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Regulation of Adrenocorticosteroid Receptor mRNA Expression in the Central Nervous System

James P. Herman[1]

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SUMMARY

1. The adrenocorticosteroid receptors are hormone-activated transcription factors that have the potential to influence gene expression in a wide variety of CNS neurons. This review summarizes the present state of knowledge regarding the localization and regulation of glucocorticoid (or type II corticosteroid) receptor and mineralocorticoid (or type I corticosteroid) receptor mRNAs in brain, from the perspective of their potential influence on a wide variety of hormone-responsive genes.

2. Corticosteroid receptors are widely but not uniformly localized in the CNS and exhibit very complex regulation by glucocorticoids, gonadal steroids, neurotransmitter systems, and endogenous circadian drive. Both receptor species are present during development, implying an ability for these transcription factors to interact with neuronal differentiation, growth, and viability, and both receptors appear to regulate with age, suggesting relationships between adrenocorticosteroid receptor populations and brain aging. Regulation of adrenocorticosteroid receptor mRNA expression at the level of polyadenylation and splicing indicates that GR and MR biosynthesis is a dynamic process susceptible to numerous classes of information.

3. Further study of GR and MR biosynthesis at the gene, mRNA, and protein level is required to determine the true meaning of the regulatory complexities seen in defined neuronal circuits.

[1] Department of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, Kentucky 40536-0084.

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INTRODUCTION

Adrenocorticosteroid receptors are responsible for transducing information regarding circulating glucocorticoid levels into changes in CNS gene transcription. Upon binding appropriate ligand, the receptor–steroid complexes act as transcription factors in the cell nucleus, binding as homodimers to pallindromic consensus elements on promoter sequences of target genes (Aronsson *et al.*, 1988; Eriksson and Wrange, 1990; Tsai *et al.*, 1988). Depending on either the nature of the DNA consensus sequence or interactions with other transcription factors, steroid–receptor complexes can act as either positive or negative regulators of gene expression (Drouin *et al.*, 1989; Pearce and Yamamoto, 1993; Tsai *et al.*, 1988). The importance of glucocorticoid-induced transcription changes to CNS physiology is underscored by the wide variety of genes expressing glucocorticoid regulatory elements and/or responsiveness to glucocorticoid hormones, some of which are depicted schematically in Fig. 1 (Antoni, 1986; Bimberg *et al.*, 1983; Castellino *et al.*, 1992; Chalmers and Watson, 1993; Chao and McEwen, 1991; Dananberg and Grekin, 1992; Day *et al.*, 1992; Foreman *et al.*, 1992; Fossom *et al.*, 1992; Herman *et al.*, 1989a; Iacopino and Christakos, 1990; Lam *et al.*, 1992; Lauterborn and Gall, 1992; Li *et al.*, 1992; Mahajan and Thompson, 1991; McGuire *et al.*, 1992; Nakagawa *et al.*, 1992; O'Callaghan *et al.*, 1991; Presse *et al.*, 1992; Schafer *et al.*, 1989; Smith *et al.*, 1992; Thai *et al.*, 1992; Tverberg and Russo, 1992; Wong *et al.*, 1992). The scope of neuronal processes susceptible to GR and/or MR regulation indicates that glucocorticoids are in a position to influence virtually all aspects of neuronal (as well as glial) function. In combination with the widespread localization of adrenocorticosteroid receptors, this property underscores the clear importance of glucocorticoid hormones in ongoing regulation of CNS physiology.

There are presently two known adrenocorticosteroid receptor molecules in brain. The "glucocorticoid receptor" (GR) was first isolated from liver and shows K_d's for corticosterone binding in the 2.5–5 n*M* range and saturation of receptors only at very high levels of circulating glucocorticoids (Reul and deKloet, 1985). This receptor population corresponds to "type II" receptors defined in the binding literature. The "mineralocorticoid receptor" (MR) binds corticosterone (CORT) with a higher affinity (0.5–1 n*M*) and as a result is highly occupied even at low circulating glucocorticoid levels (Reul and deKloet, 1985; Spencer *et al.*, 1990). The MR is responsible for "type I" adrenocorticosteroid binding. On the basis of the difference in affinity and capacity, it is clear that the actions of the two receptors are exerted in vastly different glucocorticoid contexts. Consistent with this notion is the recent documentation of differential influences of hormone-bound MR and GR on functions ranging from excitability of hippocampal neurons in culture (MR excitatory, GR inhibitory) (Joels and deKloet, 1990, 1992) to transcriptional activation by cFOS-cJUN heterodimers (MR no effect, GR inhibitory) (Pearce and Yamamoto, 1993). The picture emerging from these reports is that both the concentration of circulating glucocorticoids and the cellular availability of the adrenocorticosteroid receptor subtypes are critical in determining how individual cells interpret the glucocorticoid signal; rather than a

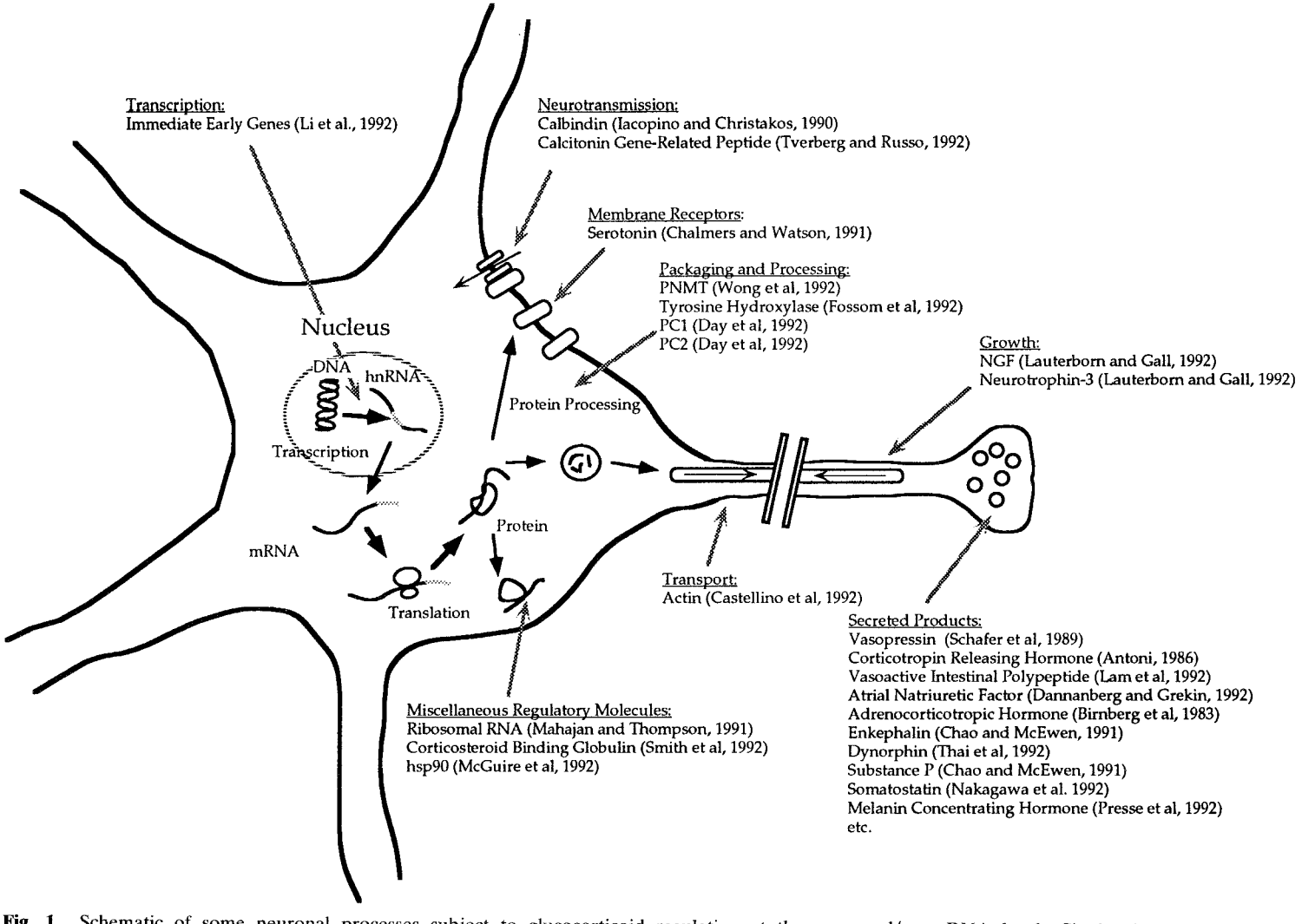

Fig. 1. Schematic of some neuronal processes subject to glucocorticoid regulation at the gene and/or mRNA level. Cited references are papers demonstrating glucocorticoid regulation of appropriate molecular species; note that these references are *examples* drawn from the literature and, in most cases, are not the sole sources of information on the particular subject.

simple "dose–response" relationship between glucocorticoids and cellular action, MR and GR are capable of radically switching the response characteristics of a given cell with changes in steroid levels (cf. Joels and deKloet, 1992).

A critical role for adrenocorticosteroid receptors in CNS function is further highlighted by the involvement of GR and/or MR systems in disease processes and aging. For example, loss of hippocampal steroid-receptive neurons has been correlated with age-related cognitive impairments and age- and chronic stress-related deficits in negative-feedback control of the hypothalamo–pituitary–adrenocortical (HPA) axis (Issa *et al.*, 1990; Sapolsky *et al.*, 1986). This neuronal loss is postulated to occur via toxic interactions between glucocorticoids and hippocampal cell function (Sapolsky *et al.*, 1988). In addition, it has been shown that antidepressant treatment can affect expression of GR and MR in brain *in vivo* and *in vitro* (Brady *et al.*, 1991; Pepin *et al.*, 1989, 1992; Seckl and Fink, 1992). Reports of dysfunctional glucocorticoid regulation of the HPA axis in human depressives (Carroll *et al.*, 1976a,b) have lead some to postulate a relationship among brain adrenocorticosteroid receptors, negative feedback, and clinical depression (Brady *et al.*, 1991; Pepin *et al.*, 1992; Seckl and Fink, 1992).

Adrenocorticosteroid receptors are in a position to govern both normal and abnormal neuronal functions. The mechanisms whereby these molecular species can influence such processes are poorly understood. Presently, considerable effort is being aimed at understanding the molecular biology, physiology, and pharmacology of GR and MR *in vitro* and *in vivo*. This article scans the literature to date concerned with adrenocorticosteroid receptor synthesis, as inferred from analysis of GR and MR mRNA expression and regulation in the CNS. Changes in mRNA levels have been directly related to functional changes in many CNS systems and clearly reflect the involvement of identified molecular species in numerous physiological processes. The studies of adrenocorticosteroid receptor mRNA expression delineated below outline considerable advances in our understanding of the role of GR and MR in the CNS, while at the same time illustrating the vast functional complexity of these molecular species.

LOCALIZATION OF GR AND MR mRNA IN THE CNS

The isolation of GR and MR cDNA clones in the late 1980s (Arriza *et al.*, 1987; Miesfeld *et al.*, 1984; Patel *et al.*, 1989) spurred a number of *in situ* hybridization mRNA mapping studies (Aronsson *et al.*, 1988; Arriza *et al.*, 1988; Herman *et al.*, 1989a; Sousa *et al.*, 1989; van Eekelen *et al.*, 1988; Yang *et al.*, 1988). Utilization of this technique bypassed problems associated with resolution of receptor subtypes by binding (lack of anatomical resolution, necessity of adrenalectomy) and immunohistochemistry (the lack, until recently, of a MR antibody), and by virtue of its molecular specificity provided the first unambiguous look at the localization of both receptor species in the mammalian CNS.

The results of several such studies are summarized in Table I. In accordance

Table I. Localization of Glucocorticoid and Mineralocorticoid Receptor mRNAs in the Rat CNS[a]

Region	GR mRNA (signal intensity)	IHC (No. cells)	MR mRNA (signal intensity	IHC (No. cells)
Telencephalon				
Neocortex	+ +	+ +	+ +	+ +
Piriform cortex	+ + +	+ + +	+ + +	+ + +
Entorhinal cortex	+ +	+ +	+ +	+ +
Subiculum	+ + +	+ +	+ + +	+ + +
Hippocampus				
CA1	+ + +	+ + +	+ + +	+ + +
CA2–CA3a	+	+	+ + +[a]	+ + +[a]
CA3b–CA4	+	+	+ +	+ +
Dentate gyrus	+ + +	+ + +	+ + +	+ + +
Septum				
Medial	+ − + +	+	+ +	+ +
Lateral	+ − + +	+ +	+ + +	+ + +
Septohypothalamic n.	+ + +	+ +	+ +	+ +
Bed nuc. of the stria terminalis	+ +	+ +	+ − + +	+ − + +
Amygdala				
Medial	+ + +	+ + − + + +	+	+ + +
Cortical	+ +	+ +	+ + +	+ +
Central	+ + +	+ + +	+ − + +	+ +
Basal	+ +	+ + − + + +	+ + +	+
Caudate-putamen	+	+ + +	n.d.	+ + +
Globus pallidus	n.d.	n.d.	n.d.	+ +
Nucleus accumbens	+	+ − + +	+ − + +	+
Thalamus				
Anterior	+ +	+ +	+	+ + +
Medial	+ + − + + +	+ +	+	+ +
Ventral	+ +	+ +	+ +	+ + +
Medial geniculate	+ + +	+ + +	+	+ + +
Lateral geniculate	+ +	+ + +	n.d.	+ + +
Reticular	n.d. − +	n.d.	+ +	+ + +
Parafascicular	+ + +	+ + +	n.d.	+ +
Paraventricular	+ +	+ + +	n.d.	+
Centromedian	+	+ − + +	n.d.	+ +
Habenula	n.d.	n.d.	+	+
Subthalamus	+ + +	+	+ + +	+ + +
Zona incerta	n.d.	+ − + +	n.d.	+ +
Hypothalamus				
Preoptic area	+	+ − + +	+	+ + +
Median preoptic n.	+ + +	+ +	n.d.	+ +
Anterior	+ +	+ +	n.d.	+ +
Suprachiasmatic	+ − n.d.[b]	+	n.d.	+
Supraoptic	n.d.[c]	n.d.	n.d.	+
Paraventricular				
Medial parvo.	+ + +	+ + +	n.d. − +	+
Posterior magno.	n.d.	n.d.	n.d.	n.d.
Arcuate	+ + +	+	n.d.	+ +
Dorsomedial	+ + +	+ +	n.d.	+ +
Ventromedial	+ +	+ + − + + +	n.d.	+ − + +
Lateral	+	+	n.d.	+ +
Mammillary	+ +	n.d. − +	n.d. − +	+ +
Midbrain				
Superior colliculus	+	+	+ +	+ +
Inferior colliculus	+ + +	+ +	+ +	+ +
Central gray	+	+ + +	+	+ +

Table I. (*Continued*).

Region	GR mRNA (signal intensity)	IHC (No. cells)	MR mRNA (signal intensity	IHC (No. cells)
Oculomotor	+	+ + +	+ + − + + +	+ +
Mesencephalic CNV	+	+ − + +	+ + − + + +	+ +
Red nucleus	+	+	+ + − + + +	+ − + +
Substantia nigra				
Zona compacta	+ + − + + +	+ + − + + +	n.d.	+ +
Zona reticulata	+	+	+ +	+
Ventral tegmental area	+ +	+ +	n.d.	+
Interpeduncular	+	+ − + +	n.d.	+ +
Pons				
Locus ceruleus	+ + +	+ + − + + +	+	+ +
Parabrachial	+	+ + +	n.d.	+ +
Dorsal raphe	+ + +	+ + − + + +	+	+ + +
Median raphe	+	+ − + +	+ +	+ − + +
Dorsal tegmental	n.r.	+	+ +	+ − + +
Trigeminal				
Sensory	+ +	+ +	+ +	+ +
Motor	n.r.	+ + − + + +	+ + − + + +	+ +
Facial	n.r.	+ − + +	+ + +	+ +
Abducens	n.r.	+	+ + − + + +	+ +
Pontine nuclei	n.r.	+	+ + +	+ − + +
Cerebellum				
Purkinje cells	+ + +	+ +	+ − + +	+ +
Ganule cells	+ + +	+ + +	+	+ +
Deep nuclei	+	+ − + +	+ + − + + +	+ − + +
Medulla				
Inferior olives	n.r.	+ + − + + +	+ +	+ +
Spinal trigeminal	n.r.	+ − + + +	+ +	+ +
Vestibular nuclei	n.r.	+ +	+ +	+ +
Nucleus tractus solitarius	n.r.	+ +	+ + − + + +	+ +
Dorsal vagal	n.r.	+	n.d.	+ +
Hypoglossal	n.r.	n.d.	+ + +	+ +
Prepositus	n.r.	+	n.d.	+ +
Raphe nuclei	n.r.	+ − + +	+	+ +
Reticular formation	n.r.	+ − + +	+ − + + +	+
Gracile	n.r.	n.d.	+ +	+ +
Cuneate	n.r.	n.d.	+ +	+ +

[a] Table I was compiled from Ahima and Harlan (1990); Ahima *et al.* (1991); Aronsson *et al.* (1988); Fuxe *et al.* (1985); Herman *et al.* (1989); Sousa *et al.* (1989); van Eekelen *et al.* (1988); and Yang *et al.*, (1988) and from maps constructed by this author. For immunohistochemistry, data are tabulated on the basis of cellular density, and for *in situ* hybridization, on the basis of intensity of hybridization signal. + + +, Highest; + +, intermediate; +, low; n.d., not detected; n.r., not specifically reported. Subjective quantitation data adapted by the author.

[b] Highest levels of MR in hippocampus.

[c] SCN contains GR-ir during the first 2 postnatal weeks but none thereafter. SON shows positive GR signal with certain fixation conditions.

with previous immunohistochemical (Fuxe *et al.*, 1985; Reul and deKloet, 1986) and binding studies (Reul and deKloet, 1986), both receptor mRNAs exhibited highest levels of expression in the limbic system, most notably in the hippocampal formation. However, coexistence in limbic system structures is not accompanied by coordinate levels of expression. For example, GR and MR show wide differences in relative level of abundance across hippocampal subfields; GR

mRNA shows highest levels of hybridization in CA1, intermediate levels in dentate gyrus (DG), and low levels in CA3, whereas MR signal is heaviest in CA2–CA3, is approximately equal in intensity in CA1 and DG, and shows lowest levels of hybridization in CA3b–CA4 (Arriza *et al.*, 1988; Herman *et al.*, 1989a; van Eekelen *et al.*, 1989). In addition, the adrenocorticosteroid receptors appear to be differentially distributed across limbic system structures; GR mRNA is abundant in the bed nucleus of the stria terminalis, septohypothalamic nucleus, and central and medial amygdaloid nuclei, whereas MR mRNA is primarily localized to the lateral septal nucleus, cortical amygdala, and amygdalohippocampal area (Table I) (Aronsson *et al.*, 1988; Arriza *et al.*, 1988; Sousa *et al.*, 1989; Yang *et al.*, 1988). In all, the differential localization of these receptor species within the limbic system implies that GR and MR may have primary actions on quite different neuronal circuits.

Perhaps more striking is the breadth of GR mRNA expression in the CNS. While GR mRNA is particularly abundant in limbic regions, it is localized in a large number of CNS nuclei (see Table I) whose significance to known domains of glucocorticoid action is less defined. This widespread distribution is in keeping with the role of the GR as a transcription factor and implies actions of glucocorticoids on numerous neural circuits. The distribution of MR mRNA is somewhat more limited; however, MR, too, is expressed outside the "traditional" limbic system (e.g., substantia nigra, numerous brainstem nuclei), again highlighting a potential role as a general transcription factor.

While GR and MR mRNAs clearly coexist in limbic system structures, physical colocalization has not been reported as yet, perhaps due to methodological difficulties in concomitantly visualizing two mRNAs. However, counterstained sections clearly reveal the presence of both receptor mRNAs in a majority of CA1 and DG neurons (Arriza *et al.*, 1988; Herman *et al.*, 1989a; van Eekelen *et al.*, 1988), rendering colocalization a foregone conclusion. Confirmation of colocalization has been verified at the protein level by Bohn and colleagues, using double-stain immunohistochemistry to show both GR and MR proteins in cultured hippocampal neurons (Bohn *et al.*, 1992). In all, it is fairly certain that a substantial number of hippocampal cells express both proteins, implying a role for both in regulation of hippocampal cellular physiology. Other regions clearly prefer one receptor over the other; for example, the hypothalamic arcuate nucleus and the parvocellular divisions of the paraventricular nucleus express primarily GR mRNA, with very little MR mRNA visible by *in situ* hybridization techniques (Table I; Aronsson *et al.*, 1988; Arriza *et al.*, 1988; Sousa *et al.*, 1989; Yang *et al.*, 1988). Unfortunately, the comparative localization of the two mRNAs in other systems has not been well studied, and it is thus difficult to determine whether GR and MR neuronal colocalization occurs in other CNS systems.

Emerging evidence suggests that in addition to neuronal localization, both GR and MR mRNAs are produced by astrocytes *in vitro* (Bohn *et al.*, 1992). In general, it has been difficult to identify glial localization by *in situ* hybridization methods in brain sections; however, given their dearth of cytoplasm, it is reasonable to suppose that if glial expression is low relative to neurons, it may be indiscernible, essentially merging with background hybridization.

Relative levels of the two adrenocorticosteroid receptor species in defined anatomical regions indicate a high relative abundance of MR mRNA relative to GR. Both *in situ* hybridization and RNAse protection data indicate a 1.5- to 5-fold greater abundance of MR mRNA in the rat hippocampus (Herman *et al.*, 1989a; Patel *et al.*, 1992; van Eekelen *et al.*, 1988). Differences in abundance varied with subfield, with the greatest differential seen in CA3 (Herman *et al.*, 1989a). At present, the significance of differential expression is not completely clear. However, the fact that the MR is expressed at higher levels in all hippocampal subfields implies a greater biosynthetic potential for MR in these neurons. In combinations with lower levels of (detectable) MR binding (relative to GR) (cf. Reul and deKloet, 1985; Chao *et al.*, 1989) and an apparently short half-life (Herman *et al.*, 1993), these results may be suggestive of a more rapid turnover of the high-affinity receptor type.

Table I also summarizes the relationship between studies of *in situ* hybridization localization with data from immunohistochemical maps. For the most part, the agreement across techniques in terms of GR localization is quite good. For example, immunohistochemical studies show a distribution of GR proteins across hippocampal subfields that agrees well with the *in situ* hybridization data (Ahima and Harlan, 1990; Fuxe *et al.*, 1985; van Eekelen *et al.*, 1988). Similarly, receptor autoradiogaphy using GR-selective ligands reveals GR and MR binding distribution profiles which roughly parallel the *in situ* data (Reul and deKloet, 1986; Sutanto *et al.*, 1988). These data therefore imply a general connection between mRNA expression and manufacture of functional receptors and indeed indicate that as expected, ongoing synthesis, protein expression, and ligand binding are closely related. However, several brain regions show differences between immunohistochemical and *in situ* hybridization GR localization that are difficult to interpret. For example, immunohistochemical studies using a monoclonal antibody against the liver GR have shown positive GR immunoreactivity in magnocellular neurons of the PVN or SON, albeit under certain fixation conditions (Kiss *et al.*, 1988), whereas little or no GR or MR mRNA signal has been observed in these nuclei in any study to date (Table I) (Sousa *et al.*, 1989; Yang *et al.*, 1988; Arriza *et al.*, 1988). These data imply either a low level of expression of GR mRNA in these neurons coupled with prolonged GR protein longevity or cross-reactivity of the GR antibody with (an)other molecular species.

The distribution of MR mRNA closely parallels binding data but is somewhat at odds with CNS localization of MR immunoreactivity (Ahima *et al.*, 1991; Arriza *et al.*, 1988; Reul and deKloet, 1986; Sutanto *et al.*, 1988). In general, MR immunoreactive neurons are more widely distributed than MR mRNA, with many regions, including several hypothalamic and thalamic nuclei and the striatum, showing protein localization without the presence of detectable mRNA levels (Table I) (Ahima *et al.*, 1991; Arriza *et al.*, 1988). The significance of this *in situ* hybridization–immunohistochemistry disagreement in presently ill defined, perhaps reflecting either very low levels of expression MR mRNA in these regions or cross-reactivity of the MR antibody with some as yet undefined steroid receptor-related epitope.

Differential distribution of GR and MR mRNA/protein in the nervous system implies a range of glucocorticoid action in the CNS, ranging from functions controlled by GR (neurons producing GR), those controlled by MR (neurons synthesizing MR predominantly), and those involving interactions between the two (i.e., GR and MR coexpressed in the same cells). In cells producing one adrenocorticosteroid receptor subtype, function may be associated with binding of receptor–ligand complexes with hormone-response elements on given genes, rendering glucocorticoid-induced functional changes a result of binding to the expressed receptor. However, in cases where GR and MR are colocalized, the similarity between their DNA-binding domains renders interactions with hormone response elements unlikely to select for function. Clearly, GR and MR must interact at levels other than DNA binding under such circumstances; this indeed appears to be the case, as Yamamoto and colleagues have recently described interactions between the N-terminal protein domains and the ability of cFOS-cJUN heterodimers to initiate transcriptional changes (Pearce and Yamamoto, 1993). Indeed, DNA-bound GR–ligand complexes seem to repress such transcriptional changes, whereas MR–ligand complexes do not. The implications of this elegant study for colocalized cells are particularly important; low levels of glucocorticoids (which see MR) allow stimulus-induced transcriptional changes (mediated by cFOS-cJUN heterodimers) to proceed, whereas higher levels (which see GR) may well inhibit the progress of such changes.

REGULATION OF CNS GR AND MR mRNA LEVELS

Methodological Considerations: Semiquantitative mRNA Determinations

The one-to-one stoichiometry between receptor mRNAs and complementary nucleic acid probes and the predictable relationship between probe radioactivity and the signal generated by it on photographic detection systems renders the Northern, RNAse protection, and *in situ* hybridization methods suitable for semiquantitative analytic procedures. This property renders mRNA analysis applicable to the study of GR and MR biosynthesis without many of the pitfalls associated with other assessment methods. For example, immunohistochemistry is excellent for receptor localization and, unlike mRNA analysis, is able to demonstrate subcellular compartmentalization of receptor proteins. This feature renders immunohistochemistry suitable for study of phenomena changing the distribution of GR or MR protein within the cell (cf. Fuxe *et al.*, 1985). However, changes in compartmentalization (i.e., nuclear vs cytoplasmic) occur only upon severe stimuli (e.g., steroid removal), and the nonquantitative nature of immunohistochemical staining do not render this technique suitable for studying more subtle regulatory changes.

Receptor binding techniques can be extremely useful for measurement of the end product of the biosynthetic cascade and thus, provide estimates of the potential for the receptor molecule to interact with its ligand. However, in most cases prior adrenalectomy has been employed to adequately visualize both

receptor subtypes (Reul and deKloet, 1985, 1986), which given steroid regulation of the receptor species can somewhat confound interpretation. In addition, the level of resolution afforded by steroid receptor *in vitro* autoradiography (e.g., film) is not suitable for detection of regulatory changes at the single-cell level.

While mRNA measures are perhaps more amenable to quantitative analysis of receptor biosynthesis than immunohistochemistry or steroid receptor binding, some caveats should be kept in mind when interpreting mRNA data. As pointed out earlier, mRNA expression is but one aspect of receptor biosynthesis, reflecting an intermediary between gene transcription and protein expression, and therefore changes in mRNA cannot be assumed to represent functional receptors in any absolute sense. In addition, mRNA does not necessarily mirror gene transcription, as mRNA levels can be influenced by cellular processes influencing mRNA stability, ribosomal loading, etc. Finally, both the GR and the MR exhibit mRNA heterogeneity, with the GR gene producing different-sized mRNAs by way of alternate polyadenylation sites (Miesfield *et al.*, 1986) and the MR gene showing several splicing variants in the 5′-untranslated region (Kwak *et al.*, 1993; Patel *et al.*, 1992). Thus, with some reservations mRNA measures allow semiquantitative analysis of subtle changes in biosynthesis and definitive attribution of these regulatory changes to the particular receptor molecule in question. However, mRNA expression does not represent a panacea for study of adrenocorticosteroid receptor regulation and is, indeed, best viewed in concert with other classes of measure.

Steroid Regulation of GR and MR mRNAs

Most receptor types are subject to autoregulation; increases in ligand binding exert inhibitory actions on biosynthesis/availability (i.e., "desensitization"), whereas decreased ligand availability increases receptor number. Given this precedent, it is not surprising that this level of regulation has been observed for both GR and MR. Adrenalectomy-induced steroid depletion elicits reliable increases in the number of hippocampal adrenocorticosteroid receptors (Tornello *et al.*, 1982). In addition, adrenocorticosteroid receptor number is significantly decreased upon administration of high doses of glucocorticoids (dexamethasone or corticosterone) or under conditions where circulating glucocorticoids are chronically elevated (Reul *et al.*, 1987; Sapolsky *et al.*, 1984b; Sapolsky and McEwen, 1985). Indeed, the concept of glucocorticoid negative feedback itself suggests self-regulation, in that glucocorticoids act to limit their own synthesis, an action requiring receptor interactions at some point along the HPA cascade.

Thus, it is reasonable to hypothesize that glucocorticoids will be involved in regulation of GR and MR mRNA expression. In the hippocampus, this appears to be the case, as up-regulation of GR mRNA following adrenalectomy has been demonstrated by numerous groups using a variety of methods, including Northern, RNAse protection, and *in situ* hybridization analyses (Herman *et al.*, 1989a; 1993; Kalinyak *et al.*, 1987; Patel *et al.*, 1992; Peiffer *et al.*, 1991b; Reul *et al.*, 1989; Sheppard *et al.*, 1990). The extent of up-regulation varies from 40 to 100%, and in all cases changes are reversed with glucocorticoid replacement.

Adrenalectomy effects on MR mRNA expression are considerably more limited, falling in the 25–50% range (Herman *et al.*, 1989a, 1993; Patel *et al.*, 1992; Reul *et al.*, 1989). Indeed, adrenalectomy-induced changes in MR message expression have not been observed by all investigators (Chao *et al.*, 1989).

While demonstrable to a greater or lesser degree in the hippocampus, glucocorticoid regulation of GR and perhaps MR mRNA is not uniform throughout the nervous system. For example, Northern analysis has shown that while GR mRNA increases in both hippocampus and amygdala following adrenalectomy, no significant changes are observed in hypothalamus (Pfeifer *et al.*, 1991b). In line with these results, *in situ* hybridization studies indicate that GR mRNA levels in neocortex, dorsomedial thalamus, hypothalamic paraventricular nucleus, and arcuate nucleus do not change with adrenalectomy (Herman *et al.*, 1993). Similarly, rats that show significant up-regulation of hippocampal MR mRNA following adrenalectomy do not show changes in neocortical MR expression (Herman *et al.*, 1993). Thus, while both GR and MR proteins have the capacity for autoregulation, the degree to which this trait is expressed shows regional variations.

The complexity of glucocorticoid regulation is further illustrated by the observation that adrenocorticosteroids differentially affect GR and MR mRNA expression in a subfield-specific fashion. For example, in response to adrenalectomy GR mRNA is up-regulated some twofold in subfield CA1, approximately 40% in the dentate gyrus, and appears unaffected in CA3 (when animals were killed during the circadian glucocorticoid nadir of the SHAM animals). Both the CA1 and the DG changes are reversed by glucocorticoid replacement with dexamethasone (Herman *et al.*, 1989a).

MR mRNA increases of 33% were observed in CA1 and CA2 following adrenalectomy, with no changes occurring in CA3 or DG (Herman *et al.*, 1989a). The low magnitude of the CA1–CA2 effect, and the fact that it was confined to two subfields somewhat explains the difficulty in detecting changes in whole hippocampal homogenates (c.f., Chao *et al.*, 1989). Interestingly, administration of dexamethasone to adrenalectomized rats partially reversed this up-regulation, suggesting either some degree of cross-talk between the receptors (i.e., ligand-bound GR regulation of the MR) or a capacity for dexamethasone to bind MR *in vivo*. In all, the hippocampus shows a functional heterogeneity of glucocorticoid GR and MR regulation that is not consistent either within or across receptor systems. The only common element observed is a coordinate GR and MR increase in CA1.

Glucocorticoid regulation of GR and MR seems pronounced in the hippocampus, while not being manifest in other brain regions examined. These findings underscore the potential importance of the hippocampus in regulation of adrenocorticosteroid regulation, indicating that hippocampal cells are particularly capable of integrating glucocorticoid signals and ongoing HPA activation. These results agree with numerous other reports demonstrating hippocampal inhibition of glucocorticoid secretion, ACTH release, and ACTH-secretagogue biosynthesis and release (Herman *et al.*, 1989b; Sapolsky *et al.*, 1984a) and thereby suggest that this structure has a privileged channel into HPA regulation. It is noteworthy

that GR mRNA localized to the hypothalamus or the hypothalamic PVN do not show steroid regulation (Herman *et al.*, 1993; Peiffer *et al.*, 1991b), in spite of the fact that GR-containing PVN neurons are the same cells that initiate ACTH release (Ceccatelli *et al.*, 1989; Uht *et al.*, 1988). Steroid insensitivity may reside at either the neuronal level, due to the necessity for the organism to rapidly decrease stress-induced changes in HPA function at this level (a capacity requiring consistent GR availability), or at the receptor level, due to the lack of MR expression in this nucleus. Whatever the explanation, the fact that adrenalectomy-induced changes occur in hippocampus but not PVN suggests different roles for the hippocampal and PVN GR-containing neurons in HPA regulation.

Regulation of CNS adrenocorticosteroid receptor mRNA can also be affected by gonadal steroids. Ovariectomized, estrogen-treated rats show significant decreases in GR and MR mRNA content in both hippocampus and hypothalamus/preoptic area (relative to oil-treated ovariectomized rats) (Burgess and Handa, 1993). Administration of the GR agonists RU28362 or the mixed agonist dexamethasone have differential effects on GR mRNA expression in ovariectomized rats, with the hippocampus and hypothalamus-preoptic area showing different patterns of down-regulation in the presence or absence of exogenous estrogen (Burgess and Handa, 1993). These studies indicate a region-specific interaction between estrogen and adrenocorticosteroid receptor expression and suggest a modulatory role for gonadal steroids in regulation of receptor synthesis.

Neural Regulation of GR and MR mRNA Expression

The fact that GR and MR mRNAs are not overtly regulated by steroids in most brain regions studied and, indeed, show subfield-specific variation in the characteristics of steroid regulation suggests that a major component of the regulatory process occurs by way of synaptic input. A series of recent studies verifies this supposition and is summarized in Table II.

Pharmacological studies indicate an involvement of monoaminergic systems in GR and MR mRNA regulation. Depletion of serotonin by intracerebroventricular administration of 5,7-dihydroxytryptamine reduces GR mRNA expression in CA1, CA2, and DG and MR mRNA in CA3 and CA4 (Seckl *et al.*, 1990). In addition, administration of antidepressants, which inhibit monoamine reuptake and thereby increase local monoamine levels, increases GR and MR mRNA levels in hippocampal homogenates (Brady *et al.* 1991; Seckl and Fink, 1992). Intracerebroventricular 6-OHDA administration, which depletes central catecholamines and in the hippocampus affects primarily norepinephrine, preferentially increases MR mRNA in CA1, CA3, CA4, DG, and parietal cortex, with no effect on GR mRNA levels (Yau and Seckl, 1992). The fact that all subfields of the hippocampus express one or more serotoninergic, adrenergic, and, indeed, dopaminergic receptor subtypes (Chalmers and Watson, 1991; Meador-Woodruff *et al.*, 1991; Zeng and Lynch, 1991) indicates that GR- and

Table II. Neural Regulations of CNS MR/GR mRNA

Treatment	Circuitry affected	mRNA	Result (region)
	Pharmalogical manipulations		
5,7-DHT	Serotonin depletion	GR	Decrease (CA1, 42%; CA2, 52%; DG, 76%)
		MR	Decrease (CA3, 56; CA4, 45%)
6-OHDA	Catecholamine dep.	GR	No change
		MR	Decrease (CA1, 23%; CA3, 34%; CA4, 25%; DG, 39%; Ctx, 29%)
Amitriptyline	Monoamines (?) (Antidepressant)	GR	Increase (hippocampal homogenates, 56%)
		MR	Increase (hippocampal homogenates, 87%)
Desipramine	Monoamines (?) (Antidepressant)	GR	Increase (hippocampal homogenates, 42%)
		MR	Increase (hippocampal homogenates, 60%)
Citalopram	Monoamines (?) (Antidepressant)	GR	No change
		MR	Increase (hippocampal homogenates, 17%)
Imipramine	Monoamines (?) (Antidepressant)	GR	No changes
		MR	Increase (all subfields, 70%)
	CNS lesions		
Med. septal lesion	Septohippocampal system (ACh)	GR	Increase (CA1, 23%; CA2, 28%; DG, 32%)
		MR	Increase (CA1, 32%; CA2, 26%; CA3, 19%; CA4, 20%)
DG lesion (icv colchicine)	Intrahippocampal circuits	GR	Decrease (dorsal CA fields only, 24–30%; DG, 100%)
		MR	Decrease (dCA1-2-63%; vCA1-2, d, vCA3-4, 26–38%, DG-100%*)
Entorhinal cortex lesion	Perforant path	GR	Transient decrease in DG, CA1 no change
		MR	No change

[a] Table II was compiled from Brady *et al.* (1992, 1991); O'Donnell *et al.* (1992); Seckl *et al.*, (1990, 1991); Seckl and Fink (1992); Yau *et al.* (1992); Yau and Seckl (1992). Percentage changes are those reported by the authors.

MR-containing hippocampal cells are likely to be responsive to all classes of monoamine input. In many cases, modulation of monoamines produces changes in GR and MR mRNA that surpass those produced by adrenalectomy or glucocorticoid administration, suggesting that these systems play a prominent role in modulating receptor expression.

The antidepressant data are rendered more interesting by the fact that administration of these agents in primary neuronal cultures can increase GR mRNA expression (Pepin *et al.*, 1989) and indeed can drive GR promoters transfected into fibroblast or neuroblastoma cell lines (Pepin *et al.*, 1992). These data indicate that antidepressant actions may not be exerted solely through alterations in monoamine reuptake *in vivo*.

As may be expected, disruption of hippocampal circuitry also changes levels

Table III. Regulation of CNS MR/GR mRNA: Development, Aging, and Diurnal Rhythms[a]

Subject	Region affected	mRNA	Result (region)
Development	Hippocampus	GR	Present at postnatal day (pd) 2; GR, MR levels comparable, CA3 hybridizes as densely as CA1 and DG
			Adult pattern by pd 12 (GR < MR, CA1 = DG ≫ CA3)
		MR	Present at pd 2; adult pattern by pd 12
	Hypothalamus	GR	Weeks 1–2–high expression in AHN, ARC, PVN, DMN, VMH
			Week 3—high expression confined to ARC, PVN
		MR	Low levels of expression
Aging			Decrease (CA3, 42%; CA4, 41%; DG, 26%)
Brown–Norway rats	Hippocampus	GR	No change
		MR	
Long Evans	Hippocampus	GR	Decrease [homogenates, 60% (6 vs. 24 mo)]
	Hypothalamus	GR	Increase [homogenates, approx. 40% (6 vs 24 mo)]
	Amygdala	GR	No changes
	Frontal cortex	GR	No changes
Fisher 344	Hippocampus	GR	Decrease (homogenates 9 vs 26 mo)
		MR	Decrease
Circadian	Hippocampus	GR	CA1 2: Unimodal rhythm (nadir: 1500 after lights)on)
		MR	All fields: Bimodal rhythm (troughs at 0300, 1500)
	Cortex	GR	No rhythm
		MR	No rhythm
	PVN	GR	No rhythm
		MR	Not detected
	Thalamus	GR	No rhythm
Circadian	Hippocampus	GR	Unimodal rhythm (nadir: 1200 after light-on) (homogenates)
		MR	Unimodal rhythm (nadir: 1200) (homogenates)
	PVN	GR	No rhythm (homogenates)
		MR	No rhythm (homogenates)

[a] Table III was compiled from Herman *et al.* (1993); Kwak *et al.* (1992); Morano and Akil (1990); Pfeiffer *et al.* (1991); and van Eekelen *et al.* (1991).

of hippocampal GR and MR mRNA expression. Lesions of the medial septum, which destroy the preeminent (and largely cholinergic) input to hippocampus from the basal forebrain, increase GR mRNA expression in CA1, CA2 and DG, and MR mRNA expression in all subfields save DG, suggesting inhibitory actions of acetylcholine on adrenocorticosteroid receptor expression (Yau *et al.*, 1992). Colchicine-induced lesions of the DG appear to affect largely MR mRNA expression in remaining viable subfields, leaving GR mRNA affected only in dorsal CA1-2 (Brady *et al.*, 1992). However, in the same study it was noted that hippocampal expression of inositol 1,4,5-triphosphate kinase is also reliably reduced, rendering it difficult to determine whether the observed effects are associated with a lack of DG or sublethal toxic damage to the pyramidal cells themselves (Brady *et al.*, 1992). Finally, entorhinal cortex lesions, which disrupt perforant path input to hippocampus (and thus eliminate cortical connections), elicit dramatic decreases in DG GR mRNA while concomitantly increasing GR

mRNA in subfield CA1; MR mRNA is not affected by this lesion. However, it should be noted that these effects are transient in nature, disappearing by 4 days following lesion, and perhaps reflect an acute neuronal response to denervation rather than tonic cortical regulation through the perforant path (O'Donnell *et al.*, 1992).

In all, the picture presented by the data to date suggests a complex regulation of hippocampal GR and MR mRNAs by neuronal inputs. Interestingly, both catecholaminergic and cholinergic lesions increase adrenocorticosteroid receptor mRNA levels in hippocampus (catecholamines affecting MR, cholinergics MR and GR). These reports suggest that these systems, which are generally associated with activational/attentional processes, may well prove inhibitory to receptor synthesis. Like direct inhibitory effects of steroids on receptor synthesis, these data imply an inverse relationship between glucocorticoid receptor expression and CNS activation.

Life-Span Regulation of GR and MR mRNA

With the recent characterization of a relationship between adrenocorticosteroid receptors and the aging process, considerable effort has been put forth to characterize regulation of GR and MR mRNA expression in aged animals. Studies to date seem to indicate that hippocampal GR mRNA levels are significantly decreased in aged Long-Evans, Fisher 344, and Brown-Norway rats (Morano and Akil, 1980; Peiffer *et al.*, 1991a; van Eekelen *et al.*, 1992). MR mRNA is also decreased in the hippocampus of aged Fisher 344 rats, but does not appear to be affected in the Brown-Norway strain (Morano and Akil, 1990; Peiffer *et al.*, 1991a; van Eekelen *et al.*, 1992). Unfortunately, cellular attrition with aging was not examined in these studies, making it difficult to determine whether the observed GR and MR mRNA changes were secondary to cell loss and whether indeed the lack of MR mRNA changes in Brown-Norway rats represented a strain-specific up-regulation in remaining viable neurons.

Developmental studies indicate that both GR and MR mRNAs can be visualized in hippocampus during the late embryonic–early postnatal period (van Eekelen *et al.*, 1991). Interestingly, the ratio between GR and MR abundance seem to change with time; in the early postnatal period (postnatal day 2), GR and MR mRNA levels are comparable, whereas MR expression appears considerably greater than GR (the adult pattern) by postnatal day 12. The significance of this differential expression during development is presently unclear. Developmental GR and MR protein expression data show interesting patterns in several brain regions; for example, GR immunoreactivity can be clearly observed in the suprachiasmatic nucleus during the first week of postnatal life, diminishes rapidly over the second and third weeks and is undetectable thereafter (Rosenfeld *et al.*, 1988; van Eekelen *et al.*, 1987). Interestingly, this observation is not reiterated by *in situ* hybridization analysis (van Eekelen *et al.*, 1991), rendering is significance rather difficult to interpret. In the brainstem and cerebellum MR immunoreactivity becomes evident prior to GR protein expression and appears to reach adult expression levels earlier, leading some to speculate a more important impact of

MR binding during the early postnatal period (Lawson *et al.*, 1992). In the diencephalon, levels of expression of GR immunoreactivity are quite high during the first postnatal week, encompassing most of the hypothalamic region; by postnatal day 16, GR localization was confined more clearly to defined nuclei (e.g., parvocellular paraventricular nucleus) (Rosenfeld *et al.*, 1988). This pattern is reiterated by GR mRNA levels (van Eekelen *et al.*, 1991), and together these data suggest a transient involvement of GR in development of several hypothalamic nuclei. In contrast, MR mRNA expression appears to obey the adult pattern as early as postnatal day 2, with no major fluctuations in relative intensity across brain regions (van Eekelen *et al.*, 1991).

Adrenocorticosteroid hormones clearly play a role in neuronal development (deKloet *et al.* 1988) and may be responsible for development of response characteristics of the HPA axis. It has been shown that handling during the perinatal period reliably increases adrenocorticosteroid receptor number and improves the efficacy of glucocorticoid negative feedback on the HPA axis (Meaney *et al.*, 1985, 1992; Sapolsky *et al.*, 1985). Handling is also capable of attenuating age-related basal hyperactivity of the HPA axis and age-related loss of hippocampal adrenocorticosteroid receptor binding (Meaney *et al.*, 1992), indicating a correlation between developmental regulation of adrenocorticosteroid receptors and changes with senescence.

In all, glucocorticoids are in a position to greatly affect processes associated with both development and aging. As transcription factors, adrenocorticosteroid receptors are capable of integrating information regarding circulating steroids into the developmental program of numerous cellular systems, prominently including the brain. In this manner, factors influencing glucocorticoid levels (such as stress) during the development of adrenocorticosteroid-receptive brain systems have the potential to affect profoundly and permanently the subsequent characteristics of these circuits.

Diurnal Rhythms of GR and MR mRNA Expression

Glucocorticoids exhibit a definitive diurnal rhythm of secretion, showing some 10- to 30-fold differences across the circadian cycle (with highest levels corresponding with the animal's active phase; in rat, the PM). Given the widespread influence of GR and MR as transcription factors, this remarkable change in circulating glucocorticoid level is clearly capable of influencing numerous aspects of organismic physiology. Potential effects of fluctuating glucocorticoid levels include regulation of adrenocorticosteroid receptor expression. Indeed, analysis of circadian patterns of GR and MR mRNA expression demonstrates a complex diurnal regulation of these mRNA species that has steroid-dependent and steroid-independent components. In hippocampus both GR (in DG and CA1) and MR (all subfields) mRNA exhibit pronounced decreases in expression around the time of dark onset, when circulating glucocorticoid levels are rising. MR mRNA expression shows an additional trough around the onset of the light phase. Steroid depletion by adrenalectomy appears to normalize GR mRNA in the DG and MR mRNA in all subfields to

peak diurnal levels, suggesting that circulating steroids play a role in down-regulating adrenocorticosteroid receptors during the rising phase of the diurnal glucocorticoid cycle. However, adrenalectomy causes pronounced increases in GR mRNA in CA1 and CA3 that far exceed normal levels of expression, indicating that steroids affect GR expression in these regions in a considerably more complicated fashion (Herman *et al.*, 1993).

Several additional points bear mention concerning this study. First, diurnal troughs of GR and MR mRNA expression coincide with increases in circulating glucocorticoids. These data suggest a direct relationship between steroid levels and gene expression in affected subfields. Second, the estimated half-life for both messages is relatively short; assuming no differential degradative processes occur across the diurnal cycle, the most conservative kinetic analysis (zero-order) assumes a half-life on the order of 8 (GR) and 6.5 (MR) hr. These estimates place regulatory changes within the time domain of fluctuating glucocorticoid levels. However, without a clear knowledge of *in vivo* half-life, it is impossible to guess from this analysis exactly when levels are changing and thus from a perspective of binding, what receptor is most likely to be involved. Finally, diurnal changes were seen only in hippocampus; GR mRNA was not altered in diurnal fashion in PVN, arcuate nucleus, thalamus or cerebral cortex, and MR message was not changed in cortex. In combination with the adrenalectomy data summarized above, these data imply a privileged regulation of hippocampal adrenocorticosteroid receptors by glucocorticoids.

GR and MR mRNA Diversity—Additional Levels of Regulation?

Cellular mRNA levels are subject to numerous levels of regulation. Two such levels, polyadenylation and alternative splicing, are directly relevant to the adrenocorticosteroid receptor family. Concerning the former, several studies using Northern analysis have revealed at least two detectable forms of GR mRNA, corresponding to 4.8- and 6.5-kB fragments. The primary difference between these forms lay in the 3′-untranslated domain and can be associated with selection of polyadenylation sites. There is circumstantial evidence that there may be a regulatory difference between these two species; in hippocampus, GR mRNA is up-regulated by adrenalectomy in studies using probes complementary to the 3′-coding region and proximal 3′-UT, which sees all message forms, while other studies using probes complementary to 3′-UT sequences distal to the first polyadenylation site, which sees only larger forms, do not show upregulation with adrenalectomy (Chao *et al.*, 1989, vs. Patel *et al.*, 1992). This apparent upregulation of the short form may have several possible explanations, including increased stability of the 4.8 form, selection of the first polyadenylation site under conditions of high transcriptional activity, or direct effects of steroids on enzymes involved in polyadenylation processes. Whatever the mechanism, the data indicate that localization of the sequence used to probe for adrenocorticosteroid receptors needs to be considered quite carefully.

Differential splicing is observed in 5′-UT sequences of the rat MR mRNA. Three distinct splicing variants have been identified in rat tissues; the alpha

variant (first described by Arriza *et al.*, 1987) appears to be heavily expressed in brain but not kidney, the beta variant (isolated by Patel *et al.*, 1987) expressed equally in brain and kidney, and the gamma variant expressed at very low levels in both tissues. Neither of the three variants incorporates any of the others, indicating that in each variant the other two sequences are spliced out as introns or components of introns. In addition, the arithmetic sum of molar estimates of alpha, beta, and gamma abundance, as determined from RNAse protection assay, does not equal that predicted using a probe recognizing all three, suggesting that other 5′ forms may exist (Patel *et al.*, 1992). All three forms are loaded on polysomes, indicating that all three are translatable (Kwak *et al.*, 1993). Interestingly, the distribution of the three variants does not coincide; the beta variant shows localization patterns similar to that seen using coding region 3′-untranslated probes, whereas the alpha variant is more homogeneously distributed among hippocampal subfields and is not seen in areas associated with the rudimentary dorsal fornix system, including the tenia tecta and induseum griseum (Kwak *et al.*, 1993; Patel *et al.*, 1992). In addition, only the alpha variant is regulated by glucocorticoids, showing a twofold increase after adrenalectomy. The physiological regulation of the three MR mRNA variants and their differential response to glucocorticoids is unclear and may be of potential importance in regulation of MR mRNA/biosynthesis.

It is notable that the GR gene appears to show a unique type of regulation whose significance is presently quite vague. Studies using GR probes complementary to 5′ coding-region sequences indicate a different pattern of GR distribution than do 3′-coding-untranslated region probes; unlike the latter, 5′ probes indicate abundant expression of GR mRNA in subfields CA3 and CA4 (J. N. Masters, personal communication; Herman *et al.*, 1989a; van Eekelen *et al.*, 1988; contrast with Whitfield *et al.*, 1990). These results suggest either that the GR mRNA is not fully transcribed in CA3–CA4 neurons or that there are presently unidentified splicing variants of GR transcripts in CA3–CA4 that select against full expression of the distal 3′-coding and -untranslated regions. These explanations have quite different ramifications, as the former possibility indicates that the 3′-coding-untranslated region UT probe is appropriate for study of GR mRNA regulation, whereas the 5′ probe measures an interesting cellular process that is not reflective of protein production. Alternative splicing, on the other hand, would indicate the production of a different form of GR mRNA that may have direct relevance to unique functions of CA3–CA4 neurons.

GR AND MR mRNA REGULATION: PERSPECTIVES

Recent advances in the study of the GR and MR further indicate that CNS adrenocorticosteroid receptor regulation is extremely complex. In addition to regulation at the level of receptor synthesis, there are several additional factors that should be considered when interpreting GR and MR regulatory data. First, the effective levels of steroids seeing these receptors in brain are modulated by corticosteroid-binding globulin (CBG), which sequesters glucocorticoids in blood

and thereby limit levels of free circulating steroid. CBG is in turn subject to negative glucocorticoid regulation (Smith and Hammond, 1992) and thus can change the availability of corticosteroids to their receptors under conditions of differential secretion. Second, the amount of adrenocorticosteroid available to a specific receptor can be regulated at the level of degradation; for example, 11-betahydroxysteroid dehydrogenase can preferentially inactive glucocorticoid hormones (Funder *et al.*, 1988), rendering mineralocorticoids the only physiologically meaningful in tissues where it is expressed. Third, once made, the regulation of the receptor itself is complex. Its path to the nucleus (where it exerts physiological effects) is critically dependent on associations with additional factors, such as the heat shock proteins. Not surprisingly, the heat shock proteins can also be regulated by glucocorticoids (negatively) (McGuire *et al.*, 1992), rendering translocation of ligand-bound receptors a process subject to adrenocorticosteroid context as well. Finally, the meaning of steroid–DNA interactions is only now becoming unveiled; whether GR-like or MR-like effects are communicated by ligand binding is likely to be interpreted in light of interactions with other DNA-binding transcription factors (Pearce and Yamamoto, 1993). These angles on GR and MR regulation are presently under intense study, and their implications for GR and MR mRNA expression and on wider issues of GR and MR functions in the CNS are obvious.

One area deserving further study is regulation of GR and MR in extrahippocampal regions. Studies to date have focused primarily on hippocampus, by and large assuming that this region regulates GR and MR mRNA in a manner representative of the CNS in general. This is definitely not the case, and indeed many studies indicate that the hippocampus is the *only* region affected by various manipulations. Neuronal regulation of these receptors is clearly complex and, given the potential influence of adrenocorticoid receptors as transcription factors in many CNS circuits, dictates careful study of regulation in the context of defined brain systems.

The importance of steroid receptors to CNS function is profound, varying from involvement in adaptive, stress-related, behavioral, developmental, aging, neuroplastic, and neurodegenerative processes, to name a few. The present focus on these receptors using modern molecular biological techniques should prove adequate to the task of defining these roles and establishing reasonable methods for management of glucocorticoid efficacy in health and disease.

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