Differential Effects of Corticosterone on the Slow Afterhyperpolarization in the Basolateral Amygdala and CA1 Region: Possible Role of Calcium Channel Subunits

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Liebmann L, Karst H, Sidiropoulou K, van Gemert N, Meijer OC, Poirazi P, Joëls M. Differential effects of corticosterone on the slow afterhyperpolarization in the basolateral amygdala and CA1 region: possible role of calcium channel subunits. J Neurophysiol 99: 958–968, 2008. First published December 12, 2007; doi:10.1152/jn.01137.2007. The stress hormone corticosterone increases the amplitude of the slow afterhyperpolarization (sAHP) in CA1 pyramidal neurons, without affecting resting membrane potential, input resistance, or action potential characteristics. We here examined how corticosterone affects these properties in the basolateral amygdala (BLA). In the amygdala, corticosterone does not change the AHP amplitude, nor any of the passive and active membrane properties studied. The lack of effect on the AHP is surprising since in both areas corticosterone increases high-voltage-activated sustained calcium currents, which supposedly regulate the sAHP. We wondered whether corticosterone targets different calcium channel subunits in the two areas because currents through only one of the subunits (Cav1.3) are thought to alter the AHP amplitude. In situ hybridization studies revealed that Cav1.2 cells express Cav1.2 and Cav1.3 subunits; corticosterone does not transcriptionally regulate Cav1.2 but increases Cav1.3 expression compared with vehicle treatment. In the BLA, Cav1.3 expression was not detectable, both after control and corticosterone treatment. Cav1.2 is moderately expressed. In a modeling study, we examined putative consequences of changes in specific calcium channel subunit expression and calcium extrusion by corticosterone for the AHP in CA1 and amygdala neurons. A differential distribution and transcriptional regulation of Cav1.2 and Cav1.3 in the CA1 area versus BLA partly explain the observed differences in AHP amplitude. The functional implication of these findings could be that stress-induced arousal of activity in the BLA is more prolonged than that in the CA1 hippocampal area, so that information with an emotional component is more effectively encoded.

INTRODUCTION

Corticosteroid hormones are secreted from the adrenal glands, particularly after stress (for review see De Kloet et al. 2005). The hormones pass the blood–brain barrier and bind to intracellular receptors in the brain. Low amounts of corticosterone (such as circulate under rest) are sufficient to activate high-affinity mineralocorticoid receptors (MRs), which are abundantly expressed in limbic areas, i.e., all hippocampal subfields and the lateral septum. Under rest the lower-affinity glucocorticoid receptor (GR) is only about 20% occupied; this receptor becomes substantially activated after stress and at the circadian peak of corticosterone release. Both receptor subtypes act as transcriptional regulators (Pascual-Le Tallec and Lombes 2005; Zhou and Cidlowski 2005) and thus slowly change the function of neurons.

Previous studies have shown that in particular voltage-dependent calcium (Ca) currents are sensitive to the stress hormone corticosterone. In the CA1 area, the amplitude of high-voltage–activated Ca currents is relatively small with predominant MR activation (i.e., in nonstressed rats), but large when GRs are activated, for example as a result of stress some hours before recording (Joëls et al. 2003; Karst et al. 1994; Kerr et al. 1992). It was shown that this effect requires binding of GR homodimers to the DNA (Karst et al. 2000) and most likely is due to an increase in the number of available L-type Ca channels in the plasma membrane (Chameau et al. 2007). In association with an increased Ca-current amplitude, corticosterone induced an increased firing frequency accommodation on depolarization as well as a larger amplitude of the slow afterhyperpolarization (sAHP) that is seen when the depolarization is terminated (Joëls and de Kloet 1989; Kerr et al. 1989). Other basal membrane properties, such as resting membrane potential and input resistance, were not affected by GR activation. The interpretation of these effects is that several hours after stress exposure, information flow through the CA1 hippocampal area is attenuated and thus earlier aroused activity normalized, through a GR-mediated mechanism (Joëls et al. 2007).

Glucocorticoids are also known to alter basolateral amygdala (BLA) function. For instance, consolidation of inhibitory avoidance behavior is promoted by glucocorticoids, acting via GRs in the BLA (Roozendaal and McGaugh 1996, 1997; Roozendaal et al. 2006). However, when we started the present investigation little was known about cellular effects of the hormone. One study described that glucocorticoids increase the amplitude of high-voltage–activated calcium currents in a delayed manner, in a very similar extent to that reported for CA1 pyramidal neurons (Karst et al. 2002); at the single-cell level, relative expression of the Cav1.2 subunit was found to be enhanced. In the present investigation we examined how corticosterone affects active and passive membrane properties of principal neurons in the BLA. We tested the hypothesis that corticosterone increases the sAHP amplitude of BLA neurons,
but does not affect other passive and active membrane properties, similar to what was reported for the CA1 hippocampal area.

**METHODS**

**Animals**

For the present study we used male Wistar rats (~150 g; Harlan CPB, Zeist, The Netherlands) or male C57Bl/6 mice (~40 g; Harlan CPB). All animals were group-housed for ≥7 days before the experiment. Food and water were provided without restriction, lights were on from 8:00 am until 8:00 pm, and the temperature and humidity were kept between 20 and 22°C and 55 ± 15%, respectively. The local committee on animal bioethics of the University of Amsterdam approved all experiments.

**Slice preparation and corticosterone treatment**

Immediately after decapitation, the brain was removed from the skull and chilled (at ~4°C) in artificial cerebrospinal fluid (aCSF) containing (in mmol/L): NaCl 120, KCl 3.5, MgSO4 5.0, NaH2PO4 1.25, CaCl2 0.2, d-glucose 10, and NaHCO3 25.0, gassed with 95% O2–5% CO2. Next, coronal slices (400 μm thick) containing the hippocampus and/or the BLA were prepared with a vibraslicer (Leica VT 1000S; Leica Instruments, Nussloch, Germany). Briefly, frontal lobes and cerebellum were removed and the caudal side of the brain was glued onto the slicing plateau. Slices were stored at room temperature in recording aCSF containing (in mmol/L): NaCl 120, KCl 3.5, MgSO4 1.3, NaH2PO4 1.25, CaCl2 2.5, d-glucose 10, and NaHCO3 25. After 1 h, half of the slices from all experimental groups were subjected to vehicle treatment (0.01% ethanol), whereas the other half received corticosterone (100 nM in 0.01% ethanol), for 20 min at 32°C. Previous studies have shown that this treatment is sufficient to observe changes in cellular properties that require homodimerization of the GR, 1–4 h later (Karst et al. 2000).

**Recording**

One slice at a time was placed in a recording chamber mounted on an upright microscope (Axioskop 2 FS plus; Carl Zeiss, Oberkochen, Germany) with differential interference contrast, water-immersion objective (×40), and ×10 ocular to identify the cells. Under both current- and voltage-clamp conditions the slices were continuously perfused with aCSF (flow rate 2–3 ml/min, temperature 32°C, pH 7.4) consisting of (in mM): 120 NaCl, 3.5 KCl, 1.3 MgCl2, 2.5 CaCl2, 25 NaHCO3, 1.25 KH2PO4, and 10 d-glucose. Patch pipettes for recording were pulled from borosilicate glass (OD 1.5 mm; Science Products, Hofheim, Germany; pipette impedance ~3–4 MΩ) on a Sutter micropipette puller (Sutter Instrument, Novato, CA) and filled with (in mM): 140 K-methane-sulfonate, 10 HEPES, 0.1 EGTA, 4 MgATP, and 0.3 NaGTP; pH 7.3 (solution #I, optimal for recording firing frequency accommodation and the sAHP amplitude; see Faber and Sah 2004). Membrane properties (except sAHP and firing frequency accommodation) were also examined with another solution (#II; in mM): 140 K-methane sulfonate, 10 HEPES, 5 EGTA, 2 MgCl2, 2 Na2ATP, and 0.3 NaGTP; this solution allows easy comparison with earlier studies from our laboratory. In some experiments Alexa hydrazin 568 (0.2 mg/ml; Molecular Probes) was added to this solution to stain cells intracellularly. After diffusion of the dye into the cell, the slices were fixed in 4% phosphate-buffered paraformaldehyde at 4°C for 30 min and rinsed three times with phosphate buffer. Slices were mounted on glass slides under a coverslip, with Vectashield (Vector Laboratories). Immunofluorescent cells in fixed sections were evaluated using a Zeiss LSM 510 (Carl Zeiss, Jena, Germany) confocal laser-scanning device equipped with a dry Plan-Neofluar ×20/0.75 lens or a fluorescent microscope (Zeiss Axioskop; Carl Zeiss, Oberkochen, Germany). Cells examined under the confocal microscope were morphologically analyzed with Image J [National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/NIH-image/ (De Simoni et al. 2003)] in combination with the Neuron_ morpho plug-in (G. D’Alessandro, University of Southampton, Southampton, UK) and LM measure (R. Scorcioni, Krasnow Institute for Advanced Studies, George Mason University, Fairfax, VA).

In the BLA we selected for recording if they displayed a pyramidal-shaped cell body, in agreement with the morphology of principal neurons in the BLA of various species (Paré et al. 1995; Rainnie et al. 1993; Washburn and Moises 1992; Yajeya et al. 1997). Interneurons, which are usually smaller and display multipolar, spineless dendrites, were avoided. Only cells with a resting membrane potential more negative than ~55 mV were included in this study. Signals were recorded using a patch-clamp amplifier (Axopatch 200B; Axon Instruments, Foster City, CA); Responses were filtered at 5 kHz and digitized at 10 kHz (Digidata 1322A; Axon Instruments). All data were acquired, stored, and analyzed on a PC using pClamp 9.0 and Clampfit 9.2 (Axon Instruments). To investigate the firing properties and the properties of the afterhyperpolarization (AHP) after corticosterone or vehicle treatment we used current-clamp conditions. Prolonged current steps (100 or 600 ms) were applied from the resting membrane potential in the range of ~200 to 400 pA with 40-pA increments. We included only reliable sAHP recordings, i.e., neurons in which the averaged sAHP for currents steps of 320–400 pA was larger than the averaged sAHP for current steps of 0–120 pA. The currents underlying the sAHP (I_{sAHP}) were further examined under voltage-clamp conditions, by giving a 200-ms voltage step to 0 mV from a holding potential of ~50 mV.

**In situ hybridization**

For in situ hybridization experiments, 16 Wistar rats were used. Animals received a single subcutaneous injection with corticosterone (10 mg/100 g body weight; dissolved in arachide oil) (cort group; n = 8) or vehicle solution (veh group; n = 8) in a total volume of 500 μl. One hour after the injection, at 9:30 am, animals were decapitated. Brains were dissected out of the skull and quickly frozen on dry ice. On a cryostat, 12-μm-thick sections containing the hippocampus and BLA were cut, put on SuperFrost Plus slides (Menzel-Glaser, Brunswick, Germany), and stored at ~80°C.

Sections were fixed with 4% paraformaldehyde for ≥30 min and subsequently washed in phosphate-buffered saline. Then, sections were acetylated for 10 min in 0.1 M triethanolamine (pH = 8.0) with 0.25% acetic anhydride, washed in 2× saline sodium citrate (SSC) for 10 min, and dehydrated in an ethanol series (50%, 80, 100, and 100%; 1 min each).

[^35S]-dATP end-labeled desoxyoligonucleotide probes were used. Sequences were 5'-gtg ggt ggt ggt ggt gat tca tgc agt agc tca att (perfect match) or 5'-gtt ggt ggt gat tca tgc gat tca tgc gat tca att (mismatch control) and specific for Cav1.2, and 5'-gtt ggt ggt gat tca tgc gat tca tgc gat tca att (perfect match) or 5'-gtt ggt ggt gat tca tgc gat tca tgc gat tca att for Cav1.3, and 5'-gtt ggt ggt gat tca tgc gat tca tgc gat tca att (per pl match) or 5'-gtt ggt ggt gat tca tgc gat tca tgc gat tca att for Cav1.1, and 5'-gtt ggt ggt gat tca tgc gat tca tgc gat tca att (perfect match) or 5'-gtt ggt ggt gat tca tgc gat tca tgc gat tca att (mismatch control) for Cav1.3 mRNA expression. After end-labeling, oligonucleotides were purified with chloroform extraction and ethanol-precipitated. Per slide, 100 μl hybridization mix containing 50% formamide, 10% dextran sulfate, 20 mM DTT, 25 mM Na2SO4, 1 mM Na-pyrophosphate, 4× SSC, 5× Denhardt’s solution, 100 μg/ml poly(A), 100 μg/ml hbsDNA, and 1 × 10^6 cpm of the oligonucleotide probe was added. Sections were then coverslipped and incubated overnight at 42°C. The next day, coverslips were removed and sections were rinsed in 1× SSC at room temperature and subsequently washed in 1× SSC twice for 30 min at 50°C, and once for 5 min at room temperature. Slides were then dehydrated in an alcohol series, air-dried, and exposed to a Kodak Biomax MR film for 6 wk.

Four sections per probe per animal were scanned and loaded into Image J (Image J 1.37v). Gray values of the BLA as well as the
hippocampal CA1 cell layer were measured and corrected for background. Per animal, the gray values for each region were averaged. After this, the values of all animals of the same group were averaged.

Computational model

The compartmental models of a CA1 pyramidal neuron and a BLA pyramidal neuron were implemented in the NEURON simulation environment (Hines and Carnevale 1997). The biophysical model of the CA1 pyramidal neuron was previously described (Sidiproulou et al. 2007). The model consists of 183 compartments and includes a variety of passive and active membrane mechanisms known to be present in CA1 pyramidal cells. We assumed a uniform membrane resistance of $R_m = 40 \text{k}\Omega\text{-cm}^2$, a uniform intracellular resistivity $R_i = 70 \Omega\text{-cm}$, and a specific membrane capacitance of $1.0 \mu\text{F-cm}^{-2}$. The resting membrane potential of the model neuron was set at $-66 \text{mV}$. Active mechanisms included two types of Hodgkin–Huxley-type Na$^+$ currents (axonal: $I_{NaA}$; dendritic: $I_{NaD}$); three voltage-dependent K$^+$ currents ($I_{Ksr}$, $I_{KCa}$, $I_{KNa}$); a fast Ca$^{2+}$- and voltage-dependent K$^+$ current ($I_{AHP}$); a slow Ca$^{2+}$-dependent K$^+$ current ($I_{sAHP}$); a hyperpolarization-activated nonspecific cation current ($I_h$); a voltage-activated calcium current ($I_{CaL}$); a persistent sodium current ($I_{Nap}$); and four types of Ca$^{2+}$- and voltage-dependent calcium currents ($I_{CaN}$, $I_{CaR}$, $I_{CaL-1.2}$, $I_{CaL-1.3}$). The $I_{CaL-1.2}$ models of the L-type Ca$^{2+}$ current carrired by channels are composed of the Cav1.2 subunits, whereas the $I_{CaL-1.3}$ models of the L-type Ca$^{2+}$ current carrired by channels are composed of the Cav1.3 subunits. Based on the available data (Marion and Tavalin 1998), the $I_{CaL-1.3}$ was colocalized with the $I_{AHP}$ as previously described (Markaki et al. 2005). Channel equations, distributions, and densities of $I_{Na}$, $I_{Ksr}$, and $I_a$ are described in more detail elsewhere (Poirazi et al. 2003).

The reconstructed BLA pyramidal neuron was based on a three-dimensional confocal representation of an intracellularly stained neuron and constituted 48 compartments (one somatic, 47 dendritic, and one axonic compartment). We assumed a uniform membrane resistance $R_m = 15 \text{k}\Omega\text{-cm}^2$ and a uniform intracellular resistivity $R_i = 70 \Omega\text{-cm}$. The resting membrane potential was set at $-66 \text{mV}$ and the input resistance (measured at $-100 \text{pA}$) at $150 \text{M}\Omega$. Active mechanisms included one type of Hodgkin–Huxley-type fast Na$^+$ current; a persistent sodium current ($I_{Nap}$); three voltage-dependent K$^+$ currents ($I_{Ksr}$, $I_{KCa}$, $I_{KNa}$); a fast Ca$^{2+}$- and voltage-dependent K$^+$ current ($I_{AHP}$); a slow Ca$^{2+}$-dependent K$^+$ current ($I_{sAHP}$); a hyperpolarization-activated nonspecific cation current ($I_h$); a low-voltage-activated calcium current ($I_{CaL}$); a persistent sodium current ($I_{Nap}$); and four types of Ca$^{2+}$- and voltage-dependent calcium currents ($I_{CaN}$, $I_{CaR}$, $I_{CaL-1.2}$, $I_{CaL-1.3}$). The $I_{CaL-1.2}$ models of the L-type Ca$^{2+}$ current carried by channels are composed of the Cav1.2 subunits, whereas the $I_{CaL-1.3}$ models of the L-type Ca$^{2+}$ current carried by channels are composed of the Cav1.3 subunits. Based on the available data (Marion and Tavalin 1998), the $I_{CaL-1.3}$ was colocalized with the $I_{AHP}$ as previously described (Markaki et al. 2005). Channel equations, distributions, and densities of $I_{Na}$, $I_{Ksr}$, and $I_a$ are described in more detail elsewhere (Poirazi et al. 2003).

Recordings were made from 68 principal neurons in mouse and rat BLA. Visual inspection under the microscope revealed that these neurons had a pyramidal-shaped cell body, from which several (two to five) spiny dendrites branched in various directions. This was confirmed in part of the neurons ($n = 22$), which were filled with the intracellular dye Alexa hydrozin 568 (0.2 mg/ml; see typical example in Fig. 1A). Occasionally ($n = 3$) and on purpose, we filled neurons without an apparent pyramidal-shaped cell body. These neurons typically showed a very high firing frequency on depolarization (means ± SE: 51 ± 4 spikes for a 0.2-nA pulse of 600-ms duration; cf. in pyramidal-shaped BLA cells: 6 ± 1 spikes, $n = 11$) and no spines on their dendrites.

Results are expressed as means ± SE and analyzed for statistical significance ($P < 0.05$) using a Student’s t-test. In the case of the sAHP amplitude and the number of spikes evoked by a depolarizing pulse, data were analyzed with a generalized model for repeated measures.

RESULTS

Membrane properties of BLA and CA1 hippocampal neurons

Recordings were made from 68 principal neurons in mouse and rat BLA. Visual inspection under the microscope revealed that these neurons had a pyramidal-shaped cell body, from which several (two to five) spiny dendrites branched in various directions. This was confirmed in part of the neurons ($n = 22$), which were filled with the intracellular dye Alexa hydrozin 568 (0.2 mg/ml; see typical example in Fig. 1A). Occasionally ($n = 3$) and on purpose, we filled neurons without an apparent pyramidal-shaped cell body. These neurons typically showed a very high firing frequency on depolarization (means ± SE: 51 ± 4 spikes for a 0.2-nA pulse of 600-ms duration; cf. in pyramidal-shaped BLA cells: 6 ± 1 spikes, $n = 11$) and no spines on their dendrites.

Resting membrane potential in mouse BLA neurons ranged from $-61$ to $-74 \text{mV}$; input resistance of all neurons incorporated in the present study was $<400 \text{M}\Omega$ (see Discussion). As is evident from Table 1, treatment with 100 nM corticosterone for 20 min, 1–4 h before recording, did not change either the resting membrane potential or the input resistance of mouse principal BLA neurons. Similarly, properties of the action potential were unaffected by hormone treatment: Values for the action potential half-width, threshold, rise time, and amplitude (from threshold) were very comparable for the vehicle- and corticosterone-treated cells (Table 1). This was confirmed in slices from rats, in two sets of experiments using different solutions in the recording pipette (Table 1). Contrary to our expectation, corticosterone also did not significantly affect the amplitude of the sAHP in BLA neurons (Fig. 1). In accordance with the current-clamp data, voltage-clamp recording of the $I_{AHP}$ revealed no difference between the corticosterone- and vehicle-treated BLA cells (Fig. 1). Also, the number of spikes elicited by a depolarizing pulse (0–400 pA, 600-ms duration) was comparable for the vehicle-treated group and corticosterone-treated cells.

Previous observations regarding the effect of corticosterone on the sAHP amplitude were made in rat hippocampal slices, with microelectrodes. To verify that these effects could be replicated under comparable recording conditions as presently used in the BLA, we examined passive and active membrane properties of CA1 neurons with whole cell recording in mouse hippocampal slices. Corticosterone treatment did not affect resting membrane potential or input resistance of CA1 neurons (Table 1). However, as reported earlier in rat cells recorded with sharp electrodes, the amplitude of the sAHP was clearly increased in mouse hippocampal CA1 neurons 1–4 h after a 20-min pulse of 100 nM corticosterone (Fig. 2). This effect of corticosterone was also seen in voltage-clamp recordings of the $I_{AHP}$, at both 200 and 500 ms after termination of the depolarizing voltage step (Fig. 2). The number of spikes, although on average for all current steps lower after corticosterone treatment, was not significantly altered.

In conclusion, high concentrations of corticosterone—such as occur after stress—slowly induce an increase in the sAHP amplitude of CA1 pyramidal neurons, whereas passive membrane properties and action potential characteristics remain unaffected. Under comparable conditions, sAHP amplitude as well as other passive and active membrane properties are not significantly affected by the hormone in principal cells of the BLA.

Expression of calcium channel subunits in the CA1 hippocampal area and BLA

The sAHP is known to be very sensitive to Ca-influx through L-type channels (Bowden et al. 2001; Lima and Marrion 2007;
Marrion and Tavalin 1998; but see Pineda et al. 1998). Because corticosterone increases high-voltage–activated sustained (and presumably L-type) calcium currents in both BLA and CA1 principal neurons by about 100%, the lack of any effect on the sAHP in the BLA was unexpected. L-type calcium channels can be composed of Cav1.2 or Cav1.3 subunits, in combination with auxiliary subunits (Arikkath and Campbell 2003; Catterall 2000). Interestingly, there is evidence that channels responsible for the sAHP are colocalized with Cav1.3 rather than Cav1.2 subunits (Bowden et al. 2001). If so, only corticosteroid effects on Cav1.3-containing channels are expected to have functional consequences for the sAHP amplitude. We wondered whether corticosterone targets different calcium channel subunits in the CA1 and BLA. Therefore we investigated the distribution of Cav1.2 and Cav1.3 subunits and their transcriptional regulation by corticosterone with in situ hybridization.

As shown in Fig. 3, Cav1.2 mRNA expression was low to moderate in the CA1 area and the BLA. High expression levels were observed in the CA3 area and the dentate gyrus. Compared with vehicle-treated animals, rats that received corticosterone 1 h before tissue collection displayed similar Cav1.2 expression in the CA1 hippocampal area. In the BLA, a 20% nonsignificant (P > 0.1) up-regulation of Cav1.2 mRNA expression was observed.

The expression levels of Cav1.3 were rather low. Clear expression was seen mostly in the dentate gyrus. Still, appreciable Cav1.3 mRNA expression was observed in the CA1 area (Fig. 3). Treatment of rats with a high dose of corticosterone increased the Cav1.3 expression by 50% (P < 0.05) compared with vehicle-injected animals in the CA1 area. In this rat strain and the present conditions, expression of Cav1.3 mRNA in the BLA was below the detection level, in both the vehicle- and the corticosterone-injected rats.

**Influence of Cav1.2 and Cav1.3 on sAHP amplitude: a modeling approach**

Electrophysiologically, it is not possible to distinguish currents generated by channels containing Cav1.2 versus Cav1.3 subunits. Thus we could not verify by electrophysiological means the subunit composition of L-type calcium channels in the BLA and CA1 hippocampal area or their corticosteroid modulation. Therefore we used a modeling approach to test whether the differential distribution of Cav1.2 and Cav1.3...
subunits in the two brain regions and their corticosteroid
dependence could explain the observed discrepancy in modu-
lation of the sAHP amplitude.

Compartmental models of a CA1 pyramidal neuron and a
BLA pyramidal neuron were implemented in the NEURON
simulation environment (Hines and Carcavale 1997; for details
see METHODS). The CA1 neuron model included both $I_{\text{CaL-1.2}}$
and $I_{\text{CaL-1.3}}$, whereas the BLA neuron model included $I_{\text{CaL-1.2}}$
and extremely low amounts of $I_{\text{CaL-1.3}}$. We questioned whether
transcriptional regulation of the Cav1.2 or Cav1.3—which in
the model was simulated by increasing the $I_{\text{CaL-1.2}}$ and the
$I_{\text{CaL-1.3}}$, respectively—could explain the discrepancy in sAHP
amplitude. To this end, $I_{\text{CaL-1.2}}$ and $I_{\text{CaL-1.3}}$ were increased by 20,
50, or 100%. These numbers were chosen based on the
range of corticosteroid-induced changes described in the present
and earlier studies, using either in situ hybridization, single-cell
RNA amplification, or electrophysiological methods (Chameau
et al. 2007; Joëls et al. 2003; Karst et al. 2002). Increasing the
$I_{\text{CaL-1.2}}$ by 20, 50, or 100% did not change the sAHP
amplitude, either in the CA1 or in the BLA pyramidal model
neuron (Figs. 4, A1 and A2). A similar increase in the $I_{\text{CaL-1.3}}$
did enhance the sAHP amplitude in the CA1 model neuron,
but only $\pm 20\%$, whereas the electrophysiologically mea-
sured sAHP amplitude was increased by 30–60% (Fig. 2).

Earlier Ca imaging and gene expression studies have shown
that Ca extrusion in CA1 pyramidal neurons is also a target for
GR modulation (Bhargava et al. 2002; Joëls et al. 1997). We
therefore next examined whether a decrease in the extrusion
rate of the Ca pump in the CA1 area could be responsible for
the discrepancy between the simulation and electrophysiological
data. Figure 4B1 shows the effect of a 20, 50, or 100%
increase of the $I_{\text{CaL-1.3}}$ in combination with a decrease in the
extrusion rate of the Ca pump in a CA1 neuron. The experi-
mentally observed 30–60% increase in sAHP amplitude could
be accurately simulated by, e.g., a 50% increase in $I_{\text{CaL-1.3}}$
in combination with a 20 to 40% decrease in the Ca extrusion
as well as by a 20% increase in $I_{\text{CaL-1.3}}$ in combination with 40 to
60% decrease in the extrusion rate of the Ca pump (Fig. 4B1).
Applying the same changes in the extrusion rate of intracellular
calcium in the BLA neurons in combination with a 20, 50,
or 100% increase in $I_{\text{CaL-1.2}}$ leads to much smaller changes in the
sAHP peak (Fig. 4B2). Traces comparing the sAHP in the CA1
and BLA model neurons under control (black line) and
“stressed” (gray line) conditions are shown in Fig. 4, C1a and
C2, a and b, respectively.

To determine whether the differential changes in the sAHP
amplitude between CA1 and BLA model neurons critically
depended on the presence/absence of $I_{\text{CaL-1.3}}$, the amount of
$I_{\text{CaL-1.3}}$ in the CA1 model neuron was largely reduced and its
colocalization with the $I_{\text{AHP}}$ was removed. Following that
change, the sAHP in the CA1 model neuron only marginally
increased under the “stressed” condition (Fig. 4C1b). The BLA
neuron was also altered, in such a way as to resemble the
biophysical mechanisms of the CA1 neuron (i.e., we increased
$I_{\text{CaL-1.3}}$ and colocalized it with the $I_{\text{AHP}}$). This was sufficient to
evoke a much larger increase in the sAHP amplitude, under
similar stressed conditions (Fig. 4C2c).

**DISCUSSION**

Corticosterone slowly increases the high-voltage–activated
L-type calcium current amplitude of principal neurons in the
BLA and CA1 hippocampal area to a similar extent (Joëls et al.
tested the hypothesis that corticosterone—similar to what was
reported earlier for the CA1 hippocampal area (Joëls and de
Kloet 1989; Kerr et al. 1989)—increases the sAHP amplitude
of BLA neurons, but does not affect other passive and
active membrane properties. Using whole cell recording in
mice, we here confirmed earlier findings (with sharp elec-
 trodes) from rat CA1 neurons that corticosterone indeed in-
creases the amplitude of the sAHP. Contrary to our expecta-
tions, we found that principal neurons in the BLA do not
exhibit a change in the amplitude of the sAHP (and the $I_{\text{AHP}}$).
We argue that this can be (at least partly) explained by a
different subunit composition of the calcium channels contrib-
uting to the L-type calcium current.

**Effects of corticosterone on membrane properties of CA1
and BLA neurons**

Earlier studies with microelectrodes in rats have shown that
administration of corticosteroid hormones does not consist-
tently change the resting membrane potential, input resistance,
or action potential properties of rat CA1 pyramidal neurons
This was confirmed in the present study using whole cell recording in mouse hippocampal slices. Similar to the CA1 hippocampal area, we also did not observe any change in these passive and active membrane properties in principal neurons of the BLA. This was found in mice as well as rats (using two different recording solutions). Contrary to our expectations, we also did not observe any change in the sAHP, $I_{sAHP}$, or the spike frequency accommodation in the BLA. The sAHP is very sensitive to the recording conditions, more specifically to the composition of the pipette solution. The lack of any corticosteroid effect on the sAHP in BLA neurons, however, was not due to suboptimal recording conditions because we readily confirmed earlier findings in CA1 pyramidal neurons. We not only observed a delayed increase in the sAHP amplitude, but also extended these observations by demonstrating an increased $I_{sAHP}$ amplitude, 1–4 h after corticosterone administration.

We have several reasons to believe that our observations in the BLA were based on a homogeneous population of pyramidal(-like) neurons. First, recordings were confined to cells that under the recording microscope exhibited a pyramidal-shaped cell body, with a restricted number of dendrites branching from the soma. The shape seen under the recording microscope was confirmed in a group of cells that was intracellularly filled after recording and analyzed with a fluorescent or confocal microscope; all of these neurons had appreciable amounts of spines on their dendrites. Second, and importantly, all cells incorporated in the present analysis showed an input resistance well below 400 MΩ and spike frequency accommodation, similar to properties reported earlier for pyramidal neurons and incompatible with interneurons. These morphological and electrophysiological characteristics are largely in line with earlier studies (Pare et al. 1995; Rainnie et al. 1993; Washburn and Moises 1992; Yajeya et al. 1997).

Very recently, a study appeared that reported that corticosterone depolarizes BLA neurons, increases their input resistance, and reduces the spike-frequency accommodation (Duvarci and Paré 2007). Small differences in recording conditions between this and our study, such as the intracellular chloride concentration, may have contributed to the discrepancies, particularly since glucocorticoids were found to shift the reversal potential of chloride-dependent conductances (Duvarci and Paré 2007). Also, it is noteworthy that the intercell variation in this recent study appeared to be larger, which may signify that perhaps various subpopulations of pyramidal neurons were included. Because it cannot be excluded that corticosterone has different effects on various subtypes of BLA neurons, less strict inclusion criteria may

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**Fig. 2.**

A: typical traces showing how the number of spikes during a 600-ms depolarizing (400-pA) pulse was determined in a CA1 pyramidal cell. B: traces illustrating at which time points the amplitude of the afterhyperpolarization was analyzed following a 400-pA depolarizing pulse of 600- ($B_1$) or 100-ms duration ($B_2$); and at which time points the $I_{sAHP}$ was established ($B_3$). C: the amplitude of the AHP following a 100-ms depolarizing current pulse is on average increased by 30% 1–4 h after corticosterone treatment of CA1 hippocampal neurons (100 nM, 20 min; black squares) compared with vehicle treatment (open squares). Symbols represent mean ± SE, based on the number of cells indicated in Table 1. D: a significant 60% increase in the amplitude of the AHP following a 600-ms depolarizing current pulse was observed ($P < 0.05$), 1–4 h after corticosterone treatment (100 nM, 20 min; black squares) compared with vehicle treatment (open squares). E: the amplitude of the $I_{sAHP}$, determined either 200 (left) or 500 ms (right) after termination of a 200-ms depolarizing step to 0 mV, was significantly ($P < 0.05$) enhanced 1–4 h after corticosterone treatment (black bar) compared with vehicle treatment (open bars). F: although the number of spikes evoked in CA1 neurons by a 600-ms depolarizing current pulse (400 pA) resulted on average in fewer spikes 1–4 h after corticosterone compared with vehicle treatment, the 2 groups did (just) not significantly differ from each other.
have led to a different outcome. The importance of strict inclusion criteria was indeed evident from our own study. Thus three pyramidal cells in the vehicle-treated control group were excluded from the overall analysis since they had a very high input resistance (means ± SE: 507 ± 27 MΩ; each cell was >3SDs removed from the mean of the remaining cells). Their overall morphological features under the recording microscope (more specifically the pyramidal-shaped cell body and number of primary branches) could not be distinguished from those of the other cells. This is reminiscent of projection neurons in the lateral amygdala, which also display two subclasses of pyramidal-shaped neurons, i.e., a prevalent subclass of cells with an average membrane resistance of 270 MΩ and an infrequently encountered subclass of cells with an average membrane resistance of 645 MΩ (Sosulina et al. 2006). If the high-input-resistance cells were included in our analysis, the control group had on average a larger sAHP amplitude and spike-frequency accommodation than those of the corticosterone-treated cells, similar to what was reported in the afore-mentioned recent paper (Duvarci and Paré 2007). This “difference” disappeared completely when more strict inclusion criteria were applied.

Relevance of calcium channel subunit distribution

The sAHP is known to be sensitive to intracellular calcium levels. For instance, the increased sAHP amplitude seen with aging is thought to develop secondary to enhanced calcium influx through L-type channels (Markaki et al. 2005; Power et al. 2002; Thibault et al. 2007). This involves enhanced levels of the Cav1.3 subunit (Herman et al. 1998; Veng et al. 2003), much more so than of the Cav1.2 subunit (Davare and Hell 2003; Herman et al. 1998), although posttranslational modification of Cav1.2 may also play a role (Davare and Hell 2003). Corticosterone has been reported to strongly increase high-voltage-activated sustained calcium currents in CA1 neurons (Karst 1994, 2000; Kerr 1992), by doubling the number of functional L-type channels in the plasma membrane (Chameau et al. 2007). Interestingly, high-voltage-activated calcium currents were increased to a similar extent by GR activation in the BLA (Karst et al. 2002). Although no pharmacological distinction was made in that study between various types of currents, there is suggestive evidence that the increase pertained to the L-type current: First, GR activation increased the sustained component, whereas the low-voltage-activated transient component was in fact decreased.
in amplitude; second, single-cell Cav1.2 mRNA expression was increased after GR activation and correlated well with the current amplitude. Despite the comparable enhancement in L-type calcium currents induced in CA1 and BLA pyramidal neurons by corticosterone, the sAHP was enhanced in the former but not in the latter cells. In view of the role of the two L-type pore-forming subunits regarding the effect of aging on the sAHP amplitude, we wondered whether corticosterone may target different L-type calcium channel subunits in the CA1 area versus the BLA.

The general distributional pattern of Cav1.2 and Cav1.3 expression in the hippocampus that we observed with in situ hybridization was very comparable to earlier reports (Ludwig et al. 1997; Tanaka et al. 1995), with high expression of Cav1.2 in the CA3 and dentate gyrus and high levels of Cav1.3 primarily in the dentate gyrus. Expression in the CA1 region was comparatively low. The available literature on expression levels of L-type channel subunits is less informative about the BLA, since a subdivision of the amygdala nuclei was not given.
(Ludwig et al. 1997; Tanaka et al. 1995). A recent immunocytochemical report showed that Cav1.2 is present in BLA principal cells and has a subcellular distribution similar to that in CA1 pyramidal neurons (Pinard et al. 2005). Accordingly, we observed moderately high expression of Cav1.2 in the BLA. However, in this rat strain and the present conditions (e.g., age) Cav1.3 expression in the BLA was low, i.e., below the detection limit with in situ hybridization.

In the CA1 area, Cav1.2 expression was not affected by corticosterone. The Cav1.3 expression was increased by 50% compared with vehicle-injected controls, although the effect was far less prominent when compared with naïve, noninjected controls (<20%; Van Gemert, personal communication). The present observations on Cav1.2 and Cav1.3 expression largely agree with an earlier study where Cav1.2 and 1.3 expression was analyzed with qPCR after treating hippocampal slices (from naïve mice) with 100 nM corticosterone; in that study no significant effects of corticosterone were seen for either subunit (Chameau et al. 2007). We tentatively conclude that in the CA1 area the 100% increase in L-type calcium current is certainly not due to transcriptional regulation of Cav1.2 subunits; possibly, it can be explained by regulation of the Cav1.3 gene, at least under specific circumstances. In the BLA, it was earlier found with single-cell RNA analysis that 1–4 h after application of a selective GR agonist the relative Cav1.2 mRNA expression in single cells is significantly increased (Karst et al. 2002). Here we found—using the mixed agonist corticosterone, and examining the expression level in the overall population of cells—a 20% (although nonsignificant) up-regulation of Cav1.2 expression. The fact that we found now looked 1 h after corticosterone application as opposed to 1–4 h after the agonist in the earlier study and 2) did not restrict investigation of transcript level to the principals cells from which electrophysiological recordings were obtained may explain why, presently, the difference did not reach significance. After fear conditioning (a highly stressful situation), Cav1.2 protein level was also increased by 66% (Chameau et al. 2007). Levels of ICaL-1.2 in our BLA model were extremely low. The ICaL-1.3 in the CA1 model cell was incorporated in the soma and at the base of dendrites, whereas ICaL-1.2 (in both CA1 and BLA) was placed in the soma as well as in clusters along the dendritic tree, as seen in immunohistochemical studies (Hell et al. 1993; Pinard et al. 2005).

As expected, increasing the abundance of Cav1.2 does not change the sAHP amplitude, either in CA1 cells or in the BLA. Doubling the Cav1.3 levels did increase the sAHP amplitude in the CA1 model cell, but only by 20%. Interestingly, glucocorticoids repress the expression of the plasma membrane calcium pump isofrom 1 in hippocampal cells (Bhargava et al. 2002). In accordance, corticosterone application to acutely dissociated CA1 hippocampal area basolateral amygdala

FIG. 5. Schematic overview with putative corticosterone-induced pathways enhancing the sAHP amplitude in the CA1 hippocampal area but not in the BLA. Left: in the CA1 area, there is no evidence that corticosterone via glucocorticoid receptors (GRs) transcriptionally regulates Cav1.2 channel subunits and thus increases the Ca2+/voltage-dependent calcium current subunit (ICaL-1.3). By targeting Cav1.3 subunits and/or the plasma membrane Ca2+-ATPase-1 (PMCA1)—here reflected by upward and downward pointing arrows, respectively—corticosterone can enhance Ca levels close to channels giving rise to the IsAHP, resulting in an enhanced sAHP amplitude (bottom). Right: in the BLA, expression levels of Cav1.3 subunits (and therefore ICaL-1.3) are below the detection limit of our in situ hybridization assay. Corticosterone may transcriptionally regulate Cav1.1 subunits, but this does not alter the function of channels underlying the IsAHP, so that in this area no effect of corticosterone on the sAHP amplitude can be discerned.
CA1 neurons delays the extrusion of Ca (Joëls et al. 1997). We therefore considered a combined effect of corticosterone on Cav1.3 expression and Ca-extrusion rate. The simulation data indicate that such combined effects can indeed fully explain the observed changes in the sAHP (see Fig. 5). Similar changes in the Ca extrusion rate in the BLA model neuron resulted in quite smaller increases in the sAHP. Since no change in the sAHP was observed experimentally after glucocorticoid modulation the function of the Ca-extrusion pump may not be altered by glucocorticoids in the BLA. However, this remains to be confirmed experimentally. Alternative explanations, of course, cannot be ruled out. It is possible that corticosterone differentially regulates channels underlying the sAHP in the CA1 region versus the BLA, although qPCR investigation of the SK1 channel—which is colocализed with Cav1.3 subunits (Bowden et al. 2001)—did not reveal any regulation by corticosteroids in the CA1 region (Y Qin, S. Spijker, P. Chameau, A. B. Smit, and M. Joels, unpublished observation). Moreover, it has been disputed whether SK channels indeed underlie the sAHP in CA1 cells (Bond et al. 2004).

Functional implications

The large sAHP amplitude and increased firing frequency accommodation seen 1–4 h after corticosterone levels peak have been interpreted as a means for CA1 cells to attenuate information flow through the CA1 area and thus to normalize local activity that is enhanced shortly after stress, due to rapid effects of catecholamines, neuropeptides, and steroids acting through nongenomic pathways (Joëls et al. 2007). If corticosterone does not change the sAHP and firing frequency accommodation in BLA principal cells this could signify that enhanced activity in these cells is less efficiently constrained several hours after stress exposure. Information with a strong emotional load, which will heavily activate BLA neurons, may therefore have longer-lasting consequences for limbic activity than neutral information.

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