Corticosteroids Alter 5-Hydroxytryptamine$_{1A}$ Receptor-effector Pathway in Hippocampal Subfield CA3 Pyramidal Cells

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ABSTRACT

Corticosteroids influence neuron activity in the hippocampus through the activation of mineralocorticoid and glucocorticoid receptors. For example, corticosteroids modulate the responses elicited by the activation of several different neurotransmitter receptors on hippocampal pyramidal cells. However, the effects of corticosteroids on the serotonin (5-HT) receptors systems in subfield CA3 are not completely known. Therefore, we used single-electrode voltage clamp techniques to examine the actions of chronic corticosteroid treatment on the 5-HT$_{1A}$ receptor-effector pathway in rat hippocampal subfield CA3 pyramidal cells. Activation of the 5-HT$_{1A}$ receptor increases the conductance of an inward rectifying potassium channel, increasing outward current. The treatment groups used in this investigation were: adrenalectomy, selective mineralocorticoid receptor activation with aldosterone, mineralcorticoid receptor and glucocorticoid receptor activation with high levels of corticosterone and SHAM. Corticosteroids altered the characteristics of the 5-HT concentration-response curve for the 5-HT$_{1A}$ receptor. The effective concentration at 50% of maximum value was smaller in cells from the adrenalectomy treatment group compared to the other treatment groups. The maximum response was smaller in cells from the high corticosterone treatment group compared to SHAM and adrenalectomy treatment group animals. G protein function was also altered by corticosterone treatment. Less current was elicited by guanosine 5'-0-13-thiotriphosphate in cells from the high corticosterone treatment group compared to the other treatment groups and in cells from the SHAM treatment group compared to adrenalectomy treatment group animals. Corticosteroid treatment did not alter the current-voltage relationship, the conductance or the reversal potential of the potassium current linked to the 5-HT$_{1A}$ receptor. We conclude that corticosteroids alter the 5-HT$_{1A}$ receptor-mediated-response in hippocampal subfield CA3 neurons at site(s) downstream of the receptor.

Activation of corticosteroid receptors in the hippocampus influences hippocampus-related behaviors (de Kloet et al., 1993) and modulates the activity of the HPA axis, which regulates the synthesis and secretion of corticosteroids (Jacobson and Sapolsky, 1991). Corticosteroid hormones (glucocorticoids and mineralocorticoids) regulate gene expression through the interaction with two receptor subtypes: MR (or type I) and GR (or type II) (Joels et al., 1994). MR and GR have a 5- to 10-fold difference in their affinity for the rat corticosteroid hormone cortisol. MR has a high affinity for cortisol and is 70 to 80% occupied at basal corticosterone plasma levels (Krozowski and Funder, 1983; Reul and de Kloet, 1985; Reul et al., 1987a). GR has a lower affinity for cortisol and its occupancy changes from 15% at basal to 90% at peak circadian or stress-induced circulating corticosterone levels (Reul and de Kloet, 1985; Reul et al., 1987a,b).

One way corticosteroids alter neural activity in the hippocampus is by modulating the responses elicited by the activation of neurotransmitter receptors (McEwen, 1996; Joels and de Kloet, 1994), including the serotonergic 5-HT$_{1A}$ receptor (Joels and de Kloet, 1994; Beck et al., 1996). Several laboratories have provided detailed pharmacological characterization of the 5-HT$_{1A}$ receptor in the CA1 and CA3 subfields of the hippocampus (Andrade et al., 1986; Andrade and Nicoll, 1987; Beck et al., 1992). The 5-HT$_{1A}$ receptor is a G protein-coupled receptor. Activation of the 5-HT$_{1A}$ receptor in the CA1 and CA3 hippocampal subfields increases an inward rectifying potassium current linked to a PTX-sensitive G protein (Andrade et al., 1986; Andrade and Nicoll, 1987; Colino and Halliwell, 1987; Beck et al., 1992; Beck and Choi, 1991; Okuhara and Beck, 1994). Corticosteroids alter the 5-HT$_{1A}$ receptor-mediated responses in hippocampal subfield CA3 pyramidal cells.

ABBREVIATIONS: MR, mineralocorticoid receptor; GR, glucocorticoid receptor; 5-HT, 5-hydroxytryptamine, serotonin; ALD, aldosterone treatment group; HCT, high corticosterone treatment group; ADX, adrenalectomy treatment group; HPA, hypothalamic-pituitary-adrenal; PTX, pertussis-toxin; AOSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; E$_{max}$, maximum response; EC$_{50}$, effective concentration at 50% of maximum; N, slope; GTP-y-S, guanosine 5'-0-13-thiotriphosphate; I-V, current-voltage.
CA1 (Beck et al., 1996; Joels et al., 1991; Joels and de Kloet, 1992). Even though the 5-HT\(_{1A}\) receptor mediates a hyperpolarization through an increase in conductance of an inward rectifying potassium channel in both area CA1 and CA3, there are important differences in the nature of the response (Beck et al., 1992; Okuhara and Beck, 1994). For example the rank order potency of the agonists are the same, but the absolute affinity of the ligands are approximately a half of a log unit lower in affinity in CA3. The antagonists spiperone and BMY 7378 are competitive antagonists in CA1, but insurmountable in CA3. The effects of corticosteroids on the 5-HT\(_{1A}\) receptor-mediated increase in potassium conductance in subfield CA3 are not known; we propose that the effects of corticosterone on the 5-HT\(_{1A}\) receptor-mediated response will be different in subfields CA1 and CA3.

Previous studies have examined the effects of corticosteroids on the number of 5-HT\(_{1A}\) binding sites and mRNA levels in the hippocampus (Chauhoff, 1995). Corticosteroids appear to alter 5-HT\(_{1A}\) receptor binding sites in subfield CA3, but not in subfield CA1 (Mendelson and McEwen, 1992a,b; Kuroda et al., 1994). Recently, we reported that chronic exposure to high levels of corticosteroids increased the protein levels of G\(_{\alpha}\), G\(_{i,\alpha}\) and G\(_{\alpha}\) subunits in the hippocampus (Okuhara et al., 1997). However, the actions of corticosteroids on G protein function in the individual hippocampal subfields and on G protein-evoked currents are not known.

In our study, we examined the effects of chronic corticosteroid treatment on different components of the 5-HT\(_{1A}\) receptor-effector pathway in hippocampal subfield CA3 pyramidal cells. Single-electrode voltage clamp techniques were used in the brain slice preparation. We hypothesized that chronic corticosteroid treatment would alter the 5-HT\(_{1A}\) receptor signal transduction system in subfield CA3. The results from these experiments will contribute to a basic understanding of how corticosteroids alter responses elicited by the activation of G protein-linked neurotransmitter receptors and help to elucidate the role of corticosteroids in hippocampus-related behaviors and HPA feedback.

**Methods**

**Animals.** Four different treatment groups were used for the experiments in this investigation (table 1). The adrenalectomy was performed as described previously (Beck et al., 1994; Birnstiel and Beck, 1995). Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) 75 to 100 g were used for all treatment groups. Bilateral adrenalectomies were performed on all treatment groups, except the SHAM group, to remove circulating corticosteroids. The animal was anesthetized with ether, a small incision was made into the abdominal cavity, just below the rib cage, and the adrenal glands carefully removed. The muscle wall was then sutured and the skin closed using wound clips. The ADX group received no further treatment. The ALD group had an osmotic minipump containing aldosterone (Alzet model 2002 from Alza Cooperation, Palo Alto, CA), implanted s.c. at the time of adrenalectomy, to selectively activate MR. The minipump delivered 10 µg/hr aldosterone (Steraloids, Inc., Wilton, NH) dissolved in propylene glycol. MR and GR were activated in another group of animals (HCT) by s.c. implanting 200 to 300 mg corticosterone pellets, 2- to 3-week release (Innovative Research, Toledo, OH), in the back of the neck at the time of adrenalectomy. The SHAM group of animals was produced by visualizing the adrenal glands but leaving them intact. After surgery the animals were maintained on a standard 12-hr light/dark cycle and rat food, ad libitum. SHAM animals were given standard drinking water while the ADX, ALD and HCT animals were given 0.9% NaCl drinking water ad libitum. At the end of 13 to 15 days, the animals were killed in the morning and hippocampal slices immediately prepared for electrophysiological recording. At the time of death, trunk blood was collected to determine the plasma corticosterone levels by radioimmunoassay (Burgess and Handa, 1992).

**Hippocampal slice preparation.** Hippocampal slices were prepared for electrophysiological recording as previously described (Okuhara and Beck, 1994). Rats were killed by decapitation and the brain rapidly removed and placed in ice cold ACSF containing in mM: NaCl 125, KCl 3, NaH\(_2\)PO\(_4\) 1.25, MgSO\(_4\) 2, CaCl\(_2\) 2.5, dextrose 10 and NaHCO\(_3\) 28. The ACSF was also supplemented with steroids, as outlined in table 1, to maintain the treatment paradigm. We previously reported that steroids must be present in the ACSF to preserve the corticosteroid-induced effects on neuron cell properties (Beck et al., 1994). The hippocampus was dissected free and the dorsal section cut in 500- to 550-µm sections on a vibratome. The hippocampal slices were then placed in a holding vial containing ACSF bubbled with 95% O\(_2\)-5% CO\(_2\) at room temperature. The slices remained in the holding vial for at least 1 hr dissection before being transferred to the recording chamber. In the recording chamber, the slice was stabilized between two nylon nets and continuously perfused with ACSF bubbled with 95% O\(_2\)-5% CO\(_2\) at a rate of 2 to 3 ml/min, at 31 to 32°C.

**Intracellular recording.** Intracellular recordings were made as previously described (Okuhara and Beck, 1994). Electrodes were pulled from borosilicate capillary tubing on a Brown and Flaming electrode puller (Sutter Instruments, Novato, CA) to a resistance of 30 to 35 MΩ (2 M KCl). Pyramidal cells were impaled with brief juxtacellular depolarizions of positive current through the electrode. The impaled cells were sealed by applying 1 nA hyperpolarizing current. Electrical signals were collected and amplified using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) and a Cyberamp 320 amplifier (Axon Instruments, Foster City, CA) and recorded on a Gould Series 3200 chart recorder (Gould Electronics, Valley View, OH). Data were collected on-line with pCLAMP software (Axon Instruments).

Discontinuous single-electrode voltage clamp technique was used under the following conditions. Briefly, the slice was perfused with ACSF bubbled with 95% O\(_2\)-5% CO\(_2\) at a rate of 2 to 3 ml/min, at 31 to 32°C.

**5-HT concentration-response.** Cells were voltage clamped at –65 mV and data for the 5-HT concentration response curves were collected by perfusing the slice with increasing 5-HT (5-HT hydrochloride, Sigma) concentrations (3, 10, 30 and 100 or 110 µM) and the maximum current response evoked during the drug application was recorded. During drug perfusion, if the voltage clamp deviated by more than 1 mV, the data were discarded.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surgery</th>
<th>Implant</th>
<th>ACSF</th>
<th>Plasma Corticosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>Intact</td>
<td>Nothing</td>
<td>1 nM corticosterone</td>
<td>1.35 ± 0.32 µg/dl (N = 15)</td>
</tr>
<tr>
<td>ADX</td>
<td>Adrenalectomy</td>
<td>Nothing</td>
<td>No steroid</td>
<td>0.06 ± 0.03 µg/dl (N = 12)</td>
</tr>
<tr>
<td>ALD</td>
<td>Adrenalectomy</td>
<td>Aldosterone (10 µg/h minipump)</td>
<td>3 nM aldosterone</td>
<td>0.07 ± 0.05 µg/dl (N = 16)</td>
</tr>
<tr>
<td>HCT</td>
<td>Adrenalectomy</td>
<td>200 to 300 mg corticosterone</td>
<td>100 nM corticosterone</td>
<td>34.0 ± 3.00 µg/dl (N = 16)</td>
</tr>
</tbody>
</table>
The magnitude of the current response was fitted to a hyperbolic function (Okuhara and Beck, 1994; Beck et al., 1996):

\[ E = E_{\text{max}}/[1 + (EC_{50}/[5-HT])^N] \]

where \( E \) is the current produced at the 5-HT concentration [5-HT], \( E_{\text{max}} \) is the maximal current response, \( EC_{50} \) is the 5-HT concentration which produces half the \( E_{\text{max}} \) response, and \( N \) is the slope index. The fitted \( E_{\text{max}}, EC_{50} \), and \( N \) values within each experimental group were used for statistical comparisons.

**Experiments with GTPγS.** The effect of corticosteroids on G protein activity was determined by measuring the magnitude of outward current evoked by 15 mM GTPγS (Boehringer Mannheim, Indianapolis, IN) which was included in the recording electrode. After the cell’s membrane potential stabilized, the cell was voltage clamped at its resting membrane potential (i.e., the potential where no current was required to maintain the voltage clamp). The voltage clamp was then moved to −60 mV and the amount of current required to maintain the clamp was recorded.

**I-V relationship.** The effect of corticosteroids on the potassium current linked to the 5-HT1A receptor was determined by plotting the current-voltage (I-V) curve for the outward current evoked by the activation of the 5-HT1A receptor. After the cell’s membrane potential stabilized, the cell was voltage clamped at its resting membrane potential (the potential where no current was required to maintain the voltage clamp). Data for I-V plots were obtained by running voltage ramps in the presence and absence of 100 μM 5-HT. Voltage ramps were run from −60 mV to +35 mV from the cell’s clamped membrane potential, i.e., approximately −120 to −25 mV, at a rate of 1 mV/sec. The I-V plot for the potassium current evoked by the activation of the 5-HT1A receptor was constructed by subtracting the control ramp values (no 5-HT) from the ramp values in the presence of 100 μM 5-HT. The reversal potential, conductance (between −105 to −60 mV) and potential where inward rectification occurred were analyzed for each curve.

**Statistical analysis.** Statistical comparisons were performed using ANOVA. The Student-Newman-Keuls method was used for post hoc tests. All values are reported as mean ± S.E.M. A P < .05 was considered significant.

## Results

**Corticosterone plasma levels.** The mean corticosterone plasma levels for the different treatment groups are listed in table 1. SHAM corticosterone plasma levels ranged from 0 to 4.13 μg/dl. ADX corticosterone plasma levels ranged from 0 to 0.22 μg/dl. ALD corticosterone plasma levels ranged from 0 to 0.56 μg/dl. The lower and upper limits of the corticosterone radioimmuno assay were 0.05 and 50 μg/dl, respectively. Adrenalecotomies that produced corticosterone concentrations ≤0.6 μg/dl were considered successful. Two SHAM, 8 ADX and 14 ALD animals had corticosterone levels less than 0.05 μg/dl. The concentration of aldosterone used in our experiments was based on an investigation by Kuroda et al. (1994). The ALD treatment decreased MR binding by 61% (n = 5 animals) in the cytoplasm (data not shown) as determined using a homogenate binding assay. HCT plasma concentrations of more than 20 μg/dl were considered successful. HCT corticosterone plasma levels ranged from 25 to >50 μg/dl.

There were no statistical differences in the resting membrane potential (SHAM −70.5 ± 0.9 mV, N = 32; ADX −68.9 ± 0.8 mV, N = 39; ALD −70.3 ± 1.1 mV, N = 26; HCT −68.6 ± 1.2 mV, N = 25) or input resistance (SHAM 50.6 ± 1.6 MΩ, N = 32; ADX 50.8 ± 1.8 MΩ, N = 39; ALD 47.8 ± 1.96 MΩ, N = 26; HCT 52.2 ± 2.1 MΩ, N = 25) of the cells recorded from the four treatment groups.

**5-HT concentration response curve characteristics for the 5-HT1A receptor.** Activation of the 5-HT1A receptor evoked an outward current in a concentration-dependent manner (fig. 1A). The 5-HT concentration-response curve characteristics recorded from cells from corticosterone treated animals were summarized in table 2. The EC50 obtained in cells from ADX was smaller compared to cells from either SHAM-, ALD- or HCT-treated animals (fig. 1B). The Emax value was also smaller in cells from HCT when compared to cells from SHAM- or ADX-treated animals (fig. 1B). Corticosterone treatment did not alter the slope index.

Perfusion of hippocampal slices with 5-HT may activate a number of different 5-HT receptor subtypes. Two 5-HT receptor subtypes that may be activated by 5-HT and thereby modulate the 5-HT1A current are the 5-HT4 and 5-HT7 receptors. Both of these receptors are located in the hippocampus (Lovenberg et al., 1993; Meyerhof et al., 1993; Ruat et al. 1993; Jakeman et al., 1994; Roychowdhury et al., 1994; Waeber et al., 1994) and are positively coupled to adenylate cyclase (Lovenberg et al., 1993; Ruat et al., 1993; Shen et al. 1993; Tsou et al. 1994). Activation of the 5-HT7 receptor produces a slow depolarizing response in subfield CA1 pyramidal cells (Andrade and Chaput, 1991). Because the 5-HT4 and 5-HT7 receptors have not been characterized in subfield CA3, we determined if activation of these receptors altered the 5-HT-induced outward current recorded in subfield CA3. The magnitude of the outward current elicited by 30 μM 5-HT was not altered by the administration of 1 μM GR113808 and ritanserin (n = 6) (fig. 2), 5-HT1A and 5-HT3 receptor antagonists, respectively (Boess and Martin, 1994; Torres et al., 1994). Perfusion of the slice with 30 μM 5-HTA while blocking the 5-HT1A receptor with either 1 μM WAY 100635 or 3 μM spiperone did not produce either a depolarizing or inward current response (n = 3) (data not shown).

**Activation of G proteins with GTPγS.** The injection of GTPγS into neurons produced a hyperpolarization of the resting membrane potential. There was no difference in the resting membrane potential between treatment groups. The relative amount of current evoked by the activation of G proteins with GTPγS was determined by voltage clamping the cell’s membrane at its resting potential (when zero current is required to maintain the voltage clamp). The voltage clamp was then moved to −60 mV for several minutes (fig. 3A). Although holding the cell at −60 mV, a saturating concentration of 5-HT (100 μM) was applied; the response to 5-HT was totally occluded when GTPγS was included in the recording pipette (fig. 3A). The amount of current required to maintain the voltage clamp at −60 mV was used as a measure of the amount of current evoked by the activation of G proteins with GTPγS. Figure 3B summarizes the results of these experiments. Significantly less current was required in cells from HCT when compared to cells from SHAM-, ADX- and ALD-treated animals. The amount of current measured in cells from SHAM was also smaller compared to cells from ADX-treated animals.

**I-V relationship.** I-V plots for the 5-HT1A evoked potassium current were analyzed in three to five cells from ADX-, SHAM- and HCT-treated animals. Representative I-V plots
in figure 4A–C demonstrate that corticosterone does not appear to alter the reversal potential nor the inward rectification properties for the potassium current linked to the 5-HT1A receptor. The reversal potential was between $-297$ to $-104$ mV and rectification started at approximately $-50$ mV. There was no significant change in the slope conductance (table 2) measured in the linear portion of the I-V plot, between $-105$ and $-60$ mV.

### Discussion

The 5-HT$_{1A}$ receptor is a member of the G protein-linked receptor family. In our study, we report the effects of chronic corticosteroid treatment on different components of the 5-HT$_{1A}$ receptor-G protein-ion channel system in hippocampal subfield CA3 pyramidal cells using electrophysiological techniques. The EC$_{50}$ value for the 5-HT-concentration-response curve was shifted to the left in cells from ADX-treated animals compared to SHAM, ALD and HCT. The E$_{max}$ value was smaller in cells from HCT animals compared to ADX and SHAM. The shift in the 5-HT$_{1A}$ response may be due to alterations at sites past the 5-HT$_{1A}$ receptor. Therefore, we compared the magnitude of the outward current evoked by the activation of G proteins with GTP$_{i}$S. Less current was evoked by GTP$_{i}$S in cells from HCT animals compared to ADX and SHAM. The shift in the 5-HT$_{1A}$ response was due to alterations at sites past the 5-HT$_{1A}$ receptor. Therefore, we compared the magnitude of the outward current evoked by the activation of G proteins with GTP$_{i}$S. Less current was evoked by GTP$_{i}$S in cells from HCT animals compared to ADX and SHAM. Furthermore, less current was evoked by GTP$_{i}$S in cells from SHAM compared to ADX animals. Finally, we determined the effects of corticosteroids on the I-V relationship of the potassium current elicited by the activation of the 5-HT$_{1A}$ receptor. Corticosteroid treat-
ment did not alter the reversal potential, conductance or inward rectification properties of the potassium current linked to the 5-HT$_{1A}$ receptor. Based on our results and those previously reported by other investigators, we conclude that corticosteroids alter the response elicited by the activation of 5-HT$_{1A}$ receptors by modulating several components of the receptor-effector cascade.

The natural neurotransmitter, 5-HT, was used in these experiments to generate the concentration-response information. If corticosterone interfered with uptake mechanisms, it is possible this would result in an alteration in the 5-HT concentration-response curve for the 5-HT$_{1A}$ receptor-mediated increase in outward current. However, it has been previously demonstrated that blocking the 5-HT uptake mechanisms with fluoxetine did not alter the characteristics of the 5-HT concentration-response curve for the 5-HT$_{1A}$ receptor-mediated hyperpolarization (Beck et al., 1992).

Alterations in the number of 5-HT$_{1A}$ binding sites may account for some of the changes we observed in the 5-HT concentration-response curve characteristics. Previous investigators reported that adrenalectomy increases the number of 5-HT$_{1A}$ receptor binding sites in subfield CA3 when compared to either SHAM, aldosterone, low or high corticosterone-treated animals (Mendelson and McEwen, 1992a, 1992b; Kuroda et al., 1994; Chalmers et al., 1993; Tejani-Butt and Labow, 1994). The increased number of 5-HT$_{1A}$ receptor binding sites may account for the shift to the left of the EC$_{50}$, with no change in E$_{max}$, for the 5-HT concentration-response curve that we observed in cells from ADX compared to SHAM- and ALD-treated animals. A shift in EC$_{50}$ with no change in E$_{max}$ may occur with spare receptors (Kenakin, 1993).

The smaller E$_{max}$ in cells from HCT-compared to SHAM-treated animals may be due to a decrease in the number of 5-HT$_{1A}$ binding sites. It has been reported that high levels of corticosterone (plasma concentrations >40 µg/dl) do not change the number of 5-HT$_{1A}$ binding sites in subfield CA3 compared to sham-treated animals (Mendelson and McEwen, 1992a). However, social and restraint stress does decrease the number of 5-HT$_{1A}$ binding sites in the hippocampus (Watanabe et al., 1993; McKittrick et al., 1995). One possible explanation for the discrepancy is the treatment length. Mendelson and McEwen (1992b) treated their animals with a corticosterone pellet implant for 7 days. The investigations that examined the effects of stress treated their animals for 14 days (Watanabe et al., 1993; McKittrick et al., 1995). It is possible that exposure to elevated corticosterone plasma levels must exceed 7 days before the down-regulation of 5-HT$_{1A}$ binding sites occurs. Alternatively, the stress response may be activating other physiological processes, in addition to

![Fig. 2.](image)

Fig. 2. The outward current elicited by the perfusion of 30 µM 5-HT in the presence and absence of 1 µM of the 5-HT$_4$ and 5-HT$_7$ receptor antagonists GR113808 and ritanserin. The drugs were applied for the duration depicted by the line above the chart record. The magnitude of the outward current evoked by 5-HT was not different (P = .141, n = 6, paired t test) in the presence or absence of GR113808 and ritanserin.

![Fig. 3.](image)

Fig. 3. The outward current evoked by GTP$\gamma$S. A, Chart recording of a cell injected with GTP$\gamma$S. The cell’s membrane potential was first voltage clamped at its resting potential (−74 mV, I = 0 pA). The clamp was then moved to −60 mV and the current recorded (I = 340 pA). Perfusion with 100 µM 5-HT (duration depicted by the line above the chart record) did not elicit a current response, indicating all the G proteins linked to 5-HT receptors were activated by GTP$\gamma$S. B, Summary graph depicting the amount of current evoked by GTP$\gamma$S in cells from corticosterone-treated animals. Treatment group and number of cells are labeled at the bottom axis. ANOVA F = 12.691; d.f. = 3, 61; P < .001. Student-Newman-Keuls, P < .05; HCT different from SHAM, ADX and ALD; ADX different from SHAM.
increasing corticosterone plasma levels, that induces the down regulation of 5-HT_{1A} binding sites.

Corticosteroids also alter the 5-HT_{1A} receptor-effector pathway downstream of the receptor. ADX increased although HCT decreased the magnitude of the current evoked by the activation of G proteins with GTPγS. Corticosteroids could be altering G protein levels, G protein coupling or the properties of the channels linked to the G proteins. However, the attenuated 5-HT_{1A} mediated-response and G protein-evoked current cannot be attributed to a decrease in G protein expression. We observed that HCT treatment increased G_{i} and G_{13,2} a-subunits levels in the hippocampus (Okuhara et al., 1997), the PTX-sensitive G proteins thought to be linked to the 5-HT_{1A} receptor (Andrade et al., 1986; Okuhara and Beck, 1994). Based on our results, we propose that the increased G protein levels may be a compensatory response to a decrease in effectual G protein function.

Interestingly, although corticosterone clearly had an effect on the magnitude of the outward current evoked by GTPγS, there was no change in the resting membrane potential of pyramidal cells between treatment groups. This is an important observation because corticosteroid induced changes in the resting membrane potential in the presence of GTPγS may not be detectable under current clamp conditions. GTPγS should activate a number of different G protein-linked channels, including inward rectifying potassium channels, inducing a decrease in membrane resistance that should shunt changes in membrane potential. Alterations in the 5-HT_{1A} receptor-mediated response and G protein-linked current could be attributed to a change in pyramidal cell resting input resistance. However, corticosteroid treatment did not alter neuron resting input resistance in subfield CA3 pyramidal cells (Okuhara and Beck, 1998). Although we were able to detect corticosteroid-induced changes in the current evoked by the activation of G proteins we do not know if corticosterone is altering G protein function or the coupling between the G protein and potassium channel.

The potassium channel linked to the 5-HT_{1A} receptor signal transduction system is probably a member of the recently cloned G protein inward rectifying potassium channel family (Spauschus et al., 1996; Kobayashi et al., 1995; Lesage et al., 1994; Ponce et al., 1996; Lesage et al., 1995). ADX, ALD and HCT treatment did not alter the reversal potential, conductance or inward rectification properties of the potassium current linked to the activation of the 5-HT_{1A} receptor. However, alterations in potassium channel number or kinetic properties cannot be ruled out.

ADX had different effects on the 5-HT concentration response curve characteristics in cells from subfield CA1 and CA3. Previous studies reported that the concentration-response curve characteristics for the 5-HT_{1A}-mediated hyperpolarization in subfield CA1 were not altered by adrenalectomy or chronic activation of MR with low levels of corticosterone as compared to sham (Beck et al., 1996; Joels et al., 1991; Joels and de Kloet, 1992). In our investigation, ADX shifted the EC_{50} value for 5-HT_{1A} mediated outward current compared to SHAM and ALD (chronic MR activation). The differences in these results could be explained in several ways. In this study, 1 nM corticosterone was included in the perfusion buffer of the SHAM-treated rats to maintain the basal levels of CT, whereas in the previous experiments no corticosterone was in the perfusion buffer of the SHAM rats. We have previously demonstrated that it is necessary to maintain the treatment paradigm by including the treatment steroid in the perfusion buffer when recording from the slices on the day of the experiment (Beck et al., 1994). Previously, we reported that the 5-HT_{1A} receptor signal transduction system is not identical between subfields CA1 and CA3 based on different 5-HT concentration-response curve characteristics for the 5-HT_{1A} receptor in the two subfields (Beck et al., 1992; Okuhara and Beck, 1994). Therefore, it is possible that corticosteroids have different effects on the 5-HT_{1A} receptor-mediated response in subfields CA1 and CA3.

In conclusion, chronic corticosterone treatment alters the response elicited by the activation of 5-HT_{1A} receptors in hippocampal subfield CA3 pyramidal cells. Some of the modulatory actions of corticosterone occur downstream of the receptor, at the G protein level. Furthermore, ADX and high corticosterone treatment have opposite effects on the 5-HT_{1A} signal transduction system. Our results provide important information toward understanding how corticosterone modulates neurotransmitter receptor-mediated responses in the hippocampus.

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