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Mineralocorticoid receptor-mediated changes in membrane properties of rat CA1 pyramidal neurons *in vitro*

(corticosterone/glucocorticoid receptor/spike accommodation/afterhyperpolarization/brain)

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ABSTRACT **Pyramidal neurons in the rat hippocampus contain mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) to which the adrenal steroid corticosterone binds with differential affinity. We have used intracellular recording techniques to examine MR-mediated effects on membrane properties of CA1 pyramidal neurons in hippocampal slices from adrenalectomized rats. Low doses of corticosterone (1 nM) applied by perfusion for 20 min decreased the spike accommodation observed during a depolarizing current pulse (0.5 nA for 500 ms) and the amplitude of the subsequent afterhyperpolarization without affecting other membrane properties tested. The decrease became apparent *ca.* 15 min after steroid perfusion was started and reached its peak value 10–20 min after the steroid perfusion was terminated. The steroid effect was blocked by the MR antagonist spironolactone and mimicked by the natural MR ligand aldosterone (1 nM). Neurons recorded 30–90 min after termination of aldosterone application still displayed a decreased spike accommodation. However, 30–90 min after corticosterone application, the decrease in spike accommodation/afterhyperpolarization appeared to be reversed. Higher doses of corticosterone (≥30 nM) induced a significant increase in accommodation and amplitude of the afterhyperpolarization, as was previously observed for selective GR ligands. The data indicate that MR and GR activations induce opposite actions on the spike accommodation/afterhyperpolarization of CA1 pyramidal neurons, an important intrinsic mechanism of these neurons to regulate their response to excitatory input. We suggest that occupation of both MR and GR by the endogenous ligand corticosterone will result in an initial MR-mediated enhanced cellular excitability, which is gradually reversed and overridden by a GR-mediated suppression of cellular activity.**

The endogenous adrenal corticosteroid of the rat, corticosterone, can cross the blood–brain barrier, bind to intracellular receptors in the brain, and, as a steroid–receptor complex, affect the expression of the genome (1, 2). Two types of corticosteroid receptors have been distinguished on the basis of molecular structure (3), binding properties, neuroanatomical topography, and physiological function (4–6): (*i*) the mineralocorticoid receptor (MR), which is thought to be involved in the control of basal activity throughout the circadian cycle, and (*ii*) the glucocorticoid receptor (GR), which participates in the feedback control of the stress response. Corticosterone binds to both receptors, displaying a 10-fold higher affinity for the MR than the GR (4).

The CA1 pyramidal neurons in rat hippocampus contain intracellular MRs and GRs (7–9). In a recent electrophysiological study on slices from adrenalectomized (ADX) rats, we showed that activation of the GR in CA1 neurons increases the amplitude and half-decay time of the afterhyperpolarization (AHP) that follows a brief depolarization (10). Other membrane properties that were tested remained unaffected by the glucocorticoids. The slow onset (1–2 hr) and long duration of the GR-mediated effects suggest that steroid-induced gene products participate in the ionic regulation.

In the present study we have used intracellular recording techniques to investigate the effect of selective MR activation on membrane properties of CA1 pyramidal neurons in hippocampal slices from ADX rats. For this purpose we studied the effect of 1 nM corticosterone, which under comparable *in vitro* conditions has been found to occupy a large proportion of the MRs but only a small part of the GRs (4), and tested if corticosterone responses were affected by the MR antagonist spironolactone. We also investigated the effects of the natural mineralocorticoid aldosterone (1 nM). Neurons were recorded continuously before (30 min), during (20 min), and after (20 min) steroid administration. Corticosterone (1 or 30 nM)- or aldosterone (1 nM)-induced actions were followed over a longer period of time by comparing membrane properties of neurons recorded before and neurons impaled 30–90 min after exposure to the steroids.

METHODS

Experiments were performed in male Wistar rats (120–170 g), ADX 5–7 days before the experiments as described elsewhere (10, 11). The animals were housed in an animal room with alternating light/dark [(0800–2000 hr)/(2000–0800 hr)] cycle and received food and saline (after ADX) ad libitum. On the day of the experiment, the rat was placed in a clean cage for 30–45 min prior to decapitation. Trunk blood was collected for measurement of plasma corticosterone levels. An animal was considered ADX if the corticosterone level was less than 1 μg of corticosterone per 100 ml of plasma. The brain was removed from the skull and dipped in ice-cold gassed (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF = 124 mM NaCl/3.5 mM KCl/1.25 mM NaH_2PO_4/1.5 mM $MgSO_4$/2 mM $CaCl_2$/25 mM $NaHCO_3$/10 mM glucose). The hippocampus was dissected and transverse slices (350 μm) were cut on a McIlwain tissue chopper. The slices were transferred to a recording chamber, submerged, and continuously perfused with warm (32°C) ACSF at a constant rate (2–3 ml/min). The standard ACSF could be switched to an ACSF containing the steroids at a known concentration. Corticosterone, aldosterone (Organon International, Oss, The Netherlands), and spironolactone (Searle) were dissolved in 100% ethanol and kept as a 1 mM stock in the freezer for 1 week at most. Just before the experimental test the steroids were diluted to the intended concentrations in oxygenated ACSF. The final ethanol concentrations for 1 nM corticosterone or aldosterone thus became 1×10^{-4} %.

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Abbreviations: MR, mineralocorticoid receptor; GR, glucocorticoid receptor; ADX, adrenalectomized; AHP, afterhyperpolarization.

Intracellular recordings of CA1 pyramidal neurons were performed with 4 M KAc-filled microelectrodes (impedance: 80–150 MΩ). 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-ms current pulses of −0.6 to 0.2 nA), spontaneous activity, and the spike accommodation/AHP evoked by a 50-ms depolarizing current pulse (0.2–1.0 nA) or a 500-ms depolarizing current pulse (0.5 nA).

Corticosterone and aldosterone were only tested in neurons that displayed stable membrane characteristics under baseline conditions for at least 30 min. These neurons were continuously recorded during a 30-min baseline period, followed by a 20-min application of the steroid and for at least 20 min after termination of the steroid application. The spike accommodation/AHP were recorded once every 5 min. Current–voltage curves were established at 10- or 20-min intervals. In experiments with the MR antagonist spironolactone, administration of the antagonist was started 20 min before the agonist was applied.

As it proved to be quite difficult to record from large groups of neurons for more than 60 min after termination of the steroid application, we used another protocol to study steroid-induced actions developing with a delay of 1–2 hr. In this protocol we compared membrane properties of neurons recorded before steroid treatment with neurons impaled 30–90 min after exposure to the steroids was terminated. If possible we recorded the same number of neurons before and after steroid treatment in each slice, preferably with the same recording electrode, to minimize the influence of variability between slices and experimental conditions.

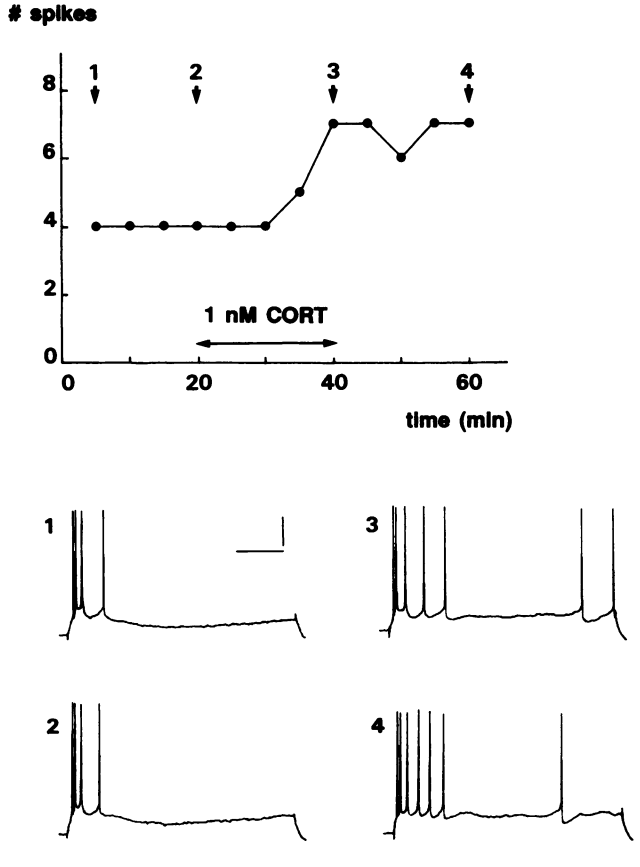

FIG. 1. Effect of 1 nM corticosterone (CORT) on the number of spikes induced in a CA1 pyramidal neuron by a depolarizing current pulse of 0.5 nA and 500-ms duration. The steroid was applied for 20 min by perfusion to a hippocampal slice from an ADX rat. The voltage traces recorded at four time points, indicated by arrows 1–4, are depicted below. (Calibration for the voltage traces: horizontal bar = 100 ms; vertical bar = 20 mV.)

RESULTS

In total, we recorded from 89 neurons in the CA1 pyramidal cell layer. The average resting membrane potential (−67.4 ± 0.3 mV), input resistance (43.1 ± 1.1 MΩ), and the low spontaneous activity of the neurons resembled the membrane characteristics described for CA1 pyramidal neurons by others (12).

In nine neurons displaying stable membrane properties for at least 30 min, we tested the effect of a 20-min application of 1 nM corticosterone. As illustrated in Fig. 1, corticosterone typically increased the number of spikes induced by a 0.5-nA depolarizing pulse of 500-ms duration. In most neurons the increase started ca. 15 min after the slice was exposed to the steroid and reached a maximum 10–20 min after the steroid

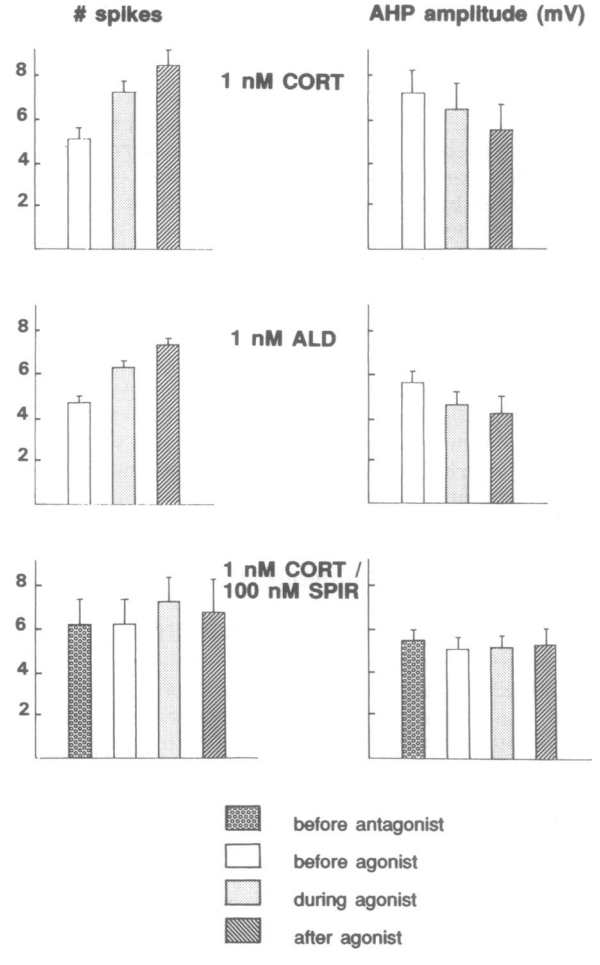

FIG. 2. Changes (mean + SEM) in the number of spikes induced in CA1 pyramidal neurons by a depolarizing current pulse (0.5 nA and 500 ms) (*Left*) and in the AHP amplitude (*Right*) during and after application of 1 nM corticosterone (CORT) (*Top*), 1 nM aldosterone (ALD) (*Middle*), and 1 nM corticosterone in the presence of 100 nM spironolactone (SPIR) (*Bottom*). The number of spikes was increased during and after a 20-min application of corticosterone ($n = 8$; $F = 8.6$; $P < 0.01$) and aldosterone ($n = 5$; $F = 5.2$; $P < 0.05$) but not if corticosterone was tested in the presence of spironolactone ($n = 5$). The AHP amplitude was only significantly decreased by corticosterone ($F = 4.0$; $P < 0.05$). Statistical significance was tested with an analysis of variance for repeated measurements.

application was terminated (i.e., 30–40 min after first exposure to corticosterone). On average, the number of spikes associated with a 0.5-nA depolarizing pulse of 500 ms was significantly ($P < 0.01$) increased by corticosterone, while the AHP after the depolarization was decreased in amplitude ($P < 0.05$; $n = 8$; Fig. 2). The resting membrane potential and input resistance of these neurons during (−67.9 ± 0.8 mV and 46.6 ± 6.2 MΩ, respectively) or after application of the steroid (−66.9 ± 1.6 mV and 44.5 ± 4.2 MΩ) were not significantly different from the pretreatment values (−66.7 ± 0.9 mV and 49.5 ± 3.4 MΩ). One neuron was not affected by corticosterone. Control experiments with ethanol (1×10^{-4} %, $n = 8$) yielded no significant changes in time for any of the parameters tested (not shown).

In the presence of the MR antagonist spironolactone (100 nM), the effects of corticosterone on spike accommodation and AHP amplitude were no longer observed ($n = 5$; see Fig. 2). The antagonist itself did not affect spike accommodation, AHP, resting membrane potential, or input resistance ($n = 5$; data not shown).

Aldosterone (1 nM) induced effects similar to those observed for 1 nM corticosterone (Fig. 2). The number of spikes associated with a depolarizing current pulse was increased by aldosterone ($P < 0.05$; $n = 6$) with a time delay comparable to corticosterone. Although the amplitude of the AHP showed a tendency to decrease in time, this effect did not attain statistical significance. Two neurons did not respond to aldosterone.

To investigate the reversibility of the MR-mediated effects, we compared membrane properties of neurons recorded before steroid application with properties of neurons that were impaled in the same slice 30–90 min after a 20-min perfusion of the steroids (Table 1). It appeared that in neurons recorded 30–90 min after exposure to aldosterone, the number of spikes induced by a 500-ms depolarizing current pulse was still raised in comparison to the neurons obtained before treatment. However, if slices were perfused with 1 nM corticosterone, the decrease in spike accommodation and AHP amplitude that was apparent 20 min after the steroid exposure was no longer observed. In contrast, there was a tendency toward an increase in spike accommodation and AHP amplitude when slices were treated with corticosterone alone or with corticosterone and spironolactone. To examine if this reversal could be due to a gradual activation of GRs, we tested the effect of a 20-min perfusion with 30 nM corticosterone, which occupies not only MRs but also most of the GRs. We observed a significant increase in the AHP amplitude (with a 50-ms pulse for 0.5 and 1.0 nA) and spike accommodation (with a 500-ms pulse) in neurons recorded 30–90 min after treatment with 30 nM corticosterone. No changes were found in neurons that were impaled 0–20 min after the steroid perfusion was terminated.

DISCUSSION

In CA1 pyramidal neurons of the rat hippocampus, MR and GR genes are expressed (7–9). Activation of the GR by a brief exposure of hippocampal slices to high concentrations of corticosterone (10, 13) or to selective glucocorticoids (10) specifically increases the AHP amplitude of CA1 pyramidal neurons 30–90 min after termination of the steroid treatment. In the present study we demonstrate that activation of the MR by aldosterone (1 nM) induces a decrease in spike-frequency accommodation of CA1 pyramidal neurons, starting ca. 15 min after exposure to the mineralocorticoid and reaching a maximal effect 30–40 min after first exposure to the steroid. Similar effects were observed for a low (1 nM) concentration of corticosterone; these effects were blocked in the presence of a MR antagonist. These data are in line with a previous observation (10) that activation of MR by treatment with 1 μM corticosterone and the selective GR antagonist RU 38486 tends to decrease the frequency accommodation.

As spike frequency accommodation and the slow AHP are both linked to a slow Ca^{2+}-dependent K^+ conductance in pyramidal cells (14–18), the present data suggest that this conductance is probably reciprocally regulated by MR- and GR-mediated events. However, other K^+ currents were also found to contribute to the amplitude of the AHP (14–18) and might thus be a target for corticosteroid action. In addition, the amplitude of the AHP is controlled by calcium entering the cell through voltage-sensitive channels, leaving the possibility that Ca^{2+} channels are a site of action for steroid hormones. Interestingly, Landfield and coworkers (13) reported that the Ca^{2+} spike in CA1 hippocampal neurons is enhanced by high doses of corticosteroid hormones.

The present study suggests that steroid-induced gene products participate in the ionic regulation underlying the accom-

Table 1. Long-term effects of steroids on hippocampal excitability

				AHP			
				50-ms pulse		500-ms pulse	
Time of recording	n	RMP, mV	R_{in}, MΩ	Spikes, no.	Ampl, mV	Spikes, no.	Ampl, mV
Before 1 nM corticosterone	7	−68.5 ± 1.1	41.7 ± 2.9	3.2 ± 0.2	3.1 ± 0.5	5.6 ± 0.5	5.3 ± 0.5
After 1 nM corticosterone	7	−67.6 ± 1.3	42.4 ± 3.7	2.6 ± 0.3	4.1 ± 1.0	4.3 ± 0.6	5.7 ± 1.3
Before 1 nM aldosterone	11	−67.2 ± 1.0	38.1 ± 2.7	2.9 ± 0.1	3.5 ± 0.6	3.8 ± 0.3	6.2 ± 0.5
After 1 nM aldosterone	6	−68.4 ± 0.7	48.8 ± 5.6	3.3 ± 0.2	3.3 ± 1.2	6.3 ± 1.2*	5.9 ± 0.7
Before 1 nM corticosterone/100 nM spironolactone	7	−66.1 ± 1.0	45.4 ± 3.5	3.0 ± 0.3	2.9 ± 0.7	5.7 ± 1.1	6.1 ± 0.7
After 1 nM corticosterone/100 nM spironolactone	5	−66.0 ± 1.7	42.0 ± 3.9	3.0 ± 0.5	4.1 ± 1.0	4.4 ± 1.0	6.3 ± 1.7
Before 30 nM corticosterone	8	−65.6 ± 1.3	40.4 ± 4.8	3.1 ± 0.2	2.8 ± 0.7	6.4 ± 0.9	5.1 ± 0.8
After (0–20 min) 30 nM corticosterone	4	−68.5 ± 1.5	38.3 ± 7.0	2.8 ± 0.3	2.1 ± 0.5	6.8 ± 0.6	3.5 ± 0.5
After (30–90 min) 30 nM corticosterone	9	−68.1 ± 0.8	41.9 ± 2.2	3.3 ± 0.2	5.2 ± 0.5*	4.0 ± 0.3*	5.8 ± 0.4

Values (mean ± SEM) were obtained for neurons recorded before or 30–90 min after steroid treatment, for resting membrane potential (RMP), input resistance (R_{in}), number of spikes, and AHP associated with a 0.5-nA depolarizing current pulse of 50-ms duration or 500-ms duration. Ampl, amplitude. For each set of data, results from control neurons (i.e., data from neurons before exposure to the steroid) were obtained in the same slices as the results recorded after exposure to the steroid. For data from experiments with 1 nM corticosterone, 1 nM aldosterone, or 1 nM corticosterone/100 nM spironolactone, statistical significance was tested with a paired t test. For the data obtained with 30 nM corticosterone, a one-way analysis of variance with the Student–Newman–Keuls test for multiple comparisons between means was used.
*$P < 0.05$.

modation and AHP. This assumption is based on the specificity of the intracellular MRs and GRs. Moreover, the effects develop and persist beyond the initial presence of the steroids. Genomic actions of the steroids have been reported to occur within the time delay of 15 min to 2 hr (19, 20), which is observed in our study. If indeed corticosteroids regulate the cellular excitability by a genomic action our electrophysiological findings can be related to a molecular model of corticosteroid action proposed by Evans and Arriza (21). Yet, direct membrane effects of the steroids cannot be fully excluded at present.

The endogenous ligand corticosterone binds to MR with a 10-fold higher affinity than to GR (4). Therefore, at the 1 nM concentration corticosterone mainly binds to MRs although a small part of the GRs will be occupied too. Accordingly, we found that at this concentration corticosterone acts like aldosterone, during and up to ca. 20 min after termination of the steroid perfusion. Yet, if the delay between treatment and recording was extended (30–90 min), corticosterone in contrast to aldosterone tended to increase the accommodation/AHP. This reversal appeared sooner (0–20 min after offset) and became more pronounced if the corticosterone concentration was raised to 30 nM, thus activating not only most of the MRs but also the GRs in pyramidal cells. If neurons were continuously recorded during and up to 20 min after termination of perfusion with 1 μM corticosterone, accommodation/AHP differed at no time from the pretreatment values (unpublished observation), although after a longer delay this dose of corticosterone markedly increases the AHP amplitude (10). We therefore conclude that the full expression of GR-mediated effects on AHP displays a longer delay in time than maximal activation of the MR. In addition, the data indicate that GR-mediated events can slowly override MR-mediated actions.

The use of ADX animals in our study was essential for distinguishing between MR- and GR-mediated effects. (*i*) Central MRs in intact animals are largely occupied with endogenous hormones, implying that MR-mediated actions will remain largely unnoticed after addition of exogenous corticosterone. (*ii*) The changes in circulating corticosterone are unpredictable and episodic resulting in variable receptor occupation. (*iii*) The steroid effects evoked *in vivo* persist *in vitro* in the brain slices (10, 13). In retrospect, the long-lasting and opposite effects on cellular excitability that we observed for the steroid action on MRs and GRs might explain the incongruencies in previous electrophysiological studies (22–25), where differences in applied corticosterone concentrations, experimental animal conditions (ADX versus intact), and delay between steroid application and the assessment of cellular effects existed. Interestingly, our data are in line with a study by Rey *et al.* (26), where a similar experimental approach was used.

The presently observed biphasic cellular effect of the endogenous ligand corticosterone by the MR and the GR might be associated with the stringent specificity of corticosterone shown previously in behavioral studies. Thus, corticosterone induced highly specific effects on conditioned behavior, which were not achieved by selective stimulation of either hippocampal MR or GR (27, 28). The observed specificity for corticosterone may be partly due to the unique sequence of MR- and GR-mediated events revealed in the present study, which can be achieved with corticosterone but not with aldosterone or selective GR ligands. However, the implication of the present findings, obtained at the cellular level in ADX animals, for behavioral functions should be interpreted with caution.

In summary, we here describe a MR-mediated decrease in the spike accommodation/AHP amplitude of CA1 pyramidal neurons, an important intrinsic mechanism of these neurons to regulate their response to excitatory input (29). The MR-mediated action is opposite to a previously described GR-mediated effect on this parameter. The data support the concept (5) that activation of the MR serves to maintain the cellular response to excitatory input, whereas GR activation suppresses the cellular response to excitation, particularly when the excitability is temporarily raised by transmitters as was shown for norepinephrine (10). In time, MR-mediated effects on spike accommodation/AHP precede the activation of GR but are gradually overridden by the GR-mediated events. We conclude that activation of the two corticosteroid receptors in rat hippocampus, each with their specific pharmacological profile and binding properties, might thus contribute to the reciprocal control of a membrane conductance that plays an important regulatory role in the cellular excitability of this brain structure.

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