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Adrenocortical Function in Deoxycorticosterone Acetate (DOCA)-Hypertensive Yucatan Miniature Swine[1] (42047)

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Abstract. Adrenocortical function was assessed in six normal and six chronic (>12 weeks), DOCA-hypertensive Yucatan miniature swine; mean arterial pressures were 115.3 ± 11.7 and 163.6 ± 27.2 mm Hg, respectively (mean ± SEM). Adrenocortical function was evaluated *in vivo* by measuring changes in plasma cortisol and aldosterone in response to exogenous ACTH (0.25 mg, iv), and *in vitro* by measuring the responses of collagenase-isolated adrenocortical cells to ACTH and angiotensin II. Corticoids were measured by specific radioimmunoassay. Basal plasma cortisol values of conscious DOCA-hypertensive swine were approximately 53% of the values of normotensive swine ($P < 0.05$). However, ACTH induced a 419% increase in plasma cortisol values in DOCA-hypertensive swine compared to a 261% increase in the normotensive swine ($P < 0.05$). These differences between the two groups were not altered by anesthesia. There were no significant differences in ACTH-induced changes in plasma aldosterone between the normotensive and DOCA-hypertensive swine. Experiments *in vitro* showed that the corticoid secretory responses of adrenocortical cells from DOCA-hypertensive animals were 6 times more sensitive to ACTH and 3.2 times more sensitive to angiotensin II than those of cells from normotensive swine. Thus, despite the possibility of adrenocortical insufficiency due to suppressed plasma renin activity and the negative feedback of DOCA on the hypothalamic–hypophyseal–adrenal axis, adrenocortical function of DOCA-hypertensive swine was hyperresponsive to trophic hormones. Results from this study suggest that the DOCA-hypertensive swine may be a valuable model in elucidating the relationship between hypertension and adrenocortical function and in investigating nonclassical control of the adrenal cortex, that is, control exerted during the hypertensive state that exists apart from or in addition to that exerted by ACTH and angiotensin II.

The adrenal cortex plays a central role in some forms of hypertension (1, 2). For example, stress (3) and stress-induced cortisol production (4) have been hypothesized as some of the many factors that may influence the development of some forms of hypertension in humans. In addition, prior to hypertension, spontaneously hypertensive rats (SHR) demonstrate hypersecretion of corticoids in response to noxious stimuli (5), altered adrenal sensitivity to ACTH, angiotensin II, and potassium (6, 7), and secrete abnormal ratios of hypertensinogenic corticoids (8, 9). In addition, some work suggests that hypophysectomy or adrenalectomy prevents the development of hypertension in SHR (10). Inhibitors of corticosteroidogenesis also have been shown to be efficacious in the treatment of some forms of human hypertension (11). Finally, administration of corticoids to intact or adrenalectomized rats has been shown to produce hypertension (12–14).

Although the SHR model has been valuable in investigating adrenocortical alterations during hypertension, it is a genetically programmed hypertension. Thus, a relationship between hypertension and altered adrenocortical function might be obscured by an intrinsic genetic defect in the adrenal cortex. However, in experimentally induced hypertension, changes in presumably normal adrenocortical function can be studied. Such information would be of value in understanding changes in adrenocortical function that might occur in forms of hypertension

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not stemming directly from the adrenal cortex itself. These changes, in turn, might exacerbate the hypertensive state.

In the work reported here, we investigated the effects of hypertension on the adrenal cortex using Yucatan miniature swine made hypertensive with deoxycorticosterone acetate (DOCA). Adrenocortical function was assessed after hypertension was fully established. We evaluated adrenocortical function *in vivo* by measuring plasma corticoids in response to ACTH and *in vitro* by measuring the response of isolated adrenocortical cells to ACTH, angiotensin II, and 8-bromo-cyclic AMP (8Br-cAMP).

Materials and Methods. *Animals.* Twelve male Yucatan miniature swine (Buckshire Farms, Perkasie, Pa.) (35–45 kg) were used for this study. Animals were maintained on a standard swine ration and permitted water *ad libitum.* Six animals were implanted with DOCA-impregnated silicone strips (100 mg/kg) as previously described (15). The remaining six animals served as controls. All studies were initiated after no less than 12 weeks of DOCA treatment.

Studies, in vivo. Experiments *in vivo* were conducted on conscious and anesthetized animals. Under sterile conditions and halothane anesthesia all animals were implanted with chronic indwelling arterial and venous catheters. The catheters were exteriorized at the back of the neck. Each animal was tested in the conscious and anesthetized state. Initially, conscious swine were studied while unrestrained in small pens. Several days later, the same animal was anesthetized with sodium pentobarbital (25 mg/kg) and the study was repeated. Swine were administered an intravenous clinical bolus (0.25 mg) of ACTH-α-1-24 (Cortrosyn; Organon Inc.). Arterial blood was collected in heparinized syringes at varying times up to 4 hr after the administration of ACTH. Blood was immediately separated by centrifugation (4°C) and the plasma was stored at −80°C until extraction for corticoids.

Studies, in vitro. We investigated the response of isolated adrenocortical cells from normotensive and DOCA-hypertensive swine to steroidogenic agents. Adrenocortical cells were isolated using 0.2% collagenase (Type II; Sigma Chemical Co.) and mechanical agitation as described previously (16). The basic cell incubation medium was Krebs-Ringer Hepes (*n*-2-hydroxyethyl piperazine ethanesulfonic acid) buffer, pH 7.4 (24.2 m*M* Hepes, 0.2 m*M* 3-isobutylmethylxanthine, 118.5 m*M* NaCl, 4.75 m*M* KCl, 7.62 m*M* $CaCl_2$, 1.20 m*M* KH_2PO_4, 1.20 m*M* $MgSO_4$, 11.1 m*M* glucose) with 0.5% bovine serum albumin (Fraction V; Sigma Chemical Co.). Additions to the basic cell incubation medium were ACTH-α-1-24 (Cortrosyn; Organon Inc.) and angiotensin II (Val-5-angiotensin II; Sigma Chemical Co.) dissolved in 0.9% NaCl, pH 2.6, containing 0.1% bovine serum albumin, and 8Br-cAMP (Sigma Chemical Co.). Incubation volumes (90% cell suspension, 10% test substances) were 500 μl; the final cell concentration was adjusted to 2×10^5 cells/ml. Isolated cell suspensions were incubated in plastic culture tubes (12 × 75 mm) in a Dubnoff metabolic shaking bath (66 oscillations/min) at 37°C for 2 hr. In each experiment at least 89% of the cells were viable after incubation as indicated by trypan blue dye exclusion (17). After incubation, cell suspensions were frozen (−20°C) until extraction for corticoids.

Radioimmunoassay for corticoids. Both plasma samples and cell suspensions were extracted with cold chloroform (sample volume to chloroform volume, 1:10). Extracts were dried in a vacuum oven (50°C). The overall recovery of tritium-labeled corticoid added to plasma samples and cell incubations after extraction and separation was 73–84%. Residues were reconstituted, and after chromatographic separation (18) and appropriate dilution, cortisol and aldosterone were measured by a modification of the radioimmunoassay procedure of Roy *et al.* (19) using specific antibodies (Miles Research Products). DOCA had little cross-reactivity with aldosterone for antibody (<0.01%).

Analysis of data. ACTH and angiotensin II dose–response data were plotted using equations modified from those presented by Sayers *et al.* (20). The data for each steroidogenic agent closely approached linearity as indicated by their coefficients of correlation measured by linear regression; no value was less than 0.91. The equations permitted the calculation of the half-maximal steroidogenic

TABLE I. COMPARISON OF BASAL AND ACTH-INDUCED PLASMA CORTISOL CONCENTRATIONS BETWEEN NORMOTENSIVE AND DOCA-HYPERTENSIVE SWINE

			Plasma cortisol (ng/ml)	
		N	Basal[a]	Maximal value after ACTH bolus[b]
Normotensive	Conscious	3	68.9 ± 18.4	172.3 ± 79.2*
	Anesthetized	3	78.7 ± 14.6	212.5 ± 78.6*
DOCA hypertensive	Conscious	3	35.6 ± 10.3**	153.2 ± 72.1*
	Anesthetized	3	42.3 ± 9.7**	173.4 ± 64.2*

[a] Mean ± SEM of nine plasma cortisol values, three values from each of three animals.
[b] Mean ± SEM of 18 cortisol values, 6 values from each of three animals. Values comprising the mean are values from the maximal plateau response after an ACTH bolus (0.25 mg).
* Values significantly different from corresponding basal values ($P < 0.05$).
** Values significantly different from corresponding normotensive values ($P < 0.05$).

concentrations (ED_{50}; slopes of the plots) of ACTH, angiotensin II, and 8Br-cAMP.

Values in the text and figures are expressed as means ± SEM and were statistically analyzed using an analysis of variance (21) followed by the Studentized Range test; differences were considered to be significant when $P < 0.05$.

Results. After 12 weeks of DOCA treatment, the DOCA-treated swine had significantly ($P < 0.05$) greater blood pressure than control animals. The mean arterial pressure for DOCA-treated animals was 163.6 ± 27.2 mm Hg, whereas, that for the control animals was 115.3 ± 11.7 mm Hg.

Table I shows the basal plasma cortisol concentrations and the maximal plateau concentrations after administration of an ACTH bolus (0.25 mg). Basal values for DOCA-hypertensive swine were approximately 53% of the values for normotensive swine. ACTH induced a rise in plasma cortisol in both groups such that the maximal plateau values for both groups were equivalent. However, when expressed as a percentage of basal value, there were differences: the plateau response for DOCA-hypertensive swine was 419% of the basal value, whereas that for normotensive swine was 261% ($P < 0.05$). This difference in response to ACTH, expressed as percentage of the basal value, was persistent over 60 min of blood sampling (Fig. 1). These differences in basal plasma cortisol values and plasma cortisol values in response to ACTH were not affected by pentobarbital anesthesia (Table I and Fig. 1).

In contrast to changes in plasma cortisol, DOCA-hypertension did not alter basal plasma aldosterone values (normotensive, 192.7 ± 47.4 pg/ml; DOCA-hypertensive, 220 ± 63.7 pg/ml). Basal aldosterone values were also not altered by anesthesia. In addition, in conscious and anesthetized states, peak

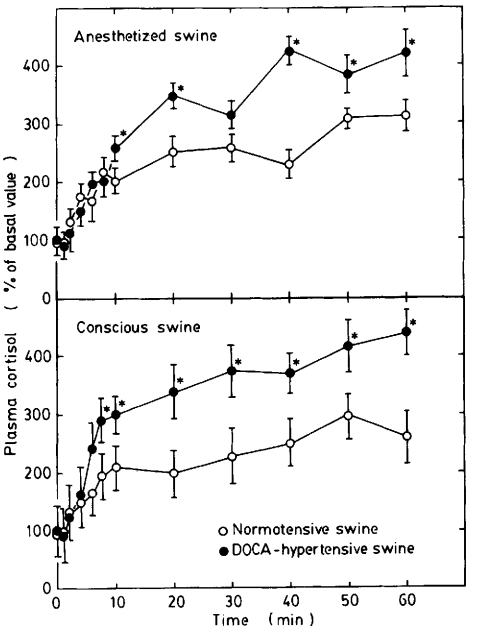

FIG. 1. Plasma cortisol responses, expressed as percentage of basal values, of normotensive and DOCA-hypertensive swine to an ACTH bolus (0.25 mg). Each symbol represents the mean of cortisol values from three pigs. SEM are represented by bars (*indicates significant difference between corresponding values; $P < 0.05$).

plasma aldosterone values in response to ACTH did not differ between the two groups (normotensive, 379.6 ± 78.7 pg/ml; DOCA-hypertensive, 325.9 ± 84.3 pg/ml).

DOCA-hypertension did not alter the temporal changes in plasma cortisol (Fig. 1) and aldosterone induced by ACTH. Peak corticoid values were attained between 30 and 60 min after the ACTH bolus, and plasma values returned to basal levels by 4 hr in both normotensive and DOCA-hypertensive swine.

In addition to the differences observed *in vivo,* there were differences observed *in vitro* using isolated swine adrenocortical cells. Fig. 2 shows the response of adrenocortical cells to varying ACTH concentrations. On an equal cell concentration basis (200,000 cells/ml), maximal ACTH-induced corticoid production of cells from DOCA-hypertensive swine was roughly 20% less than that of cells from normotensive swine. A similar DOCA-hypertension-related decrease in aldosterone production in response to angiotensin II was observed (Fig. 3). However, there was no difference in basal corticoid production of cells from normotensive *versus* DOCA-hypertensive animals (Figs. 2 and 3).

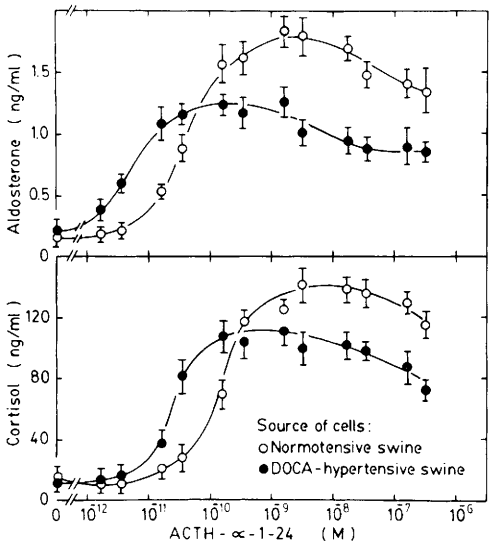

FIG. 2. ACTH-induced cortisol and aldosterone production by adrenocortical cells (2×10^5 cells/ml) isolated from normotensive and DOCA-hypertensive swine. Each symbol represents the mean of cortisol or aldosterone values from nine cell suspensions (three suspensions from each of three experiments). Adrenocortical cells were isolated from one pig for each experiment. SEM are represented by bars. Values for ACTH ED_{50} and maximal response to ACTH of cells from DOCA-hypertensive swine are significantly different ($P < 0.05$) from the values of cells from normotensive swine.

In addition to differences in maximal peptide-induced corticoid production, there were also differences in peptide potencies (Figs. 2 and 3), as indicated by the peptide ED_{50}. The ED_{50} values for ACTH-induced cortisol and aldosterone production for cells from DOCA-hypertensive swine were $(2.08 \pm 0.27) \times 10^{-11}$ *M* and $(6.02 \pm 0.31) \times 10^{-12}$ *M*, respectively, whereas for cells from normotensive swine the values were $(1.15 \pm 0.23) \times 10^{-10}$ *M* and $(4.22 \pm 0.28) \times 10^{-11}$ *M*, respectively ($n = 3$, $P < 0.05$). Thus, the potency of ACTH with cells from normotensive swine was roughly one-sixth the potency with cells from hypertensive swine. In addition, the ED_{50} values for angiotensin II were different with adrenocortical cells isolated from DOCA-hypertensive and normotensive swine (Fig. 3): the ED_{50} values were $(4.82 \pm 0.19) \times 10^{-11}$ *M* and $(1.56 \pm 0.24) \times 10^{-10}$ *M*, respectively ($n = 3$, $P < 0.05$).

In contrast to the response to peptides, cells from both types of swine had equivalent values for maximal corticoid production in response to 8Br-cAMP (Fig. 4). In addition, 8Br-cAMP had equivalent potencies (about 5×10^{-5} *M*) with cells isolated from both groups of swine.

Discussion. Work presented here suggests that DOCA-hypertensive induced changes in adrenocortical function in Yucatan miniature swine. DOCA-hypertension reduced basal plasma cortisol concentrations to about 53% of those of normotensive swine suggesting a decrease in adrenocortical function (Table I). However, in DOCA-hypertensive swine, ACTH induced a rise in plasma cortisol values over basal values that was 1.6 times the rise in normotensive swine (Table I), thus, both DOCA-hypertensive and normotensive swine attained equivalent plasma cortisol values in response to an ACTH bolus. This difference in response to ACTH, expressed as percentage of basal value, persisted throughout a 60-min sampling period (Fig. 1). In addition, sodium pentobarbital anesthesia did not alter the corticoid responses of these animals. This suggests that the differ-

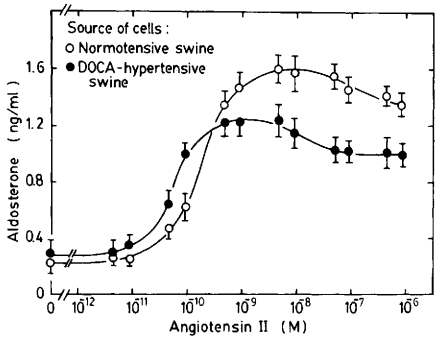

FIG. 3. Angiotensin II-induced aldosterone production by adrenocortical cells (2×10^5 cells/ml) isolated from normotensive and DOCA-hypertensive swine. Each symbol represents the mean of aldosterone values from nine cell suspensions (three suspensions from each of three experiments). Adrenocortical cells were isolated from one pig for each experiment. SEM are represented by bars. Values for angiotensin II ED_{50} and maximal response to angiotensin II of cells from DOCA-hypertensive swine are significantly different ($P < 0.05$) from the values of cells from normotensive swine.

ences in the ACTH-induced cortisol responses between DOCA-hypertensive and normotensive swine were not due to differential responses to stress. Our results are consistent with the results of other work *in vivo* with SHR which showed increased adrenal responsivity to stress and ACTH compared to normal rats (5, 6).

DOCA-hypertension did not appear to affect plasma aldosterone. Unlike plasma cortisol, basal plasma aldosterone values were equivalent between normotensive and hypertensive swine. In addition an ACTH bolus induced equivalent rises in plasma aldosterone in DOCA-hypertensive and normotensive swine. Evaluation of zona glomerulosa function, in terms of an aldosterone response, may have been better achieved by including infusions of angiotensin II and potassium.

In addition to the alterations in adrenocortical function observed *in vivo*, DOCA-hypertension also altered adrenocortical cell function as evaluated by experiments *in vitro* (Figs. 2, 3, and 4). ED_{50} values for ACTH- and angio-tensin II-induced corticoid production were 6 and 3.2 times greater, respectively, for cells from normotensive swine than for cells from hypertensive swine (Figs. 2 and 3). Since ED_{50} is a measure of cellular sensitivity (20) (the greater the ED_{50} the lesser the cellular sensitivity), these data suggest that cells from DOCA-hypertensive swine were more sensitive to these steroigenic peptides than cells from normotensive swine. In addition, although DOCA-hypertension did not affect basal corticoid production, it did significantly reduce by 20% the maximal ACTH- and angiotensin II-induced corticoid production (Figs. 2 and 3). However, DOCA-hypertension did not affect cellular sensitivity to and maximal corticoid production induced by 8Br-cAMP (Fig. 4). These data are consistent with the possibility of an increase in receptor sensitivity to ACTH and angiotensin II and a decrease in receptor number with DOCA-hypertension.

Our results showing a DOCA-hypertension-related increase in adrenocortical response to ACTH *in vivo* are consistent with other work *in vivo* using the SHR model (6). However, results showing an increased cellular sensitivity to ACTH and angiotensin II (Figs. 2 and

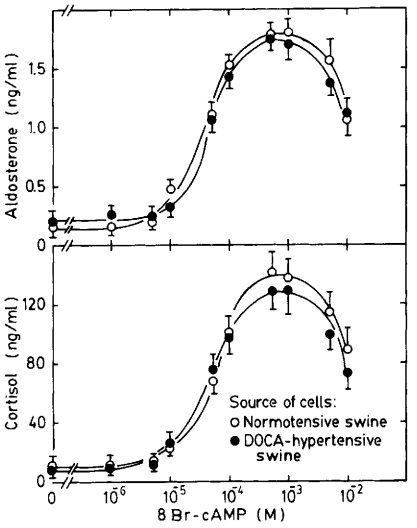

FIG. 4. 8Br-cAMP-induced cortisol and aldosterone production of isolated adrenocortical cells (2×10^5 cells/ml) from normotensive and DOCA-hypertensive swine. Each symbol represents the mean of cortisol or aldosterone values from nine cell suspensions (three suspensions from each of three experiments). Adrenocortical cells were isolated from one pig for each experiment. SEM are represented by bars. Values for 8Br-cAMP ED_{50} and maximal response to 8Br-cAMP of cells from DOCA-hypertensive swine are not different from the values for cells from normotensive swine.

3) are not in keeping with results of other work using SHR showing no change in cellular sensitivity to ACTH and decreased response to angiotensin II *in vivo* (7). These differences in cellular response to ACTH and angiotensin II may be a major distinction between the SHR and DOCA-hypertensive swine models.

We found our results from this study surprising for the following reasons: (a) DOCA-hypertensive swine have very low plasma renin activities (22). Thus, we surmised that removal of any trophic or maintenance action of angiotensin II would have resulted in a decrease in zona glomerulosa responsiveness (23). (b) DOCA is reported to have some glucocorticoid activity (24). Thus, we reasoned that the large quantity of DOCA implanted (100 mg/kg) would have suppressed adrenocortical function by inhibiting pituitary ACTH release (25) and/or by suppressing adrenocortical function directly (29). Our data suggest that any direct or indirect inhibitory effect of DOCA on adrenocortical function was overridden by physiologic events stemming from the hypertensive state.

We propose that one of the events operating in this DOCA-hypertension model and affecting adrenocortical function is the alteration in the nervous system. In many forms of genetic and experimental hypertension, there is evidence for an increased activity of central adrenalin-containing neurons (27) and increased peripheral sympathetic nerve activity (28, 29). Evidence for increased peripheral sympathetic function has been found in the DOCA-hypertensive swine model (30). Since central nervous catecholaminergic activity is associated with inhibition of ACTH release (31), we feel that the peripheral component of the sympathetic nervous system may be important in maintaining adrenocortical function in the DOCA-hypertensive pig. Although there is scant evidence for neural control of adrenocortical activity, there is evidence for β-adrenergic regulation of adrenocortical adenylate cyclase activity and corticoid production, including aldosterone production (32). In addition there is supportive evidence from ovarian innervation work which suggests a role of adrenergic nerves in modifying the actions of gonadotropins for the fine regulation of ovarian steroid secretion (33). Further evidence for neural regulation of adrenocortical secretion has been presented that, in addition, links changes in hemodynamic function with regulation of adrenocortical secretion which is rapid, partially independent of changes in plasma ACTH, and is mediated by cardiovascular stretch receptors (34). For example, moderate hemorrhage is thought to increase adrenal sensitivity to ACTH (34, 35). Possibly, with DOCA-hypertension, hemodynamic signals may be relayed to the adrenal cortex via the sympathetic nervous system and thus, induce alterations in adrenocortical function.

We feel that the DOCA-hypertensive miniature swine may be a more appropriate model than the SHR because the pig, like the human, secretes cortisol as the predominant glucocorticoid whereas the rat secretes corticosterone (36). In addition, the problems of possible genetic-induced aberrations in adrenocortical function that might exist in the SHR are absent. In our studies, presumably normal adrenocortical function has been altered by DOCA-hypertension. However, it should be pointed out that our work shows alterations in adrenocortical function that were assessed in established (after 12 weeks DOCA treatment) hypertension. Thus, the temporal relationship between the development of alterations in adrenocortical function is unknown.

Hypertensive pigs and hypertensive humans share many of the disease-induced alterations in cardiovascular parameters (37). The results of this study with DOCA-hypertensive swine suggest that in some forms of hypertension there is an increase in adrenal responsiveness to steroidogenic stimuli. Thus, the DOCA-hypertensive swine may be an invaluable model of adrenocortical dysfunction associated with hypertensive states resulting from steroid treatment, hypercortisolism, and hyperaldosteronism.

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