Corticosteroids Alter the 5-HT$_{1A}$ Receptor-Mediated Response in CA1 Hippocampal Pyramidal Cells

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The hippocampus, prolonged excessive corticosterone secretion, and the 5-hydroxytryptamine (5-HT) neurotransmitter system are implicated in the etiology and treatment of psychiatric disorders. Corticosterone regulates CA1 hippocampal physiology and the 5-HT$_{1A}$ receptor-effector pathway; however the effect of chronic stress levels of corticosterone is unknown. Bilateral adrenalectomy (ADX), adrenalectomy with high dose corticosterone replacement (HCT), or surgical sham (SHAM) treatments were for 2 weeks. Standard intracellular recording techniques were used in hippocampal slices to measure active and passive cellular properties and 5-HT$_{1A}$ receptor-mediated responses in CA1 pyramidal cells. The magnitude

and half-decay time of the slow after-hyperpolarization (sAHP) were decreased and the membrane time constant (tau) was increased by HCT treatment. The E_{max} and EC_{50}, but not the slope, of the concentration-response curve for 5-HT activation of the 5-HT$_{1A}$ receptor were reduced in cells recorded from HCT versus SHAM treated rats. The net effect of treatment with stress levels of corticosterone was to increase the excitability of the CA1 hippocampal pyramidal cell through changes in membrane properties and 5-HT$_{1A}$ receptor-mediated response.
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The glucocorticoids are endogenous steroids that are important in maintaining homeostasis (Tempel and Leibowitz 1994; Woods et al. 1998). The glucocorticoids are referred to as stress hormones since they control the physiological responses to stress and feedback regulation to return the system to normal. Chronic stress and certain mood disorders such as depression or anxiety alter the normal functions of the glucocorticoid system

and the normal functions of particular brain circuits. One of those circuits is the 5-hydroxytryptamine (5-HT) neurotransmitter system, its projections to the hippocampus and the 5-HT receptor-effector pathway.

Two receptor systems for corticosterone are present in the rat brain, the mineralocorticoid (MR) and glucocorticoid (GR) receptors (Reul and Dekloet 1985, Reul et al. 1987a, 1987b). The two receptors are distinguished pharmacologically by their relative affinity for corticosterone and other selective ligands. The affinity of corticosterone for the MR is 0.5 nM while the affinity of corticosterone for the GR is 2.5–5 nM (Reul and Dekloet 1985). MR has a 10-fold higher affinity for corticosterone than GR (Reul et al. 1987a). Because of the difference in affinity, changes in plasma corticosterone concentration alter the relative MR/GR occupation. Low plasma corticosterone levels will mainly bind to MRs, while high corticosterone levels at the circadian peak or during stress will bind to GRs as well as MRs.

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Both types of corticosterone receptors are intracellular receptors that bind to DNA and influence gene transcription (Beato 1989; Beato et al. 1996). MR binding is almost exclusively in the hippocampus (Reul et al. 1987b). GRs are also concentrated in the hippocampus but are found in other CNS areas (Reul et al. 1987b). Circulating glucocorticoid levels in rats follow a normal rhythm where plasma corticosterone levels are lowest during "lights-on," (Akana et al. 1988), peak during "lights-off," and increase in response to stress (Akana et al. 1985; Herman and Cullinan 1997). Approximately 70% of the MRs are occupied at the circadian trough (Reul et al. 1987a, 1987b). GR occupancy increases from 10% at the circadian trough to 80% at the circadian peak or following a stressful stimulus (Akana et al. 1988; Reul et al. 1987a). Stress produces plasma corticosterone levels in the range of 20–75 μg/dl depending on the type of stressor (Bhatnagar et al. 1997; Dallman et al. 1994; Galea et al. 1997; Groenink et al. 1996; Kirby and Lucki 1998; Liberzon et al. 1997; Mendelson and McEwen 1992; Raghupathi and McGonigle 1997). In pathophysiological states, such as chronic stress or depression, basal plasma cortisol levels are elevated (Dinan 1996).

In the hippocampus, corticosterone treatment alters the 5-HT_{1A} receptor-mediated response as measured using intracellular electrophysiological recording techniques. The 5-HT_{1A} receptor is linked through a pertusis toxin sensitive G-protein to a G-protein inwardly rectifying K^+ channel (GIRK) in both CA1 and CA3 subfields of the hippocampus (Andrade et al. 1986; Okuhara and Beck 1994). Activation of the 5-HT_{1A} receptor induces a membrane hyperpolarization or increase in outward current (Andrade and Nicoll 1987; Colino and Halliwell 1987; Beck et al. 1992). In the CA1 subfield administration of concentrations of corticosterone to selectively activate only MR or administration of the MR selective steroid aldosterone for several hours (short term) results in a decrease in the magnitude of the hyperpolarization (Beck et al. 1996; Hesen and Joels 1996; Joels et al. 1991). Chronic treatment (weeks) with basal levels of corticosterone to selectively activate MR (Beck et al. 1994) does not significantly alter the CA1 hippocampal pyramidal cell 5-HT_{1A} receptor-mediated hyperpolarization (Beck et al. 1994). In contrast, in the CA3 subfield of the hippocampus adrenalectomy increases the potency of 5-HT for the 5-HT_{1A} receptor-mediated outward current, chronic treatment with aldosterone has no effect and treatment with high doses of corticosterone for 2 weeks attenuates the 5-HT_{1A} response (Okuhara and Beck 1998). One previous study reported that chronic treatment with stress levels of corticosterone reduced the magnitude of the hyperpolarization elicited by 5-HT activation of the 5-HT_{1A} receptor (Karten et al. 1999). However, only one concentration of 5-HT was tested and therefore it is unknown whether the modulatory effect of 5-HT was due to a change only in the magnitude of the maximal response or due to a shift in the potency of 5-HT for the 5-HT_{1A} receptor. The goal of the experiments presented in this article was to characterize the effects of chronic treatment with stress levels of the glucocorticoid corticosterone on the basic active and passive membrane properties and on the characteristics of the 5-HT concentration-response curve for activation of the 5-HT_{1A} receptor in CA1 hippocampal pyramidal cells as compared to adrenalectomized and sham treated control animals.

METHODS

Chemicals

Chemicals used for making the artificial cerebrospinal fluid and the 5-HT were purchased from Sigma Chemical Company (St. Louis, MO). Corticosterone pellets were purchased from Innovative Research (Toledo, OH).

Adrenalectomies

Table 1 shows the three different treatment groups that were used for the experiments in this investigation. Adrenalectomy was performed as described previously (Beck et al. 1996, 1994; Okuhara and Beck 1998; Okuhara et al. 1997). Male Sprague-Dawley rats (75–100 g, Harlan, IN) were anesthetized with halothane. Bilateral adrenalectomies were performed by making a small incision (0.5 in) in the skin and muscle wall just below the rib cage. The adrenal glands were visualized and removed. The muscle wall was sutured, and the skin incision closed with wound clips. The ADX group received no further treatment. MR and GR were activated in another group of animals (HCT) by implanting subcutaneously in the back of the neck at the time of adrenalectomy two 100-mg corticosterone pellets designed for 3-week release. A small pocket was made subcutaneously with a pair of hemostats, the pellets were inserted and the incision closed with wound clips. For SHAM treated rats the adrenal glands were visualized and not removed. All rats were allowed to recover for 2 weeks. SHAM animals were given standard drinking water while the ADX and HCT groups were given drinking water containing 0.9% NaCl, ad libitum. All rats were maintained on a 12-hour light/dark cycle (7:00 A.M. to 7:00 P.M. lights on) and rat chow, ad libitum. A 2-week treatment period was chosen because it takes 2 weeks for the GR to maximally up-regulate following ADX (Reul et al. 1987a, 1987b). At the end of 14 days, the animals were sacrificed in the morning and hippocampal slices were immediately prepared for electrophysiological recording. At the time of sacrifice, trunk blood was collected to determine the plasma corticosterone levels

Table 1. Summary of Treatment Groups and Corticosterone Plasma Levels (All Values Are Expressed As Mean ± S.E.M.)

Treatment Group	Surgical Treatment	ACSF Content	Corticosterone Plasma Levels (μg/dl)
SHAM	Incision	1 nM corticosterone	2.12 ± 0.954
ADX	Adrenalectomy	No steroid	0.09 ± 0.040
HCT	Adrenalectomy	100 nM corticosterone	34.09 ± 3.054

(Table 1) by radioimmunoassay (Burgess and Handa 1992).

Hippocampal Slice Preparation

Hippocampal slices were prepared for electrophysiological recording as previously described (Beck et al. 1996, 1994; Okuhara and Beck 1998). The rats were sacrificed by decapitation and the brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (mM): NaCl 124, KCl 3, NaH$_2$PO$_4$ 1.25, MgSO$_4$ 2, CaCl$_2$ 2.5, dextrose 10 and NaHCO$_2$ 28. The ACSF was also supplemented with steroids, as outlined in Table 1, to maintain the treatment paradigm. We have 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 portion cut into 550-μM sections on a vibratome. The hippocampal slices were then placed in a holding vial containing ACSF bubbled with 95% 0$_2$-5% CO$_2$ at room temperature. The slices remained in the holding vial for at least 1 h after 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 ml/min, at 31–32°C.

Intracellular Recording

Intracellular recordings were made as previously described (Beck et al. 1996, 1994). Electrodes were pulled from borosilicilate capillary tubing on a Brown and Flaming electrode puller (Sutter Instruments, Novato, CA) to a resistance of 80–120 MΩ (2 M KMSO$_4$, 10 mM HEPES, and 10 mM KCl). Pyramidal cells were impaled with brief ejections of positive current through the electrode. The impaled cells were sealed by applying hyperpolarizing current, which was slowly reduced to zero where the cell's membrane potential stabilized (10–20 min after initially impaling the cell). Cell characteristics, i.e., resting membrane potential, slow-after hyperpolarization (sAHP), half-decay time of the sAHP, input resistance, spike amplitude, spike half width, and membrane time constant (tau), were obtained only after the cell's membrane potential stabilized with no holding current. The measurement of the cell characteristics was as previously described (Beck et al. 1992, 1994). The resting membrane potential (RMP) was the value read directly from the amplifier and was checked by withdrawing the electrode at the end of the experiment. The input resistance was calculated from the slope of the linear portion of a current-voltage plot generated from hyperpolarizing current pulses 300 ms in duration obtained under current clamp. The tau was calculated from a single exponential fit of the falling phase of the potential response to a 100- or 200-pA, 300-ms hyperpolarizing current pulse (Figure 1A). Action potential height was measured from threshold to the peak of the spike (Figure 1B) and action potential half-width was measured at a point that was 50% of the maximum height. The sAHP was elicited by a 900-pA depolarizing current pulse at a membrane potential of −62 to −65 mV, and the amplitude was measured 100 ms after the offset of the current pulse (Figure 1C). The half-decay time is the amount of time it takes for the sAHP to decay to half its peak amplitude. Only cells with an input resistance greater than 25 MΩ were used for analysis. Signals were collected and amplified using an Axoclamp 2A (Axon Instruments, Forster City, CA) and Cyberamp 320 amplifier (Axon Instruments, Foster City, CA) and recorded on a Gould Series 3200 chart recorder (Gould Instrument Company, Valley View, OH). Data were collected on line with pCLAMP 6.0 software (Axon Instruments, Foster City, CA).

5-HT Concentration Response

Data for the generation of concentration response curves were obtained by perfusing the slice with known concentrations of 5-HT in the ACSF (Figure 2). The cell was allowed to return to the resting membrane potential between each concentration of 5-HT. The 5-HT solutions were made fresh each day. Concentration-response curves were generated using the formula for a logistic function (Beck et al. 1992, 1996; Okuhara and Beck 1998): $E = E_{max}/[1+(EC_{50}/[5\text{-HT}])^N]$, where E is the response produced by the 5-HT concentration [5-HT], E_{max} is the maximal response, EC_{50} is the 5-HT concentration that elicits a half-maximal response, and N is the slope. From this analysis, estimates were obtained for E_{max}, EC_{50}, and slope.

A. Tau and RMP

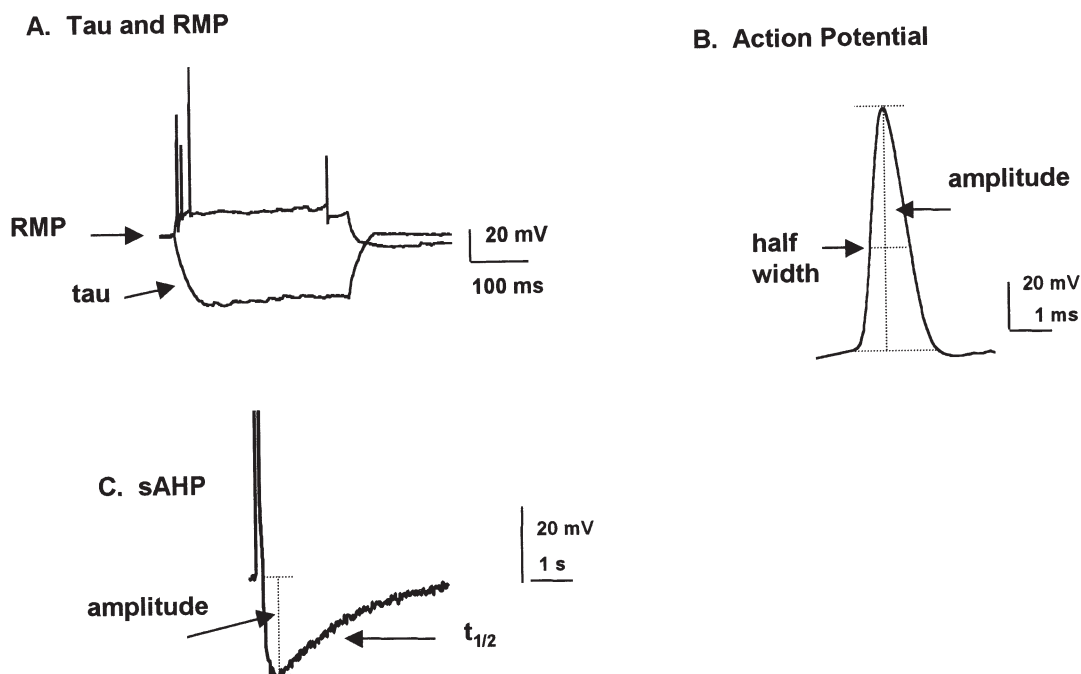**B. Action Potential**

C. sAHP

Figure 1. Measurement of CA1 hippocampal pyramidal cell characteristics. **(A)** Recording of membrane potential response to hyperpolarizing and depolarizing current pulse (300 msec). The resting membrane potential (RMP) is the membrane potential of the cell with no holding current. The tau is measured by fitting a single exponential function to the falling phase of the membrane potential response to a 100–200 pA hyperpolarizing current pulse. **(B)** Action potential generated by a depolarizing current pulse. Action potential height was measured from threshold to the peak of the spike and action potential duration was measured at a point that was 50% of the maximum height (i.e., half-width). **(C)** The sAHP was elicited by a 900-pA depolarizing current pulse at a membrane potential of −62 to −65 mV, and the amplitude was measured 100 ms after the offset of the current pulse. The half-decay time is the amount of time it takes for the sAHP to decay to half its peak amplitude.

Statistical Analysis

Statistical comparisons were performed using analysis of variance (ANOVA). The Student-Newman-Keuls method was used for *post-hoc* t-tests. All values are reported as mean ± S.E.M. A $p \leq .05$ was considered significant.

RESULTS

Corticosterone Plasma Levels

Data were collected from a total of 88 cells taken from 69 treated rats. SHAM corticosterone plasma levels ranged from 0 to 7.1 μg/dl (2.12 ± 0.95 $n = 30$). ADX corticosterone plasma levels ranged from 0 to 0.5 μg/dl (0.09 ± 0.04 $n = 17$), while HCT corticosterone plasma levels ranged from 20 to >50 μg/dl (34.09 ± 3.05 $n = 22$). The lower and upper limits of the radioimmune assay were 0.05 and 50 μg/dl, respectively. Adrenalectomies that produced corticosterone concentration ≤0.5 μg/dl were considered successful. Thirteen SHAM and

nine ADX animals had corticosterone levels less than 0.05 μg/dl. The SHAM rats had low plasma corticosterone levels because trunk blood was collected at the circadian trough when corticosterone levels are lowest. Although it may look like many of the ADX and SHAM treated rats had similar plasma corticosterone levels, this is not the case because the SHAM group experienced a normal circadian rhythm while the ADX group had negligible plasma corticosterone levels continuously.

Cell Characteristics

The magnitude and half-decay time of the slow afterhyperpolarization (sAHP) were decreased in HCT treated rats compared to SHAM (Table 2). The membrane time constant (tau) was significantly increased in the cells from the ADX treatment group versus SHAM and HCT treatment groups. There were no significant differences in the input resistance, the resting membrane potential, the spike amplitude, or the spike half
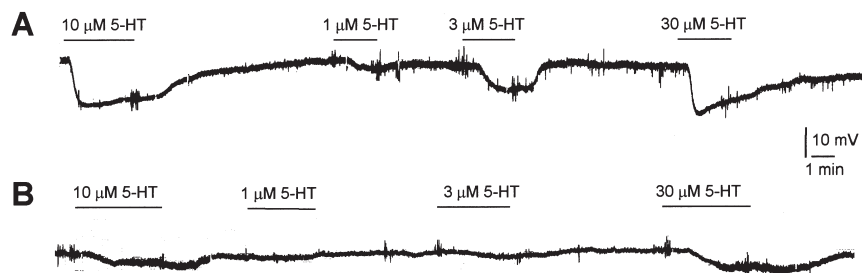
Figure 2. Chart recordings from a cell from **(A)** SHAM and **(B)** HCT treated rat. The bars above the chart depicts the amount of time that the given amount of 5-HT was in the perfusion buffer. The resting membrane potential of **(A)** was −56 mV and **(B)** −61 mV.

width of the cells recorded from the ADX, SHAM or HCT treatment groups.

5-HT Concentration Response Curve Characteristics for the 5-HT$_{1A}$ Receptor. Perfusion of the slice with 5-HT elicited a hyperpolarization. Figure 2 contains two chart recordings from a SHAM and HCT treated rats showing the response to bath administration of different concentrations of 5-HT. The lines above the chart recordings depict the amount of time that the specified concentration of 5-HT was present in the perfusion buffer. The magnitude of the hyperpolarization was determined for each 5-HT concentration tested. The data were fit to a logistic function to obtain estimates for E$_{max}$, EC$_{50}$ and slope for each neuron (see Methods). If the values were not fit well by the logistic function, the data were not used in the treatment group average. The concentration-response curve characteristics of EC$_{50}$, E$_{max}$ and slope for each neuron within a treatment group were averaged and appear in Table 3. A sum-

mary figure with the mean ± S.E.M. of the magnitude of the hyperpolarization for each concentration of 5-HT tested was generated and appears in Figure 3. The fitted curve for each treatment group is based on the mean values for EC$_{50}$, E$_{max}$ and slope for each treatment group (from Table 3).

There were no significant differences in the slope between the treatment groups (Table 3). There was a significant difference in the E$_{max}$ values according to analysis of variance, even though follow-up *t*-test found no statistically significant differences between the treatment groups. The EC$_{50}$ of the concentration-response curve for the HCT group was significantly less potent i.e., shifted to the right, as compared to SHAM (Table 3). We also conducted a two-factor repeated measures analysis of variance on the magnitude of the hyperpolarization elicited by each concentration of 5-HT tested for all of the cells tested from the HCT, ADX and SHAM treatment groups. The interaction of treatment by 5-HT concentration was significant (F = 2.15, df =

Table 2. CA1 Cell Characteristics

Parameters	SHAM	ADX	HCT
Resting membrane potential (mV)	65 ± 0.7	64 ± 0.9	65 ± 1.0
	n = 57	*n* = 27	*n* = 33
Input resistance (MΩ)	45 ± 2.0	46 ± 3.6	38 ± 2.1
	n = 56	*n* = 25	*n* = 33
Tau (ms)	20.5 ± 1.10[a]	25.6 ± 3.03	19.0 ± 1.10
	n = 56	*n* = 25	*n* = 33
Spike amplitude (mV)	80.2 ± 4.06	73.7 ± 5.09	83.0 ± 3.62
	n = 28	*n* = 14	*n* = 14
Spike half-width (ms)	0.9 ± 0.04	0.9 ± 0.07	0.8 ± 0.03
	n = 28	*n* = 14	*n* = 14
sAHP (mV)	7.6 ± 0.66	7.9 ± 0.69	5.4 ± 0.57[b]
	n = 32	*n* = 14	*n* = 23
sAHP half-decay time (ms)	1273 ± 133.0	1164 ± 151.4	856 ± 64.4[c]
	n = 32	*n* = 14	*n* = 23

Cell characteristics were determined using intracellular current clamp electrophysiology as described in Methods. All values are expressed as mean ± S.E.M.

[a] ANOVA F = 3.664, df = 2, 113, *p* = .029 SHAM is different than ADX and HCT at *p* < .05 using Student Newman-Keuls *t*-test.

[b] ANOVA, F = 3.798, df = 2, 68, *p* = .027, HCT is different than SHAM at *p* < .05 using Student Newman-Keuls *t*-test.

[c] ANOVA F = 3.282, df = 2, 68, *p* = .044; HCT is different than SHAM at *p* < .05 using Student Newman-Keuls *t*-test.

Table 3. Characteristics of Concentration-Response Curve for 5-HT activating the 5-HT$_{1A}$ receptor in CA1 Hippocampal Pyramidal Cells

Parameter	SHAM (n=13)	ADX (n=12)	HCT (n=10)
Emax(mV)			
[a]	14.36 ± 1.34	10.9 ± 1.14	10.4 ± 0.98
-log(EC$_{50}$)[b]	5.56 ± 0.08	5.45 ± 0.10	5.17 ± 0.123
Slope	1.36 ± 0.15	1.36 ± 0.15	1.29 ± 0.14

Data were obtained by adding increasing concentrations of 5-HT to the perfusion buffer. Data were fit to a curve using a logistic equation (see methods). All values are expressed as mean ± S.E.M.

[a] ANOVA F = 3.4, p = .046.

[b] ANOVA F = 4.27, p = .023; HCT is different from SHAM at $p < 0.05$ using Student Newman-Keuls t-test.

8,112, p = .037). Follow-up tests with Student Newman-Keuls t-test revealed that SHAM was significantly different from HCT and ADX at 3 μM, 10 μM and 30 μM 5-HT concentrations as indicated by asterisks in Figure 3.

DISCUSSION

Chronic HCT treatment altered both membrane properties and the 5-HT$_{1A}$ receptor-mediated response in CA1 hippocampal pyramidal cells. The membrane time constant was increased and the amplitude and half-decay
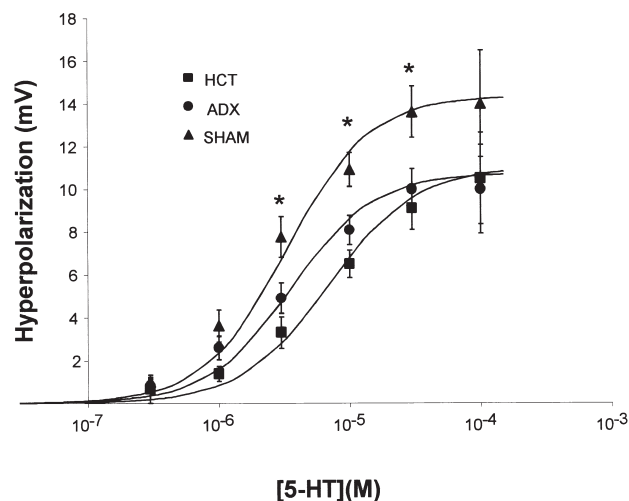

Figure 3. Magnitude of hyperpolarization elicited by 5-HT for cells recorded from HCT, ADX and SHAM treatment groups. Values are mean ± S.E.M. and number of observations was between 6–13 per data point. Analysis of variance revealed a significant interaction between treatment and 5-HT concentrations (F = 2.15, df = 8,112, p = .037). Follow-up tests with Student Newman-Keuls t-test revealed that SHAM was significantly different from HCT and ADX at 3 μM, 10 μM and 30 μM 5-HT concentrations as indicated by asterisks.

time of the sAHP were decreased. The magnitude of the maximal response and the potency of 5-HT for the 5-HT$_{1A}$ receptor-mediated hyperpolarization was decreased in the HCT group compared to SHAM.

Previously our laboratory found that 2-week treatment with basal levels of corticosterone (plasma corticosterone of 1.8 μg/dl) decreases the amplitude of the sAHP by 33–35% as compared to ADX and SHAM (Beck et al. 1994). In this study we found that increasing the dose of corticosterone so that both MR and GR (plasma corticosterone of 34 μg/dl) should be occupied also resulted in a decrease in the amplitude of the sAHP by 28–31% as compared to SHAM and ADX (even though the difference between ADX and HCT was not statistically significant due to the small sample size for ADX). In addition the decay time of the sAHP was decreased. The reduction in the amplitude and half-decay time of the sAHP by chronic corticosterone treatment allows the cell to return to its resting membrane potential more rapidly. This theoretically should make the cell more excitable and result in an increase in synaptic output from area CA1 of the hippocampus.

The sAHP is elicited by a train of action potentials and is mediated by a calcium activated K$^+$ channel (K$^+_{(Ca^{2+})}$) (see review Sah 1996). An L-type calcium channel is believed to be the source of calcium for the activation of the small conductance K$^+_{(Ca^{2+})}$ channel (Marrion and Tavalin 1998). Previously it has been shown that corticosterone decreases the number of functional K$^+_{(Ca^{2+})}$ channels present in vascular smooth muscle (Brem et al. 1999; Xie and McCobb 1998), and prevents the inhibition of channel activity induced by protein kinases (Shipston et al. 1996) or phosphatases (Pedarzani et al. 1998; Tian et al. 1998). Further experiments are needed to determine the mechanism underlying the modulatory effect of corticosterone on the sAHP characteristics in CA1 pyramidal cells.

The membrane time constant was reduced by HCT treatment as compared to ADX treatment, but was not different from the SHAM treatment group. Previously we found that chronic basal corticosterone treatment, primarily MR occupation, increases the membrane time constant as compared to ADX (Beck et al. 1994). In this study chronic occupation of both MR and GR results in tau values equal to SHAM values. The primary difference between the two studies is in the degree of occupation of the GR. Taking into account both studies, the largest effect on tau appears to be when only MR is occupied. The acute or short-term (hours) modulatory effects of corticosterone on CA1 pyramidal cell active membrane properties has been previously described by Joels as a U-shaped function, where the smallest effect is seen when only MR is occupied and the largest effects are seen when neither MR nor GR are occupied (i.e., adrenalectomized), or when both MR and GR are occupied (Joels and DeKloet 1994). The active properties

that are regulated in this manner include the sAHP amplitude, calcium currents, 5-HT$_{1A}$ and muscarinic receptor mediated responses and the anomalous rectifier potassium current I$_Q$. In contrast to this U-shaped function the magnitude of tau, a passive membrane property, appears to be greatest when primarily MR is occupied, and smallest in adrenalectomized animals or animals treated with high doses of corticosterone (i.e., an inverted U-shaped function).

Theoretically the tau is important in regulating the ability of the cell to temporally summate synaptic transmission. The longer the tau, the greater the likelihood that temporal summation will occur. Therefore, with MR occupation temporal summation should be greater in likelihood, whereas with both MR and GR occupied, temporal summation is less likely. Treatment with high doses of corticosterone should have the net effect of making the cell less responsive to synaptic input.

The other main finding of this article is that the E$_{max}$ and the potency of 5-HT acting on the 5-HT$_{1A}$ receptor in HCT treated rats were decreased. Also, the magnitude of the hyperpolarization elicited by concentrations greater than 3 μM 5-HT was less in cells recorded from the HCT group as compared to the SHAM treatment group. A higher concentration of 5-HT was required to give the same magnitude of hyperpolarization in a cell from a HCT treated animal as compared to a SHAM treated animal. Microdialysis has shown that the physiological concentration of 5-HT seen at the synapse is about 1 μM (Kreiss and Lucki 1994; Kreiss et al. 1995; Routledge et al. 1993). The magnitude of hyperpolarization elicited by low micromolar concentrations of 5-HT in a cell from a HCT treated animal would be smaller than that seen in a cell from a SHAM treated rat. Normally, a hyperpolarization of the membrane potential has an inhibitory effect, and a greater depolarizing potential is required for that cell to reach threshold and fire an action potential. In the HCT treated animal the inhibitory effect of 5-HT is diminished.

In this study, the magnitude of the maximal 5-HT$_{1A}$ receptor-mediated hyperpolarization was significantly different in the SHAM versus ADX and HCT treatment groups. Our results are in agreement with those previously published by Karten et al. (1999) who found that chronic treatment for 3 weeks with stress levels of corticosterone decreased 5-HT$_{1A}$ receptor-mediated hyperpolarization as compared to SHAM treated rats. In their study they tested only one concentration of 5-HT (i.e., 10 μM 5-HT). Statistical analysis of our data revealed a significant difference between the SHAM and HCT groups at concentrations of 3, 10 and 30 μM 5-HT. However, due to the fact that we collected data for concentration-response curve analysis, additional information was obtained. The potency as well as the maximal response elicited by 5-HT were altered by corticosterone treatment.

A decrease in the potency of 5-HT for the 5-HT$_{1A}$ receptor could be the result of corticosterone induced changes in any component of the 5-HT$_{1A}$-G protein-ion channel effector pathway. A decrease in receptor number could lead to a decrease in potency. A number of studies have looked at the effect of corticosterone treatment on 5-HT$_{1A}$ receptor number in the CA1 subfield and the results are not uniform. Both a decrease (Chalmers et al. 1993) as well as no change in 5-HT$_{1A}$ receptor number in the CA1 subfield have been reported following treatment with high doses of corticosterone (Kuroda et al. 1994; Mendelson and McEwen 1992). Chronic stress increases, decreases or has no effect on 5-HT$_{1A}$ receptor levels, depending on the nature of the stress paradigm (Fernandes et al. 1997; Kuroda et al. 1994; Mendelson and McEwen 1992; Raghupathi and McGonigle 1997).

Another mechanism for decreasing the EC$_{50}$ of a concentration-response curve is to alter the ability of the 5-HT$_{1A}$ receptor to link to its second messenger, or to directly alter the number or actions of its effector systems. The GTP sensitivity of the 5-HT$_{1A}$ receptor is decreased by high corticosterone treatment in the CA1 subfield (Beck, Magnuson and Lee, unpublished observations). We have previously found that G-protein levels are increased by treatment with high doses of corticosterone in whole dorsal hippocampus (Okuhara et al. 1997), an effect attributed to compensation for the decreased efficacy of coupling to the receptor. There is no effect of high corticosterone treatment on the number of G-protein inward rectifying potassium channels in area CA1 of the hippocampus (Muma and Beck 1999). Therefore, the cellular mechanism underlying the decreased potency of 5-HT for the 5-HT$_{1A}$ receptor-mediated hyperpolarization due to chronic treatment with high doses of corticosterone appears to be complex, but is probably at the receptor-G-protein level.

The modulatory effects of corticosterone are different in the CA1 and CA3 subfields of the hippocampus. In the CA3 subfield of the hippocampus HCT treatment decreased the magnitude of the maximal 5-HT$_{1A}$ receptor-mediated hyperpolarization compared to SHAM and ADX animals. Also, in contrast to the data obtained in CA1, adrenalectomy increased the potency of 5-HT (i.e., decreased the EC$_{50}$), as compared to SHAM and HCT treatment in the CA3 subfield (Okuhara and Beck 1998). The difference in the results collected between the two hippocampal subfields can be attributed to many factors, including differences in the identity of the G-protein that link to the 5-HT$_{1A}$ receptors in the two subfields, different affinities of the G-proteins for the 5-HT$_{1A}$ receptors or differences in the number of 5-HT$_{1A}$ receptors. Previously, we have shown that the characteristics of the 5-HT concentration-response curve for activation of the 5-HT$_{1A}$ receptor are different in area CA1 and area CA3 of the hippocampus (Beck et

al. 1992). Therefore it is not surprising to find that high corticosterone treatment would have different effects on the 5-HT$_{1A}$ receptor-mediated hyperpolarization. Both studies agree that high corticosterone treatment decreased the potency and response magnitude elicited by 5-HT activation of the 5-HT$_{1A}$ receptor.

High corticosterone affects membrane properties to make the cell more excitable. The decrease in the amplitude and half-decay time of the sAHP will allow the cell to return to its resting membrane potential quicker. However, the decrease in the membrane time constant will make the cell less likely to summate excitatory input into a response. The decrease in the maximal hyperpolarization and the decrease in the EC$_{50}$ for 5-HT acting at the 5-HT$_{1A}$ receptor also makes the cell more excitable. Therefore, we conclude that high corticosterone treatment makes the cell more excitable by multiple mechanisms and more likely to increase synaptic output from CA1 hippocampal pyramidal cells.

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