Local facilitation of hippocampal metabotropic glutamate receptor-dependent long-term depression by corticosterone and dexamethasone

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Received 29 August 2007; received in revised form 21 December 2007; accepted 23 December 2007

**Summary**

Long-term potentiation and long-term depression (LTD) of synaptic efficacy, two major forms of synaptic plasticity, are believed to underlie learning processes and memory storage. We have recently shown that acute stress, through corticosterone release and stimulation of glucocorticoid receptors (GRs), facilitates the LTD elicited by the group 1 metabotropic glutamate receptor (mGluR) agonist \((\text{R,S})-3,5\text{-dihydroxyphenylglycine (DHPG)}\) in hippocampal CA1 neurons. However, it is unknown whether sustained corticosterone release, per se, is also able to facilitate DHPG-elicited LTD in control (i.e. unstressed) conditions, and if so, whether it acts on local (i.e. hippocampal) or distant GRs. Here, we show that a brief application of 100 nM corticosterone to rat hippocampal slices lowers the threshold for DHPG-elicited LTD, an effect mimicked by the local application of the GR agonist dexamethasone. These results show that high corticosterone release facilitates hippocampal CA1 mGluR-dependent LTD, and does so through local GRs.

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1. Introduction

Synaptic plasticity, which refers to enduring activity-dependent increases and decreases in synaptic transmission, is thought to provide the neural bases for learning and memory (Martin et al., 2000). Because acute/chronic stress is endowed with profound consequences on memory storage and retrieval (Roozendaal, 2002), the observation...
that long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy, i.e. two major forms of synaptic plasticity, are sensitive to stressful events (see below) adds further support for a link between synaptic plasticity and memory processes.

The hippocampal CA1 area is one of the anatomical locations where acute stress exerts metaplasticity by modifying the thresholds needed to allow subsequent synaptic plasticity (Kim and Diamond, 2002; Alfarez et al., 2006). Specifically, acute stress impedes the ability of hippocampal pyramidal neurons to undergo high-frequency stimulation–elicited LTP whilst it facilitates the expression of LTD following low-frequency stimulation (Foy et al., 1987; Shors et al., 1989; Kim et al., 1996; Xu et al., 1997). The use of selective agonists/antagonists for corticosterone receptors, namely mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs), has demonstrated that the aforementioned effects of stress upon synaptic plasticity rely on stress-induced corticosterone release and in turn the stimulation of GRs (Xu et al., 1998; Yang et al., 2004). This result is in line with past evidence for GRs, which display low affinity for corticosterone, to be occupied by high circulating concentrations of corticosteroids, e.g. during the active phases of the diurnal cycle and during stress (De Kloet, 1991).

Both low- and high-frequency stimulation–elicited changes in synaptic plasticity in the hippocampal CA1 area are mediated by glutamatergic NMDA receptors (NMDARs) (Malenko and Bear, 2004). However, in addition to NMDAR-dependent LTD (NMDA-LTD), another form of LTD exists in hippocampal CA1 which can be evoked by the selective group 1 metabotropic glutamate receptor (mGlUR) agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) (Palmer et al., 1997; Huber et al., 2000, 2001). Indeed, this mGlUR-dependent form of LTD was also found to be facilitated by prior acute stress (Chaouloff et al., 2007). The additional observation that such a facilitatory effect of stress on DHPG-induced LTD (DHPG-LTD) was prevented by a systemic pretreatment with the GR antagonist RU38486 indicated that corticosterone stimulation of GRs was a key step in stress-induced facilitation of DHPG-LTD (Chaouloff et al., 2007).

The observation that the facilitatory effect of acute stress on DHPG-LTD was accounted for by corticosterone release leads to two complementary questions. First, does the application of a high-corticosterone concentration (as measured during stress) to slices from control animals mimic the facilitatory influence of stress-elicited corticosterone release on DHPG-LTD? If so, in keeping with the ubiquitous location of brain GRs, including in the hippocampus (De Kloet, 1991), does corticosterone act through local (i.e. hippocampal) or distant GRs? To solve these issues, the present study has examined by means of two-week-old Sprague–Dawley rat pups and their mothers (Janvier, Le Genest Saint Isle, France) were kept in temperature- and humidity-controlled quarters under a 12 h light/dark cycle (lights on at 07:00 AM). Food and water were provided ad libitum. Rats were weaned 1 week after their arrival, housed collectively by gender, and used between postnatal days (PN) 21–42, as described earlier (Chaouloff et al., 2007). All experiments were conducted in strict compliance with European directives and French laws on animal experimentation (authorization number 06369), and all efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Corticosterone and dexamethasone treatments

Rats, which were manipulated in the morning (when circulating corticosterone levels are low), were directly transferred from their home cages to an individual box saturated with isoflurane (to minimize circulating corticosterone variations). Rats were decapitated under deep anesthesia, the brains dissected out and sliced (400 μm) in the horizontal plane using a vibratome (Integraslice; Campden Instruments, Leicester, UK). Slices were collected in an ice cold dissection buffer containing (in mM) 126 NaCl, 18 NaHCO3, 2.5 KCl, 2.4 MgCl2, 1.2 CaCl2, 1.2 NaH2PO4 and 11 glucose, under constant saturation with 95% O2/5% CO2. Two cuts were applied to the slices, the first as to keep only the hippocampus and part of the cortex (auditory, temporal, perirhinal and/or entorhinal), and a second between areas CA3 and CA1 of the hippocampus to prevent epileptic activity. Slices were then transferred for 2 h in a storage chamber filled with a temperature-controlled (30 ºC) artificial CSF (ACSF) containing (in mM) 126 NaCl, 18 NaHCO3, 2.5 KCl, 1.2 MgCl2, 2.4 CaCl2, 1.2 NaH2PO4 and 11 glucose, under constant saturation with 95% O2/5% CO2. Two cuts were applied to the slices, the first as to keep only the hippocampus and part of the cortex (auditory, temporal, perirhinal and/or entorhinal), and a second between areas CA3 and CA1 of the hippocampus to prevent epileptic activity. Slices were then transferred for 2 h in a storage chamber filled with a temperature-controlled (30 ºC) artificial CSF (ACSF) containing (in mM) 126 NaCl, 18 NaHCO3, 2.5 KCl, 1.2 MgCl2, 2.4 CaCl2, 1.2 NaH2PO4 and 11 glucose, under constant saturation with 95% O2/5% CO2. This protocol was repeated twice with a 4 h period, half of the slices were treated for 20 min at 30 ºC with either 100 nM corticosterone (first series of experiments) or 10 nM dexamethasone (second series of experiments) before being returned to the initial storage chamber for another 1–4 h period.

2.3. Electrophysiological recordings

Interleaved control and corticosteroid-treated slices were successively placed in a submersion-type chamber continually perfused (2 ml/min) with a 30 ºC oxygenated ACSF containing 100 μM picrotoxin. Schaffer collateral afferents in stratum radiatum were stimulated (0.033 Hz, 100 μs duration) using bipolar tungsten electrodes. The stimulation intensity (0.20–0.25 mA) was chosen to evoke 40–60% of the maximal responses. The recording pipette was filled with ACSF and placed in CA1 stratum radiatum. Both the field excitatory postsynaptic potential (fEPSP) slope and amplitude were measured (graphs depict amplitude). An Axopatch-1D (Axon Instruments, Foster City, CA, USA) was used to record the data, which were filtered at 1–2 kHz, digitized at 5 kHz on a DigiData 1200 interface (Axon Instruments, Foster City, CA, USA), and collected on a personal computer using Clampex 9.2 (Axon Instruments, Foster City, CA, USA). These data were then analyzed using Clampfit 9.2 (Axon Instruments, Foster City, CA, USA). In all experiments, DHPG (10–100 μM) was applied for 10 min and its effects on...
fEPSPs monitored up to 50 min after the onset of that application (Rouach and Nicoll, 2003). In the text, calculations of maximal short-term depressive effects of DHPG refer to the 5-min period showing the highest inhibition of fEPSP responses whereas calculations on long-term effects of DHPG refer to the last 5 min of the washout period.

2.4. Drugs

(R,S)-DHPG was from Tocris (Fischer Bioblock, Illkirch, France). Picrotoxin, corticosterone (water-soluble 2-hydroxypropyl-β-cyclodextrin complex) and dexamethasone were from Sigma-Aldrich (Saint Quentin Fallavier, France). DHPG (20 mM) and corticosterone (1 mM) were stocked, respectively, in water and ACSF, whilst picrotoxin (100 mM) and dexamethasone (10 mM) were stocked in DMSO (all stocks kept frozen). Note that DHPG stocks were freshly prepared every week.

2.5. Statistics

All data, presented as mean±standard error of the mean (SEM), were compared using analyses of variance with repeated measures followed, if significant, by Tukey’s multiple comparison tests. In all tests, the significance level was preset to $P<0.05$.

3. Results

We first asked whether a 20 min-pretreatment (1–4 h beforehand) of the slices with 100 nM corticosterone would affect synaptic strength. As shown in Figure 1A, an analysis of the respective input–output relationships in control slices and in corticosterone-pretreated slices revealed no differences between treatments, suggesting that corticosterone did not affect synaptic strength.

We next investigated the influence of corticosterone pretreatment on the changes in excitatory synaptic transmission elicited by a 10-min application of 10–100 μM DHPG. When applied at the lowest concentration (10 μM), DHPG did not elicit long-term changes in fEPSP amplitude either in control slices or in corticosterone-pretreated slices ($F_{(1,12)} = 4.12$, NS; data not shown). On the other hand, corticosterone pretreatment affected the long-term fEPSP responses to a 25 μM application of DHPG ($F_{(1,16)} = 10.62$, $P = 0.005$) for the interaction between corticosterone pretreatment and DHPG application), as 25 μM DHPG triggered LTD in corticosterone-pretreated slices (82±4% of baseline levels, $P<0.01$), but not in control slices (Figure 1B). At higher concentrations, DHPG triggered LTD in both control slices and corticosterone-pretreated slices ($F_{(1,11)} = 33.41$, $P = 0.0001$) and $F_{(1,12)} = 82.70$, $P<0.0001$) for the respective influences of 50 and 100 μM DHPG on long-term fEPSP amplitudes.

Figure 1  Pretreatment (20 min, 1–4 h beforehand) with 100 nM corticosterone facilitates mGluR-LTD in the rat hippocampal CA1 region. (A) Corticosterone pretreatment does not affect fEPSP responses to increasing stimulus intensities. Data are represented as mean±SEM. (B) A 10-min application of 25 μM DHPG elicits LTD in corticosterone-pretreated slices, but not in control slices Inset: representative fEPSPs taken at the time indicated on the graph below. Calibration: 0.5 mV, 10 ms. (C and D) A 10-min application of 50 μM (C) or 100 μM (D) DHPG elicits LTD in both control and corticosterone-pretreated slices. Data are represented as mean±SEM, with each point being the average of two successive responses. Dashed lines represent 100% of baseline. See text for statistics.
Figure 1C and D). Actually, the amplitudes of these long-term DHPG-elicited decreases in fEPSP (P<0.05 in either slice group after 50 μM DHPG, and P<0.01 in either slice group after 100 μM DHPG) did not differ between control and corticosterone-pretreated slices (Figure 1C and D). All these results indicated that corticosterone pretreatment decreased (from 50 to 25 μM) the minimal concentration of DHPG required to observe long-term changes in synaptic efficacy; confirming, 25 μM DHPG was found to trigger LTD in only 55% of control slices, as opposed to 100% of corticosterone-pretreated slices.

Lastly, we examined whether the long-term facilitatory influence of corticosterone on DHPG-LTD (see above) was also observed after local stimulation of GRs, but not MRs. As observed with corticosterone pretreatment, pretreatment (1–4h beforehand) with 10 nM of the GR agonist dexamethasone affected the long-term fEPSP responses to the application of 25 μM DHPG ([(F1,14) = 13.72, P = 0.0024] for the interaction between dexamethasone and DHPG). Indeed, 25 μM DHPG promoted LTD in dexamethasone-pretreated slices (83 ± 4% of baseline levels, P < 0.01), but not in control slices (Figure 2). Confirmingly, all dexamethasone-pretreated slices, as opposed to only 43% of control slices, hosted DHPG-LTD. However, in contrast with corticosterone pretreatment, dexamethasone pretreatment affected also the short-term inhibitory influence of 25 μM DHPG ([(F1,14) = 18.36, P = 0.008] for the interaction between dexamethasone and DHPG). As illustrated in Figure 2, the amplitude of the early depression triggered by DHPG was stronger in dexamethasone-pretreated slices than in control slices (14 ± 2% and 26 ± 2% of baseline levels in dexamethasone-pretreated slices and in control slices, respectively; P < 0.01).

4. Discussion

As indicated above, the finding that acute stress facilitates, through corticosterone release, hippocampal CA1 DHPG-LTD (Chaouloff et al., 2007) opened the questions of the intrinsic influence of corticosterone in unstressed animals and the site of action (local versus distant), if any, of the hormone. One obvious means to answer these questions was to examine the impact of a local application of corticosterone on DHPG-LTD in control slices. To do so, we used a protocol involving a 20-min pretreatment with 100 nM corticosterone 1–4h before recordings. Actually, such a pretreatment allows the stimulation of MRs and GRs and, in turn, gene-dependent changes, i.e. through DNA-binding of GR homodimers (Karst et al., 2000). With regard to excitatory transmission in the CA1 area of the hippocampus, this protocol triggers time-dependent changes in miniature EPSPs (Karst and Joëls, 2005) as well as alterations in some forms of synaptic plasticity, as illustrated by the corticosterone-elicited reduction in primed burst potentiation (Alfaroz et al., 2002). Using such a protocol, we found that DHPG, at a concentration devoid of long-term influences in control slices (25 μM), was however able to promote LTD in corticosterone-pretreated slices. Interestingly, at concentrations effective in triggering LTD in control slices (50–100 μM), corticosterone pretreatment did not further affect the respective amplitudes of these LTDs. Such an observation is reminiscent of what was observed in slices from acutely stressed rats in that: (i) 50 μM DHPG was ineffective in control slices but sufficient to trigger LTD in slices from stressed rats whereas (ii) the DHPG-LTD that occurred with a 100 μM DHPG application was of similar amplitude in the two slice groups (Chaouloff et al., 2007). This suggests that corticosterone on the one hand, and DHPG on the other hand, share a common LTD expression pathway. Of note is the observation that the minimal DHPG concentrations required to trigger LTD in control slices differ between the aforementioned stress study and the present one, a difference probably accounted for by the respective delays between slice preparation and effective recordings (1–4 h in the first study, as opposed to 3–7 h in this study).

Circulating corticosterone concentrations and NMDA-LTP amplitude in hippocampal CA1 were initially found to follow an inverted U-shape relationship, with low and high concentrations of corticosterone, respectively, facilitating and preventing that form of plasticity (Diamond et al., 1992). In line with the ability of high-circulating concentrations of corticosterone to stimulate GRs (De Kloet, 1991), acute stress was reported to impair high frequency stimulation-induced LTP by means of these corticoid receptors (Foy et al., 1987; Shors et al., 1989; Kim et al., 1996; Xu et al., 1997; Yang et al., 2004). An opposite picture rapidly emerged with regard to the relationships between GR-mediated effects of corticosterone and stress on the
one hand, and the occurrence of NMDA-LTD on the other hand. Thus, GR stimulation (by exogenous agonists or by high-corticosterone concentrations) or acute stress were both found to facilitate hippocampal CA1 NMDA-LTD (Kim et al., 1996; Coussens et al., 1997; Xu et al., 1997, 1998; Yang et al., 2004), with stress-elicited facilitation of NMDA-LTD being prevented by GR antagonists (Xu et al., 1998; Yang et al., 2004). As indicated above, this GR-mediated facilitatory influence of stress extends to DHPG-LTD (Chaouloff et al., 2007). In keeping with this last observation and the present finding that the local application of a high concentration of corticosterone allowed DHPG to elicit LTD, it is likely that the stimulation of local GRs facilitates such a LTD. Indeed, our last series of experiments showing that the local application of the GR agonist dexamethasone transforms an ineffective concentration of DHPG into an LTD-promoting concentration reinforces this suggestion. The sole difference that could be noted between corticosterone- and dexamethasone-treated slices concerned the short-term inhibitory effects of DHPG which were amplified by dexamethasone, but not by corticosterone pretreatment. Such a difference may not be specific to dexamethasone but may be accounted for by the slice preparation as in our previous study the facilitatory effect of corticosterone on DHPG-LTD was either preceded or not by such an amplification (Chaouloff et al., 2007).

This study opens the question of the identity of the molecular link between GRs and group 1 mGluRs. Although such a quest is beyond the scope of this short study, it is relevant to indicate that: (i) DHPG-LTD has been shown to rely on several signal transduction cascades, among which the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK; Pfeiffer and Huber, 2006), and (ii) GR stimulation, either through exogenous agonist stimulation or during stress, activate the ERK/MAPK cascade (Yang et al., 2004; Revest et al., 2005). Because the ERK/MAPK cascade has been involved in the facilitatory effect of stress on NMDA-LTD (Yang et al., 2004), the involvement of that pathway, if any, in the facilitatory influence of GR stimulation on DHPG-LTD would indicate that these two forms of synaptic plasticity, which do not occlude each other (Huber et al., 2000, 2001), may do so when GRs are stimulated.

Taken together, the results of this study clearly show that DHPG-LTD, one major form of synaptic plasticity in the hippocampal CA1 region, is facilitated by the intrinsic activation of GRs. Assuming that LTP and LTD of synaptic efficacy allow, respectively, facilitation and disruption of cognitive processes such as learning and memory (Martin et al., 2000), the present results as well as our most recent ones (Chaouloff et al., 2007) indicate that in addition to the facilitation of NMDA-LTD (Kim and Diamond, 2002), facilitation of mGluR-LTD is another process through which learning and memory are impaired during GR stimulation and during stress.

Role of the funding sources

This work was funded by CNRS, INSERM, and Région Aquitaine. The funding sources had no role in the study design, analyses or interpretation of the data.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgments

We thank J.-M. Revest and F. Rougé-Pont (INSERM U862, Bordeaux) for helpful discussion at the early stage of this study.

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