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Corticosteroids in the Brain

Cellular and Molecular Actions

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Abstract

The rat adrenal hormone corticosterone reaches the brain and binds to intracellular receptors. These receptors comprise high-affinity mineralocorticoid and lower-affinity glucocorticoid receptors that, upon activation, affect the transcription rate of specific genes. The two receptor types are discretely localized in the brain, with particularly high expression levels in the hippocampus. Here we review recent studies showing that electrical properties and structural aspects of hippocampal principal neurons are specifically regulated by mineralocorticoid- or glucocorticoid-receptor activation. The molecular mechanisms by which these cellular effects could be accomplished are discussed.

Index Entries: Corticosteroid hormones; mineralocorticoid receptor; glucocorticoid receptor; signal transduction; morphology; hippocampus.

Introduction

Corticosteroid hormones are produced in the adrenal gland and released into the circulation in particularly high amounts after periods of stress (McEwen et al., 1986; Dallman, 1993). In the late 1960s it became evident that corticosteroids not only act on peripheral organs but also enter the brain compartment (McEwen et al., 1968). Within the brain they effectively bind to intracellular receptors that are structurally identical to the corticosteroid receptors in peripheral tissue (McEwen et al., 1986; de Kloet, 1991).

In later years it was found that two main types of intracellular corticosteroid receptors can be distinguished: the mineralocorticoid receptor (MR) which binds the endogenous rat hormone corticosterone (cortisol in human) with high affinity (K_d approx 0.5 n*M*), and the glucocorticoid receptor (GR) which displays approx 10-fold lower affinity for the hormone (Reul and de Kloet, 1985). These receptors are discretely localized in the brain. Most parts of

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the brain express considerably higher amounts of GRs than MRs; few areas show an excess of MRs (McEwen et al., 1986; de Kloet, 1991). Interestingly, in several parts of the limbic system MRs and GRs are coexpressed, even within individual neurons (Van Steensel et al., 1995).

Shortly after the demonstration of brain corticosteroid receptors it was found that peripherally administered steroids depress spontaneous firing of hippocampal neurons (Pfaff et al., 1971). In subsequent years, numerous studies, mostly biochemical, showed that central corticosteroid receptors mediate changes in neurotransmitter synthesis or binding (reviewed by McEwen et al., 1986; de Kloet, 1991; Meyer, 1985). It was documented only in recent years, however, that corticosteroids have profound effects on electrical properties of neurons (Joëls and de Kloet, 1992; 1994; Joëls, 1997), on their structural features (Gould and McEwen, 1993; McEwen and Sapolsky, 1995), and on behavioral processes in which these neurons are intricately involved (Luine et al., 1996; Oitzl and de Kloet, 1992). It appeared that MRs and GRs each mediate specific effects on neuronal activity, structure, and behavior.

Studies in transfected cell lines over the past decade have revealed many principles of the molecular actions mediated by corticosteroid hormone receptors (Truss and Beato, 1993). It is presently less evident, however, whether these principles also apply to steroid actions *in situ*, in this case in brain tissue. The electrical, structural, and behavioral properties that have been studied are merely the end point of a cascade of molecular and cellular events that are scarcely accessible in brain tissue, with the presently available techniques.

In this review we will describe some of the more extensively studied examples of brain corticosteroid actions and specifically discuss the presently known data concerning the molecular events that lead to the observed cellular effects. As a preamble, a concise overview is given of the characteristics of corticosteroid receptors in general, and their distribution and properties in the brain. For rapid steroid actions that are presumably mediated by membrane receptors, we refer to a number of recent reviews on this subject (Majewska, 1992; Paul and Purdy, 1992; Lambert et al., 1995).

Corticosteroid Receptors

Structural Organization of Glucocorticoid Receptors

Both the GR and MR are intracellular receptors belonging to the superfamily of steroid-activated transcription factors (Evans, 1988). Cloning of human genes and cDNAs, and subsequent deduction of the primary structure of open-reading frames revealed that the GR protein consists of 777 amino acids and the MR protein of 985 amino acids (Evans, 1988; Hollenberg et al., 1985; Arriza et al., 1987; Encio and Detera-Wadleigh, 1991; Zennaro et al., 1995). Each protein has two zinc-finger domains that are almost identical in the GR and MR, a carboxy-terminal domain involved in binding of corticosteroids, and a large, less well-conserved part with transactivation properties (*see* Fig. 1).

In the cytoplasm, the nonliganded GR is associated with heat-shock proteins, immunophilins, and possibly a number of other, as yet unidentified proteins (Smith and Toft, 1993). This multiprotein complex is thought to keep the GR in a high-affinity configuration. GR activation by corticosteroids results in dissociation of the complex, followed by phosphorylation of the ligand-activated receptor and translocation to the nucleus where it affects transcription of specific target genes (for review, *see* Bamburger et al., 1996). A similar activation mechanism is proposed for the MR, given the high structural similarity of the MR to the GR.

Molecular Mechanisms of Corticosteroid Action

Based on the molecular actions via activated GRs and MRs, different corticosteroid-responsive genes (CRGs) have been recognized. Type I CRGs are defined by the presence in their

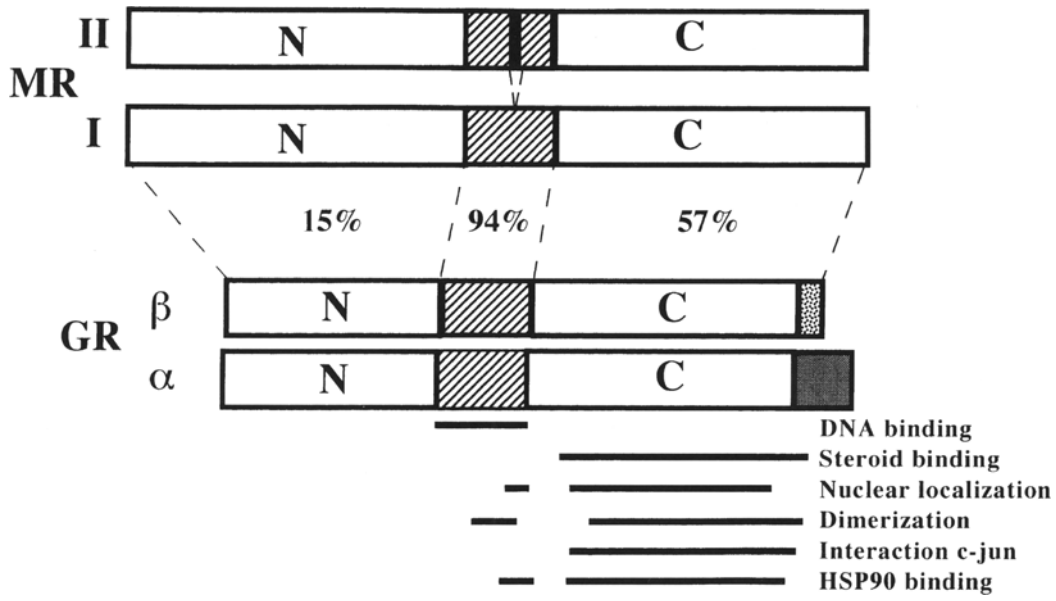

Fig. 1. Structural similarity and functional domains of the glucocorticoid (GR) and mineralocorticoid receptor (MR) and their splice variants. Both the GR and MR proteins contain three functional domains: a nonconserved (15%) N-terminal domain (N); a highly conserved (94%) DNA-binding domain (hatched); and a conserved (57%) carboxy-terminal steroid binding domain (C). Additional functional domains are represented by black horizontal bars below the GR. Though experimental evidence is lacking, a similar organization is proposed for the MR, based on structural similarity with the GR protein. Alternative splicing of the GR gene leads to two proteins: the corticosteroid-binding GRα and the nonbinding GRβ. Differences between the GRα and GRβ proteins are dotted in C. Two MR proteins have been predicted from molecular biological experiments: MR I and MR II. The difference between the two proteins is the addition of four amino acids in the DNA binding domain (black vertical bar in MR II).

promoter domain of a specific 15-nucleotide palindromic sequence, the so-called glucocorticoid-responsive element (GRE; reviewed by Bamburger et al., 1996). The DNA-binding domain of an activated monomer GR (or MR) binds to a half-site element of the palindromic sequence. This is subsequently followed by homodimerization and binding of a second GR (or MR) to the other half-site element (e.g. Lefstin et al., 1994 reviewed by Truss and Beato; Zilliacus et al., 1995). In general, this mode of action of GRs and MRs (type I activation; Fig. 2) leads to an enhanced transcription rate of the target genes, although repression sometimes also occurs (Drouin et al., 1993). Activated GRs and MRs both can bind to the same GREs in vitro, albeit with different affinity; thus far, no GREs or mineralocorticoid responsive elements that could discriminate between GR and MR action respectively have been described.

The second type of CRGs have no GRE sequence in their promoter region but instead have DNA elements that are recognized by transcription factors other than GRs or MRs. In particular, activated GRs can affect the transcription rate of type II CRGs by physically interacting with such transcription factors. For example, protein–protein interaction between GR and the immediate-early gene AP-1, consisting of c-jun and c-fos dimers, results in repression of AP-1-activated genes (for review, *see* Pfahl, 1993). As activated GR monomers are able to repress AP-1, this type of nuclear-recep-

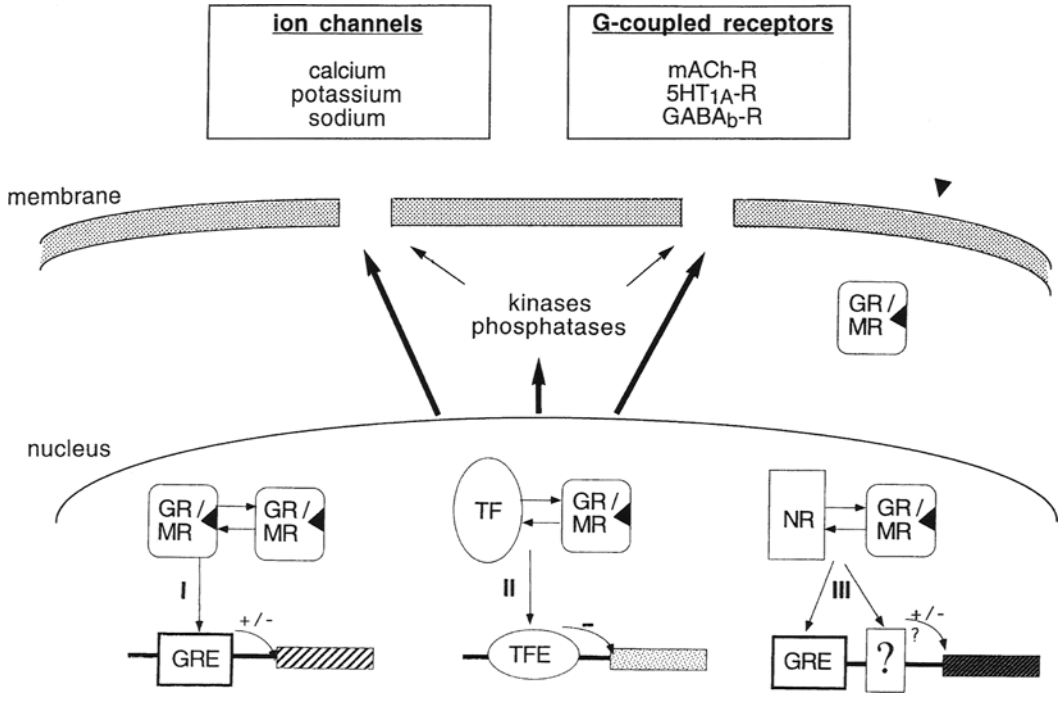

Fig. 2. Corticosteroid-hormone receptors may affect gene transcription in hippocampal cells as MR or GR homodimers (type I), by interacting with other transcription factors (TF; type II) or as heterodimers with other nuclear receptors (NR; type III activation of hormone-responsive genes). The target genes can encode for proteins with very general cell functions such as kinases or phosphatases, changing a wide array of membrane properties. Experimental evidence indicates that voltage- and ion-sensitive ion channels, and G-protein-coupled receptor cascades are clear targets for gene-mediated corticosteroid actions. Since MR and GR activation were shown to affect, with a delay of at least 1 h, many of these membrane properties in a similar way, this mode of action may underlie the relatively rapid effects of corticosteroids on hippocampal neurons. With a longer delay (> 3h up to several days), corticosteroid-receptor activation may lead to regulation of specific genes, involved in the indicated membrane properties of hippocampal cells.

tor action apparently does not depend on homodimerization (Heck et al., 1994). In addition to AP-1, the GR interacts with a number of other transcription factors including NFk-B and possibly CREB (Ray and Prefontaine, 1994; Guardiola-Diaz et al., 1996). So far, no specific repression of type II CRGs by activated MRs has been reported. However, as most nuclear receptors and other transcription factors do interact with specific partners, the repression of type II CRGs by MRs could be expected to occur.

Type II activation of CRGs may be one molecular mechanism by which divergent actions via MR and GR in the CNS can be explained. For example, the actions of AP-1 are repressed by GR but not by MR (Pearce and Yamamoto, 1993; Funder, 1993). It should be noted, though, that these studies were performed with composite-response elements allowing binding to both steroid receptors and AP-1; this does not necessarily mean that the MR does not repress classical AP-1 activity. Nevertheless the experimental data suggest that repression of AP-1-activated genes may occur only when high corticosteroid levels circulate in the blood, i.e., when GRs are activated in addition to MRs (Unlap and Jope, 1995; 1994).

Recent evidence suggests the existence of a third type of CRG activation. It was shown that heterodimers consisting of an activated GR and

MR functionally recognize GREs of reporter constructs in vitro and induce effects that differ from the actions evoked by MR and GR homodimers (Trapp et al., 1994). Positively and negatively charged amino acids in the DNA-binding domains seem to be crucial entities for this heterodimerization to occur (Liv et al., 1995). As similarly organized and highly homologous DNA-binding domains are present in other nuclear receptors—a necessary though insufficient condition for formation of heterodimers—heterodimerization may not be restricted to GRs and MRs. As yet, the presence of such additional heterodimers has not been reported. Questions regarding the natural occurrence of GR/MR heterodimers, their functional implications in vivo and whether or not they recognize specific "heterodimer-responsive elements" need to be addressed in future.

MR and GR Splice Variants

Expression of the GR and MR genes results in the generation of several splice variants (Fig. 1). Alternative splicing of exon 9A and 9B of the human GR gene creates a high abundant α-transcript encoding a protein of 777 amino acids (α-GR) and a low abundant β-transcript encoding a truncated 742 amino acid long β-GR (Hollenberg et al., 1985; Encio and Detera-Wadleigh, 1991). In contrast to the α-GR, β-GR does not bind glucocorticoids (Oakley et al., 1996). Recent evidence suggests an inhibitory role of β-GR on glucocorticoid action (Bamberger et al., 1995), possibly via heterodimerization of β-GR with α-GR (Oakley et al., 1996).

Three alternative splice variants of the MR gene are known. Two of these result from alternative splicing of the noncoding exons 1A and 1B and thus have no consequence for the structure of the MR protein (Zennaro et al., 1995). Interestingly, the expression of these two splice variants is regulated in different ways. The 1A but not 1B splice variant is under control of glucocorticoids, probably because the 1A (but not 1B) splice variant promoter region contains a GRE. The biological relevance of this divergent control of transcription is unknown but it is speculated that the two transcripts have different half lives or might be translated with different efficiency (Kwak et al., 1993). A third MR splice variant, containing 12 additional nucleotides in the DNA-binding domain, was recently found in human and rat tissue (Bloem et al., 1995). As translation of this transcript will result in four additional amino acids in a region known to be crucial for DNA binding and heterodimerization, expression of this variant may have functional implications.

All splice variants are present in the human CNS, including the hypothalamus and the hippocampus. The expression of β-GR is low compared to the α-GR expression (β/α=1/100; Oakley et al., 1996). The MR 1A and 1B variants are roughly equally abundant, but are expressed at different levels in the various substructures of the rat hippocampus (Kwak et al., 1993). Compared to the MR 1A and 1B transcripts, the third MR splice variant is expressed only at a low abundance (1:25) in the CNS and the rat hippocampus (Bloem et al., 1995; Vreugdenhil, unpublished results), indicating that its biological relevance in the CNS is probably limited.

Localization and Binding Properties

The GR protein is widely distributed in the brain, both in neurons and in glial cells (Fuxe et al., 1985; van Eekelen et al., 1987; Ahima and Harlan, 1990). Areas with particularly high expression are limbic areas such as the hippocampal formation, the paraventricular nucleus of the hypothalamus, and ascending monoaminergic neurons in the midbrain (Aronsson et al., 1988; van Eekelen et al., 1988; Chao et al., 1989). Moderately high GR expression was demonstrated in thalamic nuclei, in the central amygdala, striatum, and cortical layers.

MRs display a much more restricted distribution. The MR protein is mainly confined to some limbic areas, including the hippocampal formation, and to brain-stem sensory and motor neurons (Ahima et al., 1991); somewhat lower expression levels were found in, e.g., layer II of the cortex and in cerebellum (van Eekelen et al., 1988; Chao et al., 1989; Arriza et

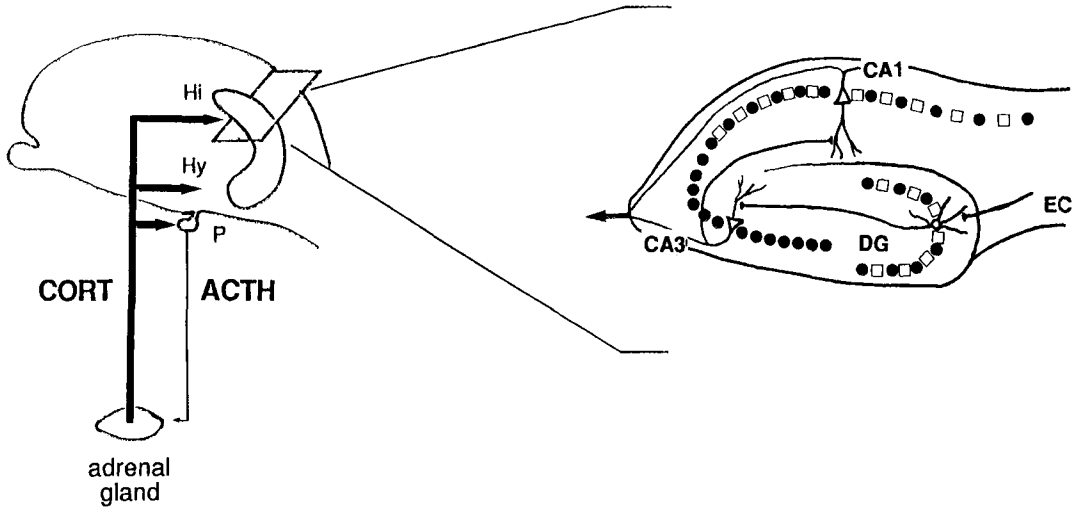

Fig. 3. *Left:* Hypothalamic (Hy) corticotropin-releasing factor production induces ACTH release from the pituitary (P) that activates corticosterone production in the adrenal gland. Corticosterone (CORT) feeds back on the brain, binding to high-affinity mineralocorticoid receptors (circles on the right) and lower-affinity glucocorticoid receptors (squares). Particularly the hippocampal formation (Hi) is enriched with these corticosteroid receptors. *Right:* Schematic transversal representation of the hippocampal formation, showing the main elements of the synaptic circuitry. Granule cells in the dentate gyrus (DG) receive a projection from the entorhinal cortex (EC) via the perforant path and, in turn, project to pyramidal neurons in the CA3 region via the mossy fibers. From the CA3 area, Schaffer collaterals project to the CA1 pyramidal neurons. With low circulating levels of corticosterone, mineralocorticoid receptors are already occupied to a considerable degree (filled symbols), whereas glucocorticoid receptors are mostly unoccupied (open symbols). With high corticosterone levels, glucocorticoid receptors become occupied too.

al., 1988; Herman et al., 1989). With confocal microscopy it was demonstrated that individual hippocampal neurons in most cases contain MRs (Van Steensel et al., 1995). Pyramidal neurons in the rat CA3 area are quite unique in that they express considerably more MRs than GRs. Principal neurons in other hippocampal subfields, i.e., the CA1 area and the dentate gyrus, exhibit comparable amounts of MR and GR (Fig. 3).

Radioligand binding studies in the 1980s (Reul and de Kloet, 1985; Coirini et al., 1985; reviewed by de Kloet 1991), that made use of selective MR and GR ligands, revealed that the affinity of hippocampal MRs for both corticosterone and aldosterone is high (K_d approx 0.5 n*M*), comparable to in vitro binding properties of the kidney MRs (Krozowski and Funder, 1983). The ED_{50} for in vivo occupancy of hippocampal MRs is approx 1 µg corticosterone/100 g body weight. In vitro, the GR displays a 10-fold lower affinity for corticosterone (K_d approx 5 nM) than the MR; the ED_{50} for in vivo occupancy amounts to approx 60 µg/100 g body weight. Synthetic analogs like dexamethasone display a high affinity for brain GRs in vitro, but poorly penetrate the brain in vivo (de Kloet et al., 1975).

Particularly for neurons in which MRs and GRs are colocalized, e.g., hippocampal CA1 neurons, these differences in binding properties of MR and GR are of great importance. Low circulating levels of corticosterone, such as occur during the circadian trough under rest, are sufficient to occupy brain MRs to a considerable (> 70%) extent, whereas only approx 10% of the brain GRs are occupied (Reul et al., 1987). When corticosteroid levels rise, e.g., after an acute stress, MR occupation is only little changed (from 70–90%), but GR occupation is consider-

ably increased (from 10–90%; Reul et al., 1987b). Consequently, under physiological conditions there is a continuous shift from predominant MR occupation to a situation in which GRs are occupied in addition to MRs. This realization has been the starting point in the late 1980s for studies addressing how specific activation of brain MRs and GRs affects neuronal properties.

Molecular and Cellular Corticosteroid Effects

Numerous studies over the past decades have demonstrated an enormous diversity of corticosteroid actions via brain MRs and GRs. These actions range from changes in chemical neurotransmission, energy metabolism, and behavioral functions to alterations in cell morphology. In line with the steroid mechanism of action involving gene transcription, the observed effects were generally slow in onset (> 1 h) and long lasting. Here we will focus on two well-investigated examples, i.e., corticosteroid modulation of electrical properties of hippocampal neurons and changes in hippocampal cell morphology resulting from the absence of corticosteroid hormones.

Ion Channels

Electrophysiological studies investigating corticosteroid actions on hippocampal cell activity reveal that virtually no effects can be seen when neurons are studied under basal conditions: Under resting conditions, both the potential and resistance over the membrane of hippocampal neurons are unaffected by hormone treatment (Joëls and de Kloet, 1989; Kerr et al, 1989; Zeise et al., 1992; Beck et al., 1994). Corticosteroid effects become visible only when neurons are shifted from their resting membrane potential, e.g., by depolarization of the membrane through current injection or by the action of neurotransmitters.

Two types of membrane conductances are a potential target for corticosteroid-receptor-mediated events (Fig. 2). The first type is the voltage-gated and ion-sensitive ionic conductances, which are in many cases not directly altered by neurotransmitters, but are indirectly altered as a consequence of depolarizations and/or influx of Ca^{2+} ions. The second type is formed by the ion conductances that are directly affected by neurotransmitter actions, either because the channels are an intrinsic part of the transmitter receptors or because the channels are modulated via receptor-activated G proteins.

Of the first type, Ca^{2+} currents were found to be particularly sensitive to corticosteroid treatment. Thus, predominant occupation of MRs in CA1 hippocampal neurons resulted in small voltage-gated Ca^{2+} currents (Karst et al., 1994). This was true for both the transient low-threshold currents and sustained high-threshold Ca^{2+} currents. When GRs were activated to a large extent, Ca^{2+}-current amplitude increased considerably (Karst et al., 1994; Kerr et al., 1992). This was particularly observed for the sustained Ca^{2+} currents. Interestingly, when corticosteroid receptors were not activated, i.e., in tissue from adrenalectomized (ADX) rats, Ca^{2+}-current amplitude was also large (Karst et al., 1994), pointing to a U-shaped dose-dependency (*see* Fig. 4; Joëls and de Kloet, 1994). Following ADX in young rats (approx 1-mo old) mainly sustained, high-threshold Ca^{2+} currents were increased in amplitude (Karst et al., 1997). In older rats (approx 6-mo old) ADX rather affected transient, low-threshold Ca^{2+} currents. The age-dependent effect of ADX in fact reflected age-related changes in Ca^{2+} currents of control rats, observed in these and also older age groups (> 12-mo old; Thibault and Landfield, 1996). Not only voltage-gated Ca^{2+} influx is affected by corticosteroid receptor occupation: Both basal and stimulus-induced intracellular Ca^{2+} levels were increased with abundant GR activation, which may be explained by steroid effects on Ca^{2+} buffering or extrusion mechanisms (Elliot and Sapolsky, 1992; 1993; Joëls et al., 1998).

Steroid modulation of Ca^{2+} homeostasis will affect Ca^{2+}-dependent phenomena in hippocampal neurons, e.g., the activation of the

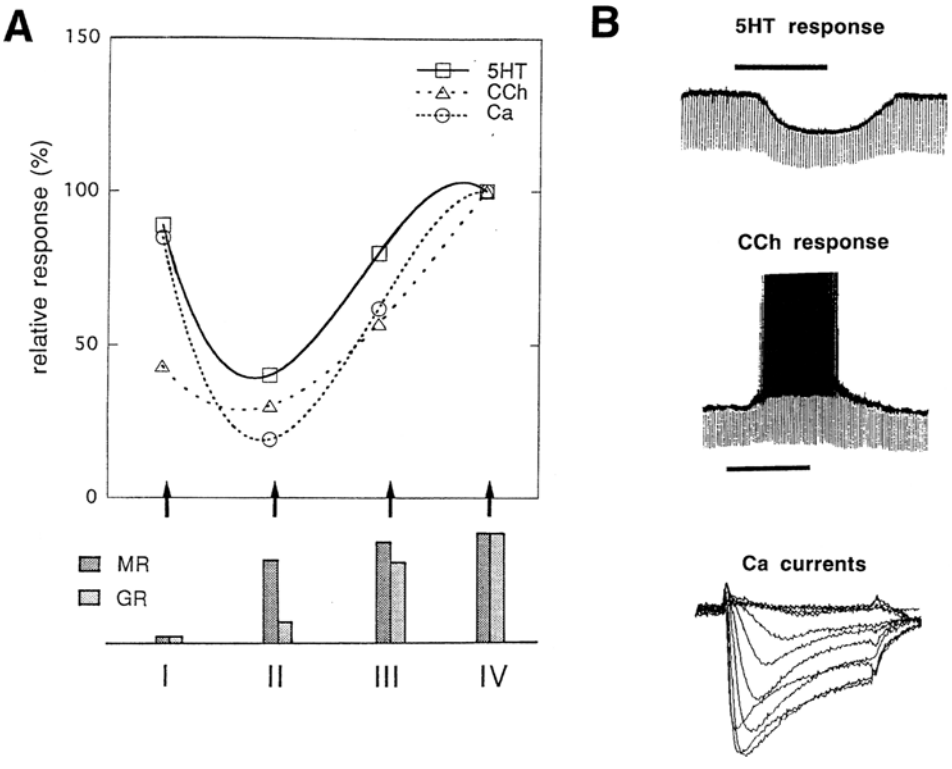

Fig. 4. **(A)** Relative calcium-current amplitude (circle), serotonin (5-HT)-induced hyperpolarization (square), and carbachol (CCh)-evoked depolarization (triangle) of hippocampal cells, expressed as a percentage of the maximal responses seen in either of the experimental conditions shown below: No steroid receptor occupation (I), predominant MR occupation (II), additional GR occupation (III), and chronic occupation of both MRs and GRs (IV). The data indicate that neuronal Ca currents and transmitter responses are small with predominant MR activation (i.e., when corticosteroid levels are low), whereas both in the absence of steroids and when neurons are (chronically) exposed to high corticosteroid levels high Ca currents and transmitter responses are observed. MR=mineralocorticoid receptor; GR=glucocorticoid receptor. **(B)** Typical serotonin-induced hyperpolarization (upper; applied during black bar), carbachol-evoked depolarization (middle, spikes truncated), and voltage-gated Ca^{2+} currents in hippocampal neurons.

slow Ca^{2+} dependent K^+-conductance (Storm, 1990). This current hyperpolarizes neurons when they receive a prolonged excitatory input, resulting in a reduction of firing (accommodation). Since deactivation of the current is very slow, an afterhyperpolarization is seen at the end of the depolarization. In agreement with the steroid effects on Ca^{2+} influx through voltage-gated channels, both the accommodation and afterhyperpolarization amplitude were found to be small with predominant MR activation (Joëls and de Kloet, 1990). Additional occupation of GRs led to an increased accommodation and afterhyperpolarization amplitude (Joëls and de Kloet, 1989; 1990; Kerr et al., 1989). For a steady excitatory input to the hippocampal CA1 area, this steroid modulation means that the CA1 hippocampal output is effectively transmitted with predominant MR activation and reduced when GRs are concomitantly activated. The corticosteroid modulation of Ca^{2+} and Ca^{2+}-dependent K^+ currents typically developed within 60–90 min and persisted for at least the duration of the experiments, i.e., 4–5 h (Joëls and de Kloet, 1990). It should be noted, though, that steroid modula-

tion of the Ca^{2+}-dependent K^+ conductance may also take place independent of the Ca^{2+} influx, e.g., at the level of the channel itself or by altering Ca^{2+} extrusion mechanisms (Elliot and Sapolsky, 1993; Knöpfel et al., 1995). This is supported by the observation that notwithstanding the large Ca^{2+} influx in tissue from ADX rats, accommodation and afterhyperpolarization amplitude were rather small (Joëls and de Kloet, 1989; Kerr et al., 1989).

Other K^+-conductances seem to be far less sensitive to central corticosteroid effects (Karst et al., 1993). Both the transient A-current and the delayed rectifier showed no steroid dependency. Only the inwardly rectifying Q-current was found to be regulated by corticosteroids, in a similar way as the Ca^{2+} currents. The functional significance of this may be that GR activation counteracts profound perturbations of the membrane potential in the hyperpolarizing direction, i.e., the voltage range in which the Q-current is active.

Voltage-dependent Na^+-conductances are also little affected by steroid-receptor occupation. Only small shifts in voltage dependency and kinetic properties were observed after adrenalectomy and subsequent steroid-receptor occupation (Werkman et al., 1997). Still, these small shifts may contribute to MR-mediated increases in the amplitude and duration of the action potential in CA1 neurons (Beck et al., 1994) and an enhanced threshold for the generation of action potentials with concomittant GR activation (Zeise et al., 1992). Thus, although K^+- and Na^+-conductances are much less a target for steroid modulation, still predominant MR activation will promote maintenance of excitatory hippocampal output to other brain regions, whereas additional GR activation reduces the hippocampal output.

What are the molecular events underlying these steroid actions on intrinsic ionic conductances, in particular on Ca^{2+} conductances? The delayed onset and persistent nature of the effects (Joëls and de Kloet, 1990) favor a role of nuclear (rather than membrane) receptors. This is supported by the effectiveness of ligands for the intracellular steroid receptors (Karst et al., 1993, 1994; Kerr et al., 1992). More direct evidence for the involvement of a nuclear receptor comes from the observation that the GR-mediated increase of both the Ca^{2+} current amplitude (Kerr et al., 1992) and the accommodation/afterhyperpolarization (Karst and Joëls, 1991) depends on *de novo* protein synthesis. Changes in gene transcription are further supported by recent observations, using the single-cell RNA amplification technique: Long-term elevation of corticosteroid levels was associated with a high expression level for the α1A and α1C/D subunit mRNA of the voltage-gated Ca^{2+} channels (Nair et al., 1998). By contrast, long-term exposure to low levels of corticosterone, occupying mainly the MRs, resulted in very low expression levels of these subunits. Still, actual proof for MR- or GR-induced changes in gene transcription is lacking to date.

In the above-mentioned examples of corticosteroid actions, intrinsic membrane properties were reduced with predominant MR occupation, compared to the situation in which no steroid receptors were activated (Karst et al., 1993; 1994; Joëls and de Kloet, 1990). This suggests that activation of MRs alters the expression of gene(s) leading to a reduction of, e.g., Ca^{2+}-current amplitude. When GRs were additionally activated, the reverse was observed. One explanation for these observations could be that the declining limb of the U-shaped curve (Fig. 4) is associated with MR dimers acting via a mineralocorticoid-responsive element, whereas the rising limb of the curve is caused by GR dimers acting on a glucocorticoid-responsive element (type I activation of CRGs). This assumes that MRs and GRs act via different hormone-responsive elements, which has so far not been demonstrated experimentally. Alternatively, there may be interactions between the MR and GR homodimers and possibly other transcription factors, at the protein or DNA level (type II activation of CRGs). The presently available data are unfortunately inconclusive on this point.

However, two sets of data strongly suggest that interactions between MRs and GRs are

required for both the declining and rising part of the U-shaped curve to develop. Recent experiments with mice lacking the GR protein through a genetic defect (Cole et al., 1995) showed that hippocampal Ca^{2+} currents in these animals resembled the currents observed in adrenalectomized mice or rats (Hesen et al., 1996), i.e., animals in whom MRs and GRs are both inactive. This suggests that for the development of MR-mediated events, the presence of at least some functional GRs is required. Similarly, the rising limb of the curve also seems to depend to some degree on MR activation. Thus, selective action of GRs in tissue from ADX rats with (moderate doses of) the GR-specific agonist RU 28362 was associated with rather small Ca^{2+}-current amplitudes (Karst et al., 1994), although much higher concentrations of RU 28362 did result in large Ca^{2+} current amplitudes (Kerr et al., 1992). These data suggest that the observed U-shaped dose dependency observed with the mixed MR/GR ligand corticosterone is not seen under conditions that only MR or GR homodimers can be formed. This favors a role of CRGs that are only activated by MR/GR heterodimers (type III activation), although further experiments are required to substantiate this hypothesis.

Neurotransmitters

Not only intrinsic membrane properties of hippocampal cells form a target for corticosteroid actions. Membrane responses induced by neurotransmitters are also modulated by activated MRs and GRs. Excitatory and inhibitory signals mediated by amino acids do display sensitivity to corticosteroids (reviewed by Joëls, 1997). However, the rapid onset of these effects (usually within 20 min) and reversibility make it questionable if they are mediated by nuclear steroid receptors. In general, the function of ionotropic receptors seems to be a target for rapidly acting 5α-reduced steroids (Majewska, 1992; Paul and Purdy, 1992; Lambert et al., 1995) rather than for the slowly acting 'classical' hormones.

Involvement of nuclear receptors in the modulation of responses to biogenic amines, in particular to serotonin, however is indicated by a number of recent studies. Serotonin (5-hydroxytryptamine, 5-HT) evokes a prominent 5-HT_{1A}-receptor-mediated hyperpolarization in CA1 pyramidal neurons, caused by opening of K^+-channels (Andrade and Nicoll, 1997). This hyperpolarization was found to be small in amplitude with predominant MR occupation (Fig. 4; Joëls et al., 1991 and de Kloet, 1992; Beck et al., 1996). Additional GR activation increased 5-HT responses. The MR- and GR-mediated events developed with a delay of 1–2 h (Joëls and de Kloet, 1992). The steroid-dependent modulation of 5-HT responses was seen both when corticosterone was applied exogenously and when it was released from endogenous sources, i.e., after an acute stress (Hesen and Joëls, 1996). Responses in the absence of steroids (i.e., in tissue from ADX rats) were relatively large, comparable to the responses with concomitant MR and GR occupation (Joëls and de Kloet, 1992). Recent studies suggest that the steroid modulation of 5-HT responses via 5-HT_{1A} (Beck et al., 1996) but also via 5-HT_4 (Birnsteil and Beck, 1995) receptors are differently regulated by steroids when animals are exposed to chronically aberrant steroid levels.

The 5-HT_{1A}-receptor-induced hyperpolarization will decrease the CA1 hippocampal excitability. Other 5-HT effects, mediated via various pre- and postsynaptic receptors, also result in an overall depression of the amino-acid-mediated synaptic transmission (*see*, e.g., Schmitz et al., 1995). It was found, however, that specifically the steroid modulation of 5-HT_{1A}-receptor-mediated effects are reflected in the local excitability. Thus, with predominant MR occupation, 5-HT_{1A}-receptor-mediated depression of the glutamatergic synaptic flow was impaired (Hesen et al., 1998). By contrast, additional GR activation, e.g., following an acute stress yielded large 5-HT-mediated reductions of excitatory synaptic flow.

Acetylcholine affects neuronal properties in various ways (reviewed by Nicoll et al., 1990).

Postsynaptically (amongst others) a depolarization of the membrane can be observed. This is caused by closure of at least two different types of K^+-conductances. Presynaptically, a large reduction of neurotransmitter release is evoked (e.g., Auerbach and Segal, 1996). It was observed that the postsynaptic depolarization induced by the metabolically stable cholinergic analog carbachol (CCh) is small with predominant MR activation (Fig. 4; Hesen and Joëls, 1993). Additional GR activation by exogenous corticosterone application resulted in large CCh responses. When endogenous corticosterone levels were enhanced because of stress, CCh responses were relatively small, indicating that stress related factors other than corticosterone may induce opposite effects on the cholinergic system (Hensen and Joëls, 1996). The relevance of this modulation for the CA1 hippocampal excitability, however, seems to be rather limited: The consequence of the CCh-evoked depolarization seems to be entirely overriden by the large depression of synaptic responses because of presynaptic cholinergic actions (Auerbach and Segal, 1996). The latter appeared to be insensitive to corticosteroid modulation (Hesen et al., 1998). Accordingly, the MR/GR-mediated modulation of CCh-induced depolarizations was not reflected in the overall CA1 excitability.

As with the effects on Ca^{2+} currents, the involvement of nuclear-hormone receptors in the steroid modulation of aminergic responses is indicated by the delayed onset and prolonged duration (Joëls and de Kloet, 1992), the pharmacological profile (Joëls et al., 1991), and the requirement of *de novo* protein synthesis (Karst and Joëls, 1991). Steroid-dependent changes in gene transcripts of the 5-HT_{1A} receptor have also been reported. Thus, removal of the adrenal glands was in most cases (Mendelsohn and McEwen, 1992; Chalmers et al., 1993; Meijer and de Kloet, 1994; but *see* Holmes et al., 1995) reported to enhance 5-HT_{1A} receptor mRNA expression in the dentate gyrus and to a lesser extent also in the CA1 region. Subsequent occupation of particularly the MRs resulted in a suppressed expression of the 5-HT_{1A} receptor mRNA. This could potentially underlie the small responses seen in the functional studies. However, two observations argue against this. First, activation of GRs in addition to MRs resulted in a further decrease of the 5-HT_{1A}-receptor mRNA expression (for review, *see* Meijer and de Kloet, 1998), wheras functional responses to 5-HT were enhanced (Joëls et al., 1991; Joëls and de Kloet, 1992). Secondly, changes in mRNA expression developed 3–6 h after steroid treatment (Meijer and de Kloet, 1998), whereas clear changes in responsiveness to 5-HT were already observed within 2 h (Joëls and de Kloet, 1992). These data suggest that responsiveness to 5-HT is altered by posttranslational modification of 5-HT_{1A} receptors, which at a later time point can be followed by transcriptional changes aiming at the 5-HT_{1A} receptor gene. Also, there may be a cumulative effect of MR- and GR-regulated proteins. More direct evidence for the nature and sequence of events can only be obtained when 5-HT_{1A}-receptor mRNA expression and electrophysiological recording of 5-HT responses are performed in the same cell.

Studies with transgenic mice indicate that the presence of some functional GRs is necessary for the development of MR-induced suppression of 5-HT and CCh responses: In homozygous GR knockouts, responses to 5-HT and CCh were indistinguishable from the responses observed in ADX mice or rats (Hesen et al., 1996). In rats, selective activation of GRs resulted in large responses (Joëls and de Kloet, 1992), similar to those seen when either no receptors were activated or when MRs and GRs were simultaneously activated. Thus, suppression of aminergic responses was only observed when the ratio of MR/GR activation was large (but not indefinite). These data suggest that some interactions between MRs and GRs could exist, such that GR activation functionally antagonizes MR-mediated effects.

Morphology in Dentate Gyrus

The steroid-induced changes in electrical properties of hippocampal cells take place in a time frame of hours. However, corticosteroids also affect hippocampal circuitry at a much slower rate. These effects mainly pertain to the morphology of the hippocampal neurons.

Recent studies have shown that excess corticosteroid levels for a prolonged period of time lead to morphological changes particularly of CA3 hippocampal neurons. The first sign is atrophy of distal dendrites that may eventually lead to overall cell degeneration if the corticosteroid excess persists (Woolley et al., 1990; Sapolsky et al., 1985; Magarinos et al., 1996). This indicates that slow corticosteroid-receptor-mediated changes in gene expression are a threat for neuronal viability. The molecular mechanisms underlying this endangerment of neurons is thought to be linked to excessive activation of metabolic genes thus leading to an energy depletion that can eventually lead to a necrotic type of cell death (Packan and Sapolsky, 1990; for extensive review on this topic, *see* Sapolsky, 1996).

However, a limited extent of corticosteroid-receptor activation also seems a prerequisite for survival of neurons. Thus, ADX animals show apoptotic-like degeneration, specifically in the dentate granule cells (Sloviter et al., 1989; 1993). Several studies support the involvement of MRs in the suppression of ADX-induced apoptosis: Administration of a low dose of corticosterone or aldosterone appeared to be sufficient to prevent the apoptotic process (Sloviter et al., 1989; Woolley et al., 1991). The role of GRs in ADX-induced apoptosis is less clear. Intraperitoneal administration of the GR-specific agonist dexamethasone to ADX animals did not prevent apoptosis (Hornsby and de Kloet, 1994; Hornsby et al., 1996; Sloviter et al., 1995). In fact, intraperitoneal administration of dexamethasone to adrenally intact rats induced rather than prevented apoptosis (Hornsby et al., 1996; Hassan et al., 1996). This process augmented with age and was counteracted by pretreatment with corticosterone (Hassan et al., 1996). However, when dexamethasone was administered via another route, opposite effects were observed. Thus, unilateral intrahippocampal infusion of dexamethasone or administration of the specific GR agonist RU28362 reduced the number of apoptotic cells (Woolley et al., 1991; Jaarsma et al., 1992), whereas addition of dexamethasone to the drinking water completely prevented apoptosis (Liao et al., 1993). It thus seems that the effect of dexamethasone depends on the way it is administered and the status and age of the animal.

The genes involved in the ADX-linked apoptosis or in the MR-mediated prevention of this phenomenon are presently under investigation. As neurotrophin (NT) and corresponding neurotrophin-receptor genes are involved in the control of hippocampal cell viability, these genes may be important targets for activated MRs and GRs. Recent data indeed support this. First, administration of NT4/NT5 but not of NT3 significantly reduced the number of apoptotic cells in ADX rats (Qiao et al., 1996). Second, transgenic mice lacking both the TrkB and TrkC neurotrophin receptors exhibited massive cell death of dentate gyrus granule neurons (Minichiello and Klein, 1996) suggesting that survival of these cells depends on activation of neurotrophin receptors. Third, the expression of neurotrophin and neurotrophin receptors, particularly of brain-derived neurotrophic factor (BDNF) and its receptor TrkB, is under corticosteroid-receptor control. For example, administration of a high dose of corticosterone to ADX rats for 7 d led to a downregulation of BDNF and other neurotrophins (Chao and McEwen, 1994). A similar downregulation was observed after immobilization stress (Smith et al., 1995). In addition, ADX attenuated kainic acid-induced upregulation of BDNF and its receptor TrkB (Barbany and Perrson, 1993). This effect was blocked by administration of dexamethasone. Administration of a single low dose of corticosterone to ADX rats led to an upregulation of the TrkB receptor but not of its ligand BDNF (Schaaf et al., 1997). The expression of other neurotrophins is also modulated by corticosteroids, both in vivo (Chao and McEwen, 1994)

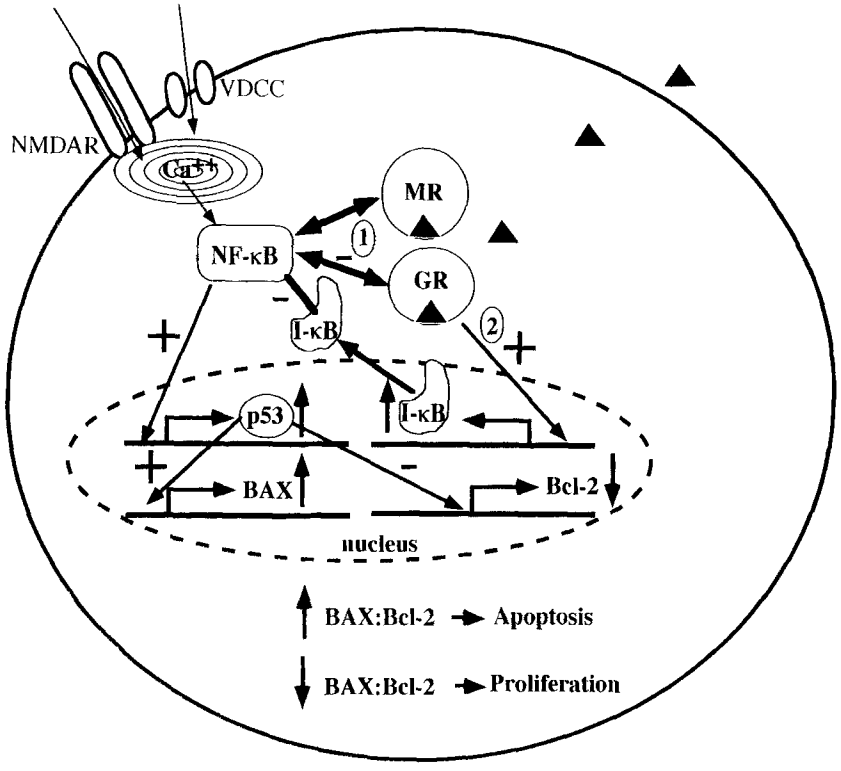

Fig. 5. Hypothetical model for adrenalectomy-induced apoptosis in the rat dentate gyrus. The absence of corticosterone (cort) upregulates p53 expression, via unknown mechanisms (Schreiber et al., 1994; Maiyar et al., 1997; Meberg et al., 1996). Activated p53 inhibits the apoptosis blocker bcl-2 (Miyashiti et al., 1994) and stimulates the apoptosis promotor bax (Miyashita and Reed), leading to cell death. For further details *see* text.

and in vitro (Schaaf et al., 1997). Although neurotrophins thus seem to be important for corticosteroid-regulated neuronal vulnerability in the rat hippocampus, their role in ADX-induced apoptosis should probably be considered as a cofactor and not as the primary factor of the apoptotic process. This is indicated by the observation that ADX by itself has no clear effect on BDNF expression (Chao and McEwen, 1994) and that hippocampal subfields other than the dentate gyrus also exhibit altered neurotrophin and neurotrophin-receptor gene expression in response to corticosteroids (Chao and McEwen, 1994; Smith et al., 1995; Schaaf et al., 1997).

The process of ADX is not only linked to cell death, but also promotes the rate of cell birth (Smith et al., 1995; Cameron and Gould, 1994). As a number of cell-cycle-related genes are capable of directing both cell division and apoptosis, these genes may also be a target of MR and/or GR action. Indeed, recent data show specific upregulation of the tumor-suppressor p53 mRNA in apoptotic cells of the rat dentate gyrus (Schreiber et al., 1994) as well as in other damaged cells of the hippocampus (Sakhi et al., 1994). Interestingly, p53 decreases the apoptosis-blocker gene bcl-2 (Miyashiti et al., 1994) in cell lines, whereas the human apoptosis-promoting bax gene is induced as a primary p53-responsive target gene (Miyashita and Reed). Since members of the bcl-2 family are involved in neuronal vulnerability, these data suggest a molecular mechanism by which corticosteroids repress p53-gene expression, thereby indirectly blocking apoptosis-promoting factors such as bax and inducing survival factors such as bcl-2. This process is summarized in Fig. 5. The mech-

anism by which corticosteroids regulate p53 expression is presently unknown. As no GREs are present in the promoter region of the p53 gene, an indirect (type II) activation mechanism may take place (Maiyar et al., 1997). This may involve various transcription factors, as was described before for the immune system (Defie et al., 1993; Meberg et al., 1996; Lipton, 1997; O'Neill and Kaltschmidt, 1997; Auphan et al., 1995).

In conclusion, corticosteroids seem to affect hippocampal cell viability via a complex mechanism involving an interacting cascade of proteins such as neurotrophins, cell cycle genes and oncogenes. Inactivation of GR and/or MR may result in inactivation of primary corticosteroid-responsive genes or may activate other transcription factors indirectly, by removal of a physical block. In this respect it is interesting to note that NMDA-receptor activation is an important factor for precipitation of the apoptotic process, not only induced by ADX but also by other, in general excitatory stimuli (Gould and McEwen, 1993; Gould et al., 1994; Sloviter et al., 1996). Thus, cell fate in the hippocampus may depend on the cross talk between GR and/or MR on the one hand and other cellular-transcription factors, e.g., activated by NMDA receptors, on the other hand.

Concluding Remarks

Experimental studies over the past decade have clearly shown that corticosteroid receptors in the brain mediate persistent effects on signal transduction and, over a longer period of time, on structure and viability of neurons. Particularly, hippocampal neurons seem to be largely modulated in function by corticosteroid hormones. In general predominant MR activation is associated with stability of hippocampal-network function and maintenance of cellular structure. Additional GR occupation restricts hippocampal excitability but also introduces instability in hippocampal networks, e.g., by reducing inhibitory transmission and impairing cellular Ca^{2+} homeostasis. When this condition persists over a longer period of time or when it is coupled to additional challenges to the tissue, e.g., during ischemic insults or epileptic episodes, the GR-mediated events may put hippocampal neurons in a vulnerable position for degeneration. A comparable increased vulnerability seems to be associated with the situation in which corticosteroid hormones are chronically absent, implying a U-shaped dose dependency of hippocampal function on corticosteroid levels. The combined electrophysiological and morphological data indicate that a condition of predominant MR activation, which is linked to the average daily level of circulating corticosteroids (Dallman, 1993), is beneficial for signal transduction and neuronal integrity. Chronically elevated or reduced corticosteroid levels, however, put neurons at risk for impaired network function and neurodegenerative processes (Joëls and de Kloet, 1992; 1994; Joëls, 1997; Gould and McEwen, 1993; McEwen and Sapolsky, 1995).

The described steroid actions will clearly affect functional processes in which hippocampal networks play a key role, e.g., learning and memory ability (Chan Palay and Kohler, 1989). This was indeed observed in a variety of learning paradigms. Thus, MR activation appears to be important for behavioral reactivity and the choice of response strategy when animals are exposed to a novel situation. Additional GR activation plays a role in storage of information (Oitzl and de Kloet, 1992). However, not only processes in which hippocampal networks are directly involved will be influenced by corticosteroid actions. Functional processes that indirectly depend on hippocampal output will also exhibit steroid dependence. One of the most intriguing examples of such processes is the activity of the paraventricular nucleus in the hypothalamus, the major negative feedback site for corticosteroids (Fig. 3; Dallman, 1993). It was found that the hippocampal formation, via transsynaptic contacts, exerts an inhibitory effect on paraventricular activity (Herman and Cullivan, 1997). Maintenance of basal hippocampal output via MR activation will therefore indirectly contribute to the con-

trol of the hypothalamo-pituitary-adrenal system. Short-lasting activation of GRs potentially increases the activity of the system, although extensive GR occupation in paraventricular neurons at the same time will counteract and probably dominate the hippocampal influence. In this way, higher brain structures can contribute to steroid feedback function (de Kloet et al., 1997).

The molecular mechanism underlying these corticosteroid effects in higher brain regions is far from being resolved (Fig. 2). The general view that activation of corticosteroid receptors leads to transcriptional activation or repression of *specific genes* does not seem to hold for the conditions that were studied. This is, e.g., suggested by the fact that mRNA changes for a specific protein, i.e., the 5-HT_{1A} receptor, seem to follow rather than precede changes in the function of the protein. Moreover, many functional responses of hippocampal neurons were found to be affected all in the same way by corticosteroid treatment, which points to a common denominator as putative target for steroid action. At present, the data suggest that corticosteroid actions on signal transduction, which develop generally within an hour, are caused by altered expression of genes involved in general cell characteristics, e.g., a phosphorylation process or the function of G proteins (Saito et al., 1989; Okuhara et al., 1997). This assumes that corticosteroids induce membrane-protein-modifying enzymes—such as kinases or protein phosphatases—affecting an array of cell features involved in signal transduction, e.g., Ca^{2+}-channel function or transmitter–receptor sensitivity. Clearly, experimental studies specifically addressing this point are required.

More prolonged aberrations of corticosteroid level, however, may lead to actions that are accomplished through a different mechanism. This was indicated by recent studies in which adrenalectomized rats chronically received either high or low corticosterone doses via the drinking water (Nair et al., 1998). This replacement regime allows daily fluctuations in corticosterone intake (linked to the drinking pattern) within the range set by the supplied corticosterone concentration. Whereas Ca^{2+}-current amplitude correlated well with the short-term, daily alterations in steroid level, mRNA expression of Ca^{2+}-channel subunits closely followed the chronic changes in overall daily corticosterone intake. This suggests that more prolonged perturbations of the corticosterone level may specifically alter the gene expression for the protein under study.

Specific changes in gene transcription also seem to take place during steroid-dependent structural changes of neurons. There is evidence that the p53 gene is one of the target genes during ADX-linked apoptosis. However, this area is only starting to be explored. For an overall impression of steroid-dependent changes in gene transcription, it will be necessary to compare gene transcripts from apoptotic versus nonapoptotic cells. Even more importantly, it will be necessary to identify neurons in an early phase of apoptosis, since the most marked changes in gene transcription are expected to take place during this phase.

Although data about the molecular mechanism underlying corticosteroid actions in the brain are still fragmentary, there is indirect evidence that these actions may not simply involve type I activation of CRGs via receptor homodimers (Fig. 2). For instance, data from genetically modified animals suggest that the presence of GRs is a prerequisite for the development of certain MR-mediated events (Hesen et al., 1996), supporting the existence of type III activation of CRGs. Other studies indicated that functional antagonism or synergism between MR- and GR-mediated actions exists (Karst et al., 1994; Joëls and de Kloet, 1992), which points to type II activation of CRGs. These hypotheses can be tested more directly in animals with genetically modified GR or MR genes, preventing interaction of GRs with hormone-responsive elements (type I activation) but leaving the possibility for protein–protein interactions (type II activation) intact. Also, it will be important to test neuronal properties in neurons that predominantly express MRs or GRs; particularly the latter is a

very relevant situation since most neurons in the brain express GRs in excess of MRs.

In conclusion, numerous studies over the past years have supplied ample evidence that brain corticosteroid receptors have profound and long-lasting regulatory effects on signal transduction and viability of neurons in the brain. These effects cannot be fully explained by the formation of MR and GR homodimers acting independently on glucocorticoid-responsive elements in the DNA. Clearly, more complex mechanisms are involved. Similarly, the nature of the genes forming a target for MR and GR actions and the extent to which these genes are affected is presently largely unknown. This is caused by the limited accessibility of neurons *in situ* and the lack of sensitive methods to measure changes in gene products in small amounts of tissue, i.e., in individual neurons. The recent development and combination of several molecular biological techniques such as differential display (Liang and Pardee, 1992), serial analysis of gene expression (Velculescu et al., 1995), inverse Northern blot analysis, single-cell RNA amplification (Eberwine et al., 1992), and hybridization of high-density DNA arrays (Chee et al., 1996) should, however, soon allow the screening of corticosteroid-related gene expression profiles in individual neurons. Preliminary studies with these approaches have already revealed some of the genes affected by corticosteroid-receptor actions (Nair et al., 1998; Vreugdenhil et al., 1996). If corticosteroid hormones, like estrogens (Yang et al., 1996), indeed make use of multiple signaling pathways in different tissues, knowledge of these processes can be very useful for the development of analogs that exert beneficial effects in brain tissue, in the absence of deleterious effects in other, peripheral organs.

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