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Mineralocorticoid Hormones Suppress Serotonin-induced Hyperpolarization of Rat Hippocampal CA1 Neurons

Marian Joëls,[1,2] Wouter Hesen,[1,2] and E. Ronald de Kloet[2]

[1]Division of Molecular Biology, Institute of Molecular Biology and Medical Biotechnology, and [2]Rudolf Magnus Institute, University of Utrecht, Utrecht, The Netherlands

Pyramidal neurons in the rat CA1 hippocampal area contain intracellular mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) to which the adrenal hormone corticosterone can bind with differential affinity. The pyramidal neurons also have high amounts of 5-HT_{1a} receptors, which mediate a membrane hyperpolarization. With intracellular recording *in vitro*, we found that selective occupation of MRs suppresses the 5-HT–induced hyperpolarization of CA1 pyramidal neurons. The suppression of 5-HT responses was observed 1–4 hr after a brief (20-min) application of the steroids. Binding properties of the 5-HT_{1a} receptor were not significantly affected by *in vitro* steroid application. Furthermore, responses to the $GABA_B$ agonist baclofen were not changed after treatment with MR ligands, implying that the K^+ conductance to which both $GABA_B$ and 5-HT_{1a} receptors are linked is also no target for the steroid action. The MR-mediated effect on 5-HT responsiveness potentially enhances cellular activity. Because activation of GRs was previously found to suppress norepinephrine-induced excitability in the same neurons, the data support the concept that cellular homeostasis in the hippocampus is under control of corticosterone via coordinative, antagonistic MR- and GR-mediated events.

Corticosteroid hormones, which are secreted by the adrenal gland, readily cross the blood–brain barrier and bind to two types of intracellular receptors in the brain: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs; Reul and de Kloet, 1985; for reviews, see McEwen et al., 1986; Funder, 1986; de Kloet, 1991). Pyramidal neurons in the rat hippocampal CA1 area are particularly rich in both MRs and GRs (Gerlach and McEwen, 1972; Fuxe et al., 1985; Arriza et al., 1988; van Eekelen et al., 1988; Herman et al., 1989). Recent electrophysiological studies with adrenalectomized (ADX) rats have shown that, while corticosteroid hormones do not alter resting membrane potential or input resistance of CA1 neurons *in vitro*, they specifically affect the spike frequency accommodation and afterhyperpolarization (AHP) associated with a short depolarizing current step (Joëls and de Kloet, 1989, 1990; Kerr et al., 1989). Thus, corticosterone induces, via MRs, a decrease in the spike frequency accommodation and AHP (Joëls and de Kloet, 1990) that is gradually overriden by a GR-mediated increase in the AHP amplitude (Joëls and de Kloet, 1989).

In vitro autoradiographical studies have shown that the CA1 area of rat hippocampus displays also a high density of binding sites for 5-HT; in particular, ligands for the 5-HT_{1a} receptor subtype bind strongly to the dorsal CA1 hippocampal region (Deshmukh et al., 1983; Marcinkiewicz et al., 1984; Pazos and Palacios, 1985). Electrophysiological data showed that 5-HT_{1a} agonists induce a hyperpolarization of CA1 pyramidal cell membranes, due to activation of K^+ conductances (Andrade et al., 1986; Andrade and Nicoll, 1987; Colino and Halliwell, 1987; for review, see Bobker and Williams, 1990). In addition to the membrane hyperpolarization mediated through the 5-HT_{1a} receptor, 5-HT also evokes a slow depolarization of the membrane that coincides in time with a decrease of the spike frequency accommodation.

Previous biochemical studies have shown that tryptophan hydroxylase activity (Azmitia and McEwen, 1974; Singh et al., 1990), 5-HT turnover (van Loon et al., 1981; de Kloet et al., 1982) and 5-HT binding site properties (Biegon et al., 1985; de Kloet et al., 1986) are affected by corticosteroids. In this study, we have investigated whether 5-HT–induced changes in electrical properties of CA1 neurons are affected by selective occupation of MRs or GRs. We focused on (1) the type of steroid receptor involved in corticosteroid control of the 5-HT responses, (2) the type of 5-HT receptor affected by the steroid action, and (3) the possible target site for the steroid in the pathway from 5-HT receptor to ion channel.

Part of these results have been published in preliminary form (Joëls and Hesen, 1990).

Materials and Methods

All the experiments (72 different experiments) were performed in male Wistar rats (120–170 gm), adrenalectomized under ether anesthesia approximately 1 week before the electrophysiological experiment, as described elsewhere (Ratka et al., 1988; Joëls and de Kloet, 1989, 1990). The animals were housed in an animal room with an alternating 12-hr: 12-hr light/dark cycle (lights on 8:00 A.M.) and received food and saline (after ADX) ad libitum.

Electrophysiology. On the day of the experiment, the rat was placed in a clean cage and decapitated after 30–60 min; trunk blood was collected for measurement of plasma corticosterone levels. All adrenalectomized animals displayed plasma corticosterone levels well below 1 μg corticosterone per 100 ml plasma. The brain was removed from the skull and dipped in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition: 124 mM NaCl, 3.5 mM KCl, 1.25 mM NaH_2PO_4, 1.5 mM $MgSO_4 \cdot 7H_2O$, 2.0 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM

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Correspondence should be addressed to M. Joëls, Department of Experimental Zoology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands.

glucose. Slices (350 μm) were prepared from the dorsal hippocampus on a McIlwain tissue chopper. The slices were placed in a perfusion system, submerged, and continuously superfused (2–3 ml/min) with warm (32°C) oxygenated (95% O_2, 5% CO_2) ACSF.

Intracellular recording was performed with 4 M KAc-filled micropipettes (impedance, 80–150 MΩ), which were placed in the CA1 pyramidal cell layer. The signals were transferred to an Axoclamp 2A amplifier (10× amplification) and continuously displayed on a Gould digital oscilloscope. The membrane potential and applied current were registered on a Gould 2200 chart recorder and, in some cases, on a Vetter videocassette recorder for later analysis. From each neuron, we recorded the resting membrane potential, input resistance (from current–voltage relationship with 150-msec current pulses of −0.6 to 0.2 nA), spontaneous activity, and spike accommodation and AHP evoked by a 50-msec depolarizing current pulse (0.2–1.0 nA) or a 500-msec depolarizing current pulse (0.5 nA). Only neurons with a stable resting membrane potential (below −60 mV) and spike amplitudes of at least 80 mV were incorporated in the study.

The standard ACSF could at any time be switched to an ACSF medium containing a transmitter or hormone in a known concentration. A 1-mM serotonin (5-hydroxytryptophan creatinine sulphate; Sigma)-containing ACSF stock solution was freshly prepared during the experiment and kept at 4°C. The stock solution was diluted to the appropriate concentration just before the experimental test. If more than one 5-HT concentration was tested, we always started with the lowest 5-HT concentration and subsequently tested increasing doses of 5-HT. The transmitter application was terminated if a steady-state level of hyperpolarization was reached or, in the case that 5-HT did not induce membrane effects, after a maximal application period of 5 min. At least 15 min were allowed to pass between the testing of successive 5-HT concentrations or as long as was necessary for the 5-HT–induced effects to normalize. The membrane resistance was monitored at regular intervals by passing current pulses (0.1–0.4 nA, 150 msec) through the recording electrode while adjusting the bridge balance. Changes in membrane resistance during 5-HT application were established while the membrane potential was adjusted to the pretreatment level by injecting positive DC current through the recording pipette. Because Andrade and Nicoll (1987) found that the decrease in spike frequency accommodation evoked by 5-HT lasts considerably longer than the hyperpolarizing response, we established 5-HT–induced effects on spike frequency accommodation (during a 0.5-nA depolarizing pulse of 500-msec duration) at the moment that the membrane hyperpolarization was just normalized. Baclofen (Ciba-Geigy, Basel, Switzerland) and 5-carboxyamidotryptamine (5-CT; Research Biochemicals Inc.) were prepared as a 1-mM stock solution in ACSF and then diluted, while spiperone (Duphar, Weesp, The Netherlands) was used at 5 μM in 0.5% ethanol. Corticosterone, aldosterone (Organon International, Oss, The Netherlands), RU 38486, RU 28362 (Roussel-Uclaf, Romainville, France), and spironolactone (Searle, Saint Louis) were dissolved in 90% ethanol (1 mM) for each experiment and kept at −20°C. Just before testing, the stock solutions were diluted to the intended concentration in oxygenated ACSF. MR and GR agonists were perfused for 20 min. CA1 neurons were recorded either before or 1–4 hr after the start of the steroid application. Application of MR and GR antagonists by perfusion was started 20 min before and terminated 20 min after the agonist administration.

Because it proved to be difficult to record electrical properties and 5-HT responses continuously before, during, and up to 4 hr after steroid application from large numbers of neurons, we usually compared the responses of neurons recorded before steroid treatment with neurons impaled 1–4 hr after steroid treatment. Unless stated otherwise, statistical evaluation was performed with a one-way analysis of variance with the Student–Newman–Keuls test for multiple comparisons between means.

Membrane binding assay. Hippocampal tissue slices (350 μm) were prepared at 4°C with a McIlwain tissue chopper. The slices were perfused for 1 hr with ACSF equilibrated with 95% O_2, 5% CO_2 at 33°C. Subsequently, 3 nM or 30 nM aldosterone was added to the ACSF. The period of steroid application was followed by 1-hr perfusion with the control ACSF solution, according to a standard protocol used for uptake of steroids in cell nuclei (de Kloet et al., 1975). Slices were then rapidly chilled on ice and homogenized in 10 vol (gm/ml) sucrose buffer (0.32 M; 4°C). The homogenate was centrifugated (10 min at 700 × *g*; 4°C); subsequently, the supernatant was centrifugated at 50,000 × *g* for 10 min (4°C). The pellet was resuspended in 10 vol Tris-HCl (50 mM, pH 7.7), incubated for 10 min at 37°C to eliminate endogenous 5-HT, and centrifugated again at 50,000 × *g* for 10 min (4°C). Membrane pellets were stored overnight at −80°C and resuspended in 50 vol of incubation buffer containing Tris-HCl (50 mM, pH 7.7), $CaCl_2$ (4 mM), ascorbic acid (0.1%), and pargyline (10 μM). The suspension was homogenized and incubated at 37°C for 15 min.

The membrane binding assay was performed as described by Gozlan et al. (1983). Aliquots of the membrane suspension (0.5 ml) were incubated for 10 min at 37°C with ^{3}H-8-hydroxy-2-(di-*N*-propylamino)tetralin (^{3}H-8OH-DPAT; New England Nuclear Chemicals, Boston, MA), with a specific activity of 128 Ci/mmol. The final concentration of ^{3}H-8OH-DPAT ranged from 0.1 to 5 nM. Nonspecific binding was determined in the presence of 10 μM 5-HT. Membranes were collected by filtration through Whatman GF/B filters. The filters were washed with 2 × 5 ml of ice-cold Tris-HCl (50 mM, pH 7.7), placed in 2 ml of soluene overnight, and counted after addition of the appropriate scintillation fluid. Nonspecific binding under these conditions was less than 15% of the total binding. In the absence of membranes, less than 1% of total radioactivity was bound by the filter. All binding assays were performed in triplicate. In a total of six experiments, we compared 5-HT_{1a} receptor binding characteristics after aldosterone treatment with binding in nontreated tissue.

Results

In total, we recorded from 126 CA1 cells, identified as pyramidal neurons as described by Schwartzkroin (1975, 1977). Average resting membrane potentials for the experimental groups (see below) ranged from −65.6 ± 2.4 to −67.6 ± 1.1 mV; input resistances were between 36.4 ± 4.2 and 48.8 ± 2.1 MΩ. With respect to resting membrane potential and resistance, no group was significantly different from any other group.

Steroid receptor

Typically, 5-HT hyperpolarized CA1 neurons recorded in slices from ADX rats (Fig. 1*A*). The hyperpolarization was associated with a decrease in resistance. As shown in Figure 1*B*, the threshold 5-HT concentration was approximately 1 μM, while near maximal responses were obtained with 10 μM 5-HT. This concentration range is in line with previous reports on 5-HT in intact animals (Andrade and Nicoll, 1987; Joëls et al., 1990).

We observed that application of 30 nM corticosterone, particularly with concomitant administration of 500 nM of the selective GR antagonist RU 38486 (Philibert, 1984; Gagne et al., 1985), markedly reduced the hyperpolarizing response to 10 μM 5-HT recorded 1–4 hr after steroid application (Fig. 1*A*). On average, application of corticosterone in the presence of the GR antagonist RU 38486, thus selectively occupying MRs, significantly suppressed both the 5-HT–induced membrane hyperpolarization and decrease in resistance (Fig. 1*C*). Similarly, a significant decrease in 5-HT–induced hyperpolarizations was obtained with 3 or 30 nM of the MR agonist aldosterone. The aldosterone-mediated effects on 5-HT responses could be effectively blocked by the MR antagonist spironolactone (300 nM). No significant changes in 5-HT responsiveness were observed with 30 nM of the selective GR agonist RU 28362 (Philibert and Moguilevski, 1983). Perfusion of spironolactone (four neurons) or RU 38486 (three neurons) by itself did not affect 5-HT responses (not shown). From this series of experiments, we conclude that MRs rather than GRs seem to be involved in the suppressive effects of corticosteroid hormones on 5-HT responses in CA1 neurons.

5-HT receptor

As reported by others (Andrade and Nicoll, 1987; Colino and Halliwell, 1987), part of the neurons in the dorsal CA1 region display a small depolarization and increase of input resistance in response to 5-HT, which coincides with a suppression of spike frequency accommodation (see, e.g., Fig. 2*A*). Although the de-

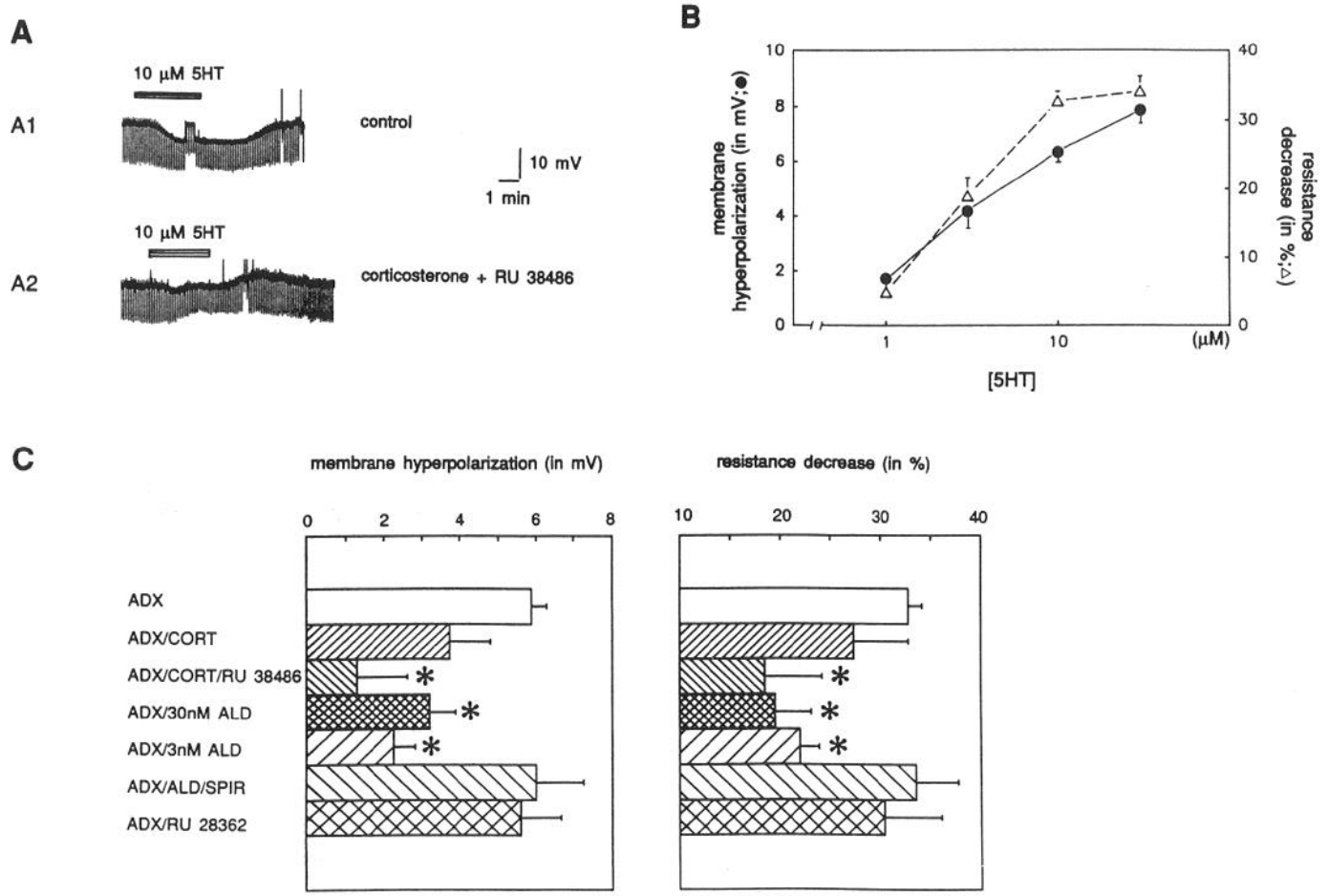

Figure 1. MR-mediated suppression of 5-HT–induced hyperpolarization in CA1 neurons. *A,* Typical responses induced by 10 μM 5-HT in CA1 pyramidal cells recorded intracellularly before treatment with a steroid (control; *A1*) and approximately 3 hr after a 20-min perfusion with 30 nM corticosterone and 500 nM RU 38486 (*A2*). Both cells were obtained in the same hippocampal slice, prepared 5 d after ADX. Downward deflections are the voltage responses to constant current pulses (0.2 nA, 150 msec). In *A1,* the membrane potential was brought to pretreatment level by injection of positive DC current, just before 5-HT administration was terminated, for correct estimation of the 5-HT–induced change in resistance. Duration of 5-HT application is indicated by the *horizontal bar. B,* Dose–response curve for the maximal membrane hyperpolarization (*circles*) and change in resistance (*triangles*) induced by 5-HT in CA1 pyramidal neurons, recorded in hippocampal slices from 5–7-d ADX rats. Data were obtained from 48 neurons. *Data points* represent mean ± SEM. *C,* Averaged maximal membrane hyperpolarization (*left histogram*) and changes in membrane resistance (*right histogram*) of CA1 pyramidal neurons in response to 10 μM 5-HT superfused on hippocampal slices from 5–7-d ADX rats. Compared to the mean ± SEM obtained before steroid application ($n = 43$), 5-HT responses were significantly (*, $p < 0.05$) reduced after a brief perfusion with 30 nM corticosterone (*CORT*) in the presence of the GR antagonist RU 38486 (500 nM; $n = 8$), with 30 nM aldosterone (*ALD*; $n = 10$) or with 3 nM aldosterone ($n = 15$). If aldosterone (3 nM) was applied in the presence of the MR antagonist spironolactone (*SPIR*; 300 nM; $n = 6$), the suppression of 5-HT responses was no longer observed. Responses obtained after treatment with 30 nM corticosterone were reduced in six of nine neurons, but on average this difference did not attain statistical significance. Application of the GR ligand RU 28362 (30 nM; $n = 5$) did not affect the 5-HT responses. Statistics were done by one-way ANOVA with the Student–Newman–Keuls test for multiple comparisons between means.

polarization is slower in onset and longer lasting than the 5-HT–induced hyperpolarization, they probably occur in part simultaneously. We therefore considered if the apparent suppression of 5-HT–induced hyperpolarizations could be ascribed to an actual decrease of the hyperpolarization or an increase of the depolarization. As shown in Figure 2*B,* 5-HT–induced depolarizations or changes in spike frequency accommodation were not significantly affected by the steroid treatment, except for an increased depolarization after treatment with 3 nM aldosterone.

To investigate this further, we studied the effect of 3 nM aldosterone on 5-HT hyperpolarization and depolarization separately: We used the 5-HT_{1a} agonist 5-CT (Beck, 1989) to examine the effect of steroid treatment on the 5-HT_{1a}–mediated hyperpolarization, and 5-HT in combination with the 5-HT_{1a} antagonist spiperone (Andrade et al., 1986) to investigate effects on the depolarizing phase of the 5-HT response. It appeared that, in neurons where 5-HT responses were largely suppressed by aldosterone (3 nM), responses to 5-CT were also significantly diminished by steroid treatment (Fig. 3). In contrast, no differences were observed for the depolarization evoked by 5-HT in the presence of spiperone between neurons recorded before (1.9 ± 0.8 mV; four neurons) and after (2.3 ± 0.3 mV; four neurons) aldosterone administration (not shown). These data suggest that corticosteroids, via MRs, suppress the 5-HT_{1a}–mediated hyperpolarization rather than enhance the subsequent depolarization.

Possible site of action for steroid

To obtain more insight into the target site for the steroid in the pathway from 5-HT_{1a} receptor to ion channel, we addressed

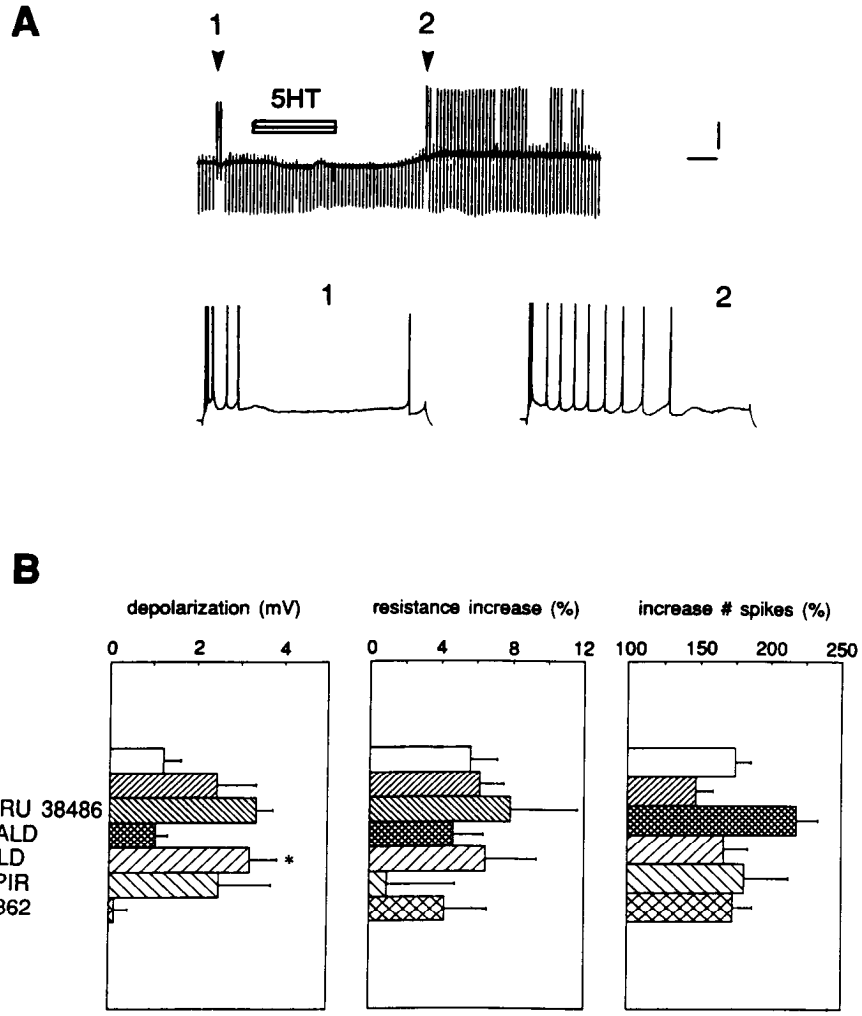

Figure 2. 5-HT–evoked depolarization of CA1 neurons is not affected by the steroids. *A,* Response of a CA1 hippocampal neuron to 10 μM 5-HT, approximately 2 hr after the application of corticosterone in the presence of RU 38486. A relatively small hyperpolarization was observed, which was followed by a depolarization of the membrane and an increase in resistance and spontaneous firing. Application of 0.5-nA depolarizing pulses 500-msec duration at the moments *1* and *2* (indicated by *arrows* in the *upper panel*) indicate that spike frequency accommodation was reduced during the depolarizing phase of the 5-HT response. The membrane potential was adjusted to pretreatment level just before termination of 5-HT application. Calibration: *upper panel,* vertical, 10 mV; horizontal, 1 min; *lower panel,* vertical, 24 mV; horizontal, 65 msec. *Downward deflections* in *upper panel* are responses to hyperpolarizing pulses of 0.4-nA and 150-msec duration. *B,* Averaged maximal membrane depolarization (*left histogram*), increase in membrane resistance (*middle histogram*), and changes in spike frequency accommodation (*right histogram*) evoked by 10 μM 5-HT in CA1 pyramidal neurons recorded in hippocampal slices from ADX rats. With the exception of the 5-HT–induced membrane depolarization after treatment with 3 nM aldosterone (*ALD*; $n = 14$), neither of the steroid treatments consistently changed these parameters when compared to the control values obtained before steroid application ($n = 42$). We did not include data on spike frequency accommodation for the group of cells treated with corticosterone (*CORT*) and RU 38486, because these neurons displayed on average (before 5-HT application) a higher number of spikes induced by a 0.5-nA depolarizing pulse of 500-msec duration than observed for the remaining groups of neurons. *SPIR,* spironolactone. *Error bars* represent SEM.

three questions: (1) do corticosteroids affect the binding properties of the 5-HT_{1a} receptors; (2) do the steroids suppress 5-HT responsiveness through 5-HT receptor desensitization; or (3) do the steroids affect the K^+ conductance linked to the 5-HT_{1a} receptor.

One possible explanation for the steroid-induced suppression of 5-HT_{1a}–mediated responses may be that the steroid decreases the binding capacity or affinity constant of the 5-HT_{1a} receptor. To investigate this, we examined binding of ^{3}H-8OH-DPAT to a hippocampal membrane fraction 1 hr following a 20-min exposure to either 3 nM or 30 nM aldosterone. We observed a slight but not significant decrease in the number of ^{3}H-8OH-DPAT–labeled sites after aldosterone treatment ($B_{max} = 1.50 \pm 0.08$ pM/mg protein; $n = 6$) when compared to the binding under control conditions ($B_{max} = 1.63 \pm 0.04$ pM/mg protein; $n = 6$; Fig. 4). No differences were found with respect to the affinity constants ($K_D = 1.48 \pm 0.15$ and 1.51 ± 0.15 nM for slices from ADX rats without and with aldosterone treatment, respectively).

An alternative explanation for the steroid action on 5-HT–induced hyperpolarizations may be that the MR ligands increase desensitization of the 5-HT receptor–mediated response. However, as was observed by others in previous studies (Andrade and Nicoll, 1987), we found that 5-HT–induced changes in membrane potential or resistance were very stable (less than 10% variation) upon repeated application. These stable responses were found both before (five neurons) and after (five neurons) steroid application (not shown). In addition, in those slices where 5-HT (10 μM) was applied only once, the average

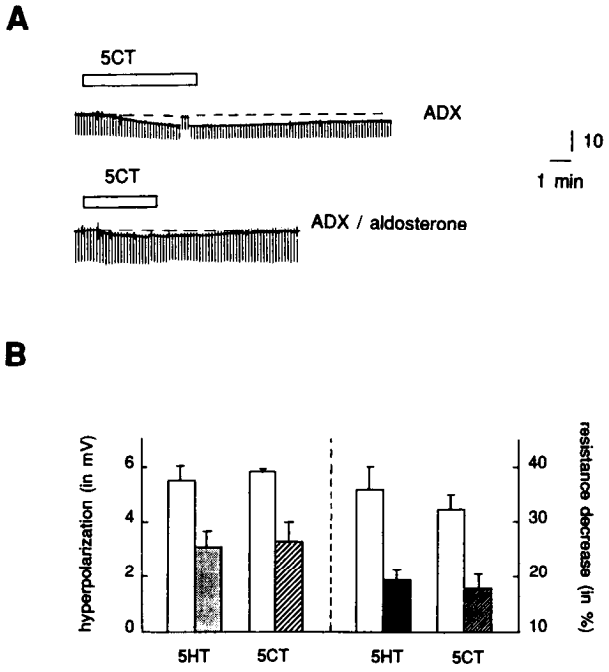

Figure 3. Responses to a 5-HT_{1a} agonist are diminished by aldosterone. *A*, The response of a CA1 pyramidal cell to the 5-HT_{1a} agonist 5-CT (0.1 μM) is diminished approximately 90 min after a 20-min perfusion with 3 nM aldosterone (*lower record*) when compared with the response obtained before steroid treatment (*upper record*). Duration of the 5-CT application is indicated by the *horizontal bar. Downward deflections* represent the voltage responses to constant current pulses (0.3 nA, 150 msec). In the *upper record,* the membrane potential was brought to the pretreatment level by injection of positive DC current just before 5-CT administration was terminated. As illustrated here, responses to 5-CT, in particular those recorded before steroid application, were not in all cases readily reversible. *B,* Membrane hyperpolarizations (*left*) and resistance decreases (*right*) obtained in the same set of CA1 pyramidal neurons ($n = 5$) by 10 μM 5-HT and 0.1 μM of the 5-HT_{1a} ligand 5-CT (mean ± SEM). Compared to the responses obtained before steroid perfusion (*open bars*), both 5-HT- and 5-CT-evoked responses (*gray/black* and *hatched bars,* respectively) were significantly reduced after a 20-min perfusion with 3 nM aldosterone. The data were tested with a Student's *t* test (significance, $p < 0.05$).

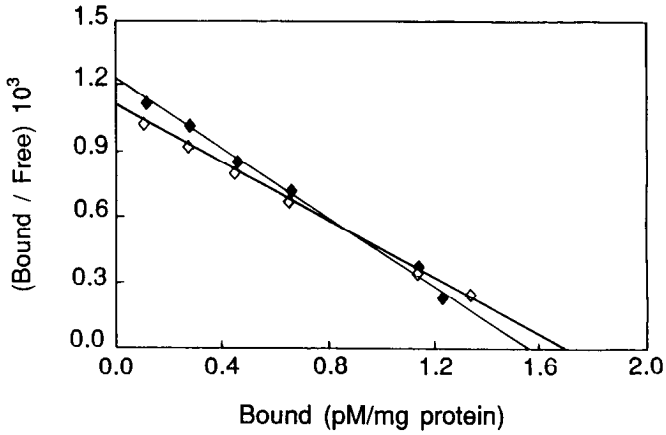

Figure 4. 5-HT_{1a} receptor binding is not changed after steroid treatment: Schatchard plot for binding of ^{3}H-8OH-DPAT to membranes of rat hippocampal slices, showing that the binding properties 1 hr after a 20-min exposure to 3 nM aldosterone are not significantly different from the binding without steroid treatment. Each *data point* is the mean of a triplicate determination. Specific binding in this experiment is total binding minus the binding persisting in the presence of 10 μM 5-HT (nonspecific binding). *Bound/Free,* bound over free ^{3}H-8OH-DPAT. Without steroid treatment: $K_D = 1.51$ nM, $B_{max} = 1.67$ pM/mg protein ($r_{corr} = 0.99$; *open diamonds*); after aldosterone application: $K_D = 1.25$ nM, $B_{max} = 1.53$ pM/mg protein ($r_{corr} = 1.0$; *solid diamonds*).

membrane hyperpolarization (2.0 ± 0.7 mV; 11 neurons) and resistance decrease (21.0 ± 2.0%) obtained after 3 nM aldosterone was still significantly (Student's *t* test, $p < 0.05$) smaller than in neurons recorded before steroid application (5.7 ± 0.4 mV and 32.0 ± 1.9% for changes in membrane potential and resistance, respectively; 24 neurons).

Andrade et al. (1986) have suggested that the 5-HT_{1a} receptor is linked directly through a G-protein to the same K^+ conductance to which the $GABA_B$ receptor is coupled. If the steroids act by decreasing the K^+ conductance linked to the 5-HT_{1a} receptor, one may expect that responses to the $GABA_B$ agonist baclofen will also be decreased. As can be seen in Figure 5, responses to baclofen were not affected by application of aldosterone, though 5-HT responses tested subsequently in the same neurons were markedly reduced.

Discussion

The present data indicate that selective MR but not GR ligands applied to hippocampal pyramidal neurons suppress the 5-HT_{1a}–mediated hyperpolarization in these neurons. The effect of the steroids seems to be specifically aimed at 5-HT_{1a}–mediated responses, because neither the depolarization nor the decrease in accommodation, which are both induced by 5-HT through a noncharacterized receptor (Andrade and Nicoll, 1987), was affected by the MR ligands. The fact that the steroid action has a slow onset and persists for many hours *in vitro* after termination of the steroid application is compatible with the notion that it involves an MR-mediated genomic action rather than a direct membrane-associated event.

In our attempt to establish the target for the steroid in the pathway from 5-HT receptor binding to opening of K^+ channels, we observed that the 5-HT_{1a} receptor–binding properties were only marginally affected by the steroid application. It seems unlikely that the small shift in B_{max} observed after aldosterone treatment can fully explain the rather large aldosterone-induced reduction of responses to 5-HT. The MR-linked effect on the 5-HT response is probably also not due to receptor desensitization, because (1) repeated application of 5-HT, either before or after steroid application, yielded very stable responses, and (2) 5-HT responses were significantly reduced even when the slice had not been exposed previously to 5-HT. Finally, it is unlikely that the steroid effect is only aimed at the K^+ conductance linked to the 5-HT_{1a} receptor. Thus, it has been proposed that the 5-HT_{1a} receptor is, through a G-protein, coupled to the same K^+ conductance as is the $GABA_B$ receptor (Andrade et al., 1986).We found that responses to the $GABA_B$ agonist baclofen were not changed after aldosterone application, though responses to 5-HT in the same group of cells were markedly reduced.

One possible explanation for the MR-mediated reduction in 5-HT responses may be that the steroid interferes with 5-HT–coupled inhibition of adenylate cyclase. This is an interesting possibility because it was recently found that chronic *in vivo* administration of estrogen enhances 5-HT responses in the hippocampal slice (Beck et al., 1989), probably by changing 5-HT–mediated inhibition of adenylate cyclase activity (Clarke and Maayani, 1990). It should be noted, though, that differences in

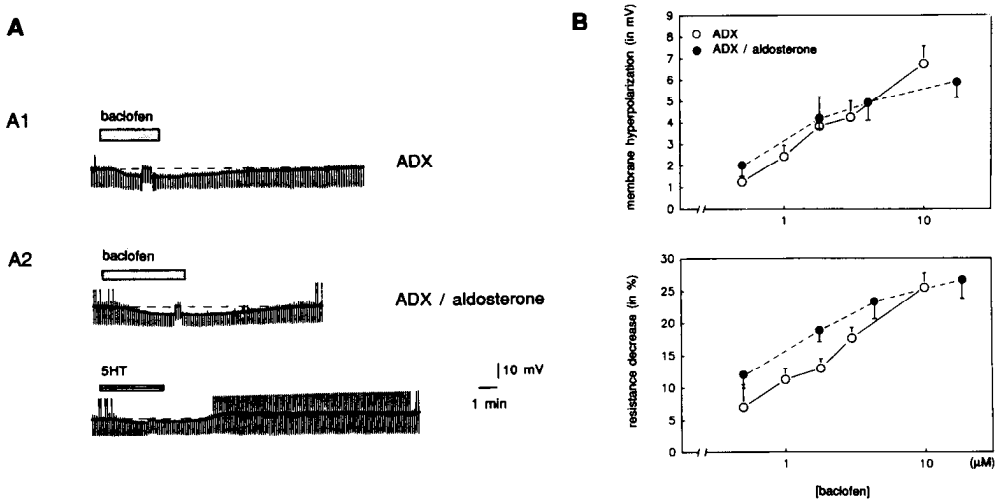

Figure 5. Responses to baclofen are not diminished by aldosterone. *A,* Response to 3 μM baclofen obtained in a CA1 pyramidal neuron recorded before steroid application (*A1*) and after a brief application of 3 nM aldosterone (*A2, upper trace*). Note that the baclofen response is not affected by aldosterone treatment, while the hyperpolarization induced by 10 μM 5-HT in the same neuron (*A2, lower trace*) is very small. Duration of transmitter application is indicated by *horizontal bars* for baclofen and 5-HT. *Downward deflections* indicate the cellular responses to constant current pulses (0.4 nA); in all recordings, membrane potential was temporarily brought to the pretreatment level for correct estimation of transmitter-induced changes in input resistance. *B,* Dose–response curves for membrane hyperpolarization (*top*) and resistance decrease (*bottom*) induced by baclofen in CA1 pyramidal neurons of ADX rats, before steroid application (*open circles*; $n = 7$) and 1–4 hr after a 20-min perfusion with 3 nM aldosterone (*solid circles*; $n = 5$). All values are means ± SEM.

time course for the estrogen- and MR-mediated effects and the fact that estrogen enhances whereas mineralocorticoids suppress 5-HT responsiveness suggest that the two steroids may well have different mechanisms of action. Alternatively, the effect of mineralocorticoids on the 5-HT response could also be caused by actions on synthesis or activation of regulatory proteins that are involved in processes between the 5-HT_{1a} receptor and the K channels linked to the 5-HT_{1a} receptor, such as G-protein–coupled phosphorylation (Saito et al., 1989).

All our experiments were performed in hippocampal slices and employed *in vitro* administration of steroids, with the advantage to study selectively steroid–transmitter interactions at the postsynaptically located CA1 pyramidal neuron. Under these experimental conditions, we were able to show that 5-HT actions on membrane properties are at least partly controlled by MR-mediated effects. Previous biochemical studies have also suggested a putative control of steroids on the 5-HT system, both at the level of the 5-HT–synthesizing neurons in the midbrain and in the hippocampus, one of the projection areas. Thus, permissive effects mediated by glucocorticoids were reported for 5-HT synthesis in the midbrain (de Kloet et al., 1983); in the forebrain and hippocampus, tryptophan hydroxylase activity (Azmitia and McEwen, 1974; Singh et al., 1990) and 5-HT turnover (van Loon et al., 1981; de Kloet et al., 1982) depended on corticosterone levels. In addition, previous autoradiographical binding studies with *in vivo* corticosterone application showed that 5-HT receptor binding in the dorsal CA1 area was reduced by the steroid (Biegon et al., 1985; de Kloet et al., 1986). The presently observed weak steroid effects on 5-HT binding may be partly explained by the fact that, in contrast to previous studies, our experiments were performed with *in vitro* application of the steroid to slices, so that (1) steroids cannot affect 5-HT synthesis in the midbrain, (2) 5-HT input fibers to the hippocampus are no longer intact, and (3) the influence of steroid-mediated actions on presynaptic 5-HT parameters will be limited due to the relatively low level of endogenous 5-HT release. In addition, the *in vivo* application of steroid hormones used in the previous studies and the presently used *in vitro* steroid administration may well have resulted in differences in time course for the MR-mediated action on the 5-HT system.

In an earlier study, we have shown that corticosterone reduces the norepinephrine-evoked reduction of cell firing accommodation in CA1 pyramidal neurons (Joëls and de Kloet, 1989). Compounds selective for GRs could mimic the effect of corticosterone on this response. Because accommodation in CA1 pyramidal neurons is linked to activation of a slow Ca^{2+}-dependent K^+ conductance, it seems likely that this conductance and probably also changes in intracellular calcium concentration (Kerr et al., 1989) play an important role in the glucocorticoid (inter)actions. In the present study, we demonstrate that selective occupation of MRs suppresses the 5-HT–induced hyperpolarization, which is caused by an increase of Ca^{2+}-independent K^+ conductances. This indicates that MR- and GR-mediated changes in membrane properties may involve completely different transmitter systems, mechanisms of action and ion conductances. The data so far indicate that MR-mediated control of the 5-HT response may result in an enhanced excitability, whereas activation of GRs with higher corticosterone concentrations may potentially reduce norepinephrine-evoked excitability in the CA1 area. The role of the two steroid receptor types involved in steroid-mediated control of neurotransmission in the CA1 hippocampal area seems therefore in line with the concept that MR activation is important for maintenance of a basal level of cellular excitability, whereas GR-mediated effects are involved in the suppression of excitability transiently raised by stress-related excitatory input (de Kloet and Reul, 1987).

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