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REGULATION OF BASAL ACTH SECRETION BY CORTICOSTERONE IS MEDIATED BY BOTH TYPE I (MR) AND TYPE II (GR) RECEPTORS IN RAT BRAIN

M. J. BRADBURY,[1]* S. F. AKANA,[1] C. S. CASCIO,[1] N. LEVIN,[2] L. JACOBSON[3] and M. F. DALLMAN[1]

[1]University of California, San Francisco, San Francisco, CA 94143-0444, [2]Fishberg Center for Neurobiology, M6 Sinai NY, NY 10029 and [3]Stanford University, Stanford, CA 94305, U.S.A.

Summary—The mechanisms involved in the physiology of the secretion of ACTH are reviewed. The secretion is regulated by the biological consequences of the occupancy of high affinity mineralocorticoid (MR) and lower affinity glucocorticoid receptors (GR) for corticosterone at specific sites of the rat brain. The regulation by this mechanism of basal secretion during the circadian rhythm, the effect of adrenalectomy and of corticosterone replacement is discussed. Experiments with RU486, a specific glucocorticoid antagonist, suggest that occupancy of both MR and GR is required for normal control of ACTH at the time of peak activity. The occupancy of the GR for a few hours per day apparently suffices to maintain steady levels of the products of GR-responsive genes throughout the body.

INTRODUCTION

Activity in the adrenocortical system exhibits 3 major characteristics: an endogenous circadian rhythm in basal activity, feedback inhibition of neurosecretory peptides and ACTH by hormonal products of the target adrenal glands, and stress responsiveness. In the following we explore the interactions between the secretion of ACTH and corticosteroid feedback under basal circadian conditions.

We review the evidence that basal ACTH secretion is controlled at all times during the circadian rhythm by corticosteroids, examining in rats the effects of adrenalectomy (ADX), corticosterone (B) replacement, the site of feedback, the probable receptor types mediating it, and the physiological consequences of the involvement of two receptors with differing affinities for B on control of ACTH.

DUAL CONTROL OF ACTH SECRETION BY CORTICOSTEROIDS

Figure 1 shows schematically the normal rhythms in ACTH and B in intact rats (left) and the consequences of adrenalectomy (ADX) without (left middle) and with steroid replacement, provided either tonically (middle right) or phasically (right). Immediately noteworthy are the low amplitude ACTH and very high amplitude B rhythms in intact rats (left). During the nadir of the rhythm, total plasma B concentrations in intact rats are indistinguishable from those we measure in ADX rats, about 3 nM; during the peak of the rhythm, B values increase by 100–200-fold, up to 600 nM. Average mean daily total plasma B values are ~170 nM [1]. This is a very consistent value that we have obtained over many years, using several strains of rats. In man, reported mean daily total circulating cortisol concentrations are similar, ranging between 150 and 250 nM [2–4]. In both species, roughly 10% of the total circulating glucocorticoid is freely diffusible into the extracellular space from plasma, and daily mean concentrations seen by cells are thus 15–25 nM. However, for approximately 25% of the day cells are bathed by corticosteroid concentrations of ~0–5 nM, and for 25% of the day the concentrations are ~30–50 nM.

Adrenalectomy results in increased activity of the hypothalamic and pituitary components of the adrenocortical system. At the level of the hypothalamic paraventricular nuclei (PVN), in CRF neurosecretory cells, *c*-fos-like immunoreactivity is expressed [5], CRF mRNA increases [6–8], peptide storage and secretion increases [9–11] and vasopressin (AVP) mRNA expression, peptide storage and secretion from the median eminence increase [10–16]. This is of consequence to the corticotroph since the combination of CRF and AVP is far more potent

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*To whom correspondence should be addressed.

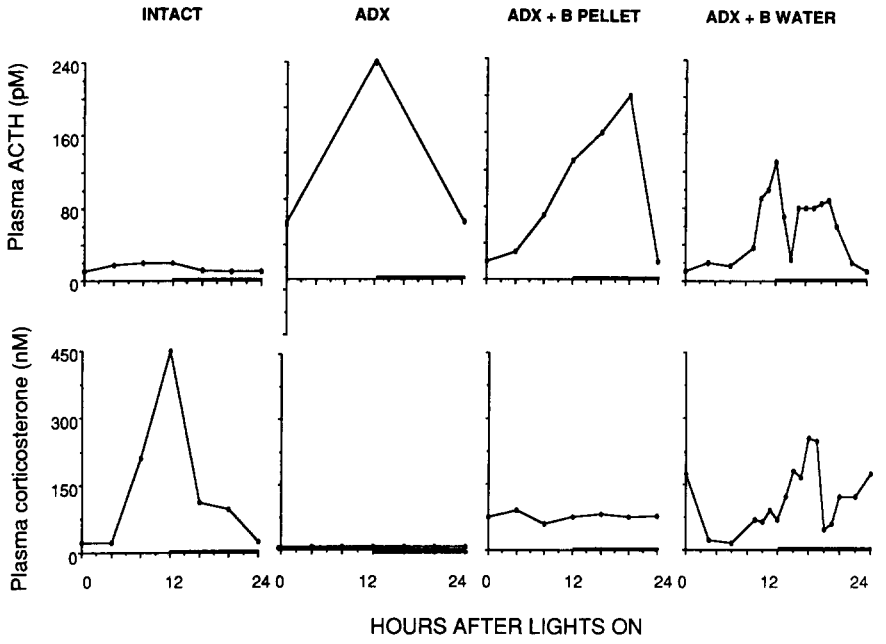

Fig. 1. Patterns of ACTH and corticosterone (B) in plasma as a function of the time of day in rats after manipulation of the corticosteroid signal. Intact (left), adrenalectomized (ADX, left center), adrenalectomized, replaced with a constant B signal (ADX + B pellet, right center), and adrenalectomized, replaced with a phasic B signal (ADX + B water, right). The 12-h daily period of darkness is indicated by the dark bar between 12 and 24 h. (Data mainly from Jacobson *et al.* [66].)

than either CRF or AVP alone on the secretion of ACTH [17]. At the pituitary, corticotroph cell number increases and POMC mRNA and its peptide products increase [18, 20]. All of these events are preventable or reversible by treatment with corticosteroids [6, 8, 11, 15, 18, 19].

The changes at the hypothalamus and pituitary resulting from the removal of endogenous B cause marked elevation of ACTH at all times of day. However, even at these high concentrations, diurnal rhythmicity in ACTH is maintained (Fig. 1, ADX). Comparison of the effects of the two B replacement regimens with that of ADX alone provides evidence that the control of ACTH by corticosterone is mediated by two distinct mechanisms. First, it is clear that ACTH concentrations during the trough of the rhythm are controlled by very low, constant circulating concentrations of B (compare ADX to ADX + B pellet); however, higher concentrations are required to inhibit peak ACTH secretion (compare B pellet to B water). Data suggesting that the corticosteroid control of peak ACTH secretion is different from that of the trough is abundant in literature detailing both clinical results and experiments on animals. In patients with adrenal enzyme defects, 17-OH progesterone concentrations are elevated during peak but not trough times of basal adrenocortical function [21]. Treatment of people with metyrapone or the glucocorticoid antagonist RU486 stimulates ACTH secretion at peak, but not trough times [22, 23]. Similarly, treatment of rats with cyanoketone (with partial blockade of corticosterone synthesis) results in persistent daily elevations in peak, but not trough ACTH concentrations [24].

At all times of the day, small perturbations in the concentrations of plasma B disrupt the tightly regulated pattern of ACTH secretion [25], providing additional evidence that different mechanisms may have evolved for the regulation of ACTH when B is low and when B is up to two log units higher. The consequences of such bimodal control are physiologically very important. If there were only an upper limit control, then, in the intact system, corticosteroid concentrations would be free to range anywhere between 0 and 600 nM during the 24-h day. One has only to see the damaging sequelae to the entire organism of constant exposure to normal daily peak levels of cortisol (~600 nM total, as in Cushing's syndrome) to realize that not only peak, but also trough concentrations of the corticosteroids must be controlled. This is achieved by controlling the secretion of ACTH during the circadian minimum. Steroid meditated inhibition of trough ACTH secretion requires a mechanism that detects and responds to very low, 0–5 nM, circulating free corticosteroid concentrations.

NEURAL STRUCTURES REQUIRED FOR A NORMAL CIRCADIAN RHYTHM IN ACTH AND B

After destruction of the medial basal hypothalamus, or cutting ventral neural tissue in anterior and lateral hypothalamus there is no change in corticosterone (B) or ACTH concentrations in plasma during the nadir of the diurnal rhythm, however the peak in the rhythm is prevented [26–30]. More directly, surgical isolation of the paraventricular nuclei (PVN) and their CRF-containing cell bodies from their output to the median eminence also results in normal basal ACTH and B concentrations in the morning, but blocks the normal diurnal rise in these hormones [30]. Results of these lesion studies are well complemented by the results of studies testing the effects of passive immunoneutralization with CRF antisera on the diurnal rhythm in adrenocortical system hormones. Administration of CRF antisera to intact rats does not alter circulating ACTH or B values during the trough of the rhythm, but blocks the normal diurnal rise in ACTH [31]. On the other hand, administration of CRF antisera to ADX rats near the time of the trough **does** decrease ACTH [32], showing that in the absence of B CRF cells actively drive ACTH during the nadir of the rhythm.

Thus the results of experiments using different techniques are in good agreement that in the presence of normal B concentrations, during the nadir of the rhythm, basal activity in adrenocortical system function occurs independently of brain activity as a consequence of what is probably constitutive ACTH secretion from the corticotrophs of the pituitary; by contrast, during the peak of the rhythm, normal basal ACTH (and B) secretion requires secretion of CRF from the axons of cell bodies located in parvocellular neurosecretory cells in the PVN.

CRF secretion which occurs during the normal diurnal peak activity in adrenocortical system function appears to be driven by structures in, or passing through, the suprachiasmatic nuclei (SCN). If the SCN are lesioned, adrenal B concentration is maintained constant, at the level of the normal circadian minimum [33]. Similarly, lesions of the SCN abolish the diurnal rhythm in plasma B [34, 35], and ACTH [35, 36]; circulating ACTH and B concentrations are maintained at the level of the normal diurnal minimum. Daily rhythms in B can be reestablished in rats with SCN lesions by placing them on a restricted feeding paradigm [37], showing that the lesions have not damaged essential inputs to (or outputs from) the PVN.

The neural pathways from the SCN to the PVN which drive CRF cell activity are still unclear; there is a sparse, direct input from the SCN to parvocellular CRF-containing cells in PVN, however there is a massive projection from the SCN to a site just ventral to PVN which may be more important for the circadian drive [38]. The adrenocortical rhythm is easily disrupted using pharmacological agents that interfere with monoamine metabolism [35]. Most of these agents also disrupt feeding cycles as well, which suggests that some of their effects on adrenocortical system function may be secondary to altered food intake [37].

SITE OF FEEDBACK INHIBITION BY GLUCOCORTICOIDS OF BASAL ACTH

Adrenalectomy stimulates marked increases in ACTH secretion under basal conditions at all times of day. This is a result of the removal of B, since ACTH can be returned to normal by B treatment. The site(s) at which B acts to inhibit ADX-induced ACTH secretion appears to be the brain, not the pituitary; where in the brain is still an open question.

Corticosteroid receptors exist in all known motor components of the adrenocortical system. *In vitro* studies of pituitary and hypothalamus have shown direct inhibition by corticosteroids of ACTH synthesis and secretion [39] and of CRF secretion [40]. Therefore, B, the endogenous ligand, could affect basal ACTH secretion at either, both or neither of these sites under basal conditions.

We performed a series of studies designed to determine whether the sites at which B acts to control ACTH secretion *in vitro* also regulate ACTH secretion *in vivo* during the trough of the diurnal ACTH and B rhythm. In the first, rats were prepared with hypothalamic lesions (or sham-lesions) that prevented endogenous secretion of CRF/AVP, and the former were infused with either CRF or vehicle. The rats in these groups were either ADX or sham-ADX. Results from lesioned rats were compared to the sham-lesioned controls to determine the effects of ADX and CRF on pituitary POMC mRNA, pituitary ACTH and plasma ACTH [29]. The results showed clearly that in the absence of stimulation by either endogenous or exogenous CRF, ADX had no effect on any measures of ACTH production or secretion, demonstrating a complete lack of corticotroph autonomy in the response to ADX. Furthermore, in the rats lesioned and given CRF, plasma and pituitary

ACTH in the sham-ADX rats were lower than those in the ADX rats, providing clear evidence for inhibition by endogenous adrenal secretions at the pituitary. However, the rate of CRF infusion in that study resulted in adrenal hypertrophy, thymic atrophy and plasma B levels which appeared fixed at the level of diurnal peak values in the sham-ADX rats, showing that inhibition by B at the pituitary could be obtained under conditions of abnormally elevated corticosteroid concentrations.

In a second set of experiments, similarly designed, all animals were ADX and replaced with a variety of concentrations of circulating B [30]. The results of those experiments revealed that in CRF-treated lesioned rats provided with a physiologically appropriate B signal (which prevented the ADX-induced increase in thymus weight in ADX rats but did not produce thymic atrophy) there was no B-induced inhibition of measures of corticotroph function compared to lesioned and CRF-treated ADX rats without exogenous B. By contrast, in sham-lesioned ADX rats treated with the same regimen of B, corticotroph activity was indistinguishable from that of intact animals. The clear conclusion from these results is that the hypersecretion of ACTH induced by ADX is a direct consequence of the lack of the normal action of B on the brain; under basal, trough conditions the low concentrations of B seen do not act at the pituitary to modulate the secretion of ACTH.

This conclusion is complemented by a number of less invasive studies of the effects of ADX on the activity of CRF cells in the PVN. ADX with systemic replacement of B at low concentrations prevents the normal ADX-induced increase in CRF mRNA in PVN [6], maintains normal POMC mRNA [18] as well as circulating ACTH concentrations measured at the time of the circadian trough [25]. Preliminary results show that the ADX-induced expression of AVP mRNA in parvocellular PVN cells is also blocked by a similar low B replacement regimen [41]. Bilateral implantation of B-containing pellets into dorsal hippocampus (but not other brain sites) decreases ADX-induced ACTH by 60%, whereas bilateral implants of dexamethasone in PVN, arcuate, septal or amygdaloid nuclei, but not dorsal hippocampus, reduce ACTH levels in ADX rats to normal [42]. The fact that B implantation was effective in reducing ACTH levels **only** when it was placed in dorsal hippocampus suggests that a primary site of action of B on inhibition of ADX-induced ACTH secretion may be extrahypothalamic, and transsynaptic to CRF neurons.

Our results agree strongly with the possibility that a major site of feedback inhibition by B is extrahypothalamic. Figure 2 shows data from studies by Levin in which lesions aimed at the PVN were found to be bilateral but ventral to the nuclei in the same anteroposterior plane [29]. The results from four groups of ADX rats are shown: sham lesion or lesions ventral to the PVN ± B replacement. In rats with complete PVN lesions ACTH concentrations were low, the expected consequence of complete removal of CRF/AVP stimulation of the pituitary [28, 29]. By contrast, the ADX rats with lesions ventral to PVN had ACTH concentrations similar to those of sham-lesioned rats, suggesting strongly that the lesion had neither damaged motor output to the corticotroph from the PVN nor resulted in chronic abnormal stimulation to CRF/AVP. In the rats with ventral lesions and replaced with B, ACTH was significantly less inhibited than in the sham-lesioned rats, although circulating B concentrations were not different in the two groups. This result suggests strongly that the lesions blocked a tonic, B-requiring inhibitory input to the PVN, implying that a major site of steroid inhibition of basal adrenocortical system activity lies outside of the PVN.

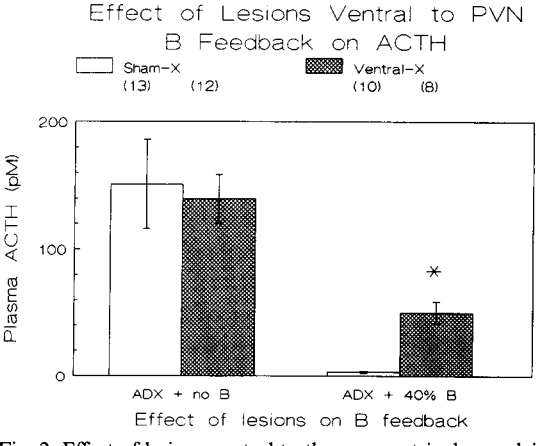

Fig. 2. Effect of lesions ventral to the paraventricular nuclei (PVN) compared to sham lesions on trough plasma ACTH concentrations in the absence (ADX + no B) and presence (ADX + 40% B) of a corticosterone (B) feedback signal. Although the lesions did not affect ACTH in the ADX rats in the absence of a B signal, the lesions caused the B signal to be significantly less effective on ACTH than it was in sham-lesioned controls (right bars). Plasma total B concentrations were not different in sham and lesioned groups (136 ± 17 vs 167 ± 17 nM, respectively). These results indicate that feedback control of ACTH by B during the diurnal trough is mediated at least in part by association with B at sites remote from CRF cells in the PVN. (Data redrawn from Levin *et al.* [30].)

Feedback inhibition of central drive to pituitary secretion by the genomically active hormonal products of peripheral target glands is common to the thyroidal [43], gonadal [44], and adrenocortical systems. In the latter systems, which are both characterized by marked cyclic changes in basal function, it appears that the site of feedback is not directly on the cells that produce the releasing factors; rather, estradiol localizes in GABAergic neurons which impinge on LHRH neurons [44], and, as reviewed above, present evidence favors a tonic action of B on cells which are remote from the CRF cells.

In terms of system function, the implications of a remote site of feedback on releasing factor secretion are considerable. The results suggest that in the presence of the target gland hormone, there is tonic, transsynaptic inhibition of the final motor neuron which limits the synthesis and secretion of releasing factors. However, because it is not exerted directly at the releasing factor cell, the inhibition can be overridden by other, directly stimulatory, inputs, thus allowing a degree of flexibility and rapid responsiveness in the system that would be impossible were the action of the tonic hormonal feedback exerted directly on the genome of the releasing factor cell. In addition, a long transsynaptic inhibitory pathway provides the possibility at multiple points for modulation effected by other inputs and transmitters. At least for the adrenocortical system, it is likely that the profound atrophy and non-responsiveness of the system which is occasioned by prolonged treatment with supraphysiological concentrations of corticosteroids results from the genomically-mediated inhibitory effects of the steroids exerted directly on CRF neuroendocrine neurons [40] and corticotrophs [39].

CORTICOSTEROID RECEPTOR TYPES AND CONTROL OF ACTH

There are two known receptors that bind with relatively high affinity to the natural glucocorticoids, corticosterone and cortisol. In rats, the high affinity mineralocorticoid receptor (MR) exhibits a K_d for corticosterone of 0.5 nM, and the lower affinity glucocorticoid receptor (GR) has a K_d for corticosterone of 2.5–5 nM. It is logical to propose that occupancy of MRs by B controls ACTH levels at the trough of the diurnal rhythm and that occupancy of GRs by B controls ACTH levels at the peak of the rhythm. The latter has been proposed by de Kloet and his coworkers [45–48] and by us [1, 49, 50].

However, a direct test of this hypothesis, comparing the efficacies of B and dexamethasone (DEX) on inhibition of ACTH in adrenalectomized rats at trough and peak times of the diurnal cycle reveals that DEX is less effective than B at both times of day (Fig. 3, from [50]). Both steroids bind to both MR and GR, however B exhibits higher affinity for MR than GR and DEX exhibits higher affinity for GR than for MR [51, 52]. If peak ACTH secretion were controlled primarily by the association of B with

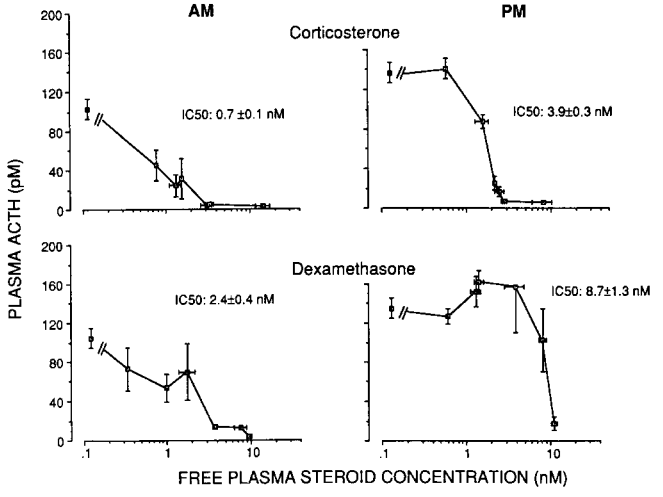

Fig. 3. Relationships between circulating free corticosteroid concentrations and ACTH as a function of the time of day in ADX rats provided with steroid replacement at surgery and sampled in the AM (trough) or PM (peak) 5 days later. Data are plotted as mean results from groups of 4–5 rats tested at each of 6 levels of steroid (either corticosterone or dexamethasone) replacement. IC_{50} values were estimated from best fit of the data by either Hill or Michaelis–Menten equations. There is a marked and significant shift to the right in the inhibitory efficacy of the corticosteroids on ACTH between AM and PM, and corticosterone is more effective than dexamethasone at both times of day. (From Dallman *et al.* [50].)

GR rather than with MR, then DEX should have been relatively more effective than B at the diurnal peak.

The shift in IC_{50} for B on ACTH from 0.7 nM at the trough to 3.9 nM at the peak, suggests that the hypothesis is correct, and that occupancy of GR as well as MR by B at the circadian peak is required for control of ACTH. However, the relative ineffectiveness of DEX at both times of day is puzzling, and militates against the hypothesis. It may be that DEX does not reach the brain sites involved in control of ACTH. Autoradiographic studies of the uptake of DEX into brain of ADX rats have shown a high degree of localization in vascular elements and significantly less uptake into neuronal structures than was observed for B [53].

In recent studies testing the hypothesis that control of ACTH is mediated by association of B with MR at the trough and with MR + GR at the peak of the rhythm, we have employed MR and GR antagonists in the presence of a constant B signal in ADX rats [54]. In ADX rats provided with a relatively high B signal and then given the MR antagonist, spironolactone, ACTH concentrations were significantly elevated at both the peak and the trough of the circadian rhythm. Such results support the notion that occupancy of MR by B is important for control of ACTH at all times during the 24-h period.

In the next experiment, we used spironolactone and the highly specific GR antagonist RU486 separately and together in ADX rats without and with low level B replacement. Spironolactone treatment had no effect on ACTH in ADX rats without B, and tended to elevate ACTH during the trough of the rhythm. To our surprise RU486 acted as a glucocorticoid agonist rather than as an antagonist in this experiment (Table 1). Either without or with B replacement, ADX rats treated with RU486 had lower ACTH concentrations at both times of day than the vehicle controls. The agonist effect of RU486 also caused thymic atrophy and a decrease in transcortin (CBG) concentrations, classic glucocorticoid effects. Agonist effects of RU486 at similar or lower doses have been shown in humans, primates and within rat hippocampal neurons [55–57].

Because we had used a single, large dose of RU486 in the above experiment, we next tested the effect of a variety of lower doses of RU486 (Fig. 4). Again, the high dose, but not lower doses of the drug acted as a glucocorticoid agonist on ACTH in ADX rats not treated with B. When ADX rats were provided with B which maintained plasma B at the low total concentrations of 72 nM (concentrations which occupy the MR nearly completely but occupy the GR by $<20\%$) agonist effects of RU486 were observed at all doses of the drug provided, in a dose–response pattern. The effect was particularly striking at the time of peak ACTH secretion. There was a clear interaction between the low constant concentrations of B and the agonist effect of RU486. In this experiment, the results suggested that occupancy of both MR and GR is important for control of ACTH secretion at the time of peak activity in the adrenocortical system. Subsequent experiments (in progress) confirm this notion; occupancy of the MR appears to be required for normal control of ACTH in the morning and occupancy of both MR and GR appears to be required for normal control of ACTH in the evening. In the AM, forced occupation of GR, or antagonism of exogenous B at concentrations sufficient to occupy GR is superfluous for the control of trough ACTH.

Table 1. The agonist effect of RU38486 on glucocorticoid sensitive targets in ADX rats with or without B replacement

Treatment	ACTH (pM) a.m.	ACTH (pM) p.m.	Thymus (mg/100 g)	CBG (nM)
SHAM	14 ± 1	15 ± 2	311 ± 10	716 ± 75
ADX, 0%B				
Vehicle	92 ± 11	268 ± 20	383 ± 15	873 ± 125
RU486	25 ± 6*	177 ± 30*	301 ± 16*	507 ± 35*
ADX, 20%B				
Vehicle	13 ± 3	237 ± 53	315 ± 5	454 ± 56
RU486	10 ± 2	44 ± 20*	262 ± 10*	368 ± 31

*Values from RU-treated rats are lower than those in vehicle-treated rats.

The distribution of MR and GR in brain differs markedly. Neurons containing appreciable concentrations of MR are primarily localized in hippocampus and lateral septum [58] whereas neurons containing GR are found throughout the brain, including CRF-containing neurosecretory cells in the PVN [59]. This anatomically discrete distribution of MR is obviously compatible with the physiological data favoring tonic, transsynaptic inhibitory control of CRF cells by occupancy of MR. At the moment it is not clear where the neurons containing GR which inhibit CRF/ACTH secretion during the circadian peak reside. Preliminary results by Bradbury and Dallman [60] suggest that dorsal hippocampus may be involved with GR-mediated inhibition of peak ACTH concentrations.

Arriza and Evans have shown, in an elegant series of experiments using cells transfected with

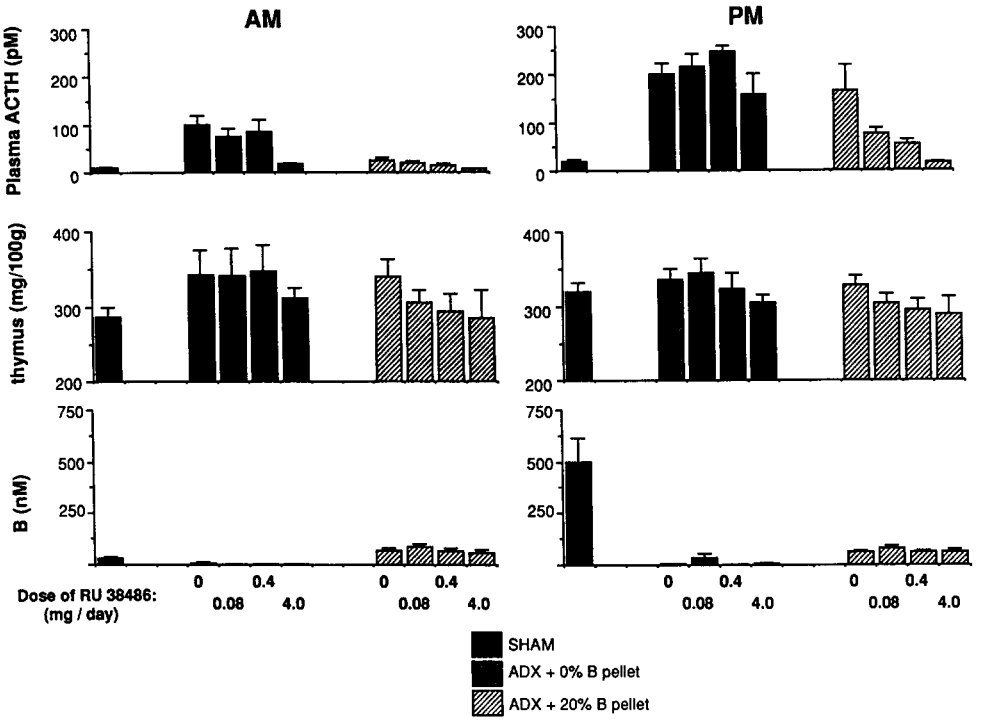

Fig. 4. RU38486, the synthetic glucocorticoid antagonist, acts as a GR agonist on ACTH and thymus weight in ADX rats without and with corticosterone (B) replacement (also see Table 1). Rats were sham-ADX or ADX, injected s.c. twice daily for 4–5 days with various doses of RU38486 without or with low level B replacement and sampled during the trough (a.m.) or peak (p.m.) of the basal rhythm. There was an interaction between the effects of RU38486 and B on ACTH; together the steroids were far more potent than separately, particularly in the p.m. These results provide strong evidence that occupancy of both MR and GR is required for normal control of ACTH at peak times of the basal ACTH rhythm.

MR, GR and reporter genes containing GREs, that occupancy of MR by steroid exerts a smaller, but similar biological effect to the occupancy of GR by steroid [61]. Based on these results, these authors have proposed that the function of MR is simply to extend the range of steroid concentrations of which a biological effect is achieved, and have pointed out the probability that neurons in the dorsal hippocampus contain both MR and GR [62]. An implication of their proposal and findings is that a single neuronal cell group, containing both MR and GR—and thus with an expanded range of sensitivity to corticosteroids—is responsible for the control of basal ACTH through a single biological output mechanism.

Alternatively, basal ACTH concentrations throughout the diurnal cycle could be under dual control through occupancy of MR by B in cells that contain only MR and not GR, and occupancy of other cells that contain GR and, possibly, not MR. The findings that either chronic [54] or acute [48] treatment of rats with MR antagonists has been shown to result in increased ACTH output favors the notion that there are separate sites (and, thus, mechanisms) mediating the effects of B occupancy of MR and GR. If the mechanism of inhibition of ACTH by B were identical for the two receptor types, then blocking MR occupancy, under conditions in which endogenous B was sufficiently high to assume occupancy of GR, should not have overridden the GR effect. Although these results do not definitively favor separate feedback sites for MR and GR, they suggest strongly that feedback control of basal ACTH is not exerted by a single group of neurons containing both receptor types.

CONCLUSIONS

The secretion of basal ACTH is normally regulated at all times of day by the biological consequences of occupancy of MR and GR by B at sites in the brain.

The major effect of occupancy of MR by B can be selectively and readily observed during the nadir of the circadian rhythm when, in the presence of a normal steroid milieu, CRF secretion does not occur. Because of their high

affinity for corticosterone, the effect of occupancy of MR saturates at very low circulating B concentrations, making this effect eminently suited for control of the adrenocortical system at the time of the diurnal minimum. B concentrations sufficient to occupy fully MR but not GR are sufficient in ADX rats to maintain normal levels of expression of CRF and AVP mRNA in neurosecretory neurons of PVN. This action of B limits the maximum activity of the basal adrenocortical system during the trough of the rhythm; the consequence is tight control of mean daily corticosteroid concentrations. Most experiments suggest that B acts at a site distant from the PVN to promote tonic transsynaptic inhibition of the capacity of neurosecretory CRF cells to synthesize CRF and AVP mRNAs. Appreciable occupancy of GR does not appear to be required to maintain normal levels of basal activity in the adrenocortical system during the time of trough activity.

By contrast, B concentrations sufficient to occupy both MR and GR are required for normal control of CRF and ACTH during the time of peak basal activity. There is a clear shift in the inhibitory efficacy of B on ACTH secretion between nadir and peak times of the basal rhythm. Adequate occupancy of GR is required to limit the maximum activity of the adrenocortical system during peak times. In the case of GR as well as MR, their location may be at sites remote from the PVN, and their occupancy may diminish stimulatory input to CRF cells from the SCN. It is probable that there is not a single neuronal cell group, containing both MR and GR which mediates the inhibition of circadian ACTH rhythms by B: Fig. 5 summarizes these conclusions.

To our surprise, RU486, the specific glucocorticoid antagonist acted in our hands as an agonist in ADX rats. We were able to take advantage of this effect to show that RU486 strongly interacted with low levels of B in the PM to inhibit ACTH, whereas it was marginally effective by itself. This result suggests strongly, as do those from our other studies, that occupancy of both MR and GR is required for normal control of ACTH at the time of peak activity in the adrenocortical system.

The consequence of the inhibition of ACTH being mediated by two receptor systems for B is tight regulation of basal activity throughout the 24-h day. Such control allows periodic occupancy of the GR by B to occur only at peak times of the rhythm. At the time of the trough

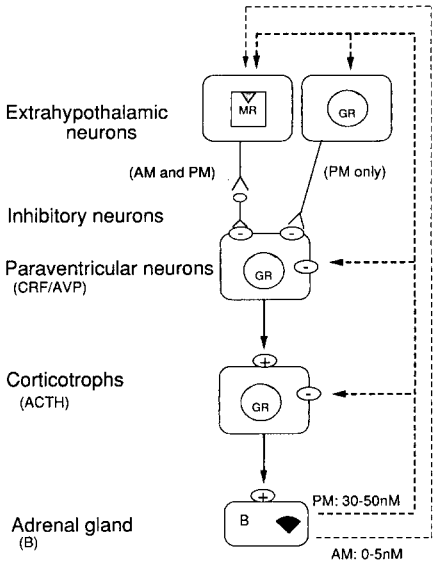

Fig. 5. Model for control of basal ACTH by corticosterone (B) under normal conditions. In the a.m., trough, plasma free B concentrations range between 0 and 5 nM, sufficient to occupy mineralocorticoid receptors (MR), but not glucocorticoid receptors (GR). MR are found in extrahypothalamic brain sites, occupancy of MR is required for control of basal ACTH; the effect of MR occupancy by B is on the level of expression of corticotropin-releasing factor (CRF) and vasopressin (AVP) mRNA, is tonic, and is mediated transsynaptically. By contrast, in the PM, peak, plasma free B concentrations range between 30 and 50 nM, sufficient to occupy extensively both MR and GR. At the time of the peak in the rhythm, adequate occupancy of both MR and GR is required for normal control of ACTH. There are multiple neuronal sites in which GR occur, and inhibition of ACTH may occur through occupancy of GR at remote neural sites, in paraventricular neurons and/or at the corticotroph.

in B, circulating concentrations are maintained (through association of B with MR) at concentrations too low for appreciable occupancy of the GR. The daily period of GR occupancy provides the required stimulation to the multiple sets of GR-responsive genes throughout the body so that their transcription rates are maintained at mean levels that are optimal for basal cellular function. Cellular signal smoothing effects would occur by virtue of the lags between transcription of DNA and the production of products as well as relatively long mRNA half-life; sustained occupancy of GRs for a few hours, once daily, apparently suffices to maintain steady state levels of the gene products. A substantial degree of occupancy of GRs continuously almost certainly results in excessive activity (either under- or over-) of steroid-responsive genes (for review, see Ref. [1]), despite the capacity for receptor down regulation [63–65]. Thus, an effective and very neat solution to the requirement for enough, but not too much, daily exposure to the potent

glucocorticoids has evolved through the use of high and low affinity receptors for B which control the basal secretion of CRF.

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