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Corticosterone Alters 5-HT$_{1A}$ Receptor-Mediated Hyperpolarization in Area CA1 Hippocampal Pyramidal Neurons

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The 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor in the CA1 region of the hippocampus is linked through a G protein to an inwardly rectifying potassium conductance. Activation of the 5-HT$_{1A}$ receptor results in a membrane hyperpolarization and decreases neuronal firing rate. The hippocampus contains a high concentration of the mineralocorticoid (MR) and glucocorticoid (GR) corticosterone (CT) receptor subtypes. Some laboratories have reported that CT modulates 5-HT$_{1A}$ receptor binding density and mRNA levels in area CA1 of the hippocampus; however, others have reported no change. Previous electrophysiological studies have demonstrated that acute (1 to 4 hour) MR activation in slices from adrenalectomized (ADX) rats attenuates the 5-HT$_{1A}$ receptor-mediated hyperpolarization, while acute MR + GR or GR activation alone did not alter the 5-HT$_{1A}$ response. Our results confirm that the 5-HT$_{1A}$ response was attenuated 2 to 8 hours following MR activation. However, we found that GR activation alone decreased the potency, but not the maximal response to 5-HT. Chronic (2-week) treatment with basal levels of CT did not alter the 5-HT$_{1A}$ response. Administration of high concentrations of CT in vitro to neurons from chronically treated ADX rats decreased the magnitude of the 5-HT$_{1A}$ receptor-mediated hyperpolarization. We conclude that the 5-HT$_{1A}$ receptor-effector system in CA1 hippocampal pyramidal neurons is modulated by CT in a dose- and time-dependent manner. ***[Neuropsychopharmacology 14:27–33, 1996]***

KEY WORDS: *5-Hydroxytryptamine; Corticosterone; Glucocorticoid; Hippocampus; Intracellular electrophysiology*

The subfields of the hippocampus receive extensive innervation from the 5-hydroxytryptamine (5-HT) cell bodies in the dorsal and median raphe (Azmitia and Segal 1978; Molliver 1987; Oleskevich and Descarries, 1990). In area CA1 of the hippocampus 5-HT elicits a hyperpolarization due to an increase in an inwardly rectifying potassium conductance (Andrade and Nicoll 1987; Colino and Halliwell 1987). The hyperpolarization has been extensively studied, and the receptor mediating this response has been identified as the 5-HT$_{1A}$ receptor (Andrade and Nicoll 1987; Beck et al. 1992; Colino and Halliwell 1987).

There is some evidence that the 5-HT$_{1A}$ receptor-mediated response may be altered by the steroid corticosterone (CT). The hippocampus contains a high density of CT receptors. The CA1 region of the hippocampus contains both the mineralocorticoid (MR) and the glucocorticoid (GR) receptors (Reul and DeKloet 1985, 1986; Sarrieau et al. 1986, 1988). CT has a tenfold higher affinity for the MR than for the GR (Reul and DeKloet 1985, 1986; Reul et al. 1987a, b). Under normal physiological conditions, the basal plasma CT levels are sufficient to occupy approximately 80% of the MR and

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less than 10% of the GR (Reul and DeKloet 1985; Reul et al. 1987a, b). The 5-HT_{1A} receptor density and mRNA levels in area CA1 are increased following adrenalectomy (ADX) and decreased to normal levels following CT replacement treatment for 1 day or 1 week (Biegon et al. 1985; Chalmers et al. 1993). On the other hand, others have reported no change in 5-HT_{1A} receptor density in area CA1 following ADX or CT treatment (Mendelson and McEwen 1992a, b). In one study the magnitude of the maximal hyperpolarization elicited by 5-HT was attenuated in area CA1 pyramidal neurons recorded from ADX rats following acute (1- to 4-hour) MR activation (Joels et al. 1991). GR activation alone did not alter the magnitude of the 5-HT_{1A} receptor-mediated hyperpolarization, whereas MR + GR activation decreased the hyperpolarization with a return to control levels after 2 hours (Joels and DeKloet 1992).

In the normal rat the MR are chronically activated by low circulating levels of plasma CT. Therefore, we investigated the effect of chronic activation (2 weeks) of CT receptors using replacement CT pellets following ADX. We present evidence that short-term MR activation in vitro decreased 5-HT_{1A} response magnitude and that short-term GR activation decreased the potency of 5-HT for the 5-HT_{1A} receptor-elicited hyperpolarization. Chronic predominant basal activation of MR (i.e., the normal physiological state) did not alter the 5-HT_{1A} receptor-mediated response. Short-term administration of high concentrations of CT to neurons from chronically treated rats decreased the maximal 5-HT_{1A} response.

METHODS

Adrenalectomies

Male Sprague-Dawley rats (75–150 g) were anesthetized with ether. Bilateral ADX 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. A placebo pellet, a 12.5-mg CT pellet, or a 100-mg CT pellet designed for two-week release was implanted in the back of the neck at the time of ADX. A 1-cm incision was made with scissors, and a pair of hemostats were used to make a small pocket subcutaneously for the pellet. The pellet was inserted and the incision closed with wound clips. ADX rats were allowed to recover for two weeks and were given standard laboratory chow and drinking water containing 0.9% NaCl, ad libitum. For sham treated rats the adrenal glands were visualized and not removed. All rats were maintained on a twelve hour light/dark cycle (7:00 A.M. to 7:00 P.M. lights on). A two week treatment period was chosen because it takes two weeks for the GR to maximally upregulate following ADX (Reul et al. 1987a,b).

Hippocampal Slice Preparation

On the day of the experiment, rats were decapitated between 8:00 and 10:00 A.M. Trunk blood was collected for determination of plasma CT concentrations (Burgess and Handa 1992). The brain was rapidly removed and rinsed in ice-cold artificial cerebrospinal fluid (ACSF) containing (mM): NaCl (124), KCl (3), NaH_2PO_4 (1.25), $MgSO_4$ (2), $CaCl_2$ (2.5), dextrose (10), $NaHCO_3$ (28). The hippocampus was dissected free and starting at the dorsal/septal tip, 500 to 600 μ sections were cut on a vibratome. Slices were placed in a holding vial containing room-temperature ACSF bubbled with 95% O_2/5% CO_2 to maintain the pH at 7.4. After at least 1 hour a slice was transferred to the recording chamber where it was perfused continuously with ACSF at 32° ± 1°C, bubbled with 95% O_2/5% CO_2 at a flow rate of 2 to 3 ml/minute. CT and RU 28362 were made up in alcohol in stock solutions of 1 mM. The buffer was made fresh daily and the steroids diluted to the desired concentration by addition to the stock buffer. The final concentration of alcohol in the stock buffer was 0.001% or less. The tested steroid was continuously present in the buffer during the experiment.

Standard intracellular recordings were made as previously described (Beck et al. 1992). Electrodes were pulled from borosilicate capillary tubing (1.2 mm OD, 0.69 mm ID, Sutter Instruments, Novato, CA) on a Brown and Flaming electrode puller (Sutter Instruments, Novato, CA) to obtain resistances of 40 to 140 MΩ (2 M KCl or 2 M KCH_3SO_4). Pyramidal cells in area CA1 were impaled by briefly (10–50 ms) increasing the capacity compensation or by increasing positive current ejection through the recording electrode. The impaled neuron was hyperpolarized to facilitate sealing. Only neurons with a resting membrane potential less than −55 mV, input resistance ⩾ 20 MΩ, and action potential overshoot of at least 15 mV were used for experiments. Once the neuron had stabilized at the resting membrane potential (RMP), 5-HT was added to the ACSF in increasing concentrations. Electrical signals were amplified using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA), stored on disk for later analysis and recorded online on a Gould chart recorder (series 2200 and 3200, Gould Inc., Valley View, OH).

Treatment Groups

There were four treatment groups: (1) intact or sham (plasma CT levels = 2.47 ± 1.03 μg/dl, n = 20 rats), (2) ADX with a placebo pellet (12.5 mg cholesterol; plasma CT levels = 0.17 ± 0.3 μg/dl, n = 35 rats), (3) ADX with a CT pellet (12.5 mg) that produced a plasma CT concentration of 1.8 ± 0.6 μg/dl, n = 25 rats, and

Table 1. Treatment Groups[a]

Group	Treatment	
	In Vivo	In Vitro
Sham/naive	Visualized adrenal glands/none	No steroid
ADX	ADX	No steroid
ADX – MR[b]	ADX	1 nM CT
ADX – GR[c]	ADX	10 nM RU 28362
ADX + CT	ADX with 12.5 mg CT pellet	1 nM CT/no steroid (see Methods)
ADX + CT – MR + GR[d]	ADX with 100 mg CT pellet	100 nM CT

[a] The surgical treatment and absence or presence of steroid in the ACSF bathing the hippocampal slice is outlined for each treatment group.
[b] ADX – MR refers to the predominant MR stimulation by 1 nM CT in vitro.
[c] ADX – GR refers to the selective activation of GR by RU 28362 in vitro.
[d] ADX + CT – MR + GR refers to chronic treatment with CT and MR + GR activation in vitro.

(4) ADX with a CT pellet of 100 mg, which produced a plasma CT concentration of 4.81 ± 0.4 μg/dl, $n = 7$ rats. On the day of the experiment the treatment groups were divided into experimental groups based on the absence or presence of steroid in the ACSF. No steroid, CT or the selective GR agonist RU 28362, were included in the stock buffer used for the preparation of the slices, incubation, and perfusion of the slice preparation (Table 1). For half of the ADX + CT-treated rats 1 nM CT was included in the ACSF on the day of the experiment; however, since there was no difference between these two ADX + CT-treated groups, the data were combined into one group. For the ADX + CT – MR + GR group, the tissue was taken from a chronically treated rat and 100 nM CT was added to the ACSF on the day of the experiment. Since MR receptors were chronically activated, this treatment should result in short-term GR activation. The amount of time to obtain a cell varied from 2 to 8 hours following decapitation; therefore for the ADX – MR, ADX – GR, and ADX + CT – MR + GR experimental groups, the amount of time that the neuron was exposed to CT or RU 28362 varied from 2 to 8 hours. The treatment and experimental groups are summarized in Table 1.

Concentration-Response Curves

Data for the generation of 5-HT concentration-response curves were obtained by perfusing the slice with known concentrations of 5-HT in the ACSF. The 5-HT solutions were made fresh each day. Four to seven concentrations of 5-HT were tested in half-log unit increments. Concentration-response curves were generated using the formula for a hyperbolic function: $E = E_{max}/[1 + (EC_{50}/A)^N]$, where E is the response produced by A, the concentration of drug, E_{max} is the maximal response, EC_{50} is the concentration of drug that elicits a half-maximal response, and N is the slope. From this analysis estimates were obtained for E_{max}, EC_{50}, and slope.

Statistical Analyses

Often data from more than one neuron were obtained per rat. Only one neuron was recorded per slice. The assumption is that the experimental treatment affects all hippocampal neurons in all rats within a treatment group in a similar manner; each neuron was assumed to be an independent sample. Analysis of Variance (ANOVA) and post hoc Student Newman-Keuls *t*-tests were done. The geometric means of the EC_{50} values were used for statistical comparisons. A $p < .05$ was considered significant.

Chemicals and Drugs

The chemicals for making the ACSF and 5-HT hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). CT was purchased from Steraloids (Wilton, NH), and RU 28362 was generously donated by Roussel-UCLAF (Romainville, France). Placebo and CT pellets were purchased from Innovative Research (Toledo, OH).

RESULTS

Data were collected from a total of 127 hippocampal cells that displayed the electrophysiological characteristics of CA1 pyramidal neurons (Schwartzkroin 1977). The mean RMP ranged between −62 to −65 mV, and the input resistance between 49 and 56 MΩ; there was no difference in the RMP and input resistance between the different treatment groups (Beck et al. 1994). Perfusion of the slice with 5-HT elicited a hyperpolarization, as can be seen in Figure 1 for a neuron recorded from an intact rat. The magnitude of the hyperpolarization was determined for each 5-HT concentration tested and used to generate concentration-response curves. The data were fitted to a hyperbolic function to obtain estimates for EC_{50}, E_{max}, and slope for each neuron (see Meth-