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CHAPTER III

Corticosterone time-dependently modulates β-adrenergic effects on long-term potentiation in the hippocampal dentate gyrus

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Previous experiments in the hippocampal CA1 area have shown that corticosterone can facilitate long-term potentiation (LTP) in a rapid non-genomic fashion, while the same hormone suppresses LTP that is induced several hours after hormone application. We here elaborated this finding by examining whether in the dentate gyrus too corticosterone exerts opposite effects on LTP depending on the timing of hormone application. Moreover, we tested rapid and delayed actions by corticosterone on β-adrenergic dependent changes in LTP. Unlike the CA1 region, our in vitro field potential recordings show that rapid effects of corticosterone do not influence LTP induced by mild tetanization in the hippocampal dentate gyrus, unless GABA_A receptors are blocked. By contrast, the β-adrenergic agonist isoproterenol does initiate a slow-onset, limited amount of potentiation. When corticosterone was applied concurrently with isoproterenol, a further enhancement of synaptic strength was identified, especially during the early stage of potentiation. Yet, treatment with corticosterone several hours in advance of isoproterenol fully prevented any effect of isoproterenol on LTP. This emphasizes that corticosterone can regulate β-adrenergic modulation of synaptic plasticity in opposite directions, depending on the timing of hormone application.

Introduction

Living organisms like mammals experience stressful situations regularly in their lives. Stress, be it of a physical or psychological nature, potentially disrupts physiological homeostasis. Two systems are subsequently activated, which help to adapt to environmental changes and restore physiological balances: activation of the autonomous nervous system (ANS) triggers the release of adrenaline from the adrenal medulla; the hypothalamo-pituitary-adrenal (HPA) axis elicits secretion of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal cortex. Although glucocorticoids can cross the blood-brain barrier easily, adrenaline does not readily reach the central nervous system; however it can stimulate peripheral vagal afferents and lead to central release of noradrenaline from both the nucleus of the solitary tract and the locus coeruleus (Roosevelt et al., 2006). These hormones target many areas, including those involved in processing of the information about the stressful event, i.e. the hippocampus and the amygdala. Each of these structures is specialized in formulating specific aspects of the ongoing event; yet, their outputs integrate and underlie the memory of an overall picture of the experienced “stress” (Kim and Diamond, 2002; McGaugh, 2004; Richter-Levin, 2004).

Typically, the amygdala and the hippocampus are extensively involved in behavioral tasks like inhibitory avoidance or fear conditioning (Richter-Levin, 2004; Roozendaal et al., 2006). These behaviors are affected by corticosterone and noradrenaline. Thus, post-training microinjection of noradrenaline or β-adrenergic agonists into the basolateral amygdala enhances memory consolidation in inhibitory avoidance task (Ferry and McGaugh, 1999; Lalumiere and McGaugh, 2005), as well as in contextual fear conditioning (LaLumiere et al., 2003) and in spatial water maze task (Hatfield and McGaugh, 1999). Likewise, glucocorticoid receptor (GR) agonists micro-infused into the basolateral amygdala (Roozendaal and McGaugh, 1997b) or the hippocampus (Roozendaal

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and McGaugh, 1997a; Oitzl et al., 2001) enhance memory retention. It was subsequently shown (Roozendaal et al., 2002) that noradrenergic activation is essential for the memory-enhancing effects, and that glucocorticoids play a permissive role in noradrenergic actions thus promoting memory formation. Notably, it was suggested that the interaction between the two hormones should occur within an interval of 30 minutes, which is rather a short time-interval for gene-mediated events to occur (Makara and Haller, 2001; Roozendaal, 2003).

Yet, glucocorticoids can also suppress noradrenaline-mediated regulation. Thus, adrenalectomy impairs memory in an inhibitory avoidance task; this deficit could be rescued by post-training administration of adrenaline (Borrell et al., 1984). Pretreatment with corticosterone one hour before adrenaline dramatically reduced the efficacy of adrenaline. A similar result was also observed at the cellular level in the hippocampus (Joels and de Kloet, 1989), where noradrenaline increases the excitability of CA1 pyramidal neurons from adrenalectomized animals; treatment of tissue with GR agonists 1 – 4 hours prior to noradrenaline attenuated such increases. Given the delayed action of corticosterone, it is likely that these corticosteroid effects involve gene-mediated mechanisms (Reichardt and Schutz, 1998; Vreugdenhil et al., 2001; de Kloet et al., 2005; Zhou and Cidlowski, 2005).

Collectively, these observations have led to the hypothesis that corticosterone can enhance memory formation or boost noradrenergic actions when these elements coincide in time, whereas the hormone suppresses these activities when it reaches the same circuits some hours in advance of a learning event (Joels et al., 2006). This is in line with recent observations in the hippocampal CA1 area, showing that corticosterone can enhance long-term potentiation (LTP) – presently the best described neurobiological substrate of learning and memory (Martin and Morris, 2002; Morris, 2003), when the hormone is applied around the time of LTP induction (Wiegert et al., 2006); while it suppresses LTP when given several hours in advance (Alfarez et al., 2002; Wiegert et al., 2005).

The present study aimed to acquire further evidence for this hypothesis in the rat hippocampal dentate gyrus (DG). To this end, we first examined the effects of corticosterone alone on LTP, under conditions where the hormone was either present during tetanic stimulation or several hours in advance. Next, we tested the effects of corticosterone on noradrenergic regulation of LTP, based on the known fact that noradrenergic activation generally facilitates LTP in the dentate gyrus (Stanton and Sarvey, 1985, 1987; Dahl and Sarvey, 1990; Chaullk and Harley, 1998; Bronzino et al., 2001; Frey et al., 2001; Straube and Frey, 2003). We specifically tested: 1) if corticosterone administration around the time of LTP induction and application of the β-adrenergic agonist isoproterenol promotes the β-adrenergic action on LTP; and 2) if corticosterone administration more than 2 hours earlier suppresses the isoproterenol-mediated effect.

Materials and Methods

Animals
The Animal Committee for Bioethics of University of Amsterdam approved all of the experiments.
Male Wistar rats (Harlan CPB, the Netherlands) were housed in groups, with food and water ad libitum available. A 12 hr:12 hr light-dark cycle (light-on at 8.00 am) was maintained, and the temperature kept at 20 – 22 °C, the humidity at 55 ± 15 %. After arrival, the rats were not disturbed for at least one week before the experiments started. The body weights ranged between 200 g and 300 g at the time of the experiment.

**In vitro slice preparation**

The animals were decapitated early in the morning – between 9:25 and 10:15 hours, when plasma corticosterone levels are still quite low. The brain was rapidly removed from the skull and immersed in chilled (4 °C) dissection buffer which consists of 120 mM NaCl, 3.5 mM KCl, 5.0 mM MgSO₄·7H₂O, 1.25 mM NaH₂PO₄, 0.2 mM CaCl₂·2H₂O, 10 mM glucose and 25 mM NaHCO₃, oxygenated with 95 % O₂ and 5 % CO₂. 400 μm-thick horizontal slices were made with a vibroslicer (Leica VT1000S). Slices were kept in artificial cerebrospinal fluid (aCSF) containing 120 mM NaCl, 3.5 mM KCl, 1.3 mM MgSO₄·7H₂O, 1.25 mM NaH₂PO₄, 2.5 mM CaCl₂·2H₂O, 10 mM glucose and 25 mM NaHCO₃, oxygenated with 95 % O₂ and 5 % CO₂. Slices remained in aCSF at room temperature for 2 hours before being transferred to the recording chamber.

**Electrophysiology**

One slice at a time was transferred to the recording chamber, where the temperature was maintained at 30 – 32 °C. For recordings of field excitatory postsynaptic potentials (fEPSPs) in the hippocampal dentate gyrus, a bipolar stimulation electrode (60 μm in diameter, stainless steel, insulated except for the tip) was placed at the medial perforant pathway and a glass microelectrode (impedance 2 – 5 MΩ, filled with aCSF) was positioned within the middle third of the molecular layer in the suprapyramidal blade, serving as the recording electrode.

At the beginning of each experiment, an input-output relationship was established by gradually increasing the stimulus intensity until the maximally evoked responses were obtained. The relationship was fit with a sigmoidal function: \[ R(i) = \frac{R_{\text{max}}}{1 + \exp((i - i_h)/(-S))} \], where \( R(i) \) represents the response at the intensity \( i \), \( R_{\text{max}} \) the maximal response, \( i_h \) the intensity at which the half maximal response is observed, and \( S \) represents an index proportional to the slope of the stimulus-response curve. The intensity that evoked the half-maximal response was applied throughout the experiments. The magnitudes of the responses were assessed by measurement of both the slopes and the amplitudes of the fEPSP signals (Alfarez et al., 2003; Krugers et al., 2005). Both parameters yielded comparable results; we here only report on the slope of the fEPSP.

In most of the experiments, electrophysiological recordings were done in aCSF. In some experiments, GABAergic transmission was inhibited with 10 μM (-)-bicuculline methiodide (Sigma-Aldrich) added to the aCSF.
Protocols and drug application

In the groups of slices that were tested for rapid drug effects, baseline synaptic transmission at half maximal stimulation intensity was monitored for 10 minutes, which was followed by the perfusion of either: 1) corticosterone (Sigma-Aldrich) 100 nM, 2) the β-adrenergic agonist (-)-isoproterenol (+)-bitartrate (Sigma-Aldrich) 1.0 μM, 3) a combination of 1.0 μM (-)-isoproterenol (+)-bitartrate with 100 nM corticosterone, or 4) vehicle solution, as the control, into aCSF for 15 minutes. Perfusion co-terminated with the tetanus, which consisted of theta-burst stimulation (TBS): a burst of 4 pulses at 100 Hz, repeated 200 ms later by another 4 pulses at 100 Hz; this sequence was repeated 5 times, with an inter-train interval of 30 seconds (Alfarez et al., 2003). After TBS, synaptic responses were further monitored for 60 minutes.

For the groups of slices that were tested with corticosterone pretreatment, slices were incubated with 100 nM corticosterone at 32 °C for 20 minutes starting one hour after decapitation. These slices then remained in aCSF at room temperature for at least two hours before being transferred to the recording chamber. For one group, baseline transmission was monitored during 10 minutes, followed by a 15-minute period of perfusion with 1.0 μM (-)-isoproterenol (+)-bitartrate; this co-terminated with TBS, and synaptic responses were further observed during 60 minutes. For the other group, baseline transmission was monitored during 25 minutes in total without any perfusion period, this followed by TBS; after TBS, synaptic responses were monitored during 60 minutes.

Data analysis

Synaptic potentiation after tetanus was expressed as percentual change from the baseline; the average of the measurements during the 25 minute pre-tetanus period served as the baseline value. Changes in synaptic response beyond ± 10% from the baseline were considered not related to spontaneous fluctuation and were physiologically meaningful. A two-tailed, paired Student’s t-test was used to compare synaptic responses before versus after TBS within each group. The general linear model for repeated measures (GLM) was performed for between-group comparisons of overall differences in LTP, followed by post hoc least significant difference (LSD) multiple comparison tests. Two-tailed, unpaired Student’s t-tests were additionally conducted to examine the significances. Between-group comparisons were performed 1) for the entire 60 minutes post-tetanus period, 2) for the initial post-tetanus phase (0’ – 10’), and 3) for the later phase after tetanus (30’ – 60’). All data are expressed as average ± S.E.M.; n indicates the number of animals. P-values < 0.05 were accepted as significantly different.

Results

LTP induction in the dentate gyrus with theta burst stimulation

We observed that theta burst stimulation (TBS) alone was insufficient to evoke LTP in the dentate gyrus, as illustrated in the control group where the vehicle medium of corticosterone was perfused for 15 minutes and co-terminated with TBS (Figure 1A). In this control group, TBS resulted in an insignificant increase in the slope of the field excitatory postsynaptic potentials (mean slope fEPSP ± SEM over the post-tetanus 60 minutes: 107 ± 3 %, n = 10) compared to the pre-TBS baseline.
response. The early component of potentiation (here defined as the average fEPSP slope over post-tetanus 0’ – 10’) was 96 ± 3 % of the baseline value while the later component (defined as the average fEPSP slope during post-tetanus 30’ – 60’) amounted to 108 ± 4 %.

As suggested by earlier reports, TBS can become effective in the dentate gyrus when GABA<sub>A</sub> inhibitory activity is prevented (Alfarez et al., 2003; Boekhoorn et al., 2006). This was confirmed in the current study if the vehicle group was tested in the presence of 10 µM (-)-bicuculline methiodide. With bicuculline supplemented to aCSF, the mean fEPSP slope ± SEM over 60 minutes, subsequent to TBS, was 124 ± 5 % (n = 7, P < 0.01) of the baseline level (Figure 2A). Early phase of potentiation (post-tetanus 0’ – 10’) amounted to 104 ± 8 % and later phase (post-tetanus, 30’ – 60’) to 128 ± 5 % (P < 0.01). We conclude that TBS alone is insufficient to induce LTP <em>in vitro</em> in the dentate gyrus unless GABA<sub>A</sub> receptors are blocked simultaneously.

**Effects of corticosterone on LTP**

Application of 100 nM corticosterone (in the absence of bicuculline), just before and during TBS, did not significantly influence synaptic responses after the tetanus. Synaptic responses over the post-tetanus 60 minutes amounted to 115 ± 9 % (mean slope fEPSP ± SEM, n = 11) of the baseline value (Figure 1A), with 105 ± 3 % and 115 ± 10 % in the early (post-tetanus 0’ – 10’) and later (post-tetanus 30’ – 60’) phases respectively. In comparison with the vehicle treated slices, perfusion of corticosterone around the time of TBS did not result in changes in synaptic responses with regard to both early and later components of potentiation (Figure 1B). Apparently, corticosterone cannot potentiate synaptic responses in the DG when linked to a sub-threshold protocol of LTP.

Next, slices were incubated for 20 minutes with 100 nM corticosterone more than 2 hours before TBS. Over the post-synaptic 60 minutes, synaptic responses amounted to 111 ± 2 % (n = 4) of its pre-tetanus value, which is very similar to the values seen in vehicle treated control slices (Figure 1A). Also, synaptic responses during both the early phase and later phase after TBS were indistinguishable from those of slices perfused with vehicle only (Figure 1B).

We considered the possibility that corticosterone can become effective under conditions that GABAergic transmission is suppressed and LTP is induced, i.e. in the presence of 10 µM bicuculline. Previously, we reported that under those conditions a brief pulse of corticosterone 1 – 4 hours in advance of TBS tends to suppress potentiation, but this effect did not reach significance (Alfarez et al., 2003). We presently completed these observations in the presence of bicuculline by examining putative non-genomic effects of corticosterone in the DG, i.e. when corticosterone was administered just before and during TBS. Interestingly, we observed that in the presence of bicuculline, such application of corticosterone results in a clear form of LTP over 60 minutes (mean slope fEPSP ± SEM: 130 ± 9 %, n = 8, P < 0.05) (Figure 2A) compared to the pre-tetanus values. Early phase of potentiation (post-tetanus 0’ – 10’) was 123 ± 10 % (P = 0.055) of the baseline value and later phase (post-tetanus 30’ – 60’) was 129 ± 9 % (P < 0.05). Compared with the vehicle-treated slices in bicuculline subjected to TBS, a significant enhancement in synaptic response was
found in the corticosterone- versus vehicle-treated group with respect to the early \( (P < 0.05) \) but not the later phase of potentiation (Figure 2B).

**Figure 1** Effect of corticosterone on the slope of the fEPSP evoked in the dentate gyrus by perforant path stimulation. (A) In aCSF (with vehicle perfusion, as indicated by the horizontal bar, \( n = 10 \)), theta burst stimula-
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tion (TBS) does not induce LTP (the upper graph). Brief perfusion of 100 nM corticosterone just before and during TBS resulted in a slight increase in synaptic responses, but this did not reach statistical significance (the middle graph, n = 11). Pre-incubation with 100 nM corticosterone for 20 minutes > 2 hrs prior to TBS did not modify post-tetanus synaptic responses (the lower graph, n = 4). The symbols represent the mean (+ SEM) slope of the fEPSP. VEH: vehicle; CORT: corticosterone. The representative analogue traces of recordings are shown on the right, respectively; the asterisk indicates the stimulus artifact. (B) Mean (+ SEM) synaptic responses during the early phase after TBS (here defined as post-tetanus 0' – 10'; the left graph) or the later phase after TBS (post-tetanus 30' – 60'; the right graph) revealed no significant effect of corticosterone treatment compared to vehicle treatment (white bars). The absence of any effect was seen both when the hormone was applied just prior to and during tetanic stimulation (gray bars; using the protocol as indicated in the middle graph in A), and when corticosterone was briefly perfused > 2 hrs before TBS (dark bars; using the protocol as indicated in the lower graph in A).

**Figure 2** Effects of corticosterone on synaptic potentiation in the DG, in the presence of bicuculline. (A) When 10 μM bicuculline (BIC) was continuously present during recording, TBS stimulation did result in a significant
and lasting potentiation of the fEPSP slope with vehicle (the upper graph, n = 7). Clear synaptic potentiation was also seen with TBS after brief perfusion with 100 nM corticosterone (the lower graph, n = 8). The symbols represent the mean (+ SEM) slope of the fEPSP. The representative analogue traces of recordings are shown on the right, respectively; the asterisk indicates the stimulus artifact. #, ##: $P < 0.05$, $P < 0.01$, compared with the pre-tetanus baseline. (B) The mean (+ SEM) fEPSP slope in the early phase after TBS (post-tetanus 0’ – 10’, the left graph) was not increased in the vehicle treated animals (diagonally striped bar) in bicuculline. However, synaptic potentiation was significantly enhanced by perfusion of corticosterone (gray bar). In the later phase after TBS (post-tetanus 30’ – 60’, the right graph), both groups revealed a comparable degree of synaptic potentiation without between-group difference. *: $P < 0.05$, comparison with the vehicle treatment.

To examine the effect of corticosterone on isoproterenol-mediated actions (next section), we chose to record under conditions that are as close as possible to the "natural" situation, i.e. with intact GABAergic activity. As reported above, corticosterone by itself – when applied shortly before TBS or when given more than 2 hours in advance – does not significantly affect synaptic potentiation induced by TBS under those circumstances.

**Effect of corticosterone on isoproterenol-mediated actions**

The $\beta$-adrenergic agonist (-)-isoproterenol (+)-bitartrate (1.0 $\mu$M) was rapidly applied by perfusion for 15 minutes before and during TBS; this was done to test whether $\beta$-adrenoceptor activation could facilitate LTP induction by TBS. Results show that after a brief administration of isoproterenol, mild potentiation was observed over the post-tetanus 60 minutes (mean slope fEPSP ± SEM: 118 ± 7 %, n = 11, $P < 0.05$) (Figure 3A). Potentiation was not manifest during the first 10 minutes after tetanus; however, it slowly developed over time. Early responses (post-tetanus 0’ – 10’) amounted to 100 ± 5 % of the baseline value, and later responses (post-tetanus 30’ – 60’) to 122 ± 10 % ($P < 0.05$). These data indicate that \( \beta \)-adrenergic activation can mildly facilitate synaptic responses after TBS; this facilitation particularly pertains to the later phase of LTP.

Interestingly, when 100 nM corticosterone was co-applied with 1.0 $\mu$M isoproterenol, synaptic potentiation was already observed during the early phase after TBS. Synaptic responses over the post-tetanus 60 minutes were 126 ± 8 % of the baseline value (mean slope fEPSP ± SEM, n = 9, $P < 0.05$) (Figure 3A). Average fEPSPs were significantly enhanced with respect to the early phase (post-tetanus 0’ – 10’: 119 ± 6 %, $P < 0.05$) and the later phase (post-tetanus 30’ – 60’: 125 ± 11 %, $P < 0.05$) in comparison to the baselines. Early phase of potentiation – when corticosterone and isoproterenol were co-applied – was significantly different from that of the slices perfused with isoproterenol only ($P < 0.01$), with corticosterone only ($P < 0.05$), or with vehicle only ($P < 0.01$); however, no significant differences were identified among these groups with regard to the later phase of potentiation (Figure 3B).

In order to examine if isoproterenol-mediated effects on LTP can be differently modulated by corticosterone via a delayed action, 100 nM corticosterone was briefly (for 20 minutes) applied to the slices more than two hours in advance of isoproterenol perfusion; isoproterenol perfusion co-terminated with TBS. An interval of 2 hours was chosen to ensure that gene-mediated effects can take place (Karst and Joels, 2005; Wiegert et al., 2005; Morsink et al., 2006). Contrary to what was
seen with isoproterenol only or with concurrent perfusion of both hormones, isoproterenol did not affect synaptic responses after TBS if corticosterone was applied to the same slice a few hours earlier. In that case, the mean fEPSP slope over the post-tetanus 60 minutes was 97 ± 4 % (mean slope fEPSP ± SEM, n = 6) of the baseline (Figure 3A), comparable to the control group not subjected to any hormonal treatment (Figure 1A). During the early post-tetanus phase (post-tetanus 0' – 10'), average responses (101 ± 2 %) were significantly smaller than those of slices where corticosterone and isoproterenol had been co-applied (P < 0.01), but did not differ from those of slices perfused with isoproterenol only (Figure 3B). With regard to the later phase (post-tetanus 30' – 60'), synaptic responses (96 ± 5 %) were significantly reduced in comparison to the slices perfused with both hormones (P < 0.05), or perfused with isoproterenol only (P < 0.05) (Figure 3B).

**Baseline transmission with stress hormones**

Baseline synaptic responses in response to half-maximal stimulation during the perfusion period with: 1) corticosterone, 2) isoproterenol, 3) isoproterenol together with corticosterone, or 4) corticosterone incubation > 2 hours before isoproterenol perfusion, did neither demonstrate within-group differences compared with their (10 minutes) pre-perfusion baselines, nor between-group differences compared with the vehicle-perfused group (Figure 4). These data suggest that synaptic basal transmission was not altered by a brief administration of stress hormone(s) at the present dosages.

**Discussion**

In this study we tested the hypothesis that corticosterone time-dependently affects LTP and β-adrenergic actions in the rat DG. Based on earlier findings in the hippocampal CA1 area (Wiegert et al., 2005; Wiegert et al., 2006), we presumed that in the DG corticosterone – in a rapid non-genomic fashion – promotes LTP and noradrenergic efficacy while the same hormone has suppressive effects when acting through slow gene-mediated pathways. We observed that in the DG, corticosterone can enhance the early phase of synaptic potentiation only when GABA A-receptor mediated transmission is blocked; delayed suppression by the hormone, though, was not seen using the present stimulation protocol. Isoproterenol potentiated particularly the later phase of LTP. When corticosterone was co-applied with isoproterenol, a significant enhancement of the early but not the later LTP phase was observed. Finally, brief administration of corticosterone several hours before application of isoproterenol markedly suppressed the efficacy of isoproterenol to potentiate the later phase of LTP. These observations are largely in line with the hypothesis, although the effects of corticosterone were in some aspects unexpected.

**Effect of corticosterone on LTP in the DG**

Under in vitro recording conditions, mature DG granule cells exhibit a quite negative resting membrane potential (Liu et al., 1996). Moreover, these neurons are subject to a strong GABAergic inhibitory tone (Nusser and Mody, 2002; Chandra et al., 2006). Consequently, it is not easy to induce robust synaptic potentiation in mature DG cells in vitro (Wang et al., 2000). This was confirmed in the present experiments. Under those conditions, corticosterone was unable to facilitate synaptic potentiation after TBS in a rapid non-genomic fashion. However, when the GABAergic
inhibitory tone was relieved by applying bicuculline, a rapid facilitatory effect of corticosterone particularly in the first 10 minutes after TBS became apparent. Earlier studies in the CA1 area also revealed that corticosterone applied just before and during high frequency stimulation has particularly strong effects during the first phase of potentiation, while later phases seem somewhat less affected (Wiegert et al., 2006); it should be noted, though, that in the CA1 area as opposed to the DG, corticosterone administered around the time of high-frequency stimulation still significantly enhanced synaptic potentiation over the entire recording period.

The fact that rapid effects of corticosterone in the DG (as opposed to the CA1 area) can only be seen in the presence of bicuculline could signify that the hormone induces sub-threshold actions which do not become evident when cells are far removed from the firing threshold, but can become essential when these neurons are in a more depolarized state. We cannot exclude this possibility with the current extracellular recording approach; yet, the fact that corticosterone by itself did not change the basal synaptic response in any respect seems to argue against it. Similarly, it is unlikely that bicuculline-induced enhancement of endogenous noradrenaline release (Barik and Wonnacott, 2006) can explain the findings, in view of the continuous perfusion prior to the start of electrophysiological recording thus washing out most of the endogenously released transmitters. An interesting possibility is that corticosterone could exert rapid effects on GABAergic transmission which counteract putative facilitatory effects on TBS-induced synaptic potentiation. In support of this view, recent experiments revealed that neurosteroids potentiate tonic GABAergic inhibition in the DG, an effect that involves GABAA receptor δ-units (Stell et al., 2003). If corticosterone is partly metabolized, the actions of these metabolites could mask putative facilitatory actions of the hormone. This differs from the situation in the CA1 area where δ-subunits (which play a key role in the steroid-induced enhancement of tonic GABAergic inhibition) are much less expressed (Pirker et al., 2000; Peng et al., 2002).

In contrast to the CA1 area, brief administration of corticosterone several hours in advance of high-frequency stimulation did not affect synaptic potentiation, neither in the absence (this study) nor in the presence of bicuculline (Alfarez et al., 2003). In the former case this could be simply due to the fact that synaptic potentiation with TBS is so weak that suppressive effects by corticosterone cannot be discerned; however, this cannot explain the findings in the presence of bicuculline where appreciable LTP is observed. As argued earlier, the apparent lack of gene-mediated effects on LTP may be linked to the TBS protocol (Alfarez et al., 2002; Alfarez et al., 2003). It should be noted, though, that an apparent lack of gene-mediated corticosteroid effects in the DG – presumably via the low affinity glucocorticoid receptor (GR) – is not unprecedented, as a similar protocol of corticosterone administration also failed to change calcium currents (van Gemert and Joels, 2006) and AMPA-receptor mediated responses (Karst and Joels, 2003) in granule cells, while these parameters are clearly altered by the hormone in the CA1 region (Kerr et al., 1992; Karst et al., 2000; Karst and Joels, 2005). As DG granule cells, like CA1 pyramidal cells, abundantly express GRs, this could signify that DG granule cells i) express different receptor variants, ii) are exposed to lower intracellular levels of corticosterone, or iii) contain a different set of cellular proteins that determine the transcriptional activity of the GR (Joels, 2006).
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**Figure 3** Time-dependent effects of corticosterone on β-adrenergic actions. (A) Brief perfusion of 1.0 μM isoproterenol (ISO) just before and during TBS (indicated by horizontal bar) induced a slow-onset potentiation of the fEPSP slope (the upper graph, n = 11). Concurrent perfusion of isoproterenol and corticosterone (the middle graph, n = 9) immediately evoked LTP after TBS. Brief administration of corticosterone (20 minutes, CORT)
> 2 hours in advance of isoproterenol perfusion completely prevented the occurrence of LTP (the lower graph, n = 6). All symbols represent the mean (+ SEM) slope of the fEPSP. The representative analogue traces of recordings are shown on the right, respectively; the asterisk indicates the stimulus artifact. #: $P < 0.05$, compared with the pre-tetanus baseline. (B) Co-application of corticosterone and isoproterenol (gray bar) during perfusion enhanced the mean (+ SEM) fEPSP slope during the early phase after TBS (post-tetanus 0’ – 10’, the left graph) when compared to the slices perfused with isoproterenol only (white bar). No differences were found between the groups with respect to the later phase after TBS (post-tetanus 30’ – 60’, the right graph). Pretreatment of corticosterone >2 hours in advance of isoproterenol application (dark bar) prevented synaptic potentiation as could be found in the co-application group during the early phase, and abolished the potentiation as could be found in both co-application and isoproterenol alone groups during the later phase after TBS. *, **: $P < 0.05$, $P < 0.01$, based on between-group comparisons.

**Figure 4** Baseline transmission during drug perfusion was not affected by stress hormones. The mean (+ SEM) slope of the fEPSP induced by half-maximal stimulation of perforant path afferents was not significantly altered by perfusion of corticosterone and/or isoproterenol. This was true when signals were compared to the average of the pretreatment baseline signal for each condition shown below, and when signals recorded during the various drug applications were compared to each other. The observations were based on the following number of animals for each condition: VEH, white bar, n = 11; CORT, gray bar: n = 12; ISO, white striped bar: n = 11; CORT+ISO, gray striped bar: n = 9; CORT > 2hrs before ISO, dark bar: n = 6.

All in all, we conclude that under the present recording conditions corticosterone does not affect synaptic potentiation in the DG, unless the GABAergic inhibitory tone is relieved to reveal a rapid facilitatory effect by the hormone.

*Effects of isoproterenol and its modulation by corticosterone*

In contrast to corticosterone, the β-adrenergic agonist isoproterenol does rapidly facilitate synaptic potentiation induced by a weak stimulation protocol in the DG. This is in line with most previous studies in the DG (Stanton and Sarvey, 1985, 1987; Dahl and Sarvey, 1990; Chaulk and Harley,
A temporal perspective on stress hormones and memory

1998; Bronzino et al., 2001; Frey et al., 2001; Straube and Frey, 2003), as well as in the CA1 region (Katsuki et al., 1997). Several other studies have identified a form of long-lasting potentiation in the DG by applying β-adrenergic agonists without tetanization, which resembles LTP and is NMDA receptors- and protein synthesis-dependent (Stanton and Sarvey, 1987; Sarvey et al., 1989; Dahl and Sarvey, 1990). Another study using in vivo microdialysis established an association between increases in hippocampal noradrenaline level and tetanization of the medial perforant pathway (Bronzino et al., 2001). These studies suggest that noradrenaline can act as an endogenous mediator of DG LTP and suffices to result in long-term enhancement of synaptic strength. In our recording conditions, isoproterenol-mediated regulation of baseline activity was not observed. This may be due to the fact that in our set-up isoproterenol levels peaked only briefly, which may be too short to evoke long-lasting potentiation.

The most remarkable finding of our study is that corticosterone can bi-directionally modulate the isoproterenol-mediated effect. When corticosterone was applied several hours in advance of isoproterenol, the former fully prevented the facilitatory actions of the latter. In view of the time delay and the dose of corticosterone that was applied, it seems likely that these actions are mediated via intracellular GRs. By contrast, when corticosterone was co-applied with isoproterenol, the steroid enhanced the early phase of synaptic potentiation, similar to what was seen when bicuculline was perfused (in the absence of isoproterenol). Possibly, corticosterone enhances the availability of the β-adrenergic agonist, through mechanisms that resemble its interference with extrasynaptic catecholamine uptake (Grundemann et al., 1998). Also, it cannot be excluded that corticosterone in the short term increases isoproterenol-induced cAMP accumulation, as has been described in thymocytes (Durant et al., 1983). Both pathways, however, would be expected to enhance isoproterenol effects not only during the early but also during the later phase of LTP. The data rather seem to indicate that corticosterone and isoproterenol enhance synaptic responses independently and through different pathways. Isoproterenol (like bicuculline) may relieve the inhibitory tone in the DG just enough to enable rapid facilitatory effects of corticosterone to develop.

The dichotomy fits well with earlier studies at the behavioral and cellular level. In an inhibitory avoidance task, corticosterone interacted with β-adrenergic activation to facilitate memory consolidation (Roozendaal et al., 2002). In view of the time window in which corticosterone was active, a rapid non-genomic mechanism seems to be indicated (Roozendaal, 2003), although the effectiveness of the selective GR agonist RU 283862 argues against this notion. Delayed effects of corticosterone were examined in another study in adrenalectomized rats (Borrell et al., 1984). Post-training application of adrenaline could rescue the memory deficit in inhibitory avoidance behavior seen after adrenalectomy. If corticosterone was given more than 1 hour before adrenaline, the dose-response relationship was altered and the efficacy of adrenaline was significantly reduced. Along the same line, at the cellular level noradrenaline was shown to enhance cellular excitability in hippocampal CA1 pyramidal neurons from adrenalectomized rats; when corticosterone was transiently applied more than 1 hour in advance, the β-adrenergic enhancement of activity was suppressed (Joels and de Kloet, 1989). While these earlier studies suggest evidence for a time-dependent effect of corticosterone, the present study is the first to demonstrate that in a controlled
experimental setting corticosterone acts in the same direction as a β-adrenergic agonist when the two compounds are present in the circuit around the same time, while corticosterone exerts an opposite effect when cells have been exposed to this hormone several hours before β-adrenoceptors are activated.

A similar bidirectional modulation with time was also found in vivo in studies which explored the role of the amygdala on DG LTP. Thus, basolateral amygdala stimulation enhanced DG LTP when amygdala activation was closely linked in time to DG stimulation, while it impaired LTP when amygdala stimulation preceded DG stimulation by more than 1 hour (Akirav and Richter-Levin, 2002; Vouimba and Richter-Levin, 2005; Vouimba et al., 2006). Importantly, in both cases, noradrenaline- and corticosterone-mediated mechanisms appeared to be involved.

**Functional implications**

The presently observed effects can have consequences for DG-dependent encoding and consolidation of information under stressful circumstances. When an organism is “stressed”, levels of corticosteroids, noradrenaline and neuropeptides in – among others – limbic regions such as the amygdala and hippocampal subfields will be raised, i.e. in areas that are enriched with receptors for these factors. The present data indicate that at least in the DG, patterned input that is transferred along specific afferent fibers will be strengthened by the hormones, so that the ensuing synaptic potentiation (which is weak when hormone levels are not elevated) becomes appreciable shortly after arrival of the patterned input and until at least up to 60 minutes later. This may promote encoding of the information associated with the stressful event.

At that time, however, corticosterone will also initiate a gene-mediated cascade which changes cell functions several hours later. Based on the present data, it is expected that unrelated information arriving at the same place some hours after the initial stressful event will become difficult to be encoded, especially when it involves a combination of patterned input and elevated noradrenaline levels. This would preserve the initial information and thus promote the consolidation of that event.