Corticosteroid effects on glutamatergic transmission and fear memory
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Chapter 6
Corticosterone regulates NMDA receptor mediated synaptic function via GluN2B subunits

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In preparation
Abstract

Corticosteroid hormones, within minutes, increase AMPA receptor mediated synaptic transmission and facilitate long-term potentiation (LTP). Here we examined in hippocampal primary cultures whether corticosterone also alters NMDA receptor function which is critical for activity-dependent changes in synaptic transmission. We report that brief application of corticosterone (50-100 nM) rapidly increases the surface expression and synaptic content of GluN2B but not GluN2A subunits in primary hippocampal cell cultures, an effect that remained stable for at least 30 minutes. Furthermore, corticosterone enhanced the amplitude as well the area under the curve of spontaneous NMDA receptor currents, but did not affect the frequency or decay time. These physiological effects were absent in cells which were treated with the GluN2B antagonist Ro 25-6981. Overall, the results suggest that corticosteroid hormones rapidly increase NMDA receptor function via regulation of the GluNR2B subunit. Since this subunit determines calcium permeability of NMDA receptors and facilitates LTP, this may provide a cellular mechanism via which corticosteroid hormones facilitate synaptic plasticity.
Introduction

Exposure to stressful experiences increases the release of corticosteroid hormones from the adrenal glands (de Kloet et al., 2005). Corticosteroids (cortisol in humans, corticosterone in rodents) activate the high-affinity mineralocorticoid receptor (MR) and when plasma corticosteroid levels rise they also activate the lower affinity glucocorticoid receptor (GR) (de Kloet et al., 2005). Via activation of MRs and GRs, corticosteroids facilitate behavioural adaptation to stressful experiences which involves a.o. selection of adaptive behavioural strategies (Schwabe et al., 2010) and enhanced memory consolidation of emotionally arousing experiences (Roozendaal et al., 2009).

Glutamatergic synaptic transmission is critical for activity-dependent changes in synaptic function in networks which underlie behavioural adaptation such as memory formation (Malinow and Malenka, 2002; Kessels and Malinow, 2009). Corticosterone dynamically regulates hippocampal glutamatergic synaptic transmission; within minutes after administration. The hormone increases neurotransmitter release probability, AMPA receptor (AMPAR) mobility and facilitates synaptic long-term potentiation (LTP) in hippocampal neurons (Karst et al., 2005; Wiegert et al., 2006; Groc et al., 2008; Martin et al., 2009). Hours after a brief administration, corticosterone increases AMPAR postsynaptic transmission via a process that requires activation of GRs and protein synthesis, promotes retention of AMPARs and suppresses LTP, both in hippocampal and prefrontal neurons (Alfarez et al., 2002; Karst and Joëls, 2005; Wiegert et al., 2005; Groc et al., 2008; Yuen et al., 2011).

Postsynaptic NMDA receptors (NMDAR) are required for activity-dependent synaptic plasticity such as LTP and various forms of learning and memory (Tsien et al., 1996; Kessels and Malinow, 2009; Bliss and Collingridge, 2013). NMDARs are hetero-tetramers comprising various combinations of GluN1, GluN2A-D, and GluN3 subunits (Paoletti et al., 2013). In the hippocampal formation, the most abundant NMDAR subtypes are composed of GluN1 subunits associated with GluN2A and/or GluN2B subunits (Paoletti et al., 2013). The ratio between GluN2A and GluN2B-NMDARs is not uniform and can be
rapidly altered; it may play a role in metaplasticity (Tovar and Westbrook, 2002; Groc et al., 2006; Bellone and Nicoll, 2007; Zhao et al., 2008; Matta et al., 2011).

Recent studies have suggested that corticosterone alters NMDA receptor mediated synaptic transmission and plasticity in hippocampus (Tse et al., 2011) and prefrontal cortex (Yuen et al., 2009; 2011), possibly through altering the synaptic GluN2A/GluN2B ratio. We investigated here whether corticosterone, within minutes after application, alters NMDA receptor function in hippocampal primary cultures. We focused on changes in GluN2B subunits localization and functionality following corticosterone exposure, using live cell imaging and electrophysiological approaches.

Materials and Methods

Primary hippocampal culture

The experiments were carried out with permission of the local Animal Committee of the University of Amsterdam. Primary hippocampal neurons were prepared from E18 pregnant Wistar rats. The hippocampus was dissected from E18 embryos and digested with 2.5% trypsin (Sigma, USA). Neurons were plated in Neurobasal medium (Invitrogen USA) supplemented with 2% B27 (Invitrogen USA), 0.5 mM glutamax and penicillin/streptomycin (GIBICO USA) and 5% Fetal Bovine Serum (FBS) (GIBICO, USA, only for the first day) on 12-mm glass coverslips pre-coated with 0.1 mg/mL poly-L-lysine. Neurons (40,000 cells per coverslip) were fed once a week for 3 weeks in Neurobasal medium supplemented with 2% B27 and penicillin/streptomycin. 5-Fluoro-2’-Deoxyuridine (FUDR) 10 μM was used to inhibit glial growth. All experiments were carried out in cultures DIV14-DIV21. For live imaging, neurons were transfected at 7 to 14 DIV using the effecten transfection kit (QIAGEN), using the provider’s protocol.

Time-lapse imaging

Neurons co-transfected with Homer1c-DsRed and either GluN2A-SEP, GluN2B-SEP or GluN1-SEP were placed on the heated stage (37°C) of an inverted confocal spinning-disk microscope (Leica, Germany). To test the population of surface GluN subunits-SEP,
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We used a low pH-solution adjusted to pH 5.4 which quenched all the fluorescence confirming that SEP allows the specific visualization of surface receptors. Fluorescence was excited using a monochromator, and cluster fluorescence intensity was followed over time to assess synaptic receptor content. Corticosterone (50 nM) was applied after a 5 min baseline; next, the medium was carefully replaced by new equilibrated and heated medium after the protocol application. Clusters were imaged over a total period of 35 minutes. Fluorescence intensity was measured using Metamorph software (Universal imaging, USA) and corrected for photobleaching and background noise.

Electrophysiology (mEPSCs)
Coverslips were placed in a recording chamber mounted on an upright microscope (Zeiss Axioskop 2 FS Plus, Germany). We used Mg2+ free extracellular solution which contained the following component (in mM): 140 NaCl, 5 KCl, 3 CaCl2, 10 Glucose, 10 HEPES, 0.0025 TTX, 0.02 bicuculline, 0.005 NBQX, pH 7.4 (310 mOsm), and kept the coverslip fully submerged. Corticosterone (100 nM) or vehicle solution (<0.1% ethanol) was added directly into the extracellular solution while recording. In a separate series of experiments, testing the involvement of GluN2B subunits, the selective GluN2b-blocker Ro 25-6981 (3 μM, Tocris) or vehicle (DMSO) was also directly added into the extracellular solution while recording. The two series unfortunately could not be combined, because recordings became too unstable when the solution was altered several times in succession (data not shown).

Whole cell patch clamp recordings were made using an AXOPATCH 200B amplifier (Axon Instruments, USA), with electrodes from borosilicate glass (1.5 mm outer diameter, Hilgerberg, Malsfeld, Germany). The electrodes were pulled on a Sutter (USA) micropipette puller. The pipette solution contained (in mM): 120 Cs methane sulfonate; CsCl (17.5); HEPES (10); BAPTA (5); Mg-ATP (2); Na-GTP (0.5); QX-314 (10); pH 7.4, adjusted with CsOH; pipette resistance was between 3–6 MΩ. Under visual control (40X objective and 10X ocular magnification) the electrode was directed towards a neuron with positive pressure. Once sealed on the cell membrane (resistance above 1 GΩ) the membrane patch under the electrode was ruptured by gentle suction and the
cell was kept at a holding potential of −70 mV. The liquid junction potential caused a shift of no more than 10 mV, which was not compensated during mEPSCs recording. Recordings with an uncompensated series resistance of <15 MΩ and <2.5 times of the pipette resistance and with a shift of <20% during the recording were accepted for analysis. Data acquisition was performed with PClamp 8.2 and analyzed off-line with MiniAnalysis 6.0.

**Statistics**

Statistical analyses were calculated using Prism 5 (GraphPad software, Inc). Data are expressed as mean ± S.E.M. Unpaired and paired Student’s t-tests were performed when required.

**Results**

*Corticosterone acutely alters GluN2B but not GluN2A-NMDAR surface distribution and dynamics.*

In order to explore the dynamic interplay between corticosterone exposure and the trafficking of GluN2-NMDAR subtypes in live hippocampal neurons, we first expressed in cultured hippocampal neurons the GluN2A or GluN2B subunit fused to a Super Ecliptic pHluorin (SEP) at its extracellular N-terminus (GluN2B-SEP). SEP is a pH sensitive variant of GFP that only emits fluorescence at neutral pH; fluorescence is quenched at acidic pH, such as in intracellular vesicles. This tool allowed us to study surface GluN2A/B-NMDAR separately from intracellularly located subunits.

We imaged the surface clusters of GluN2A- and GluN2B-NMDAR before and during exposure to 50 nM corticosterone (Figure 1A). For GluN2A-NMDAR surface clusters (colocalizing with synaptic markers), there was no significant change in the cluster fluorescence intensity over time (Figure 1A-C), indicating that the synaptic content of GluN2A-NMDAR remains stable after an acute exposure to corticosterone. For GluN2B-NMDAR, however, there was a rapid increase in the cluster fluorescence intensity, already observed 5 min after corticosterone incubation (Figure 1A-C). In addition,
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the corticosterone-induced GluN2B-NMDAR synaptic content increase remained stable over a period of more than 30 min (Figure 1B). Together, these data show that corticosterone acutely favors the clustering of surface GluN2B-NMDAR, likely changing the GluN2A/GluN2B-NMDAR synaptic ratio.

To directly address this point, we then transfected hippocampal neurons with GluN2A and GluN2B subunits containing different extracellular tags in order to measure their relative content in synapses (labeled by the expression of Homer 1c-DsRed) (Figure 1D). The application of corticosterone rapidly increased the synaptic content of GluN2B and significantly reduced the 2A/2B synaptic ratio (Figure 1E).

Corticosterone increases amplitude and charge area of NMDAR mEPSCs

To examine the effect of corticosterone on NMDA receptor function, we applied corticosterone (100 nM) to hippocampal cultures neurons. Traces of NMDA mEPSCs
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Figure 2: Corticosterone increases amplitude and charge of NMDARs-mEPSCs.
A. Representative traces (at two time resolutions, see calibration bars) of NMDARs-mEPSCs after vehicle (<0.1% ethanol) and corticosterone (100 nM). B. Cumulative percentage distribution and histograms showing the interval of NMDARs-mEPSCs after vehicle (n=14 cells) and corticosterone (n=19 cells) treatment. C. Cumulative percentage distribution and histograms showing the peak amplitude of NMDARs-mEPSCs after vehicle (n=14 cells) and corticosterone (n=19 cells) treatment. D. Cumulative percentage distribution and histograms showing the decay of NMDARs-mEPSCs after vehicle (n=14 cells) and corticosterone (n=19 cells) treatment. E. Cumulative percentage distribution and histograms showing the charge of NMDARs-mEPSCs (area under the curve) after vehicle (n=14 cells) and corticosterone (n=19 cells) treatment.

Figure 3: Corticosterone effects on NMDARs function via modulating GluN2B subunit. A. Representative traces (at two time resolutions, see calibration bars) of NMDARs-mEPSCs after Ro 25-6981 (3 μM) and Ro 25-6981 (3 μM) plus corticosterone (100 nM). B. Cumulative percentage distribution and histograms showing the interval of NMDARs-mEPSCs after Ro 25-6981 (n=9
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are shown in Figure 2A. Analysis of the data indicates that corticosterone increases the peak amplitude and the area under the curve (charge) of NMDAR mEPSCs when compared to vehicle treated cells (Figure 2C, E). Corticosterone did not affect the frequency (Figure 2B) or decay time (Figure 2D) of NMDAR mEPSCs.

Corticosterone effects on NMDAR function occur via GluN2B

We next investigated how corticosterone regulates NMDA receptor function. By using Ro 25-6981, which is a potent and selective activity-dependent blocker of NR2B containing NMDA receptors, we tested whether corticosterone effects on NMDA receptor function are mediated via GluN2B. Traces of NMDA mEPSCs are shown in Figure 3A. In the presence of Ro 25-6981 corticosterone did not alter the frequency, amplitude, decay time or area of NMDA mEPSCs (Figure 3C-E).

Discussion

NMDA receptor activation is critical for activity-dependent changes in synaptic plasticity such as long-term potentiation (LTP) (Kessels and Malinow, 2009) as well as learning and memory (Tang et al., 1999; Shimizu, 2000; Lisman et al., 2012). Corticosteroid hormones strongly regulate AMPA receptor-mediated synaptic transmission and LTP (Karst et al., 2005; Wiegert et al., 2006). Corticosterone, within minutes, increases synaptic transmission and facilitates synaptic plasticity (LTP) ((Karst et al., 2005; Wiegert et al., 2006; Groc et al., 2008). Here we report that corticosterone, also within minutes, increases the amplitude of NMDA receptor-mediated currents as well the area under the curve of NMDA currents. This agrees with a recent report indicating that exposure to corticosterone increases hippocampal NMDA receptor-mediated synaptic transmission within 0-30 minutes after administration of the hormone (Tse
et al., 2011). Our effects were absent in cells treated with the GluN2B antagonist Ro 25-6981 suggesting that corticosteroid hormones increase NMDA receptor function via regulation of the GluNR2B subunit. Accordingly, the relatively fast effects by corticosterone on receptor function were accompanied by a fast redistribution and increase in synaptically localized GluN2B-NMDAR, as observed with high-resolution at the single molecule level. This supports the view that corticosterone rapidly reorganizes synaptic NMDA receptors in hippocampal networks to promote synaptic alterations.

It remains to be determined how corticosterone rapidly regulates NMDA receptor function. Recent studies have shown that corticosterone within minutes affects AMPAR mediated synaptic function (Karst et al., 2005; Groc et al., 2008) via activation of mineralocorticoid receptors (MRs). In line with this, preliminary evidence suggests that the effects of corticosterone on NMDARs are also mediated by MRs (Laurent Groc, personal communication). The signalling pathway between (putative) MR activation and NMDAR mobility and/or function, however, still needs to be entirely resolved.

Taken together, recent data suggests that corticosterone via MRs and within minutes is able to regulate glutamatergic synaptic transmission via multiple pathways, at least in the hippocampus: via neurotransmitter release probability, AMPAR (particularly GluA2) mobility, NMDAR enrichment in the synaptic fraction and NMDA receptor function, the latter two mainly involving GluN2B. Importantly, the GluN2B subunit largely determines the calcium permeability of NMDA receptors (Lisman et al., 2012) and enhanced GluN2B expression facilitates LTP and memory formation (Tang et al., 1999). The presently observed increase in GluN2B function may therefore provide a molecular mechanism to explain how corticosterone, within minutes after administration, is able to facilitate long-term potentiation (Wiegert et al., 2005), possibly in concert with increased neurotransmitter release (Karst et al., 2005) and enhanced AMPAR mobility (Groc et al., 2008). It is tempting to speculate that these rapid changes in synaptic function and synaptic plasticity reflect adaptations in neuronal network to promote storage of relevant information and facilitate behavioural adaptation to stressful experiences.
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