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Steroids and Electrical Activity in the Brain

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Corticosteroid hormones can enter the brain and bind to two receptor subtypes: the high affinity mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) with approximately 10-fold lower affinity. Under physiological conditions the degree of receptor occupation will range from a predominant MR occupation (at the beginning of the inactive period, under rest) to concurrent activation of MRs and GRs (at the circadian peak and after stress). With in vitro electrophysiological recording techniques we observed that neuronal excitability in the CA1 hippocampal field is under a long-term control of MR- and GR-mediated events. The predominant occupation of MRs is associated with a stable amino acid-carried synaptic transmission; calcium- and potassium-currents are small, as are the responses to biogenic amines. Occupation of GRs in addition to MRs results in a gradual failure of CA1 neurons to respond to repeated stimulation of amino acid-mediated input; ionic conductances and responses to biogenic amines are large. In general, electrical properties recorded when both MRs and GRs are unoccupied (i.e. after adrenalectomy) resemble the responses observed when both receptor types are activated. The corticosterone dependency of electrical properties is thus U-shaped. We conclude that MR occupation may be responsible for the maintenance of information processing in the CA1 field and the stability of the circuit. Additional activation of GRs will initially suppress synaptic activity, but may eventually result in an increased instability and even vulnerability of the neuronal networks.

INTRODUCTION

The rat adrenocortical hormone corticosterone is released into the circulation in a circadian pattern, with low levels at the beginning of the inactive period (i.e. in the morning, for the rat) and high levels at the start of the active period [1, 2]. Periods of stress also result in high adrenocortical activity. The hormone reaches peripheral organs such as the liver, kidney and colon but also interacts with the immune system [3]. In general, corticosterone enhances the availability of glucose in the blood, induces breakdown of proteins and promotes anti-inflammatory processes. The mineralocorticoid hormone aldosterone, which is also produced in the adrenal cortex, serves to retain body salts [4].

In the late 60s, it became apparent that the adrenocortical hormones do not only bind to receptors in peripheral organs but also in the brain [5]. Due to their lipophilic nature the hormones can easily pass the blood-brain barrier. McEwen and co-workers showed that [3H]corticosterone is retained within the brain, most notably in the hippocampus [5]. Some years later Pfaff et al. demonstrated that subcutaneous injection of corticosterone to a hypophysectomized rat resulted, with a delay of ca. 30 min, in a depression of hippocampal cell firing [6]. At that time it was concluded that corticosterone binds to specific receptors in the hippocampus and consequently inhibits neuronal firing.

In the mid-80s, however, de Kloet and co-workers reported that corticosterone binds to at least two receptor types in the brain [7]. With the use of new synthetic steroids [8, 9] binding properties and localization of these receptor types were evaluated. The binding studies showed that corticosterone could bind to a high affinity (Kd ca. 0.3 nM) site and to a site with approximately 10-fold lower affinity [7]. The high affinity site also effectively bound aldosterone and mineralocorticoid antagonists such as spironolactone and RU 28318;
binding affinity for the synthetic glucocorticoid antagonist RU 38486 was negligible. The properties of this brain receptor type therefore closely resembled those of the peripheral mineralocorticoid receptor (MR) in the kidney; however, in contrast to the latter, the high affinity brain corticoid receptor bound corticosterone equally well as aldosterone [10]. Due to this difference, the high affinity brain corticosteroid receptor was at that time named the type I (corticosterone-selective) receptor type. This high affinity receptor was found to be mainly localized in limbic structures, e.g. in the septal region and hippocampus. The receptor population with lower affinity for corticosterone displayed negligible affinity for aldosterone and RU 28318, but high affinity for the glucocorticoid analogues dexamethasone, RU 38486 and RU 28362. The binding profile thus was similar to the glucocorticoid receptor in peripheral organs such as the liver. This lower affinity site in the brain was designated as the type II receptor. In contrast to the high affinity receptor it was found to be quite widespread in its distribution.

A few years later, the mineralo- and glucocorticoid receptors were cloned and their primary structure was resolved [11, 12]. It appeared that the protein for the brain type I and the peripheral MR were identical. Similarly, the type II receptor in the brain and the glucocorticoid receptor (GR) in peripheral organs displayed the same structure. In the following we will therefore refer to the type I receptors as MRs and to the type II receptors as GRs. The apparent different binding properties of the peripheral and central MR may be due to the presence/absence of other binding proteins or tissue-specific enzymes. It was shown that one of the mechanisms yielding aldosterone preference to the MR in the kidney is linked to the presence of the enzyme 11β-OH-steroid dehydrogenase [13, 14]. This enzyme converts corticosterone to a metabolite (11-dehydrocorticosterone) with much lower affinity for the MR. Consequently, the MR in the kidney will be preferably occupied by aldosterone, notwithstanding the more than 100-fold excess of circulating corticosterone over aldosterone. The biological activity of this enzyme in hippocampus, in vivo, seems to be very low. If the enzyme is indeed less active in the brain than in the kidney, this may partly explain why brain MRs in the hippocampus bind corticosterone and aldosterone equally well.

In the original study of McEwen et al., in which they showed that [3H]corticosterone binds to receptors in the brain, particularly in the hippocampus [5], the dose of the tracer was very low. This amount of steroid was later found to occupy the high affinity MRs rather than the GRs [7]. Were the effects of corticosterone on neuronal firing shown by Pfaff et al. mediated by these MRs? That was not clear, since the dose used in the electrophysiological study was such that not only MRs but also GRs were activated [6]. Clearly, a new question emerged: How do corticosteroids, via specific activation of MRs and GRs, affect the most unique feature of neurons, i.e. the propagation and integration of electrical signals?

**METHODOLOGICAL CONSIDERATIONS**

In our experimental approach to this question, three aspects of steroid action were taken into consideration [15].

First, corticosteroid hormones act on the genome [16]. Thus, binding of the steroid to the receptor results in activation of the steroid receptor complex and nuclear translocation. The steroid receptor complex binds to hormone responsive elements in the DNA and subsequently acts as a transcription factor. Transcription and translation will lead to an altered cellular protein content. The steroid-sensitive proteins may be involved in various aspects of signal transduction, e.g. properties of voltage-gated ion channels or the sequence of events leading to a neurotransmitter response. Due to this genomic mechanism of action it is to be expected that the steroid modulation of neuronal excitability will be slow in onset and long-lasting. The experimental protocol was chosen such that these slow but persistent actions could be adequately monitored (see Fig. 1). Thus, we recorded neuronal properties of neurons before steroid application and compared these with the characteristics observed in neurons recorded 1-4 h after a brief steroid application *in vitro* (see also below). In some cases, we monitored the properties of a single neuron during this entire 4 h period.

Second, steroid actions have often been described as conditional, meaning that they only become apparent if the system is shifted from its set-point [17]. This may also be true for putative steroid effects on neuronal activity. If so, one may expect to see steroid effects particularly when the membrane properties of the neuron are driven from their resting level. This can be best studied when there is a good voltage control over the neuronal membrane, a situation that can be optimally realized with voltage clamp recording. However, recording of neuronal membrane properties, particularly under voltage clamp conditions, is not feasible *in vivo*, due to the pulsatory movement of the brain. We therefore selected an *in vitro* brain preparation, i.e. the hippocampal slice, of which at least part of the synaptic circuitry is intact; in preliminary studies we found that under our recording conditions we still observed corticosterone binding in the slice.

Third, in order to study MR- and GR-mediated events separately, we should be able to occupy these receptor subtypes selectively. Most of the experiments were performed in rats that were adrenalectomized (ADX) ca 7 days before the experiment. At the day of the experiment, steroids were applied *in vitro*, for 20 min at 32°C (Fig. 1). The action of antagonists was tested by applying the antagonist first for 20 min alone, then for 20 min together with the agonist and in most cases for another 20 min after the agonist had been
washed out. Selective MR occupation was induced by a low dose of corticosterone (1 nM), by aldosterone (3 nM) or by corticosterone in the presence of a GR-antagonist [7]. Spironolactone was used as an MR-antagonist. GR occupation was induced with the very selective GR-analogue RU 28362. Occupation of both MRs and GRs was achieved by using high (>~ 30 nM) doses of corticosterone [7]. In all series of experiments we always included a sham-operated control group. Since all animals were exposed to a novel environment just before the preparation of the brain slices, plasma steroid levels in the sham-operated rats were relatively high (ca 10 µg/100 ml plasma).

The advantage of this approach is, evidently, that one can monitor the effect of selective steroid receptor occupation on electrical properties of a single neuron, under very stable and controlled recording conditions. However, it should be emphasized that the data, in terms of functional implications for brain activity in vivo, should be carefully interpreted. This is (amongst other things) related to (1) the fact that neuronal activity in vitro represents only one (fixed) level of activity of the wide range of neuronal conditions that can occur in vivo; (2) the fact that ADX rats may not merely be regarded as animals in which the MRs and GRs are free from the endogenous ligand, but also as animals which have developed adaptational changes during the 1-week period following the surgery; and (3) the fact that in vitro conditions may determine the degree to which steroid effects develop, due to the restraint of fuels and building blocks required for the genomic actions. Notwithstanding these limitations, we observed a fairly consistent array of steroid-mediated influences on neuronal activity, using the experimental protocol described above.

RESULTS

In current clamp recordings, we found that corticosteroids do not affect resting membrane potential or input resistance of CA1 pyramidal neurons [18]. However, when the membrane potential is removed from its resting level, steroid actions become apparent.

Under voltage clamp conditions, using patch electrodes in hippocampal slices, calcium (Ca) currents were evoked by short depolarizing voltage steps. The total Ca-current comprised (at least) a transient current, which activated around -70 mV and peaked around -40 mV, and a sustained Ca-current, which activated around -30 mV and peaked around -10 mV. These two currents may correspond to the T-type and L-type Ca-currents, respectively, described in other preparations before. When compared to the Ca-currents recorded in the sham-operated control group, Ca-currents in tissue from ADX rats were rather large [19] (see Fig. 2). In particular, the T-type Ca-current was significantly enhanced after ADX.

In vitro occupation of the MRs greatly reduced both the T- and L-type Ca-currents, when compared to the untreated ADX group or the sham controls. Voltage dependency and kinetics of the Ca-currents were not affected by the hormone treatment. Concurrent occupation of MRs and GRs in vitro resulted in Ca-current amplitudes that were comparable to those observed in the sham-operated controls. This GR-linked restoration of the Ca-conductance may depend on activation of MRs, since only high [20] but not low

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**Fig. 1.** Timing protocol for in vitro evaluation of steroid-mediated actions. At the start of the experiment all rats are subjected to a novelty stress for ca 30 min. After decapitation, slices are prepared (4°C) and kept in a holding chamber, at room temperature; trunk blood is evaluated for corticosterone levels with a radioimmunoassay. One slice at a time is transferred to the recording chamber (32°C). Neuronal properties are recorded after an equilibration period of at least 1.5 h. Next, steroids are applied for 20 min in vitro, via the perfusion medium. After 1-4 h, properties of neurons in the same slice are recorded and compared with those observed before steroid application.
doses of the selective GR-agonist RU 28362 were able to induce large Ca-currents.

While this series of experiments focussed on steroid-mediated modulation of the Ca-influx in CA1 neurons rather than overall steroid modulation of Ca-homeostasis, the effects described above may, nevertheless, have implications for Ca-dependent phenomena. Thus, at a short time scale, reduced Ca-influx may result in a depression of Ca-dependent conductances, e.g. the Ca-dependent K-conductance. Indeed, it has been observed that conditions of predominant MR occupation result in a small accommodation and afterhyperpolarization of CA1 neurons, two phenomena which are linked to the slow Ca-dependent K-conductance [21]; additional GR occupation is associated with a large accommodation/afterhyperpolarization [18, 22, 23]. However, it should be noted that effects of steroids on this conductance, independent of modulatory events on the Ca-influx, may also occur. At a longer time scale, steroid-induced actions on Ca-influx may have more severe implications. Thus, it is widely accepted that large increases in intracellular Ca-level may, over time, result in delayed cell death [24, 25]. It is conceivable that the GR-linked increase of Ca-currents, particularly when combined with other demanding cellular events such as ischemia, could over time enhance the vulnerability of hippocampal neurons [26].

Other K-conductances that we tested appeared to be less sensitive to the pattern of steroid receptor occupation [27]. The transient outward K-conductance \( I_A \) and the delayed rectifier, evoked in hippocampal CA1 neurons by depolarizing steps, were not significantly affected by steroid treatment. Yet, one voltage-dependent K-conductance, i.e. the inwardly rectifying \( I_{\text{f}} \), did show sensitivity to steroid treatment. Thus, the \( I_{\text{f}} \) amplitude was small with predominant MR activation, whereas simultaneous activation of MRs and GRs was associated with a much larger \( I_{\text{f}} \) amplitude, comparable to the current evoked in sham-operated control tissue. As with the Ca-current, the \( I_{\text{f}} \) was only large when both MRs and GRs were activated: selective activation of GRs did not result in a large \( I_{\text{f}} \) amplitude. Since the \( I_{\text{f}} \) is only activated at relatively negative membrane potentials (< -80 mV), the functional significance of steroid modulation may be limited.

The main stream of input to the CA1 area is carried by the glutamatergic Schaffer pathway [28]. In addition, both feed forward and feedback inhibition via GABAergic interneurons are (indirectly) activated by stimulation of the Schaffer afferents. As a result electrical stimulation of the Schaffer fibers will evoke a glutamate-induced excitatory postsynaptic potential (EPSP), followed by a \( \text{GABA}_A \) receptor-mediated fast inhibitory postsynaptic potential (fIPSP) and a slower \( \text{GABA}_B \) receptor-dependent IPSP (sIPSP). With strong stimulus intensities, an action potential can be superimposed on the EPSP. With intracellular current clamp recording we observed that there were no differences for this sequence of synaptic responses between
tissue of ADX animals and the adrenally intact controls [29]. However, with repeated stimulation, neurons in slices from ADX rats gradually failed to transmit the signal: the probability of evoking an action potential decreased and the amplitude of the sIPSP (but not fIPSP) diminished over time (see Fig. 2). Brief in vitro exposure to the mineralocorticoid aldosterone prevented this gradual failure. By contrast, treatment with the mixed MR/GR ligand corticosterone was not able to relieve the tissue from gradual synaptic attenuation: the probability of evoking an action potential and the amplitude of the sIPSP decreased with repeated stimulation and, in addition, the amplitude of the EPSP was significantly reduced over time.

Extracellularly, stimulation of the Schaffer fibers evokes, in the CA1 pyramidal cell layer, a field population spike superimposed on a field EPSP. The field EPSP is a reflection of the EPSP amplitudes induced by stimulation in a large population of CA1 neurons; the population spike is a measure for the proportion of CA1 neurons which responds to stimulation with an action potential. The influence of steroid receptor activation on the synaptically evoked field responses resembled the pattern observed with intracellular recording. Thus, in untreated tissue from ADX rats repeated stimulation of the Schaffer afferents resulted in a gradual decline of the field population spike but not the field EPSP [30]. Low doses of corticosterone were able to prevent this decline, but with high doses of corticosterone a decline of both the field EPSP and the population spike amplitude was observed over time. From the intracellular and extracellular data we conclude that predominant occupation of MRs is an optimal condition for maintenance of the synaptic transmission in the CA1 area, while both in the absence of steroids and after exposure to high steroid levels (MRs and GRs simultaneously occupied), transmittal of synaptic input fails with repeated stimulation.

Apart from the amino acid-carried input, CA1 pyramidal neurons also receive modulatory inputs via, e.g., serotonin (5-HT), acetylcholine and noradrenaline [28]. In CA1 neurons, the most apparent effect evoked by 5-HT concerns a large hyperpolarization which is mediated via 5-HT$_{1a}$ receptors [21]. Carbachol, a metabolically stable cholinergic analogue, induces (1) a depolarization of the membrane, (2) a reduction of the cellular afterhyperpolarization and accommodation and (3) a depression of the synaptic responses, probably via a presynaptic mechanism; all of these responses are mediated by muscarinic receptors [21]. It appeared that in the absence of steroids (ADX rats), the 5-HT$_{1a}$ receptor-mediated hyperpolarization [31] and the depolarization evoked by carbachol [32] were not significantly different from the responses observed in tissue from the sham-operated control group (see Fig. 2).

However, with a predominant MR occupation induced with 3 nM aldosterone, responses to 5-HT and carbachol were markedly reduced. Concurrent activation of MRs and GRs with 30 nM corticosterone was associated with relatively large responses to 5-HT and carbachol. Other membrane effects of serotonin and carbachol were not sensitive to corticosteroid treatment.

Noradrenaline can bind to a number of receptor subtypes in CA1 neurons. Binding to the $\beta_1$-adrenergic sites results in a small depolarization and, most notably, in a reduction of depolarization-induced accommodation and afterhyperpolarization [21]. It appeared that in tissue from ADX rats the $\beta_1$ receptor-mediated responses were large when compared to the tissue from the adrenally intact controls [18]. Treatment of ADX slices with high doses of corticosterone or with a selective GR-agonist significantly reduced the noradrenergic responses. This was also reflected in the noradrenergic modulation of the amino acid-carried synaptic input to the CA1 area [33]. Thus, noradrenaline via its effect on $\beta_1$ receptors enhances the amplitude of the synaptically induced field population spike. The noradrenergic effect on the amplitude of the population spike was found to be enhanced in slices from ADX rats, when compared to tissue from either sham-operated controls or to slices from ADX rats treated with a high dose of corticosterone.

**DISCUSSION**

One of the most striking findings of our studies was that MR-mediated effects on neuronal activity are in most cases different from and often opposite to GR-mediated events. The knowledge about the dependency of MR/GR occupation on corticosterone levels [7] and the use of selective MR- and/or GR ligands [8, 9], both of which became available in the mid-80s, has helped tremendously to differentiate between the effects on electrical properties mediated via MRs and via GRs; with this knowledge it also became possible to interpret the findings in earlier studies. For instance, the depression of hippocampal firing observed in the early paper by Pfaff et al. [6] was probably due to concurrent activation of MRs and GRs and thus not only due to occupation of the (mineralocorticoid) receptors demonstrated by McEwen a few years earlier [5].

From our studies a general picture of corticosteroid actions on neuronal properties emerged [34, 35]. We found that predominant occupation of MRs is associated with a stable flow of amino acid-carried fast synaptic transmission; the depolarizations evoked by synaptic stimulation are, however, not accompanied by a large Ca-influx. Modulatory inputs carried by biogenic amines are reduced. When GRs are occupied in addition to MRs an almost opposite pattern can be observed: the fast amino acid-mediated synaptic flow is initially not different from the situation observed with predominant MR activation, but the neuronal responsiveness starts to decline with repeated stimulation. The synaptic depolarization is linked to large
Ca-influxes. With limited exposure to high corticosteroid levels, the relatively large Ca-signals may help to attenuate the synaptic response through activation of the slow Ca-dependent K-conductance. The responses to biogenic amines are in general large, with the exception of the responses mediated via β1 receptors.

The shift from predominant MR occupation to concurrent MR/GR activation is physiologically relevant. Thus, at the beginning of the inactive period (under rest) corticosterone levels are very low; yet, these levels are sufficient to activate a considerable degree of the MRs, but not GRs [36]. When corticosteroid levels in the blood rise, due to circadian rhythmicity or after stress, GRs become occupied in addition to MRs [36, 37]. Based on our findings we predict that ionic conductances and transmitter responsiveness may therefore vary during the day and after stress, via a coordinated MR- and GR-mediated process.

The data also indicate that more extreme shifts in the steroid receptor occupation, such as they may occur during chronic absence of or over-exposure to corticosteroids, could also have marked consequences for cellular excitability. Quite unexpectedly we found that in the absence of corticosterone, ionic conductances and transmitter responsiveness resembled the situation of combined MR/GR occupation. This could indicate that under these conditions, too, maintenance of stable transmission in the CA1 area is not guaranteed. These effects of ADX on neuronal activity in the CA1 area imply that the dose dependency of cellular events on corticosterone concentrations is U-shaped (see Fig. 3).

A comparable dose dependency has also been shown with respect to steroid actions on LTP, in the CA1 area and dentate gyrus [38, 39].

The regulation by steroids, particularly of Ca-currents, may have long-lasting consequences, under the above-mentioned conditions of chronic absence of or over-exposure to corticosteroid hormones. In the absence of corticosterone (ADX), Ca-currents are very large. This regards in particular the low threshold activated Ca-current. We have shown that this current is at least partly generated in the dendrites [40]. Small depolarizations may thus give rise to considerably high local Ca-levels in the dendrites of CA1 neurons. Interestingly, CA1 neurons do not display morphological changes (e.g. in their dendrites) after prolonged periods of ADX [41, 42]. By contrast, granule neurons in the dentate gyrus, which also possess high levels of MRs and GRs are known to become pycnotic after ADX [41, 42]. It is conceivable that comparable modulation of Ca-currents by corticosteroids in dentate granule cells may contribute to this process of degeneration. The difference between CA1 and dentate granule cells in their sensitivity to steroid-dependent neuronal degeneration is presently unclear, but could be due to a difference in the whole array of neuronal properties which protect neurons against over-excitation, such as the properties of the inhibitory networks and of the (Ca-dependent) K-conductances.

![Fig. 3. Schematic representation of the relative ionic conductances and transmitter responses of CA1 hippocampal neurons in vitro, as a function of the MR/GR occupation ratio. The effects are expressed as a percentage of the maximal response. With predominant MR occupation, the conductances and transmitter responses are generally small, and synaptic transmission is optimally maintained (stable circuit); with simultaneous MR and GR occupation the responses are relatively large, while synaptic transmission fails with repeated stimulation (unstable). In most cases, the membrane responses are also quite large in the absence of steroids, implying a U-shaped dose-response relationship.](image-url)
High corticosterone levels also result in large Ca-currents, particularly of the high threshold type. The latter evoke large Ca-influxes during prolonged and strong depolarizations. Prolonged depolarizations, as occur during ischemic and epileptic insults, may therefore result in very large Ca-signals when taking place under conditions of high corticosteroid levels. This could explain why corticosteroids, and more specifically GR activation, can become endangering during these prolonged excitatory events, as was found by Sapolsky [26]. In this situation, too, the sensitivity of neurons to steroids in the process of degeneration should be regarded in conjunction with other neuronal properties, e.g. the characteristics of the local excitatory and inhibitory networks and the localization of ion channels.

In conclusion, at this moment it is clear that corticosteroid hormones exert slow and persistent actions on electrical properties of neurons. The overall steroid effect will be partly determined by the relative activation of brain MRs and GRs; transactivational interactions with other transcription factors are also important for the net result of steroids on neuronal firing [43, 44]. The coordinated control via MRs and GRs over neuronal excitability adds an essentially new regulatory mechanism of brain function to the communicatory pathways mediated by "classical" transmitters: the steroids represent a humoral response to environmental challenges, which reaches the brain. At those sites where receptors are present, the hormone exerts a gene-mediated, persistent control over transmission of electrical signals. The challenge for the future will be to validate these extrapolations from in vitro to in vivo conditions and to determine how (patho) physiological fluctuations in circulating steroid levels affect the function of brain circuitry and, thus, of behavioral patterns.

REFERENCES


