Characterization of Corticosterone Feedback Regulation of ACTH Secretion[a]

M. F. DALLMAN, S. F. AKANA, L. JACOBSON, N. LEVIN, C. S. CASCIO, AND J. SHINSAKO

Department of Physiology
University of California, San Francisco
San Francisco, California 94143-0444

The regulation of adrenocortical system function exhibits 3 major characteristics: a circadian rhythm in basal activity, responsiveness of the system to stressors, and feedback control of ACTH secretion by the steroid products of the adrenal cortex. Of these 3, the feedback effects of corticosteroids on ACTH secretion are quantitatively the most prominent. Opening the feedback loop by adrenalectomy causes a massive increase in the drive to ACTH secretion by central neural components of the system, of greater magnitude and longer duration than any other single maneuver that has been studied.

During the last few years, we have determined the effects of presenting a constant corticosterone signal on ACTH secretion in rats. Use of adrenalectomy with provision of a constant feedback signal has allowed us to determine new information about how the entire system is regulated by corticosteroids. In this paper, we will first describe the known effects of adrenalectomy, and then the characteristics of the circadian drive to ACTH secretion. The effects of corticosterone on these drives to ACTH will be discussed. From the results of our experiments, and characteristics of the adrenocortical system described by others, it appears that the site of action of corticosteroids in inhibiting adrenalectomy-induced and circadian ACTH secretion is on the activity of central neural components of the system, rather than at the corticotroph directly. Moreover, the feedback by corticosterone appears to be mediated by association of the steroid with a high affinity, type I, corticosterone-preferring receptor. *In vivo,* dexamethasone is less effective than corticosterone on the inhibition of ACTH, and aldosterone is still less effective.

ADRENALECTOMY-INDUCED CHANGES IN ACTH SECRETION

Bilateral adrenalectomy induces a nearly immediate hypersecretion of ACTH compared to sham-adrenalectomized controls.[1-3] Examination of the rate of transcription of POMC mRNA has also shown a rapid increase in the rate of synthesis after bilateral adrenalectomy.[3,4] However, because there is a time lag of 6–8 h between increased POMC mRNA synthesis and the increase in cytoplasmic POMC mRNA concentrations, and consequently the rate of ACTH synthesis, during the first 24 h after adrenalectomy, the ACTH synthesis rate does not keep pace with the secretory rate, and pituitary stores of ACTH are depleted.[1,3,5] By 10 days after adrenalectomy, a new steady-state is achieved, in which plasma ACTH concentrations are elevated

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10–40-fold above normal, the percentage of anterior pituitary cells that stains for ACTH doubles,[6,7] pituitary ACTH content is increased 3–5-fold, and POMC mRNA levels are increased 2–3-fold.[3,8]

The changes in pituitary and plasma ACTH that occur after bilateral adrenalectomy require input from the central nervous system.[8,9] The paraventricular nuclei (PVN) of the hypothalamus contain parvocellular neurons which synthesize corticotropin-releasing factor (CRF) and which have axons that end in the external layer of the median eminence.[10] Lesions of the PVN and CRF cell bodies[8] or CRF axons,[9] prevent adrenalectomy-induced increases in POMC mRNA levels, pituitary and plasma ACTH concentrations. Moreover, stimulation of the pituitaries of rats with such lesions with a constant infusion of CRF allows adrenalectomy-induced increases in POMC mRNA and ACTH of nearly normal magnitude to occur.[9]

There are marked changes in the parvocellular CRF-containing neurons in PVN that occur after adrenalectomy. The level of mRNA for prepro-CRF increases by 50–200% after adrenalectomy,[11,12] and AVP mRNA can be visualized in parvocellular cells that immunostain for CRF.[13] Under normal conditions, AVP immunostaining is not apparent in parvocellular neurons of the PVN that do immunostain for CRF; however, after adrenalectomy, immunostaining for both AVP and CRF is apparent.[14,15] At the axonal terminals of these cells in the external layer of the median eminence, there is normally visible staining for both CRF and AVP; however, after adrenalectomy, AVP immunostaining increases markedly.[16] The content of AVP in the zona externa of the median eminence increases after adrenalectomy, as does the release of AVP from median eminence fragments in response to high K^+.[17,18]

Thus, adrenalectomy and removal of corticosteroids results in marked increases in the drive exerted by the CRF neurons in the parvocellular PVN on the corticotrophs of the anterior pituitary. Not only is more CRF synthesized and secreted by these cells, but there is an increase in AVP mRNA expression and AVP secretion. The effect of the increase in the combined peptide signals would, from results of studies *in vitro* and *in vivo*, be expected to increase synergistically the synthesis and secretion rate of ACTH above that achieved by CRF alone. All of these changes are prevented by replacement of corticosteroids at the time of surgery.

To determine whether corticosterone inhibits ACTH secretion after adrenalectomy at the brain or at the pituitary, Levin has compared the effects of corticosterone on plasma ACTH in adrenalectomized rats prepared with sham hypothalamic lesions or prepared with hypothalamic lesions and infused with CRF.[19] Concentrations of plasma ACTH in sham-lesioned rats and in lesioned, CRF-driven rats in the absence of steroid were approximately the same. In sham-lesioned rats, small increases in corticosterone concentration decreased ACTH concentration to normal, and the concentrations of corticosterone that were effective in reducing ACTH did not result in thymic atrophy. By contrast, in lesioned, CRF-driven rats the concentrations of corticosterone that were required to restore ACTH concentration to normal also caused thymic atrophy. The low circulating corticosterone concentrations that restored ACTH concentration to normal in sham-lesioned rats were ineffective in the lesioned rats. We conclude from the results of these experiments that corticosterone acts on the brain to reduce adrenalectomy-induced increases in ACTH secretion, and that only at supranormal concentrations of corticosterone is a pituitary-mediated effect observed.

THE CIRCADIAN RHYTHM IN BASAL ACTH SECRETION

At the time of day of the nadir in ACTH secretion, lesions that destroy CRF- and AVP-containing axons to the external layer of the median eminence have no effect on

basal ACTH and corticosterone levels, compared to sham-lesioned controls.[9,20] Moreover, at the time of the nadir in the circadian adrenal rhythm, the system appears to be turned off; *i.e.*, plasma ACTH concentrations are low, and plasma corticosterone concentrations cannot be distinguished from those obtained in adrenalectomized rats.[2] These results suggest strongly that, under truly basal conditions, the hypothalamus does not drive the anterior pituitary during the time of the nadir in adrenocortical system activity. By contrast, at the time of peak basal activity in the adrenocortical system, the hypothalamus does drive secretion of ACTH from the corticotroph. Plasma ACTH concentrations remain at the circadian minimum levels in lesioned rats sampled at the time of the circadian maximum.[9,20]

Relying on the fact that AVP is a weak ACTH secretogogue by itself, but markedly potentiates CRF-driven ACTH secretion, Salata *et al.*[21] have derived information about the circadian regulation of ACTH secretion in man that is similar to the evidence obtained by more direct experimentation in rats. These investigators have tested the magnitude of ACTH responses to an injection of AVP administered either during the nadir or the peak of the adrenocortical rhythm in man. AVP did not stimulate ACTH secretion when given at the time of the nadir, but stimulated a marked ACTH response when given in the morning, at the time of the circadian peak in activity. These results are interpreted by the authors to suggest that during the time of peak adrenocortical activity, CRF is driving ACTH secretion from the corticotroph, thus allowing AVP to potentiate the action of CRF; during the nadir in adrenocortical activity, AVP is ineffective because there is no CRF signal from the hypothalamus for AVP to potentiate.

The circadian drive to CRF-secreting neurons in the PVN appears to be supplied by cells in, or fibers passing through, the suprachiasmatic nuclei (SCN). Lesions of the SCN have been found to decrease rhythms in both plasma ACTH[22,23] and corticosterone[22–24] concentrations throughout the day and set them at values normally observed at the time of the circadian minimum of adrenocortical system activity. Although the circadian input theoretically could act to inhibit CRF secretion during the nadir of the rhythm, the recent experiments of Cascio *et al.*[23] showed that in the absence of a normal input from the SCN, circulating ACTH concentrations, amplified by our experimental paradigm, were low at the time of normal peak activity, confirming and clarifying the results of others (FIGURE 1).

Thus, under normal conditions, it appears that the pituitary is not stimulated by CRF during the time of trough activity in the adrenocortical system, but during the time of peak basal activity, drive from neurons in, or passing through the SCN activates CRF secretion from neurons in the PVN.

ADRENALECTOMY AND CIRCADIAN MINIMUM ACTH SECRETION

It is clear that after adrenalectomy there is increased activity in the CRF neurons, and that the increased AVP and CRF synthesis and secretion results from lack of the normal concentrations of corticosteroids in the circulation. It is not at all clear where in the central nervous system the receptors are that, when normally occupied by corticosterone, maintain CRF cells in a state of normal excitability and cause them to synthesize CRF and AVP at the normal rates and in the ratios that are characteristic of the intact rat.

Two types, and distributions, of corticosterone receptors have been described in rats. Type I, high affinity, aldosterone-preferring receptors (K_d = 0.5 nM) have been described in high abundance in the hippocampus and lateral septum,[25–27] whereas type II, lower affinity, glucocorticoid receptors (K_d = 2.5–5 nM) have been described in

good abundance in various parts of the brain, including the PVN.[27,28] A high affinity, corticosterone-preferring receptor has been described in pituitary,[29] however, the specific distribution of this high affinity binder has not yet been described in the central nervous system.

Replacement of bilaterally adrenalectomized rats with subcutaneous pellets containing corticosterone at the time of surgery prevents the marked changes in pituitary and plasma ACTH concentrations that usually accompany adrenalectomy.[2,30] We have studied such rats 5–7 days after adrenalectomy to determine the characteristics of corticosterone feedback on basal ACTH secretion in the morning, at the time of the

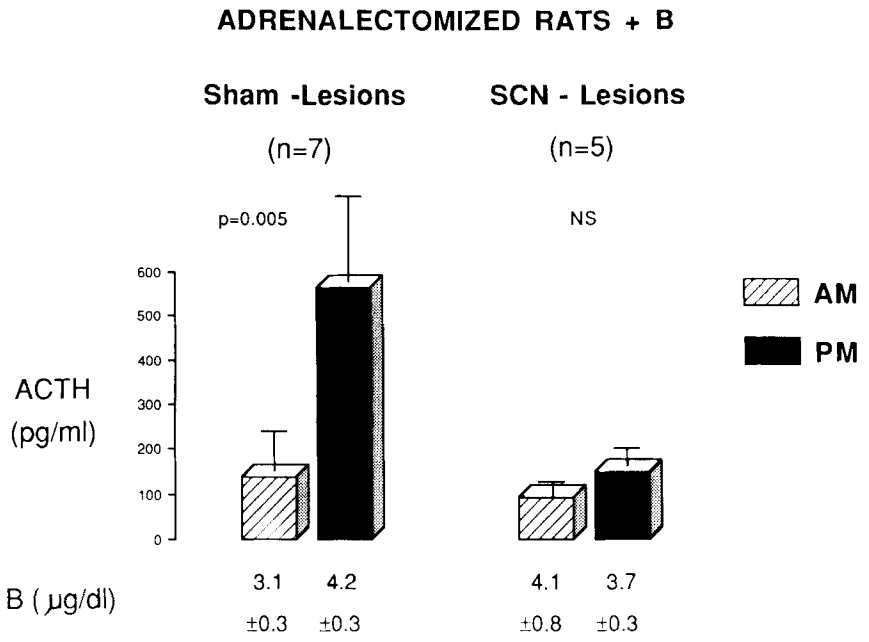

FIGURE 1. Plasma ACTH concentrations obtained within 2 h of lights on (AM) or lights off (PM) from rats with sham lesions of the suprachiasmatic nuclei (SCN) or from rats with SCN lesions. All rats were provided with indwelling femoral arterial cannulas prior to lesion placement and adrenalectomy with subcutaneous insertion of a fused pellet of 40% corticosterone:cholesterol. Because of the constant corticosterone concentrations, the diurnal rhythm in ACTH is amplified,[38] revealing clearly that normally structures in, or passing through the SCN stimulate (CRF and) ACTH secretion at the time of the circadian peak.[31]

nadir in circadian activity. We have routinely found that when plasma corticosterone concentrations are maintained at relatively constant values of 2–4 μg/dl (~100 nM total corticosterone), morning plasma ACTH concentrations are in the range observed in sham-adrenalectomized controls (FIGURE 2). The estimated free concentration of corticosterone (measured by an ultrafiltration technique, at 37°C) which provides 50% inhibition of ACTH from the high concentrations observed in adrenalectomized, unreplaced rats, is 0.8 + 0.05 nM. This concentration of corticosterone is lower than that which decreased by 50% the concentration of corticosteroid-binding globulin

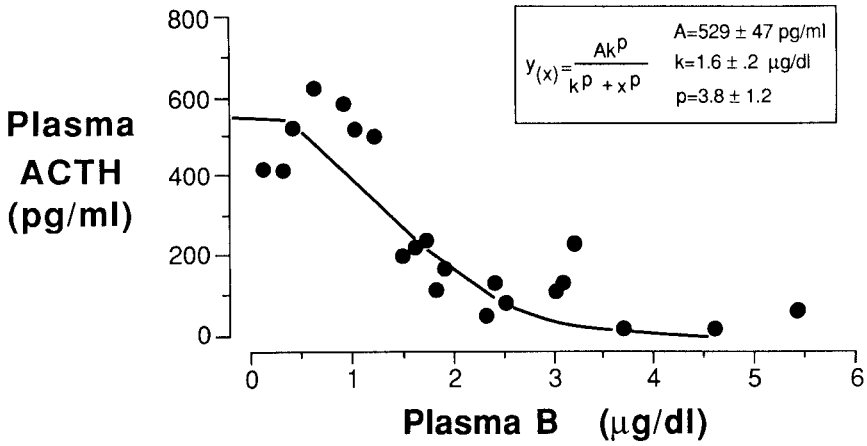

FIGURE 2. Plasma ACTH in adrenalectomized, corticosterone (B)-replaced rats in the morning. Plasma ACTH is plotted as a function of plasma B in individual rats. The line represents the best fit of the data to the Hill function:

$$y_{(x)} = \frac{Ak^p}{k^p + x^p}$$

where A = the theoretical ACTH concentration
when B = 0; k = the concentration of corticosterone at which ACTH values are half-maximally reduced; and, p = the Hill coefficient.

(CBG) in the circulation, and reduced thymus wet weight by 50% (ca. 4.5 nM; FIGURE 3). The latter two endpoints are classical glucocorticoid targets, and we tentatively concluded that corticosterone might inhibit ACTH secretion via an association with type I, high affinity corticosteroid receptors, whereas the inhibition of CBG and thymus wet weight was compatible with an action of corticosterone mediated by type II, glucocorticoid receptors.[31]

We next tested the effectiveness of corticosterone, compared to dexamethasone (a potent synthetic glucocorticoid) and aldosterone, a mineralocorticoid, on the inhibition of adrenalectomy-induced ACTH secretion. In these experiments also, we compared the relative glucocorticoid potencies of corticosterone and dexamethasone using circulating CBG concentrations and thymus wet weight. Adrenalectomized rats were replaced at the time of surgery with either pellets of corticosterone:cholesterol[30] or miniosmotic pumps that delivered dexamethasone or aldosterone. Five days later the rats were killed in the morning and plasma ACTH and CBG concentrations and thymus wet weight were measured. The results of these experiments show that for infusions of corticosterone and dexamethasone which were equally effective on the suppression of thymus weight, corticosterone was more effective than dexamethasone in the suppression of ACTH (FIGURE 4). With the infusion rates used in these experiments, aldosterone did not affect either thymus weight or ACTH (not shown).

In subsequent experiments, corticosterone was found to be approximately 2–5-fold more potent than dexamethasone or aldosterone in inhibiting the secretion of ACTH when determined by measurement of circulating steroid concentrations (ultrafilterable, free corticosterone, total dexamethasone and aldosterone). Dexamethasone and corticosterone were roughly equipotent on thymus wet weight and CBG concentra-

tions, and aldosterone was ineffective on these glucocorticoid-sensitive endpoints at circulating concentrations up to 30 nM.

We conclude from the above sets of experiments that the central neural drive to ACTH secretion caused by adrenalectomy is normally kept in check by association of corticosterone with high affinity, type I corticosterone receptors, rather than with lower affinity, type II glucocorticoid receptors. In addition to the IC50 for corticosterone of ca. 0.8 nM, which is compatible with the known K_d for the type I receptor of 0.5 nM, aldosterone infusions resulted in inhibition of ACTH secretion at concentrations of ca. 3 nM, whereas this steroid had no effect on either of the peripheral glucocorticoid targets that we measured. Although both dexamethasone and corticosterone altered CBG concentrations and thymus wet weight, these peripheral effects were exerted at higher circulating steroid concentrations than their effects on ACTH.

At first glance, this conclusion seems to be incompatible with the results of 2 sets of recent studies that have examined the effectiveness of treatment of adrenalectomized rats with steroids either infused systemically,[32] or implanted locally over the PVN.[33,34] The results of both sets of studies led to the conclusion that the inhibition of adrenalectomy-induced increases in CRF and AVP in parvocellular cells of the PVN are prevented by dexamethasone better than by corticosterone.

Sawchenko[32] has shown that systemic infusions of both corticosterone and dexamethasone, but not deoxycorticosterone or aldosterone, prevent adrenalectomy-

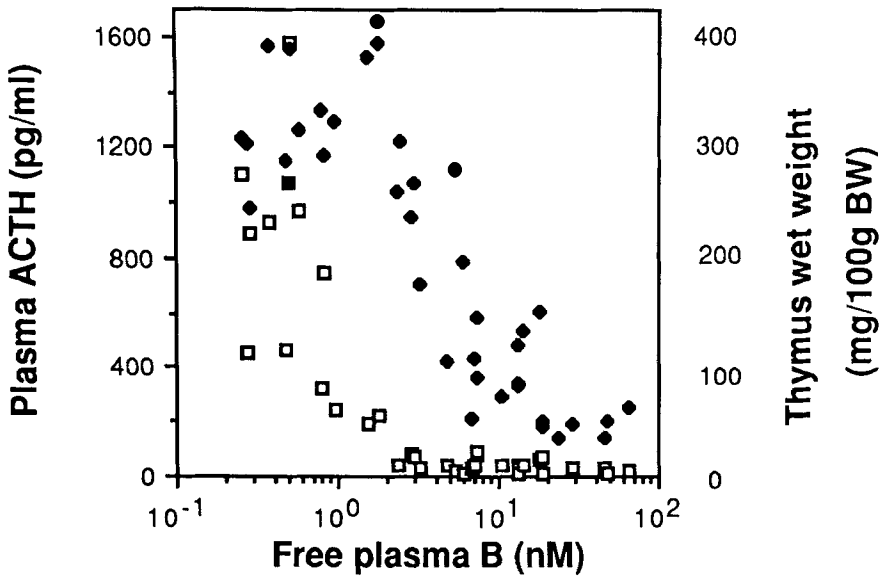

FIGURE 3. The relationships between plasma ACTH (*open squares*) and thymus wet weight (*closed diamonds*) and free plasma corticosterone (B) concentrations in adrenalectomized rats 5 days after adrenalectomy and replacement with B. Free B concentrations were calculated as the product of the percent steroid ultrafiltered at 37°C[46] and the total plasma B concentrations. ACTH secretion is inhibited by lower concentrations of plasma B than is thymus wet weight. The IC50 for ACTH is 0.8 nM, compatible with an effect mediated by association of B with type I corticosteroid receptors, whereas the IC50 for thymus wet weight is 4.4 nM, compatible with an effect mediated by the association of B with type II glucocorticoid receptors.

induced changes in AVP- and CRF-immunostaining in the PVN. The same infusion rate of each steroid was provided for 5 days to adrenalectomized rats. Because dexamethasone has a much longer half-life than corticosterone, and is not bound in the circulation to transcortin, as is corticosterone, this treatment regimen must have

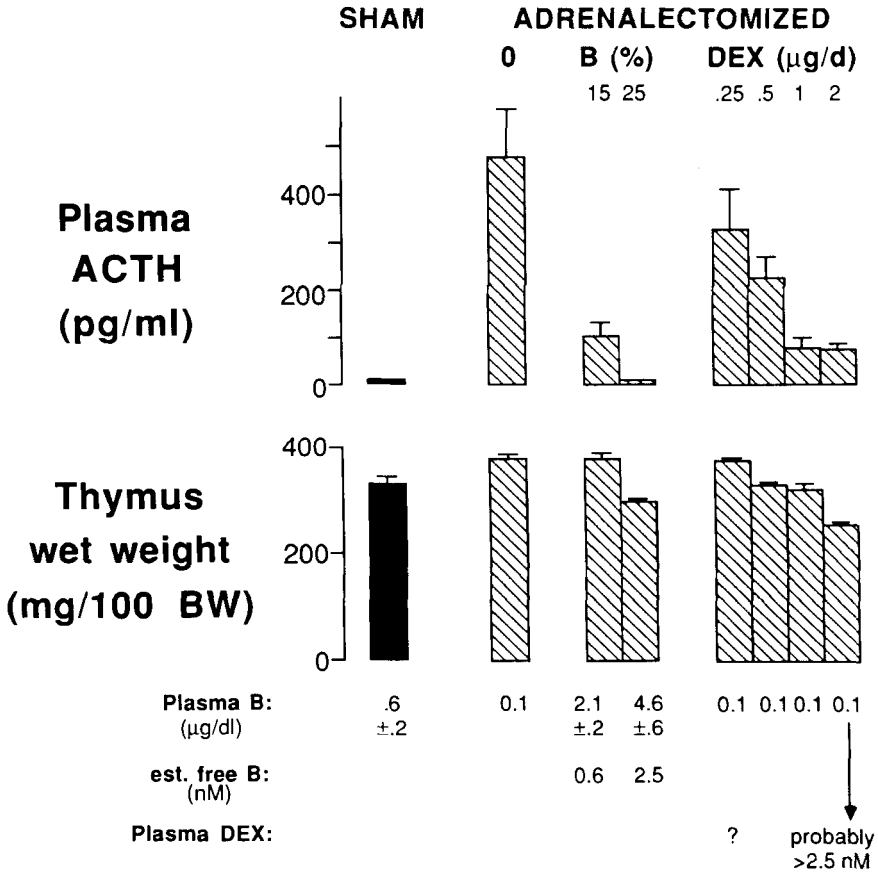

FIGURE 4. Plasma ACTH concentrations (*top*) and thymus wet weight (*bottom*) in the morning 5 days after surgery in sham-adrenalectomized rats (*black bars*), and in adrenalectomized rats with no replacement, or replaced at surgery with constant infusions of corticosterone (B) or dexamethasone (DEX). Plasma B concentrations are indicated at the *bottom* of each group of 6 rats. Although B and DEX infused at these rates are approximately equipotent on the inhibition of thymus wet weight, B is significantly more effective on plasma ACTH then DEX. An estimate of plasma free B and DEX concentrations is indicated at the *bottom* of the figure.

resulted in much higher effective circulating concentrations of dexamethasone than corticosterone. Nonetheless, corticosterone treatment prevented the effects of adrenalectomy on CRF and AVP staining in the PVN nearly as well as dexamethasone. The fact that neither deoxycorticosterone nor aldosterone appreciably affected the adrenal-

ectomy-induced expression of the peptides is compatible with the relatively high concentrations of aldosterone that we have found are required for inhibition of adrenalectomy-induced ACTH secretion, as well as with the short half-life of these steroids in plasma. Thus, in this study, a much lower effective dose of corticosterone was nearly as potent as a very large dose of dexamethasone in inhibiting the changes induced by adrenalectomy, in agreement with our findings that corticosterone is more effective than dexamethasone on the inhibition of ACTH secretion after adrenalectomy.

Kovacs and Makara have reported that crystalline implants of dexamethasone placed in the vicinity of the PVN prevent the normal increases in AVP and CRF staining that occur after adrenalectomy.[33] The effect appears to be exerted directly on the PVN cells, since it could be obtained ipsilaterally after a unilateral implant. However, of considerable interest, implants of corticosterone did not prevent the increased staining in PVN after adrenalectomy, although these implants were quite large. These results suggest either that the site of corticosterone receptors in brain that control basal activity in the PVN is geographically removed from the PVN *per se,* or, that there was insufficient delivery of corticosterone to the PVN from the implant site. It seems likely in those studies that dexamethasone inhibited CRF and AVP staining by its interaction with type II glucocorticoid receptors in or near PVN neurons. Similar results have also been reported by Sawchenko.[34]

If there are only two receptors in brain and other tissue that recognize corticosterone, a high affinity receptor that recognizes aldosterone and corticosterone equally and dexamethasone less well, and a lower affinity receptor that recognizes dexamethasone best, but also corticosterone and aldosterone, then how can the results of our experiments which show clearly that corticosterone is by far the most effective of the 3 steroids on ACTH secretion be rationalized? Sheppard and Funder have examined the same question in a variety of tissues which contain high affinity corticosteroid receptors. These authors have shown that the binding of 3H-corticosterone and 3H-aldosterone is equivalent in renal, parotid and colonic cytosolic preparations, but that when the labelled steroids are presented *in vivo,* in the presence of a saturating dose of an unlabelled synthetic glucocorticoid agonist, there is selective uptake of 3H-aldosterone by a cytosolic binder in these tissues, and very little uptake of 3H-corticosterone.[35] These results suggest that there are tissue-specific factors which allow discrimination between the uptake of aldosterone and corticosterone by cells which contain a cytosolic binder that does not distinguish between the 2 steroids. From the experiments of Sheppard and Funder, it does not seem that the specificity is provided by preferential binding of corticosterone by transcortin since the same *in-vivo* results were obtained in adult rats, which have normal concentrations of transcortin, and in 10 day old rat pups, in which transcortin concentrations are extremely low.

CORTICOSTEROID CONTROL OF CIRCADIAN MAXIMUM ACTH SECRETION

There appears to be a reset in the sensitivity of controlling elements of the adrenocortical system to feedback inhibition by corticosteroids between the time of the nadir and the peak of the circadian rhythm in man[36] and rats.[2,37,38] The shift in feedback sensitivity is clearly shown by the marked increases in circulating ACTH concentrations at the time of day of the circadian maximum in adrenalectomized rats maintained on a constant replacement of corticosterone.[38] We proposed recently,[2] that the shift in corticosteroid feedback efficacy that occurs between the circadian nadir and peak could be accounted for if feedback effects of corticosteroids on ACTH

secretion in the morning (nadir for rats) were mediated by association of the steroid with the type I, high affinity receptors, and if feedback in the evening (peak for rats) were mediated by association of the steroid with the lower affinity, type II receptors.

We have tested this hypothesis by comparing the efficacy of corticosterone, dexamethasone and aldosterone signals on ACTH in the morning and evening in adrenalectomized rats. If there is a shift in the receptor type used for the feedback control of ACTH between nadir and peak of the circadian rhythm, then dexamethasone would be expected to be less potent than corticosterone during the time of the nadir (when type I receptors mediate the steroid-induced inhibition), and at least as potent as corticosterone during the time of the peak (when it is proposed that type II receptors mediate the steroid-induced inhibition). The results of these experiments showed that corticosterone was more potent in the inhibition of ACTH secretion of adrenalectomized rats both in the morning and evening than either dexamethasone or aldosterone (Dallman, Levin, Akana, Cascio, Jacobson, Kuhn and Siiteri, unpublished). Therefore, the inhibition of basal ACTH secretion is mediated by association of corticosterone with high affinity, type I receptors at both times of day.

This conclusion is at variance with that of Reul and De Kloet[27] who propose that the high affinity corticosteroid receptors in rat brain are 80% or more occupied at all times of day. The conclusion of Reul and De Kloet was arrived at as the result of experiments in which the authors examined the degree of type I receptor occupancy 60 min after the injection of unlabelled corticosterone. Receptor occupancy was determined and then related to the circulating concentrations of corticosterone at the time of sacrifice.[27] It seems very likely from results reported by Hodges and Jones,[39] that after s.c. injections of corticosterone at 0 time, circulating corticosterone concentrations were considerably higher 7.5 to 30 min after injection than they were at 60 min. Because there is uptake and retention of corticosterone by its receptors, it seems likely that the corticosterone signal that occupied the type I receptors in the studies of Reul and De Kloet was greater than that they measured 1 hour after injection. The demonstration by Reul and De Kloet of 90% occupancy of type I receptors in resting rats in the morning assumes that the period of 4 min required for manipulation of the rats before collection of hippocampal tissue did not cause appreciable corticosterone secretion. Clearly, the conclusion that type I receptors are nearly fully occupied at all times of day in the intact rat will have to be carefully reexamined. In our experiments, we are unable to detect a difference in basal morning corticosterone concentrations between intact and adrenalectomized rats,[2] suggesting that, at least under these conditions, the type I receptors are not occupied. The steroid specificity and concentrations at which these decrease ACTH concentrations in the morning and evening in adrenalectomized rats also suggest that changes in type I receptor occupancy probably occur throughout the day in intact rats to result in the modulation of ACTH secretion.

If basal ACTH secretion during the morning and evening is regulated in part by the consequences of association of corticosterone with type I receptors, what accounts for the shift in sensitivity of animals to the feedback effect of corticosterone between the circadian nadir and peak in activity? A possible explanation for the altered effectiveness of corticosteroid feedback between peak and trough times of ACTH secretion is that there is drive from the SCN to CRF secretion during peak ACTH secretion, whereas there is not drive to CRF secretion from other brain structures at the time of trough ACTH secretion. If the neural pathway that transmits circadian peak drive to CRF secretion contains a feedback element, then in the evening one would see the results of the effects of corticosterone acting on 2 feedback elements in series (one in, or between, the SCN and the CRF neuron, the other acting on the CRF neuron, *per se*). In the morning, one would only see the results of the effects of

corticosterone acting on 1 feedback element (the one that acts on the CRF neuron). We have previously discussed the multiplicative effects that would be expected of series feedback elements.[40] In our previous,[38] as well as our present studies, we have found that maximal inhibition of ACTH secretion in adrenalectomized, corticosterone-replaced rats occurs at the same concentration of corticosterone in plasma in the morning and evening, and that it is only the sensitivity of the corticosterone effect on ACTH that is higher in the morning than the evening. This result would be expected, if both the drive to CRF secretion and the number of series feedback elements were increased at night.

A consequence of the tight regulation of ACTH, and thus corticosterone, secretion by the effects of association of corticosterone with the high affinity, type I corticosteroid receptors, is that in the absence of stress, the mean circulating free corticosterone

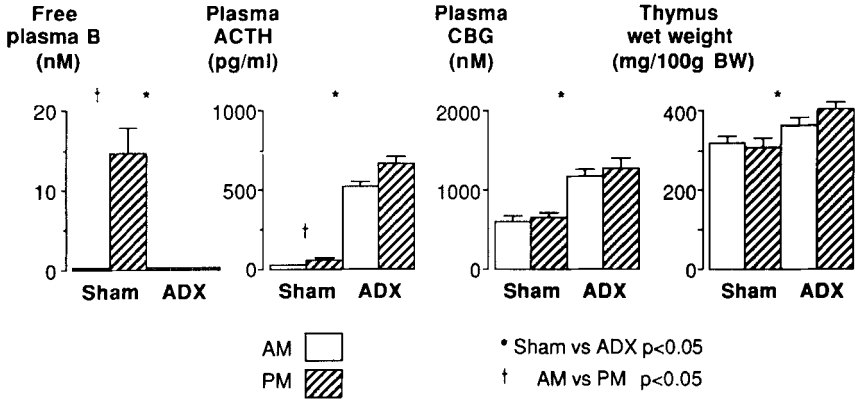

FIGURE 5. Plasma free B (*left*), ACTH (*left middle*), CBG (*right middle*) concentrations and thymus wet weight (*right*) in the AM and PM in rats 5 days after sham-adrenalectomy (sham) or adrenalectomy (ADX). There is a marked rhythm in B and a small rhythm in ACTH between AM and PM but no changes in CBG or thymus weight as a function of the time of day in the sham-adrenalectomized rats (n = 9–10/group, sham). Plasma B concentrations are significantly decreased in ADX rats only in the PM; all other variables are significantly increased in the ADX rats compared to sham, and there were no significant AM-PM differences in any variable (n = 10–11/group, ADX).

concentrations averaged over a 24-hr period are maintained at approximately 1–1.5 nM. This concentration of steroid would be predicted to occupy only a low percentage of the type II, glucocorticoid receptors, thus maintaining glucocorticoid target tissues at the low end of the glucocorticoid dose-response curve. A considerable increase in the occupation of the type II receptors would be expected to occur following prolonged stress.

That the peripheral glucocorticoid targets, thymus wet weight and CBG concentrations, appear to integrate the daily concentrations of corticosterone is shown in FIGURE 5. Although both circulating corticosterone and ACTH concentrations exhibit a diurnal rhythm in sham adrenalectomized rats, there is no AM-PM difference in either thymus wet weight or CBG concentration (first pair of bars in each panel, FIGURE 5; the second pair of bars in each panel of the figure shows the effect of removal of corticosterone by adrenalectomy). Thus, the peripheral target tissues for corticoste-

rone do not appear to respond to the marked daily excursions in circulating corticosterone concentrations in rats with endogenous rhythms in the steroid; these endpoints are clearly responsive to mean circulating corticosterone concentrations experienced over a period of several days (FIGURE 5, last 2 panels, compare sham-adrenalectomy to adrenalectomy; and FIGURES 3 and 4, thymus wet weight.[2,30])

CONCLUSIONS

Recent work has shown that the corticosteroid feedback that normalizes adrenalectomy-induced ACTH secretion is exerted specifically on the brain and not at the pituitary. Our further studies have provided strong evidence that the effects of corticosterone on parvocellular neurons in the PVN are mediated by association of the steroid with high affinity, type I corticosteroid receptors. Because high concentrations of type I receptors have been reported to be localized in only hippocampus and lateral septum,[25,27] it is possible that the effect of the steroid on CRF/AVP synthesis and secretion in PVN is mediated transsynaptically. Finally, the corticosterone-induced inhibition of ACTH secretion appears to be exerted through association of corticosterone with the high affinity, type I receptors during both the diurnal trough and peak in basal activity in the adrenocortical system.

These findings increase our understanding of the basic operating principles in the adrenocortical system. They reveal a sophisticated control system in which the association of steroid with high affinity receptors in brain ultimately regulates adrenocortical secretion. This control, in combination with the fact that at least several peripheral target tissues are responsive to corticosterone via association of the steroid with lower affinity, glucocorticoid receptors, ensures a eucorticoid state even when minor stresses occur. Transient, stress-induced increases in adrenocortical function will provide a feedback signal to central control elements which probably acts to decrease activity in the system for the next few hours (*e.g.,* REFERENCE 40). In this way, mean circulating corticosteroid concentrations over periods of days are maintained in the normal low range, providing an appropriate, carefully defended, low level of occupation of the type II glucorcorticoid receptors.

These studies also have considerable implications for a set of human disease states which are characterized by elevated ACTH and corticosteroid concentrations throughout the day. These include hypothalamic Cushing's disease, depression, anorexia nervosa, and bulemia.[41] Aging has also been associated with increased mean corticosteroid levels.[42] In each of these conditions, not only is basal ACTH secretion increased, but the feedback regulation of ACTH has also been suggested or shown to be abnormal.[42–44] Our findings would suggest that in each of these states there is an accompanying, if not causal, specific abnormality of the association (or its result) of corticosteroids with the high affinity type I, rather than the type II corticosteroid receptors. Our results suggest that treatment of patients with hypothalamic Cushing's disease, or other of the above abnormalities, with steroids which are designed with specific agonist activity for the high affinity corticosteroid receptor might effectively ameliorate the hypercortisolemia and deleterious target effects of excess glucocorticoids. Conversely, highly specific glucocorticoid agonists currently exist (*e.g.,* REFERENCE 45). Use of these for therapy might decrease, to some extent, the profound depression of the adrenocortical control elements which is an undesirable side effect of glucocorticoid therapy. These notions, as well as the potential application of basic work to clinical problems, are in the tradition so well established and supported by Dr. Krieger.

SUMMARY

Adrenalectomy-induced increases in ACTH secretion in rats are returned to normal by an action of corticosterone on the brain, not on the pituitary. Five days after adrenalectomy with constant steroid replacement, the concentration of free corticosterone in plasma which reduces plasma ACTH by 50% is approximately 0.8 nM. By contrast, the concentration of free plasma corticosterone required for 50% reduction of thymus wet weight or plasma transcortin concentration (both targets for glucocorticoid action) is about 4.5 nM. These results suggested that the inhibition of ACTH by corticosterone might be mediated by association of the steroid with high affinity, type I corticosteroid receptors, whereas the inhibition of thymus weight and transcortin might be mediated by association of the steroid with lower affinity, type II receptors. The results of studies comparing the ability of corticosterone, dexamethasone and aldosterone to inhibit adrenalectomy-induced ACTH secretion support the hypothesis that basal ACTH secretion in rats is mediated by association of corticosterone with type I receptors.

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