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Sensitivity to Corticosterone-Induced Cleft Palate Is Not Associated with H-2[1] (41835)

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Abstract. The frequency of cleft palate (CP) and corticosterone levels in maternal plasma and amniotic fluid were determined in pregnant C57BL/10 (H-2^b) and congenic B10.A (H-2^a) mice after the ip injection of repository ACTH, corticosterone acetate, or desoxycorticosterone acetate (DOCA) on the 11th through 14th day of gestation. It was found that ACTH (a) induces CP in B10.A but not C57BL/10 mice, (b) induces CP in B10.A mice at about the same frequency noted when diluent alone is injected, and (c) produces an elevation in maternal plasma corticosterone levels 1.5- to 2.0-fold lower and amniotic fluid levels 3- to 5-fold lower than those found after the injection of 5 mg corticosterone acetate, a dose which induces a comparable frequency of CP in B10.A mice. The injection of 5 mg corticosterone acetate produced CP frequencies in C57BL/10 and B10.A mice of 4.9 and 3.3%, respectively, and increasing the dose to 9.2 mg resulted in significant increases in CP to 23.8 and 24.7%, respectively. DOCA at two dose levels induced CP in B10.A fetuses at about the frequency noted when diluent alone has been given. These findings show that susceptibility to corticosterone induced CP is not associated with the major histocompatibility complex of the mouse, H-2, as is the case with glucocorticoids (e.g., cortisone, dexamethasone), and they raise the possibility that factors other than or in addition to corticosterone may be involved in spontaneous or ACTH- or stress-induced CP.

Over 25 years ago an animal model for isolated cleft palate was developed in mice (1). It was shown that clefting of the developing secondary palate could be induced by the administration of cortisone to pregnant mice between the 11th and 14th days of gestation. Further, it was found that inbred strains of mice had different degrees of susceptibility to cortisone-induced cleft palate ((1,2); e.g., over 90% of the progeny of A/J and DBA/1 mice given 2.5 mg of cortisone developed cleft palate (CP), whereas only 20–25% of the offspring of similarly treated C57BL/6 or CBA/J mice had the defect). Subsequently, it has been shown that inbred strains of mice respond similarly when other glucocorticosteroids are given (3–5) and that several of the factors which determine susceptibility to glucocorticosteroid-induced CP are linked to the major histocompatibility complex of the mouse, H-2 (3, 4, 6).

Thus, cortisone, hydrocortisone, and a variety of their synthetic analogues induce CP in fetal mice, and to a great degree susceptibility is determined by the H-2 type of the mother and fetus, (3, 4). However, in the mouse corticosterone, a mineralocorticoid with weak gluconeogenic and anti-inflammatory properties, is the primary adrenal corticoid, and cortisol has not been detected in the plasma ((7, 8) and unpublished observations using HPLC, M. L. Tyan, J. J. Bonner, E. Canalis). The conventional wisdom has been that corticosterone is the agent responsible for "spontaneous" and stress-induced CP in the mouse. In support of this, it has been shown that corticosterone (9) and ACTH (10) can induce CP in A/J mice, and in the one published report where CP was induced in mice by the stress of transport the frequency varied significantly among the strains studied: A/J, 27%; DBA/2, 3%; CBA/J and C57BL/6, 0% (11). However, it has not been demonstrated that susceptibility to the teratogenic action of these agents is linked to the major histocompatibility complex as it has been with glucocorticoids.

To investigate this, pregnant congenic mice, C57BL/10 and B10.A, were injected with ACTH or corticosterone, corticosterone levels were measured in maternal plasma and amniotic fluid, and the incidence of CP was determined in the progeny. It was found that (a) plasma and amniotic fluid corticosterone

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levels are similar in the two strains under basal conditions and after the injection of ACTH or corticosterone, (b) susceptibility to ACTH-induced CP appears to be associated with H-2, but (c) susceptibility to corticosterone-induced CP clearly is not. These findings suggest that corticosterone may not be the teratogenic agent in "spontaneous," or ACTH- or stress-induced CP.

Materials and Methods. Breeding pairs of C57BL/10Sn(H-2^b) and congenic C57BL/10.ASgSn(H-2^a) mice were purchased from Jackson Laboratory, Bar Harbor, Maine. Stock mice used in these experiments were bred in this Laboratory. The mice were fed Purina Laboratory Chow, and they were given acidified tap water.

In the experiments one male and two virgin 10- to 12-week-old females were placed in each cage. The day a vaginal plug was detected was considered Day 0 of pregnancy. On Days 11 through 14 the mice were injected ip with ACTH, corticosterone acetate, or desoxycorticosterone acetate (DOCA). The mice were killed on the 18th day of pregnancy, and the number of living fetuses and resorptions were recorded (dead fetuses, regardless of age were recorded as resorptions). Living fetuses were weighed and examined for gross external malformations. The oral cavity was inspected for the presence of CP, and the internal organs were examined for defects and to determine sex.

Repository corticotropin (HP Acthar Gel, 80 U.S.P. units/ml: diluent; 16% gelatin, 0.5% phenol, 0.1% cysteine) was obtained from Armour Pharmaceutical Company, Phoenix, Arizona. Corticosterone acetate was purchased from Sigma Chemical Company, St. Louis, Missouri and suspended (25 mg/ml) in an aqueous medium containing 0.45% NaCl, 0.2% polysorbate 80, 0.25% sodium carboxymethylcellulose, and 0.9% benzyl alcohol. DOCA (5 mg/ml; diluent; 0.08% methylparaben, 0.1% propylparaben in sesame oil) was obtained from Organon, Inc., West Orange, New Jersey. Control pregnancies were given ip punctures with a sterile needle on Days 11 through 14.

Corticosterone levels were determined in plasma and amniotic fluid by the microadaption of the acid fluorescence method described by Glick *et al.* (12). In the mouse which does not produce cortisol this method measures primarily corticosterone. Comparisons of means were made using Students' *t* test (13).

The sampling unit in the CP studies is the litter. The proportion of CP in each litter was transformed to an arcsine by using the table of Freeman–Tukey arcsine transformations for small sample sizes which was calculated by Mosteller and Youtz (14). This transformation has a weighting factor for litter size and for 0 and 100%. The proportion of CP per litter was transformed so that parametric analysis could be used. Means were compared using the Tukey–Kramer method (13).

Results. For comparative purposes, previously published data are presented in Table I showing the frequencies of CP obtained when C57BL/10 and B10.A mice were given 2.5 mg cortisone acetate on Days 11–14 or dexamethasone (80 mg/kg) or its diluent (creatinine 0.8%, sodium citrate 1%, methylparaben 0.15%, and propylparaben 0.02% in distilled water) on Day 12 (15, 16). It can be seen that B10.A mice are significantly more sensitive to the induction of CP by the glucocorticoids than are C57BL/10 mice. Further, B10.A mice appear to be more sensitive to CP induction by the specific or nonspecific effects of the diluent used with dexamethasone.

In the first experiments presented here C57BL/10 and B10.A pregnant dams were injected ip with 5 or 9.2 mg corticosterone acetate on Days 11 through 14 (Table II). It was found that the lower dose level produced resorption rates not significantly different from those noted when the same strains were injected with cortisone or dexamethasone as shown in Table I; however, the higher dose produced a twofold increase in the resorption rates. In neither case, was there a significant difference between the two strains. These results together with unpublished dexamethasone dose–response studies in C57BL/10, B10.A, B10.A(5R), B10.A(2R), B10.D2, B10.BR, and B10.A(18R) mice (J. J. Bonner and M. L. Tyan) suggest that the glucocorticoids, cortisone acetate and dexamethasone, have little effect on the normal resorption rate even at a relatively large dose (e.g., 400 mg (1.0 m*M*)/kg dexamethasone); however, corticosterone acetate at a dose of approximately 200 mg (0.6 m*M*)/kg doubles the rate in C57BL/10 and B10.A fetuses.

The injection of 5 mg corticosterone acetate

TABLE I. FREQUENCY OF ISOLATED CLEFT PALATE AMONG THE PROGENY OF CONGENIC C57BL/10 AND B10.A DAMS GIVEN ip INJECTIONS OF CORTISONE ACETATE ON DAYS 11 THROUGH 14 OR DEXAMETHASONE ON DAY 12

Strain	Corticosteroid (mg)	Number of litters	Total implants	Percentage resorption	Percentage cleft palate
C57BL/10	Cortisone acetate (2.5)[a]	14	98	12.5	36[c]
B10.A		18	147	13.0	92[c]
C57BL/10	Dexamethasone (80/kg)[b]	15	121	14.0	19.2[d]
B10.A		20	161	11.2	46.2[d]
C57BL/10	(220/kg)[b]	30	199	19.1	47.8[e]
B10.A		17	135	16.3	78.8[e]
C57BL/10	Diluent, Day 12[f]	13	100	26.0[g]	0.0[h]
B10.A		12	93	11.8[g]	2.4[h]

[a] Ref (15).
[b] Ref (16).
[c] C57BL/10 vs B10.A, $P < 0.01$.
[d] C57BL/10 vs B10.A, $P < 0.01$.
[e] C57BL/10 vs B10.A, $P < 0.01$.
[f] Ref (23).
[g] C57BL/10 vs B10.A, $P > 0.10$.
[h] C57BL/10 vs B10.A, $P < 0.05$.

produced comparably low frequencies of CP in the two strains studied (4.9 and 3.3%), and increasing the dose to 9.2 mg resulted in significant and equal increases in the rates (23.8 and 24.7%, Table II). These results suggest that barring complex differences in maternal–fetal metabolism and/or placental transport these two strains do not have genetic differences associated with H-2 which modulate susceptibility to corticosterone-induced CP. In the sham-injected controls the frequency of CP was significantly higher in B10.A mice (0.0% vs 3.6%), but the resorption rates were nearly identical.

DOCA is a mineralocorticoid with approximately seven times the sodium-retaining potency of corticosterone and essentially no anti-inflammatory activity. It has been reported that even at very high doses DOCA does not induce CP in susceptible strains of mice (5). To confirm this C57BL/10 and B10.A pregnant dams were injected ip with 1.0 or 2.5 mg DOCA on Days 11–14 (Table III). At the higher dose the mice received 0.5 ml of sesame oil ip; because of the absence of diluent controls it is not known if this contributed to the somewhat high resorption rates noted in these experiments. Two fetuses with CP were found

TABLE II. FREQUENCY OF ISOLATED CLEFT PALATE AMONG THE PROGENY OF CONGENIC C57BL/10 AND B10.A DAMS GIVEN ip INJECTIONS OF CORTICOSTERONE ON DAYS 11 THROUGH 14

Strain	Corticosterone (mg)	Number of litters	Total implants	Percentage resorption	Percentage cleft palate
C57BL/10	None[a]	15	109	27.5	0.0[b]
B10.A	None[a]	15	115	27.8	3.6[b]
C57BL/10	5	13	102	20.6[c]	4.9[e]
B10.A	5	13	113	19.5[d]	3.3[f]
C57BL/10	9.2	18	143	41.2[c]	23.8[e]
B10.A	9.2	21	176	49.4[d]	24.7[f]

[a] These mice were stuck in the lower abdomen with a sterile needle on Days 11 through 14.
[b] C57BL/10 vs B10.A, $P < 0.01$.
[c] C57BL/10, 5 mg vs C57BL/10, 9.2 mg, $P < 0.01$.
[d] B10.A, 5 mg vs B10.A, 9.2 mg, $P < 0.01$.
[e] C57BL/10, 5 mg vs C57BL/10, 9.2 mg, $P < 0.01$.
[f] B10.A, 5 mg vs B10.A, 9.2 mg, $P < 0.01$.

TABLE III. FREQUENCY OF ISOLATED CLEFT PALATE AMONG THE PROGENY OF C57BL/10 AND B10.A DAMS GIVEN ip INJECTIONS OF DESOXYCORTICOSTERONE (DOCA) ON DAYS 11 THROUGH 14

Strain	DOCA (mg)	Number of litters	Total implants	Percentage resorptions	Percentage cleft palate (No./total)
C57BL/10	1.0	8	57	10.4[b]	3.9 (2/51)[c]
B10.A[a]	1.0	11	83	27.7[b]	6.7 (4/60)[d]
C57BL/10	2.5	4	32	25.0	0.0 (0/24)
B10.A	2.5	9	66	30.3	2.2 (1/46)

[a] One litter was completely resorbed. If this pregnancy is not included, there were 74 implants of which 18.9% were resorbed.
[b] $P < 0.01$.
[c] Two affected fetuses were found in one pregnancy.
[d] Affected fetuses were found in three pregnancies.

in 1 of 12 C57BL/10 litters. The frequency of CP among B10.A litters was low and unrelated to dose (6.7 and 2.2%). These results suggest that DOCA has little or no ability to induce CP at pharmacological doses and that the CP induced in B10.A mice was most likely the result of the stress of injection as was noted in the dexamethasone diluent and sham injected controls (Tables I and II, respectively).

A major component of the response to stress in mice is the release of ACTH from the pituitary which results in adrenal stimulation and increased corticosterone secretion. If stress plays a role in the etiology of spontaneously occurring CP and if corticosterone the primary adrenal corticoid in the mouse is the agent through which the effect is mediated, then from the results reported above it would be expected that C57BL/10 and B10.A mice would be equally sensitive to CP induction by ACTH. It was found, however (Table IV), that (a) B10.A mice are significantly more sensitive to CP induction by ACTH than are C57BL/10 mice (5.2 and 7.7% vs 0%), (b) 8 and 16 units of ACTH induced essentially the same rate of clefting in B10.A mice, and (c) the rate of clefting was only slightly higher but not significantly different from that noted in the control groups (Tables I and II; no statistical comparison was made between the dexamethasone diluent controls and the ACTH group because of the differences in the diluents and the injection schedules). Therefore, it is not clear from these experiments whether the CP noted were induced by the physiological action of ACTH or if the effect was the result of the multiple injections or of a component of the diluent (not likely because again there was no dose effect). Whatever the case, B10.A mice are significantly more likely to develop CP under these conditions than are C57BL/10 fetuses.

Virgin C57BL/10 and B10.A mice were injected ip with diluent or 5.0 or 9.2 mg corticosterone acetate, and they were bled prior to and 2 and 6 hr after injection (Table V). It was found that the resting plasma corticosterone levels of the two strains were not

TABLE IV. FREQUENCY OF ISOLATED CLEFT PALATE AMONG THE PROGENY OF CONGENIC C57BL/10 AND B10.A DAMS GIVEN ip INJECTIONS OF REPOSITORY ACTH (HP ACTHAR GEL, ARMOUR) ON DAYS 11 THROUGH 14

Strain	ACTH (IU)	Number of litters	Total implants	Percentage resorptions[d]	Percentage cleft palate
C57BL/10	8	15	130	38.0	0[a]
B10.A	8	15	113	32.7	5.2[a,c]
C57BL/10	16	8	69	43.4	0[b]
B10.A	16	8	72	27.7	7.7[b,c]

[a] C57BL/10 vs B10.A, $P < 0.01$.
[b] C57BL/10 vs B10.A, $P < 0.05$.
[c] B10.A, 8 units ACTH vs 16 units, not significantly different.
[d] C57BL/10 vs B10.A, not significantly different at either dose level.

TABLE V. PLASMA CORTICOSTERONE LEVELS IN C57BL/10 AND B10.A VIRGIN MICE GIVEN CORTICOSTERONE ACETATE ip

Strain	Corticosterone (mg)	N	Plasma corticosterone (μg/100 ml) 0 hr	2 hr	6 hr
C57BL/10	None[a]	3	19.9 ± 18.4[b,c,d]	51.9 ± 19.8[c]	89.8 ± 12.0[d]
	5.0	3		959 ± 378	209 ± 87
	9.2	3		>2000	889 ± 89
B10.A	None[a]	3	36.1 ± 17.3[b,e,f]	58.4 ± 10[e]	66.5 ± 16.7[f]
	5.0	3		933 ± 52	178 ± 51
	9.2	3		>2000	991 ± 81

[a] These mice were bled at 0 time and 2 and 6 hr later.
[b] This value was derived from the nine samples obtained from this strain at 0 time. $P > 0.05$.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] $P < 0.05$.
[f] $P < 0.05$.

significantly different and that bleeding alone produced significant and comparable elevations of these levels. Plasma corticosterone levels were very similar in the two strains 2 and 6 hr after the injection of 5.0 and 9.2 mg corticosterone acetate.

Next pregnant C57BL/10 and B10.A mice were injected ip on the 11th or 13th day of gestation with 5 mg corticosterone acetate, and corticosterone levels were determined in maternal plasma and the amniotic fluid 2 hr later. It was found that the injection of corticosterone on the 13th day of gestation produced comparable maternal plasma and amniotic fluid levels in the two strains and that on the 11th and 13th days corticosterone levels in the amniotic fluid were approximately one-half those of the maternal plasma (Table VI).

In the last series of studies corticosterone levels were determined in the plasma of nonpregnant mice and in the maternal plasma and amniotic fluid of pregnant mice under basal conditions and 2 hr after the ip injection of 8 units of repository ACTH (Table VII). Again it was found that under basal conditions B10.A and C57BL/10 mice have comparable plasma corticosterone levels whether pregnant or not. Basal plasma corticosterone levels more than tripled between the 11th and 13th day of pregnancy and remained at this level to the 17th day as has been reported (17). The injection of ACTH produced a 2-fold increase in plasma corticosterone in nonpregnant mice, a 3-fold increase on the 11th day of gestation, and a 4-fold increase on the 13th day (the mice were injected on Days 11–13). The response of the two strains to ACTH were indistinguishable. Corticosterone levels in the amniotic fluid on the 13th day of pregnancy were approximately one-half maternal plasma levels and the injection of the mother with ACTH doubled fetal levels while increasing maternal plasma levels 4-fold. This suggests that a significant barrier exists to the equilibration of maternal plasma and amniotic fluid corticosterone levels. The injection of mice on the 13th day of gestation with 5 mg corticosterone produced maternal plasma corticosterone levels 1.5- to 2.0-fold greater and amniotic fluid levels 3- to 5-fold greater than those induced by 3 days of treatment with 8 units of repository ACTH.

TABLE VI. CORTICOSTERONE LEVELS IN MATERNAL PLASMA AND AMNIOTIC FLUID OF C57BL/10 AND B10.A MICE 2 hr AFTER THE ip INJECTION OF 5 mg CORTICOSTERONE ACETATE

Strain	Day of pregnancy	N	Corticosterone (μg/100 ml) Maternal blood	Amniotic fluid
B10.A	11	3	1475 ± 152[a]	791 ± 258[a]
C57BL/10	13	4	1052 ± 285[b]	535 ± 100[b]
B10.A	13	4	812 ± 328[c]	388 ± 212[c]

[a] $P < 0.01$.
[b] $P < 0.02$.
[c] $P > 0.05$.

Discussion. It has been demonstrated convincingly that the glucocorticoids, cortisone,

TABLE VII. PLASMA CORTICOSTERONE LEVELS IN PREGNANT AND NONPREGNANT C57BL/10 (B10) AND B10.A MICE UNDER BASAL CONDITIONS AND 2 hr AFTER THE ip INJECTION OF 8 UNITS OF REPOSITORY ACTH

Strain	Pregnant?	Day of gestation	ACTH?	N	Corticosterone (μg/100 ml) Plasma	N	Amniotic fluid
B10	No		No	16	47 ± 20[b]		
B10.A	No		No	10	48 ± 19[c]		
B10	No		Yes	9	110 ± 22[b]		
B10.A	No		Yes	8	105 ± 27[c]		
B10	Yes	11	No	3	35 ± 18[d]		
B10.A	Yes	11	No	6	40 ± 17[e]	2	36 ± 5
B10	Yes	11	Yes	9	97 ± 50[d]		
B10.A	Yes	11	Yes	8	133 ± 61[e]		
B10	Yes	13	No	6	133 ± 54[f,h]	4	48 ± 19[h]
B10.A	Yes	13	No	4	139 ± 24[g,i]	5	63 ± 26[i]
B10[a]	Yes	13	Yes	13	500 ± 115[f,j]	10	109 ± 52[j]
B10.A[a]	Yes	13	Yes	9	579 ± 122[g,k]	9	115 ± 55[k]
B10	Yes	16–17	No	3	134 ± 35		
B10.A	Yes	16–17	No	5	149 ± 40		

[a] These mice were given repository ACTH (8 units) ip on Days 11, 12, and 13.
[b–k] $P < 0.01$. Peak responses were obtained 2 hr after the injection of ACTH.

cortisol, dexamethasone, and others produce cleft palate in mice and that the degree of sensitivity to CP induction is determined to a great degree by genes linked to the major histocompatibility complex (1–6). However, these glucocorticoids do not exist naturally in the mouse, and, therefore, they can play no role in spontaneous or stress-induced disease.

Corticosterone is the major adrenal steroid found in the mouse, and it has been demonstrated here and previously that this agent can induce CP when administered in doses comparable to those used to induce CP with glucocorticoids (9, 18). However, unlike the glucocorticoids, sensitivity to corticosterone-induced CP has been shown here to be the same in C57BL/10 and B10.A mice, suggesting that H-2-associated genes may play little or no role in CP induced by this agent. Measurements of maternal plasma and amniotic fluid corticosterone levels at rest and after the administration of ACTH or corticosterone strongly suggest that there are no major differences between the two strains studied under basal conditions, in response to ACTH, in placental transport and/or in the metabolism of corticosterone, and from this, in tissue sensitivity. Although previous studies have shown that both resting and poststress plasma corticosterone levels are under genetic control and can vary significantly between strains, the reported results reveal no correlation with H-2 type and/or sensitivity to glucocorticoid induced CP (19, 20).

In addition, it has been reported that when maternal plasma corticosterone levels during restraint or food deprivation on the 14th day of gestation were compared with those following the injection of a dose of corticosterone (2.5 mg) sufficient to induce a comparable frequency of CP in the progeny (corticosterone 2.5%; restraint, 5%; food deprivation, 7%), it was found that (a) levels were slightly lower in the restrained and food-deprived mice and (b) although there was a significant correlation between high maternal corticosterone levels and the frequency of CP in the offspring of the restrained mice, there was no correlation in the food-deprived group (18). In the studies reported here a near identical dose–response relationship in the frequency of CP was noted when corticosterone was given to the two congenic strains; however, there is no correlation between plasma and amniotic fluid corticosterone levels and CP frequency when comparisons are made between (a) ACTH-treated C57BL/10 and B10.A mice or (b) the ACTH- and corticosterone-treated groups (Table VIII).

The stresses of restraint, food and/or water deprivation, and shipment clearly produce elevations of plasma corticosterone and low frequencies of CP (11, 18, 21, 22). However, as noted above, the elevation of plasma corticosterone produced by stress consistently has

TABLE VIII. COMPARISON OF MATERNAL PLASMA AND AMNIOTIC FLUID CORTICOSTERONE LEVELS (μg/dl) AND CP FREQUENCY IN C57BL/10 AND B10.A DAMS GIVEN CORTICOSTERONE ACETATE, 5 mg,OR ACTH, 8 UNITS ON DAYS 11 THROUGH 14[a]

	C57BL/10			B10.A		
	Maternal plasma	Amniotic fluid	Percentage CP	Maternal plasma	Amniotic fluid	Percentage CP
Corticosterone, 5 mg	1052 ± 285	535 ± 100	4.9	812 ± 328	388 ± 212	3.3
ACTH, 8 units	500 ± 115	109 ± 52	0.0	579 ± 122	115 ± 55	5.2
None	133 ± 54	48 ± 19	0.0	139 ± 24	63 ± 26	2.4

[a] Corticosterone levels were determined on Day 13.

been reported to be lower than that noted when corticosterone is given at a dose which induces the same frequency of clefting. Further, sensitivity to stress-induced CP appears to be strain dependent ((11), and sham-injected controls, Table 11), but the evidence presented here suggests that sensitivity to corticosterone-induced CP may not be.

Similarly, ACTH (a) causes elevations of plasma and amniotic fluid corticosterone levels which are significantly lower than those produced by a dose of corticosterone which produces a comparable CP frequency in H-2[a] mice (10), and (b) does not induce CP in a strain which is resistant to glucocorticoids (C57BL/10). Further, ACTH induces CP in susceptible strains at about the frequency noted in diluent, saline, or sham-injected groups, and no dose–response relationship could be demonstrated in the experiments reported here. This suggests that the effect may not be the result of the physiological action of ACTH but rather that of just another physiologically undefined stress.

When viewed together these and other observations raise the possibility that factors other than or in addition to corticosterone may be involved in spontaneous, ACTH-, or stress-induced CP. The evidence for this is summarized as follows: (a) at a given dose, corticosterone induces CP in C57BL/10 and B10.A mice at the same frequency, but B10.A mice are more susceptible to stress or ACTH induced CP; (b) basal, poststress, and post-ACTH maternal blood and amniotic fluid corticosterone levels are nearly identical in C57BL/10 and B10.A mice; (c) there is no consistent correlation between the level of plasma or amniotic fluid corticosterone levels and the frequency of CP (compare ACTH- vs corticosterone-treated groups, restraint vs food deprivation (18), and ACTH-treated C57BL/10 vs B10.A); (d) the frequency of CP induced in B10.A mice by ACTH is not significantly different from that induced by saline, diluent, or sham injection; (e) stress-induced suppression of lymphocyte stimulation by PHA but not stress-induced lymphopenia can be demonstrated in adrenalectomized rats indicating that stress-induced modulation of immunity is a complex phenomenon involving several mechanisms (24); (f) ACTH and β-endorphin are coreleased from the anterior pituitary in animal stress models (25); and (g) Plasma corticosterone levels are temporally related to immunological hyporeactivity but not to hyperactivity which may be modulated by any number of pituitary neurohumors (26). To sum stress-induced CP does not appear to be caused by the direct action of corticosterone, ACTH-induced CP is most likely caused by the stress of injections rather than the pharmacologic action of the agent, and recent reports raise the possibility that nonadrenal factors may play a role in stress-induced CP.

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