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MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS IN THE BRAIN. IMPLICATIONS FOR ION PERMEABILITY AND TRANSMITTER SYSTEMS

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ABBREVIATIONS

11 β -OHSD	11 β -Hydroxysteroid dehydrogenase	EPSP	Excitatory postsynaptic potential
5HT	5-Hydroxytryptamine, serotonin	GABA	γ -Amino butyric acid
ACh	Acetylcholine	GPDH	Glycerol phosphate dehydrogenase
ACTH	Adrenocorticotropin hormone	GR	Glucocorticoid receptor
ADX	Adrenalectomy, adrenalectomized	HPA-axis	Hypothalamo-pituitary-adrenal axis
AHP	Afterhyperpolarization	IPSP	Inhibitory postsynaptic potential
AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	KA	Kainic acid
AVP	Arginine vasopressin	LTP	Long-term potentiation
CBG	Corticosteroid binding globulin	MR	Mineralocorticoid receptor
CRH	Corticotropin releasing hormone	NMDA	N-Methyl-D-aspartate
DA	Dopamine	PVN	Paraventricular nucleus
		VIP	Vasointestinal polypeptide

1. INTRODUCTION

The adrenal cortex of the rat produces several steroid hormones, including the mineralocorticoid aldosterone and the glucocorticoid corticosterone (cortisol in humans). Aldosterone has a number of well described peripheral actions, of which sodium retention in the kidney is the best known example. Corticosterone is particularly implicated in the energy metabolism and the defense reaction of the body against stressful stimuli. Potential disruption of homeostatic control, e.g. by trauma, pain or environmental challenges, activates the autonomic nervous system, and initiates a cascade of humoral events in the hypothalamo-pituitary-adrenal (HPA) axis. Peptides from the hypothalamus, such as corticotropin releasing hormone (CRH) and vasopressin (AVP), stimulate the release of pro-opiomelanocortin derived peptides including adrenocorticotropin hormone (ACTH) from the pituitary gland. In turn, ACTH activates the adrenocortical production of corticosterone, a hormone that exerts a wide array of peripheral actions, including e.g. the suppression of prostaglandin synthesis and glucose utilization. Corticosterone also exerts a negative feedback action on the production of releasing hormones in the hypothalamus and ACTH in the pituitary gland. For further details about the peripheral and neuroendocrine regulation by corticosteroid hormones we refer to excellent reviews (Munck *et al.*, 1984; Dallman *et al.*, 1987, 1992).

Due to their lipophilic nature corticosteroid hormones readily enter the brain and, as a true hormone, are only retained in those cells that contain corticosteroid receptors (McEwen *et al.*, 1968). Over the past 5–10 years the knowledge about brain corticosteroid receptors has tremendously increased. Receptors for the corticosteroid hormones belong to

the superfamily of nuclear receptors, which act as transcription factors in the regulation of gene expression. At least two types of corticosteroid receptors have been described in the brain, i.e. the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Corticosterone, which circulates in ~100-fold higher concentrations than aldosterone, binds to both receptors albeit with different affinity. The molecular and binding properties of corticosteroid receptors, their localization and regulation are described in Section 3 of this review.

The slow, genomic effects resulting from MR and GR activation by corticosteroid hormones potentially affect cellular properties in the brain. These may comprise general cell features such as metabolic homeostasis, but also characteristics that are directly or indirectly related to the conductance of the cell membrane, which is an exceedingly important property for the transmission of signals. Recently developed electrophysiological techniques have made a more indepth investigation of steroid-induced changes of membrane conductance possible. These and other cellular effects of steroids are reviewed in Section 4. The final section discusses how coordinated MR- and GR-mediated cellular actions affect the excitability in the brain and what the consequences may be under physiological and pathological conditions.

2. CORTICOSTEROIDS: BIOSYNTHESIS AND SYNTHETIC ANALOGUES

The adrenocorticosteroids are derived from cholesterol. The structure of some of the compounds discussed in this review is depicted in Fig. 1. Biological activity depends on the presence of the 4,5 double bond and a ketone group at C-3, which is primarily responsible for the tight binding to corticosteroid

FIG. 1. Structure of the endogenous hormones corticosterone, aldosterone, the synthetic GR agonist dexamethasone and the GR antagonist RU 38486.

receptors. The presence of a hydroxyl group at C-11 conveys glucocorticoid activity to corticosterone, the principal glucocorticoid of rodents; absence of the hydroxyl group results in predominant mineralocorticoid (deoxycorticosterone) activity of the steroid. Addition of a hydroxyl group at C-17 yields cortisol, which is the glucocorticoid secreted from the human adrenal cortex. The mineralocorticoid aldosterone differs from corticosterone in that it contains an aldehyde group at C-18. At least five isomers of aldosterone circulate, each with different biological potency.

The pituitary hormone ACTH is the predominant regulator of corticosterone and cortisol secretion, while angiotensin II regulates aldosterone secretion. However, it was recently shown that factors released from the adrenal medulla such as the catecholamines, but also CRH and AVP directly affect steroid synthesis and blood flow in the adrenal gland (Hinson, 1990; Charlton, 1990; Van Oers *et al.*, 1992). The steroids are not stored. Upon stimulation, cholesterol is taken up from the plasma, converted to the steroid hormones by a series of enzymatic steps and subsequently secreted. The secretion displays a circadian rhythm, with low levels at the start of the inactive period (i.e. in the morning for the rat) and high levels at the start of the active period (evening). In addition to mineralo- and glucocorticoid hormones, the adrenal gland also secretes small amounts of progesterone, androstenedione, dehydroepiandrosterone sulphate and testosterone.

Numerous analogues of corticosterone and aldosterone have been synthesized over the past decades, including e.g. the potent GR agonist dexamethasone. However, all of these potent glucocorticoid analogues still displayed appreciable affinity towards MRs, which hampered the discrimination between MR- and GR-mediated events. In the beginning of the 1980s a new class of selective GR agonists and antagonists became available, which turned out to be very instrumental in quantifying MRs and GRs independently and in studying MR- and GR-mediated effects independently (Philibert and Moguilevski, 1983; Philibert, 1984; Gagne *et al.*, 1985). This new class of analogues includes the synthetic glucocorticoid agonist RU 28362 and the mixed glucocorticoid and progesterone antagonist RU 38486 (see Fig. 1). Mineralocorticoid antagonists that are now commonly used include spironolactone, the 7 α -propylspiro lactone (RU 26752) and its potassium salt (RU 28318). RU 28318 shows very little binding affinity to MRs *in vitro*. However, upon infusion RU 28318 is readily converted to an active moiety, which suppresses [3 H]-aldosterone retention in the brain (McEwen *et al.*, 1986a).

3. CORTICOSTEROID RECEPTORS

3.1. MEMBRANE RECEPTORS

Classically, corticosteroid hormones are thought to act via intracellular receptors that mediate slow genomic actions. However, over the past decades several studies have shown that rapid steroid effects also occur. Membrane rather than intracellular steroid

receptors may be implicated in these rapid effects. Saturable binding of [3 H]-corticosterone to partially purified synaptic neuronal membranes was indeed found some 10 years ago (Towle and Sze, 1983), but the affinity of this site was quite low (K_d 120 nM). Recently, a recognition site for corticosterone with much higher affinity (K_d 0.5 nM) was described for neuronal membranes from amphibian brains (Orchinik *et al.*, 1991). The affinity for aldosterone and synthetic glucocorticoids was very low. The binding of [3 H]-corticosterone appeared to be affected by nonhydrolyzable guanyl nucleotides, suggesting that this membrane corticosteroid receptor is coupled directly to G-proteins (Orchinik *et al.*, 1992). Whether or not a similar high affinity membrane receptor for corticosterone will also be found in mammalian brain tissue awaits further research. So far, membrane receptors for corticosteroid hormones in mammals have only been observed outside of the central nervous system, including an aldosterone selective membrane receptor on lymphocytes (see for review Wehling *et al.*, 1993).

3.2. INTRACELLULAR RECEPTORS

3.2.1. Primary Structure

In contrast to the putative non genomic pathway mediated by membrane receptors for corticosteroid hormones, much is known nowadays about the genomic mechanism of action. In short, binding of the corticosteroid hormone to its receptor induces a conformational change which leads to dissociation of the receptor from the attached heat shock protein (HSP90; Baulieu, 1987), activation of nuclear translocation signals and dimerization of activated receptor complexes. The receptor dimer binds to hormone responsive elements of the nuclear DNA and transcription is initiated. As a consequence translation of mRNAs to certain proteins is affected, which may eventually result in steroid-induced alterations of e.g. membrane characteristics or general cell features such as metabolic homeostasis.

With molecular biological techniques many steps in this intracellular pathway have been further elaborated. The primary structure of the MR and GR has been elucidated, the nucleotide sequence that is essential for some of the hormone responsive elements is known and recent studies have even focussed on the two and three dimensional representation of the steroid protein-DNA complex (see for reviews e.g. Gustafsson *et al.*, 1987; Beato, 1989; Schwabe and Rhodes, 1991; Gronemeyer, 1992; King, 1992). Important for this progress was the purification of the GR in the early eighties (Westphal and Beato, 1980; Wrange *et al.*, 1984). Subsequently, the cDNAs for the GR and MR were cloned (Hollenberg *et al.*, 1985; Arriza *et al.*, 1987). It appeared that the MR and GR are structurally related to each other but also to other hormone receptors, particularly the progesterone and androgen receptor, and to a lesser degree also the estrogen and thyroid hormone receptor: They all belong to the superfamily of nuclear receptors (Evans, 1988). The evidence so far also indicates that the MR and GR proteins in the brain are structurally similar

FIG. 2. Schematic representation of the six regions (A–F) of corticosteroid receptors. Indicated (lower part) is the approximate location of sequences that are important for association to the HSP90, the dimerization, nuclear translocation and transactivational activity, within the N-terminal domain, the DNA-binding domain and hormone binding domain. The upper part of the scheme indicates the homology for the three domains between rat or human MR and GR, the affinity of rat MR and GR for corticosterone and the approximate range of occupancy for the two receptor subtypes.

to the receptors in peripheral organs (Patel *et al.*, 1989).

Corticosteroid hormone receptors display a modular structure of six conserved regions (see Fig. 2). The function of the regions was pursued with sequence alignments, deletion studies, site-directed mutagenesis and domain-swap experiments in transfected cell lines; in many cases the promoter for the mouse mammary tumor virus was used as part of the reporter gene. Most of the data were obtained for the GR, but considering the high degree of homology between the MR and GR, it is thought that most of the findings for GR also apply to the MR.

It was observed that the regions comprise a number of functional domains (Giguere *et al.*, 1986; Carlstedt-Duke *et al.*, 1987; Hollenberg *et al.*, 1987; Rusconi and Yamamoto, 1987): (1) The C-terminal steroid binding domain (220–250 amino acid residues), which probably contains a pocket into which the ligand fits. Contained in this area is also one of the dimerization domains and the HSP90 association site. In addition, it contains a hormone-inducible nuclear localization signal; the latter inhibits the formation of an active complex in the absence of a ligand (Picard *et al.*, 1990). (2) The DNA binding domain which consists of 70–80 amino acid residues. The domain comprises two zinc fingers, each consisting of about 20 amino acids, with four cysteine residues placed in a way that they tetrahedrally coordinate zinc and thereby form a loop (Severne *et al.*, 1988; Freedman *et al.*, 1988; Evans and Hollenberg, 1988). There is a large degree of homology for the DNA binding domains of the hMR and hGR (>90%, Arriza *et al.*, 1987), but also for domains of other receptors belonging to the same superfamily. However, a certain degree of specificity is retained in the N-terminal zinc finger: For instance, three amino acid residues in this zinc finger are essential for the specificity of the DNA binding domain of the GR and the estrogen receptor

(Green *et al.*, 1988; Mader *et al.*, 1989; Danielsen *et al.*, 1989; Umesono and Evans, 1989). The N-terminal zinc finger binds to specific nucleotides of the hormone responsive element while the C-terminal zinc finger is more important for stabilization of the protein–DNA complex (Chalepakakis *et al.*, 1988; Hard *et al.*, 1990; Luisi *et al.*, 1991). The DNA binding domain of the GR also contains a second nuclear translocation signal. It is probably due to this second translocation signal that, in the absence of corticosterone, GRs are detected in the cytosolic compartment (Picard and Yamamoto, 1987), in addition to the nuclear compartment (Brink *et al.*, 1992). Still, even in the absence of the ligand, binding to DNA is possible but the efficacy and kinetics of this process are much reduced (Becker *et al.*, 1986; Willmann and Beato, 1986; Schauer *et al.*, 1989; Power *et al.*, 1992). Finally, a second dimerization region partly overlaps with the DNA binding domain. (3) The N-terminal region (~400 amino acid residues), of which the function is not clearly defined. Most of the antibodies are raised against this part of the receptor protein (Carlstedt-Duke *et al.*, 1982; Westphal *et al.*, 1982).

While the DNA binding domain in itself is sufficient to activate transcription, the total transcriptional activity of the whole receptor protein is many times larger. Transactivational domains were identified in the C-terminal and N-terminal regions of the receptor (Giguere *et al.*, 1986; Webster *et al.*, 1988; Hollenberg and Evans, 1988; Pearce and Yamamoto, 1993). These domains are important determinants of the final transcriptional efficacy (Arriza *et al.*, 1988). Recent studies indicate that the transactivational domains also play a role in the interactions with other transcription factors. The best documented example concerns interactions with the AP1 complex, which like steroid hormone receptors bind to the DNA in heterodimers of the oncogenes *c-jun* and *c-fos* or a homodimer of *c-jun*. GR enhances transcription from

a hormone responsive element when AP1 is composed of *c-jun* homodimers, while it represses transcription in the presence of *c-jun/c-fos* heterodimers (Diamond *et al.*, 1990; Lucibello *et al.*, 1990; Yang-Yen *et al.*, 1990; Jonat *et al.*, 1990; Schule *et al.*, 1990; Pearce and Yamamoto, 1993). In a recent report it was shown that MR and GR differentially affect the activity of the AP1 complex: Under conditions where GR repressed AP1-stimulated transcription from a hormone responsive element, MR was found to be ineffective (Pearce and Yamamoto, 1993). An N-terminal transactivational domain of the GR was required for the interaction with AP1. While the function of transactivational regions is already intriguing for the *in vitro* conditions in which these experiments are performed, their relevance for the situation *in vivo* is enormous. In the *in vitro* situation, the elements necessary for the subsequent steps of steroid-induced gene transcription are overexpressed, but *in vivo* this is probably not the case. Synergism but also antagonism may be the result, as was indeed suggested for MR- and GR-dependent interactions (Evans, 1989). In addition to transcription factors such as *c-jun*, matrix proteins probably also determine the transcriptional activity of the steroid receptors (Van Driel *et al.*, 1991).

The hormone responsive element with which MRs and GRs interact was also investigated. The most important feature is the occurrence of a sequence of six nucleotides which is then, with a certain spacing, repeated in an inverted form (palindrome). This sequence can be activated not only by GRs but also (albeit with much lower efficiency) by MRs and even receptors for progesterone and androgens (Strahle *et al.*, 1987; Arriza *et al.*, 1988; Cato and Weinmann, 1988; Ham *et al.*, 1988). However, some sort of selectivity is retained since e.g. the glucocorticoid responsive element differs at three points from the responsive element for the estrogen receptor (Klock *et al.*, 1987; Nordeen *et al.*, 1990).

In summary, many characteristics of the MR and GR structure have been elucidated over the past 10 years: The cDNAs for the receptors are cloned, functional domains of the receptors have been defined and there is a consensus responsive element both for GR-induced and GR-suppressed transcription (Sakai *et al.*, 1988). Even though there is a large degree of similarity for the various steps of the steroid-induced transcriptional activation via MRs, GRs and other steroid receptors, there are several ways in which specificity can be guaranteed at the transcriptional level: (a) The specificity of the receptor DNA binding domain and the consensus sequence of hormone responsive element. Both elements are important for the discrimination between MRs and GRs on the one hand and other hormone receptors on the other hand, but may be less relevant for discrimination between MRs and GRs themselves. So far there is no evidence that separate hormone responsive elements exist for MRs and GRs, but this possibility can not be ruled out for MR and GR responsive elements in the brain. (b) Transactivational interactions, mutually between receptors but also with other transcription factors. The latter interactions have shown that even when MRs and GRs interact with the same hormone responsive element, different transcriptional effects may occur.

This opens the possibility that MRs and GRs mediate different effects in the brain and that synergism or antagonism between the two receptors will occur.

3.2.2. Binding Characteristics and Localization

Studies in the late 1960s and early 1970s (McEwen *et al.*, 1968; Gerlach and McEwen, 1972) showed that [³H]-corticosterone administered to ADX rats was retained predominantly in pyramidal and granule cells of the hippocampus. The potent synthetic glucocorticoid analogue dexamethasone, however, was not retained by these sites suggesting that the hippocampal receptors were unlike GRs in the pituitary (de Kloet *et al.*, 1975; Birmingham *et al.*, 1984; Stumpf *et al.*, 1989). This was explained by studies in the 1980s (Coirini *et al.*, 1985; Reul and de Kloet, 1985; Maraginos *et al.*, 1990) which determined the binding properties of corticosteroid receptors in the brain, with the use of selective mineralocorticoids and glucocorticoids. With radioligand binding assays it was shown that the affinity of MRs for both corticosterone and aldosterone is high ($K_d \sim 0.2$ nM). In this respect the hippocampal MR appeared to be identical to the MR in the kidney (Krozowski and Funder, 1983). The affinity of GRs for corticosterone is about one order of magnitude lower ($K_d \sim 3$ nM), while the GR affinity for aldosterone is still lower. These observations about the relative binding affinity of MRs and GRs to corticosterone have a number of implications. First, it was shown that the ED_{50} for *in vivo* occupancy of hippocampal MRs and GRs amount to 0.9 and 60 μ g corticosterone/100 g body weight respectively. This means that the high retention of the 'glucocorticoid' corticosterone in the hippocampus after a peripheral administration of ~ 1 μ g corticosterone to ADX rats, as described in the early studies (McEwen *et al.*, 1968; Gerlach and McEwen, 1972), represented binding to MRs rather than GRs, since the affinity of GRs is too low for extensive labelling. Second, during the circadian trough in the morning, when plasma corticosterone levels are below 25 nM and aldosterone levels below 1 nM, MRs in the hippocampus are occupied for more than 70%, while only about 10% of the GRs are occupied. Consequently, changes in plasma corticosterone concentrations from basal up to peak levels (induced by stress or due to circadian variation) are accompanied by relatively small differences in MR occupancy (range: 70%–90%), while occupancy of GRs can vary considerably (from 10%–90%). Third, it is recognized that an 'average' level of circulating corticosterone of about 15 nM is sufficient to maintain corticosteroid-dependent functions (Dallman *et al.*, 1992). This 'average' corticosterone concentration is associated with a predominant MR occupancy (>80% versus only 20–30% GR occupancy).

Autoradiography of radiolabelled brain sections, immunocytochemistry and *in situ* hybridization procedures have been used to study the topography of MRs and GRs. MR immunoreactivity (Ahima *et al.*, 1991) and MR mRNA (Arriza *et al.*, 1988; Van Eekelen *et al.*, 1988; Chao *et al.*, 1989; Herman *et al.*, 1989a) are highly expressed in the brain, both in neurons and glial cells, but display a non-uniform distribution: High MR densities are confined to the

neurons of the hippocampal formation, lateral septum, medial and central amygdala, olfactory nucleus, layer II of the cortex, cerebellum and in brain stem sensory and motor neurons. The highest neuronal MR density occurs in area CA2 and in the dorsomedial subiculum, followed by area CA1, then CA3 and CA4 of the hippocampal formation; the dentate gyrus and the cerebellum contain scattered strongly labelled cells among cells with intermediate nuclear labelling. MR mRNA is also present in the anterior hypothalamus, subfornical organ and choroid plexus. The MR mRNA signal in the hypothalamic regions however is very low, although it can be increased after glucocorticoid treatment (Swanson and Simmonds, 1989).

The distribution of GR immunoreactivity (Fuxe *et al.*, 1985; Van Eekelen *et al.*, 1987a; Cintra *et al.*, 1991; Ahima and Harlan, 1990) and GR mRNA (Aronsson *et al.*, 1988; Van Eekelen *et al.*, 1988; Chao *et al.*, 1989) is much more widespread, in neurons and glial cells throughout the brain. Particularly high GR concentrations are found in the limbic system (hippocampus, septum) and in the parvocellular neurons of the PVN in the hypothalamus where glucocorticoids suppress the stress-induced synthesis of CRH and AVP. GRs are present in relatively high concentrations in the ascending monoaminergic neurons of the brain stem. Moderately high GR levels are also found in many thalamic nuclei, in a patch-like distribution within the striatum and in the central amygdaloid nucleus, as well as throughout the cortical hemispheres.

Hippocampal neurons of the CA1 and CA2 subfields and the dentate gyrus express large amounts of both MR and GR mRNA (Van Steensel *et al.*, in preparation). Pyramidal CA3 neurons contain relatively more MR than GR mRNA in the rat. In other species, such as the hamster (Sutanto and de Kloet, 1987) and the dog (Reul *et al.*, 1990) MRs and GRs are also highly expressed in the hippocampal subfields.

3.2.3. Receptor Specificity Conferring Mechanisms

Given the high affinity of brain MRs for both corticosterone and aldosterone it is not surprising that most of the cellular actions described so far (see Section 4) can be induced effectively with both hormones. However, corticosteroid-mediated regulation of blood pressure (Van den Berg *et al.*, 1990) and salt appetite (Denton, 1984) via the brain appear to be aldosterone specific. The latter aldosterone selectivity can be explained by recently elucidated steroid specificity conferring mechanisms. One of these mechanisms involves the corticosteroid binding globulin (CBG), which binds corticosterone with a rather high affinity ($K_d \sim 15$ nM; cf MR: $K_d \sim 0.2$ – 0.5 nM). CBG circulates in blood as a transport protein for corticosterone but not aldosterone (Pardridge, 1987; Hammond, 1990; Rosner, 1990). Interestingly, the K_d of CBG (i.e. half-maximal occupation) is similar to the 'average' level of total circulating corticosterone over a period of 24 hr. The binding capacity of CBG is very high: With a concentration of 15 nM corticosterone in the blood, only 5% circulates in the unbound (free) form. The amount of free steroid increases linearly, when circulating corticosterone exceeds concentrations of 30 nM (Dallman *et al.*, 1987). CBG is not

only present in the blood but also in e.g. the pituitary, where it may serve as an intracellular competitive factor for the sequestration of corticosterone (de Kloet *et al.*, 1977; Hammond, 1990). However, CBG is absent in the brain.

Aldosterone specificity can also be conferred to MRs by the enzyme 11β -hydroxysteroid dehydrogenase (11β -OHSD; Edwards *et al.*, 1988; Funder *et al.*, 1988). This enzyme catabolizes corticosterone to its 11-oxo metabolite, 11-dehydrocorticosterone, which binds with much lower affinity to MRs or GRs. 11β -OHSD activity has been found in abundance in the classical aldosterone target tissues, e.g. the kidneys and parotid glands. This explains the preference of the renal MRs for aldosterone notwithstanding a 100–1000-fold excess of circulating corticosterone. The biological significance of this converting enzyme for the aldosterone selective binding in the kidney was revealed in animals that were pretreated with the licorice component glycyrrhizic acid, a blocker of 11β -OHSD. In the presence of this blocker, corticosterone was not metabolized and binds to MRs like aldosterone (Funder *et al.*, 1988; Edwards *et al.*, 1988).

In the rat hippocampus, cortex, cerebellum, hypothalamus and anterior pituitary the 11β -OHSD gene is transcribed and an immunoreactive form of the enzyme protein is present (Lakshmi *et al.*, 1991; Moisan *et al.*, 1992). Furthermore, catabolic enzyme activity can be demonstrated *in vitro*; however, little evidence is presently available for *in vivo* conversion of corticosterone in the hippocampus. This is supported by the observation that the aldosterone concentration in hippocampal cell nuclei varies between 10 and 50 pg/mg DNA, while that of corticosterone varies between 100 and 1000 pg/mg DNA. Yet, in the anterior hypothalamus and cerebellum, aldosterone is concentrated to a much larger extent than corticosterone (Yongue and Roy, 1987), suggesting aldosterone selectivity in these areas. The anterior hypothalamus is indeed a target for aldosterone in the control of salt appetite and cardiovascular regulation (Brody and Johnson, 1980; McEwen *et al.*, 1986a).

In summary, MRs bind corticosterone and aldosterone with similar affinity, yet some neurons in the brain display aldosterone selectivity (e.g. in the anterior hypothalamus but not in the hippocampus). The appreciation of steroid selectivity conferring mechanisms has partly elucidated this phenomenon. One of these mechanisms concerns the catabolic enzyme 11β -OHSD, which through conversion of corticosterone into a weakly active compound lends aldosterone selectivity to local corticosteroid actions. Although this enzyme is widely distributed in the brain, its function in the maintenance of corticosteroid homeostasis or in aldosterone specificity of MRs in e.g. the hippocampus has not been established.

3.2.4. Regulation

The binding properties and localization of MRs and GRs are not fixed entities but they are subject to regulation. Some of the regulatory mechanisms are described below.

As described in Section 3.2.2, the cDNA for the rat hippocampal MR has been cloned (Patel *et al.*, 1989)

and appears to be similar to the human kidney MR (Arriza *et al.*, 1987), at least in the coding region. Subsequent studies have revealed the presence of at least two additional types of MR cDNAs, with different sequences in the untranslated 5' region (Castren and Damm, 1993). The three forms of MR mRNA have been identified and are differentially localized in hippocampal pyramidal cell fields (Kwak *et al.*, 1992a). The studies suggest that multiple promoters control the expression of MR genes in the hippocampus and allow the generation of different mRNAs by alternative promoter usage or alternative splicing. Expression of the recently cloned mouse GR gene is organized in a similar way as the MR gene: Three promoters control the expression of the GR gene, resulting in three different 5' non-coding exons, which are alternatively spliced (Strahle *et al.*, 1992).

The promoters which direct the expression of the receptors are G- and C-rich and do not contain TATA or CAAT elements. The absence of TATA boxes is common for promoters of 'housekeeping' genes; these genes often have multiple transcription initiation sites (Valerio *et al.*, 1985). The transcription of a single gene from multiple promoters has important consequences for the fine regulation of gene expression, e.g. with respect to tissue specificity or developmental stages. The 5'UTR sequence is highly conserved in the rat and in the human MR- and GR-cDNA, suggesting a specific function of this region in the regulation of mRNA stability and translational efficiency (Kozak, 1989).

While these characteristics of the genomic organization are indicative of multiple regulatory mechanisms for the transcription and translation of the corticosteroid receptor genes, the presently available evidence for such regulation is mainly derived from radioligand binding, immunocytochemical and hybridization studies. Most of the regulatory changes of steroid expression and properties were described in conjunction with *in vivo* manipulations (e.g. of the HPA-axis), which makes the interpretation of these data rather complex: At the organismic level it is hard to distinguish between primary effects of conditions and drugs on the one hand and adaptive changes of receptor properties secondary to changing circulating corticosteroid levels on the other hand.

3.2.4.1. Homologous regulation

Corticosteroid binding in the brain depends on the level of endogenous corticosteroids. In the absence of corticosteroid hormones (ADX), binding of labelled corticosterone initially (up to 11 hr) rises, indicating that the endogenous hormone no longer occupies the receptors which then become available for the exogenously administered ligand (McEwen *et al.*, 1974). A second rise in steroid binding sites starts at 18 hr after ADX; this rise probably represents steroid-dependent changes in MRs and GRs.

MRs and GRs are differentially regulated by endogenous and exogenous corticosteroids. Thus, high levels of synthetic GR agonists or corticosterone down regulate GRs, but increase MR capacity (Swanson and Simmonds, 1989; Reul *et al.*, 1987b). Mineralocorticoids down regulate both receptor types, while the antagonist spironolactone results in

the opposite (Brinton and McEwen, 1988; Luttgé *et al.*, 1989a; Sutanto and de Kloet, 1991). These changes in receptor binding are preceded by transient changes in mRNA levels, suggesting that the corticosteroids affect the turnover rate of the receptors (Reul *et al.*, 1989). There is also a circadian rhythm in steroid receptor capacity, with maximal MR binding in the evening, whereas GRs are down regulated at that time (Reul *et al.*, 1987a; Stephenson and Funder, 1987). However, the latter changes in binding were not paralleled by changes in receptor mRNA levels; MR and GR mRNA display little variation throughout the circadian cycle, and only minor ultradian variations (Kwak *et al.*, 1992b).

3.2.4.2. Ontogeny

The steroid receptors display considerable age-dependent changes. Only low levels of GRs are present around the time of birth (Rosenfeld *et al.*, 1988a, 1988b; Sarrieau *et al.*, 1988; Van Eekelen *et al.*, 1991; see Fig. 3). The concentrations rise slowly, and do not achieve adult levels until the third week of life. GR affinity for corticosterone is higher perinatally than at later ages. The receptor microdistribution also changes during ontogeny. Certain regions, such as the suprachiasmatic nucleus of the hypothalamus, only express high levels of receptor during the first week of life (Van Eekelen *et al.*, 1987b). Furthermore, GRs show impaired capacity to undergo transformation and/or nuclear translocation during the second postnatal week (Rosenfeld *et al.*, 1988b; Lawson *et al.*, 1991).

Early life events are also important for the receptor characteristics, with consequences for the adult and even aged animal. For instance, handling of rats during the first week of life (Levine and Mullins, 1966) results in a permanent increase of GR capacity (Meaney and Aitken, 1985; Meaney *et al.*, 1988, 1989, 1991), less hippocampal damage in the aged animal and improved cognitive functions (Meaney *et al.*, 1988; Issa *et al.*, 1990). Long-lasting changes in corticosterone binding have been observed in the offspring of pregnant rats that were either stressed (Szuran *et al.*, 1992) or adrenalectomized (Angelucci *et al.*, 1983). Neonatal corticosterone administration causes pronounced changes in the development of the hippocampus (Bohn, 1984; de Kloet *et al.*, 1988) and may result in permanent changes of corticosterone binding (Angelucci *et al.*, 1985).

Most of the studies mentioned above included MR in their analyses. MRs are present in very low concentrations during the first days of life (Turner, 1978; Van Eekelen *et al.*, 1991). Binding capacity rises rapidly thereafter, and resembles the capacity of adult animals by the end of the first week. Neither binding affinity *in vitro* nor overall distribution change with age. In the young animals, as in the adult, low doses of corticosterone *in vivo* bind mainly to the MRs (Rosenfeld *et al.*, 1990). This is particularly relevant for these young animals since the levels of corticosterone are low and relatively unperturbable in the adrenally intact infant during the first two weeks of life (Sapolsky and Meaney, 1986). This period is called the stress hyporesponsive period. In view of the

very low levels of circulating corticosterone, it is likely that during the stress hypo-responsive period most of the physiological actions of the hormone are mediated by the MR. Preliminary observations suggest that the condition of maternal deprivation during the stress hypo-responsive period leads to adrenocortical activation. Following maternal deprivation the adrenal gland becomes responsive to exogenous ACTH at a time that it is normally quiescent (Levine *et al.*, 1991). We found that the condition of maternal deprivation leads in male pups to a permanent reduction of GR capacity in hippocampus, which can be enhanced by ACTH administration (Rosenfeld, Sutanto, de Kloet and Levine, unpublished observation).

3.2.4.3. Aging

Pronounced changes in receptor density have been described during senescence in rats and other species (de Kloet, 1992). The alterations for the two receptor types partly depend on the rat strain that was used for the studies. MR binding in hippocampi of 17.5, 24 and 30 month old Brown Norway or Wistar rats was found to be consistently decreased by 30%–60% (Reul *et al.*, 1988; Van Eekelen *et al.*, 1991); similar decreases occurred in other species such as the dog (Rothuizen *et al.*, 1993). However, MR mRNA density was not altered much.

Down regulation of GRs in brain and pituitary is commonly observed in aged Wistar and Fischer-344 rats, which show obvious signs of hypercorticism (Reul *et al.*, 1988; Landfield *et al.*, 1992; Sapolsky *et al.*, 1986); one report showed an impaired upregulation of the GRs (Eldridge *et al.*, 1989). GR mRNA was found to be decreased, particularly in the CA2, CA3, CA4 cell fields and the dentate gyrus (Van Eekelen *et al.*, 1991). However, GR affinity in the hippocampus and pituitary was reported to be increased (Landfield and Eldridge, 1989; Van Eekelen *et al.*, 1991). The age-induced changes of MRs, but not those of GRs, are reversible after treatment with a potent neurotrophic peptide (Reul *et al.*, 1988; Rügter *et al.*, 1984).

3.2.4.4. Stress and toxic insults, drug treatment

Receptor properties also change as a result of stressful and toxic insults, drug treatment and denervation. Exposure to stress results in a chain of probably adaptive events that leads from initial upregulation of both receptor types in the hippocampus towards ultimately down regulation of MRs and GRs after several weeks (Maccari *et al.*, 1991a, b; Van Dijken *et al.*, 1993; Sapolsky *et al.*, 1986). Denervation of the dorsal hippocampus or transection of the pituitary stalk transiently up regulates particularly GRs, probably due to proliferation of GR-containing glial cells (De Ronde *et al.*, 1986; Seger *et al.*, 1988). Neurotoxic lesions of the 5HT innervation lead to up (Angelucci *et al.*, 1981) or down regulation (Seckl *et al.*, 1990) of particularly GR binding and mRNAs in the dentate gyrus, and to a lesser extent of MRs depending on the severity of the lesion. 6OH dopamine lesions of the dorsal bundle resulted in an increase of the MR binding capacity (Maccari *et al.*, 1990). Conversely, chronic adminis-

tration of reserpine results in a decrease (Lowy, 1990), while anti-depressants increase MR mRNA levels in the hippocampus (Brady *et al.*, 1991; Seckl and Fink, 1992). Intracerebroventricular treatment with endotoxins or interleukin-1 results in a pronounced reduction of the MR affinity and cell nuclear retention of corticosterone by MR in the hippocampus. There are no cellular data available which can be related to altered activity of the receptors. In general, the view emerges that decreased affinity and/or capacity of the MRs correlate with enhanced basal and stress-induced activation of either ACTH, corticosterone, or both.

In conclusion: Binding affinity and capacity of MRs and GRs are not a fixed entity but, rather, subject to regulation during development and aging. They change as a result of variations in endogenous corticosteroid hormone level, drug treatments, gender (Turner, 1992) or strain (Walker *et al.*, 1989; Sutanto *et al.*, 1989). It should be kept in mind that a certain level of circulating steroid therefore not invariably results in a defined MR/GR occupancy ratio. Many of the cellular actions that will be described in the next section should therefore be interpreted with some caution, since in most cases the properties of MRs and GRs were not established along with the parameter under study. The use of selective synthetic analogues for the MRs or GRs then becomes an important tool to distinguish between MR- and GR-mediated events.

4. CELLULAR EFFECTS OF CORTICOSTEROID HORMONES

The fact that corticosteroid hormones reach the brain and subsequently bind to intracellular receptors, or perhaps to membrane receptors, naturally led to the question how these hormones will affect cell properties within the brain. In the following sections we will review most of the cellular actions that have been observed so far (see Fig. 4). We will distinguish between (i) actions that result in altered ionic conductances, (ii) steroid effects that concern the efficacy of transmitter systems, ranging from alterations in transmitter synthesis, turnover, release, uptake and receptor properties, to changes in functional responses, and (iii) actions that involve general cell features such as metabolic properties and cell morphology. The time course of the steroid-induced effects displays a great variability. On the one hand there are steroid actions that take place within minutes after exposure to the hormone. The fast onset of these responses seems to preclude a genomic mechanism of action and consequently the involvement of membrane receptors is indicated. On the other hand, many studies report steroid-mediated events that develop after hours and persist throughout the period of investigation. In most cases the steroid specificity of these effects and particularly the considerable delay in onset is compatible with a genomic mechanism of action, but proof for the involvement of gene transcription or protein synthesis was supplied in only a few cases. The review will particularly highlight these delayed events.

FIG. 3. Developmental changes in the localization of ^{35}S -labelled antisense cRNA probes hybridized with MR mRNA (left panel) and GR mRNA (right panel) in the dorsal hippocampus, at postnatal day 2, pnd 8, pnd 12 and in adulthood. Middle panel represents thionine stained hippocampal cells. Photomicrographs show the different subfields of the hippocampus, i.e. CA1, CA2, CA3 and the dentate gyrus (DG). From Van Eekelen *et al.*, 1991.

FIG. 4. Binding of corticosterone (triangle) to intracellular MRs and GRs affects DNA transcription and subsequent mRNA translation. As a result of the altered protein synthesis, properties of the neuronal membrane may be affected. These properties comprise (1) ionic conductances through voltage gated K-channels (I_K), Ca-dependent K-channels ($I_{K(Ca)}$) and voltage gated Ca-channels (I_{Ca}); (2) ionic conductances through ligand gated ion channels (R_I); and (3) processes linked to activation of G-protein coupled transmitter receptors (R_G). Not only membrane properties, but also general cell characteristics such as glucose metabolism may be altered. In addition to the genomic mechanism of action, direct steroid effects via membrane receptors (R_S) may occur.

4.1. IMMEDIATE ACTIONS

Many of the early investigations used an experimental approach in which corticosteroid hormones were microinjected or iontophoretically applied while simultaneously, extracellular single unit activity in anaesthetized animals was monitored. Due to this design, rapid effects will be easier to discern than delayed actions. While most of these studies prove that steroid actions can take place within minutes, they are less conclusive about the possible development of secondary, delayed steroid-mediated events.

In the paraventricular nucleus, the majority of neurons appeared to be, within seconds, inhibited by iontophoretically applied cortisol or corticosterone, both *in vivo* (Saphier and Feldman, 1988; Chen *et al.*, 1991) and *in vitro* (Kasai and Yamashita, 1988; Chen *et al.*, 1991). The inhibitory effects were blocked by the GR antagonist RU 38486; however, the synthetic glucocorticoid dexamethasone excited rather than inhibited the neuronal activity (Chen *et al.*, 1991). Neurons in the brainstem, i.e. in the raphe nuclei (Avanzino *et al.*, 1984) and pontine region (Dubrovsky *et al.*, 1985), were predominantly excited by corticosterone. Local application of low doses of dexamethasone was found to rapidly inhibit multi-unit activity in the dorsal hippocampus of urethane anaesthetized female rats (Michal, 1974). However, no immediate changes in firing activity were observed when corticosterone was iontophoretically applied to hippocampal neurons under similar conditions in male rats (Ben Barak *et al.*, 1977).

Clearly, the results with this approach were variable although in most cases corticosteroid hormones appeared to depress the firing activity of neurons rapidly. Unfortunately, the lack of control over local steroid concentrations and over the baseline activity of the neuron associated with this experimental approach do not permit a further interpretation of the

underlying mechanism of action. With the technique of intracellular recording one can further pursue the nature of the cellular actions exerted by corticosteroids.

In isolated guinea pig coeliac ganglia, cortisol induced a rapid membrane hyperpolarization, which became prominent with concentrations exceeding 100 nM (Hua and Chen, 1989). The input resistance was decreased and the hyperpolarization persisted in the presence of low Ca/high Mg (Chen *et al.*, 1991). These data indicate that corticosteroid hormones evoke a rapid postsynaptic increase of K-conductances. Interestingly, the steroid effects were effectively blocked by RU 38486 (Hua and Chen, 1989), an antagonist for the intracellular GR. In the spinal cord too, large intravenous doses of the glucocorticoid methylprednisolone acutely changed membrane properties. Thus, cat lumbar motor neurons were hyperpolarized by prednisolone, the action potential threshold was elevated, the action potential zero overshoot diminished and repolarization enhanced (Hall, 1982). These phenomena also point to a steroid-evoked increase of K-conductances. The data may be partly explained by a glucocorticoid-dependent fast increase of the Na/K-ATPase activity in the spinal cord (Braugher and Hall, 1981). It should be noted that the above mentioned *in vivo* experiments were all carried out in animals with an intact HPA-axis; given the stress associated with the anaesthesia that was applied in many cases, we can assume that most of the intracellular steroid receptors were already activated by the endogenous ligand at the start of the experiment, another argument that the rapid effects induced by exogenous steroids are not mediated via these 'classical' steroid receptors.

Rapid corticosteroid actions on GABA-mediated responses have also been described. GABA responses of primary afferent neurons in isolated bullfrog spinal ganglia were diminished in the presence of high doses

(5 μM –1 mM) of natural or synthetic glucocorticoids (Ariyoshi and Akasu, 1987). This depression was not due to diminished GABA uptake or facilitated desensitization but rather to non-competitive antagonism of the GABA-induced Cl-conductance.

Apparently, glucocorticoid hormones are not very potent in their modulatory actions on the GABA_A receptor. However, another class of steroid hormones, i.e. the steroids with a reduced A-ring including some of the neurosteroids, are very potent, rapid modulators of GABA-mediated responses. For details we recommend excellent, recent reviews on this subject (Baulieu and Robel, 1990; Lambert *et al.*, 1987; Majewska, 1992; Paul and Purdy, 1992; Schumacher, 1990; McEwen, 1991; Simmonds, 1991). In short, endogenous and synthetic steroids with a reduced A-ring at the C-5 position and a hydroxyl group at the 3 α -position act as allosteric agonists at the GABA_A receptor; e.g. tetrahydroprogesterone, tetrahydrodeoxy-corticosterone and androsterone increase the binding of the benzodiazepine flunitrazepam, potently enhance GABA_A-mediated Cl-conductances and, at higher concentrations, even induce Cl-conductances by themselves. In excised outside-out patches from cultured mouse spinal cord neurons it was shown that the enhancement of GABA receptor current is due to an increase in channel-opening frequency and an increase in the probability of longer channel openings; the prolongation of average open duration but not open frequency is also seen with barbiturates, suggesting that the effector mechanism for the A-ring reduced steroids and the barbiturates is not identical (Twyman and MacDonald, 1992). There are however also A-ring reduced steroids acting as non-competitive antagonists rather than allosteric agonists on the GABA_A-receptor complex; pregnenolone sulfate and dehydroepiandrosterone are the most prominent examples. The data so far indicate that there may be multiple recognition sites on the GABA_A-receptor complex (or even not associated with this receptor) mediating the actions of the A-ring reduced steroids. The array of rapid cellular effects described for this class of steroids however is ever expanding. Recent reports include an increase of the NMDA receptor-mediated calcium influx in cultured hippocampal neurons by pregnenolone sulfate (Irwin *et al.*, 1992) and a rapid depression of high threshold calcium currents in dissociated hippocampal neurons by most of the A-ring reduced steroid compounds (Ffrench-Mullen and Spence, 1991).

Summarizing, rapid effects of corticosteroid hormones generally concern an inhibition of neuronal firing induced with rather high steroid concentrations. Several arguments support the involvement of a putative membrane receptor rather than the 'classical' intracellular receptor for corticosteroids. It seems unlikely that these rapid corticosteroid effects are directly linked to the enhancement of GABA-mediated inhibition observed for A-ring reduced steroids, since corticosteroid hormones themselves are usually very weak allosteric agonists while the very short latency of their inhibitory effects does not allow for extensive metabolic conversion to more potent A-ring reduced compounds.

4.2. DELAYED ACTIONS

4.2.1. Ionic Conductances

In contrast to the extracellular recording studies in which corticosteroid hormones were applied by iontophoresis, some investigations employed peripheral steroid injection combined with *in vivo* single unit recording. This method allows the examination of more delayed steroid actions, which are compatible with a genomic mechanism of action. For instance, the firing rate of hippocampal neurons was suppressed by corticosterone, with a delay of at least 30 min (Pfaff *et al.*, 1971). More variable responses of both hippocampal single units (Dafny *et al.*, 1973) and cells in the rat lateral septum and preoptic area (Saphier, 1987) were observed with peripherally injected cortisol.

The variable results indicated that the delayed steroid-mediated events perhaps depend on the background activity of the local circuit and the membrane potential of the recorded cell. Therefore, recent studies have employed *in vitro* recording techniques which allow control over the external medium of the tissue and the membrane potential of the neuron. In these studies, the experimental design still allowed examination of delayed steroid effects: One approach is to manipulate the HPA-axis of animals *in vivo* and subsequently establish the neuronal properties in slices from these animals; this approach depends on the assumption that genomic steroid receptor-mediated events *in vivo* will persist for hours, even *in vitro*. Another approach makes use of a brief *in vitro* application of steroids, comparing cell properties in neurons recorded before the steroid administration with properties of neurons recorded several hours after the application; since the ligand has probably been washed out of the tissue after this long delay, persisting steroid actions can be studied in the absence of possibly immediate effects.

Application of corticosteroid hormones appeared to have no delayed effects on passive membrane properties of hippocampal neurons, such as resting membrane potential or input resistance (Joëls and de Kloet, 1989; Kerr *et al.*, 1989; Beck *et al.*, 1992). Steroid modulation of electrogenic pumps is therefore unlikely to play a prominent role in the brain, since this would certainly affect the resting ionic gradients of the neurons. This was confirmed in voltage clamp studies where steroids did not affect the capacity or leak conductance of the membrane (Karst *et al.*, 1993). Only when the cells were shifted towards a depolarized or hyperpolarized voltage level, did steroid-induced effects become apparent. Thus, the inwardly rectifying K-current I_K in hippocampal CA1 neurons *in vitro*, which is activated at relatively negative membrane potentials (below -80 mV), was found to be affected by steroid treatment (Karst *et al.*, 1993; see Fig. 5). The I_K amplitude is small when recorded 1–3 hr after predominant MR activation and increases when GRs are additionally occupied, through a protein synthesis requiring process (Karst *et al.*, 1993). Interestingly, selective activation of GRs only with RU 28362 was not able to enhance the I_K amplitude, showing that cooperativity of MR- and GR-mediated events is necessary to evoke a large I_K conductance. Other

FIG. 5. (A) Example of the inwardly rectifying K-conductance I_Q in a CA1 hippocampal neuron. The inward current, measured as the difference between the instantaneous (IS) and steady state (SS) current, is activated by very negative voltage steps (see voltage protocol on the lower right hand side). (B) The I_Q recorded for voltage steps to -130 mV is small when only MRs or only GRs are activated (ADX/CT/RU 38486 or ADX/RU 28362 respectively), but large when both receptor subtypes are simultaneously activated (Sham, ADX/30 nM CT or ADX/CT/ORG 31169 (ORG 31169 is a progesterone but not a GR antagonist)). The neurons in untreated slices from ADX rats displayed on average a large I_Q ; however, the majority of the neurons had small I_Q amplitudes, whereas some of the neurons had extremely large I_Q amplitudes, resulting in a median (white stippled line) that is very different from the average value (adapted from Karst *et al.*, 1993).

voltage-dependent K-conductances did not depend on MR/GR occupation: The transient outward current I_A and the delayed rectifier in hippocampal neurons are similar in tissue from ADX or sham operated controls, or in tissue from ADX rats in which only MRs, GRs

or both receptor types are occupied (Karst *et al.*, 1993).

Calcium-dependent K-conductances are also subject to steroid regulation. So far this was only indirectly demonstrated in current clamp studies. Depolariz-

FIG. 6. (A) Depolarizing current pulses activate a slow Ca-dependent K-conductance I_{AHP} ; at the end of the pulse the I_{AHP} is slowly deactivated. As a result of the activation of I_{AHP} , the firing of the cell slowly attenuates (accommodation); the deactivation underlies a transient afterhyperpolarization. (B) The number of action potentials evoked by a 500 msec depolarizing current (0.5 nA) pulse at various moments after steroid application, expressed as a percentage of the number of action potentials recorded from the same neurons before steroid application. A persistent increase in the number of action potentials (i.e. a decrease of the accommodation) is observed after a brief application of 1 nM of aldosterone, which activates only MRs. However, administration of 1 nM of corticosterone, which activates MRs but also part of the GRs, results only in a transient increase of the number of action potentials. Simultaneous occupation of MRs and GRs (30 nM corticosterone) suppresses rather than enhances the number of action potentials, with a delay of 1–2 hr.

ation of hippocampal neurons induces several action potentials (see Fig. 6), but after 50–100 msec the firing slows down and eventually ceases; this phenomenon (accommodation of cell firing) is due to the activation of a slow Ca-dependent K-conductance (Hotson and Prince, 1980; Gustafsson and Wigstrom, 1981; Madison and Nicoll, 1984; Lancaster and Adams, 1986). The deactivation of this current at the end of the depolarization results in a lingering hyperpolarization of the membrane, the so called afterhyperpolarization (AHP). Both phenomena potentially attenuate the transmission of excitatory input in the CA1 area. It appeared that neurons in slices from ADX rats display a significantly smaller AHP amplitude and duration than neurons from the sham operated controls (Joëls and de Kloet, 1989; Kerr *et al.*, 1989), while other cell parameters such as resting membrane potential,

resistance and spike threshold are not affected. Selective occupation of MRs and GRs provided insight into putative variation of AHP/accommodation throughout the day, as a function of MR and GR occupation: Selective activation of MRs induces a reduction of the AHP/accommodation amplitude (Joëls and de Kloet, 1989) and AHP duration (Beck *et al.*, 1992) compared to the untreated ADX tissue, peaking around 1 hr after steroid application (Joëls and de Kloet, 1990; see Fig. 6). Simultaneous GR occupation overrides and eventually (after 2 hr) reverses this action and results in an increase of the AHP/accommodation (Joëls and de Kloet, 1989, 1990; Kerr *et al.*, 1989). The latter effect can also be achieved with GR occupation only (Joëls and de Kloet, 1989) and depends on *de novo* protein synthesis (Karst and Joëls, 1991); the effect only develops after a delay of at least 1 hr (Joëls and de Kloet, 1990, 1993) and not at shorter intervals (Zeise *et al.*, 1992; Joëls and de Kloet, 1993).

Is the modulation of the Ca-dependent K-conductance directly aimed at the K-channel or secondary to steroid-dependent actions on Ca homeostasis? While the first needs to be investigated, evidence for the second possibility was recently supplied. Current clamp investigations revealed that the high threshold Ca-spike in CA1 pyramidal neurons is enhanced in amplitude and duration in neurons from rats with an intact HPA-axis vs ADX rats (Kerr *et al.*, 1989); similarly, high doses of the GR agonist RU 28362 administered *in vitro* increased the Ca-spike (Kerr *et al.*, 1992). RU 28362 also increased the N- and L-type Ca-conductances in hippocampal CA1 neurons through a protein synthesis requiring process, while voltage dependency and steady state inactivation characteristics remained unaltered (Kerr *et al.*, 1992). The MR-mediated role in Ca-conductances was not directly apparent from this study. Therefore, we recently performed a study with whole cell voltage clamp in hippocampal slices from ADX rats in which the role of MR activation on Ca-currents was specifically studied. We found that 1–3 hr after a brief application of 30 nM corticosterone in the presence of the GR antagonist RU 38486 both low threshold inactivating and high threshold non-inactivating Ca-conductances were depressed (Karst *et al.*, 1994). Simultaneous MR and GR activation restored the Ca-conductances to the level of the sham operated controls. GR activation alone was not sufficient to yield large Ca-currents, pointing to a mechanism of MR and GR cooperativity. Therefore, the data that are available so far indicate that Ca-conductances are small with predominant MR activation and increase when GRs are additionally activated.

Thus, the steroid-mediated effects on ionic conductances evaluated so far show a general principle in that they are (1) only apparent at depolarized or hyperpolarized membrane potentials and (2) 'at the low end of the scale' with predominant MR occupation, as occurs in situations of low adrenocortical activity, and large with simultaneous MR and GR occupation, as occurring at the peak of the circadian cycle or after stress. Whether the latter principle will hold for other ionic conductances, e.g. for Na or Cl, awaits further investigation.

4.2.2. Transmitter Systems

4.2.2.1. Excitatory amino acids

By now, there is quite some evidence showing that corticosteroid hormones affect the efficacy of excitatory amino acid-mediated transmission; glutamate is the most prominent member of this class of amino acids, taking care of most of the fast excitatory synaptic transmission in the brain.

In the brain, basal and stimulus-induced extracellular glutamate levels are enhanced after treatment of ADX rats with high corticosterone doses (Stein-Behrens *et al.*, 1992). This may be due to an enhanced release of the amino acid and/or to a decreased uptake in neuronal or glial tissue. The latter was indeed demonstrated in a preparation of cultured astrocytes (Virgin *et al.*, 1991). The activity of glutamine synthetase, and therefore the availability of glutamate, is not regulated by corticosteroids in the brain (Tombaugh and Sapolsky, 1990), as it is for instance in peripheral tissue (Max *et al.*, 1988).

The results about steroid-dependent changes in amino acid receptor characteristics are not very consistent. With a membrane binding assay for [³H]-glutamate in whole hippocampi, corticosterone treatment to ADX rats was found to decrease the $B_{\max}$ for glutamate binding in the dorsal hippocampus (Halpain and McEwen, 1988). However, a study under comparable neuroendocrine conditions but using *in vitro* autoradiography reported only minimal effects of ADX or corticosterone treatment on the binding properties of AMPA, KA and NMDA receptors in the hippocampus (Clark and Cotman, 1992). Clearly, these studies should be carefully interpreted: The membrane binding assay may have inadvertently included information about glutamate uptake sites rather than receptors and also yields an overall picture of all of the hippocampal regions; in the *in vitro* autoradiographical study the choice of the concentration of the radioactively labelled ligand may have been such that possible steroid effects remained unnoticed. Acute stress increases AMPA receptor binding properties specifically in the hippocampus, (Tocco *et al.*, 1991), but this increase depends on opioids rather than corticosteroids (Shors *et al.*, 1991).

Several studies have demonstrated that the electrical response to stimulation of glutamatergic fibers depends on corticosteroid levels. Most of the studies were performed *in vitro* in the CA1 area of the hippocampus, where the Schaffer collaterals convey an excitatory, glutamatergic message to the CA1 pyramidal neurons (reviews Nicoll *et al.*, 1990; Lopes da Silva *et al.*, 1990). Low frequency stimulation, a condition in which AMPA rather than NMDA receptors are activated, yields an EPSP in the dendritic layer which after propagation to the soma can result in the generation of an action potential. Extracellularly these signals will be recorded as a compound EPSP in the dendritic area and a population spike in the cell layer.

Low doses of corticosterone applied *in vitro* to slices from ADX rats, thereby probably establishing a predominant occupation of MRs, stabilized (Joëls and Fernhout, 1993) or even enhanced (Reiheld *et al.*, 1984; Rey *et al.*, 1987, 1989) the amplitude of the

FIG. 7. (A) Repeated synaptic activation (for 70 min) of the glutamatergic projection to CA1 pyramidal cells in ADX rats leads to a run-down of the extracellularly recorded field potential amplitude, particularly with half-maximal stimulation intensity. (B) The field potential amplitude, expressed as a percentage of the amplitude at the start of the experiment, displays a gradual decrease in slices from ADX rats (MRs and GRs unoccupied), in slices from sham-operated controls treated with 1 μ M corticosterone (MRs and GRs fully activated), but not in slices from sham-operated controls which had a moderate plasma corticosterone level (1–5 μ g/100 ml plasma, occupying predominantly MRs) at the start of the experiment.

synaptically-induced population spike in the CA1 area (see Fig. 7). By contrast, high corticosteroid concentrations, resulting in occupation of both MRs and GRs, reduced the amplitude of the population spike without affecting the cEPSP (Rey *et al.*, 1987, 1989; Joëls and Fernhout, 1993). The attenuating effect on the population spike amplitude via GRs was also supported by the observation that application of high corticosterone levels to slices from adrenalectomized rats, thus occupying the available GRs, reduced the population spike within 20 min (Vidal *et al.*, 1986; Rey *et al.*, 1987; Joëls and Fernhout, 1993). The GR-mediated depression of synaptic transmission displays to some extent calcium dependency. It was shown that the sensitivity to corticosterone was largely enhanced when extracellular calcium concentrations were elevated (Talmi *et al.*, 1992), indicating that enhanced Ca²⁺-influx is a factor in

the final corticosterone effect. This is supported by a recent study showing that both basal and KA-induced elevations in $[Ca]_i$ are enhanced by prolonged treatment of hippocampal cultures with high corticosteroid concentrations (Elliot and Sapolsky, 1992, 1993), a phenomenon that not only involves changes in Ca-influx but also in Ca-extrusion. In addition, Ca-entry through voltage gated Ca-channels, which open secondary to the amino acid-mediated depolarization, is also elevated when GRs are activated (Kerr *et al.*, 1992; Karst *et al.*, 1994).

Interestingly, synaptic transmission in slices from ADX rats, i.e. in the absence of corticosteroids, was also attenuated (Joëls and Fernhout, 1993). Initially the field potentials were normal in their appearance, but repeated stimulation gradually decreased the signal. The failure may be related to metabolic processes or changes in Ca-influx. Perhaps this gradual failure to transmit excitatory input underlies a recent observation (Doi *et al.*, 1991) that population spikes in slices from the dorsal hippocampus of ADX rats are much reduced when compared to the signals in adrenalectomized controls.

Intracellular studies have largely confirmed the dose-dependent corticosterone actions on glutamate transmission in the hippocampus (Joëls and de Kloet, 1993). In the absence of steroids or with doses occupying both MRs and GRs the probability for inducing synaptically driven action potentials declined with repeated stimulation; in the absence of steroids the EPSP amplitude remained stable, while with simultaneous MR and GR activation, the EPSP was also diminished (Joëls and de Kloet, 1993), though not in adrenalectomized animals (Zeise *et al.*, 1992). All of these changes occurred in the absence of steroid effects on resting membrane potential, membrane resistance or spike threshold. Under conditions of predominant MR activation, synaptic transmission remained stable during the entire (> 1 hr) recording period. Therefore, both the extracellular and intracellular studies indicate that the dose-response relationship for corticosterone-mediated effects on glutamate transmission displays an inverted U-shape.

Are these observations relevant for endogenous variations in corticosterone concentrations, e.g. as occurs after stress? In the dentate gyrus, baseline synaptic responses were increased following a mild (novelty) stress (Sharp *et al.*, 1985), while uncontrollable shock decreased the synaptically evoked response with a very short latency (Henke, 1990). In the CA1 area, the threshold for inducing population spikes or cEPSPs was reduced after chronic stress (Kerr *et al.*, 1991). The lack of information about circulating corticosterone levels at the moment of the test and possible changes in MR and GR binding properties in the chronic stress study makes it hard to interpret these findings. However, even when more information about the circulating steroid levels during the various stress paradigms was available, discrepancies between the effects of high plasma corticosteroid levels *per se* and exposure to stress (resulting in similar plasma steroid levels) may occur (Dallman, 1993).

While the above reviewed studies mainly focussed on AMPA receptor-mediated transmission, the influence of steroids on a combined AMPA and

NMDA receptor phenomenon has also been investigated. Thus, high frequency or burst stimulation of e.g. the Schaffer collaterals or the perforant path yields long term potentiation of synaptic responses in the CA1 area and dentate gyrus respectively; at least for the induction of the potentiation, NMDA receptors are important (Bliss and Collingridge, 1993). In the CA1 area, primed burst potentiation in anesthetized rats was found to depend on circulating corticosterone concentrations. Adrenalectomy yielded relatively inefficient potentiation (Diamond *et al.*, 1992). Low levels of corticosterone (pellet implantation), corresponding with activations of MRs, enhanced the degree of primed burst potentiation (Diamond *et al.*, 1992), whereas increasing corticosterone concentrations (stress or pellet implantation), thus additionally activating GRs, reduced primed burst potentiation (Diamond *et al.*, 1990; Bennett *et al.*, 1991; Diamond *et al.*, 1992). A similar inverted-U relationship was also observed in a series of studies, where LTP was recorded *in vitro*, subsequent to *in vivo* manipulation with the HPA-axis: Adrenalectomy resulted in relatively weak LTP (Shors *et al.*, 1990a). In the dentate gyrus, high frequency potentiation is also controlled by corticosteroids. One study reported relatively fast depressant effects of corticosterone on potentiated cEPSPs in anesthetized ADX rats (Dubrovsky *et al.*, 1990). In another study corticosterone levels in anaesthetized, adrenalectomized intact animals were inversely related to the degree of potentiation of the population spike rather than the cEPSP (Pavlidis *et al.*, 1993). In ADX rats, selective occupation of MRs enhanced whereas occupation of GRs decreased synaptic potentiation (Pavlidis *et al.*, 1992). Stress also largely reduced the degree of LTP (Foy *et al.*, 1987; Shors *et al.*, 1989). However, the influences of stress (particularly when applied for a prolonged period) on LTP involve factors other than corticosterone, such as endogenous opioids (Shors *et al.*, 1990b). Finally, the experimental protocol (e.g. the time of the day) is an important factor for these studies on LTP in the hippocampus, since it was shown that the circadian rhythmicity for LTP in the dentate gyrus is reversed after adrenalectomy (Dana and Martinez, 1984).

In summary, the data about steroid modulation of either AMPA receptor-mediated transmission or processes involving both AMPA and NMDA receptors, yield the following picture: Predominant MR activation stabilizes or enhances the amino acid-mediated responses, whereas additional GR occupation reduces the responses. Most of the effects of corticosteroids on excitatory amino acid-mediated transmission were quite rapid in onset and not very persistent (Joëls and de Kloet, 1993). Therefore, it is not yet clear, whether or not these influences are mediated via a genomic pathway; so far, the pharmacological profile rather than the timing support the involvement of intracellular receptors.

4.2.2.2. Inhibitory amino acids

Data about delayed corticosteroid actions on inhibitory amino acids are relatively sparse, when compared to the wealth of information about rapid modulation of GABA_A receptor characteristics by

corticosteroid metabolites or other neurosteroids (see Section 4.1).

Adrenalectomy generally increases binding of benzodiazepines, GABA or muscimol in striatum, midbrain (Kendall *et al.*, 1982), cortex (Acuna *et al.*, 1990) and hypothalamus (Majewska *et al.*, 1985; DeSouza *et al.*, 1986). However, the latter may be secondary to a rise in ACTH levels after ADX (Kendall *et al.*, 1982; DeSouza *et al.*, 1986). In cortex, the binding characteristics were normalized with corticosterone treatment (Acuna *et al.*, 1990).

Intracellular recording from CA1 pyramidal neurons in the rat hippocampus showed that repeated stimulation of the Schaffer input resulted in a gradual decline of the slow GABA_B receptor-mediated IPSP (Joëls and de Kloet, 1993). With predominant MR occupation in slices from ADX rats the amplitude of the slow IPSP remained much more stable. However, increasing the corticosterone dose to a level where both MRs and GRs were occupied yielded again a gradual decline of the slow IPSP amplitude. Administration of supramaximal doses of corticosterone to slices from adrenalectomized rats also resulted in a decrease of the slow IPSP in CA1 hippocampal and neocortical neurons (Zeise *et al.*, 1992). This inverted-U relationship between corticosterone concentrations and the amplitude of the slow IPSP is probably not due to modulation of the GABA_B receptor characteristics, since membrane effects of the GABA_B agonist baclofen were not sensitive to *in vitro* steroid treatment (Joëls *et al.*, 1991b). Since the slow IPSP is particularly sensitive to metabolic disturbances (Krnjevic *et al.*, 1991), steroid-dependent effects on metabolism (see Section 4.3) rather than membrane characteristics *per se* may underlie this phenomenon. The GABA_A receptor-mediated fast IPSPs were not altered after repeated synaptic stimulation in slices from ADX rats or with subsequent corticosterone treatment (Joëls and de Kloet, 1993). This dissociation between steroid modulation of GABA_A and GABA_B receptor-mediated IPSPs seems to preclude a steroid-mediated reduction of GABA release. However, a presynaptic modulation can not be excluded with very high steroid levels, since 1–10 μ M corticosterone suppressed both the fast and slow IPSPs in CA1 hippocampal and neocortical cells within 10 min (Zeise *et al.*, 1992). Notwithstanding the reduction of GABAergic responses observed particularly with high corticosterone doses in the CA1 area, the consequence of this reduction for the input–output relationship in this area may be limited: In none of the extracellular field potential recordings did corticosterone induce multiple population spikes, a phenomenon that is observed with GABA_A antagonists such as bicuculline or picrotoxin (Knowles and Schwartzkroin, 1981).

Taken together, the data indicate that modulatory effects of corticosteroids on inhibitory amino acids are probably limited. Changes in the binding characteristics after ADX may be secondary to alterations of the ACTH level. The maintenance of the GABA_B-mediated slow IPSP depends on MR occupation and could be linked to a metabolic process. Only extremely high steroid levels are able to suppress GABA-mediated responses, perhaps via a presynaptic mechanism of action.

4.2.2.3. Noradrenaline, dopamine and acetylcholine

The fact that GRs are present in neurons of the brainstem which produce monoamines and in neurons to which the monoaminergic areas project (Fuxe *et al.*, 1985; Harfstrand *et al.*, 1986) suggests that steroids interact with the central monoaminergic system. This is indeed true for some aspects, though not all, of the monoaminergic systems.

The activity of tyrosine hydroxylase activity, the rate limiting step in NA-synthesis, does not seem to be affected by corticosteroids in the adult mouse locus coeruleus (Markey *et al.*, 1982). The data concerning NA turnover are somewhat conflicting. In general, turnover was increased 1 week after ADX, particularly in the hypothalamus, a process that was reversed by substitution with moderately high corticosterone levels (Jhanwar-Uniyal *et al.*, 1989; see review Meyer, 1985). Nevertheless, the NA content remained unaltered after ADX or subsequent corticosterone treatment (Jhanwar-Uniyal *et al.*, 1989); the latter may be due to an increase in NA uptake (Maas and Mednieks, 1971) although changes in NA uptake have not been demonstrated for physiologically relevant corticosteroid hormone levels (Lieberman *et al.*, 1980). No steroid-dependent changes in NA synthesis were observed for the cerulo–hippocampal system (see for review McEwen, 1987). However, stressful stimuli do affect the availability of NA (see review Stone and McCarty, 1983). Recently, it was shown with microdialysis that acute immobilization stress increases NA release in the PVN of ADX or repeatedly stressed rats (Vertrugno *et al.*, 1993; Pacak *et al.*, 1992).

Changes in steroid levels *per se* do not affect the binding characteristics of α 1- or β -adrenergic receptors (Roberts and Bloom, 1981; Mobley and Sulser, 1980; Kendall *et al.*, 1982; Mobley *et al.*, 1983). However, one study reported an increase of the β -adrenergic binding capacity in conjunction with a lesion of the dorsal noradrenergic bundle (Roberts and Bloom, 1981); the ADX-dependent increase was normalized in rats treated for 1 week with relatively high doses of corticosterone. By contrast, α 2-receptor binding seems to be directly subject to regulation by corticosteroids: α 2-receptor binding was decreased in the paraventricular nucleus and increased in the supraoptic nucleus 1 week after ADX; these effects were reversed by *in vivo* corticosterone treatment (Jhanwar-Uniyal and Leibowitz, 1986). Properties of G-proteins coupled to adrenergic receptors also alter, depending on the steroid levels. Thus, implantation of corticosterone pellets in ADX rats increased G α mRNA, immunoreactivity and ADP ribosylation in cortical tissue, while G α mRNA and immunoreactivity decreased (Saito *et al.*, 1989). Chronic corticosterone treatment also increased the mRNA for ADP-ribosylation factors in cortical tissue (Duman *et al.*, 1990). These findings may underlie the observation that adrenalectomy (for more than 1 week) increases the efficacy of NA to stimulate cAMP in cortex (Mobley and Sulser, 1980; Mobley *et al.*, 1983) and hippocampus (Roberts *et al.*, 1984). Administration of high levels of corticosterone for a period of at least 1 week decreased the NA-dependent cAMP formation (Nakagawa and Kuriyama, 1976;

Mobley and Sulser, 1980; Mobley *et al.*, 1983; Roberts *et al.*, 1984; Duman *et al.*, 1989). While cAMP formation is mediated by β -adrenergic receptors, selective activation of β -receptors by isoproterenol was not always sensitive to steroid treatment (Mobley *et al.*, 1983; Duman *et al.*, 1989). Apparently, the mixed agonist NA is necessary to accomplish the interaction with steroids. It was proposed that the corticosterone-induced decrease of NA-dependent cAMP formation in the cortex is due to desensitization of the α 1-receptors, perhaps via an intermediate activation of calmodulin (Gannon and McEwen, 1990), which then indirectly activate β -receptors (Stone, 1987; Stone *et al.*, 1987). Although not specifically investigated, the steroid receptor involved in these actions on cAMP formation appears to be the GR rather than the MR, considering the high levels of corticosterone required to induce the effects.

Consistent with these findings is an intracellular electrophysiological study showing that ADX enhanced a cAMP-dependent response of hippocampal neurons to NA (Madison and Nicoll, 1986) compared to neurons in the adrenally intact controls, whereas treatment of slices from ADX rats with high levels of corticosterone or the GR agonist RU 28362 reversed this enhancement with a delay of > 1 hr (Joëls and de Kloet, 1989; see Fig. 8). A subsequent extracellular recording study revealed that the effect of NA was indeed inversely related to the plasma corticosterone levels of the animals at the start of the experiment (Joëls *et al.*, 1991a).

Another catecholamine that is modulated by corticosteroids is DA. The turnover of DA appears to be increased by glucocorticoid analogues in mouse brain (Iuvone *et al.*, 1977) and in the mesolimbic system and the arcuate nucleus/median eminence area of the rat (Versteeg *et al.*, 1983). Very rapid increases in DA levels were observed after dexamethasone treatment in cat spinal cord (Hall and McGinley,

1982); however, ADX or chronic (7 days) corticosterone treatment did not affect DA content or utilization in rat brain (Jhanwar-Uniyal *et al.*, 1989). The rapid effects of glucocorticoids on DA content congrue with relatively fast and transient effects on release and uptake. Thus, *in vivo* microdialysis studies showed that stress and high corticosterone doses induce a transient increase of DA release in the nucleus accumbens and cortex, which peaks after 20 min (Imperato *et al.*, 1989). DA uptake measured in septal or striatal synaptosomes was also increased after stress, but not after *in vivo* corticosterone injection (Gilad *et al.*, 1987). However, *in vitro* incubation of the synaptosomal preparations with methylprednisolone rapidly reduced DA uptake, both in stimulated and unstimulated conditions (Gilad *et al.*, 1987). Perhaps as a consequence of these changes in the availability of DA, long term changes in steroid content also affect DA receptor characteristics. ADX generally decreased D1 receptor density in the striatum, nucleus accumbens and substantia nigra; D2 receptor density was decreased in part of the striatum (Biron *et al.*, 1992). Dexamethasone treatment starting 14 days after ADX reversed these effects or even enhanced the binding capacity. The functional significance of all of these findings at the cellular level is presently hard to fathom, since electrophysiological data about the steroid-DA interaction lack to date.

Modulation of the cholinergic system in the brain by corticosteroids is less obvious. Brain regions containing ACh-producing cells are quite sparse in their GR content (Fuxe *et al.*, 1985). However, chronic (2 months) treatment of rats with high corticosterone levels, but not stress, reduced the number of ACh-esterase positive neurons in the medial septal nuclei (Tizabi *et al.*, 1989); indirect effects of the steroid treatment can not be excluded. In contrast to these very slow effects on ACh-producing cells, very rapid modulation of choline uptake and ACh release

FIG. 8. Application of noradrenaline (NA) diminishes the accommodation of hippocampal CA1 neurons during a steady (500 msec) depolarizing (0.5 nA) input. The NA-induced effect on accommodation is more pronounced in neurons from ADX rats (left) than in neurons treated with a GR-agonist (right).

was observed in rat hippocampus but not caudate, both *in vivo* (Imperato *et al.*, 1989) and *in vitro* (Gilad *et al.*, 1985, 1987). The onset of the effects (within 10 min) is so fast as to be probably not mediated by genomic actions. High affinity uptake of choline was enhanced after chronic *in vivo* treatment with corticosteroid hormones in the caudate putamen (Riker *et al.*, 1979), but not in the hypothalamus or hippocampus (Riker *et al.*, 1979; Jhanwar-Uniyal *et al.*, 1989). Finally, there are some reports about corticosterone or stress-induced changes in ACh receptor binding. Nicotinic receptor binding in mouse brain was decreased by chronic administration of high corticosterone doses (Pauly *et al.*, 1990), both in adrenalectomized and ADX animals. By contrast, in adrenalectomized rats chronic stress was found to increase the B_{max} (but not K_d) for muscarinic receptor binding in a synaptosomal preparation from the hippocampus (Finkelstein *et al.*, 1985); in rat, removal of the adrenals did not alter the muscarinic receptor binding when compared to sham operated controls (Kendall *et al.*, 1982).

A recent study performed in our laboratory (Hesen and Joëls, 1993) revealed that carbachol-induced depolarizations in CA1 hippocampal neurons via muscarinic receptor activation are small in hippocampal slices with predominant MR occupation, while additional GR activation significantly increases the carbachol-induced depolarization; cooperativity of the MR and GR seems to be required to achieve this increase. Other membrane actions evoked by carbachol, including a decrease of the synaptically evoked EPSP and IPSPs (possibly via a presynaptic mechanism of action) were not consistently altered by the steroid treatments. Since the latter carbachol action is probably the most important effect of muscarinic receptor activation for the propagation of signals in the CA1 area, the functional relevance of the steroid-induced effects on carbachol responsiveness may be limited.

We conclude that steroids particularly interfere with the NA system in the brain. GR activation appears to reduce the functional outcome of the adrenergic system, while ADX evokes the opposite. The role of MRs in this interaction has not been specifically investigated.

4.2.2.4. Serotonin

Mutual interactions between corticosteroid hormones and the serotonergic (5HT) system in the brain have been documented (Azmitia, 1992; Jacobs and Azmitia, 1992; Chaouloff, 1993). In this review we will only focus on the modulation by corticosteroid hormones of the raphe-hippocampal 5HT system.

Corticosteroid effects on 5HT synthesis have been studied with several approaches: (i) Changes in tryptophan hydroxylase activity, the rate limiting step in the 5HT synthesis; (ii) changes in 5HT synthesis rate or turnover (usually assessed as the 5HT accumulation after blockade of the breakdown); and (iii) effects on 5HT content. A detailed analysis of the findings is given by Meyer (1985). In short, tryptophan hydroxylase activity is enhanced by glucocorticoids and decreased after ADX (Azmitia and McEwen,

1969, 1974; Yanai and Sze, 1983; Singh *et al.*, 1990), particularly under conditions of stress. The steroids do not affect the enzyme quantity, but rather exert a permissive role on its catalytic activity. In agreement with this are data showing that shortly (1–2 hr) after ADX, 5HT turnover is reduced in the hypothalamus, midbrain and hippocampus; treatment with low doses of corticosterone administered at the time of ADX restores the 5HT turnover (Van Loon *et al.*, 1981; de Kloet *et al.*, 1982, 1983). While in one study (Van Loon *et al.*, 1981) dexamethasone was also able to restore the ADX-induced changes of 5HT turnover, we observed that only low doses of corticosterone and not dexamethasone or aldosterone given at the time of ADX were able to restore the reduced 5HT synthesis rate in the raphe area and the dorsal hippocampus 1 hr after the stressful surgical procedure (de Kloet *et al.*, 1982, 1983). In fact, dexamethasone treatment after ADX even further reduced the 5HT turnover, and pretreatment 1 hr before ADX with dexamethasone or aldosterone prevented, in the raphe-hippocampal system, the reduction of 5HT turnover due to ADX. In addition to these effects reported for ADX rats, 5HT turnover in adrenalectomized animals turned out to be stimulated shortly after glucocorticoid treatment (Millard *et al.*, 1972; Neckers and Sze, 1975). Dexamethasone furthermore inhibits 5HT uptake (Sze *et al.*, 1976).

The dissociation between effects of low corticosterone doses and dexamethasone treatment on 5HT turnover, suggest that activation of MRs vs GRs may differentially affect 5HT synthesis. In agreement with this idea are reports that low corticosterone doses result within 30 min in an increase of the 5HT concentration in hypothalamic areas, midbrain and amygdala, whereas high doses produce the opposite result (Telegdy and Vermes, 1975; Kovacs *et al.*, 1977). This dose-dependency may partly explain incongruencies in other reports, which make use of different species and design (e.g. Green and Curzon, 1968; Hall and McGinley, 1982). Apart from these acute effects on 5HT content in the brain, long term ADX was found either not to affect 5HT concentration or induce a glucocorticoid reversible reduction of the 5HT concentration (Telegdy and Vermes, 1975; Rastogi and Singhal, 1978).

Not only 5HT synthesis is altered shortly after ADX. The 5HT₁ receptor density was found to be increased in the dorsal subiculum, parts of the dentate gyrus, substantia nigra and dorsal raphe, 1 hr after ADX. Substitution with low doses of corticosterone but not aldosterone restored the binding capacity of these regions and decreased the binding in the CA1 area. Scatchard analyses of 5HT binding in a membrane binding assay revealed that the corticosterone manipulations altered the B_{max} , without changing the K_d (de Kloet *et al.*, 1986). One study reported that 1 week after ADX, binding of [³H]-5HT to 5HT₁ sites in the CA1 area was still enhanced as compared to the adrenalectomized controls (Biegon *et al.*, 1985). This increase was partially reversed by 3–5 days of corticosterone treatment (moderate levels). The 5HT_{1A} receptor is abundantly expressed in dentate gyrus and CA1 pyramidal neurons and this subtype is a prominent candidate for the effects of corticosterone on [³H]-5HT binding. In fact, it was

FIG. 9. Neurons in slices from ADX rats display a hyperpolarization to 30 μ M 5HT which is comparable to the response observed in the adrenally intact controls (upper). 1–4 hrs after application of 3 nM of aldosterone, resulting in predominant MR activation, the response to 5HT is very small (middle). Pretreatment with the GR agonist RU 28362 prevents the effect of aldosterone (lower).

recently confirmed that binding of radiolabelled 8-OH-DPAT, a specific 5HT_{1A} ligand, does increase after ADX and that this effect is due to lack of corticosterone (Mendelsohn and McEwen, 1992a). Not only 5HT₁ but also 5HT₂ binding sites in the hippocampus were found to be increased after a prolonged period of ADX, an effect that could be restored with moderate levels of corticosterone (Martire *et al.*, 1989). In addition, ADX resulted in an apparent desensitization of 5HT autoreceptors in the hippocampus and hypothalamus, resulting in an increased stimulus-evoked 5HT release (Martire *et al.*, 1989).

Stress-induced changes of the 5HT system are not necessarily identical to changes evoked by high corticosteroid levels *per se*. Thus, the effects measured by de Kloet *et al.* (1982, 1983), that concerned acute, stress-induced changes shortly after ADX revealed a selective increase of 5HT synthesis and concomitant 5HT₁ receptor down regulation by low doses of corticosterone. Mendelsohn and McEwen (1991) studied the effect of chronic restraint stress in intact rats and noticed that these conditions, which produce elevated corticosterone levels, also resulted in increased 5HT_{1A} receptor binding. However, chronic corticosterone *per se* down regulated 5HT_{1A} receptors (Mendelsohn and McEwen, 1992b). Interestingly, chronic administration of corticosterone has also been shown to reduce 5HT₁-dependent behavioural (Dickinson *et al.*, 1985) and endocrine responses (Bagdy *et al.*, 1989), while repeated exposure to stressors resulted on the behavioural and neuroendocrine level in 5HT₁ supersensitive responses (Kenneth *et al.*, 1985). These findings from behavioural pharmacology are consistent with the effects of steroid and stress at the level of 5HT_{1A} receptors.

The cloning of the genes for the 5HT receptor types now allows another approach to study the effect of corticosterone on the raphe-hippocampal system. Using a riboprobe directed at part of the 5HT_{1A} receptor we found that 1 week after ADX the 5HT_{1A} mRNA expression is increased selectively in the dentate gyrus (Meijer and de Kloet, 1994). Replacement with constant levels of circulating corticosterone released from a subcutaneously implanted pellet reduced the 5HT_{1A} receptor mRNA; the reduction

was a linear function of the corticosterone concentrations, from extremely low (0.5 nM) to very high (1000 nM) levels of free circulating corticosterone. This finding suggests that MRs and GRs synergistically control 5HT_{1A} receptor expression in the dentate gyrus of the hippocampus.

Recently, we studied the effect of ADX and *in vitro* corticosterone treatment on 5HT receptor-mediated electrical responses in the CA1 hippocampal area. The most prominent effect of 5HT in the CA1 area of the hippocampus is a hyperpolarization of the pyramidal neurons (see for review Nicoll *et al.*, 1990); this effect is mediated by 5HT_{1A} receptors. ADX did not alter the 5HT_{1A} receptor-induced hyperpolarization of CA1 neurons compared to the sham operated controls (Joëls *et al.*, 1991). However, *in vitro* occupation of MRs in slices from ADX rats largely suppressed 5HT-evoked hyperpolarizations. This phenomenon develops with a delay of *ca.* 2 hr (Joëls and de Kloet, 1992b) and requires protein synthesis (Karst and Joëls, 1991). Actions induced by 5HT via other receptor subtypes were not affected by the steroid treatment. Interestingly, pretreatment of the slices with a selective GR agonist, RU 28362, prevented the development of the MR-mediated 5HT response suppression (Joëls and de Kloet, 1992b; see Fig. 9). The net result is that low doses of corticosterone suppress 5HT-mediated hyperpolarization, thereby maintaining the cellular excitability, whereas high corticosterone levels yield a 5HT response that is comparable to the response in ADX rats (Joëls and de Kloet, 1992b). In conclusion, the data indicate that alterations in the availability of corticosteroid hormones affect the raphe-hippocampal 5HT system at a rather short time scale (hours). ADX usually reduces the 5HT synthesis, while these effects are restored by low levels of corticosterone, sufficient to occupy the MRs. Tryptophan hydroxylase activity in the raphe neurons is enhanced by GR-mediated actions. In the hippocampus slice *in vitro*, the postsynaptic 5HT_{1A} receptor-mediated hyperpolarization response of CA1 pyramidal neurons is blocked by low doses of corticosterone activating predominantly MRs. Higher levels of corticosterone reverse this MR-mediated inhibition by activation of colocalized GRs. Thus, high levels of corticosterone activate the raphe-hippo-

campal system both by stimulation of 5HT synthesis and 5HT responsiveness of the hippocampal neurons.

4.2.2.5. Peptides

Many peptidergic neurons in the brain also contain GRs (Fuxe *et al.*, 1985). A number of studies have therefore focussed on the question whether GR activation controls peptide production. The majority of these investigations concerns the steroid-mediated regulation of CRH and AVP production in the hypothalamus, an important feature in the negative feedback exerted by steroids on ACTH releasing factors. ADX enhances CRH immunoreactivity and mRNA in parvocellular neurons of the paraventricular nucleus (PVN) in the hypothalamus (Merchenthaler *et al.*, 1983; Paull and Gibbs, 1983; Swanson *et al.*, 1983; Young *et al.*, 1986). The changes in CRH immunoreactivity and mRNA were restored effectively with high levels of dexamethasone or corticosterone, but not by aldosterone (Sawchenko, 1987; Kovacs and Mezey, 1987), pointing to a GR-mediated event. Considering the quite marked changes of CRH-producing neurons in the PVN after steroid treatment, it is remarkable that binding properties of CRH receptors in the brain were steroid-independent (Hauger *et al.*, 1987).

Following ADX, CRH synthesizing parvocellular neurons start to produce AVP (Silverman *et al.*, 1981; Tramu *et al.*, 1983; Kiss *et al.*, 1984; Sawchenko *et al.*, 1984; Davis *et al.*, 1986; Swanson and Simmonds, 1989; Imaki *et al.*, 1991). The change in AVP synthesis was reversible by pretreatment with dexamethasone or corticosterone (Silverman *et al.*, 1981; Davis *et al.*, 1986; Swanson and Simmonds, 1989; Imaki *et al.*, 1991). The effect of stressful stimuli on the synthesis of CRH and AVP in the PVN does not parallel the findings with high levels of exogenously applied corticosteroids. It was found that, as with ADX, chronic stimulation of the HPA-axis often induces an increased AVP-CRH ratio, resulting in more ACTH secretion from the pituitary corticotropes (review Dallman, 1993).

Messenger RNA but not immunoreactivity for enkephalins decreased in the parvocellular neurons of the PVN after ADX, but increased in the striatum and hippocampus (Sawchenko, 1987; Swanson and Simmonds, 1989). Chronic intermittent (Imaki *et al.*, 1991) or hypertonic saline stress (Lightman and Scott Young, 1987; Harbuz and Lightman, 1989) increased mRNA for both CRH and proenkephalin A in the PVN, which could be prevented by dexamethasone administration. Other peptides in the hypothalamus, e.g. ACTH (Krieger *et al.*, 1979; Van Dijk *et al.*, 1981) or neurotensin (Sawchenko, 1987) did not show apparent sensitivity to manipulation of the HPA-axis, although β -endorphin levels are increased after long term ADX (Lee *et al.*, 1980).

Not only in the hypothalamus, but also in higher brain areas peptidergic systems are regulated by the corticosteroid hormones. For example, ADX enhanced CRH levels not only in the hypothalamus but also in e.g. the cortex and amygdala (Sawchenko, 1987). The mRNA expression for opioids (preproenkephalin or preprodynorphin) were decreased 7 days after ADX in hippocampus and striatum (Iglesias

et al., 1991). The endogenous level of corticosterone at the moment of ADX appeared to be very important, since both a single injection of corticosterone prior to ADX or ADX in the evening yielded opioid mRNA expression comparable to the adrenalectomized rats. The cholecystokinin or neuropeptide Y mRNA expression in the hippocampus, striatum or hypothalamus was not altered in any of the experimental groups (Iglesias *et al.*, 1991). Similarly, restraint stress did not affect neuropeptide Y levels in medulla, hypothalamus and cortex (Rivet *et al.*, 1989). By contrast, another study reported a corticosterone reversible decrease after ADX of neuropeptide Y mRNA in striatum and hypothalamus, but not in cortex and hippocampus (Dean and White, 1990). Other peptidergic effects following ADX comprise (1) a decrease of somatostatin and increase of substance P and calcitonin gene related peptide content in the dorsal root ganglion (Smith *et al.*, 1991) and (2) a reduction of the ANG precursor angiotensinogen in the preoptic area, anterior hypothalamus and periaqueductal grey (Wallis and Printz, 1980; Deschepper and Flaxman, 1990). The latter may be related to the central steroid control of salt appetite (Nitabach *et al.*, 1989).

Of the other peptides in the brain, steroid-dependent modulation of VIP is the best studied example. It was shown that the VIP concentration in the hippocampus decreases after ADX, an effect that can be restored by dexamethasone or corticosterone treatment (see for more details McEwen *et al.*, 1986b). In addition, ADX increased VIP-induced cAMP accumulation in the hippocampus, amygdala and septum (Harrelson *et al.*, 1987). Dexamethasone or corticosterone administration reversed these ADX-induced effects within 48 hr. The direct functional relevance of these actions has not been evaluated, but may relate to a VIP-mediated, corticosterone sensitive modulation of 5HT₁ receptor characteristics in hippocampus (Rostene *et al.*, 1985).

In conclusion, although several studies have focussed on central effects of corticosteroids on peptidergic systems, the data so far predominantly relate to the neuroendocrine regulation by steroids of CRH and AVP production in the hypothalamus. It will be interesting to elaborate possible interactions at higher brain centers, e.g. the hippocampus, both with respect to the synthesis of peptides, the properties of their receptors and their functional responsiveness.

4.3. METABOLICALLY REGULATED CHARACTERISTICS

Corticosteroid-mediated actions not only concern changes in intrinsic membrane properties or transmitter-dependent characteristics but also involve 'general' cell functions which are important for homeostasis. In fact, studies about steroid-induced alterations of protein synthesis, dating back to the late seventies (Etgen *et al.*, 1979) have so far supplied evidence for a role in several of these general cell processes: (1) The synthesis of GPDH was found to be increased by acute stress or corticosterone, with a delay of only 2 hr (Nichols *et al.*, 1988, 1989; Schlatter *et al.*, 1990); (2) In hippocampal tissue glucocorticoids enhanced the amount of synapsin-1, a phosphoprotein which is involved in the control of neurotransmitter

release (Nestler *et al.*, 1981); the effect was apparent after 1 day, but reached a maximum after 7 days. By contrast, the mRNA for GAP43, another protein associated with transmitter release (Dekker *et al.*, 1989), was not changed after 4 days corticosterone treatment (Nichols and Finch, 1991); (3) Corticosteroid hormones may play a role in free radical formation, since they were shown to inhibit prostaglandin synthesis in the brain (Weidenfeld *et al.*, 1987); however, lipid peroxidation was not altered (Koide *et al.*, 1986). In peripheral tissues, NO synthase activity was inhibited (Moncada and Palmer, 1991). While the functional implications of steroid-mediated changes in synapsin-I formation for transmitter release has not been studied in detail, and the effects on free radical formation have been related mainly to peripheral steroid-induced phenomena, the role of steroids in metabolic processes has been extensively studied (see for comprehensive review Sapolsky, 1992).

The increased synthesis of GPDH mediated by GRs (Nichols *et al.*, 1988, 1989; Schlatter *et al.*, 1990) potentially enhances catabolic processes in neurons and glial cells. Enhancement of the cellular glycolysis by itself may have limited consequences, but the ensuing shortage of glucose or glycogen is probably aggravated by a GR-dependent inhibition of glucose uptake (Landgraf *et al.*, 1978; Horner *et al.*, 1990; Schasfoort *et al.*, 1988; Virgin *et al.*, 1991). This reduction in glucose uptake was seen both in glial and neuronal cultures and develops within 4–8 hr; conversely, shortly after adrenalectomy (5 hr) glucose utilization *in vivo* was enhanced in numerous brain areas, including the hippocampus (Kadekaro *et al.*, 1988). Clearly, severe energy deprivation is a threatening condition. Initially, there may be protective mechanisms which can curtail the deleterious effects of glucose and ATP deprivation, such as activation of ATP-dependent K-conductances, reduction of Na-conductance, inactivation of Ca-currents, enhanced release of adenosine, mild acidosis and micro-environmental regulation by glial cells (see for reviews Walz, 1989; Miller, 1990; Rudolph *et al.*, 1992; Haddad and Jiang, 1993). However, eventually the energy shortage will become damaging and the ability of the tissue to cope with any additional challenge will be largely reduced. The deadly dance of increased glutamate activity, particularly via NMDA receptors, and intracellular calcium accumulation has started, which through activation of e.g. calpains, endonucleases, phospholipase C, oxygen radical formation and severe acidosis eventually leads to delayed neuronal death (Siesjö and Bengtsson, 1989; Choi, 1988, 1990; Meyer, 1989; Erecinska and Silver, 1989). Glucocorticoid hormones do not seem to reduce cellular metabolism to an extent that they are damaging by themselves, but they can clearly exacerbate the vulnerability to excitotoxins (Sapolsky, 1985), hypoxia/ischemia (Sapolsky and Pulsinelli, 1985; Koide *et al.*, 1986) and hypoglycemia (Sapolsky, 1985) in hippocampal tissue. They do so regardless of their peripheral catabolic actions, since the aggravation of induced lesions was also observed in isolated brain preparations (Sapolsky *et al.*, 1988; Tombaugh *et al.*, 1992). Additional administration of energy sources, such as glucose or mannose, effectively protected

against the deleterious effects of glucocorticoids (Sapolsky, 1986). Considering the implication of increased excitatory amino acid transmission, particularly through NMDA receptors, and a steady elevation of intracellular [Ca] in neuronal damage due to energy shortage, it is not surprising that the glucocorticoid-induced exacerbation of neurotoxic agents shows NMDA and Ca dependency: (1) Corticosterone enhanced 3AP toxicity was reduced by a NMDA antagonist (Armanini *et al.*, 1990); rapid changes in glucose utilization after mild stress are also NMDA-dependent (Schasfoort *et al.*, 1988). (2) Kainic acid-evoked rises in [Ca]_i in a hippocampal neuronal culture are enhanced after 24 hr incubation with 1 μ M corticosterone (Elliot and Sapolsky, 1992), at least partly due to an impaired Ca-extrusion mechanism (Elliot and Sapolsky, 1993).

In summary, the combination of a corticosteroid-induced increase in glucose metabolism and inhibition of cellular glucose uptake, which develops over the course of several hours in neurons and glial tissue, potentially exacerbates the vulnerability of brain tissue to additional challenges such as ischemia or hypoxia. It should be noted though that apart from the usual (temporary) defense mechanisms in situations of energy deprivation, corticosteroids also evoke several actions which may initially help to avert the damage. Thus, glucocorticoids induce the synthesis of Calbindin-D28k in hippocampal tissue (Iacopino and Christakos, 1990), resulting in an increased capacity to bind calcium intracellularly. If nevertheless [Ca]_i rises, corticosteroids enhance the Ca-dependent K-conductances, resulting in the attenuation of cellular excitability (Joëls and de Kloet, 1989; Kerr *et al.*, 1989). In a next stage, glucocorticoid-induced inhibition of the prostaglandin synthesis (Weidenfeld *et al.*, 1987) potentially decreases the amount of damaging free radicals.

Most of the above described effects on cell damage were observed after exposure to very high levels of corticosterone. Accordingly, the actions were attributed to GR-mediated events. MR-mediated actions were in most cases not specifically investigated. Our observations about corticosterone effects on e.g. Ca-influx (see Section 4.2.1) suggest that MR-mediated events may be protective rather than damaging to neurons. Interestingly, rats treated with metyrapone, which does not block basal (MR-occupying) levels of corticosterone but prevents the stress-induced corticosterone production, showed reduced hippocampal damage evoked by kainic acid (Stein and Sapolsky, 1988). Therefore, it is very possible that the deleterious corticosteroid actions reviewed above will only occur under conditions where persistent, very high corticosterone levels coincide with additional severe challenges to the tissue.

4.4. MORPHOLOGY

There are indications that prolonged elevation of plasma corticosterone, in contrast to the limited exposure discussed above, even without additional major challenges to the brain, result over time in neuronal cell damage. Chronic treatment of rats with high corticosterone levels resulted after 3 weeks in atrophy of the dendritic tree of CA3 hippocampal

neurons (Woolley *et al.*, 1990) and after a 3 month period even in the actual loss of neurons in this area (Sapolsky *et al.*, 1985). The neuronal loss was reminiscent of the cell loss observed in aged animals. Indeed, adrenalectomy was shown to be protective with regard to the age-related loss of hippocampal neurons (Landfield *et al.*, 1981). However, it should be noted that in the latter study animals received low doses of corticosterone in their drinking water, probably enough to occupy the MRs to a considerable extent. It is therefore likely that MR occupation rather than adrenalectomy exerts a protective action.

Another line of research showed that the absence of steroids (ADX) may actually be a damaging rather than protective condition. Thus, removal of the adrenal glands results in a large cell loss in the dentate gyrus (Sloviter *et al.*, 1989, 1993a, 1993b; Roy *et al.*, 1990; Sapolsky *et al.*, 1991; Jaarsma *et al.*, 1992), as early as 3–4 days after adrenalectomy (Gould *et al.*, 1990; Jaarsma *et al.*, 1992) or even faster when corticosterone replacement after ADX is suddenly withdrawn (Sloviter *et al.*, 1993a). Ultrastructural analysis of the degeneration of dentate granule cells suggests that apoptosis is involved (Sloviter *et al.*, 1993b), reminiscent of the steroid-dependent apoptosis in lymphocytes (Compton and Cidlowski, 1986); however, a central role of steroids in apoptosis is not generally indicated (Masters *et al.*, 1989). The cell loss after ADX is mainly restricted to the granule cells in the dentate gyrus although occasional cell death is observed in the CA3 area (Jaarsma *et al.*, 1992; Sloviter *et al.*, 1993a). The fact that corticosteroid receptors (particularly GRs) are widespread in the brain seemingly precludes a direct role for steroids in the very localized cell death. However, *in vivo* treatment of ADX animals with low doses of corticosterone (Gould *et al.*, 1990) or aldosterone but not with the GR agonist RU 28362 (Woolley *et al.*, 1991) prevents the cell death in the dentate gyrus. It is conceivable that a combination of characteristics, e.g. the localization and properties of Ca- and Cl-conductances in addition to the steroid receptor content, may underlie the extreme sensitivity of dentate granule cells to the absence of particularly MR ligands.

Thus, both in the absence of corticosteroid hormones and with chronic over exposure relevant for e.g. the condition of chronic stress, neuronal tissue is subject to degeneration. By contrast, occupation of MRs seems to guarantee survival of neuronal networks.

5. GENERAL CHARACTERISTICS OF CELLULAR STEROID ACTIONS

The data presented in the previous sections show that corticosteroid hormones evoke sometimes rapid but in most cases delayed effects on (1) ionic conductances, (2) transmitter systems, and (3) general cell properties such as metabolism. Are the corticosteroids just another group of compounds affecting neuronal activity? What makes these adrenal hormones so special?

5.1. DIVERGENCE IN SPACE, TIME AND RESPONSE

An outstanding feature of corticosteroid hormones is that they represent a link between the body and the brain. They are synthesized in response to a pituitary signal and transported as a humoral factor. In the body as well as the brain they act as a true hormone: The spatial specificity is not contained in a point to point distribution, as is the case for transmitters and peptides, but rather by the localization of steroid binding receptors. The focus of this review is on cellular effects of steroids in the brain; this may have inadvertently fostered the idea that these actions can be regarded independently from the other endocrine messages conveyed by the hormones. However, it is important to realize that whatever the steroids do in the brain, they simultaneously induce effects in peripheral organs as well as the hypothalamus/pituitary as part of the neuroendocrine negative feedback pathway, in other words it is a pleiotropic hormone.

Another interesting feature is the fact that corticosteroid hormones can be converted to active or inactive metabolites. The former implies that the secretion of one hormone from the adrenal gland may, after metabolic conversion, result in an array of neuroactive compounds, each with its own recognition sites and functional implications; this is relevant for the formation of rapidly acting neurosteroids in the brain. The conversion to inactive compounds is also important for the functional significance of the hormone, since the discrete localization of inactivating enzymes such as 11 β -OHSD may lend a steroid receptor specificity which is not apparent from the primary structure of the receptor or its binding properties.

Finally, steroids generally bind to intracellular receptors which in activated form serve as transcription factors for the genome. Recent evidence suggests that corticosteroid hormones also interact with membrane receptors in the brain, which could mediate the fast effects of corticosteroids that have been occasionally described. If so, steroid-mediated events could take place over a wide period of time, with a delay anywhere between minutes and many hours. Such a wide effective timespan probably also exists for other steroid hormones, e.g. estrogens and progesterone.

Summarizing, the feature that really distinguishes corticosteroid hormones from other neuroactive compounds is the fact that the hormones represent an endocrine signal from the body to the brain, which displays divergence with respect to the spatial and temporal distribution and to the resulting cellular effects.

5.2. MRS AND GRs: TWO OF A KIND?

Interesting as the rapid steroid-mediated events may be, the delayed gene-mediated effects are quite unique, in that very few compounds have the potential to exert delayed and long lasting control over neuronal excitability. Many of the delayed cellular actions of steroids in the brain were already known 5–10 years ago (see e.g. Meyer, 1985). What really helped to sort out the sometimes paradoxical findings is the

TABLE 1. CORTICOSTEROID RECEPTOR-MEDIATED EFFECTS ON MEMBRANE CONDUCTANCES AND TRANSMITTER RESPONSES IN CA1 PYRAMIDAL NEURONS

	MR	GR	MR + GR
I _Q	↓	↓	○
I _{Ca}	↓	↓	○
AHP	↓	↑	↑
5HT _{1a}	↓	○	○
ΔFP*	○	↓	↓
ΔEPSP	○	↓	↓
ΔsIPSP	○	↓	↓

*Firing probability for synaptically evoked action potentials.

Only those Parameters of which the effects of MRs occupation only, GRs only, and simultaneous MR/GR activation, were established are represented in the table. The changes (↑: increase; ↓: decrease; ○: no change) are expressed against the values obtained in adrenalectomized controls. In some cases (I_Q and I_{Ca}), MR and GR activation induced comparable effects while simultaneous MR/GR occupation resulted in different changes of the response. The data suggest that a degree of cooperativity (synergism or antagonism) between MR- and GR-mediated events can occur.

recognition of at least two intracellular corticosteroid receptors, i.e. the MR and GR, mediating the delayed cellular influences. Furthermore, new experimental approaches allowed a functional study of delayed MR- and/or GR-mediated events.

At first glance it is not at all obvious why the fact that corticosterone activates MRs and GRs should help to understand the often paradoxical findings of the past decades. After all, the two receptors are structurally similar, they bind to the same hormone responsive elements and induce qualitatively (though not quantitatively) comparable messages, at least in transfected cell systems. Yet, functional studies of the past 5 years have supplied many examples that different effects do occur after activation of MRs and of GRs, in cell transfection systems, but particularly *in situ* in brain cells. How is this possible? One possibility is that other transcription factors partly determine the efficacy of MRs and GRs to affect gene transcription. Due to conformational differences between MRs and GRs, interactions with other transcription might be different for the two steroid receptor subtypes, as was indeed shown e.g. for interactions with *c-fos* and *c-jun*: In the presence of *c-fos/jun* GRs alter transcription, whereas MRs do not. Similarly, one may reason that mutual interactions between MRs and GRs may occur,

although this has not been shown to date. The interactions between MRs, GRs and other transcription factors which bind to closely related parts of the DNA potentially lead to synergism or antagonism. Functional studies on brain tissue do indeed support this (see Table 1). An even more straight forward explanation for the diverging functional effects mediated by MRs and GRs in the brain is the existence of different hormone responsive elements for MRs and GRs but, again, evidence for this has not been provided.

If MR activation on the one hand and additional GR activation on the other hand indeed evoke different effects on gene transcription, then it is much easier to understand how differential occupation of the two receptor subtypes can result in a wide scale of cellular responses. In that case the relative MR/GR occupation ratio becomes extremely important (de Kloet and Reul, 1987; de Kloet, 1991). This ratio depends (amongst other things) on the circulating level of the steroid, the affinity of the two receptors for the ligand, the total amount of MRs and GRs, and the presence of local enzymes such as 11β-OHSD, which allows selective occupancy of MRs by aldosterone. All of these factors are subject to plasticity, under physiological conditions but even more so under pathological conditions. In other words, the balance between MR and GR activation is very important for the net result of corticosterone on a given cellular property.

5.3. TARGETS FOR DELAYED STEROID ACTIONS

Are all of the neuronal properties, such as ionic conductances and transmitter responsiveness, a target for delayed regulation by corticosteroid hormones? Even though many properties have not even been investigated yet, it is now already clear that there is a certain degree of specificity in this regard with respect to (i) the ionic conductances, (ii) neurotransmitter systems and (iii) link in the neurotransmitter response that is affected.

Thus, Ca and Ca-dependent conductances are very sensitive to steroid treatment, much more so than K-conductances. The extent of this specificity naturally awaits new observations concerning steroid actions on Na- and Cl-conductances. Some degree of specificity also exists with respect to the neurotransmitter systems that are under steroid control: The central noradrenergic and serotonergic systems are largely modulated by steroid treatment, while e.g. the dopamine and acetylcholine systems display very few delayed and persistent changes as a result of variations in corticosteroid levels. The interactions with the excitatory amino acids are ambiguous and may develop secondary to changes in glucose metabolism; inhibitory amino acids do not seem to be greatly altered by physiological shifts in steroid levels, although metabolic conversion of the steroids yielding A-ring reduced compounds, could lead to membrane interactions with the GABA_A receptor complex.

Which elements in the neurotransmitter systems are most likely to be influenced by the steroids? In

the case of e.g. 5HT (and NA), both synthesis and receptor properties, are affected by the hormone, one perhaps as a result of the other; electrical responses to exogenously applied 5HT are also altered. By contrast, effects on transmitter release and uptake are far less established. Whether this is a general rule can be doubted: For instance, release and uptake rather than synthesis and receptor properties for excitatory amino acids are altered after steroid treatment. Unfortunately, relatively little is known about the corticosteroid influences on G-proteins and second messenger systems. At least some of the G-proteins are under steroid control (Saito *et al.*, 1989); indirectly, this will affect the activation of second messengers. Yet, basal second messenger activity was usually not affected by steroid treatment; only transmitter-induced second messenger accumulation was subject to steroid regulation. Whether or not steroids directly interfere with G-proteins and second messengers is an important issue, since these intracellular components are a common denominator for many transmitter systems in the brain. If steroids affect these elements of convergence, they have yet another powerful tool to control transmitter responses in the brain.

5.4. REGULATION OF ELECTRICAL ACTIVITY

Recent studies have provided insight into the membrane characteristics that are altered by corticosteroids. With *in vitro* electrophysiological techniques it was possible to study steroid modulation of intrinsic and transmitter-activated ionic conductances. Several general principles have emerged from these electrophysiological investigations (see also Joëls and de Kloet, 1992a).

The first important principle is that corticosteroid actions are conditional. In other words, under resting conditions treatment with steroids will be ineffectual; only when the membrane is hyperpolarized (e.g. for modulation of the 5HT response, the sIPSP and the I_Q) or depolarized (e.g. for effects on NA-responses, Ca-dependent K-conductances or Ca-conductances) modulation by steroids will become apparent. This is in line with studies about steroid-mediated endangerment of hippocampal neurons, where the corticosteroids alone do not directly damage the neurons but exacerbate the lesions evoked by neurotoxins or ischemia. The *in vitro* conditions were essential to recognize the conditional nature of steroid effects, since these circumstances allow a stringent control over the membrane potential, intracellular and extracellular fluid composition and the concentration of steroids and transmitters that are applied. The lack of such control *in vivo* may explain the variable positive and negative results with corticosteroids in earlier studies. However, it is quite important to realize that the latter *in vivo* condition with its great variation in background activity, input and consequently membrane potential is the actual situation in which steroids are active, so that eventually the role of steroid-mediated control of excitability can only be fully appreciated when studied in the adrenalectomized rat *in vivo*.

The second rule is that predominant MR activation on the one hand and simultaneous MR and GR occupation on the other hand usually induce different effects. As shown in Fig. 10, predominant MR activation generally results in small transmitter responses and ionic conductances, whereas MR plus GR activation evokes large transmitter responses and ionic conductances. The one exception so far to this rule is the GR-mediated suppression of noradrenergic responses in CA1 hippocampal neurons. This transition from predominant MR activation to MR plus GR activation is probably the variation in relative receptor occupancy that takes place as part of the circadian rhythmicity and due to the stress-induced changes in corticosteroid levels. The associated transmitter responses and conductances may therefore represent the range of daily variation.

Two other conditions, which may develop under more extreme circumstances, are also depicted in Fig. 10, i.e. the situation where MRs are not occupied and the situation where either very high doses or prolonged administration of corticosteroids were applied. Removal of the adrenal glands without steroid substitution results in variable effects; however, for most parameters the responses are quite comparable to the responses observed with MR plus GR activation. Exposure of tissue from ADX or

FIG. 10. Schematic representation of the relative ionic conductances and transmitter responses of CA1 hippocampal neurons *in vitro*, as a function of the MR/GR occupation ratio. The effects are expressed as a percentage of the maximal response. With predominant MR occupation, the conductances and transmitter responses are generally small; with simultaneous MR and GR occupation the responses are relatively large. In most cases, the membrane responses are also quite large in the absence of steroids, implying an U-shaped dose-response relationship. The figure illustrates that the effect of steroid application on membrane properties depends on the occupation of the steroid receptors before steroid addition and on the applied steroid concentration. Not incorporated in this figure are membrane characteristics that were not altered by corticosteroids, the effects on the β -adrenergic responses (for which the MR influences have not been investigated) and the steroid-induced control of the EPSP (stable in tissue from ADX rats without MR and GR occupation or with predominant MR occupation, attenuated with high corticosterone levels).

adrenally intact animals to very high steroid levels usually yields the same or even stronger effects as occupation of MRs plus GRs with physiological doses of the steroids. As a result, the steroid-induced modulation of intrinsic membrane conductances or transmitter responses yields a dose response curve with a U-shape. This explains why the net result of steroid application depends on the steroid receptor occupation before application and on the applied concentration. A shift by less than one order of magnitude along this dose-response curve may be the difference between a decrease or an enhancement of a particular membrane response.

5.5. PHYSIOLOGICAL IMPLICATIONS

What is the physiological relevance of these phenomena? First of all, not all of the described phenomena are necessarily important for the local transmission of signals. For instance, ACh induces amongst other things a depolarization and a suppression of the synaptic input. The latter is most important for the input-output relationship in the CA1 area. However, only the first is subject to steroid regulation. Therefore, although corticosteroids modulate cholinergic responses in the CA1 area, the net effect for the conveyance of messages may be limited. Secondly, the steroid modulation of ionic conductances, transmitter responses and metabolic disturbances were quite often studied separately and information about a more integrated level, even in a local neuronal network such as the CA1 area, is very sparse. It is actually quite likely that some steroid-mediated effects are related to others and in some cases may have a common underlying mechanism of action. For instance, metabolic disturbances and changes in Ca-influx could explain the failure of hippocampal neurons to respond to repeated synaptic activation. Changes in Ca-influx, particularly in the thin distal dendrites of CA1 neurons where most of the synaptic input impinges, may after repeated stimulation give rise to an extensive build-up of calcium; due to the metabolic problems energy- and ATP-demanding processes such as Ca-extrusion or sequestration may no longer be very active. This may start a serious condition where initially synaptic transmission is reduced but on a longer time scale neurons are endangered.

Three MR-mediated actions seem to be important for the CA1 hippocampal area: The suppression of 5HT hyperpolarizations, the stability of amino acid-mediated synaptic transmission and the decrease of Ca and Ca-dependent conductances. As a result MR activation will guarantee the maintenance and stabilization of the excitability in the area. At a longer time scale the latter is also reflected in the MR-mediated protection of neuronal integrity in the dentate gyrus. Without trying to unify all of the cellular effects observed so far, it is clear that MR occupation is important for the survival and ongoing activity in the hippocampus (Table 2). GR-Mediated actions on the other hand may, at short term, reduce local excitability, but on a longer time scale become damaging. This concept is not new but in fact much in line with the original formulation by Selye (1950) of the general adaptation syndrome.

The coordinated control of local excitability by MRs and GRs in the hippocampus has evidently, consequences for functional processes in which the hippocampus plays a prominent role. One prediction is that spatial learning and memory, for which e.g. LTP in the hippocampus is of great relevance (Bliss and Collingridge, 1993), will be affected by coordinated MR- and GR-mediated events. This was indeed demonstrated recently (Oitzl and de Kloet, 1992). Another function of the hippocampus, i.e. as a predominantly inhibitory controller of hypothalamic/pituitary ACTH production (Sapolsky *et al.*, 1984; Herman *et al.*, 1989b), will also be regulated by the joint MR- and GR-dependent control of hippocampal excitability.

Clearly, the fact that most areas in the brain contain GRs indicates that steroid-mediated cellular actions all over the brain will contribute to the final functional outcome. Since most brain regions do not contain MRs, the coordinated control over excitability by MR and GR observed in the hippocampus may be the exception rather than the rule in the brain. Since very little is known about corticosteroid modulation of membrane properties in the areas with only GRs, it is hard to predict the nature of steroid actions there. It is possible that the local excitability in these parts of the brain is only affected under conditions of high adrenocortical activity, e.g. after a period of stress.

5.6. RELEVANCE FOR PATHOLOGICAL CONDITIONS

The cellular actions of corticosteroids may get an entirely different appearance under pathological conditions. This may be due to (i) changes in circulating corticosterone levels, (ii) changes in the steroid receptor properties or (iii) disturbances in the local excitability. These three alterations are not necessarily independent phenomena. A well documented example concerns the hypercorticism and increased GR affinity observed in the hippocampus of Fischer rats at old age, which correlates well with a GR-mediated increase of the Ca-influx through voltage-activated calcium channels (Kerr *et al.*, 1992; Landfield *et al.*, 1992).

Many diseases are indeed associated with changes in circulating corticosterone levels. The etiology of some involve decreased CRH production and hypocorticism, as occurs with fibromyalgia (Griep *et al.*, 1993), chronic fatigue syndrome, atypical depression, obesity or post traumatic stress disorder (King, 1988; Sternberg, 1993). These diseases are characterized by an increased vulnerability to inflammation. Other pathological conditions, comprising anorexia nervosa and malnutrition, melancholic depression, excessive exercise, chronic disease and chronic alcoholism are associated with increased CRH production (and hypercorticism; Gold *et al.*, 1988a, 1988b; Holsboer, 1989). They correlate with an increased vulnerability to infections. While data about alterations of the corticosteroid-dependent cellular actions in men in relation to these pathological conditions are not available, animal studies indicate that the corticosteroid levels and the MR and GR properties are indeed important factors for the local excitability. For example, both hypercorticism (chronic stress) and

TABLE 2. OVERVIEW OF THE CELLULAR ACTIONS IN VARIOUS HIPPOCAMPAL SUBFIELDS, ASSOCIATED WITH PREDOMINANT MR (UPPER PART) OR MR + GR OCCUPATION (LOWER PART OF THE TABLE)

MR	ACh _m	Less depolarization	
	5HT _{1a}	Less binding/hyperpolarization	Less modulatory input
	EPSP/FP	Stable with repeated stimulation	
	IPSP	Ibid	Stable amino acid transmission
	AHP	Less accommodation/AHP	
	Ca	Less influx	Neuroprotective
MR+GR	ACh _m	More depolarization	
	5HT _{1a}	More synthesis, turnover; hyperpolarization restored	
	NA	Less cAMP/more accommodation	Excitability initially reduced
	EPSP/FP	Attenuated with repeated stimulation more free glutamate	
	IPSPs	Decreased	
	AHP	More accommodation/AHP	
	Ca	More influx, less extrusion	Delayed increase of vulnerability for neurodegeneration
	Glucose	Less uptake/breakdown	

It should be noted that the MR/GR occupation ratio is not a rigid measure, but dependent on the availability of the endogenous ligand, the receptor characteristics and liable to regulation by e.g. chronic stress, aging or antidepressants.

Predominant MR occupation may be important for steady transmission of the fast excitatory and inhibitory signals carried by amino acid transmitters; the responsiveness to modulatory transmitters (e.g. ACh and 5HT) is much reduced; voltage-dependent Ca-influx is small, which means that the effect of continuous, excitatory challenges will be limited. The latter could be neuroprotective, on the long run.

When MRs and GRs are both activated, the local excitability will initially be reduced: The excitatory input carried by glutamate and NA is attenuated, the accommodation/AHP enhanced and the hyperpolarizing response to 5HT is restored (but note that the ACh-evoked depolarization is enhanced and the sIPSP decreased!). Continuous exposure to high steroid levels however may be an endangering condition, due to the delayed increase of Ca-conductances, of free glutamate and the decrease of glucose availability.

removal of steroids (ADX) alter steroid receptor properties but also the cellular actions mediated via these receptors and eventually the integrity of the tissue. Still, extrapolation of the results from these animal studies, in which the effect of controlled alterations in corticosteroid levels was established, to conditions of hypo- or hypercorticism should be carefully interpreted.

For the role of steroids during pathological conditions the local excitability is also an important factor. This is probably due to the fact that steroid actions are generally conditional: When the membrane potential is shifted from its resting level steroid-mediated events become apparent. Steroid-dependent cellular actions that are not prominent under physiological circumstances may become important when the activity of transmitter systems, ionic conductances or metabolic processes are altered during pathological conditions. For instance, the steroid effects on 5HT responses may become important in conjunction with changes in the 5HT system during depression (Gold *et al.*, 1988a, 1988b; Holsboer, 1989); genomic steroid actions on membrane properties could be of significance if transmitter responses and ionic conductances are disturbed as a result of epilepsy (Feldman, 1966; Holmes, 1991), or corticosteroid-dependent alterations of the glucose metabolism and Ca-influx that can normally be

controlled, might become deleterious when they occur in combination with ischemia (Sapolsky, 1992).

Clearly, the importance of cellular actions of corticosteroid hormones in the brain for the etiology of the above mentioned diseases is only starting to be explored. With specific knowledge about these cellular processes the potential of selective corticosteroid hormone analogues for the treatment of these diseases can be further elaborated.

6. SUMMARY

In this review we have argued that corticosteroid hormones represent an endocrine signal that can influence neuronal communication. The steroids bind to intracellular receptors in the brain, resulting in slow effects that involve gene transcription, but they may also evoke rapid effects via membrane receptors. The signal carried by the corticosteroids is therefore divergent with respect to the dimension of space and time.

Within the rat brain, at least two intracellular receptor subtypes, i.e. MRs and GRs, bind corticosterone. The affinity, density and localization of the MRs is different from the GRs, although the actual properties may vary somewhat depending on the

condition of the animal. In general, due to the difference in affinity, low corticosteroid levels result in a predominant MR occupation, while higher steroid levels additionally occupy GRs. Recent studies indicate that predominant MR occupation is important for the maintenance of ongoing transmission in certain brain regions and for neuroprotection. By contrast, additional GR occupation (for a limited period of time) results in an attenuation of local excitability; yet, prolonged exposure to high steroid levels may become an endangering condition for neurons. Since predominant MR occupation on the one hand and additional GR occupation on the other hand induce different cellular actions, the ratio of MR/GR occupation is an important factor determining the net effect of corticosteroid hormones in the brain. How coordinated MR- and GR-mediated effects control neuronal communication under various physiological and pathological conditions will be a challenge for future research.

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