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Extreme Posture Elevates Corticosterone in a Forced Ambulation Model of Chronic Stress in Rats

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HULSE NEUFELD, J., L. BREEN AND R. HAUGER. *Extreme posture elevates corticosterone in a forced ambulation model of chronic stress in rats.* PHARMACOL BIOCHEM BEHAV 47(2) 233-240, 1994.—The adrenocorticotropin (ACTH), corticosterone, and corticosterone-binding-globulin (CBG) levels in rats stressed chronically by a method designed to induce biomechanical stress were investigated in order to determine the relationship between degree of behavioral stress and evoked change in posture during forced ambulation and pituitary-adrenocortical hormone secretion. The method consists of forcing rats to ambulate and compares rats forced to ambulate in a normal (control group) and extreme (experimental) posture. In this method an extreme posture of relative extension was assumed by rats forced to ambulate inside a rotating cylinder. The numbers of faecal boli produced during forced ambulation were determined as a behavioral measure of stress. Rats forced to ambulate in cylinders produced more faecal boli while they ambulated than rats forced to ambulate on a flat surface. Rats forced to ambulate in cylinders had higher resting levels of corticosterone and lower levels of CBG than rats forced to ambulate on a flat surface. The higher corticosterone levels were not associated with increased ACTH levels. Because adaptation to forced ambulation resulted in higher corticosterone and lower CBG levels when exercise is performed in an extreme posture, it is suggested that extreme posture is stressful.

Chronic stress Forced ambulation Extreme posture Rat

NEUROENDOCRINE disorders such as hypercortisolism have been described in many forms of psychiatric illness (3). An animal model for the study of hypercortisolism is, therefore, desired to determine the role of neuroendocrine abnormalities in depressive and other psychiatric illnesses and would be useful in determining behavior correlates with glucocorticoid levels. However, it has been difficult to develop an animal model characterized by high glucocorticoid levels, since the hypothalamo-pituitary-adrenal (HPA) hormone hypersecretion during stress typically decreases with time during the adaptation recovery period. For example, although acute stress results in a rise in corticotrophin-releasing factor (CRF), adrenocorticotropin (ACTH), and corticosterone (5), repeated stress exposures cause adaptation of the HPA axis resulting in a gradual decrement in the release of ACTH in contrast to the large ACTH response characteristic of acute stress response (17,20). Feedback inhibition by corticosterone of CRF and/or ACTH secretion has been described in some models to explain in part the habituation of ACTH to repeated exposure to stress (29). Since the ACTH response to a superimposed novel stressor has been reported to be enhanced by repeated chronic stressors (4,14), the habituation of ACTH responsiveness during chronic stress may involve regulatory changes in hypothalamic CRF drive and/or behavioral adaptation rather than decrease in the pituitary's secretion capacity (23). For example, the release of ACTH in response to a novel, superimposed stressor is significantly greater in streptozotocin (STZ)-diabetic rats (31) and following chronic restraint stress (20) than the release in control rats. Therefore, the adaptive decline in ACTH secretion during chronic exposure to one stressor is selective, and the hypersecretion of corticosterone induced by STZ-diabetes does not inhibit ACTH response to a novel stress. These findings suggest that a "facilitatory trace" may emerge during the chronic stress characterized by HPA axis hyperresponsiveness to new stimuli rather than a desensitization of the HPA axis (1).

The ACTH response in the presence of artificially elevated corticosterone levels has been studied using implanted SC corticosterone-releasing pellets in intact rats (2). The results of exposure to increased levels of corticosterone from implanted pellets showed that, within a range of 0.4 to 10 μg/dl corticosterone, restraint stress in the morning induced an ACTH

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and corticosterone response that was progressively less than the control response. At a corticosterone level of 10 μg/dl the HPA did not respond to a novel restraint stimuli. However, in other studies (11) when corticosterone levels were elevated by stressful stimuli, the facilitatory trace allowed for normoresponsiveness to the new stressor.

The effects on the HPA responses by corticosterone levels elevated by the chronic stress of STZ-diabetes (31) and by chronic immobilization (20) were observed with only 5 and 16 days of chronic stress, a time during which changes in body weights were occurring. The chronically stressed rats either failed to gain weight at a normal rate (31) or even lost weight (20) during the period of chronic stress. These changes in normal body weight gain might represent an endogenous source of novel stress which could interact with the applied novel stressor to initiate an augmented ACTH response. Therefore, a model of chronic stress was sought where elevated levels of corticosterone could be found after the period of weight adjustment.

Since exercise has been proposed as a model of chronic stress adaptation (21,22,23), we have examined the chronic effects of biomechanical loading during forced ambulation (exercise) (19). In previous research on the forced exercise model, 10 days of 15 min/day of forced ambulation in a wheel resulted in partial habituation of pituitary cAMP (22) but no habituation of corticosterone release when measured immediately after the end of the last stress (22,23). The work reported here uses an animal paradigm that incorporates long periods (one and a half months) of intermittent (1 h/day) stress where no loss of body weight occurs. Experimental animals forced to walk in cylinders (extreme posture) were compared to rats forced to walk on a flat surface (normal posture). This article reports levels of ACTH, corticosterone, and corticosterone-binding-globulin (CBG) in tonic and stressed states of this rat model of biomechanical stress and correlates HPA axis changes to behavioral stress experienced in this exercise mode.

METHODS

Design and Variables

Two groups were compared: rats that were forced to ambulate on a flat treadmill and maintain a normal posture (AF), and rats that were forced to ambulate in rotating cylinders and maintain an extreme posture (AC) (19). Both groups of rats were forced to walk for 1 h a day for six weeks. The data from rats not forced to ambulate (NA) is also given for the purpose of comparison with literature values. The variables measured and reported include tonic and acute-stress-driven plasma levels of ACTH, corticosterone, and CBG and numbers of faecal boli produced during forced ambulation.

Animals: Care and Euthanasia

The Sprague-Dawley male rats (175–200 g) used in these experiments were from Harlan Laboratories (San Diego). All procedures for care and experimental treatments were approved by the Animal Use Subcommittee and conformed to the guidelines for humane care of research animals. The rats, one and a half months of age and 206 ± 1 g after two to three days of care in the animal care facility, were housed two to three per home cage for one and a half months. The rats had continuous access to food and water except during times of experimental treatments and during various behavior studies (18). The animals were maintained at a constant temperature under a 12-h white light 0600 to 1800, 12-hour dark cycle. At the time of euthanasia the animals were three months old and weighed more than 300 g (AF, 347 ± 18 g; AC, 346 ± 17 g; and NA, 345 ± 22 g). All animals were euthanized by decapitation or perfusion after a two-week period of daily conditioning to the euthanasia procedure (see below).

Experimental Treatments: Rats Forced (AF and AC) and Not Forced (NA) to Ambulate

The home cages of rats forced to ambulate were carried to the procedure room and the rats were placed in the motor-driven apparatus and forced to ambulate for 1 h a day at 6 ± 1.5 cm/s for one and a half months, five to seven days a week. The rats in groups stressed biomechanically by ambulation in a cylinder (AC, $N = 43$) were forced to tread inside a motor-driven, turning cylinder whose axis was horizontal (19). There were two cylinders which had diameters of 25.5 cm (LD, larger diameter) or 19 cm (SD, smaller diameter) and held 10 (LD cylinder) and 12 (SD cylinder) rats at a time. The rats in groups stressed by ambulation on a flat surface (AF, $N = 39$) were forced to tread in parallel with the rats in the AC group by use of a motor-driven treadmill which held 10 rats at a time. Each of the two batches had rats randomly selected for forced ambulation on the flat treadmill (AF) and in the large (LD AC) and smaller (SD AC) diameter cylinders. Each experimental treatment period of forced ambulation consisted of loading the flat treadmill with 10 rats, loading a cylinder, and then loading another cylinder. After the rats were forced to walk for 1 h, the flat treadmill was unloaded and loaded again with 10 more rats. The order in which rats went first or second into the apparatus and the order within the apparatus was rotated daily.

The rats not forced to ambulate (NA, $N = 64$) were housed in the home room with the rats forced to ambulate.

Habituation to the Decapitation Procedure

Before collection of blood the rats were habituated for 10 to 14 days to the procedure used for euthanasia. The rats were taken, while near the end of their dark cycle, to the conditioning room, which had the lights on, and left for 1–3 h (trough period) before being placed individually into an anesthesia induction chamber (8″ × 5″ × 5″). After 10 to 30 s the induction chamber was carried into another room (the holding room) where the rats were placed into a holding cage. When the rats from a home cage had been conditioned, the rats were returned to the home cage in the holding room. After all the rats had been conditioned, the home cages were returned to the home room in the animal care facility. Later in the day the groups of rats were submitted to their experimental protocol. On the day the blood was taken the rats had not been forced to ambulate for more than 20 h.

Sample Collection

Collection of blood: Tonic, weak, and strong ACTH drive. Blood was collected under three different conditions during the trough period to stimulate different levels of ACTH drive to the adrenal glands. Some rats were decapitated with no anesthesia within 1 min of being removed from their home cages (tonic levels of corticosterone and ACTH—i.e., tonic ACTH drive). Other rats were decapitated within 5 to 10 min of isoflurane gas anesthesia (weak ACTH drive). Still others were anesthetized with isoflurane gas anesthesia, and the pumping heart was surgically exposed prior to perfusion (see below); this blood was also collected within 5 to 10 min of initiating the anesthesia (strong ACTH drive).

Adrenal glands. In one experiment both adrenal glands from decapitated rats (AF, $N = 7$; AC, $N = 7$; NA, $N = 21$) were isolated, cleaned, and weighed as soon as possible after decapitation.

Faecal boli. Because faecal boli production is associated with acutely stressful situations (6,7), faecal boli were collected after each of the 1-h periods of forced ambulation and counted. The number of boli produced by 10 rats during each period of forced ambulation was determined for each of both cylinders (SD and LD) and also for each of both runs of the flat treadmill. The number of boli produced by 10 AC rats in the smaller diameter cylinder (SD) was calculated from the raw number with multiplication by 10/12 because the SD cylinder held 12 rats and the LD cylinder and flat treadmill each held 10 rats. The average number of boli produced by 10 rats was calculated from four values for the AC group and four values for the AF group. The difference between the faecal boli output of the AC rats forced to ambulate in the LD and SD cylinders was determined from the average boli output of three sequential 10- to 11-day periods. The average of three consecutive daily faecal boli outputs by 10 rats in each apparatus was determined using data from the three days just prior to conditioning for decapitation and compared to the mean corticosterone levels from the same 10–12 rats.

Perfusion Procedure

The rats that underwent the acute stress of the perfusion procedure (AF, $n = 9$; AC, $n = 15$; NA = 17) were sedated with isoflurane and surgically opened to expose the pumping heart. A catheter was placed into the heart and blood was withdrawn with a syringe prior to pumping Karnovsky's fixative into the arteries (19).

Assay for ACTH, Corticosterone, and CBG

Truncal blood was collected by decapitation, and an intracardiac blood sample was obtained via cardiac puncture after surgically opening the chest during the perfusion procedure. Approximately 5 ml of blood was combined in plastic conical centrifuge tubes containing 200 μl of a solution of 50 mg/ml EDTA and 500 KIU of aprotonin (Sigma Co., St. Louis). Plasma ACTH was measured with an immunoradiometric assay (IRMA) (28,33). Limit of detection was 1 pg/ml, and intra-assay coefficient of variation was $<5\%$. Plasma corticosterone was measured by radioimmunoassay (RIA) as previously described (20). CBG was analyzed by the laboratory of Dr. Hammond using a binding capacity assay (15). No correction for interassay variations were made.

Statistical Analysis

The effects of the method of blood collection (ACTH drive) and group (AF vs. AC) were determined with two-way analysis of variance (ANOVA) after logarithmic transformation of the ACTH, corticosterone, and CBG data. The ratio of ACTH/corticosterone and linear regression analysis were used to express the relationship of transformed corticosterone to transformed ACTH data with the corticosterone data as the dependent variable. Multiple linear regression analysis was used to determine the significance of difference in the slopes of the relationships of corticosterone to ACTH (8). The significance of the difference in faecal boli output between the rats in the SD and LD cylinders was determined with two-way ANOVA using repeated-measure design. Linear regression analysis was used to show that the groups of rats with highest faecal boli output also had highest corticosterone levels using boli count per 10 rats and mean corticosterone level in the

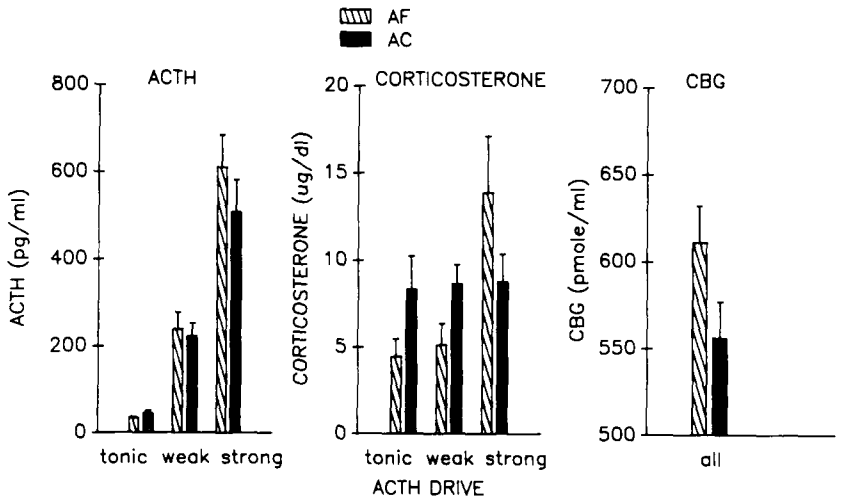

FIG. 1. Tonic and stimulated ACTH, corticosterone, and CBG levels in rats forced to ambulate for 1 h a day for six weeks on a flat surface (AF) and in cylinders (AC). Stimulation occurred by decapitation only (tonic ACTH drive), by exposure to isoflurane gas anesthesia for 5 to 10 min prior to decapitation (weak ACTH drive), or by isoflurane gas anesthesia for 5 to 10 min accompanied by surgical exposure of the heart prior to cardiac puncture for blood sampling (strong ACTH drive). Each bar is the mean ± SE; tonic ACTH drive $n = 12$–13, weak ACTH drive $n = 16$–17, and strong ACTH drive $n = 9$–15 animals/group. By ANOVA for ACTH (left), effect of ACTH drive, $F(2, 76) = 134, p < 0.001$, group p = NS, interaction p = NS; for corticosterone (middle), effect of ACTH drive, $F(2, 76) = 4.13, p < 0.02$, group p = NS, interaction, $F(2, 76) = 4.24$, $p < 0.02$; for CBG (right), effect of group, $F(1, 80) = 4.06, p < 0.05$.

same 10–12 rats. Results were expressed as serum levels of ACTH, corticosterone, and CBG and as faecal boli count ± SE. Statistical analysis of the data was performed by computer using the BMDP statistical software package (13). A p value of less than 0.05 was chosen to be significant.

RESULTS

Three methods of blood collection were used to induce three different levels of ACTH to drive the adrenal glands to secrete corticosterone. Blood was collected by decapitation with no anesthesia, by decapitation under anesthesia, and by cardiac puncture after surgical exposure of the heart under anesthesia. The three levels of ACTH drive were called tonic, weak, and strong ACTH drives because exposure to isoflurane gas anesthesia resulted in increased ACTH levels (231.2 ± 24.2 pg/ml, $N = 33$) compared to rats not exposed (39.7 ± 3.3 pg/ml, $N = 25$), $F(1, 56) = 143.8$, $p < 0.001$. A higher ACTH level (546.6 ± 54.0 pg/ml, $N = 24$) was obtained by surgical exposure of the heart under isoflurane gas anesthesia and cardiac puncture. There was no difference in the ACTH response with these methods of blood collection between rats forced to ambulate on a flat surface (AF) and rats forced to ambulate in cylinders (AC) (Fig. 1, left). The significant change in ACTH levels with the various methods of blood collection, $F(2, 76) = 134$, $p < 0.001$, was not, however, accompanied by a parallel change in corticosterone levels. The corticosterone levels in the AC rats did not change with the ACTH drive (Fig. 1, middle). In analyzing corticosterone levels, there was a significant interaction of group and ACTH drive, $F(2, 76) = 4.24$, $p < 0.02$, which was not observed for the ACTH data. Because there was no significant effect on corticosterone levels of blood collected by decapitation without (tonic ACTH drive) and with (weak) anesthesia, the corticosterone levels with weak ACTH drive are assumed to represent mainly the tonic state. With an analysis of the corticosterone data for conditions of the tonic and weak ACTH drives, rats forced to ambulate in cylinders had higher levels of corticosterone, $F(1, 56) = 8.92$, $p < 0.005$, than rats forced to ambulate on a flat surface. Complementing the corticosterone data, the AC rats had lower levels of CBG, $F(1, 80) = 4.06$, $p < 0.05$ (Fig. 1, right).

The values for ACTH and corticosterone for the NA group were greater than for the AF group under weak ACTH drive—ACTH, $F(1, 33) = 13.7$, $p < 0.001$; corticosterone, $F(1, 33) = 13.9$, $p < 0.001$—but the same in conditions of tonic and strong ACTH drives. Also, the CBG levels were the same in the NA and AF groups (Table 1).

TABLE 1

ACTH, CORTICOSTERONE, AND CBG LEVELS IN RATS NOT FORCED TO AMBULATE (NA)

ACTH Drive	ACTH (pg/ml)	Corticosterone (μg/dl)	CBG (pmol/ml)
Tonic ($n = 29$)	46 ± 4	5.43 ± 0.79	
Weak ($n = 18$)	385 ± 27	10.42 ± 1.17	
Strong ($n = 17$)	638 ± 62	13.49 ± 2.01	
All drives* ($n = 64$)			590 ± 17

Each value represents the mean ± SE in blood collected under tonic, weak, and strong ACTH drive. *The mean value ± SE after combining the values obtained after tonic, weak, and strong ACTH drive.

The ACTH and corticosterone results (Fig. 1) can be expressed as a significant difference in both the ratio of ACTH/corticosterone (Fig. 2, left) and in the relationship of ACTH to corticosterone in linear regression analysis (Fig. 2, middle and right). The difference between the linear regression equations was significant, $F(2, 29) = 6.06$, $p < 0.01$, and the difference resided in the y-intercept. Using multiple linear regression analysis of the corticosterone and ACTH data obtained with blood collected under tonic, under weak, or under strong ACTH drives, no significant difference in the slopes of the relationships could be found between the AF and AC groups.

Although there was no difference in initial and final body weights between the NA, AF, and AC groups, there was a significant difference in the weights of the adrenal glands, $F(2, 32) = 5.57$, $p < 0.01$, and in the weights of the adrenal glands corrected for body weight, $F(2, 32) = 5.04$, $p < 0.02$ (Table 2). The adrenal glands of both groups of rats forced to ambulate were heavier than the adrenal glands from rats not forced to ambulate. There was no difference between the AF and AC groups in body weight or in adrenal weight.

Rats forced to ambulate in cylinders produced more faecal boli than rats forced to ambulate on a flat surface (Fig. 3, left). Rats stressed by ambulation in the larger diameter cylinder (LD AC) produced more faecal boli than rats stressed by ambulation in the smaller diameter cylinder (SD AC) (Fig. 3, middle). Groups of rats with higher corticosterone levels produced more faecal boli (Fig. 3, right).

DISCUSSION

The finding in this model is that biomechanical stress during chronic intermittent forced ambulation is accompanied by increased resting levels of plasma corticosterone and faecal pellet output evidence of behavioral stress. Based on the plasma corticosterone levels, this model would therefore be included among those with "more complex stressors" (16) such as adjuvant-induced arthritis (30) and streptozotocin-induced diabetes (31). Because differences in hormone levels under putative tonic conditions may be the result of different states of arousal, blood was collected under both tonic and weak ACTH drive conditions to determine if stimulation would effect the findings. The experimental paradigm generated three different conditions of blood collection (i.e., tonic, weak, and strong ACTH drive). A relatively weak stimulation was achieved by only exposure to isoflurane gas anesthesia and resulted in ACTH levels elevated six times above tonic conditions and corticosterone levels elevated 1.1 times (i.e., the conditions of weak ACTH drive) (Fig. 1). This weak stimulation occurred within 5 to 10 min before blood collection and did not allow the fuller expression of the adrenal response seen in the AF group with the strong ACTH drive where the ACTH levels were elevated 15 times above tonic conditions and corticosterone levels 3.1 times. Overall, in both conditions, with tonic and with weak ACTH drive, the rats forced to ambulate intermittently in cylinders (AC) for one and a half months had higher levels of corticosterone than rats forced to ambulate on a flat surface (AF), even though ACTH levels were the same. Therefore, under tonic and at least early under weak ACTH drive, the AC rats have higher corticosterone values than AF controls. Also, consistent with other studies using rats submitted to daily restraint (24), crowding stress, (12) or corticosterone-releasing pellets (2), the higher resting level of corticosterone in the AC rats may have blunted the corticosterone response to strong ACTH drive. However, because a complete time course for the pituitary-adrenal re-

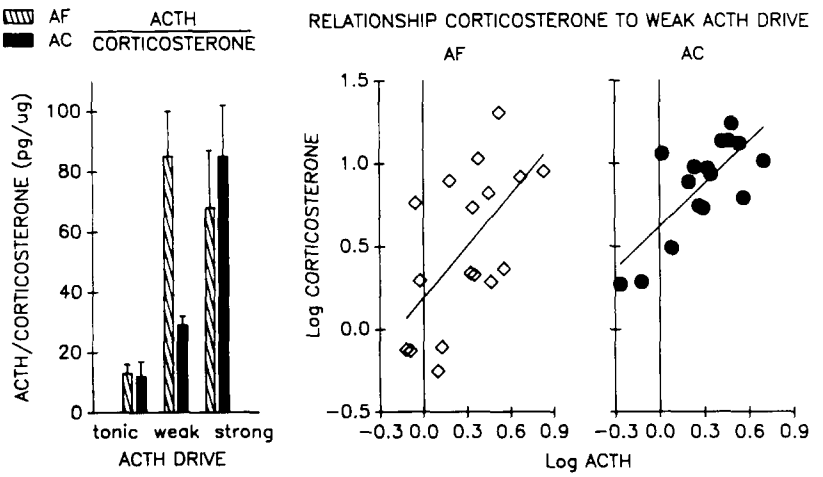

FIG. 2. The ratio of ACTH/corticosterone as in the legend to Fig. 1 and the relationship of ACTH to corticosterone under weak ACTH drive in AF and AC rats. By ANOVA the ratio ACTH/corticosterone (left), effect of ACTH drive, $F(2, 76) = 42.7$, $p < 0.001$, group p = NS, interaction, $F(2, 76) = 3.34$, $p < 0.05$, and with weak ACTH drive AF is unequal to AC, $F(1, 31) = 12.67$, $p < 0.002$. By linear regression AF $n = 17$ animals/group (middle), $y = 1.0x + 0.2$ with $F(1, 15) = 8.9$, $p < 0.01$, $r = 0.6$; AC $n = 16$ animals/group (right), $y = 0.9x + 0.6$ with $F(1, 14) = 17.8$, $p < 0.001$, $r = 0.7$.

sponse was not obtained, it is not known if AC rats would develop a corticosterone response to strong ACTH drive later in the response period (e.g., after 10 min).

Since it was shown that the AC rats had elevated corticosterone levels and thus represented a group more stressed than the AF rats, the weight of the adrenal glands and total body weight were examined to determine if these parameters likewise expressed a difference. However, neither the adrenal weight, body weight, nor adrenal weight corrected for body weight showed a difference between the AF and AC groups (Table 2). However, forced ambulation in either posture resulted in heavier adrenal glands than those from rats not forced to ambulate. The differences in adrenal weights between rats forced to ambulate and NA rats are similar to the findings reported for adrenal glands from rats forced to ambulate 6 h/day for two weeks (32). However, in that study at 28 and 42 days of forced ambulation the difference in the weights of the adrenal glands could not be seen. Clearly, in adrenal weight, adaptation to forced ambulation is occurring over extended periods of time as a component of neuroendocrine changes. In the model presented here, forced ambulation in both normal and extreme posture resulted in an increased adrenal gland weight.

The relationship between ACTH and corticosterone in the plasma has been examined in other studies (17,25). With two weeks of immobilization stress the ratio of ACTH/corticosterone decreased because the resting corticosterone level increased and the ACTH levels were unaffected (25). In that study (25), one explanation for the dissociation between ACTH and corticosterone was an increased sensitivity of the adrenal cortex to ACTH. In a different study (17), the ratio ACTH/corticosterone in rats after chronic intermittent restraint stress increased in response to an acute ether experience because the ACTH hyperresponded and the adrenal cortex

TABLE 2

INDICES OF CHRONIC STRESS IN RATS FORCED TO AMBULATE ON A FLAT SURFACE (AF) AND IN CYLINDERS (AC), AND THOSE NOT FORCED TO AMBULATE (NA)

	Treatment Group		
Variable	AF (n = 7)	AC (n = 7)	NA (n = 21)
Initial body weight (g)*	207 ± 5	214 ± 3	209 ± 2
Final body weight (g)	380 ± 5	381 ± 8	374 ± 6
Adrenal weight (mg)†	46 ± 3‡	47 ± 3‡	37 ± 2
Adrenal weight (mg/100 g body weight)§	12.2 ± 0.7	12.3 ± 0.7¶	9.9 ± 0.5

Values are the mean ± SE. Final body weight and adrenal weights were measured one and a half months after arrival in the animal care facility. *Initial body weight was measured two to three days after arrival at the animal care facility. †$F(2, 32) = 5.57$, $p < 0.01$. ‡AF > NA and AC > NA by post hoc test ($p < 0.05$, Bonferroni procedure). §$F(2, 32) = 5.04$, $p < 0.02$. ¶AC > NA by post hoc test ($p < 0.05$, Bonferroni procedure).

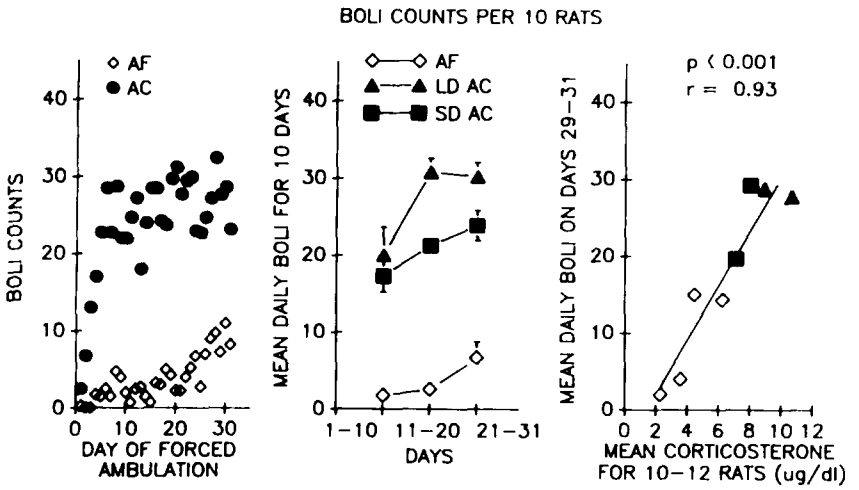

FIG. 3. Faecal boli produced by 10 rats during 1 h of forced ambulation. Daily counts of boli per 10 rats comparing AF to AC rats (left). Each data point is the mean of four determinations. By ANOVA with repeat-measure design the effect of group, $F(1, 6) = 96.9$, $p < 0.001$; effect of day, $F(30, 180) = 4.53$, $p < 0.001$; interaction, $F(30, 180) = 2.11$, $p < 0.002$. The average of 10 consecutive daily counts of boli per 10 rats (middle), comparing AC rats in the larger diameter cylinder (LD) to AC rats in the smaller diameter cylinder (SD). Each data point is the mean ± SE of two determinations. By ANOVA with repeated-measure design the effect of group LD vs. SD, $F(1, 2) = 2613$, $p < 0.001$; effect of period of days, p = NS; interaction p = NS. The relationship between the average of three consecutive daily counts of boli per 10 rats and mean corticosterone levels for the same 10–12 rats (right). Using linear regression analysis, $F(1, 6) = 40.4$, $p < 0.001$, $r = 0.93$.

appeared desensitized to ACTH. In the work reported here, to explain the different relationship of ACTH to corticosterone between the AF and AC groups under weak ACTH drive (Fig. 2) it was hypothesized that either the adrenal glands of the AF rats were unresponsive to ACTH or a suppressor of corticosterone activity in the AF rats interfered with the feedback suppression of ACTH secretion. Because the slopes of the lines in Fig. 2 are a measure of the responsiveness of the adrenal glands to ACTH, a statistical test for parallelism in slopes was used to determine the significance of any difference in corticosterone response to ACTH. No difference between the slopes of AF and AC groups could be detected. Therefore, the corticosterone response to a change in ACTH was the same in AF and AC rats. It was concluded that no difference in the corticosterone responsiveness of the adrenal glands to ACTH could be found. This was the conclusion in diabetic rats about responsiveness of adrenal glands to ACTH drive (31). Also, under strong ACTH drive the adrenal glands of the AF rats released as much (corticosterone as) or more corticosterone than the adrenal glands of the AC rats (Fig. 1). Therefore, the adrenal glands of AF rats were not unresponsive to ACTH, and a difference in adrenal responsiveness cannot explain the relatively low corticosterone levels in the AF rats.

Alternatively, it has been shown that corticosterone regulates the production of CBG in an inverse manner (10). Since the AF rats had higher levels of CBG than the AC rats (Fig. 1), the CBG data supported, in an independent manner, the finding that AC rats have higher corticosterone levels than the AF rats. These concentrations of CBG would accentuate or increase the difference in corticosterone activity between the AF and AC rats even more than the total corticosterone levels indicate. CBG would decrease free corticosterone in the AF rats and possibly lead to depressed occupation of corticosterone receptors in the brain. This in turn would increase ACTH secretion and lead to the observed increase in the ratio of ACTH/corticosterone for the AF rats (Fig. 2, left).

The values for resting levels of ACTH and corticosterone in the NA group were similar to baseline measurements reported in the literature for control groups of Sprague-Dawley rats maintained for one week or longer (4,9,14,23), and the CBG levels were similar to those reported for the mean of sham-operated rats (10). Rats maintained for long periods and used as controls for studying the effects of chronic stress treatments have been shown to be no different than the treatment groups with continuous and discontinuous heat exposure (9) and with the variable stress exposure paradigm (4). However, elevated resting corticosterone levels were observed in experimental groups treated by intermittent forced ambulation for 10 days in a motor-driven wheel (22,23) and by chronic intermittent mild restraint for 21 days (27). In the report on chronic severe restraint, control and treatment groups were the same in corticosterone levels because both the control and experimental group levels were elevated (27). The ACTH and corticosterone levels for the NA group were similar or even greater than the AF group values. Since both AC and AF rats were forced to ambulate, and because there is an acute corticosterone response to forced ambulation (23), the difference between AC and AF groups must be viewed as a difference in recovery from or habituation to the initial elevation in corticosterone level associated with being forced to ambulate. It is suggested that the extreme posture prevented the corticosterone levels in the AC rats from adapting to the low levels seen in the AF rats.

While the rats were forced to ambulate, the AC rats pro-

duced more faecal boli than the AF rats (Fig. 3). Because numbers of faecal boli are markers of behavioral stress (7) and because physical restraint increases defecation (6), the increased defecation in the AC rats could be viewed as restraint-induced. However, in the animal model presented here the restraint was not as severe as in restraining tubes. The volumes of three dimensional space available in the apparatus were calculated to be, for an individual rat in the AF group on the treadmill, 2976 cm^3; in the AC group in the larger diameter cylinder, 510 cm^3; and in the AC group in the smaller cylinder, 283 cm^3. Furthermore, the number of faecal boli produced by the rats in the larger diameter cylinder (LD AC) was greater than that produced by the rats in the smaller diameter cylinder (SD AC) (Fig. 3, right). Also, the mean level of corticosterone in the LD rats (10.00 ± 1.18 μg/dl) was greater compared to SD rats (7.50 ± 1.49 μg/dl), but not significantly so. Therefore, it is unlikely that restraint was the stress that caused faecal pellet output in this model. The possibilities for the cause of the increased faecal pellet output include the biomechanical stress and/or disorientation associated with a turning environment. As indicated by faecal boli output, the AC rats were more stressed during forced ambulation than the AF rats, but it is not known why AC rats in the larger diameter cylinder produced more faecal boli than AC rats in the smaller diameter cylinder. As indicated by resting corticosterone levels, the AC rats were more stressed during times of rest. Because there was a mean difference in boli count and in corticosterone level, the correlation of boli count and corticosterone level was significant, but this does not mean that the boli count was induced by the corticosterone level. Rather, it only demonstrates that there was a mean difference between groups in both boli count and resting corticosterone level.

In conclusion, this model of elevated corticosterone associated with biomechanical stress may be useful in the study of hypercortisolism. However, the response to repeated stress in this model is unique in that, although the tonic ACTH level is habituated after one and a half months to control levels, the corticosterone levels do not reflect the ACTH drive. This shifts the mechanism in alteration in the relationship of corticosterone to ACTH to after the pituitary's activity. Possibilities include changes in metabolic turnover and/or adrenal response. Since no significant difference in response of adrenal glands to the ACTH level could be found between groups of rats, the mechanism of alteration is hypothesized to be the rate of turnover of corticosterone by liver metabolism. In this working hypothesis the lower level of CBG in the AC group would prevent efficient transfer of corticosterone to the liver for catabolic clearance (26), resulting in elevated resting levels of corticosterone.

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REFERENCES

1. Akana, S. F.; Dallman, M. F.; Bradbury, M. J.; Scribner, K. A.; Strack, A. M.; Walker, C.-D. Feedback and facilitation in the adrenocortical system: Unmasking facilitation by partial inhibition of the glucocorticoid response to prior stress. Endocrinology 131:57–68; 1992.
2. Akana, S. F.; Scribner, K. A.; Bradbury, M. J.; Strack, A. M.; Walker, C.-D.; Dallman, M. F. Feedback sensitivity of the rat hypothalamo-pituitary-adrenal axis and its capacity to adjust to exogenous corticosterone. Endocrinology 131:585–594; 1992.
3. Altemus, M.; Gold, P. W. Neuroendocrinology and psychiatric illness. Front. Neuroendocrinology 11:238–265; 1990.
4. Armario, A.; Restrepo, C.; Castellanos, J. M.; Balasch, J. Dissociation between adrenocorticotropin and corticosterone responses to restraint after previous chronic exposure to stress. Life Sci. 36: 2085–2092; 1985.
5. Axelrod, J.; Reisine, T. D. Stress hormones: Their interaction and regulation. Science 224:452–459; 1984.
6. Barone, F. C.; Deegan, J. F.; Price, W. J.; Fowler, P. J.; Fondacaro, J. D.; Ormsbee, H. S., III. Cold-restraint stress increases rat fecal pellet output and colonic transit. Am. J. Physiol. 258: G329–G337; 1990.
7. Bruell, J. H. Genetics and adaptive significance of emotional defecation in mice. In: Tobach, E., ed. Experimental approaches to the study of emotional behavior, vol. 159. New York: New York Academy of Sciences; 1969:825–830.
8. Chatterjee, S.; Price, B. Multiple regression model. In: Bradley, R. A.; Hunter, J. S.; Kendall, D. G.; Watson, G. S., eds. Regression analysis by example. New York: John Wiley & Sons; 1977: 51–73.
9. Cure, M. Plasma corticosterone response in continuous versus discontinuous chronic heat exposure in rat. Physiol. Behav. 45: 1117–1122; 1989.
10. Dallman, M. F.; Akana, S. F.; Cascio, C. S.; Darlington, D. N.; Jacobson, L.; Levin, N. Regulation of ACTH secretion: Variations of a theme of B. Rec. Prog. Horm. Res. 43:113–173; 1987.
11. Dallman, M. F.; Jones, M. T. Corticosteroid feedback control of ACTH secretion: Effect of stress-induced corticosterone secretion on subsequent stress responses in the rat. Endocrinology 92:1367–1375; 1973.
12. Daniels-Severs, A.; Goodwin, A.; Keil, L. C.; Vernikos-Danellis, J. Effect of chronic crowding and cold on the pituitary-adrenal system: Responsiveness to an acute stimulus during chronic stress. Pharmacology 9:348–356; 1973.
13. Dixon, W. J., ed. BMDP statistical software manual. Berkeley: University of California Press; 1985:109, 237.
14. Garcia-Marquez, C.; Armario, A. Chronic stress depresses exploratory activity and behavioral performance in the forced swimming test without altering ACTH response to a novel acute stressor. Physiol. Behav. 40:33–38; 1987.
15. Hammond, G. L.; Lahteenmaki, P. L. A. A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin binding capacities. Clin. Chim. Acta 132:101–110; 1983.
16. Harbuz, M. S.; Lightman, S. L. Stress and the hypothalamo-pituitary-adrenal axis: Acute, chronic and immunological activation. J. Endocrinol. 134:327–339; 1992.
17. Hauger, R. L.; Lorang, M.; Irwin, M.; Aguilera, G. CRF receptor regulation and sensitization of ACTH responses to acute ether stress during chronic intermittent immobilization stress. Brain Res. 532:34–40; 1990.
18. Hulse Neufeld, J. Rat low back dysfunction associated with ambulation in an abnormal posture. Soc. Neurosci. Abstr. 17(Part1):292; 1991.
19. Hulse Neufeld, J. Induced narrowing and back adaptation of lumbar intervertebral discs in biomechanically stressed rats. Spine 17:811–816; 1992.
20. Irwin, M. R.; Hauger, R. L. Adaptation to chronic stress: Temporal pattern of immune and neuroendocrine correlates. Neuropsychopharmacology 1:239–242;1988.
21. Kant, G. J.; Anderson, S. M.; Dhillon, G. S.; Mougey, E. H.

Neuroendocrine correlates of sustained stress: The activity-stress paradigm. Brain Res. Bull. 20:407–414; 1988.
22. Kant, G. J.; Bunnell, B. N.; Mougey, E. H.; Pennington, L. L.; Meyerhoff, J. L. Effects of repeated stress on pituitary cyclic AMP, and plasma prolactin, corticosterone and growth hormone in male rats. Pharmacol. Biochem. Behav. 18:967–971; 1983.
23. Kant, G. J.; Eggleston, T.; Landman-Roberts, L.; Kenion, C. C.; Driver, G. C.; Meyerhoff, J. L. Habituation to repeated stress is stressor specific. Pharmacol. Biochem. Behav. 22:631–634; 1985.
24. Keller-Wood, M. E.; Shinsako, J.: Dallman, M. F. Inhibition of the adrenocorticotropin and corticosteroid responses to hypoglycemia after prior stress. Endocrinology 113:491–496; 1983.
25. Marti, O.; Gavalda, A.; Jolin, T.; Armario, A. Effect of regularity of exposure to chronic immobilization stress on the circadian pattern of pituitary adrenal hormones, growth hormone, and thyroid stimulating hormone in the adult male rat. Psychoneuroendocrinology 18:67–77; 1993.
26. Pardridge, W. M.; Sakiyama, R.; Judd, H. L. Protein-bound corticosteroid in human serum is selectively transported into rat brain and liver in vivo. J. Clin. Endocrinol. Metab. 57:160–165; 1983.
27. Pittman, D. L.; Ottenweller, J. E.; Natelson, B. H. Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: Chronic stress and habituation. Physiol. Behav. 43:47–55; 1988.
28. Raff, H.; Findling, J. W. A new immunoradiometric assay for corticotropin evaluated in normal subjects and patients with Cushing's syndrome. Clin. Chem. 35:596–600; 1989.
29. Rivier, C.; Vale, W. Diminished responsiveness of the hypothalamic-pituitary-adrenal axis of the rat during exposure to prolonged stress: A pituitary-mediated mechanism. Endocrinology 121: 1320–1328; 1987.
30. Sarlis, N. J.; Chowdrey, H. S.; Stephanou, A.; Lightman, S. L. Chronic activation of the hypothalamo-pituitary-adrenal axis and loss of circadian rhythm during adjuvant-induced arthritis in the rat. Endocrinology 130:1775–1779; 1992.
31. Scribner, K. A.; Walker, C.-D.; Cascio, C. S.; Dallman, M. F. Chronic streptozotocin diabetes in rats facilitates the acute stress response without altering pituitary or adrenal responsiveness to secretagogues. Endocrinology 129:99–108; 1991.
32. Taché, Y.; Du Ruisseau, P.; Ducharme, J. R.; Collu, R. Pattern of adenohypophyseal hormone changes in male rats following chronic stress. Neuroendocrinology 26:208–219; 1978.
33. Zahradnik, R.; Brennan, G.; Hutchison, J. S.; Odell, W. D. Immunoradiometric assay of corticotropin with use of avidin-biotin separation. Clin. Chem. 35:804–807; 1989.