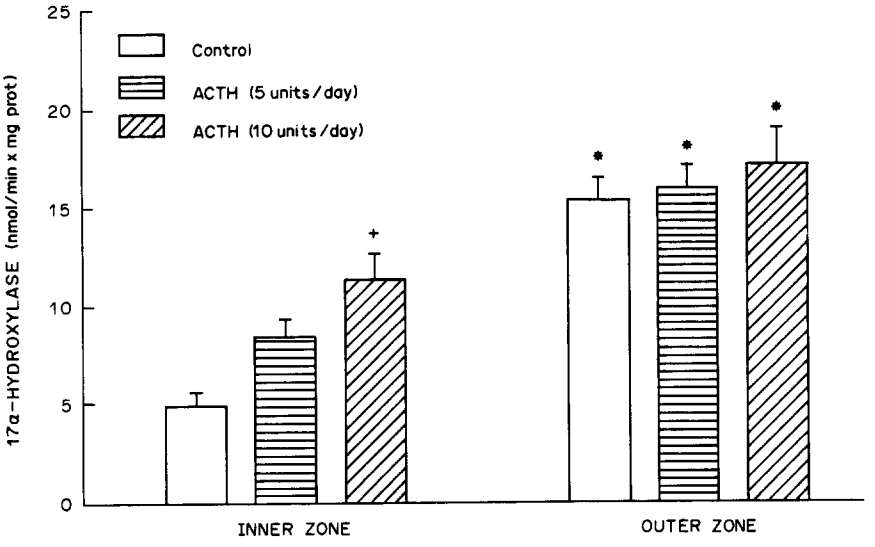

Fig. 1. Effects of ACTH treatment on microsomal 17 α-hydroxylase activities in the inner and outer zones of the guinea pig adrenal cortex. Animals were treated and enzyme activity determined as described in the Experimental section. Values are means ± SE of 5–7 animals. $^{*}P < 0.05$ (vs corresponding inner zone value); $^{+}P < 0.05$ (vs corresponding control value).

reported previously [19], ACTH decreased microsomal protein concentrations in the inner but not the outer adrenal zone. There were also dose-dependent declines in inner but not outer zone microsomal *P*-450 concentrations resulting from ACTH treatment. Martin and Black [19] noted a smaller ACTH-induced decline in inner zone microsomal *P*-450 than reported here which is probably attributable to the shorter duration of ACTH treatment in their studies.

We have previously reported that in untreated guinea pigs, steroid 17α-hydroxylase activity is far greater in outer than inner zone microsomes, but for 21-hydroxylase activity the opposite pertains [11]. Similar results were obtained in the present studies (Figs 1 and 2). However, treatment with ACTH tended to diminish the zonal differences in the activities of both enzymes. ACTH caused a dose-dependent increase in inner zone 17α-hydroxylase activity, but had little effect on activity in the outer zone (Fig. 1). By contrast, ACTH treatment decreased 21-hydroxylase activity in inner zone microsomes to a far greater extent than in the outer zone. As

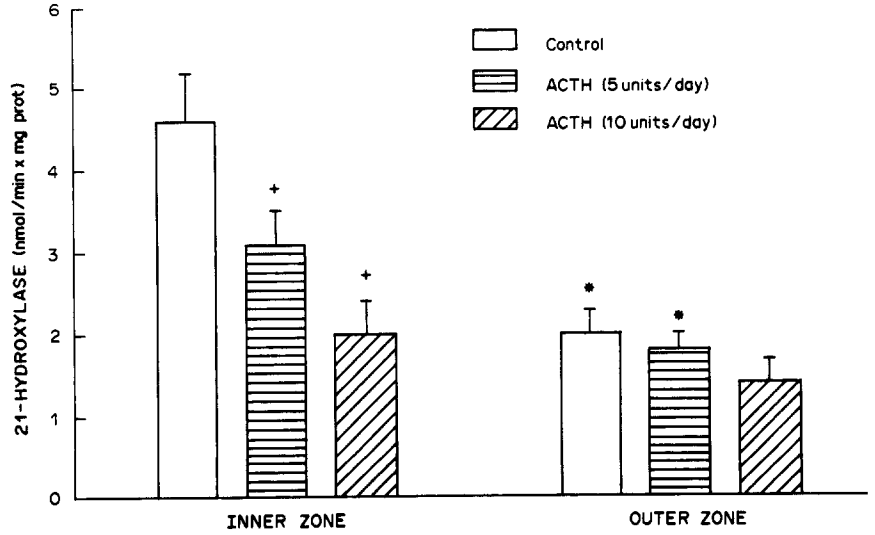

Fig. 2. Effects of ACTH treatment on microsomal 21-hydroxylase activities in the inner and outer zones of the guinea pig adrenal cortex. Animals were treated and enzyme activity determined as described in the Experimental section. Values are means ± SE of 5–7 animals. $^{*}P < 0.05$ (vs corresponding inner zone value); $^{+}P < 0.05$ (vs corresponding control value).

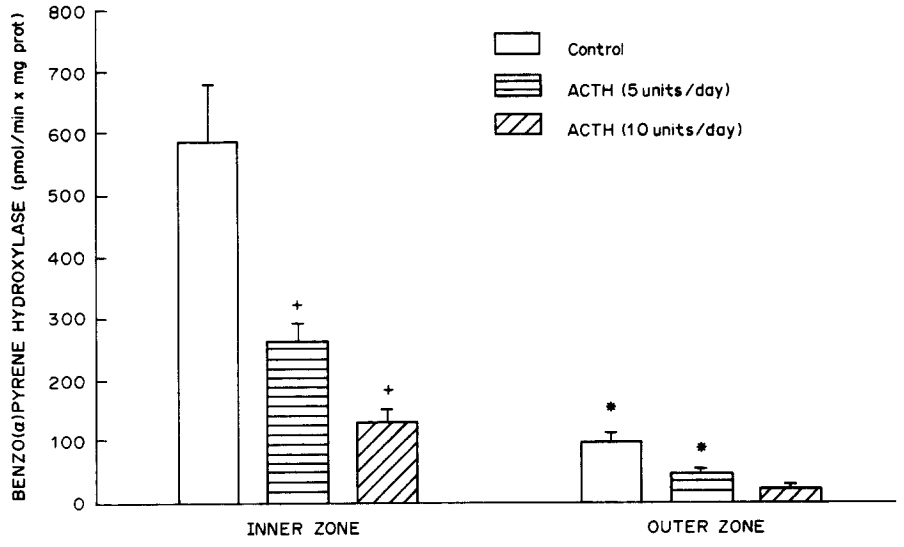

Fig. 3. Effects of ACTH treatment on microsomal benzo[*a*]pyrene hydroxylase activities in the inner and outer zones of the guinea pig adrenal cortex. Animals were treated and enzyme activity determined as described in the Experimental section. Values are means ± SE of 5–7 animals. $^{*}P < 0.05$ (vs corresponding inner zone value); $^{+}P < 0.05$ (vs corresponding control value).

a result, at the higher dose of ACTH, 21-hydroxylase activities in the two zones were not significantly different.

Although outer zone 17α- and 21-hydroxylase activities were relatively unresponsive to ACTH treatment, ACTH did affect other cytochrome *P*-450-catalyzed reactions in the outer zone. For example, ACTH inhibited benzo[*a*]pyrene hydroxylase activity in outer zone microsomes. It is known that cytochrome *P*-450-catalyzed xenobiotic metabolism is far greater in inner than outer zone microsomal preparations [8, 19, 20]. The latter is illustrated by the high ratio of inner to outer microsomal benzo[*a*]pyrene hydroxylase activity in the guinea pig adrenal (Fig. 3). However, ACTH treatment caused proportionately similar decreases in enzyme activities in both zones, resulting in approx. 80% decreases at the higher dose of ACTH.

ACTH treatment also decreased mitochondrial 11β-hydroxylase activity in the outer cortical zone (Fig. 4). As noted previously, outer zone activity is greater than that in the inner

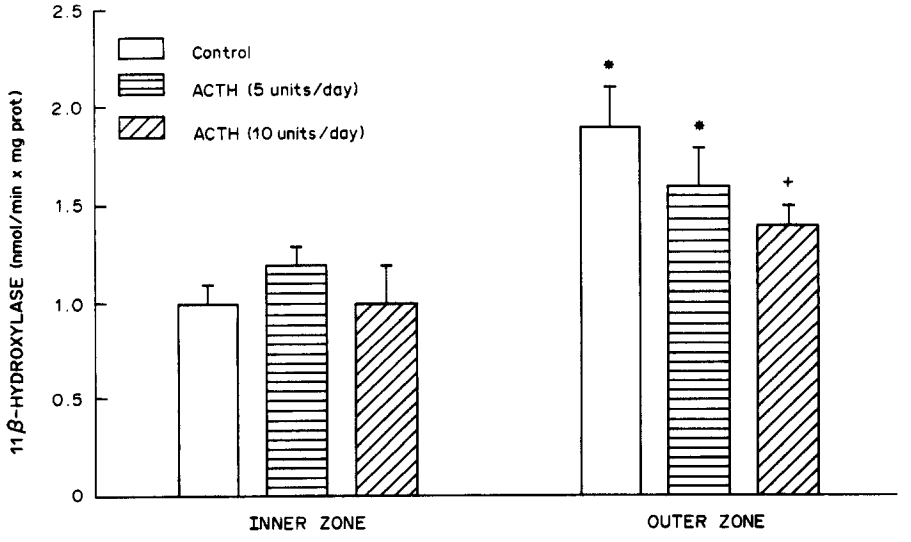

Fig. 4. Effects of ACTH treatment on mitochondrial 11 β-hydroxylase activities in the inner and outer zones of the guinea pig adrenal cortex. Animals were treated and enzyme activity determined as described in the Experimental section. Values are means ± SE of 5–7 animals. $^{*}P < 0.05$ (vs corresponding inner zone value); $^{+}P < 0.05$ (vs corresponding control value).

zone in adrenal glands from untreated animals [12]. However, ACTH caused a decline in activity in the outer zone (Fig. 4), eliminating the zonal differences in 11β-hydroxylase activity at the higher dose.

DISCUSSION

The results presented in this communication indicate that ACTH has both zone- and isozyme-selective effects on *P*-450-catalyzed reactions in the guinea pig adrenal cortex. ACTH increased 17α-hydroxylase and decreased 21-hydroxylase activity in the inner zone but did not significantly affect the activity of either enzyme in the outer zone. By contrast, outer zone 11β-hydroxylase activity was decreased as a result of ACTH treatment but activity in the inner zone was unaffected. Shinzawa *et al.* [21] have also reported that ACTH selectively decreases 21-hydroxylase activity in the inner zone of the guinea pig adrenal cortex, but Martin and Black [19] did not find a significant decline in enzyme activity in either zone. The reason for the apparently conflicting results is probably related to differences in the duration of treatment and/or dose of ACTH employed. Zonal differences in ACTH actions on 17α- or 11β-hydroxylase activities have not been reported previously.

The overall effect of ACTH treatment on *P*-450-mediated steroid metabolism in the guinea pig adrenal cortex was to diminish the enzymatic differences between the inner and outer zones. For all of the reactions studied, ACTH treatment made the two zones functionally more similar to one another. Martin and Black [19] previously noted that ACTH treatment altered the macroscopic distinction between the inner and outer zones of the guinea pig adrenal cortex, an observation which we have also made on various occasions. Thus, the effects of ACTH on enzyme activities in the two zones provide a functional correlate to earlier morphologic findings. These actions of ACTH may be mechanistically related to its effects on cultured ZG cells, resulting in their acquisition of ZF-specific functions [see 2].

The mechanisms responsible for the zonal differences in enzyme activities in the guinea pig adrenal cortex as well as for the zone-specific responses to ACTH have yet to be resolved. Studies in recent years by Waterman, Simpson, and others [26, 27] have established that the chronic effects of ACTH on adrenocortical function are cAMP-dependent and include the synthesis and maintenance of steroidogenic enzymes. Particular emphasis has been placed on the regulation of adrenal cytochromes *P*-450 because of their prominent roles in steroidogenesis. It has been demonstrated that ACTH increases the rates of synthesis of adrenal *P*-450_{scc} (cholesterol sidechain cleavage), *P*-$450_{11\beta}$ (11β-hydroxylase), *P*-450_{C21} (21-hydroxylase) and *P*-$450_{17\alpha}$ (17α-hydroxylase) in cultured adrenocortical cells via cAMP-mediated enhancement of gene expression for these enzymes [26, 27]. However, there is not always a corresponding increase in enzyme activity [28]. In addition, virtually all of these studies have been done with mixed populations of ZF and ZR cells, precluding the possibility of defining actions of ACTH that are specific to each zone.

The tissue-specific expression of cytochromes *P*-450 seems to play a major role in establishing the enzymatic differences responsible for uniqueness of steroid secretory profiles in different endocrine organs. However, there is evidence that post-transcriptional modulation of steroid hydroxylases contributes to zonal differences in enzyme activities in the guinea pig adrenal [21, 29, 30]. For example, despite large differences in cholesterol sidechain cleavage, 17α-hydroxylase, and 21-hydroxylase activities between ZF and ZR cells in the guinea pig adrenal cortex [11, 12], enzyme apoprotein concentrations are similar in the two zones [21, 29, 30]. These observations implicate regulatory mechanisms beyond apoprotein synthesis. Results from one laboratory indicate that there is a surplus of *P*-450 apoproteins relative to holoenzymes in guinea pig adrenal microsomes [21]. Thus, differences in the assembly or turnover of *P*-450 isozymes may contribute to zonal differences in steroid hydroxylase activities. Further studies are needed to pursue these and other possibilities.

In contrast to the regulation of adrenal steroid hydroxylases, differences in *P*-450 apoprotein levels do seem to account for the zonal differences in adrenal xenobiotic metabolism. Findings in several laboratories, including our own, indicate that the ZR is the major site of xenobiotic metabolism within the guinea pig adrenal cortex [8, 19, 20]. Studies by Black *et al.* [20, 29] demonstrated the presence of a *P*-450 isozyme in ZR microsomes which is highly correlated with the metabolism of foreign compounds and is immunochemically related to *P*-450IA1 and/or IA2. Although it has not been

unequivocally established that this isozyme catalyzes adrenal xenobiotic metabolism, suppression of xenobiotic metabolism by ACTH treatment is associated with a decline in the concentration of this apoprotein [20, 29]. It is not known if the decrease in quantity of apoprotein is the result of a decline in synthesis or increase in turnover.

Studies are now needed to pursue the mechanism(s) involved in the differential effects of ACTH on steroid hydroxylase activities in the inner and outer zones of the adrenal cortex. It has been demonstrated that ACTH stimulates cAMP production to a similar extent in the two zones [14], suggesting that post-cAMP phenomena are involved. In fact, as noted above, some of the actions of ACTH may involve post-translational mechanisms. The guinea pig adrenal cortex should serve as an excellent model for studies on the regulation of steroid hydroxylase activities in the ZR and ZF since the zones can be readily separated and are sufficiently large to provide ample amounts of tissue for investigation. The information derived from such studies may also contribute to a fuller understanding of the mechanisms involved in the establishment and/or maintenance of the functional zonation in the adrenal cortex.

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