Modulation of serotonin responses in the rat hippocampus by corticosteroids: Mechanism and functional implications.

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Chapter 3

Serotonin and carbachol induced suppression of synaptic responses in rat CA1 hippocampal area: Effects of corticosteroid receptor activation \textit{in vivo}

Y.J.G. Karten, W. Hesen and M. Joëls

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ABSTRACT

Previous studies have shown that corticosteroids affect the changes in membrane potential evoked in CA1 hippocampal neurons by serotonin and the metabolically stable cholinergic analogue carbachol: Low corticosteroid levels induced by steroid administration to adrenalectomized rats or obtained in adrenally intact rats were associated with small transmitter responses. High corticosteroid levels induced by exogenous corticosteroid application or by an acute stress in adrenally intact rats generally evoked large transmitter responses. In the present study we investigated the consequences of this steroid modulation for the main stream of synaptic information in the CA1 hippocampal region, which is carried by amino acids. To this purpose the effect of serotonin and carbachol administration on both extracellularly and intracellularly recorded synaptic responses to Schaffer collateral stimulation were investigated. The data show that the effect of in vivo activation of corticosteroid receptors on the serotonin-induced hyperpolarization of the membrane responses is clearly reflected in the inhibitory effect of serotonin on synaptic responsiveness in the CA1 area. Low circulating levels of corticosterone or selective mineralocorticoid receptor activation reduced the serotonin mediated inhibition of synthetically evoked responses, whereas high corticosterone levels were associated with strong serotonin mediated suppression of synaptic responses. This steroid modulation seems to be specifically aimed at serotonin neurotransmission, as the cholinergic effects on excitatory synaptic transmission were not affected by the hormone treatment.

INTRODUCTION

Corticosteroid hormones are synthesized in the adrenal cortex and secreted into the blood in a circadian rhythm. After stress, corticosteroid levels are particularly high. Due to their lipophylic character, these hormones pass the blood-brain barrier (McEwen et al., 1986; De Kloet, 1991). In the brain, corticosteroids can induce rapid effects, probably through membrane-bound receptors (Hua and Chen, 1989; Orchinik et al., 1991). However, in general, corticosteroids evoke slow and long-lasting effects through activation of intracellular receptors that act via the genome (Beato et al., 1989). Two intracellular corticosteroid-receptor subtypes have been recognized: the mineralocorticoid receptor (MR; Kd,cort ~ 0.5 nM) and the glucocorticoid receptor (GR; Kd,cort ~ 5 nM) (Reul and de Kloet, 1985). Co-localization of MRs and GRs at the cellular level was recently demonstrated in hippocampal neurons using immunofluorescent confocal microscopy (van Steensel et al., 1996). The tenfold difference of affinity for corticosterone between these two receptor types will have important consequences for the relative occupation of MR and GR: at low to moderate levels of corticosterone, prior to the inactive period, predominantly MRs are activated. MRs and GRs are simultaneously activated when high levels of corticosterone
levels are circulating i.e. at the circadian peak (prior to the active period) or during stress (De Kloet and Reul, 1987; Reul et al., 1987a; Reul et al., 1987b; Spencer et al., 1990).

Experimental data indicate that differential activation of corticosteroid receptors influences the excitability of hippocampal neurons by modulation of intrinsic properties or synaptic connectivity (for review see: Joëls and de Kloet, 1994). Previously, the sensitivity of CA1 pyramidal neurons to neurotransmitters such as serotonin (5-HT) and the cholinergic analogue carbachol (CCh) was shown to be affected by differential activation of MRs and GRs: the 5-HT$_{1A}$ mediated hyperpolarization and the CCh-induced depolarization were small with predominant activation of MRs compared to conditions when both MRs and GRs were activated (Joëls et al., 1991; Hesen and Joëls, 1993; Beck et al., 1996). Because the postsynaptic responses of CA1 neurons to 5-HT and CCh are thus clearly modulated by corticosteroids we here investigated the putative consequences of these effects for the main inputs to the CA1 area, via the Schaffer collaterals and local interneuronal networks.

CA1 pyramidal cells receive a strong excitatory amino acid mediated input via the Schaffer collaterals (for review see Lopes da Silva et al., 1990). Electrical stimulation of these afferents moreover results in feedforward and feedback inhibition mediated by GABA (Lopes da Silva et al., 1990; Buhl et al., 1995). Earlier reports revealed that the excitatory glutamatergic transmission in the CA1 area is reduced by both 5-HT and cholinergic analogues (Segal, 1980; Valentino and Dingledine, 1981; Segal, 1982; Beck and Goldfarb, 1985; Segal, 1988).

In the present study, we investigated the functional significance of MR and GR mediated actions on 5-HT and cholinergic responses, for the synaptic efficacy in the CA1 area. For a correct interpretation it was necessary to first establish the effect of MR and GR activation per se on synaptic responses in the CA1 area. Synaptic responses were studied with extracellular field recording and conventional intracellular recording techniques. The experiments were designed to mimic the circadian or stress-related variations in MR and GR occupation balance. First, effects of 5-HT and CCh were recorded in slices from adrenalectomized rats which received different amounts of corticosterone in vivo. The objective of these experiments was to show that in vivo administration of corticosterone to ADX animals affected neurotransmitter responses recorded subsequently in vitro. Next, neurotransmitter effects were recorded in slices from adrenally intact rats in which plasma corticosterone levels were temporarily elevated by either applying stress or by exogenous administration of corticosterone.
METHODS

Surgery and drugs
Apart from the recording techniques, the experimental conditions were similar as described in previous studies (Joëls et al., 1991; Joëls and de Kloet, 1993). In short, male Wistar rats (120-220g; Harlan CPB, The Netherlands) were housed under standard conditions with alternating 12h light/dark cycles (lights on at 8:00h). Part of the animals were bilaterally adrenalectomized (ADX) under ether anesthesia, two to seven days before the experiment. Food and tap water (0.9% NaCl for ADX-rats) were provided ad libitum.

Animals were subjected to different treatments at the day of the experiment, as shown in Figure 1. ADX rats received subcutaneous injections with corticosterone (1, 10, 30, 100 or 1000 µg/100g body weight) or vehicle, 60 to 90 min before decapitation. Corticosterone (cort; Organon Int., Oss, The Netherlands) was dissolved in ethanol and subsequently diluted in peanut oil. From each animal, trunk blood was collected for determination of plasma corticosterone levels by means of a radio-immunoo assay. Untreated ADX rats were considered to be properly adrenalectomized when the plasma corticosterone level was less than 1 µg/dl plasma. For data analysis three experimental groups were considered: no or very low cort (0-1 µg corticosterone / 100g body weight), moderate cort (10-30 µg/100g) and high cort (100-1000 µg/100g). The plasma corticosterone levels of these animals were 0.5 ± 0.3, 4.6 ± 2.6 and 19.7 ± 3.5 µg corticosterone / dl plasma respectively.

Adrenally intact rats received either corticosterone (1mg / 100g body weight) or were subjected to an ether stress (30 sec) 60-90 min before decapitation, to increase the plasma corticosterone level. While on average high plasma corticosterone levels were observed in both groups (33.8 ± 16.4 and 30.3 ± 4.7 µg corticosterone / dl plasma respectively), one corticosterone-injected animal showed an extremely low plasma corticosterone value (0.4 µg/dl). We concluded that this animal did not receive the intended corticosterone dose and analyzed the data from this animal separately. Part of the stressed animals were pretreated with the GR-antagonist RU 38486 (1 mg / 100g body weight, dissolved in acidified saline; Roussel Uclaf, Romainville, France), 90 min before exposure to the ether stress (see Fig.1). Plasma corticosterone levels in this group amounted to 11.5 ± 2.4 µg/dl. The control group consisted of adrenally intact rats that were not subjected to any treatment or received a subcutaneous vehicle injection at 1 hour before decapitation. Considering the circadian rhythmicity of adrenal corticosterone release (for review: McEwen et al., 1986) and the time at which these control animals were sacrificed, we expected to see moderately low plasma corticosterone levels (~5 µg/dl) in the control group. While most (n=11) animals indeed showed moderate corticosterone levels (8.6 ± 2.1 µg/dl; significantly lower value than obtained in the corticosterone injected or acutely stressed groups), three animals exhibited elevated levels (>15 µg/dl). In retrospect, we conclude that these animals had probably been stressed (by reasons unknown to the experimenter)
just before they were carried from the animal house. The data of these animals were analyzed separately from the remainder of the control group.

Dorsal hippocampus slices (300 μm) were prepared with an McIlwain tissue chopper and stored at room temperature in carbogenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid (ACSF) that consisted of (mM) NaCl (124), KCl (3.5), NaH₂PO₄ (1.25), MgSO₄ (1.5), NaHCO₃ (25), CaCl₂ (2) and glucose (10). After an equilibration period of at least 30 min, one slice at a time was transferred to the recording chamber and continuously perfused with warm (34°C), carbogenated ACSF (pH = 7.4). The slice was fixed between two nylon meshes and kept fully submerged. Serotonin (5-HT; 5-hydroxytryptamine creatine sulphate; Sigma, USA) and carbachol (CCh; carbamylcholine-chloride; Sigma, USA) were applied to the bath, by switching the bath perfusion medium to ACSF containing the transmitter. After approximately 1.5 minutes, the transmitter containing medium reached the slice (bath volume= 0.75 ml). Both transmitter ligands were diluted in ACSF prior to bath perfusion from stock solutions (1 mM) which were kept at -20 °C. For the tests with 5-HT we selected a concentration of 10 μM, since previous experiments (Joëls et al., 1991) showed that near maximal 5-HT responses were obtained with this concentration (both in extra- and intracellular recordings), allowing investigation of steroid modulation. For the same reason CCh was tested at a 3 μM concentration (Hesen and Joëls, 1993; Hesen and Joëls, 1996a).

Stimulation and recording

A bipolar stimulation electrode (60 μm diameter) was placed in the stratum radiatum to activate fibers of the Schaffer collateral pathway through constant current pulses (150 μs; Neurolog NL-80 and Axon Instruments SI-10 stimulus isolators) with an intensity up to 300 μA. Extracellular field potentials were recorded with glass, ACSF-filled micropipettes (1-8 MΩ) placed in the CA1 stratum pyramidale. Extracellularly, stimulation of the Schaffer collaterals results in an excitatory postsynaptic potential recorded in stratum pyramidale, with a superimposed population spike (PS) (see Fig. 2A2). The amplitude of the population spike reflects the number of pyramidal cells responding to synaptic activation with an action potential. In our study, only those experiments were included where the evoked field potential amplitude was constant for at least 15 min before data acquisition was started. Maximal population spike amplitudes (3-10 mV) were evoked with a stimulus intensity of 300 μA. In all experiments, data were obtained with at least two stimulation intensities i.e. stimulus intensities that evoked half maximal and maximal population spike amplitudes respectively.

Simultaneously, intracellular responses to synaptic stimulation, before and during 5-HT or CCh application, were recorded. Intracellular recordings were obtained from CA1 pyramidal neurons with conventional methods using glass microelectrodes filled with 4M KAc (set to pH 7.4 with HCl) yielding impedances of 80-130 MΩ. Voltage signals were passed to an NPI sec1L/H amplifier, displayed on a Gould digital oscilloscope and fed to
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an Atari's computer for later data analysis. The membrane potential and current injections were continuously registered on a chart recorder. Depolarizing and hyperpolarizing currents were passed through the microelectrode to determine the input resistance, afterhyperpolarization and accommodation properties. Neurons included in this study displayed stable resting membrane potentials between -60 and -73 mV, membrane resistances of at least 30 MΩ and spike amplitudes of at least 80 mV. In intracellular recording experiments, stimulation of the Schaffer collaterals evoked a fast EPSP, followed by a biphasic IPSP sequence which consisted of a fast IPSP and a more slowly developing IPSP (slow IPSP) (Lopes da Silva et al., 1990; Buhl et al., 1995); see Fig. 2A1).

**Figure 1** A. Adrenalectomized (ADX) animals were subcutaneously injected with different doses of corticosterone (1, 10, 30, 100 or 1000 µg/100g body weight) or vehicle. Neurotransmitter responses were recorded at least 1 hour after slice preparation (sp). B. In the second series, experimental groups (B1-B4) comprised adrenally intact rats. (B1) One group of rats was sacrificed early in the morning (9:30h). Some animals received subcutaneously a vehicle injection at least 1 hour before sacrifice. (B2) The second group comprised rats that received a subcutaneous injection with a high dose of corticosterone (1000 µg/100 g body weight). (B3) The third group was stressed by exposure to ether for approximately 30s. Because of the small amount of ether and the short incubation period, no signs of anesthesia were observed. After this, animals were placed in another cage and sacrificed one hour afterwards. (B4) The last group of rats received a subcutaneous injection with the GR-antagonist RU38486 (1mg/100g body weight) before they were subjected to ether stress.

**Experimental protocol**

At the start of the experiment (t=0 min), the stimulation intensities that yielded half maximal and maximal population spikes were determined from input-output curves that comprised 9 stimulus intensities delivered with an interval of 10 s. Also, for intracellular recordings, a stimulus intensity was selected at t=0 min. which was just subthreshold for generation of an action potential, yielding the maximal EPSP amplitude that could be reliably analyzed; extracellular signals recorded in the same slices generally exhibited half
maximal responses with this stimulus intensity. At t= 15 min. a second control series of synaptic responses to subthreshold, half maximal and maximal stimuli was obtained. At t= 20 min, perfusion with 5-HT or CCh was started. After approximately 7 minutes (t= 27 min.) of perfusion, synaptic responses during drug perfusion were determined. The stimulus protocol was subsequently repeated while injecting DC through the intracellular electrode, to bring the resting membrane potential back to pre-drug perfusion potential. Subsequently, the ACSF containing the drug was switched back to control ACSF. After 20 min of washout (t= 57 min) the stimulus protocol was started to record control synaptic responses after drug application. The stimulation protocols, data acquisition and analysis of the extracellularly and intracellularly recorded evoked responses were performed with an Atari computer with in-house developed software.

All data is presented as mean ± standard error of the mean (S.E.M.). Statistic analysis was performed by a one-way analysis of variance (ANOVA). If statistical significance (P < 0.05) was attained, an unpaired Student's t-test was applied to determine the level of significance between the experimental groups.

RESULTS

Corticosteroid treatment and synaptic responses
In the first series of experiments, synaptic potentials were recorded extracellularly and intracellularly in the CA1 area of dorsal hippocampal slices obtained from adrenalectomized rats that received a subcutaneous injection of corticosterone (1-1000 µg/100 g body weight), 60 to 90 minutes prior to sacrifice. For data analysis three experimental groups were considered: (no or) very low cort (0-1 µg corticosterone/100 g body weight), moderate cort (10-30 µg/100 g) and high cort (100-1000 µg/100 g).

Intracellular recordings were made from CA1 pyramidal neurons in hippocampal slices from rats belonging to either the very low, moderate or high corticosterone treated group. Figure 2A shows a typical example of the intracellularly recorded synaptic responses evoked by stimulation of the Schaffer collaterals in a slice from an untreated ADX rat. Schaffer collateral stimulation typically evokes an EPSP which is followed by a short lasting fast IPSP and a delayed but persistent slow IPSP. Averaged amplitudes of the EPSP, fast IPSP and slow IPSP for the very low, moderate and high corticosterone treated groups are shown in Figure 2B. From this graph it is clear that the concentration of injected corticosterone is negatively correlated to the amplitude of the EPSP: In slices obtained from rats that received vehicle or very low amounts of corticosterone averaged EPSP amplitudes were significantly larger when compared to responses determined in slices from rats that received high (P<0.01) doses of corticosterone. In contrast, the averaged amplitudes of the fast IPSP and the slow IPSP were not significantly different between the very low, moderate and high corticosterone treated groups.
Table I Averaged values for the resting membrane potential (RMP) and input resistance (Rin).

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The number of rats and the number of recorded cells per group where RMP and Rin could be reliably determined, are shown. Data are in part based on groups of cells tested for 5-HT and CCh responsiveness, by Hesen and Joels (1996a and 1996b). Data are represented as mean ± Standard Error of the Mean (S.E.M.). From a limited number of cells no reliable value for the RMP and/or the Rin could be established. These cells are not included in this table.

The procedure by which the EPSP amplitude was determined, i.e. by using the stimulus intensity that is just subthreshold for the induction of an action potential, critically depends on the resting membrane potential, input resistance and firing threshold of the cell. However, the observed difference in EPSP amplitude between the three experimental groups can not be explained by a steroid dependent effect on resting membrane potential or input resistance, since these parameters were not significantly affected by different doses of corticosterone (Table IA). The values in Table IA are similar to those described previously for CA1 pyramidal neurons (Segal, 1982; Dutar and Nicoll, 1988; Kør et al., 1989; Hesen and Joels, 1993; Joëls and Fernhout, 1993). Similarly, the firing threshold was not significantly different between the very low, moderate and high cort groups (-52 ± 2, -54 ± 1 and -54 ± 1 mV respectively). Accordingly, the depolarization required to evoke an action potential from resting membrane potential was also comparable for the three groups (11 ± 1, 10 ± 1 and 10 ± 1 mV for the very low, moderate and high cort treatment respectively). Interestingly, the percentage of neurons in which no action potential could be evoked with (maximal) synaptic activation -though still with current injection- was highest in the high cort group (28%), compared to the moderate (14%) or very low cort (20%) groups.
Figure 2 A. Typical examples of a subthreshold synaptic response of a CA1 pyramidal neuron (1) and a maximal population spike recorded in stratum pyramidale (2), evoked through stimulation of the Schaffer collateral pathway in a slice from an untreated ADX-animal. The amplitude of the population spike is measured as indicated (PSA). The amplitudes of the intracellular EPSP and fast and slow IPSP are measured at the points indicated by the arrows. B. The graph shows the averaged (Mean ± S.E.M) amplitudes of the intracellularly recorded EPSP, fIPSP and sIPSP and of the extracellularly recorded population spike (PS). For the population spike amplitude, data were averaged per rat in case recordings were made from more than one slice. The number of rats were 7, 8 and 9 for the very low, moderate and high corticosterone treated groups respectively. The number of cells for the very low, moderate and high corticosterone treated groups were for the EPSP: 17,17,15, for fIPSP: 17,17,19 and for sIPSP 20,19,23 respectively. Statistical analysis was as described in the Methods section; **: P<0.01.

The likely consequence of corticosterone mediated suppression of EPSP amplitudes is a reduced probability to evoke an action potential, provided the firing threshold is not affected by the corticosterone treatment. If so, this might be reflected in the synaptic field responses recorded from the pyramidal layer of the CA1 area. A typical example of a field response, evoked by stimulation of the Schaffer collaterals, is shown in Figure 2A. Although a similar trend towards a dose-dependent reduction of the averaged population spike amplitude by corticosterone was apparent, no statistically significant differences were observed between the three experimental groups (Fig. 2B).
In conclusion, corticosterone administered to ADX rats has an inhibitory effect on the maximal amplitude of the synaptically evoked EPSP, which may influence the effects of neurotransmitter (such as 5-HT and acetylcholine) mediated actions within the CA1 area, as will be discussed later. To minimize this influence, transmitter induced effects on synaptic responses will be expressed relative to the baseline synaptic responses.

![Figure 3](image.png)

*Figure 3* Averaged synaptic responses from adrenally intact rats with variable MR and GR activation. The graph shows the averaged (Mean ± S.E.M) amplitudes of the extracellularly recorded population spike and the intracellularly recorded EPSP, fIPSP and sIPSP. For the population spike amplitude, data were averaged per rat in case recordings were made from more than one slice. The number of observations were 10, 6, 10 and 7 for the control, high corticosterone treated, stressed and the stress+RU 38486 treated groups respectively. For the intracellular data, the number of cells for the four experimental groups were: 17, 10, 15, 12 respectively for the EPSP, for fIPSP: 13, 11, 14, 8 and for sIPSP 19, 12, 15, 14. Statistical analysis was as described in the Methods section; *: P<0.05.

In the second series of experiments we examined the effect of manipulation of steroid levels in adrenally intact rats. Therefore intracellular synaptic responses were recorded from CA1 pyramidal neurons of adrenally intact rats belonging to one of the following four experimental groups: As a control we used rats that were sacrificed early in the morning; some of these rats received a subcutaneous vehicle injection at least 1 hour before decapitation. While most (n=11) of the control animals exhibited moderately low plasma corticosterone levels resulting in a predominant MR occupation (Reul et al., 1987a) as expected under the present conditions, some (n=3) animals were found to have clearly elevated plasma corticosterone levels (>15 µg/dl). These animals were excluded from the control group and the data from these rats will be presented separately in the text. The second group consisted of rats which received a subcutaneous injection with a high dose of corticosterone (1 mg/100g body weight), resulting in elevated plasma corticosterone levels. One animal, however, showed a very low circulating corticosterone level (0.4 µg/dl); we excluded this animal, where the corticosterone injection apparently had failed.
to induce a rise in plasma corticosterone level, from the group and will report the data separately in the text. Animals in the third group were stressed by exposure to ether one hour before decapitation, which resulted in an increased plasma corticosterone level. The corticosterone levels in the stressed and in the corticosterone-injected animals were such that not only MRs but also most of the GRs will be occupied (Reul et al., 1987b). The last group consisted of rats which received a subcutaneous injection with the GR-antagonist RU 38486 (1mg/100g body weight) before they were subjected to the ether stress.

Intracellularly recorded EPSPs and fast IPSPs were not different between the four experimental groups (Fig. 3A). The averaged amplitude of the slow IPSP was significantly larger in the group of animals subjected to ether stress compared to the averaged slow IPSP from the control group (P<0.05). Resting membrane potential and input resistance were not significantly different between the four experimental groups (Table 1B). In agreement, averaged population spike amplitudes were similar for all four experimental groups. Apparently, variation of corticosteroid levels in adrenally intact rats does not largely affect the amino acid mediated synaptic transmission in the CA1 area. This was also found in the four animals that were excluded from the experimental groups, i.e. the three control animals which exhibited high plasma corticosterone levels (>15 \(\mu g/dl\)) and in the one corticosterone injected animal which had a very low plasma corticosterone level.

Modulation of synaptic responses by 5-HT and CCh; influence of steroids in ADX animals

As observed earlier the mean 5-HT mediated hyperpolarization was significantly suppressed in slices from rats that received moderate doses of corticosterone (10-30 \(\mu g/100g\)) when compared to rats that received relatively high doses of corticosterone (100-1000 \(\mu g/100g\); Fig. 4A). On average, the response of the former group amounted to only 70% of the response observed in the latter. When no or very low amounts of corticosterone (0-1 \(\mu g/100g\) body weight) were administered, responses were not different from responses determined in slices from rats that received high doses of corticosterone.

The 5-HTA1 receptor evoked hyperpolarization (Andrade and Nicoll, 1987) will shift neurons away from the firing threshold, so that the probability to evoke an action potential with synaptic stimulation will be reduced. Accordingly, in almost all experiments, bath application of 10 \(\mu M\) 5-HT induced a gradual and reversible suppression of the population spike amplitude (Fig.4B). Yet, additional effects of 5-HT on synaptic responses, including presynaptic actions, may also contribute to the observed 5-HT induced reduction of synaptic responses. This was further investigated by studying 5-HT effects on intracellularly recorded EPSPs and IPSPs. If 5-HT only reduces synaptically evoked field responses because it causes a membrane hyperpolarization, one would expect to see no reduction by 5-HT of the EPSP amplitude, since the driving force for the
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cations contributing to the EPSP increases rather than decreases by a hyperpolarization; for the same reason, a marked decrease of the fast IPSP amplitude and a small decrease of the slow IPSP is expected. If 5-HT affects excitability through additional, possibly presynaptic mechanisms, a considerable decrease of the EPSP and IPSP amplitudes may be found. As shown in Figure 4C, the EPSP amplitude was very little affected by 5-HT, while both the fast and slow IPSP amplitude were largely suppressed. These data suggest that 5-HT reduces CA1 excitability via a postsynaptic 5-HT1A receptor mediated hyperpolarization, while it may exert additional (presynaptic) effects mostly on GABA receptor mediated transmission.

\[ \text{Figure 4} \] Effects of 5-HT on membrane hyperpolarization but not on intracellularly recorded synaptic responses depend on circulating corticosteroid levels. Field potentials also show steroid dependency, but the differences are not statistically significant. A. Averaged (Mean ± S.E.M) 5-HT (10 μM) induced membrane hyperpolarization in tissue from ADX rats treated with corticosterone. B. Averaged 5-HT (10 μM) induced decrease of the maximal population spike amplitude. (Based on: 8,7,9 (A) and 7,7,9 (B) animals). C. Bar graphs showing the effect of corticosteroids on 5-HT mediated reduction of intracellularly recorded EPSP, fast IPSP and slow IPSP in slices from adrenalectomized animals. Synaptic responses were recorded after 7 min drug perfusion at resting membrane potential. The 5-HT mediated effects on amplitudes of the synaptic responses are expressed as a percentage of the control value. (Based on 9,8,8 (EPSP) 11,10,14 (fIPSP) and 12,11,18 (sIPSP) cells). Statistical analysis was as described in the Methods section; **: P<0.01.
Figure 5 A. Averaged CCh (3 μM) induced membrane depolarization in tissue from ADX rats treated with corticosterone, showing clear steroid dependency. B. No such dependency was observed for averaged CCh (3 μM) induced decrease of the maximal population spike amplitude. For graphs A and B, data were averaged per rat in case recordings were made from more than one slice. (Based on 9,8,9 (A) and 7,7,8 (B) animals). C. No effect of corticosteroids was seen on CCh mediated reduction of intracellularly recorded EPSP, fast IPSP and slow IPSP in slices from adrenalectomized animals. The CCh induced effects on amplitudes of the synaptic responses were expressed as a percentage of the control value. (Based on 5,11,8 (EPSP), 8,10,11 (fIPSP) and 8,14,13 (sIPSP) cells). Statistical analysis was as described in the Methods section; * : P<0.05, ** : P<0.01.

Since the intracellularly obtained data suggest that the reduction of the field potential is probably mainly caused by the 5-HT$_{1A}$ receptor mediated hyperpolarization, a similar steroid dependency of the two phenomena can be expected. Indeed, on average a similar corticosterone dose dependency was observed for the 5-HT induced suppression of the population spike amplitude as found for the 5-HT mediated hyperpolarization, recorded in the same slices (Fig. 4B): The 5-HT induced inhibition of the population spike amplitude in slices from animals treated with moderate corticosterone doses amounted to only 73% of the effect observed in animals treated with a high amount of corticosterone. However, for the results with population spikes statistical significance was not reached; this may have been partly caused by the larger degree of variability in the field potential recordings.

As argued above the reduction by 5-HT of the EPSP amplitude, but particularly of the IPSPs is probably not linked to the 5-HT$_{1A}$ receptor mediated hyperpolarization. It
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appeared that neither the EPSP nor the IPSPs amplitude was sensitive to steroid treatment. Similarly, when the membrane potential during 5-HT was restored to the pretreatment value by injecting DC, thus allowing to study 5-HT effects on synaptic transmission in the absence of a membrane hyperpolarization, no effect of steroid treatment was observed (data not shown).

A similar study was performed to investigate the effect of CCh on synaptic responses. Most of the CA1 pyramidal neurons tested in this study were depolarized by 3 µM CCh. As is shown in Figure 5A, the CCh-induced depolarization was similarly affected by corticosterone treatment as the 5-HT-induced hyperpolarization (part of these data have been published before: (Hees and Joëls, 1996a)). The averaged membrane depolarization, recorded after 7 minutes of CCh application, was significantly larger when recorded in slices from rats that received a very high dose of corticosterone, compared to rats treated with moderate amounts of corticosterone. On average, the responses of the group treated with a moderate corticosterone dose amounted to only 5% of the responses in the high corticosterone treated group.

Depolarization of the membrane brings cells closer to the firing threshold. The CCh induced depolarization would therefore result in an enhanced amplitude of the population spike. As shown in Figure 5B, however, CCh markedly suppressed the population spike amplitude. This indicates that additional, possibly presynaptic CCh actions affect the CA1 excitability. This was also supported by intracellular recordings: While only marginal effects of the CCh-evoked depolarization are expected to be seen on the EPSP and IPSP amplitudes, CCh in fact reduced the EPSP and IPSPs considerably (Fig.5C).

Since the CCh action on the field potential appears to depend only marginally on the CCh evoked depolarization, the steroid dependence of the latter may not be reflected in the CCh effects on field potentials. Indeed, the CCh mediated suppression of the population spike amplitude was not significantly modulated by corticosteroids (Fig.5B). In contrast to what was seen for the CCh evoked depolarization, tissue from animals treated with a moderate corticosterone dose exhibited on average larger (110%) rather than smaller CCh mediated suppressions of the field potentials than the group treated with a high amount of corticosterone. Intracellular recording studies revealed a similar pattern for the EPSP, both recorded at the resting membrane potential during CCh treatment (Fig. 5C) and when the membrane potential was restored to the pretreatment level by DC injection (data not shown). The reduction of synaptic responses with very high corticosterone doses was even significant when studying the fast and slow IPSP amplitudes (fig. 5C). This was also seen when the membrane potential during CCh application was restored to the pretreatment level by DC injection (level of significance: p<0.05; data not shown). The latter observation and the fact that the GABA_A receptor mediated fast IPSP and the GABA_B receptor mediated IPSP were affected to a similar extent suggest that the observed steroid modulation may be aimed at a presynaptic effect of CCh.
Chapter 3

Figure 6 A. Averaged 5-HT (10 μM) induced membrane hyperpolarization in tissue from adrenally intact rats. Responses are increased in stressed animals compared to either controls or stressed rats pretreated with RU38486. (Based on 11,9,9,8 cells). B. The averaged (Mean ± S.E.M) 5-HT (10 μM) induced decrease of the maximal population spike amplitude shows a similar steroid dependency. For graph B, data was averaged per rat in case recordings were made from more than one slice. (Based on 6,6,6,7 animals). C. Averaged (Mean ± S.E.M) 5-HT (10 μM) induced suppression of EPSP, fIPSP and sIPSP amplitudes was not affected by the various MR/GR activation. (Based on 11,5,8,9 (EPSP), 5,5,6,7 (fIPSP) and 12,6,7,11 (sIPSP) cells). Statistical analysis was as described in the Methods section; * : P<0.05, ** : P<0.01.

Modulation of synaptic responses by 5-HT and CCh; influence of steroids in intact animals

In the next series of experiments, we manipulated corticosterone levels in adrenally intact rats by applying stress or by injecting corticosterone and evaluated effects of this manipulation on 5-HT- and CCh-mediated actions. EPSP and IPSP amplitudes in the presence of the neurotransmitter are expressed as a percentage of the EPSP and IPSP amplitudes prior to drug perfusion.

The corticosterone dose-dependency of 5-HT induced hyperpolarizations in neurons from intact rats is shown in Figure 6A (part of these data have been published before: Hesen and Joëls, 1996b). On average, 5-HT responses were relatively large in slices from rats in which corticosterone levels were elevated by acute stress or rats that received an injection with a high dose of corticosterone (Fig. 6A). Administration of RU 38486 before subjecting animals to the stress event significantly reduced the large 5-HT
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responses recorded after stress (Fig. 6A). For all experimental groups basal membrane properties were not significantly affected (Table 1B). Interestingly, one animal that was excluded from the control group since it exhibited an elevated plasma corticosterone level, showed relatively large 5-HT responses (7.3mV, based on 2 cells); conversely, a corticosterone injected animal which was excluded because it showed a very low plasma corticosterone level, showed rather small 5-HT responses (5.8mV, 2 cells). The findings in these animals are in line with the U-shaped dose-dependency of 5-HT mediated hyperpolarizations on plasma corticosterone level (Hesen and Joëls, 1996b).

The corticosterone dependency of the 5-HT induced suppression of the population spike amplitude (Fig. 6B) appeared similar as the corticosterone dose dependency of the 5-HT mediated hyperpolarization (Fig. 6A): On average the 5-HT responses were relatively large in slices from rats in which corticosterone levels were clearly elevated by acute stress or rats that received an injection with a high dose of corticosterone (Fig. 6B). Administration of RU 38486 before subjecting animals to the stress event significantly reduced the large 5-HT responses recorded after stress (Fig. 6B). In agreement with this, the 5-HT-induced decrease of the population spike amplitude in the one corticosterone-injected animal which failed to show an elevated plasma corticosterone level was extremely low (4.3%).

The 5-HT induced reduction of the intracellularly determined EPSP amplitude showed no significant differences (Fig. 6C). Similarly, when the membrane potential during 5-HT application was brought to the pretreatment level by DC injection, no significant effects of steroid treatment were observed on the averaged amplitudes of the EPSP (data not shown). The 5-HT mediated inhibition of slow and fast IPSP amplitudes (with or without DC correction) was also not changed by the various experimental treatments (Fig. 6C and data not shown).

As shown in Figure 7A, administration of a high dose of corticosterone resulted in relatively large CCh induced depolarizations compared to the control rats; the difference was just not significant. In accordance, 2 control animals with rather high (>15 μg/dl) plasma corticosterone levels showed CCh responses (2.8mV, based on 4 cells) which were above the average of the control group; the responses obtained in a corticosterone-injected animal which showed a very low corticosterone level were however comparable to the mean of the entire corticosterone receiving group. Elevation of corticosterone levels by a stress-stimulus was associated with small rather than large CCh induced responses. The CCh responses observed after an acute stress were significantly reduced compared to the responses obtained after injection with a high corticosterone dose.

As shown in Figure 7B, CCh induced suppression of the population spike amplitude was not significantly modulated by corticosteroids. In accordance, intracellular recordings show that the CCh induced reduction of the EPSP amplitude was not changed by steroid treatment (Fig. 7C). Similarly, the CCh mediated inhibition of the fast IPSP was not affected. However, CCh mediated suppression of the slow IPSP amplitude was
significantly more reduced by pre-treatment of stressed animals with RU 38486 compared to non-treated stressed animals (Fig. 7C). Moreover, while injecting DC, stressed animals pre-treated with RU 38486 exhibited an increased CCh-evoked effect not only on the amplitude of the slow IPSP, but also of the fast IPSP. Mean (± S.E.M.) values of the control, high cort, stressed and stressed+RU 38486 groups are for the fast IPSP during DC injection: 49.8 ± 13.9, 63.0 ± 12.3, 51.4 ± 15.3, 126 ± 20.4 % respectively (n=8,4,8,5 cells) and for the slow IPSP during DC injection: 66.9 ± 14.3, 53.1 ± 8.6, 63.0 ± 11.7, 91.9 ± 7.9 % respectively (n=8,5,9,9).

Figure 7 A. Averaged CCh (3 μM) induced membrane depolarization hyperpolarization in tissue from adrenally intact rats. (Based on 14,12,10,10 cells) B. Averaged CCh (3 μM) induced decrease of the maximal population spike amplitude. Data was averaged per rat in case recordings were made from more than one slice. (Based on 8,4,8,5 animals) C. CCh mediated reduction of intracellularly recorded EPSP, fast IPSP and slow IPSP in slices from intact animals. CCh mediated effects on amplitudes of the synaptic responses are expressed as a percentage of the control value. (Based on 11,5,10,5 (EPSP), 9,4,7,4 (fIPSP) and 10,4,9,7 (sIPSP) cells). Statistical analysis was as described in the Methods section; * : P<0.05.
DISCUSSION

Corticosteroid treatment and synaptic responses

The aim of the present study was to investigate the functional consequences of MR and GR mediated actions on 5-HT and CCh responses, for the amino acid mediated synaptic transmission in the hippocampal CA1 area. For the interpretation of the results it was important to know if MR and/or GR activation in vivo, as was used in the present experimental design, already modulated the synaptic responsiveness in the CA1 region. Such a modulatory role was indeed suggested by previous studies where MR and GR activation was achieved by in vitro application of steroid receptor agonists to slices from ADX or adrenally intact animals (Reiheld et al., 1984; Vidal et al., 1986; Rey et al., 1987; Zeise et al., 1992; Joëls and Fernbout, 1993; Bimstiel and Beck, 1995). Thus, activation of MRs was associated with stable or even enhanced synaptic responses whereas activation of GRs resulted in decreased synaptically evoked potentials in the CA1 hippocampal area. These effects were relatively rapid in onset and reversible within one hour.

In the present study we found that corticosterone administration in vivo to ADX rats, 60 to 90 minutes prior to decapitation, also affects synaptic responses subsequently recorded in vitro: The EPSP (but not the IPSP) amplitude was dose-dependently reduced by corticosterone. This was also reflected in the averaged amplitudes of the maximal population spike amplitudes, recorded extracellularly in the same slice, although here no significant changes were observed. Given the fact that neither the resting membrane potential, the input resistance nor the firing threshold were significantly altered by the corticosterone treatment, we tentatively conclude that the corticosterone-dependent decrease of the EPSP is caused by a diminished availability or efficacy of synaptically released excitatory amino acids.

The presently observed effect of in vivo corticosterone administration in ADX animals differs from previous findings in several aspects. First, we did not observe marked reductions in the field potential or EPSP amplitude in untreated ADX rats, as was reported in one earlier study (Doi et al., 1991). However, it confirms other studies, where synaptic responses in tissue from ADX were found to be comparable to responses in adrenally intact animals, at least at the start of the experiment (Kerr et al., 1989; Joëls and de Kloet, 1993; Joëls and Fernbout, 1993; Bimstiel and Beck, 1995). Second, the presently observed effects of in vivo administered corticosterone were of a persistent nature, since they were seen at least 2-3 hours after injection of the hormone. By contrast, the previously observed effects of in vitro applied corticosterone were generally reversible within 1 hour (Reiheld et al., 1984; Vidal et al., 1986; Joëls and de Kloet, 1993). And finally, in vitro administration of corticosteroids resulted in an enhanced responsiveness with moderate amounts of the hormone, while high doses of corticosterone led to a depression of synaptic responsiveness. With in vivo administration only the reduction of synaptic responses with
high doses of the hormone was observed; no enhanced responses with moderate hormone levels were found.

The data therefore indicate that corticosterone applied \textit{in vivo} to ADX rats affects synaptic transmission in the CA1 area recorded subsequently \textit{in vitro}. Corticosterone evokes a dose-dependent reduction of the excitatory transmission. The dose-dependency and persistent nature of this effect differs from previously described actions of \textit{in vitro} applied corticosterone. No modulatory effects of \textit{in vivo} administered corticosterone were observed in adrenally intact rats, even though the plasma corticosterone levels of the control group were in the range of the ADX moderate cort group and the plasma corticosterone levels of the corticosterone-injected or stressed groups were even higher than those observed in the ADX high cort group. This may signify that the modulation of synaptic responsiveness observed after \textit{in vivo} administration of corticosterone to ADX rats to some extent depends on adaptive processes linked to the process of ADX. The functional significance of the findings in ADX rats should therefore be considered with caution. For the present study, however, it is important to realize that modulation of 5-HT or CCh effects on synaptic transmission by corticosteroids in ADX animals (though not in intact rats) is superimposed on an effect of the hormone itself. To minimize the consequences hereof we expressed all effects of 5-HT and CCh on synaptic responses as percentual changes. However, we can not exclude that the corticosteroid effects on the absolute range of the synaptic responses may have affected the ability of 5-HT or CCh to modulate synaptic responsiveness within this range.

\textit{Modulation of synaptic responses by 5-HT}

Application of 5-HT induces several actions on synaptic transmission in the hippocampal CA1 area. The first action concerns the activation of 5-HT$_{1A}$ receptors which will result in a membrane hyperpolarization, causing a suppression of the population spike (Beck and Goldfarb, 1985; Ropert, 1988; Dijcks \textit{et al.}, 1991; Schmitz \textit{et al.}, 1995b). The hyperpolarization will little affect the EPSP, however, since the driving force for the cations involved in the EPSP is only slightly altered. For the same reason a marked decrease of the fast IPSP and a small decrease of the slow IPSP may be expected. The hyperpolarization is caused by opening of K-channels, resulting in a decreased resistance of the membrane. When the EPSPs and IPSPs are generated in the distal dendrites the decreased membrane resistance may attenuate the EPSPs and IPSPs recorded in the soma (Hestrin \textit{et al.}, 1990). Since the EPSP amplitude, however, was found to be hardly affected we tentatively conclude that the 'shunting' caused by 5-HT seems to be of limited importance. Although it may have contributed to the 5-HT-induced reduction of the IPSPs. The second action of 5-HT is the activation of other, possibly presynaptic 5-HT receptors, which will induce additional effects on synaptic responses, e.g. due to changes in the release of amino acids involved in the synaptic responses. If these effects exist they are likely to change the amplitude of both the population spike and the EPSP / IPSPs.
The present study shows that 5-HT effectively reduced the population spike, but had little effect on the EPSP amplitude. This indicates that under our experimental conditions the excitatory transmission in the CA1 area was mainly affected by the 5-HT1A receptor mediated hyperpolarization. The IPSPs amplitudes, however, were considerably reduced by 5-HT. In fact, fast and slow IPSPs were reduced to a similar extent. The latter supports the idea that, in addition to changes in the IPSP amplitude due to 5-HT1A receptor activation, presynaptic effects of 5-HT on GABA release may also play a role in the suppression of IPSPs.

In the present study we confirmed and extended earlier findings (Joëls et al., 1991; Hasen and Joëls, 1996b) that a predominant MR activation in experimentally treated ADX or adrenal intact rats (Reul and de Kloet, 1985), is associated with small 5-HT1A receptor mediated hyperpolarizations. High corticosterone levels due to in vivo administered corticosterone or due to stress, were linked to relatively large 5-HT responses. Since we argued above that the 5-HT evoked suppression of the population spike is mainly caused by the 5-HT1A receptor mediated hyperpolarization, it can be expected that the steroid modulation of postsynaptic 5-HT responses is also reflected in 5-HT modulation of the population spike. This was indeed observed, most markedly so in the adrenal intact rats where 5-HT effects on the population spike were very small in RU 38486 pretreated stressed rats resulting in selective MR activation. Since the effects of 5-HT on the EPSP or IPSP amplitudes showed no steroid dependence the present data suggest that effects of 5-HT on synaptic transmission mediated via other (non-5-HT1A) receptors are not sensitive to modulation by the hormones.

**Modulation of synaptic responses by CCh**

Activation of muscarinic receptors in the CA1 area can evoke many different effects, including a depolarization of the membrane due to closure of K-conductances (Madison et al., 1987; Benson et al., 1988). The depolarization brings neurons closer to the firing threshold for an action potential and thus potentially enhances the excitability. The CCh induced depolarizations, however, are rather small, so that EPSP and IPSP amplitude will be only little affected.

As observed in the present study, CCh application resulted in a marked reduction rather than an enhancement of the population spike amplitude, suggesting that CCh actions additional to the depolarization contribute largely to the modulation of synaptic transmission. This is also supported by the intracellularly recorded synaptic responses obtained in the same slices, showing a marked reduction of both the EPSP and IPSP amplitudes. These data confirm earlier findings that muscarinic receptor induced depolarization on the one hand and depression of synaptic responses on the other hand are mediated by different receptor subtypes (Valentino and Dingledine, 1981; Dutar and Nicoll, 1988; Haltiwell, 1990). In accordance, it was recently shown that the muscarinic receptor mediated reduction of excitatory transmission in the CA1 hippocampal area is mediated through

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presynaptic muscarinic receptors (Auerbach and Segal, 1996). We therefore conclude that the CCh induced depolarization does not contribute clearly to the cholinergic modulation of synaptic transmission in the CA1 area.

This may explain why steroid modulation of CCh evoked membrane depolarizations was not reflected in the modulation of the synaptic transmission. Thus, we here extended earlier observations (Hesen and Joëls, 1996a) that in vivo occupation of MRs resulted in relatively small CCh evoked depolarizations, while additional occupation of GRs by exogenous corticosterone (but not by stress) was associated with rather large effects, both in ADX and in adrenally intact animals. The small CCh responses in the group with predominant MR occupation versus the group with concomitant MR and GR occupation, was not observed for the CCh mediated actions on synaptic transmission, both extracellularly and intracellularly. This indicates that the corticosteroid modulation of postsynaptic cholinergic responsiveness may be of limited consequence for the CA1 excitability.

While the steroid modulation of postsynaptic CCh evoked depolarizations was not reflected in the excitatory transmission, consistent effects of the hormone were observed on the CCh induced reduction of the IPSP, mediated by other, possibly presynaptic muscarinic receptors. In ADX animals, both the fast and slow IPSP were dose-dependently inhibited by corticosterone. In adrenally intact rats this suppression was not apparent, although pretreatment with RU 38486 resulted in a significant enhancement of the CCh modulation of IPSP amplitudes. The characteristics of this steroid modulation were very similar to the effect of corticosterone on the EPSP amplitude, outlined above. It is presently unclear how the steroid modulation of CCh actions on IPSPs is accomplished. The fact that the fast and slow IPSP were affected similarly suggests that the effect of corticosterone is aimed at a presynaptic site of action for CCh. Since these actions were particularly apparent in ADX rats adaptive changes due to the removal of the adrenals may play a role. The consequences of the steroid action on CCh reduced IPSPs may therefore be limited in adrenally intact animals.

In conclusion, in vivo activation of corticosteroid receptors clearly influences the inhibitory effect of 5-HT on excitatory neurotransmission in the CA1 area. Physiological fluctuations of corticosterone levels may thus, via modulation of 5-HT responsiveness, influence the main stream of synaptic information in the CA1 hippocampal area. Predominant MR occupation associated with moderately low circulating corticosterone levels may relieve the CA1 neurons from 5-HT mediated inhibition of neuronal excitability. By contrast, high corticosterone levels, as circulating during stress, may enhance 5-HT mediated action which reduces the probability that neurons reach the firing threshold and thus attenuates the transmission of excitatory input. This steroid modulation is specifically aimed at 5-HT neurotransmission, as the cholinergic effects on excitatory synaptic transmission were not affected.
References are shown in the back of this thesis.

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