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Genetic-Dependent Alterations in Adrenal Stress Response and Adrenocortical Cell Function of the Domestic Fowl (*Gallus domesticus*)[1] (42392)

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Abstract. Strain-dependent differences in adrenocortical function were investigated in male White Leghorn domestic fowl. Adrenocortical function of Cornell K strain (K) (genetic control), autosomal dwarf strain (ADW), and sex-linked recessive dwarf strain (SLD) was evaluated *in vivo* by measuring plasma corticosterone and *in vitro* by measuring acute (2 hr) corticosterone production by enriched adrenocortical cell populations. Regardless of strain, there was an age-dependent decrease (27–57%) in plasma corticosterone from 1 to 12 weeks of age. However, there was a tendency for plasma corticosterone values of ADW and SLD to be, respectively, greater and less than that of K. In addition, at 12 weeks of age, plasma corticosterone responses of ADW and SLD to transient heat stress (50°C, 30 min) were, respectively, 22.8% greater and 15.9% less than that of K. Strain differences in adrenal weight and relative adrenal weight (mg% body wt) were also apparent. At 12 weeks of age, adrenal weights of ADW and SLD were, respectively, 33 and 42% less than that of K, whereas relative adrenal weights were, respectively, 27.6% greater and 22.4% less than that of K. In addition there were strain-dependent differences in adrenocortical function at the cellular level. Although there were no consistent strain differences in basal and maximal corticosterone production by cells, there were strain differences in cellular sensitivity to ACTH and pregnenolone. On an equal cell concentration basis, the half-maximal steroidogenic concentrations (ED_{50} values or effective doses for 50% maximal effect) of ACTH for ADW and SLD adrenocortical cells were, respectively, 0.23 and 2.07 times the ED_{50} value for K cells. In addition, the ED_{50} value of pregnenolone for ADW cells was 0.46 times that for K and SLD cells. Since ED_{50} values are a measure of cellular sensitivity (the greater the ED_{50} value the lesser the cellular sensitivity), the order of sensitivity to ACTH was ADW > K > SLD and the order of sensitivity to pregnenolone was ADW > K = SLD. However, there were no strain differences in ED_{50} values of 8-bromo-cyclic AMP. These data suggest that strain differences in plasma corticosterone response to stress are, in part, due to differences in relative adrenal weight and differences in adrenocortical cell function.

Abnormal glucocorticoid secretion in response to stressors can result from alterations in any of the modulating factors operating at the levels of the brain–pituitary–adrenal axis. Thus, research has been focused at each level in order to provide a basis for other work aimed at elucidating the mechanisms underlying aberrant glucocorticoid response to stressors. For example, a major portion of recent work has been focused on the interaction and combined actions of factors that regulate ACTH secretion (1, 2). In addition, other work has been focused at the adrenal level in order to elucidate nonadrenocorticotropic mechanisms involved in the regulation of glucocorticoid secretion in response to stressors. The latter body of recent work focused at the adrenal level has provided strong evidence supporting the notion that apart from or in addition to alterations in ACTH secretion, alterations in adrenocortical function can modulate glucocorticoid response to stressors (3–5). However, there have been no reports of studies focused on the relationship between altered adrenocortical cell function per se, and altered glucocorticoid response to stressors.

Genetic dwarf strains of domestic fowl have been shown to exhibit abnormalities in plasma concentrations of some growth-related hormones. For example, compared with those of a genetic control strain [Cornell K strain (K)], plasma 3,4,3′-triiodothyronine concentrations of the autosomal dwarf (ADW) and the sex-linked recessive dwarf (SLD) strains (dwarf strains derived from the K strain) were de-

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pressed by 33–67% (6), and plasma immunoreactive somatomedin-C concentrations were depressed 50–60% (7) after 12 weeks of age. In contrast, plasma growth hormone concentrations tended to be elevated 53–331% after 12 weeks of age (K = 100%). However, glucocorticoids, well-established suppressors of poultry growth (8–11), were not measured in these studies.

In the present study we measured some parameters of adrenocortical function of normal and dwarf domestic fowl strains including plasma corticosterone responses to transient heat stress and corticosterone production by isolated enriched adrenocortical cell populations. We show that adrenocortical function of ADW and SLD strains is abnormal compared to the genetic control strain (K). Moreover, we present evidence that suggests that abnormal glucocorticoid response to stressors is, in part, the result of alterations in adrenocortical cell function.

Materials and Methods. *Animals.* Male dwarf and genetic control strains of White Leghorn domestic fowl were housed at 20 ± 1°C under a 16-hr light: 8-hr dark photocycle. They were fed a standard commercial diet and water *ad libitum.*

Birds (1, 3, 6, and 12 weeks old) from the different strains were collected together in a plastic carrying crate, transported to a surgery room, and then quickly killed at random by decapitation and exsanguinated. Blood was collected in chilled heparinized plastic culture tubes and quickly centrifuged (1000*g*, 15 min, 4°C) to prepare plasma. Adrenal glands were quickly removed, trimmed free of connective tissue, and weighed.

In other studies, birds (12 weeks old) were subjected to transient heat stress to evaluate strain differences in plasma corticosterone response to stress. Heat stress was induced by using modifications of procedures described previously (12, 13). Cockerels from the different strains were collected together in a plastic carrying crate and transported to a room housing a double-chambered incubator. Cockerels were individually placed under inverted wire cages on wooden pallets. Cages provided enough space for birds to stand. Birds were allowed 30 min to acclimate to the cages. Then, the pallet supporting the caged birds was moved to the floor of an incubator and the birds were subjected to 50°C for 30 min. Cockerels subjected to identical conditions, except that they were moved to an incubator maintained at room temperature, served as controls. Birds were then quickly decapitated at random and blood was collected for the preparation of plasma. In initial studies, plasma corticosterone values were determined at 30, 60, and 90 min of heat stress. Plasma corticosterone values for 60 and 90 min were not significantly higher than those at 30 min regardless of strain. In addition, some deaths occurred at 60 and 90 min of heat stress. Thus, the remainder of the studies were conducted at 30 min of heat stress to minimize trauma to birds.

Adrenocortical cell isolation and incubation. Adrenal gland from 12-week-old cockerels were diced into small pieces (about 2 mm^3) for cell isolation. The number of birds used for adrenocortical cell isolation varied from 15 to 25, depending on the amount of adrenal gland tissue required to yield a sufficient quantity of cells to test cell responses to steroidogenic agents and to achieve equal cell concentrations for each strain studied. Cell yields per adrenal for K, ADW, and SLD strains were (means ± SEM from three experiments) 625,580 ± 58,520, 531,250 ± 47,325, and 406,230 ± 42,960 cells, respectively.

Adrenocortical cells were prepared essentially as described in detail previously (14, 15) using collagenase (0.2%) (Type I, Sigma Chemical Co.) and mechanical agitation followed by Percoll (Pharmacia Fine Chemicals) continuous density gradient centrifugation. Enriched adrenocortical cells were suspended in a Krebs–Ringer Hepes (*n*-2-hydroxyethylpiperazine ethanesulfonic acid) buffer (24.2 m*M* Hepes, 118.5 m*M* NaCl, 4.75 m*M* KCl, 2.54 m*M* $CaCl_2$, 1.20 m*M* KH_2PO_4, 1.20 m*M* $MgSO_4$, pH 7.5) containing 11.1 m*M* glucose and 0.5% bovine serum albumin. Aliquots of cell suspensions of equal cell concentrations (60,000 cells/ml) were incubated with various concentrations of ACTH [ACTH-α-1-24 (Cortrosyn; Organon, Inc.)], 8-bromo-cyclic AMP (8Br-cAMP), and pregnenolone (Sigma Chemical Co.) at 39.5–41.0°C for 2 hr. The incubation volumes were 200–500 μl (90% cell suspension; 10%, a solution containing a steroidogenic agent). Prior to the addition of the

agents to cell suspensions, ACTH was dissolved in a solution of 0.9% NaCl, pH adjusted to 2.6 with 1.0 *N* HCl, containing 0.1% bovine serum albumin, whereas 8Br-cAMP and pregnenolone were made up in the incubation medium. In each experiment at least 87% of cells were viable as indicated by trypan blue dye (4%) exclusion.

Radioimmunoassay for corticosterone. Aliquots of plasma (200 μl) were extracted with dichloromethane (1 vol plasma:10 vol dichloromethane) and then the dried residue from the extract was processed for radioimmunoassay for corticosterone. Recoveries were routinely >68%. Cell suspensions were assayed directly for corticosterone. Corticosterone, the major glucocorticoid secreted by the domestic fowl adrenal gland (16), was measured by a modification of the radioimmunoassay procedure of Roy, Jr. *et al.* (17) using specific antibody (Miles Research Products). Cross-reactivity with pregnenolone was low (≤0.67%). As little as 0.1 ng/ml corticosterone could be detected. Radioimmunoassay of aliquots of the same pooled plasma samples or cell suspensions (performed with each radioimmunoassay) showed intraassay and interassay coefficients of variation of 5.6 and 10.3%, respectively.

Analysis of data. The response of isolated adrenocortical cells to steroidogenic agents was analyzed by fitting the data comprising the sigmoidal dose-response curves shown in this report using a four-parameter logistic equation model that is available as a computer program (ALLFIT, Biomedical Computing Technology Information Center, Vanderbilt Medical Center, Nashville, Tenn.). This method allowed the simultaneous testing of a family of dose-response curves for optimally efficient data analysis (18). ED_{50} and maximal production values were calculated and statistically analyzed using this program.

Other data (adrenal gland weights, plasma corticosterone values, etc.) were statistically analyzed by analysis of variance (19). Data are expressed as the means ± SEM; means were deemed significantly different at $P \leq 0.05$.

Results. *Effect of genetic strain on adrenal growth.* Table I shows the effect of genetic strain on domestic fowl body weight, adrenal weight, and relative adrenal weight (adrenal weight expressed as mg% body wt) at 1, 3, 6, and 12 weeks after hatching. At each age, body weights of ADW and SLD were significantly lower than those of K. Compared to K body weights, body weights of ADW and SLD were decreased, 29–47 and 16–26%, respectively, over the ages measured. Similarly, adrenal weights of ADW and SLD were, respectively, 20–33 and 24–48% lower than corresponding K values. However, there was a tendency of ADW and SLD relative adrenal weight to be, respectively, higher and lower than corresponding K values: At 12 weeks of age, ADW relative adrenal weight was 27.6% higher, whereas SLD relative adrenal weight was 22.4% lower than K relative adrenal weight.

Effect of genetic strain on plasma concentrations of corticosterone. Figure 1 shows trunk plasma concentrations of corticosterone. There was a tendency for ADW and SLD birds to have, respectively, higher and lower concentrations than K birds, at the ages measured.

TABLE I. BODY WEIGHT, ADRENAL WEIGHT, AND RELATIVE ADRENAL WEIGHT OF AUTOSOMAL DWARF, SEX-LINKED DWARF, AND GENETIC CONTROL STRAINS OF MALE DOMESTIC FOWL

	Body weight (g)			Adrenal weight (mg)			Relative adrenal weight (%) $\left(\frac{\text{adrenal weight (mg)}}{\text{body weight (g)}} \times 100\%\right)$		
Age (weeks)	Genetic control	Autosomal dwarf	Sex-linked dwarf	Genetic control	Autosomal dwarf	Sex-linked dwarf	Genetic control	Autosomal dwarf	Sex-linked dwarf
1	56.5 ± 3.6	40.2 ± 3.1*	47.2 ± 4.0*	18.2 ± 1.4	14.6 ± 1.1*	9.5 ± 0.8*	32.5 ± 2.5	36.6 ± 2.7	20.1 ± 1.7*
3	133.8 ± 11.5	97.8 ± 10.2*	113.9 ± 10.7*	33.9 ± 2.0	29.2 ± 1.4*	19.0 ± 1.2*	25.4 ± 1.5	29.8 ± 1.5**	16.7 ± 1.1*
6	349.4 ± 18.7	216.5 ± 20.2*	277.7 ± 19.5*	72.6 ± 4.3	63.6 ± 3.2*	55.1 ± 1.5*	20.8 ± 1.2	29.4 ± 1.5**	19.9 ± 0.6
12	912.0 ± 27.9	479.5 ± 21.3*	677.1 ± 21.6*	155.3 ± 9.2	104.2 ± 5.9*	89.6 ± 3.7*	17.0 ± 1.0	21.7 ± 1.2**	13.2 ± 0.5*

NOTE. Values are the means ± SEM. $N = 8$.

* Values significantly lower than corresponding genetic control values ($P \leq 0.05$).

** Values significantly greater than corresponding genetic control values ($P \leq 0.05$).

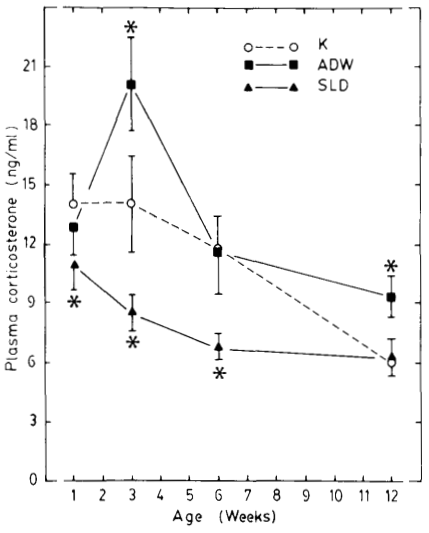

FIG. 1. Plasma corticosterone concentrations in domestic fowl at various ages after hatching. Each symbol represents the mean of trunk plasma corticosterone values from eight birds. SEM are represented by bars (* indicates values that are significantly different from the corresponding value of K birds, $P \leq 0.05$).

To evaluate the effect of genetic strain on plasma corticosterone further, birds (12 weeks after hatching) were subjected to transient heat stress (50°C, 30 min) under controlled conditions. The results are shown in Fig. 2. There were no differences in basal plasma corticosterone concentrations between the strains, whereas there were differences in plasma corticosterone responses to heat stress. ADW and SLD birds had heat stress-induced plasma corticosterone concentrations that were, respectively, 22.8% greater and 15.9% less than those of K birds.

Effect of genetic strain on adrenocortical cell function. Genetic strain affected adrenal growth and plasma corticosterone response to stress. To learn if these alterations in adrenocortical variables were manifest at the cellular level, we measured the influence of strain on corticosterone production by isolated adrenocortical cells. Figure 3 shows the results of experiments in which adrenocortical cells, isolated from 12-week-old K, ADW, and SLD birds, were incubated with various concentrations of ACTH, 8Br-cAMP, and pregnenolone. These agents stimulated cellular corticosterone production in a dose-dependent manner over approximately two log orders of concentration regardless of fowl strain. However, the data of Fig. 3 suggest that the responsivity of cells isolated from the strains to these agents might be different. Accordingly, the data of Fig. 3 were fitted and statistically analyzed using the ALLFIT computer program (see Materials and Methods), and maximal corticosterone production induced by each agent (Table II) and the ED_{50} for each agent (Table III) were calculated.

In the absence of agents (basal corticosterone production), there were no consistent strain-dependent differences in corticosterone production (Table II). In the presence of ACTH and 8Br-cAMP, maximal corticosterone production was roughly the same for all strains. However, in the presence of pregnenolone alone, maximal corticosterone production of ADW cells was 36–44% greater than those of K and SLD cells. In addition, there were differences in cellular sensitivity to agents between the strains, as indicated by the differences in ED_{50} values of agent (the greater the ED_{50} value, the lesser the cellular sensitivity) (Table III). On an equal cell concentration basis, the ED_{50} values of ACTH for ADW and SLD cells were 0.23 and 2.07 times the ED_{50}

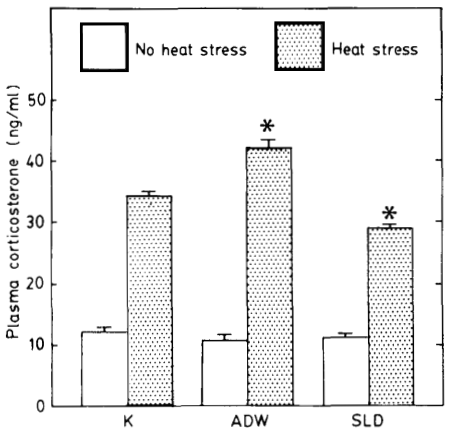

FIG. 2. Plasma corticosterone responses of domestic fowl subjected to transient heat stress (50°C for 30 min). Each column represents the mean of plasma corticosterone values from 15 birds. SEM are represented by bars on the columns (* indicates values that are significantly different from corresponding value of K birds, $P \leq 0.05$). For each fowl strain, heat stress values are significantly greater than corresponding control values ($P \leq 0.05$).

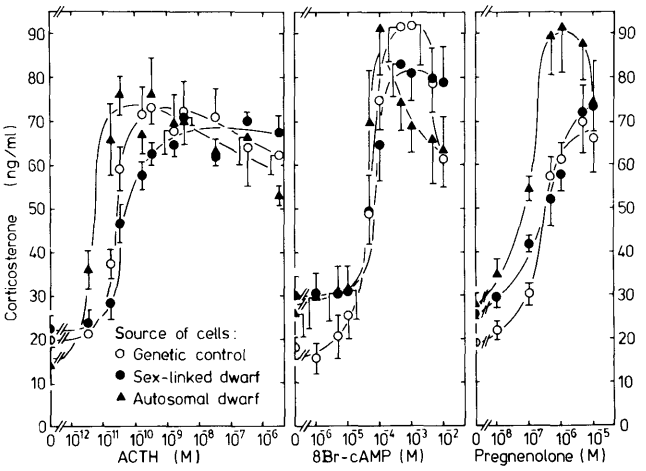

FIG. 3. Acute (2 hr) corticosterone production by enriched adrenocortical cell populations isolated from the adrenal glands of the various strains of domestic fowl. Adrenocortical cells (60,000 cells/ml) were incubated with various concentrations of ACTH, 8Br-cAMP, and pregnenolone. Each symbol represents the mean of corticosterone values from nine cells suspensions (three suspensions from each of three experiments). SEM are represented by bars drawn through the symbols.

value for K cells, respectively. Thus, the order of cellular sensitivity to ACTH was ADW > K > SLD. Differences in cellular sensitivity were also seen with pregnenolone. The ED_{50} value of pregnenolone for ADW cells was 0.46 times the ED_{50} value for K cells. However, the ED_{50} values of pregnenolone for K and SLD cells were the same; thus, the order of sensitivity was ADW > K = SLD. Yet, in contrast to the strain-dependent differences in cellular sensitivity to ACTH and pregnenolone, there were no differences in cellular sensitivity to 8Br-cAMP as indicated by the equivalent ED_{50} values for cells from the different strains (Table III).

Discussion. Data presented here indicate that genetic dwarf strains of domestic fowl have abnormal stress-induced plasma corticosterone concentrations. In addition the present study suggests that the strain-dependent differences in plasma corticosterone response to stress are, in part, due to differences in relative adrenal weight and adrenocortical cell function.

First, although the adrenal weights of ADW and SLD birds were, respectively, 33 and 42% lower than those of K birds at 12 weeks of age, the relative adrenal weights were, respectively, 27.6% higher and 22.4% lower than those of K birds (Table I). These differences in adrenal

TABLE II. MAXIMAL CORTICOSTERONE PRODUCTION OF ISOLATED DOMESTIC FOWL ADRENOCORTICAL CELLS IN RESPONSE TO STEROIDOGENIC AGENTS

Source of cells: Fowl strain	No additions	ACTH	8Br-cAMP	Pregnenolone
Genetic control (K)	19.9 ± 2.7	73.3 ± 3.5	92.3 ± 6.8	67.9 ± 10.6
Autosomal dwarf (ADW)	24.9 ± 5.4	77.6 ± 3.3	82.8 ± 7.9	98.1 ± 5.5*
Sex-linked dwarf (SLD)	25.5 ± 3.9	67.0 ± 3.4	87.7 ± 8.6	71.2 ± 8.3

NOTE. The data for cellular corticosterone production stimulated by various concentrations of ACTH and 8Br-cAMP and that supported by pregnenolone (Fig. 3) were fitted and statistically analyzed using the ALLFIT computer program (see Materials and Methods). Each value (ng/ml) is the calculated mean ± SEM (ALLFIT) from the data of three experiments.

* Value significantly different from corresponding K and SLD values ($P \leq 0.05$).

TABLE III. HALF-MAXIMAL STEROIDOGENIC CONCENTRATION (ED_{50}) OF A STEROIDOGENIC AGENT FOR ISOLATED DOMESTIC FOWL ADRENOCORTICAL CELLS

Source of cells: Fowl strains	ACTH (*M*)	8Br-cAMP (*M*)	Pregnenolone (*M*)
Genetic control (K)	$(2.24 \pm 0.31) \times 10^{-11}$	$(5.86 \pm 0.76) \times 10^{-5}$	$(3.48 \pm 1.22) \times 10^{-7}$
Autosomal dwarf (ADW)	$(5.23 \pm 1.04) \times 10^{-12}$*	$(4.63 \pm 1.25) \times 10^{-5}$	$(1.48 \pm 0.21) \times 10^{-7}$*
Sex-linked dwarf (SLD)	$(4.64 \pm 1.18) \times 10^{-11}$*	$(7.51 \pm 1.86) \times 10^{-5}$	$(5.36 \pm 1.57) \times 10^{-7}$

NOTE. The data for cellular corticosterone production stimulated by various concentrations of ACTH and 8Br-cAMP and that supported by pregnenolone (Fig. 3) were fitted and statistically analyzed using the ALLFIT computer program (see Materials and Methods). Each ED_{50} value is the calculated mean ± SEM (ALLFIT) from the data of three experiments.

* Values significantly different from corresponding K values ($P \leq 0.05$).

weight to body weight ratio might, in part, explain the differences in plasma corticosterone concentrations between the strains (Figs. 1, 2). Presumably, the greater this ratio, the greater the plasma corticosterone concentration, provided that the proportion of cortical tissue in the adrenal gland is fairly constant between the strains.

Second, in addition to differences in adrenal weight to body weight ratio, there were differences in adrenocortical function at the cellular level. On the one hand, basal corticosterone production and maximal ACTH- and 8Br-cAMP-induced corticosterone production of equal concentrations of adrenocortical cells isolated from the different strains were equivalent (Fig. 3, Table II). On the other hand, maximal pregnenolone-supported corticosterone production of ADW cells was about 40% greater than those of K and SLD cells, thus suggesting that ADW cells had a greater unstimulated steroidogenic enzyme pool than did K and SLD cells.

In addition, there were strain-dependent differences in cellular sensitivity to ACTH as indicated by the differences in ACTH ED_{50} values (Table III) [the greater the ED_{50} value, the lesser the cellular sensitivity (20)]. An index of sensitivity can be obtained by taking the reciprocal of the ratios of the ACTH ED_{50} values for ADW and SLD cells to the ACTH ED_{50} value for K cells. Thus, ADW cells were 4.4 and 9 times more sensitive to ACTH than were K and SLD cells, respectively. Moreover, ADW cells were 2.2 times more sensitive to pregnenolone than were K and SLD cells. However, there were no differences in cellular sensitivity to 8Br-cAMP. The data suggest that the strain-dependent differences in cellular sensitivity may have been due to differences in ACTH-cell interaction (possibly including ACTH receptors) and differences in steroidogenic enzyme affinity for substrates.

Other *in vivo* studies, using turkeys (21) and chickens (22) suggest an enhanced adrenal sensitivity to exogenous ACTH in birds selected for high plasma corticosterone response to stress. Our *in vitro* work corroborates and extends the *in vivo* work of others: ADW birds which had greater stress-induced plasma corticosterone concentrations than K and SLD birds had adrenocortical cells that were more sensitive to ACTH than cells of K and SLD birds. Moreover, our work is the first to demonstrate alterations in adrenocortical function at the cellular level in a genetic-dependent, stress response model. In addition, our work suggests that alterations in adrenocortical cell function and stress response can be coselected with the selection of traits other than a high corticosterone response to stress as, in this case, selection for dwarfism.

The mechanisms underlying the alterations in adrenocortical cell function presented here are unknown. However, at least two plausible hypotheses deserve consideration: (i) The alterations are intrinsic and genetically programmed within the adrenocortical cell, and (ii) the alterations are the result of the abnormal secretion of other factors which in turn affect adrenocortical cell function. The latter hypothesis is supported by the work of others, indicating abnormal plasma concentrations of 3,5,3′-triiodothyronine, growth hormone, and immunoreactive somatomedin-C in the dwarf strains (6, 7). Importantly, in this connection, our recent work with hypophysectomized cockerels suggests that 3,5,3′-triiodothyronine

and growth hormone are important modulators of adrenocortical cell steroidogenic capacity and sensitivity to ACTH (15).

In summary, our work and the work of others strongly suggest that genetic-dependent differences in plasma corticosterone response to stress are due, in part, to differences in adrenocortical function. Moreover, the present study indicates that these differences are due to functional alterations at the adrenocortical cell level. Thus, genetic-dependent alterations in adrenocortical function may operate in concert with alterations in the modulation of pituitary ACTH secretion in the frank expression of abnormal glucocorticoid secretion in response to stress.

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