CORTICOSTERONE SLOWLY ENHANCES MINIATURE EXCITATORY POSTSYNAPTIC CURRENT AMPLITUDE IN RAT CA1 HIPPOCAMPAL CELLS

Henk Karst and Marian Joëls
SILS-CNS, Swammerdam Institute of Life Sciences, University of Amsterdam, The Netherlands.

Running title: Slow enhancement of AMPA responses by glucocorticoids…

Correspondence to:
Henk Karst
SILS-CNS
University of Amsterdam
Kruislaan 320
1098 SM Amsterdam
The Netherlands
Phone: 00-31-20-5257646
Fax: 00-31-20-5257709
E-mail: karst@science.uva.nl
Abstract

Corticosteroid hormones are released in high amounts after stress and bind to intracellular receptors in the brain, which in activated form function as transcription factors. We here tested the effect of a high dose of corticosterone on AMPA receptor mediated transmission in the CA1 hippocampal area, which is enriched in corticosteroid receptors. To focus on slow gene-mediated effects of the hormone, excitatory postsynaptic currents were measured at least one hour after a brief application of 100 nM corticosterone to slices from adrenally intact adult mice. The amplitude but not frequency of miniature postsynaptic excitatory currents was found to be significantly enhanced. These effects were mimicked by 100 nM RU 28362, a selective agonist for intracellular glucocorticoid receptors. Evoked AMPA responses at the single cell were significantly enhanced when measured 2-4 hours after application of 100 nM corticosterone, but not at earlier moments nor with a longer delay. In summary, the present results show that activation of hippocampal glucocorticoid receptors induces a slow enhancement of AMPA receptor mediated responses, at the single cell level.

Key words: Glucocorticoid, mEPSC, eEPSC, AMPA receptor, patch clamp
Introduction

Adrenal corticosteroid hormones (cortisol in man, corticosterone in rats) are released into the circulation in high amounts after a stressful event. Hormone levels peak within 15-30 minutes and gradually normalize within 2 hrs, due to a negative feedback regulation via the hypothalamus and pituitary gland (Dallman et al., 1987; Makino et al., 2002). Corticosteroids enter the brain and bind to discretely localized high affinity mineralocorticoid receptors (MRs) as well as lower affinity glucocorticoid receptors (GRs) (reviewed by McEwen et al., 1986; De Kloet et al., 1998). Hippocampal CA1 pyramidal neurons co-express both receptor subtypes (Van Steensel et al., 1996). Due to the difference in affinity, basal levels of corticosterone under resting conditions will predominantly activate MRs; when steroid levels rise, e.g. after a stress, GRs will also become substantially activated (reviewed by de Kloet et al., 1998). Activation of corticosteroid receptors is known to regulate transcription of responsive genes (Aranda and Pascual, 2001; Datson et al., 2001).

Earlier studies have shown that functional properties of CA1 pyramidal neurons are slowly but persistently changed through these genomic actions, following a temporary rise in hormone level (for review Joëls, 2001). Thus, voltage-dependent calcium currents of CA1 neurons were found to be enhanced in amplitude with a considerable delay in time, i.e. 1-4 hrs after a brief (20 min) application of high doses of corticosterone (30-100 nM; Kerr et al., 1992; Karst et al., 1994, 2000) or following stress (Joëls et al., 2003). It was shown that this is indeed a gene-mediated process, involving DNA-binding of GR homodimers (Karst et al., 2000) and protein synthesis (Kerr et al., 1992). Other voltage-dependent currents were found to be less sensitive to GR activation (see for review Joëls, 2001). G-protein coupled signal transduction also appears to be a target for steroid modulation. For instance, CA1 pyramidal hyperpolarizations mediated by the serotonin-1A receptor are small with low levels of corticosterone (1 nM) that will predominantly activate MRs (Joëls et al., 1991; Beck et al., 1996) but increased 1-4 hrs after an acute stress or GR activation in vitro (Joëls et
al., 1991; Hesen and Joëls, 1996), again requiring GR-homodimer binding to the DNA (Karst et al., 2000) and protein synthesis (Karst and Joëls, 1991).

Surprisingly, to date little is known at the cellular level about slow GR mediated effects on transmission via hippocampal ionotropic receptors, in particular via the AMPA receptor which mediates the main basal excitatory flow of information in the CA1 area (Meng et al., 2003). In the present study we therefore examined if brief application of corticosterone to hippocampal slices slowly changes functional properties of AMPA receptors involved in excitatory projections onto the CA1 area. We examined miniature excitatory postsynaptic potentials (mEPSCs) as well as evoked (e)EPSCs in CA1 pyramidal neurons, before and several hours after a 20 min application of 100 nM corticosterone in vitro. To specifically investigate the role of the GR, slices were also exposed to 100 nM of the highly selective GR agonist RU 28362 (Philibert and Moguilevski, 1983).

**Materials and Methods**

*Animals and slice preparation*

C57Bl/6 mice, 6 weeks of age, were group-housed in cages with a light/dark cycle of 12 h (lights on at 08.00 h). Food and water were given ad libitum. The experiments were carried out with permission of the local Animal Committee (DED 91). The mice were decapitated under rest, around 9.30 am, i.e. when plasma corticosterone levels are low (see e.g. Karst et al., 2000; Alfarez et al., 2002). Thus, decapitation was always done within 2 min after taking the animal from its home cage, which is too short a time to induce any discernable rise in plasma corticosterone concentration. Accordingly, the plasma corticosterone levels of animals decapitated under such circumstances are very low, i.e. 2-3 µg/dl (Karst et al., 2000; Alfarez et al., 2002). The brain was removed from the skull and stored in continuously gassed (95% O2, 5 % CO2) ACSF containing (in mM): 124 NaCl, 3.5 KCl, 1.25 NaH2PO4, 1.5 MgSO4, 2 CaCl, 25 NaHCO3 and 10 glucose; pH 7.4, at room temperature. The osmolarity (300 mOsm) of this ACSF was
adjusted with a Wescor Inc. 5100C vapor pressure osmometer. Transverse slices of the hippocampus were made with a tissue chopper. The slices were stored at room temperature. After a delay of one hour, some of the slices were treated with 100 nM corticosterone (Sigma) or 100 nM RU28362 for 20 min in ACSF at 32°C. After this treatment, the slices were moved to a storage bath with normal ACSF at room temperature and left undisturbed for at least another 40 min. The same procedure was carried out for the vehicle treated controls.

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 ml/s) and kept fully submerged. Bicuculline Methiodide (20 µM, Sigma) was added to the buffer to prevent GABAa receptor mediated inhibition that could be activated by pathway stimulation. GABAb receptor mediated components were blocked by QX-314 which was added to the pipette solution (see below; Perkins and Wong, 1996).

Debris on the surface of the CA1 cell layer was removed with a cleaning pipette. Whole-cell patch clamp recordings were made with an Axopatch 200B amplifier (Axon instruments, USA) using electrodes from borosilicate glass (1.5 mm outer diameter, Hilgenberg, Malsfeld, Germany) with an impedance of approximately 3-6 MΩ. The electrodes were pulled on a Sutter micropipette puller. The intracellular pipette solution contained (in mM): 120 Cs methane sulfonate, 17.5 CsCl, 10 Hepes, 2 MgATP, 0.1 NaGTP, 5 BAPTA, 10 QX-314; pH was 7.4, adjusted with CsOH. The osmolarity of the pipette fluid was 295 mOsm. BAPTA was obtained from Molecular probes (The Netherlands); the sodium channel blocker QX-314 from Alomone (Jerusalem, Israel). All other chemicals were obtained from Sigma, The Netherlands. Cesium salts are commonly used to record mEPSCs in mammalian brain cells (see e.g. Li and Prince, 2002; Hendricson et al., 2004). Under visual control (40x objective and 10x ocular
magnification) the electrode was directed towards a CA1 neuron using positive pressure. Once a patch electrode was sealed on the cell (~1 GΩ) the membrane patch under the electrode was ruptured and the cell was kept at a holding potential of -70 mV. The liquid junction potential caused a shift of 8 mV at most. We did not compensate for this potential shift. Recordings with an uncompensated series resistance of less than 2.5 times the pipette resistance were accepted for analysis. Series resistances were typically between 4 and (at the very most) 15 MΩ. In view of the small current amplitudes, the recordings were not corrected for series resistance. Input resistances ranged from 100-300 MΩ, which is comparable to resistances reported earlier for rat CA1 neurons with a Cs-salt in the pipette (see e.g. Staff et al., 2000). Data acquisition was performed with PClamp (version 8.2) and analyzed with Clampfit (version 8.2) and WCP (whole cell program) Strathclyde software (J. Dempster, University of Strathclyde, Glasgow, UK, [2002, Feb 23]).

Stimulus evoked EPSCs

A bipolar stainless steel stimulus electrode (60 µm diameter, insulated except for the tip) was placed in the Schaffer collaterals. Biphasic stimuli (250 µs) were applied through a Neurolog stimulus isolator (NL 800) driven by PClamp 8.2. Input-output curves of EPSCs evoked in CA1 neurons were made at holding potential (-70 mV) by increasing stimulus intensities from 16 µA to 500 µA, given once every 10 seconds. Evoked (e)EPSCs were recorded with a sampling frequency of 10 kHz. Signals were stored and off line corrected for leak. Input-output curves were fit with a Boltzmann equation: \( R(i) = \frac{R_{\text{max}}}{1 + \exp\left(\frac{i - i_H}{I_C}\right)} \), where \( R_{\text{max}} \) is the maximal evoked current, \( i_H \) the half maximal stimulus intensity and \( I_C \) proportional to the slope. Based on this curve the half maximal stimulus intensity was determined. This intensity was used to determine voltage-dependent properties by evoking EPSCs at holding potentials between -80 to +50 mV, increasing the voltage in subsequent steps by 10 mV and using intervals of 10 seconds. During
these recordings D-(-)-2-Amino-5-phosphonopentanoic acid (APV, Sigma; 50\(\mu\)M) was perfused to block the NMDA receptor. We also studied possible changes in the AMPA versus NMDA responses after corticosterone treatment, by applying a protocol described by Saal et al. (2003). In brief, AMPA receptor mediated responses were determined by the (peak) signal at +40 mV in the presence of APV. By subtracting this signal from the overall response, the NMDA receptor mediated component could be obtained.

**Miniature EPSCs**

Miniature (m) EPSCs were recorded either following the recording of eEPSCs or without prior recording of eEPSCs, by adding tetrodotoxin (0.5 \(\mu\)M, Latoxan, Rosans, France) to the perfusate. At a holding potential of –70 mV, mEPSCs were recorded for 5 minutes. During some recordings the non-NMDA receptor blocker CNQX (10 \(\mu\)M, Tocris) or the selective AMPA receptor blocker GYKI 53655 (50 \(\mu\)M, Bleakman et al., 1996; Vignes and Collingridge, 1997; LY300168, obtained through M. Eder, München) was perfused to confirm that the mEPSCs were indeed mediated by AMPA receptors. The digitized data (stored on PC via Digidata interface) were off-line analyzed using Strathclyde software with detection threshold levels set above 5 pA. The currents were identified as mEPSCs when the rise time was faster than the decay time. Of all cells measured, the following mEPSC characteristics were determined: inter-mEPSC interval, the frequency, rise time, peak amplitude and tau of decay. The decay of each mEPSC was fitted with a mono- and bi-exponential curve in WCP. This program uses the Levenberg-Marquardt algorithm to iteratively minimize the sum of the squared differences between the theoretical curve and data curve. As criterion for the goodness of the fit the residual standard deviation should be less than 0.3. Fitting with a bi-exponential instead of a mono-exponential curve did not increase goodness of the fit (data not shown).
Statistics

Delayed effects (examined in different sets of cells) were analyzed with a two-tailed unpaired Student's t-test. The distribution of mEPSC amplitudes between control and treatment conditions was performed with a Kolmogorov Smirnov Test. Input-output curves for eEPSCs were tested with an analysis of variance for repeated measures (MANOVA). In all cases, significance was set at p<0.05.

Results

Miniature excitatory postsynaptic potentials (mEPSCs) were recorded in CA1 neurons under conditions that GABAα receptors were blocked by bicuculline and spontaneous release was prevented by the addition of tetrodotoxin. Typical mEPSCs are shown in the trace of figure 1A. These mEPSCs are mediated by AMPA receptors since: 1) All recordings (unless stated otherwise) were carried out at a holding potential of -70 mV, when NMDA receptors are blocked by Mg-ions (Nowak et al., 1984). 2) Introduction of the non-NMDA receptor antagonist CNQX (n=4) or the specific AMPA receptor blocker GYKI 53655 (n=3) completely abolished the mEPSCs (figure 1B and C). Removal of the antagonists reversed the blockage of the receptors (67.5 ± 1.5%, data not shown).

In cells recorded at least one hour after a brief (20 min) treatment with 100 nM corticosterone in vitro, the amplitude but not the frequency of mEPSCs was significantly (p = 0.0001) enhanced (see Table I). Typical traces are shown in figure 2A. Figure 2B furthermore shows the normalized frequency histogram of the mEPSC amplitude and ln(amplitude), in cells from corticosterone and vehicle treated slices. The corticosterone-induced shift of the distribution to larger amplitudes is also very clear from the cumulative frequency distribution of the amplitudes (figure 2C). The delayed effect of the hormone on mEPSC amplitude was clearly mediated by the GR. As shown in figure 3, the highly selective GR-agonist RU28362 induced very similar, delayed effects on the mEPSC amplitude. Especially in the case of the ln(amplitude), the distribution of both
experimental groups was not different from a normal distribution and could be well fitted with a single Gaussian. Neither corticosterone nor RU 28362 affected the kinetic properties of the mEPSCs (Table I). That is, no significant GR-dependent changes were observed in the mean rise time and the time constant of the decay for the mEPSCs.

The increased mEPSC amplitude in combination with a lack of effect on the frequency suggests that the delayed effects of corticosterone involve postsynaptic aspects of glutamatergic transmission. In subsequent experiments we examined the putative changes in synaptically evoked EPSCs and the ratio between AMPA and NMDA receptor mediated responses. In figure 4A, averaged input-output curves of the eEPSCs are depicted for the control group and cells recorded at least 1 hr after a 20 min.

administration of 100 nM corticosterone. All data were obtained at a holding potential of -70 mV and therefore presumably represent AMPA receptor mediated responses. The data shows that particularly with strong stimulation intensities, corticosterone induced a ~25% increase in the maximal eEPSC amplitude compared to the control amplitudes. Over the stimulation range of 300-500 µA, a significant (p = 0.04) enhancement was seen after corticosterone treatment (n = 22), compared to control (n = 26). The responses induced by half maximal stimulus intensity, though, were not different between the vehicle and corticosterone treated slices. Interestingly, maximal eEPSC amplitudes seemed to change with time (figure 4B). The largest increase in amplitude was observed between 2 and 4 hrs after steroid treatment, when the corticosterone treated cells responded significantly stronger to high intensity stimulation than the control cells recorded in the same time interval (mean ± SEM; corticosterone group: -1.75 ± 0.12 nA, n=17; control: -1.26 ± 0.15 nA, n=15; p=0.02). Relatively small amplitudes were observed at short (<2 hrs) intervals after corticosterone treatment and particularly after long intervals (>4 hrs, see figure 4B). In the control group, such clear decline after long intervals was not observed (mean ± SEM: 2-4 hrs: -1.26 ± 0.15 nA, n=15; >4 hrs: -1.14 ± 0.22 nA, n=7).
We also investigated the voltage-dependency of the eEPSCs. To this end, eEPSCs were recorded at various potentials in the presence of APV, thus isolating AMPA-receptor mediated events. Current-voltage relationships of the eEPSCs (normalized to the response obtained at -70 mV) were comparable for the two experimental groups in the voltage range from -70 mV to +50 mV, indicating that voltage-dependency was not changed by corticosteroid treatment (figure 4C). The paired pulse response ratio was also not changed after corticosterone treatment (figure 4D).

In this study we focused on AMPA receptor mediated responses. To get a first impression about the selectivity of the corticosteroid effects we examined to what extent the hormone affected NMDA receptor mediated synaptic events. For each cell, AMPA responses were analyzed as the peak response evoked by synaptic stimulation at +40 mV, in the presence of APV (example in figure 5A). NMDA receptor mediated responses were determined by subtracting this signal from the overall synaptic response before APV treatment. As shown in figure 5B, the NMDA receptor mediated component of the synaptic responses was nearly identical in vehicle and corticosterone treated cells. By contrast, AMPA receptor mediated components recorded in the same set of cells were significantly enhanced. A similar lack of effect on NMDA receptor mediated responses was also inferred from the overall response at +40 mV, at 100 ms after stimulation. Earlier studies showed (Otmakova et al., 2002; Karst and Joëls, 2003) that at this timepoint AMPA responses are in general completely inactivated, so that the recorded currents are nearly exclusively mediated by NMDA receptors. Thus analysed signals showed no difference between the vehicle treated cells (0.16 ± 0.02 nA) and corticosterone treated cells (0.17 ± 0.01 nA).

Discussion
Over the past decade several groups have shown that glucocorticoids have slow but persistent effects on the function of various voltage-dependent ion channels as well as
G-protein coupled receptor signaling pathways in the CA1 hippocampal area (see for review Joëls, 2001). Surprisingly little is known, at the cellular level, about corticosteroid actions on transmission mediated by glutamate, one of the major excitatory transmitters in the hippocampus. We here show that glutamate transmission can be consistently changed by temporary activation of the glucocorticoid receptor.

Thus, several hours after a brief (20 min) corticosterone treatment, the amplitude of AMPA receptor mediated mEPSCs was significantly enhanced. The mEPSC frequency and kinetic properties remained unaltered. Moreover, an increase in the amplitude of eEPSCs was observed, particularly between 2 and 4 hrs after corticosteroid treatment. This contrasts to earlier findings in the dentate gyrus, where eEPSC amplitude was unaffected by 100 nM of corticosterone in control rats, though similarly enhanced as in CA1 cells in granule cells from chronically stressed animals (Karst and Joëls, 2003). A similar dichotomy between effects of corticosterone in CA1 and dentate cells was earlier also observed for field potential responses (Stienstra et al., 2000) as well as responses mediated by the serotonin-1A receptor (Karten et al., 2001).

The corticosterone-induced increase in synaptic responses presently seen in the CA1 area was restricted to the AMPA receptor mediated component as the NMDA receptor mediated component appeared to be unaffected. The latter was determined by subtracting the APV-insensitive eEPSC from the overall response. Since not all NMDA receptor mediated effects seem equally sensitive to APV-blockade (Rosenblum et al., 1997) such putative APV-insensitive NMDA currents could have escaped our attention when using this subtraction approach. However, the second method of analysis for NMDA currents, determining currents 100 ms after depolarization to +40 mV, by which time AMPA currents are inactivated (Otmakova et al., 2002; Karst and Joëls, 2003), yielded similar results, i.e. that the NMDA receptor mediated component of the eEPSC is not affected by corticosterone. The paired pulse response ratio was also unchanged. Collectively, the data indicates that corticosterone slowly increases the responses mediated by AMPA receptors in the postsynaptic density.
The presently observed effects seen several hours after corticosterone application are probably accomplished via the intracellular GR, given the effectiveness of the highly selective GR agonist RU 28362 (Philibert and Moguilevsky, 1983). Incidentally, the fact that RU 28362 yielded highly comparable results as seen with corticosterone in a different (independently recorded) population of CA1 neurons supports that variations introduced by the non-homogeneity of the CA1 pyramidal cells did not confound our results. The influence of this presumed non-homogeneity was also limited by recording from relatively large number of cells per group (at least 15 cells in the control and corticosterone treated groups). The timecourse and involvement of the GR are compatible with a gene-mediated process, much like the effects on calcium current amplitude and 5-HT-1A receptor mediated responses described earlier (Joëls et al., 1991; Karst and Joëls, 1991; Kerr et al., 1992; Karst et al., 1994). The effects on calcium currents and 5-HT-1A receptor mediated responses, using the same experimental protocol, were found to involve DNA-binding of GR homodimers (Karst et al., 2000). This may also be the case for the present actions on AMPA receptors, although protein-protein interactions of the GR with other transcription factors cannot be ruled out. Transcriptional regulation may be targeted directly at AMPA receptor subunit genes, but also at proteins that are involved in the recruitment of AMPA receptors in the postsynaptic density, like Homer-1a/Vesl-1S (Hennou et al., 2003). Interestingly, very recently swim stress was shown to induce a GR-dependent increase in AMPA receptor mediated synaptic responses of dopaminergic neurons in ventral tegmental area slices, prepared 24 hrs after the stress (Saal et al., 2003). It was demonstrated that this effect of stress in midbrain neurons requires the presence of GluR1 receptors (Dong et al., 2004). Possibly, GR activation leads to insertion of GluR1-subunit containing receptors in the postsynaptic density. If so, this did not lead to discernable changes in rectification properties (Lerma et al., 1994) under the present recording conditions.

By this delayed action, corticosterone could potentially enhance postsynaptic aspects of glutamatergic transmission in the CA1 area in a slow manner. We observed,
however, that the eEPSC amplitude associated with maximal stimulus intensity was only enhanced within a time-window of 2-4 hrs after a brief corticosterone administration. Thus, within the first hours responses were somewhat variable and no consistent enhancement was observed. Probably, at these early timepoints gene-mediated events are not yet fully developed. Non-genomic actions, however, may take place at this timepoint, particularly when one applies very high concentrations of corticosterone; this may be one of the explanations why some earlier studies observed a fast and rapidly reversible change in the field EPSP response while applying a high concentration of corticosterone to the slice (e.g. Vidal et al., 1986). At later moments (>4hrs) the eEPSC amplitude was also not enhanced and even showed a tendency towards a decrease. Indeed, earlier field potential studies reported either no effect or even a decline of the maximal field response after corticosterone treatment (e.g. Alfarez et al., 2002). It should be noted that these data were often based on recordings obtained with a relatively long delay (>3 hrs) after corticosterone treatment. We speculate that fibers cut by the slicing procedure and synaptic terminals as well as distal dendrites slowly deteriorate and become dysfunctional in our preparation (Schurr et al., 1984). This effect may be exacerbated by corticosterone treatment. Also, GR activation may slowly lead to dendritic retraction, which could influence our present recordings. Three observations support this speculation. First, slices subjected to GR activation showed a gradual decline in synaptic responses with repeated stimulation, suggesting a stronger decline in synaptic machinery after glucocorticoid exposure (Joëls and de Kloet, 1993). Second, ionic currents which are generated in distal dendrites of CA1 neurons slowly decline after glucocorticoid treatment or stress (but not control conditions), as does the membrane capacitance which forms an indicator for the total cellular membrane surface (Joëls et al., 2003). Finally, preliminary data in organotypic hippocampal slice cultures shows that corticosterone through a GR-dependent mechanism reduces the apical dendritic surface of CA1 pyramidal neurons (Alfarez et al., 2004). Alternatively, other differences between the present and earlier recording situations, e.g. the blockade of
GABAa receptors applied in the present series, may explain the discrepancies with respect to glucocorticoid actions. To allow a better comparison between whole cell patch clamp and field potential recordings with regard to corticosteroid effects, it will be helpful to study the effect of corticosterone on field potentials in the presence of bicuculline, in future.

In summary, the present study shows that postsynaptic aspects of AMPA receptor mediated synaptic responses in CA1 hippocampal neurons are slowly increased after a brief activation of glucocorticoid receptors through a delayed, presumably gene-mediated mechanism. This effect lasts for several hours in the slice preparation but, at least in vitro, seems to decline with longer intervals. In this way, stress may—for a restricted period of time—effect the overall output of the hippocampal formation, including the trans-synaptic inhibition of the hypothalamic feedback site (Herman and Cullinan, 1997), as well as behavioral processes which critically depend on glutamatergic transmission in the hippocampus such as learning and memory.

References


Bleakman D, Ballyk BA, Schoepp DD, Palmer AJ, Bath CP, Sharpe EF, Woolley ML, Bufton HR, Kamboj RK, Tarnawa I and Lodge D. Activity of 2,3-benzodiazepines at

**Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L and Levin N.**


**Hesen W and Joëls M.** Modulation of 5HT1A responsiveness in CA1 pyramidal neurons by in vivo activation of corticosteroid receptors. *J Neuroendocrinol* 8: 433-438, 1996.


Table I

Averaged (mean ± SEM) values for mEPSC amplitude, frequency, rise time and time constant of the decay in the control group (n=15) and in cells treated with either 100 nM corticosterone (n=19, CORT) or RU 28362 (n=6) for 20 min, 1-4 hrs prior to recording. Data were tested with a Student’s t-test (* p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CORT</th>
<th>RU 28362</th>
</tr>
</thead>
<tbody>
<tr>
<td>mEPSC amplitude</td>
<td>21.5 ± 1.6</td>
<td>29.5 ± 1.0 *</td>
<td>30.8 ± 2.2 *</td>
</tr>
<tr>
<td>mEPSC frequency</td>
<td>0.69 ± 0.10</td>
<td>0.80 ± 0.14</td>
<td>0.87 ± 0.13</td>
</tr>
<tr>
<td>mEPSC rise time</td>
<td>1.52 ± 0.09</td>
<td>1.53 ± 0.04</td>
<td>1.57 ± 0.05</td>
</tr>
<tr>
<td>mEPSC τ of decay</td>
<td>13.8 ± 0.8</td>
<td>10.8 ± 0.6</td>
<td>12.7 ± 0.3</td>
</tr>
</tbody>
</table>
Legends

Figure 1
Miniature excitatory postsynaptic potentials (mEPSCs) recorded at -70 mV in CA1 hippocampal neurons are mediated by AMPA receptors.

A. Typical trace showing the frequency and kinetic properties (inset) of mEPSCs in a CA1 neuron.

B. mEPSCs recorded at -70 mV were blocked by the non-NMDA receptor antagonist CNQX (10 µM). The numbers (1 through 6) indicate the order of the consecutive traces. Application of CNQX is indicated by the stippled line.

C. The AMPA receptor blocker GYKI 53655 (50 µM) rapidly blocked mEPSCs. The numbers (1 through 6) indicate the order of the consecutive traces. Application of GYKI 53655 is indicated by the stippled line.

Figure 2
Corticosterone induces a delayed enhancement of the mEPSC amplitude.

A. Typical traces illustrating that mEPSC amplitude was enhanced at >1 hr after a 20 min corticosterone application to hippocampal slices.

B. Frequency histogram for the distribution of the amplitude (left) or ln(amplitude) of mEPSCs in CA1 pyramidal neurons after control treatment (gray bars) or treatment with 100 nM corticosterone (open bars, fat lines). A shift towards larger amplitudes was observed after hormone treatment. The histograms are based on 3960 events in cells from vehicle (n=10) and 4216 events in cells (n=10) from corticosterone treated slices.

C. The cumulative frequency histogram shows a marked shift towards larger amplitude mEPSCs after corticosterone treatment.

Figure 3
The GR-selective agonist RU 28362 enhances the mEPSC amplitude.

A. Frequency histogram for the distribution of the amplitude (top) or ln(amplitude) of mEPSCs in CA1 pyramidal neurons after control treatment (gray bars) or treatment with 100 nM RU 28362 (open bars, fat lines). A shift towards larger amplitudes was observed after treatment with the selective GR agonist. The histograms are based on 2161 events in cells from vehicle (n=6) and 2226 events in cells (n=7) from RU 28362 treated slices.
B. The cumulative frequency histogram shows a shift towards larger amplitude mEPSCs after RU 28362 treatment, comparable to what was seen after corticosterone administration.

**Figure 4**
Corticosteroid effects on AMPA receptor mediated eEPSC.

A. The averaged input-output curve constructed at a holding potential of -70 mV shows increased amplitude of eEPSCs 1-4 hrs after corticosterone treatment (n=22) compared to the control situation (n=26). Over the range of 278 to 498 \( \mu \)A, the difference attained statistical significance (p=0.04).

B. As shown in the scatter plot, a time-dependent change in eEPSC amplitude seemed to occur after corticosterone treatment. Relatively large responses were observed between 2 and 4 hrs after corticosterone administration (individual data represented by a closed square), while responses recorded at earlier time-points but particularly at a longer delay were small. The average of the control responses, which did not display such a time-dependency (see text), is indicated by the striped line.

C. Voltage dependency of the evoked AMPA receptor mediated currents was not affected by corticosteroid treatment (vehicle: n=13; corticosterone: n=14). A small rectification at depolarized membrane potentials was seen in both the control and hormone treated groups. Responses were induced by half maximal stimulus intensity. The currents were normalized to the response obtained at -70 mV.

D. The ratio of the response amplitude induced by the second stimulus divided by the response to the first stimulus in a double pulse stimulation protocol with half maximal intensity (interval 100 ms, typical example on top) was comparable in corticosterone treated cells (n=11) and vehicle treated cells (n=11). We selected a 100 ms interval, so that the first synaptic response was normalized before the second stimulation was delivered.

**Figure 5**
Glucocorticoid effects on glutamate transmission are selective for the AMPA receptor.

A. AMPA receptor mediated responses were determined by the peak amplitude of the response evoked at +40 mV in the presence of APV. By subtracting this signal from the overall response before APV administration the NMDA receptor mediated responses were obtained.
B. AMPA receptor mediated responses were enhanced (p=0.004) at >1 hr after corticosterone treatment (n= 10 cells; left), compared to the control group (n= 10 cells). By contrast, no change was observed for the NMDA receptor mediated responses (right).

Figure 1
Figure 2

A

vehicle

corticosterone

10 pA
10 s

B

normalized frequency

amplitude (-pA)

C

cumulative frequency

amplitude (nA)

vehicle
corticosterone
Figure 3
Figure 4
Figure 5