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Long-term Control of Neuronal Excitability by Corticosteroid Hormones

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Hippocampal CA1 neurons express both mineralocorticoid and glucocorticoid receptors. Due to the difference in affinity of the two receptor types for corticosterone and variations in endogenous steroid levels, occupation of the receptors will range between a situation of predominant mineralocorticoid receptor activation and conditions where both receptor types are occupied. It was observed that local signal transduction is regulated by activation of the corticosteroid receptors. Particularly, transmission mediated by biogenic amines appears to be sensitive to steroid control. The data indicate that cholinergic and serotonergic responses are small with predominant mineralocorticoid receptor activation, while additional glucocorticoid receptor activation results in large responses; the reverse has been found for noradrenalin. The steroid-dependent control over transmission by biogenic amines will influence local excitability and therefore functional processes in which the hippocampal system is involved.

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INTRODUCTION

The rat steroid hormone corticosterone is produced in the adrenal gland, in response to ACTH [1]. The hormone is released in a circadian pattern, with high levels around the start of the active period and low levels at the start of the inactive period. In addition to this circadian variation, corticosteroid levels in blood can also change in response to environmental factors: after stressful experiences circulating corticosteroid levels are transiently raised. The biological availability of corticosterone, however, is not only determined by the adrenal secretion, but also by the properties of corticosterone binding proteins [2] and the presence of corticosterone converting enzymes [3, 4].

Corticosterone reaches many peripheral organs; it is retained at places where selective corticosteroid receptors are present. Two receptor subtypes have been recognized, based on the primary structure and the binding properties *in vitro*. First, the glucocorticoid receptor (GR), which is abundant in e.g. liver and colon [5–8]. This receptor binds corticosterone relatively well ($K_d \sim 5$ nM), can be selectively activated by dexamethasone and RU28362 [9] and antagonized by RU38486 [10]. Secondly, the mineralocorticoid receptor (MR), which can be found e.g. in the kidney [11]. *In vitro*, the MR in the kidney binds corticosterone with a high affinity ($K_d \sim 0.3$ nM), similar to aldosterone [12]. *In vivo*, though, kidney MRs are primarily activated by aldosterone, notwithstanding the prevalence of corticosterone in the blood. It was shown that the aldosterone-preferring character of the kidney MRs is conferred by the presence of 11β-OH steroid-dehydrogenase, which converts corticosterone to its 11oxo metabolite which has very low affinity for the MR [3, 4].

In the late sixties McEwen and coworkers demonstrated that corticosterone is also highly retained in the brain, particularly in the hippocampal formation [13]. Subsequent studies in the brain revealed that corticosterone binds to steroid receptors of which the proteins are identical to the MRs and GRs in peripheral organs [14, 15]. The binding properties of the brain MRs and GRs also resemble the binding observed in peripheral tissue, with one exception: in most brain nuclei MRs bind corticosterone equally well as aldosterone, *in vitro* and *in vivo*. It is thought that the biological activity of the 11β-OH-steroid-dehydrogenase in most (though not all) brain areas is low, so that the prevailing hormone corticosterone can occupy the receptors [16]. Consequently, corticosterone is a mixed agonist for MRs and GRs in the brain, as opposed to the periphery.

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Due to the variations in circulating corticosterone levels and difference in affinity of brain MRs and GRs for corticosterone, variable degrees of MR and GR occupation may occur. When corticosteroid levels are low, i.e. at the start of the inactive period (under rest), mainly high-affinity MRs will be activated, whereas only a small part of the GRs are occupied [17]. When corticosteroid levels rise, e.g. to circadian peak levels or after periods of stress, not only MRs but also most of the GRs will be occupied [18]. Under physiological conditions this implies that corticosteroid receptor occupation varies between predominant MR occupation on the one hand and concurrent MR/GR occupation on the other hand [19]; importantly, there is a daily shift from one situation to the other. However, more extreme variations in steroid receptor activation may occur, often in association with chronic disorders and diseases. This comprises situations of chronic hyper- or hypocorticism, in some cases accompanied by steroid resistance pointing to a steroid receptor defect [20–22]. The net result of these pathological conditions is that effects mediated by brain MRs and/or GRs are chronically reduced or overexpressed.

Binding of corticosterone to its receptor results in dissociation of a heat-shock protein from the receptor, nuclear translocation of the activated steroid–receptor complex, dimerization and binding of the receptor dimer to hormone responsive elements in the nuclear DNA [23]. The activated steroid receptor complexes, acting as transcription factors, thus enhance or reduce the expression of certain cellular proteins. The steroid sensitive proteins may play a role in general cell processes, e.g. metabolism, but also in more specific functions, such as signal transduction, which are particularly important for neuronal tissue. Several features of the signal tranduction pathway may form a target for steroid effects: one possibility is that corticosterone affects intrinsic membrane properties which determine the "state" of the cell when it is reached by signals from other sources. These properties include the voltage-independent and voltage-gated ion conductances. Another possibility refers to steroid actions on neurotransmitter systems. Potential targets are transmitter release mechanisms, transmitter receptors, second messengers and G-proteins coupled to the transmitter receptors, and ion channels ((in)directly) gated by transmitters.

Previous studies from our laboratory and other groups revealed that corticosteroids exert slow and persistent effects on several of the above mentioned targets in signal transduction [24–26]. In particular, voltage-gated calcium conductances and inputs mediated by biogenic amines are modulated by corticosteroid hormones. In this paper we will review recent information regarding interactions between corticosteroids and biogenic amines.

MATERIALS AND METHODS

To study long-term effects of corticosteroid hormones on signal transduction in the brain a number of methodological considerations need to be made.

The first important aspect is the choice of the experimental method, which is dictated by the nature of the question. Steroid actions on transmitter release can be investigated with biochemical methods. *In vivo*, microdialysis probes or push–pull cannulas can be used to examine the changes in neurotransmitter release induced by corticosteroid receptor occupation; *in vitro*, one can also collect the superfusion fluid and determine transmitter content. Steroid actions on transmitter receptor properties may involve changes in the binding affinity or capacity of the receptor protein. This can be investigated with membrane binding assays or with *in vitro* autoradiography. Changes in binding capacity may point to an altered mRNA expression for the transmitter receptor protein, a possibility that can be studied with *in situ* hybridization. Eventually, steroid-induced alterations in transmitter release, binding or subsequent steps in the signal transduction pathway will be translated to changes in functional transmitter responses. The latter can be studied with electrophysiological methods, *in vivo* and *in vitro*. The (dis)advantages of the various electrophysiological approaches have been discussed in detail elsewhere [27]. In short, steroid actions are often conditional in that they are not apparent under resting conditions but only when the membrane potential is shifted from its resting level. The study of these effects is highly improved when there is a good control over the ion composition of the cellular environment and voltage control over the membrane. This can be optimally achieved with voltage clamp conditions, *in vitro*, using either microelectrodes or patch clamp electrodes. The data which will be reviewed below were obtained with either one of these techniques.

The second important methodological aspect concerns the occupation of the corticosteroid receptors. It is feasible that activation of MRs affects signal transduction differently than activation of (MRs and) GRs. Therefore selective activation of these corticosteroid receptors is indicated. This is difficult to achieve with the natural ligand corticosterone, since it acts as a mixed agonist on MRs and GRs. Natural fluctuations in corticosteroid levels will thus result in variable degrees of MR/GR activation. To control the degree of corticosteroid receptor occupation two approaches can be followed. One approach is to adrenalectomize (ADX) rats and substitute them with known concentrations of corticosterone or selective agonists. Though eliminating effectively the endogenous steroid by this procedure, other essential compounds (like adrenaline) are also removed from the system. This may introduce extensive deficiencies and trigger complex adaptational processes in the organism. The alternative approach

is to treat adrenally intact rats with selective (ant)agonists.

A third methodological aspect that is inherent to working with steroid hormones regards the timespan over which effects develop and persist. By acting on the genome, corticosteroid hormones induce slow actions on signal transduction. It is therefore imperative to study aspects of signal transduction for at least 1–2 h (and preferably longer) after a change in steroid receptor occupation has been evoked. It is not always feasible to follow processes for such a long period of time. As an alternative, one could compare parameters studied before steroid receptor occupation with the values obtained at least 1–2 h after receptor occupation. Since these values are (in most cases) obtained from different populations (of cells, tissue, animals), variables which are not controlled for may disturb the result. The study should therefore preferably involve large numbers of observations.

Recent advances in methodology, availability of selective MR/GR (ant)agonists and increased knowledge of the mechanism of action for steroids has allowed a more in-depth investigation of steroid actions on signal transduction in the brain.

RESULTS

Acetylcholine

Data concerning the effect of selective corticosteroid receptor occupation on the cholinergic system are sparse. However, since several studies have shown that (acute) stress affects cholinergic uptake/release and binding properties of cholinergic receptors, there is indirect evidence supporting that corticosteroids influence the cholinergic system in the brain.

Thus, stressful conditions such as chronic immobilization and exposure to noise decrease the Na-dependent high affinity uptake of choline in various brain regions [28–30]; brief exposure to immobilization, however, temporarily raised choline uptake [28]. The reports about acetylcholine (ACh) release are conflicting. In a number of studies a brief rise in ACh release was observed in limbic structures during acute [28, 31] and chronic stress [28]; yet, others found no change [30]. Following acute stress, muscarinic ACh receptors appeared to be changed, mostly showing an increased receptor affinity in several brain areas including the CA1 hippocampal region [28, 30, 32, 33]. Chronic (immobilization) stress induced a significant increase in the maximal number of muscarinic receptors; this was found in brain areas such as the cortical layers, the caudate-putamen and the CA1 hippocampal region [34]. In general, these data point to a stimulation of the brain cholinergic system after stress, possibly through corticosterone.

Data from electrophysiological studies support this. CA1 pyramidal neurons in hippocampal slices respond to cholinergic agents (i.e. carbachol) with (1) a depolarization of the membrane, (2) a suppression of the accommodation and afterhyperpolarization associated with a brief membrane depolarization and (3) suppression of synaptic excitatory and inhibitory potentials [35]. It appeared that the membrane depolarization induced by carbachol is small subsequent to treatment of slices from ADX rats with aldosterone *in vitro*, which will mainly occupy MRs (see Fig. 1). Occupation of both receptors *in vitro* results in large depolarizations [36]. When both receptors are unoccupied (in untreated ADX rats) carbachol responses were also relatively large. Other effects of carbachol were not consistently affected by steroid treatment. Similar results were obtained following *in vivo* treatment with the natural, mixed agonist corticosterone: low to moderate doses of corticosterone, which mainly occupy MRs, are associated with small carbachol responses, while both very high and extremely low corticosterone concentrations result in large carbachol-induced depolarizations (Hesen and Joëls, in preparation). Whether stressful experiences, leading to high corticosterone levels, can also evoke similar large cholinergic responses in hippocampal neurons is currently under investigation.

Noradrenaline

The rate of noradrenaline (NA) synthesis or release does not appear to be very sensitive to corticosteroid treatment [37–39]; stressful stimuli, though, were shown to alter the local availability and release of NA [40–42], perhaps through a corticosterone-independent mechanism. Accordingly, α_1- and β-adrenoceptor characteristics were also not affected by changes in corticosteroid levels [43–46]. This is not the case for α_2-adrenoceptors: binding to this receptor type is decreased in the paraventricular nucleus and enhanced in the supraoptic nucleus 1 week after ADX [47]. Corticosterone treatment *in vivo* can prevent these effects.

The most apparent actions of corticosteroid hormones on the noradrenergic system involve steps in the signal transduction pathway which are beyond the receptor, i.e. the G-proteins and second messengers coupled to adrenoceptors. It was found that chronic corticosterone treatment of ADX rats increases Gsα mRNA, immunoreactivity and ADP ribosylation in cortical tissue; by contrast, Giα mRNA and immunoreactivity decreased [48]. These effects may contribute to the fact that adrenalectomy increases the efficacy of NA to stimulate cAMP formation in cortical and hippocampal tissue [44, 49]. Conversely, high doses of corticosterone, which will occupy both MRs and GRs, suppress the NA-induced cAMP formation. There are indications that these steroid effects on second messengers involved in noradrenergic signal transduction not only concern β_1- but also α_1-adrenoceptors [50].

The biochemical data indicate that steroid concentration and the efficacy of the (nor)adrenergic signal transduction are inversely related. This is also reflected in the functional outcome of the signal transduction pathway, as recorded with electrophysiological methods. Noradrenaline, through β_1-adrenoceptors, suppresses the firing frequency accommodation and afterhyperpolarization of a CA1 hippocampal neuron which are linked to a brief period of cellular depolarization [35]. This effect is most apparent in tissue from untreated ADX rats [51]. Treatment of the tissue with high doses of corticosterone or GR-agonists suppresses the efficacy of noradrenaline to alter electrical properties of the cells. Synaptic responses, recorded extracellularly in the hippocampal CA1 field, are increased in amplitude by noradrenaline; this effect arises from adrenergic actions on several receptors (including the β_1-receptor). Here too, adrenergic actions were most prominent in the absence of corticosteroids, while small adrenergic responses were observed with very high levels of corticosterone (see Fig. 2, ref. [52]).

Serotonin

The brain serotonergic system is strongly regulated by corticosteroid hormones. Almost all steps in the signal transduction pathway (as far as they have been investigated) display strong sensitivity to circulating steroid levels.

Serotonin (5HT) availability appeared to be reduced shortly after ADX [53]. This reduction may be caused by (1) a decrease in tryptophan hydroxylase activity [54–56] and/or (2) a decrease of 5HT turnover [57–59]. The steroid effects on tryptophan hydroxylase probably involve a GR-mediated effect; however, ADX-induced decreases of the turnover could be most effectively reversed by low doses of corticosterone, primarily occupying MRs [58, 59].

The expression of 5HT receptors was recently investigated with *in situ* hybridization. In hippocampal subfields, particularly the dentate gyrus, $5HT_{1a}$ receptor mRNA expression was enhanced after ADX [60, 61]. This was observed both 1 day and 1 week after ADX. Replacement with low doses of corticosterone resulted in expression levels which were comparable to the values of adrenally intact controls. Binding to 5HT receptors was similarly affected by corticosteroids. Thus, $5HT_1$ receptor density was increased in some of the hippocampal subfields and midbrain areas, 1 h [62] and 1 week after ADX [63]. The increase can be reversed by corticosterone substitution. Similarly, binding of radiolabelled 8-OH-DPAT, a selective $5HT_{1a}$ ligand, increases after ADX, due to the lack of corticosterone [64]. Apart from $5HT_{1a}$ receptors,

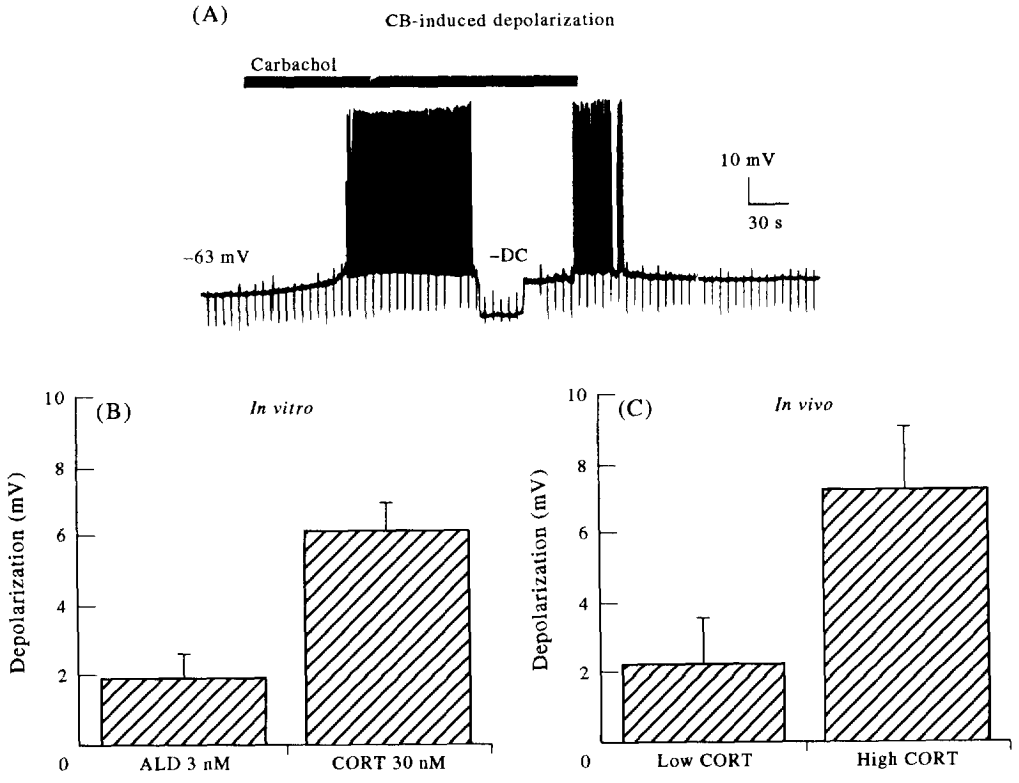

Fig. 1. (A) Typical membrane response of a rat CA1 pyramidal neuron to the cholinergic analogue carbachol (10 μM), showing depolarization of the membrane and the appearance of action potentials. (B) *In vitro* occupation of MRs by 3 nM aldosterone and of MRs + GRs by 30 nM corticosterone (left) results in small and large carbachol-induced depolarizations respectively. Similar effects are observed following *in vivo* injection (right) of low and high corticosterone concentrations (10–30 μg/100 g rat and 1 mg corticosterone/100 g rat).

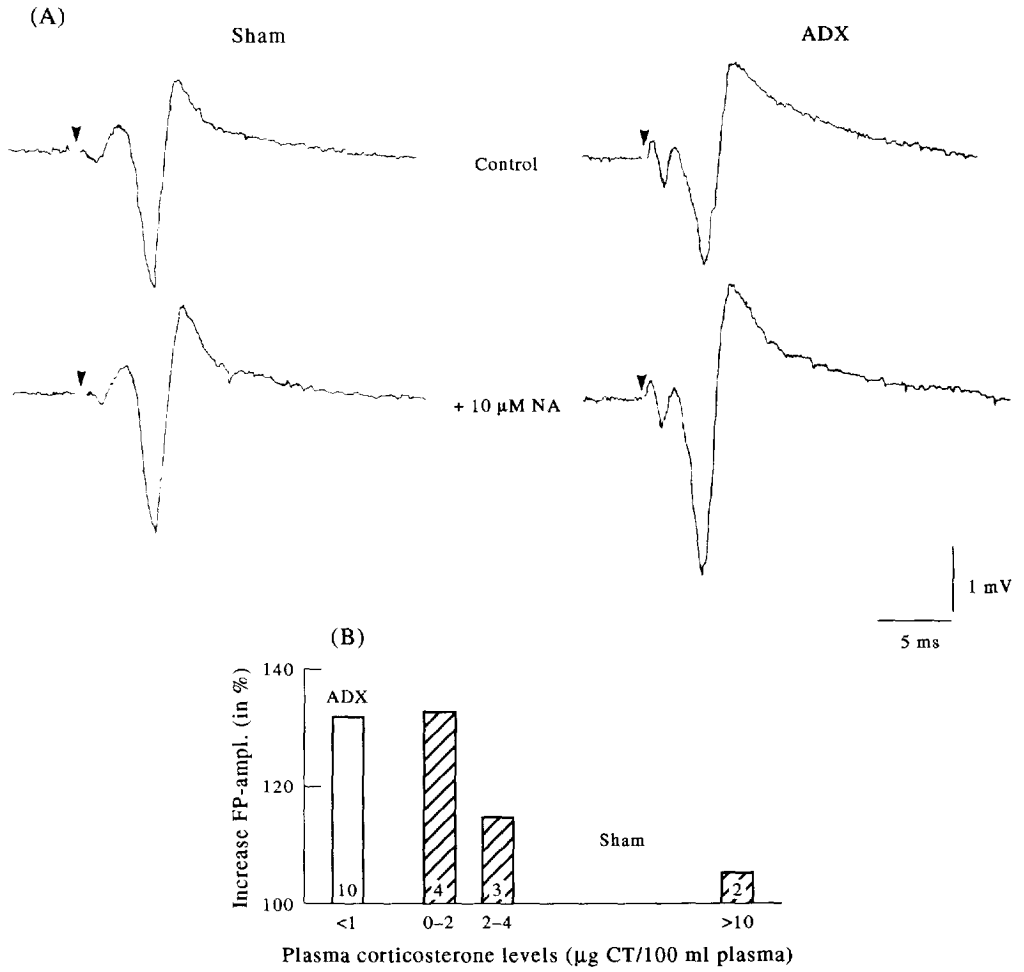

Fig. 2. (A) Field response evoked in the pyramidal layer of the CA1 hippocampal area by synaptic activation of the Schaffer collaterals. The amplitude of the half-maximal response is markedly increased by *in vitro* application of noradrenaline (10 μM) in ADX but not in sham operated control rats. (B) Plasma corticosterone levels obtained in adrenally intact rats prior to sacrifice are inversely related to the increase in field potential amplitude induced by 10 μM noradrenaline.

properties of the $5HT_2$ sites in hippocampus are also affected by ADX and corticosterone treatment [65].

As summarized above, variations in corticosteroid level do affect various aspects of the brain serotonergic system. Stress was also found to change synthesis and binding of serotonin to its receptors. However, the effects induced by high levels of corticosterone and by stress (which temporarily induces high corticosteroid concentrations) are not necessarily the same. For instance, chronic restraint stress was found to increase $5HT_{1a}$ receptor binding in hippocampus [66]. Yet, chronic administration of corticosterone down regulated $5HT_{1a}$ receptors [67].

Recent electrophysiological studies have shown that 5HT-evoked changes in ionic conductances, which take place at the end of the signal transduction pathway, display clear modulation by corticosteroid hormones. In hippocampal CA1 neurons, 5HT hyperpolarizes the membrane through the opening of K-channels, via the $5HT_{1a}$ receptor [35]. In hippocampal tissue treated *in vitro* with low doses of corticosterone or with aldosterone, thus predominantly occupying MRs, 5HT-induced hyperpolarizations were found to be small [68, 69]. Both in untreated tissue (MRs and GRs unoccupied) and in tissue where MRs and GRs were fully activated 5HT responses were relatively large. This effect typically developed 1–2 h after steroid treatment was initiated (see Fig. 3) and could be prevented by a protein synthesis inhibitor, pointing to the genomic origin of the steroid modulation [70]. Other responses to 5HT which are mediated via non-$5HT_{1a}$ receptors were not affected by corticosteroid treatment. It is not quite clear which point of the signal transduction pathway for 5HT is the exact target for corticosteroids. It seems unlikely, however, that the K-conductance, which is at the end of the pathway, forms the target: responses to the $GABA_B$ agonist baclofen, which are due to opening of the same K-channels [35], were not affected by corticosteroid treatment [68]. Preliminary data show that the steroid modulation of 5HT responses observed after perfusion of hippocampal slices with MR- and/or GR

(ant)agonists can also be observed with *in vivo* administration of the natural, mixed agonist corticosterone (Hesen and Joëls, in preparation).

DISCUSSION

Since the early observation by McEwen *et al.* [13] that corticosterone is highly retained in the hippocampal formation, many studies have focused on the role of corticosteroid hormones in one of the most striking features of neuronal tissue, i.e. transduction of electrical signals. Some developments facilitated this research. First, the appreciation that corticosteroid actions in the brain are mediated by at least two types of receptors; the availability of selective (ant)agonists for these receptors allowed to distinguish between MR and GR mediated actions. Secondly, the applicability to brain tissue of a number of methods with high resolution permitted studies of steroid actions at the level of a single cell or even at the molecular level, *in situ*.

Studies over the past 10 years have clearly shown that corticosteroid hormones affect both the intrinsic membrane properties of brain cells and their responses

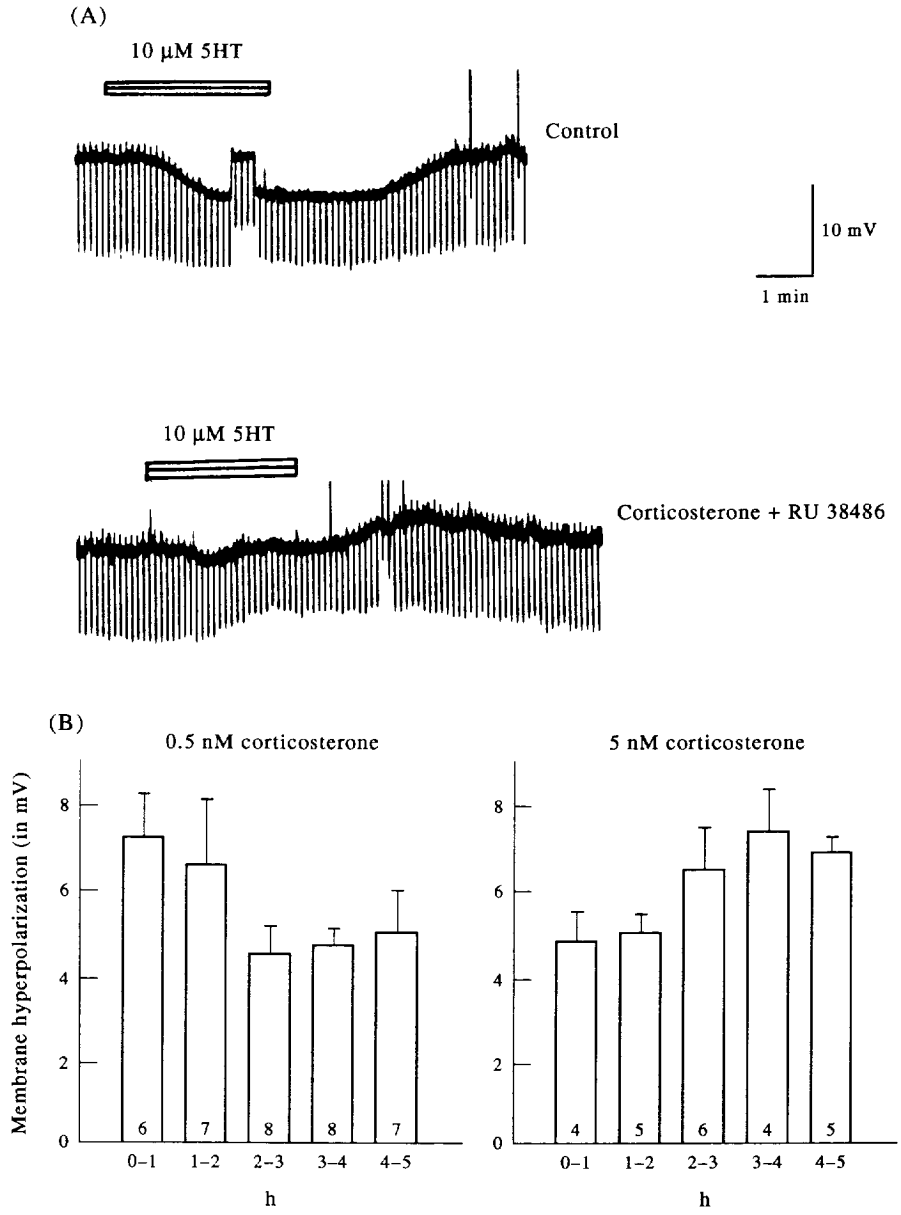

Fig. 3. (A) Typical response of CA1 pyramidal cells to perfusion with 10 μM 5HT (upper). After treatment of the tissue with corticosterone/RU 38486, thus selectively occupying MRs, only a small response to 5HT is observed. (B) Response to 10 μM serotonin obtained at various moments after continuous superfusion with 0.5 nM (left) and 5 nM (right) corticosterone was started. The figure shows that depression of 5HT responses by low (but not high) doses of corticosterone develops with a delay of 2–3 h, a delay similar to the normalization of the 5HT responses with high corticosterone levels. Bars represent mean ± SEM for each group of cells (number of cells indicated below).

to neurotransmitters [19, 26]. Of the latter, the responses to biogenic amines appeared to be markedly controlled by the degree of MR and GR occupation. Electrophysiological methods allow study of the endpoint of the signal transduction pathway for transmitters. While responses of the biogenic amines reviewed here, i.e. acetylcholine, noradrenaline and serotonin, all depended on the relative MR and GR activation, the nature of this dependency was not always the same. Thus, responsiveness to noradrenaline was inversely related to the plasma corticosterone concentration: noradrenaline responses were strongest in the absence of corticosterone (MRs and GRs unoccupied), moderate for corticosteroid levels occupying mostly MRs and strongly reduced when GRs were activated (regardless of prior MR activation). A similar inverse relationship was found with respect to expression of $5HT_{1a}$ receptor mRNA. However, the electrical responses mediated by $5HT_{1a}$ receptors displayed a different steroid dependency. Here, reduced 5HT responses were observed under conditions of predominant MR occupation, while both in the absence of corticosterone or with corticosteroid levels occupying MRs and GRs simultaneously 5HT responses were large. A similar U-shaped dose–response relationship was observed for electrical responses mediated by muscarinic ACh receptors. The differences in dose–response relationship may point to a different mechanism of action underlying the steroid modulation of neurotransmitter responsiveness.

At this moment, little is known about the molecular mechanism of action underlying the steroid actions in the brain. Although steroid effects on gene expression have been described for several proteins [71, 72], and steroid modulation of signal transduction—when investigated—was found to depend on *de novo* protein synthesis [25, 70], functional actions of corticosterone have not yet been directly linked to molecular processes in the same cell. Much of what we presently know about the molecular mechanism of action for corticosteroids is based on studies in transfected cell systems. It will be one of the challenges for future research to translate these molecular test systems to physiologically more relevant models, i.e. the cells for which functional steroid responses have been described.

The dependency of neurotransmitter responses on corticosteroid receptor occupancy implies that during the day and after stress the efficacy of signal transduction varies. Under physiological conditions, MR/GR occupation will range from a situation of predominant MR occupation (in the morning, under rest) to a situation where GRs are largely occupied in addition to MRs (at the circadian peak and particularly after stress, refs. [1, 19]). Accordingly, the responses to ACh and 5HT range from small (with mainly MRs occupied) to large (both receptors activated); the reverse is observed for noradrenaline. Since synaptic inputs mediated by these neurotransmitters add to the membrane potential of the receiving neuron, the excitability of the neuron is expected to be influenced by corticosteroid actions. An important aspect of the corticosteroid actions on local excitability is the fact that, due to the genomic mechanism, steroid modulation will be slow in onset and delayed; this is clearly different from the fast and short-lasting effects of neurotransmitters. A second aspect that underlines the unique role of corticosteroids in signal transduction is the pleitropic character of the hormonal actions: Both the intrinsic neuronal properties and many aspects of neurotransmitter systems are regulated by the corticosteroids [26].

Since corticosteroid hormones exert a long-term control over excitability under physiological conditions, we can expect that chronic over- or under-activation of the brain corticosteroid receptors also has profound implications for excitability. These aberrant activation levels of the steroid receptors may occur as a result of chronic hypo- or hypercorticism, in some cases associated with steroid resistance, as can be seen during several disorders and diseases [20–22]. The data so far indicate that in the absence of corticosterone (in ADX animals) excitability often displays features which resemble the situation observed when MRs and GRs are fully occupied. The effects of prolonged (over)exposure to corticosteroids on signal transduction have not been extensively studied. Whether or not chronic exposure to high levels of corticosterone induces similar effects as chronic submission to stress remains to be resolved; however, biochemical studies already indicate that these two conditions, which are both associated with chronic hypercorticism, do not necessarily result in comparable actions on signal transduction [66, 67]. Establishing how chronic aberrations in brain corticosteroid receptor activation affect local excitability may help to understand how corticosteroids can affect hippocampal function in cognition and mood, during (affective) disorders.

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