Effect of Chronic Stress on Synaptic Currents in Rat Hippocampal Dentate Gyrus Neurons

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Karst, Henk and Marian Joëls. Effect of chronic stress on synaptic currents in rat hippocampal dentate gyrus neurons. J Neurophysiol 89: 625–633, 2003; 10.1152/jn.00691.2002. We investigated the effect of chronic stress on synaptic responses of rat dentate granule cells to perforant path stimulation. Rats were subjected for 3 wk to unpredictable stressors twice daily or to control handling. One day after the last stressor, hippocampal slices were prepared and synaptic responses were determined with whole-cell recording. At that time, adrenal weight was found to be increased and thymus weight as well as gain in body weight were decreased in the stressed versus control animals, indicative of corticosterone hypersecretion during the stress period. In slices from rats with basal corticosteroid levels (at the circadian trough, under rest), no effect of prior stress exposure was observed on synaptic responses. However, synaptic responses of dentate granule cells from chronically stressed and control rats were differently affected by in vitro activation of glucocorticoid receptors, i.e., 1–4 h after administration of 100 nM corticosterone for 20 min. Thus the maximal response to synaptic activation of dentate cells at holding potential of −70 mV [when N-methyl-D-aspartate (NMDA) receptors are blocked by magnesium] was significantly enhanced after corticosterone administration in chronically stressed but not in control animals. In accordance, the amplitude of α-amino-3-hydroxy-5-methylisozolace-4-propionic acid (AMPA) but not of NMDA receptor-mediated currents was increased by corticosterone in stressed rats, over the entire voltage range. Corticosterone treatment also decreased the time to peak of AMPA currents, but this effect did not depend on prior stress exposure. The data indicate that following chronic stress exposure synaptic excitation of dentate granule cells may be enhanced when corticosterone levels rise. This enhanced synaptic flow could contribute to enhanced excitation of projection areas of the dentate gyrus, most notably the CA3 hippocampal region.

INTRODUCTION

The rat adrenal hormone corticosterone is secreted in high amounts after acute stress (review by Dallman et al. 1994). Corticosterone can enter the brain and bind to the high-affinity mineralocorticoid receptor (MR) and to the glucocorticoid receptor (GR) with 10-fold lower affinity (Reul and de Kloet 1985). Principal neurons in the CA1 area and dentate gyrus (DG) of the hippocampus express high amounts of both receptor types; CA3 pyramidal neurons contain high amounts of MR but much lower levels of GR (review by McEwen et al. 1986). Previous studies have shown that differential activation of hippocampal MR and GR affects functional characteristics of CA1 and DG neurons. It has been proposed that signal transfer is maintained at a stable level under conditions of predominant MR activation, such as occurs under rest at the circadian trough; perturbations of hippocampal activity following stress are normalized by activation of the GR (review by Joëls 1997). These effects of the hormone add to its adaptational role following stress exposure.

Chronic exposure of animals to stress though is often associated with maladaptation. It is well documented that exposure of animals to elevated corticosteroid levels for several weeks results in atrophy of CA3 pyramidal cells (Magarinos and McEwen 1995a,b; Sapolsky et al. 1985; Watanabe et al. 1992b). Based on pharmacological studies, it was concluded that excitatory amino acid mediated transmission, in particular through N-methyl-D-aspartate (NMDA) receptors, plays a role in the stress-induced atrophy. Thus pretreatment of animals with the amino acid release compound phenytoin or the competitive NMDA receptor blocker CPG 43487 both prevented atrophy (Magarinos and McEwen 1995a; Watanabe et al. 1992a). Presynaptic as well as postsynaptic elements of excitatory amino acid transmission could be involved. With respect to the latter, short-term manipulation of corticosterone level rather than chronic stress appeared to increase NMDA receptor binding and subunit expression (Watanabe et al. 1995; Weiland et al. 1997). More recently, however, RT–PCR in hippocampal tissue revealed that chronic stress increases expression of the GluR1 (α-amino-3-hydroxy-5-methylisozolace-4-propionic acid; AMPA) subunit while the NMDA-R1 subunit expression was unaffected (Schwendt and Jezova 2000). With respect to the presynaptic glutamate-mediated input, bilateral lesion of the entorhinal cortex, which projects to the CA3 neurons directly but also indirectly via the dentate gyrus (Steward 1976), fully prevented dendritic atrophy in the CA3 region after chronic stress (Sunanda et al. 1997). Also, mossy fiber terminals from DG cells exhibited changes after chronic stress, showing more densely packed vesicle clusters localized in the vicinity of active zones (Magarinos et al. 1997). The latter suggests that DG cell function and output to CA3 pyramidal neurons may alter during chronic stress exposure.

We here investigated the effect of chronic stress on glutamate-mediated entorhinal input to DG cells, which in turn provide a major glutamatic input to the CA3 area via mossy fibers (Crawford and Connor 1973). Rats were subjected to

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chronic unpredictable stress or control handling for 3 wk. This paradigm was earlier shown to be associated with apparent corticosterone hypersecretion during the stress period (Herman et al., 1995; Paskitt et al., 2000) and to result in CA3 dendritic atrophy (Magarinos and McEwen 1995b). Hippocampal slices were prepared 1 day after the last stressor or control treatment. At that time, gain in body weight during the stress period, basal corticosterone level, and weight of the adrenals and thymus were determined. Apart from basal cell characteristics (input resistance and membrane capacitance), the responsiveness of granule cells to perforant path stimulation was examined with whole-cell patch-clamp recording at a holding potential (−70mV) close to the resting membrane potential. In addition, the conductance, voltage dependency, and kinetic properties of both the NMDA and AMPA receptor–mediated components of synaptic responses were investigated. Since chronic stress was reported to either attenuate or sensitise central responses to subsequent GR activation, depending on the stressor used or parameter tested (Lanfumey et al. 1999; Nisenbaum et al., 1991), we here also tested the efficacy of GR activation in dentate neurons, in chronically stressed and control animals. To this end, all animals entered the experiment in the morning when they display basal plasma corticosterone levels (i.e., with predominant MR activation) (Reul and de Kloet 1985); responses under these conditions were compared with those recorded 1–4 h after GRs activation, induced in vitro by a 20-min perfusion with 100 nM corticosterone.

METHODS

Stress paradigm

Young adult male Wistar rats (n = 23, Harlan) of ±150 g were housed two (or, in one case, 3) in a cage, with a light/dark cycle of 12 h (lights on at 0800). Food and water were given ad libitum. At the start of the stress exposure, rats were randomly assigned to a group receiving chronic unpredictable stress (n = 11) or to the control group (n = 12). The experiments were carried out in the local Animal Experiment Committee (DED protocols 79 and 80). Seven different stressors were given randomly during a period of 21 days, twice daily, i.e., in the early morning and in the afternoon: 1) the rats were forced to swim in cold water (10 °C) for 1 h; 2) they were immobilized at room temperature in a restrainer tube for 4 h; 3) they were immobilized in room temperature in a restrainer tube for 4 h; 4) they were kept at a holding potential of 1 h; 5) shaker for 1 h on a horizontal shaker 30 cm; 6) crowded for 1 h (4–6 per cage); 7) isolated for 1 h. Control rats were handled daily. During this period of 21 days, the stressed and control rats were weighed every morning.

Rats entered the electrophysiological experiment 1 day after the last stressor and were decapitated around 0930. Trunk blood was collected to determine plasma corticosterone levels with a RIA. The adrenals and thymus were removed and weighed. The brain was removed from the skull and stored for 3 min in ice-cold artificial cerebrospinal fluid (ACSF) containing low calcium and high magnesium of the following composition (in mM): 124 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 5.0 MgSO₄, 0.2 CaCl₂, 25 NaHCO₃, and 10 glucose; pH 7.4 gassed with 95% O₂-5% CO₂. The osmolarity (300 mOsm) of this ACSF was adjusted with a Wescor 5100C vapor pressure osmometer. Horizontal slices of the brain were made with a vibratome (Campden Instruments, Sibley, UK). The slices containing the hippocampus were stored in continuously gassed (95% O₂-5% CO₂) ACSF containing (in mM): 124 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 1.5 MgSO₄, 2 CaCl₂, 25 NaHCO₃, and 10 glucose; pH 7.4, at room temperature. After a delay of 1 h, some of the slices were treated with 100 nM corticosterone (Sigma) for 20 min in ACSF of 32°C. After this treatment the slices were moved to a storage bath with normal ACSF at room temperature. The same procedure was carried out for the vehicle-treated controls. Before moving the slice to the recording chamber, an incision was made between the DG and the CA3 area.

Electrophysiology

One slice at a time was placed in a recording chamber mounted on an upright microscope (Nikon Optiphot-2). Slices were continuously perfused with ACSF (32°C, 2–3 mls) and kept fully submerged. Bicuculline methiodide (20 μM, Sigma) was added to the buffer to prevent GABA-mediated inhibition that could be activated by pathway stimulation.

The surface of the suprapyramidal blade of the DG was cleaned with a cleaning pipette. Patch-clamp electrodes for recording (1.5 mm OD, borosilicate glass; impedance approximately 3–4 MΩ) were pulled on a Sutter micropipette puller and placed above the slice. The intracelular pipette solution contained (in mM) 120 Cs methanesulfonate, 17.5 CsCl, 10 Heps, 2 MgATP, 0.1 NaGTP, 5 BAFTA, and 10 QX-314; pH 7.4, adjusted with CsOH. The osmolarity of the pipette fluid was 295 mOsm. BAFTA was obtained from Molecular Probes (Leiden, The Netherlands); the sodium channel blocker QX-314 was from Alomone (Jerusalem, Israel). Under visual control (40× objective and 10× ocular magnification) the electrode was directed toward a granule neuron using positive pressure. Once a patch electrode was sealed on the cell (approximately 1 GΩ) the membrane patch under the electrode was ruptured and the cell was held at a holding potential of −70 mV. Signals were fed into an Axopatch 200B amplifier (Axon Instruments). Data acquisition and analysis was performed with an Atari computer with in-house−developed software (courtesy T. Juta and W. Wadman). Series resistance was compensated to ±70%.

A bipolar stainless steel stimulus electrode (60 μm diam, insulated except for the tip) was placed in the perforant path (Stienstra et al. 1998). Biphasic stimuli (250 μs) were applied through a Neurolog stimulus isolator (NLS 800) driven by a homemade software program. Input−output curves of excitatory postsynaptic currents (EPSCs) evoked in DG neurons were made at holding potential by increasing stimulus intensities from 7 to 600 μA (see example in Fig. 2), given once every 10 s. EPSCs were recorded with a sampling frequency of 1 or 10 kHz, depending on whether the focus was on slow or fast (e.g., risetime) components, respectively: signals were stored and off-line corrected for leak. Fast components of the synaptic currents (e.g., rise time) were studied separately with a 10-KHz sampling frequency. Input−output curves were fit with a Boltzmann equation R(t) = R_{max}(1 + exp[(i - i_0)/k_{C}]), in which R_{max} is the maximal evoked current, i_0 the half-maximal stimulus intensity, and k_{C} proportional to the slope. Based on this curve the half-maximal stimulus intensity was determined. This intensity was used to evoke EPSCs at holding potentials between −90 and +40 mV, increasing voltage in subsequent steps by 10 mV, using intervals of 10 s. During approximately half of the recordings t→(−)-2-amino-5-phosphonopentanoic acid (APV, Sigma; 50μM) was perfused to block the NMDA receptor; in a limited number of neurons, CNX (10 μM, Sigma) was applied to block AMPA receptors.

Statistics

Statistical analysis of the input−output curves, the current-voltage (I-V) relationship, and the gain in weight were tested with ANOVA for repeated measurements (MANOVA). Other data sets were tested with ANOVA and a nonparametric Mann−Whitney U test (P < 0.05),
RESULTS

Neuroendocrine parameters

Several standard procedures were carried out to examine the effectiveness of the chronic stress procedure. One of the most indicative parameters is the gain in weight of the adrenals. As shown in Fig. 1A, the increase in absolute weight of the adrenals and the weight corrected for body weight (per 100 g) was significantly increased in chronically stressed compared with control animals. Another index of chronic stress is the weight of the thymus. Following chronic stress, the absolute thymus weight (549 ± 22 mg) was significantly reduced compared with the control group (658 ± 38 mg), although this difference did not attain statistical significance when corrected for body weight (Fig. 1). Gain in body weight (relative to the body weight when animals started to be exposed to stress or handling) was attenuated in stressed compared with control rats (Fig. 1). The plasma corticosterone level of the stressed group was somewhat elevated [control: 2.2 ± 1.3 μg/100 ml; chronically stressed: 4.7 ± 2.5 μg/100 ml, based on observations in part of the control (n = 4) and stressed (n = 7) animals]. These data support that rats subjected to unpredictable stress indeed experienced prolonged corticosterone hypersecretion prior to the electrophysiological experiments.

Effect of chronic stress and in vitro corticosterone on basal properties and input–output curves

In total, 72 DG granule cells were recorded with whole-cell recording. Input resistance (R_in) was comparable for DG cells of chronically stressed and control rats, both before and after corticosterone treatment. Interestingly, corticosterone treatment increased R_in by approximately 30%. The corticosterone-induced increase of R_in was significant in control rats though not in chronically stressed rats (Table 1). Capacitance of the cells was unaffected by chronic stress and/or corticosterone treatment (Table 1).

Excitatory synaptic currents in DG granule cells (recorded in vitro and in vivo)

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TABLE 1. Corticosteroid effects on input resistance and membrane capacitance in dentate granule cells of chronically stressed and control rats

<table>
<thead>
<tr>
<th></th>
<th>No. of Observations</th>
<th>Input Resistance, MΩ</th>
<th>Capacitance, pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, no CORT</td>
<td>17</td>
<td>128 ± 15</td>
<td>14.2 ± 0.8</td>
</tr>
<tr>
<td>Control CORT</td>
<td>17</td>
<td>215 ± 33*</td>
<td>13.9 ± 0.9</td>
</tr>
<tr>
<td>Stressed, no CORT</td>
<td>21</td>
<td>160 ± 17</td>
<td>14.7 ± 0.9</td>
</tr>
<tr>
<td>Stressed, CORT</td>
<td>17</td>
<td>215 ± 29</td>
<td>12.7 ± 0.8</td>
</tr>
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</table>

Values are means ± SE. CORT, corticosteroid. * Significantly (P < 0.05) different from untreated control group.
the presence of bicuculline) involve activation of NMDA and AMPA receptors, while the role of kainate receptors is negligible (Behr et al. 2001). At a holding potential of −70 mV NMDA receptors are blocked by magnesium (see following text), so that synaptically evoked currents are supposedly mediated by AMPA receptors. Figure 2A shows an example of AMPA receptor-mediated currents evoked in DG granule cells at a holding potential of −70 mV by perforant path stimulation, using increasing stimulus intensities. Maximal amplitude was reached with stimulus intensities higher than 400 μA. With the Boltzmann equation $R(i) = R_{\text{max}}/[1 + \exp((i - i_H)/I_C)]$, the maximal evoked current ($R_{\text{max}}$), the half-maximal stimulus intensity ($i_H$), and a factor $I_C$ proportional to the slope factor were calculated. In Fig. 2A, an example of a Boltzmann plot is shown.

Averaged input–output curves for all the experimental groups are shown in Fig. 2B. No effect of chronic stress was seen on the input–output relationship under basal conditions when corticosterone levels are low. In vitro treatment of the slices of control rats with 100 nM corticosterone had a marginal but nonsignificant [$F(1,36) = 2.56; P = 0.119$] effect on the input–output curves. However, a major and significant change in the input–output curve [MANOVA: $F(3,50) = 4.588; P = 0.0065$] was seen when slices of chronically stressed rats had been treated with 100 nM corticosterone for 20 min, 1–4 h prior to recording. The main change was a significant increase in the maximal amplitude of synaptically evoked EPSCs (Fig. 2C). No change was observed with respect to the $i_H$ (Fig. 2D) or $I_C$ (Fig. 2E). Subsequent testing of the cells was performed at half-maximal stimulus intensity.

**NMDA receptor- and AMPA receptor-mediated components of the EPSCs**

Depending on the holding potential, EPSCs evoked in granule cells of the DG by stimulation of the perforant path contained one or two components: a fast component was seen at all holding potentials while a slow component appeared at holding potential...
potentials more depolarized than −40 mV (Fig. 3A). The slow currents disappeared in the presence of the APV, indicating that they were mediated by the NMDA receptor (Fig. 3B). The remaining fast current was fully blocked when CNQX was added in addition to APV. The fast current therefore represents the AMPA receptor-mediated component. Since no currents were evoked in the presence of the two blocking agents, we conclude that only NMDA and AMPA receptor-mediated currents take part in the synaptically evoked currents.

In a first series of experiments, we analyzed fast peak currents and slow synaptic currents 50 ms after stimulation (Fig 3A, a and b, respectively). The slow current displayed a nonlinear I-V relation (Fig. 3F, b), with no apparent conductance up to −40 mV, increasing conductance between −40 and −20 mV and linear characteristics at levels more depolarized than −20 mV. Two observations indicate that the slow currents measured at b are mediated by NMDA receptors only. First, the slow currents were abolished in the presence of the NMDA receptor blocker APV (Fig 3B, e). Second, by subtracting AMPA currents (recorded in the presence of APV) from the total synaptic currents, presumably NMDA receptor-mediated currents could be studied in isolation (Fig. 3C, d). The I-V relation of signal d was indistinguishable from that of b (Fig. 3F). We conclude that currents measured 50 s after stimulation are a reliable indicator of NMDA receptor-mediated synaptic activity.

Interpretation of the peak total synaptic current was less straightforward. Thus the I-V relationship of the evoked currents (Fig. 3E, a) was nonlinear: A clear deviation was seen, starting from a holding potential of −40 mV. The nonlinearity was clearly caused by activation of NMDA receptors since a linear I-V relationship was obtained for the peak amplitudes in the presence of the NMDA receptor antagonist APV (Fig. 3E, c). In a second series of experiments we therefore studied AMPA receptor-mediated currents in isolation, during administration of APV.

**Effect of chronic stress and in vitro corticosterone on I-V relation**

EPSCs were evoked with half-maximal stimulus intensities at holding potentials varying from −90 to +40 mV. In the first series of experiments, currents were determined in the absence of any blockers (Fig. 4A). The averaged I-V plot of the peak current is shown in Fig. 5A, the I-V plot of the slow, NMDA receptor-mediated currents is shown in Fig. 5B. Under basal corticosterone conditions, no differences were observed between the I-V relations of synaptic responses evoked in the DG neurons of chronically stressed animals and control rats (typical examples in Fig. 4A). However, 1–4 h after a brief (20 min) in vitro application of 100 nM corticosterone, peak currents over a voltage range of −90 to 0 mV were significantly enhanced in chronically stressed but not control animals (Figs. 4A and 5A). No differences between experimental groups were observed for the NMDA receptor-mediated currents [Fig. 5B; MANOVA for currents between −90 and 0 mV; F(3,31) = 0.59; P = 0.63]. This suggested that AMPA rather than NMDA currents are evoked from perforant path stimulation. Traces show superimposed currents recorded at holding potentials between −90 and +40 mV. Fast and slow currents can be distinguished. Here, the fast AMPA receptor-evoked current has a maximal amplitude at the holding potential of −90 mV. The slow, NMDA receptor-mediated current is evoked at holding potentials of −40 mV and higher. In the presence of the NMDA receptor blocking agent APV (50 μM) only the fast AMPA receptor evoked currents remain. C: subtraction of the AMPA currents from the total currents of A, yields the slow NMDA receptor-mediated currents. D: additional application of an AMPA receptor blocking agent CNQX (10 μM) reveals that the evoked EPSCs consist of only AMPA and NMDA receptor-mediated currents. E: when plotting the peak amplitudes of the currents in A (a), the nonlinear character of the I-V relation indicates that NMDA currents appearing from −40 mV onward interfere with linear relation of the AMPA currents. This is supported by the observation that blocking the NMDA currents (see B) yields a linear relation for the remaining AMPA receptor-mediated currents (peak amplitude = c). F: at 50 ms after the onset of the EPSCs in A (b) when the fast AMPA currents are deactivated, NMDA receptor currents can be distinguished. The I-V plot shows that the currents are evoked from −40 mV onward, when the voltage-dependent Mg2+ block is removed. The role of the NMDA receptor is confirmed by the fact that the currents disappear in the presence of an NMDA receptor blocking agent, APV (e). Moreover, the peak amplitudes of the subtracted NMDA currents, yielding the NMDA component in isolation (C), have a comparable I-V relationship as the currents of (b).
With 100 nM corticosterone are increased in amplitude compared with receptor-mediated EPSCs evoked in the slices of chronically stressed rats. From these examples it is obvious that the AMPA NMDA currents, in the presence of 50 μM APV, only fast AMPA currents are evoked by stimulation. From these examples it is obvious that the AMPA receptor-mediated EPSCs evoked in the slices of chronically stressed rats treated with 100 nM corticosterone are increased in amplitude compared with the other groups.

NMDA receptor components were affected by corticosterone treatment in tissue from chronically stressed rats.

To address this specifically, synaptic currents were studied in a second series of experiments while perfusing APV, yielding AMPA receptor-mediated currents in isolation (typical traces in Fig. 4B). As shown in Fig. 5C, the AMPA receptor-mediated currents showed very similar sensitivity to corticosterone and stress as the peak current in the absence of APV. Thus no changes by chronic stress were seen under basal conditions. While corticosterone did not significantly alter the I-V relation of AMPA receptor-mediated responses in DG cells of control rats, marked enhancement of the current amplitude was seen after corticosterone treatment in chronically stressed rats, in the voltage range of −90 to 0 mV. We therefore conclude that corticosterone specifically enhances AMPA receptor-mediated synaptic responses of DG cells in chronically stressed but not in control rats.

**Effect of chronic stress and in vitro corticosterone treatment on rise time and decay of synaptic currents**

We looked at two aspects of the kinetic properties of AMPA receptor-mediated currents. First, the effect of corticosterone treatment on the rise time (interval between 10–90% of the peak amplitude) was studied (Fig. 6A). Second, we examined the effect on the decay time constant (r) of the AMPA currents (Fig. 6B).

Typically, granule cells in untreated slices from control rats displayed a rise time of 4.5 ms (Fig. 6C). This is comparable to the values published by others in various hippocampus cell types (Obokata et al. 1997; Rammes et al. 1999; Xie et al. 1997). The rise time is rather slow compared with the rise times recorded from dendrites (Benke et al. 1998), which can be explained by dendritic filtering of the currents (Major et al. 1994). Corticosterone treatment consistently shortened the rise time of the AMPA current. The histogram of Fig. 6C shows that the mean rise time was reduced in corticosterone-treated slices of both the control and the chronically stressed rats. In stressed as well as nonstressed groups, corticosterone treatment reduced the rise time by approximately 20%.

Decay time constants were obtained by fitting the current traces after the peak with a single exponential. In nearly all cells this yielded a good fit (r > 0.90). In contrast to the rise time, no group differences were observed with respect to the decay time (Fig. 6D). We conclude that corticosterone decreases the rise time of the AMPA receptor-mediated synaptic response, without affecting the decay time.

**DISCUSSION**

In this study we investigated the effect of chronic stress on DG granule cell responses to perforant path stimulation. The model of chronic unpredictable stress was selected since this paradigm induces dendritic atrophy in CA3 pyramidal neurons and shows less habituation to the stressor than, e.g., found after 3 wk of daily restraint stress (Magarinos et al. 1995b). In agreement with the presumed chronic hypercorticism of this model, animals included in the present study displayed increased adrenal weight, somewhat decreased thymus weight (only significant if not corrected for body weight), and attenuated body weight gain, compared with the control animals, which were only handled. The changes were relatively small, as was also reported earlier for this model (Cullinan and Wolfe 2000), indicating that the animals probably did not experience severe stress.

In thus stressed rats, the conductance, voltage dependency, and kinetic properties of NMDA receptor and AMPA receptor-mediated synaptic currents induced by perforant path stimulation in DG granule cells were not altered under basal conditions, i.e., when corticosterone levels are quite low. Only when GRs were substantially activated by in vitro administration of a high dose of corticosterone did DG granule cells respond in a different way to synaptic input. Thus the amplitude of AMPA but not NMDA receptor-mediated synaptic currents of granule cells was markedly enhanced after GR activation in stressed but not in control rats. It should be noted that we can presently not fully exclude that we missed an effect on the NMDA receptor-mediated currents: NMDA receptor-mediated currents were measured at 50 ms after stimulation, i.e., during the period when the channels are closing. No apparent shifts in voltage dependency could be discerned. A consistently faster rise time of the AMPA receptor-mediated synaptic current was observed after GR activation, but this effect was seen in stressed as well as in control rats and therefore apparently is not linked to chronic stress.

Although clear changes were thus observed in association
with chronic stress and corticosterone treatment, these effects need to be interpreted with some caution since voltage control over DG granule cells in situ is incomplete, although less so than in CA1 or CA3 pyramidal neurons (Jaffe and Carnevale 1999). This is relevant since lateral perforant path input mostly impinges on distal dendrites, while we here recorded synaptic currents in the soma. If the cable properties of the dendrites are comparable between experimental groups, one can assume that all groups are equally affected by the incomplete voltage control and filtering of signals (due to the cable properties), so that quantitative rather than qualitative aspects of group differences may be influenced. However, we observed in dentate

FIG. 5. A: with half-maximal stimulus intensities, EPSCs were evoked at holding potentials varying from −90 to +40 mV. No difference was seen between stressed and control when corticosteroid levels were basal. However, after corticosterone treatment, peak current amplitudes observed in chronically stressed rats were increased compared with the untreated slices in the voltage range from −90 to 0 mV [F(3,64) = 3.9; P < 0.05]. Number of cells: untreated control, n = 17; CORT-treated control, n = 21; untreated stress, n = 21; CORT-treated stress, n = 13. B: NMDA receptor-mediated synaptic currents (amplitudes b in Fig. 3A) were neither affected by chronic stress nor by corticosterone treatment of the slices. C: increased amplitude of peak currents in the presence of APV, yielding AMPA currents, was seen after corticosterone treatment of slices from chronically stressed rats [F(3,41) = 3.3; P < 0.05]. Number of cells: untreated control, n = 10; CORT-treated control, n = 11; untreated stress, n = 9; CORT-treated stress, n = 6. D: in all experimental groups, current amplitudes at 50 ms after EPSC onset were completely blocked by APV (e in Fig. 3B).

FIG. 6. A: two characteristics of the kinetic properties of the AMPA current were calculated: the rise time, i.e., the interval between 10 and 90% of the peak amplitude of the current evoked with a stimulus intensity of 250 μA, and the decay time constant τ, here fit with a single exponential (stippled line). B: typical examples showing that the rise time of AMPA receptor-mediated currents was decreased by corticosterone treatment of slices from control animals. C: corticosterone treatment of slices from control as well as from chronically stressed rats decreased the rise time of the AMPA receptor currents (ANOVA, Mann–Whitney U test; P < 0.05). Data from control animals were obtained with a sampling rate of 10 kHz. Results in control animals when using a sampling rate of 1 kHz were very comparable (data not shown). Data shown here for the chronically stressed groups were obtained at 1 kHz. D: no effect of corticosterone treatment or chronic stress was observed on the decay time constant. Number of cells: untreated control, n = 14; CORT-treated control, n = 16; untreated stress n = 15; CORT-treated stress, n = 14.
cells that (contrary to CA1 neurons) the input resistance is increased after corticosterone treatment in the nonstressed controls. It is therefore necessary to consider whether the observed effects of chronic stress and/or corticosterone treatment can be merely explained by changed cable properties and incomplete voltage control. Several observations argue against this possibility. First, although reversal potentials of AMPA receptor and NMDA receptor-mediated synaptic currents were rather depolarized—which is expected in case of incomplete voltage control—and was also seen in earlier studies using comparable recording conditions (e.g., Otmakhova et al. 2002)—there was no clear difference between the four experimental groups. Second, an increased input resistance such as seen after corticosterone treatment in nonstressed animals is expected to result in a decreased amplitude and slowing down of the rise time as well as the decay of synaptic currents recorded in the soma. Contrary to this prediction, synaptic currents were either not altered (nonstressed group) or even increased in amplitude (stressed group) after corticosterone treatment. Also, corticosterone caused a faster rather than slower rise time in the stressed as well as the control group, while it did not affect the decay of synaptic currents. We tentatively conclude that the presently observed differences in synaptic currents between the experimental groups cannot be explained (and if anything are masked) by the changes in basic cell properties in combination with incomplete voltage control.

Interestingly, very little effect of prior chronic stress exposure was observed when synaptic currents were recorded in slices obtained from animals that at the start of the experiment were at the circadian trough of corticosterone release and at rest. Plasma corticosterone level of the stressed group was somewhat elevated, but the influence of this rise was apparently limited, probably since, in both the stressed and the control animals, extensive GR activation can be ruled out. The lack of functional differences also argues against major changes in MR function and slow stress-induced adaptational changes in basal cellular and network properties, e.g., in morphology and innervation.

Prior stress exposure, however, markedly altered the response of synaptic currents to in vitro perfused corticosterone. Thus, in control animals, GR activation had little effect on the amplitude of the synaptic currents. A lack of effect of GR (in addition to MR) activation on synaptic responses in the DG of nonstressed rats was earlier observed in extracellular recording studies investigating the field response evoked by perforant path stimulation (Stienstra et al. 2000). Yet, a clear enhancement of synaptic currents by GR was seen in chronically stressed rats, pointing to increased GR activity after chronic stress. If this is translated to the protein level, it seems unlikely that increased GR capacity underlies the observed phenomena. Recently, it was reported that chronic stress prolongs GR interaction with the DNA (Kitchener et al. 2001), which could enhance GR functional activity. Clearly, extensive studies addressing transcriptional regulation after chronic stress, including the role of all proteins involved (not only GR but also, e.g., coactivators and represors), will be necessary to resolve the molecular mechanism underlying the here observed enhanced excitatory responses after GR activation.

In theory, the enhanced effect of GR on synaptic responses in chronically stressed rats could involve pre- or postsynaptic targets determining synaptic responsiveness. If GR-mediated actions change entorhinal innervation of the DG and/or glutamate release this would be expected to affect NMDA and AMPA receptor-mediated responses to an equal extent. The present findings do not strongly point in that direction. Postsynaptically, GR effects could involve altered functionality of AMPA receptors. Corticosterone treatment was indeed found to change AMPA receptor binding (Clark and Cotman 1992; Watanabe et al. 1995), while modulation of NMDA receptor binding and subunit expression still is equivocal (Watanabe et al. 1995; Weiland et al. 1997). Expression of the Glu-R1 AMPA receptor subunit was also significantly increased in hippocampal tissue after chronic stress, while the NMDA-R1 subunit was not changed, as recently demonstrated with competitive RT–PCR (Schwendt and Jezova 2000), although not with in situ hybridization (Watanabe et al. 1995). Functionality of the AMPA receptor, however, could also be altered by posttranslational modification following GR activation, as was earlier proposed for GR effects on other membrane properties (Karten et al. 1999). A distinct possibility is that GR activation leads to recruitment of existing AMPA receptors, similar to that assumed in the case of long-term potentiation (Shi et al. 1999).

The functional consequence of increased granule cell responsiveness to entorhinal input after chronic stress can be considerable: It is likely to enhance the excitation of these cells at least during part of the day, i.e., after activation of GR by an acute stressor or during the circadian peak of corticosterone release. If not counteracted by local inhibitory processes, such altered DG granule cell firing properties could possibly contribute to the earlier described changes in mossy fiber terminals (Magarinos et al. 1997) and to the presumed increased excitation of CA3 pyramidal neurons during stress (Watanabe et al. 1995b). It may also at least partly explain why lesioning of the entorhinal cortex protects against stress-induced dendritic atrophy in the CA3 region (Sunanda et al. 1997). Importantly, the present study for the first time shows that chronic stress results in functional changes in hippocampal signal transfer, not only in the CA3 region but also in the DG, which provides a major afferent pathway to the CA3 area.

REFERENCES


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