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# EFFECT OF CORTICOSTEROID HORMONES ON ELECTRICAL ACTIVITY IN RAT HIPPOCAMPUS

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**Summary**—Pyramidal neurons in the rat CA1 hippocampal area contain both mineralocorticoid (MR) and glucocorticoid receptors (GR) which bind the endogenous adrenal steroid corticosterone with differential affinity. With intracellular electrophysiological recording techniques we have investigated how corticosterone affects the membrane properties of these cells. We observed that low doses (1 nM) of corticosterone or aldosterone can, through MR, reduce the spike frequency accommodation and afterhyperpolarization (AHP) evoked by a short depolarizing current in pyramidal neurons. As the accommodation/AHP can be considered as an intrinsic mechanism of CA1 neurons to attenuate transmission of excitatory input, the MR-mediated action might potentially enhance cellular excitability in the CA1 area. Higher doses of corticosterone or selective glucocorticoids were able to reverse the MR-mediated effect on accommodation/AHP, eventually increasing particularly the amplitude of the AHP. GR-mediated events may thus potentially suppress excitability in the hippocampal CA1 area. Not only current- but also transmitter-induced membrane effects were affected by the steroids. Firstly, GR-ligands were able to suppress a temporary noradrenaline-evoked decrease in accommodation/AHP. Secondly, membrane hyperpolarizations induced by serotonin were reduced by MR-agonists. We propose that cellular excitability in the hippocampus is at least partly under control of coordinative, antagonistic MR- and GR-mediated effects on electrical activity.

The steroid hormones of the rat adrenal cortex can cross the blood–brain barrier and bind to two intracellular receptor-types in the brain: the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) [1–3]. According to the concept of steroid action, the steroid receptor complex can subsequently alter the expression of cell-specific genes [2]. Biochemical studies have shown that the MR displays a 10-fold higher binding affinity for the endogenous rat adrenal steroid corticosterone than GR [1]. The MR is discretely localized in parts of the limbic system, and motor and sensory neurons in the brainstem. In contrast, the distribution of GRs over the brain is much more ubiquitous. Pyramidal neurons in the rat CA1 hippocampal area were shown to encode for and contain both the MR and GR [4–7]. We have used electrophysiological recording techniques to examine how selective occupation of GR or MR affects electrical properties and transmitter-induced responses of CA1 pyramidal neurons.

All the experiments were performed in male Wistar rats (120–170 g), adrenalectomized under ether anesthesia approx. 1 week before the electrophysiological experiment [8]. At 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. The brain was removed from the skull, dipped in icecold artificial cerebrospinal fluid (ACSF) and slices (350 $\mu$m) were prepared from the dorsal hippocampus on a McIlwain chopper. The slices were placed in a perfusion system, submerged and continuously superperfused with warm (32°C), oxygenated (95% $O_2$ + 5% $CO_2$) ACSF to which drugs could be added by switching to an ACSF solution containing the drug at a known concentration. Steroid hormones were always freshly prepared as a 1 mM solution in 90% ethanol and diluted to the correct concentration just before application. MR- or GR-agonists were applied for 20 min. Steroid antagonists were administered for 20 min before, during and 20 min after the application of the agonist. In a limited number of neurons we recorded

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neuronal characteristics before, during and up to 30 min after steroid administration. However, in most experiments we compared properties of neurons recorded before steroid administration with neurons recorded 30–90 min after termination of the steroid perfusion. Intracellular recording electrodes (KAc-filled, impedance 80–150 MΩ) were placed in the CA1 pyramidal cell layer. Signals were amplified, filtered and stored according to conventional intracellular recording techniques. Only neurons with a stable resting membrane potential below −60 mV and a spike amplitude of at least 80 mV were incorporated.

We found that administration of the endogenous MR-ligand aldosterone (1 nM) or low doses of corticosterone (1 nM) significantly suppresses the spike frequency accommodation and the amplitude of the afterhyperpolarization (AHP) which are associated with a depolarizing pulse (0.5 nA and 500 ms duration) in CA1 neurons [9]. The accommodation and AHP are linked to a slow $Ca^{2+}$-dependent $K^+$-conductance and thought to play a regulatory role in cellular excitability [e.g 10]. The steroid effect starts approx. 15 min after the onset of steroid application and reaches a maximal effect 10–20 min after termination of the steroid perfusion, i.e. *ca* 30 min after the tissue is first exposed to the steroid (see Fig. 1). The effect of corticosterone could be effectively blocked by the MR-antagonist spironolactone. The resting membrane potential or input resistance of the CA1 neurons was not affected by the steroids. The data indicates that selective occupation of the MR in CA1 pyramidal cells suppresses the spike frequency accommodation/AHP in these cells, reaching a peak effect with a delay of at least 30 min.

Interestingly, the suppression of spike frequency accommodation/AHP observed *ca* 30 min after exposure of the slice to low doses of corticosterone gradually reverses if the delay between steroid application and recording of a CA1 neuron progresses (see Fig. 1). Thus, neurons recorded approx. 90 min after first exposure of the slice to 1 nM corticosterone, but not 1 nM aldosterone, display a spike frequency accommodation that is comparable with the accommodation observed in neurons recorded before steroid treatment. This phenomenon of a reversal is even stronger when a higher corticosterone concentration is applied: with 30 nM corticosterone, thus not only occupying most of the MR but also of GR, spike frequency accommodation/AHP is increased 90 min after onset of

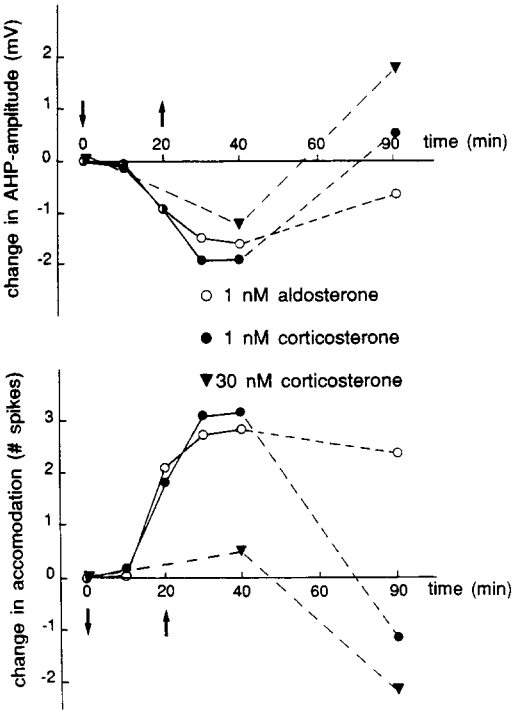


Fig. 1. Averaged steroid-induced changes in the amplitude of the AHP (upper graph) and the number of spikes (lower graph) associated with a 0.5 nA depolarizing current step of 500 ms duration. Aldosterone (1 nM, ○, $n = 6$), 1 nM cortocosterone (●, $n = 7$) or 30 nM corticosterone (▼, $n = 4$ to 9) were applied for 20 min by superfusion, starting at $t = 0$ (↓) and terminating at $t = 20$ min (↑). Values obtained during continuous recordings from the same neuron are connected by a solid line. Values obtained in different groups of neurons recorded at various timepoints after steroid application are connected by a dotted line. As can be seen in the figure, the effects of aldosterone remain in the same direction, over a period of 90 min after first exposure of the slices to the steroid. In contrast, the actions of 1 nM corticosterone and in particular 30 nM corticosterone gradually reverse.

the steroid. This fact and the observation that the reversal is not observed with aldosterone, suggests that occupation of the GR and MR may actually have opposite effects on the accommodation/AHP of CA1 neurons. Accordingly, we [11] and others [12] found that with high concentrations of corticosterone (up to 1 μM) the amplitude of the AHP is markedly enhanced. This effect can be blocked by the selective GR-antagonist RU 38486 [11]. Likewise, administration of the selective GR-agonist RU 28362 dose-dependently increased the amplitude of the AHP. We therefore concluded that the occupation of the GR in CA1 hippocampal neurons leads to an increase in the amplitude of the AHP. The GR-mediated effect is opposite to that of the MR, more gradual in onset and eventually overrides the MR-mediated effect.

The effects of selective MR- and GR-occupation described above may explain observations on neurons obtained in slices from adrenalectomized vs sham-operated rats [11, 12]. On average, the neurons from these two groups of animals did not differ with respect to resting membrane potential, input resistance or spontaneous activity. However, the AHP induced by a short depolarizing current pulse in CA1 neurons from sham-operated rats is significantly increased in comparison with the AHP recorded in neurons from adrenalectomized rats. This agrees with the fact that (under the present experimental conditions) part of the GRs in sham-operated animals will be occupied, thus eventually leading to an increased amplitude of the AHP. Our results emphasize the importance of using controlled experimental conditions in the investigation of steroid effects on electrical activity in the brain. Thus, variability in experimental design, e.g. the use of adrenalectomized vs sham-operated rats, concentration of the steroids to be applied or delay between steroid application and recording of steroid-induced effects could greatly affect the observed steroid actions.

The differential control by MR and GR of spike frequency accommodation/AHP potentially affects the excitability in the hippocampal CA1 area [10]. Thus, occupation of MR may enhance cellular excitability, while GR-mediated actions may lead to a suppression of temporarily raised cellular excitability. This coordinative, antagonistic control of the steroid hormones over cellular excitability in the hippocampus is also reflected in the steroid effects on transmitter-induced actions in this area. This will be illustrated on two examples. First, noradrenaline is known to suppress the spike frequency accommodation/AHP in CA1 pyramidal neurons via the cAMP-coupled $\beta$-adrenergic receptor [13–15]. We found that neurons in slices from adrenalectomized rats display a stronger $\beta$-adrenergic response than neurons in sham-operated rats [11]. In contrast, *in vitro* application of corticosterone or a selective GR-ligand on slices from adrenalectomized rats diminishes the $\beta$-adrenergic response of CA1 neurons. The functional implication of this observation may be that occupation of GR can reduce temporarily (stress-induced) raised cellular excitability. The second example concerns the interaction between corticosteroid hormones and serotonin in the CA1 area. Serotonin primarily hyperpolarizes CA1 neurons by increasing $K^+$-conductances, a response which is probably mediated by the 5HT1a receptor [16, 17]. We observed that corticosterone, particularly when applied in the presence of the GR-antagonist RU 38486, suppresses the hyperpolarizing responses to serotonin [18]. This effect can also be induced with aldosterone (3 or 30 nM) and blocked with the MR-antagonist spironolactone. In contrast, administration of the GR-agonist RU 28362 does not affect the serotonin response. Thus, while noradrenaline-evoked cellular excitability is suppressed by a GR-mediated event, serotonin-induced membrane hyperpolarization is selectively diminished via the MR.

In conclusion, we have observed that corticosteroid hormones selectively affect the spike frequency accommodation and AHP of CA1 neurons with a delay of at least 30 min, while the passive membrane properties of these cells remain unchanged by the steroids. These steroid actions therefore differ from previously described actions which were characterized by a fast onset and may be mediated by membrane-associated steroid receptors [19, 20]. The accommodation/AHP is under the differential control of the steroid receptors, since occupation of MR diminishes this membrane property, while it is enhanced if part of the GR are occupied. Since the accommodation/AHP may be considered as an intrinsic cellular property to regulate excitability [10], MR-mediated events are potentially maintaining excitability, while GR-mediated events are suppressive for the electrical activity in the CA1 region. Transmitter-evoked effects in CA1 pyramidal neurons also appear to be selectively regulated by MR- and GR-mediated events. Thus, while the 5HT-dependent reduction in cellular excitability of CA1 neurons is attenuated by an MR-mediated action of corticosterone, the steroid suppresses, via GR, noradrenaline-evoked excitability. The role of the two steroid receptor types in neurotransmission of the CA1 hippocampal area is therefore in line with the concept that MR is important for maintenance of a basal level of cellular excitability, whereas GR is involved in the suppression of excitability transiently raised by stress-related excitatory input [21].

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