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Research Report

Corticosteroid receptor-dependent modulation of calcium currents in rat hippocampal CA1 neurons

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Abstract

Pyramidal CA1 neurons in the rat hippocampus contain mineralocorticoid (MRs) and glucocorticoid receptors (GRs) for corticosterone, which, in activated form, act as transcription factors of the genome. The relative MR and GR occupation changes throughout the day, with predominant MR occupation under rest in the morning and additional GR occupation in the evening and after stress. We examined the effect of MR and GR activation on Ca currents in hippocampal slices from adrenalectomized (ADX) rats under whole-cell voltage-clamp conditions. In slices from ADX rats, where MRs and GRs are unoccupied, Ca currents (particularly in the low-voltage range) were larger than in neurons from the sham-operated controls; these effects became apparent with a delay of ≥ 3 days after ADX. Selective occupation of MRs in tissue from ADX rats greatly (by 70%) and persistently (up to 3 h) reduced transient but also sustained Ca conductances. Voltage dependency and kinetic properties of the currents were not affected. Occupation of GRs as well as MRs by corticosterone (30 nM) resulted in relatively large Ca currents, comparable to those recorded in tissue from mildly stressed sham-operated control animals. Interestingly, exclusive occupation of GRs with 30 nM RU 28362 was not sufficient to induce large Ca currents. The data suggest that the changes in MR and GR occupation throughout the day, related to circadian and stress-induced corticosterone release, are linked to marked alterations in Ca currents, with small Ca currents in the morning and large currents in the evening or after stress. Corticosteroid hormones may, thus, exert a persistent gene-mediated control over Ca conductances in the brain, affecting the excitability of neurons.

Key words: Corticosterone; Mineralocorticoid receptor; Glucocorticoid receptor; Calcium current; Hippocampus; CA1 neuron; In situ patch clamp; Rat

1. Introduction

The rat adrenocortical hormone corticosterone is released in a circadian rhythm with low plasma levels in the morning, rising to high levels in the evening [7]; the levels are also high during periods of stress. The hormone passes the blood–brain barrier and binds to intracellular corticosteroid receptors in the brain [31,32]. Within the brain, at least two corticosteroid receptor subtypes can be distinguished: the high-affinity mineralocorticoid receptor (MR; $K_d \sim 0.5$ nM) and the glucocorticoid receptor (GR) with a ~ 10 -fold lower affinity for corticosterone [8,32,40]. Hippocampal CA1

pyramidal cells express both MRs and GRs [1,2,5,12,50]. The MRs and GRs will be differentially occupied, depending on the circulating level of the hormone [41]. Thus, with low corticosterone levels, as occurs during rest at the trough of the circadian cycle, mainly MRs are occupied, while GRs will become fully occupied at the peak of the cycle and particularly during periods of stress. After binding of the steroid to the intracellular receptor, the activated steroid–receptor complex affects DNA transcription and consequently protein synthesis [3]. Due to the genomic pathway, the resulting steroid-induced cellular effects are slow in onset and long-lasting.

Previous studies have shown that corticosteroids affect several intrinsic membrane properties of CA1 neurons, including a low Ca-dependent K conductance [16,17,20,24,25]. Activation of this Ca-dependent K

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current attenuates the generation of action potentials during a steady depolarizing input (accommodation) and results in a transient afterhyperpolarization at the end of the depolarization (AHP) [11,13,26,29]. It appeared that predominant MR occupation results in a small AHP amplitude and weak accommodation [17] while additional or exclusive GR occupation is linked to a large AHP amplitude and strong accommodation [16,17,24]. Considering the strong Ca dependency of this K conductance [26,27], we wondered if the steroid-mediated changes in accommodation/AHP are secondary to alterations in voltage-dependent Ca conductances. Recent data indeed support the idea that GR-dependent effects in the hippocampus involve alterations in Ca influx [25,48]. A long-lasting gene-mediated control of Ca influx by corticosteroid hormones would not only affect the Ca-dependent K conductance but potentially also the excitability and viability of the neurons.

In the present study, we employed the *in situ* patch-clamp technique in hippocampal slices [9] to record Ca conductances of CA1 neurons. This method was selected since (1) the use of the slice preparation allows a comparison with earlier steroid-mediated effects recorded under identical experimental conditions [16,17,24,25]; and (2) since the voltage control achieved with the whole-cell recording configuration is better than with the switch-clamp technique with microelectrodes [e.g. 30]. We recorded Ca currents in slices from adrenalectomized (ADX) rats and compared these with the currents in tissue from adrenally intact controls. In slices from ADX rats, we next examined the Ca currents under conditions of (1) predominant MR occupation, (2) simultaneous MR and GR activation or (3) exclusive GR activation. This design allows a prediction about changes in Ca conductances during the physiologically relevant transition from predominant MR to concurrent MR and GR activation.

2. Materials and methods

The experiments were carried out in male Wistar rats (100–170 g). 1–7 days before the experiments, the rats were quickly ADX or sham-operated under ether anaesthesia as described elsewhere [38]. The animals were housed in an animal room with alternating light/dark (08:00–20:00/20:00–08:00) cycle and received food and water (sham) or saline (after ADX) *ad libitum*. Before decapitation, the rats were placed in a novel environment (clean cage) for 30–60 min. After decapitation under ether anaesthesia, trunk blood was collected for measurement of plasma corticosterone levels with a RIA. Animals were considered to be effectively ADX when the corticosterone levels were $< 1 \mu\text{g}/100 \text{ ml}$ plasma. Sham-operated rats were, due to the experimental procedure, mildly stressed and, therefore, had probably most of their MRs and a considerable degree of the GRs activated at the moment of decapitation [41]. The brain was quickly removed from the skull and dipped in ice-cold

carbogenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid (ACSF, in mM: 124 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 1.5 MgSO₄, 2 CaCl₂, 25 NaHCO₃ and 10 glucose; pH 7.4, ~300 mOsm). The hippocampus was dissected and transverse slices (120 μm thick) were cut on a tissue chopper (McIlwain). The slices were stored at room temperature in a holding chamber. After an equilibration period of at least 1 h, one slice at a time was transferred to the recording chamber, submerged and continuously perfused (2 ml/min) with warm ($32 \pm 1^\circ\text{C}$) carbogenated ACSF.

Steroid-receptor activation in slices from ADX rats was achieved by perfusion of specific steroid analogues *in vitro* for 20 min at 32°C , as described earlier [17,20]. MRs were activated by perfusion of the mixed MR and GR agonist 30 nM corticosterone in the presence of the GR antagonist [36] RU 38486 (500 nM); to ensure the effectiveness of the antagonist, RU 38486 application was started 20 min before the corticosterone treatment. GRs alone were activated by the selective GR agonist [37] RU 28362 (30 nM). Activation of both MRs and GRs was established with 30 nM of the mixed agonist corticosterone [40]. Neurons were recorded with a delay of 0.5–3 h after steroid application was terminated, to allow enough time for the induction of gene-mediated actions. All steroids were dissolved as a 1-mM stock solution in 90% ethanol at the beginning of the experiment and kept in the freezer. Dilution to the final concentration was performed just before testing. Final ethanol concentrations used for the steroid-agonist applications were $< 0.003\%$. Corticosterone was obtained from Organon (Oss, The Netherlands); RU 28362 and RU 38486 were from Roussel-Uclaf (Romainville, France).

Hippocampal pyramidal CA1 neurons could be distinguished and selected for recording with a light microscope (Nikon 104), using a magnification of $400\times$, i.e., $40\times$ water-immersion objective and $10\times$ ocular. With a patch pipette (pulled on a Mecnex BBCH or Sutter micropipette puller from 1.5-mm o.d. borosilicate glass; 1.5–3.0 M Ω) the selected cell was approached. Positive pressure ensured that the tip of the electrode was kept clean and that the surface of the membrane was freed from surrounding neuropil [9]. When the tip of the electrode was placed on the membrane, a giga seal could be established by application of light suction. Subsequently, the whole-cell configuration was achieved by additional suction. The pipette solution consisted of (in mM): 100 CsF, 0.5 CaCl₂, 2 MgCl₂, 2 MgATP, 0.1 NaGTP, 10 hepes, 10 EGTA, 20 creatinephosphate, 50 U/ml creatinephosphokinase, 0.1 albumine and 20 TEACl; pH 7.4, 300 mOsm. The solution was frozen and kept on ice during the day of the experiment.

Whole-cell currents were measured under voltage-clamp conditions using a Biologic RK 300 amplifier. Data was collected with an Atari PC, at 5-kHz sampling rate [cf. 51]. Voltage-step protocols were generated by the acquisition program. Each cell was subjected to the same sequence of timed voltage protocols. Activation and inactivation properties of the Ca currents were recorded once every 5 min. Typically, the Ca-current amplitude increased over the first 5-min period after breaking into the cell. The current reached a maximum after 10 min and remained stable over the next 10 min; a gradual decline was observed thereafter. For reasons of comparison between experimental groups, we standardly analysed the currents recorded 10 min after establishing the whole-cell configuration. Access resistance compensation was not applied; considering the electrode resistance ($\sim 2 \text{ M}\Omega$) and the relatively small currents ($< 1 \text{ nA}$), the effect on voltage characteristics will only be limited ($< 4 \text{ mV}$ offset). Correction for linear leak-current, as estimated from sequential depolarizing and hyperpolarizing voltage steps of 5 mV and 50-ms duration at holding potential, was performed. All experiments were carried out in the presence of 0.5–1.0 μM tetrodotoxin to block Na currents; K conductances were blocked by extracellular addition of tetraethylammonium HCl (10 mM), 4-aminopyridine (5 mM) and 5 mM CsCl and by Cs in the patch pipette.

Statistical analysis of the differences between the various experimental groups was performed by an one-way ANOVA ($P < 0.05$),

followed by a posthoc unpaired Student's *t* test. No more than two neurons were recorded per animal.

3. Results

3.1. Properties of Ca currents in adrenally intact rats

Ca currents were activated in CA1 neurons by a 200-ms depolarizing potential, preceded by a 3-s hyperpolarization at -130 mV. The depolarizations ranged

from -100 to 0 mV, with an increment of 10 mV (Fig. 1A, inset; interval between successive sweeps 10 s). Holding potential was at -65 mV.

The total Ca current ($I_{Ca,tot}$) in neurons from adrenally intact rats (sham) displayed a transient and a sustained component (Fig. 1A, upper panel). The $I-V$ relationship of the peak amplitude of $I_{Ca,tot}$ (Fig. 1B) revealed a small current, activated around -80 , which increased considerably around -50 mV. The maximal amplitude for the $I_{Ca,tot}$ was observed at -30 mV and amounted to 870 ± 233 pA (mean \pm S.E.M., $n = 9$).

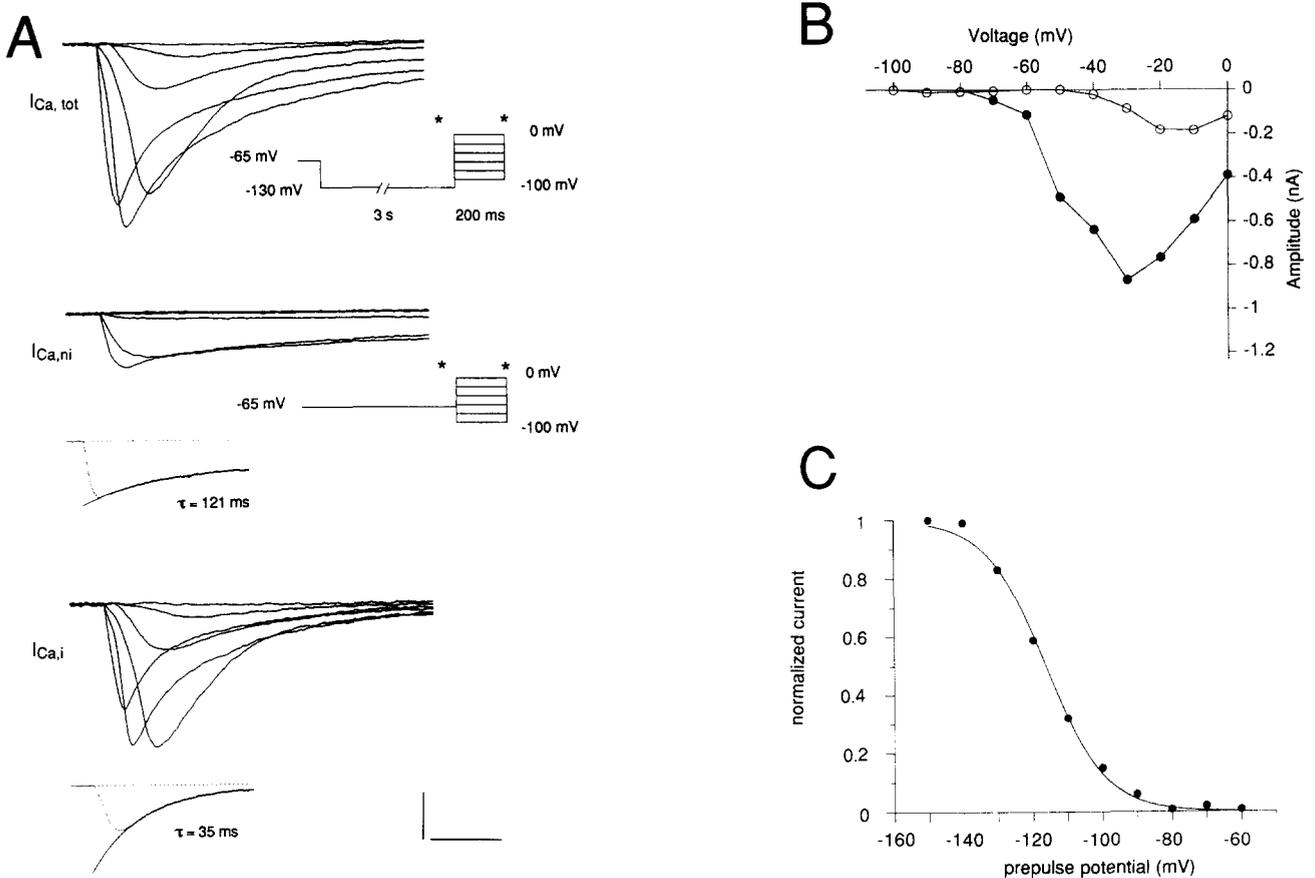


Fig. 1. A, upper panel: representative Ca currents ($I_{Ca,tot}$) activated in a CA1 neuron by 200-ms depolarizing command potentials from a 3-s prepulse at -130 mV, according to the voltage protocol as depicted in the inset; asterisks mark the part of the protocol for which the current traces are shown. The picture shows currents activated at -100 , -80 , -60 , -40 , -20 and 0 mV, as recorded with the in situ patch-clamp technique. Holding potential was -65 mV, the interval between successive depolarizations was fixed at 10 s. middle panel. In the same neuron, Ca currents were evoked by command potentials directly from the holding potential, at -65 mV (see voltage protocol). A sustained Ca current ($I_{Ca,ni}$) can be seen, which activates around -40 mV and is maximal around -10 mV. The inset below depicts a current evoked at -10 mV, fitted with a single exponential function. A, lower panel: the current traces shown here were obtained by subtraction of the traces obtained in the middle panel from the total Ca currents as evoked in the upper panel. This yielded the Ca current with a voltage-dependent inactivation ($I_{Ca,i}$). For the subtraction protocol, the voltage steps were alternately applied from -130 and -65 mV. Inset below shows subtracted current obtained by a voltage step to -60 mV, which was used for the calculation of the time constant for the decay. Calibration bar for the currents: vertical, 0.3 nA; horizontal, 40 ms, and 80 ms for the insets. B: $I-V$ relationship for the total Ca currents in the adrenally intact group ($I_{Ca,tot}$, \bullet , $n = 8$), as evoked with the protocol depicted in A (upper panel), and the high-threshold current ($I_{Ca,ni}$, \circ), as evoked by the voltage protocol depicted in A (middle panel). Represented are the mean values of the peak amplitudes recorded for each current trace in the individual cells. C: voltage-dependent inactivation of $I_{Ca,tot}$ was calculated from currents evoked by a command potential to -40 mV, with prepulses of variable potentials (-150 to -60 mV, in steps of 10 mV). The values of the peak amplitudes for each current trace, normalized to maximal value, as a function of membrane potential, were fitted with a Boltzmann equation (solid line). The curve shows that the conductance responsible for the transient phase (see e.g. upper panel in A) is completely inactivated at -65 mV, i.e., when the command potential is directly applied from the holding potential.

The $I_{Ca,tot}$ showed a voltage-dependent inactivation, which was measured by varying the potential of the 3-s hyperpolarizing prepulse. The voltage dependency of the steady-state inactivation could be described with the Boltzmann equation, $g(V)/g_{max} = 1/[1 + \exp\{(V - V_H)/V_C\}]$, where $g(V)/g_{max}$ is the relative conductance at membrane potential V , V_H is the voltage of half-maximal inactivation and V_C is the slope factor of the curve. For the sham group, the V_H was -117 ± 2 mV (Fig. 1C).

Due to inactivation, the transient component of $I_{Ca,tot}$ was absent when the depolarizing step was applied from -65 mV, i.e., directly from the holding potential (Fig. 1C). However, depolarization from -65 mV did reveal a high-threshold current, which activated around -40 mV and reached a maximum amplitude at -10 mV (195 ± 71 pA; Fig. 1A, middle panel; Fig. 1B). This current displayed only little voltage-dependent inactivation ($I_{Ca,ni}$). The decaying phase was

fitted by a single exponential function (Fig. 1A, inset of middle panel), with a mean time constant (τ_{ni}) at -10 mV of 121 ± 25 ms ($n = 9$), for the group of adrenally intact controls.

The presence of the high-threshold Ca current $I_{Ca,ni}$ implies that the $I_{Ca,tot}$ activated by steps from -130 mV to voltages more positive than -40 mV comprises at least two different components: the high-threshold $I_{Ca,ni}$ and a low-threshold Ca current that shows a marked voltage-dependent inactivation ($I_{Ca,i}$). The $I_{Ca,i}$ was separated from $I_{Ca,ni}$ by subtracting $I_{Ca,ni}$ from the total Ca current $I_{Ca,tot}$ (see example in Fig. 1a, lower panel). The voltage-dependent properties of the peak of $I_{Ca,i}$ resembled those of $I_{Ca,tot}$ although, as expected, the $I-V$ relationship for the former was steeper between -40 and 0 mV. The maximum current (820 ± 209 pA) was observed at -30 mV. The time constant for the decay (τ_i) at -60 mV was 35 ± 8 ms (Fig. 1A, inset of lower panel).

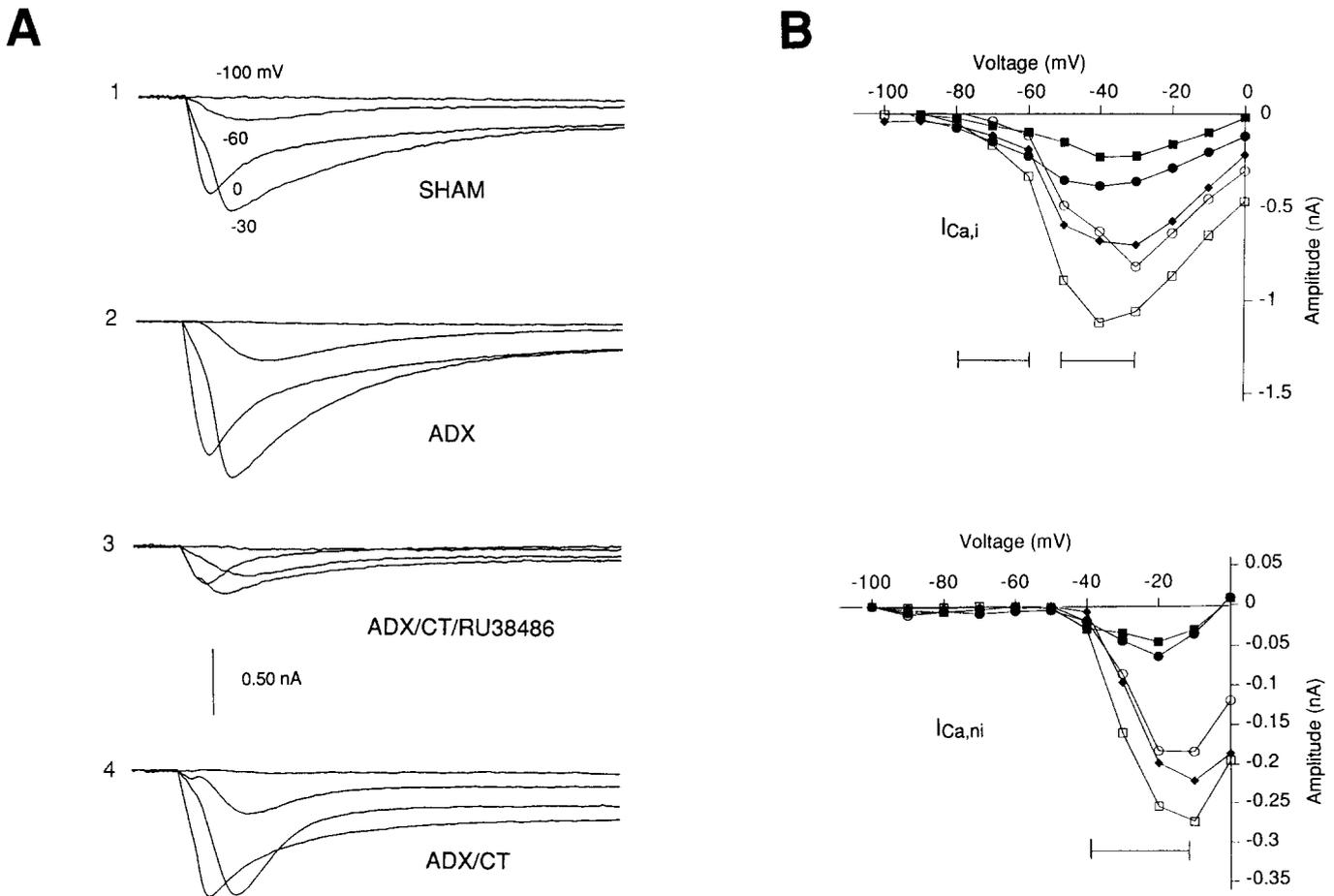


Fig. 2. A: typical Ca currents ($I_{Ca,tot}$, protocol as depicted in Fig. 1A, upper panel) activated in a CA1 neuron in slices from a sham-operated rat (1); in slices from a rat 7 days after ADX (2); in slices from ADX rats treated with 30 nM corticosterone in the presence of the GR antagonist RU 38486, thus, occupying predominantly MRs (3); and in slices from ADX rats treated with 30 nM corticosterone only, thus, occupying both MRs and GRs (4). Only currents evoked by command steps to -100 , -60 , -30 and 0 mV are shown. B: $I-V$ relationship for the peak amplitudes of $I_{Ca,i}$ (upper panel) and $I_{Ca,ni}$ (lower panel), in neurons from sham-operated rats ($n = 9$ cells, \circ), from ADX rats ($n = 18$ cells, \square), slices from ADX rats treated with corticosterone/RU 38486 ($n = 7$ cells, \blacksquare), ADX slices treated with corticosterone ($n = 6$ cells, \blacklozenge) and ADX slices treated with the GR agonist RU 28362 ($n = 8$ cells, \bullet).

3.2. Effect of adrenalectomy on Ca currents

Ca currents in neurons from ADX rats tended to be larger than in the sham-operated controls (Fig. 2A). As shown in Fig. 2B, both the $I_{Ca,i}$ and $I_{Ca,ni}$ were, on average, over the whole voltage range larger for the ADX group than for the sham controls. For a further comparison, we evaluated currents in three different voltage ranges. (1) The voltage range of $I_{Ca,i}$ between -80 and -60 mV, where a small conductance was activated; in this range, the Ca currents evoked in neurons from ADX rats were significantly larger than in the adrenalectomized controls (Fig. 3, upper panel). (2) The voltage range of $I_{Ca,i}$ between -50 and -30 mV, where the activated conductance was large; although the Ca currents for the ADX group were, on average, larger than the controls, the difference did not attain significance. (3) The voltage range of $I_{Ca,ni}$ between -40 and -10 mV, which is indicative of the high-threshold Ca current; in this voltage range, the Ca currents for the ADX were also not different from the values in the sham controls. Other properties of the cells and of the Ca currents, such as the input resistance R_{in} , V_H for steady-state inactivation and τ_i , were not significantly different between the ADX and the sham control group (Table 1). The τ_{ni} for the ADX group was, on average, much smaller than in the sham-operated controls although the difference did not attain statistical significance.

The above-mentioned data were obtained from rats that were ADX 1–8 days before the experiment. It appeared that the delay between removal of the adrenals and the electrophysiological recording (partly) determined the amplitude of the Ca currents. Thus, for each cell, we calculated the maximal value of $I_{Ca,tot}$. Up to 3 days after ADX, the averaged maximal value of $I_{Ca,tot}$ was relatively small (868 ± 170 pA, $n = 8$), comparable to the value obtained in the group of sham-operated controls. However, with a longer delay, the Ca current increased significantly to 1395 ± 162 pA ($n = 15$; $P < 0.05$ with unpaired t test). Other cell properties did not change consistently over this period. The maximal amplitude of $I_{Ca,tot}$ in the matched sham controls did not change significantly ($P < 0.14$) over the 8-day period.

3.3. Effect of *in vitro* corticosteroid treatment on Ca currents

Application of 30 nM corticosterone in the presence of the GR antagonist RU 38486, thus, presumably only activating MRs [36], resulted 0.5–3 h later in a large depression of the Ca currents. The maximal amplitude of both the $I_{Ca,i}$ and the $I_{Ca,ni}$ were significantly ($P < 0.05$) reduced, compared with the untreated ADX

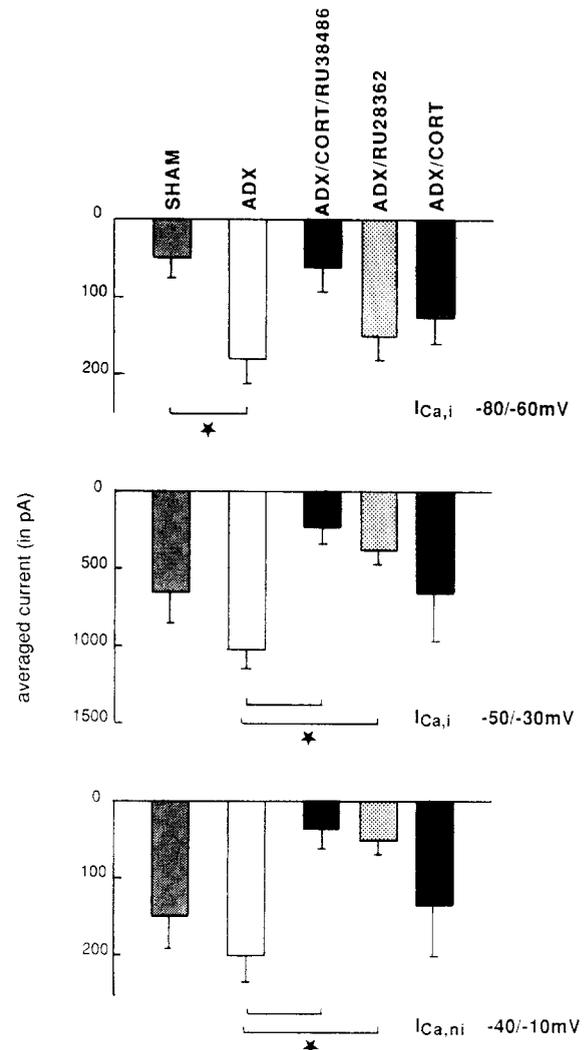


Fig. 3. For individual neurons, we averaged the currents evoked with the protocol for $I_{Ca,i}$ in the voltage range from -80 to -60 mV and the voltage range between -50 and -30 mV and for the $I_{Ca,ni}$ in the voltage range between -40 and -10 mV. The mean \pm S.E.M. values for all neurons are depicted in the upper, middle and lower panels, respectively. For statistical comparison, we first employed a one-way ANOVA ($P < 0.05$), followed by a posthoc unpaired Student's t test ($*P < 0.02$) of the sham control group or the steroid-treated ADX groups against the untreated ADX group.

group (Fig. 2A, middle panel; 2B). Treatment with RU 38486 alone ($n = 3$) was ineffective.

When we differentiated between the currents for the three voltage ranges described above, the following was observed (Fig. 3): (1) the $I_{Ca,i}$ between -80 and -60 mV was, on average, reduced after corticosterone/RU 38486 treatment, but the difference with the ADX group was not significant ($P < 0.07$); (2) both the $I_{Ca,i}$ between -50 and -30 mV and the $I_{Ca,ni}$ between -40 and -10 mV showed a significant reduction after corticosterone/RU 38486 application. The R_{in} , V_H for steady-state inactivation, the τ_i and τ_{ni}

were not different (Table 1) although the time constants for the decay seemed, on average, to be small.

Considering the gradual change in Ca-current characteristics with increasing delay after removal of the adrenals, we wondered if these MR-mediated effects took place only in slices that were prepared > 4 days after ADX. Yet, even in slices prepared within 24 h after ADX surgery, the maximal amplitude of the $I_{Ca,i}$ was small after treatment with corticosterone/RU 38486 (293 ± 84 pA, $n = 4$).

When we applied only 30 nM corticosterone (i.e., without the GR antagonist), thus, occupying GRs in addition to MRs [40], Ca conductances were enhanced in comparison to those observed in the slices, where MRs only were activated. The currents were comparable to those recorded in tissue from the mildly stressed sham-operated controls (Fig. 2A, lower panel; 2B). Within the three voltage ranges described above, no differences were observed between the $I_{Ca,i}$ or $I_{Ca,ni}$ in the corticosterone-treated group and the sham group (Fig. 3). R_{in} , V_H for steady-state inactivation and τ_i were also not different (Table 1). The τ_{ni} was, on average, quite small but statistical differences could not be demonstrated.

Interestingly, selective activation of GRs with 30 nM of the GR agonist RU 28362 [36] yielded Ca currents that resembled those observed after treatment with corticosterone/RU 38486 rather than with corticosterone alone (Fig. 2B). The $I_{Ca,i}$ between -50 and -30 mV and the $I_{Ca,ni}$ between -40 and -10 mV were significantly smaller than in the untreated ADX group; $I_{Ca,i}$ between -80 and -60 mV was not different (Fig. 3). In this group of cells, R_{in} was found to be quite variable.

4. Discussion

4.1. Ca currents

The data in this study were collected, with the in situ patch-clamp technique, in hippocampal slices. The motivation for this choice was two-fold. (1) We preferred to use a slice preparation rather than dissociated or cultured neurons because (a) this allows a comparison with earlier studies by us and others [16–21,24,25] performed in hippocampal slices (continuously superfused at 32°C) and (b) because the morphology of the adult pyramidal neurons in slices approaches the situation in vivo. (2) We used patch-clamp recording rather than the switch-clamp method with microelectrodes since the first method allows more accurate measurements of ion currents in isolation [30].

While an extensive characterization of the Ca currents in adult CA1 neurons with the in situ patch-clamp technique was not the purpose of this study, a stand-

ardized analysis of the currents was nevertheless required evaluate steroid modulation. This analysis was necessary given the differences in recording conditions with previous studies on Ca-current characteristics [4,22,23,33–35,46,47,49,52], e.g., the presently intact dendritic morphology and the perfusion with an external medium at 32°C that resembles the cerebrospinal fluid on the one hand (as opposed to most studies with patch electrodes) and the reasonable voltage control, efficient blockade of ion channels but also the possible dialysis of cytoplasmic factors on the other hand (as opposed to studies with microelectrodes).

The total Ca current ($I_{Ca,tot}$), which we describe in this study had a low threshold for activation, reached a maximal amplitude around -30 mV and displayed a voltage-dependent inactivation with a half maximal value around -110 mV. The Ca current comprised a transient and a sustained component. At membrane voltages above -65 mV, the transient component was completely inactivated and the sustained Ca current could be studied in isolation. This current, $I_{Ca,ni}$, yielded a threshold for activation of -40 mV, peaked around -10 mV and showed relatively little decay. Preliminary studies ($n = 4$) show that that the $I_{Ca,ni}$ can be effectively blocked by nifedipine (H. Karst et al., unpubl. data). In its voltage dependency and pharmacological profile, the current resembles the sustained L-type Ca current described before for isolated adult CA1 hippocampal neurons in rat [49] and guinea pig [22] and in cultured CA1 neurons [33,52]. It has also been recorded with the switch-clamp technique, in CA1 neurons [4]. The relatively small decay observed in the current can probably be attributed to Ca (rather than voltage-)dependent inactivation, which is characteristic for the L-type Ca current. The amplitude of the presently recorded $I_{Ca,ni}$ was quite small although not unlike the amplitude previously recorded in cultured rat CA1 neurons with 2 mM Ca^{2+} as the charge carrier [33]. The amplitude may have been reduced by CsF in the patch pipette, which is known to attenuate phosphatase activity and particularly the activation of the L-type Ca channels [23].

Subtraction of the sustained $I_{Ca,ni}$ from the total Ca current yielded a transient Ca conductance ($I_{Ca,i}$), with a threshold for activation of -80 mV, peak between -40 and -30 mV, marked inactivation with a small time constant for decay. The existence of such a low-threshold current was quite unexpected in CA1 neurons from adult rats since it was previously mainly observed in acutely dissociated CA1 neurons from young rats [49] and in CA1 neurons cultured from fetal or neonatal tissue [33,52]. In a recent study with the in situ patch-clamp technique, we described that a similar transient Ca conductance in CA1 cells, sensitive to amiloride, was no longer recorded (in the soma) when the dendrites were cut at a distance of < 150 μm from

the soma. From this observation and concurrent changes in kinetics and voltage dependency (of, e.g., the inactivation), we concluded that the current represents a T-type low-threshold Ca conductance generated in the distal dendrites [21]. Recent model studies incorporating the cable properties of CA1 neurons indeed support this interpretation [14,34]. Therefore, we assume that the $I_{Ca,i}$ consists at least partly of a dendritic low-threshold Ca current with, in the dendrites, voltage and kinetic characteristics comparable to the T-type current in young, cultured [33,35,52] or dissociated CA1 neurons [47,49]. These voltage and kinetic properties in the dendrites do allow activation of the current within a physiological range of membrane potentials.

4.2. Effects of adrenalectomy and steroid treatment on Ca currents

We observed that $I_{Ca,i}$ increased during the first week after ADX. The critical interval between ADX and recording session was ~ 4 days. In neurons from rats that were ADX < 4 days earlier, Ca currents were similar to the sham controls. With longer intervals, $I_{Ca,i}$ in the low-voltage range increased. This dependence of the $I_{Ca,i}$ amplitude on the delay between ADX and the recording session almost precludes that our data were much influenced by the problems attached to the patch-clamp technique, such as dialysis of cytoplasmic factors. As pointed out above, part of the low-threshold Ca conductance probably has a dendritic origin. Even though the currents in the low-voltage range are small, influx of Ca^{2+} into the thin distal dendrites through voltage-gated Ca channels may give rise to high local Ca concentration, especially when combined with the dendritic glutamate-gated Ca channels [39].

A previous current clamp study by Landfield and coworkers showed that the duration of a high-threshold Ca spike in tissue from 2–11-day ADX rats is reduced when compared with the sham-operated controls [24]. These current clamp data suggest that the inactivation kinetics of a high-threshold Ca current may be altered by ADX. While we here show that the τ_{ni} in the ADX group is, on average, indeed smaller than in the sham-operated control group, this difference did not attain statistical significance; it should be noted though that we observed considerable variation for the τ_{ni} in the sham-operated control group. The fact that the transient Ca current was enhanced in our preparation could very well contribute to an increased Ca-dependent inactivation of the high-threshold Ca current.

While changes in the Ca-current amplitude after ADX developed with a delay of > 3 days, in vitro application of MR and GR ligands altered the currents within only a few hours. This indicates that the mechanism underlying the changes in Ca currents may be

different for these two conditions. This is supported by the fact that ADX mainly affected currents in the low-voltage range while in vitro steroid application particularly altered currents in the high-voltage range. Our present study does not supply direct evidence for a genomic mechanism of action in both cases. However, the considerable delay between steroid modulation of Ca currents (even in vitro) and the effectiveness of steroid analogues, which act specifically on the intracellular steroid receptors, support a gene-mediated rather than putative membrane receptor-induced action. Clearly, the nature of the steroid actions after ADX and steroid application needs to be further elaborated. Possible effects include the synthesis of new Ca channels or structural changes of existing channels.

Very small Ca currents were observed after treatment of slices with corticosterone/RU 38486, irrespective of the delay between ADX and recording. The corticosterone/RU 38486 treatment probably results in a predominant MR occupation [36]. Accordingly, preliminary findings show that treatment with 30 nM of the mineralocorticoid aldosterone/RU 38486 also results in relatively small Ca currents (562 ± 155 pA, $n = 4$, for a command potential to -30 mV). Occupation of GRs in addition to MRs enhanced the Ca conductances, to the level of the mildly stressed sham-operated controls. Consequently, the physiologically relevant transition from predominant MR occupation (as occurs at the trough of the circadian cycle) to concurrent MR and GR occupation (as occurs at the peak of the cycle and after stress) may be associated with a shift from small to large Ca currents, respectively. Whether lowering of circulating steroid levels subsequent to the circadian peak or periods of stress results in a delayed decrease of the Ca currents still needs to be established.

The increase in Ca influx with occupation of GRs in addition to MRs may have results for other properties of CA1 neurons. Thus, the shift could explain the previously described GR-dependent increase of the Ca-dependent K conductance [16,17,24]. However, direct effects of steroids on the Ca-dependent K conductance cannot be excluded. The latter is also stressed by the fact that Ca-current amplitudes in untreated tissue from 4–7 (but not 1–3-)day ADX rats are large while the Ca-dependent K conductance was found to be relatively small [16,24]. In the long run, the level of voltage-activated Ca influx could also have implications for the survival of cells [6]. Predominant MR activation can be expected to be neuroprotective while the enhanced Ca influx associated with additional GR activation (or the absence of steroids) could potentially lead to disruption of neuronal circuits and, eventually, degeneration. The former may have consequences for the transmittal of synaptic input [15,19]. With respect to the latter, it was found that CA1 neurons, as opposed

to CA3 pyramidal cells and granule cells in the dentate, are quite resistant to degeneration as a result of chronically elevated levels or absence of corticosteroids, respectively. This emphasizes that the overall effect of an increased Ca influx can not be evaluated regardless of additional factors, such as the inhibitory input to CA1 neurons and activation of K channels.

Two recent reports indicate that GRs affect Ca influx in the CA1 area. One study, employing field potential recording in response to stimulation of Schaffer collaterals, showed that the mixed agonist corticosterone suppresses field potential amplitudes via a GR-dependent mechanism [41], particularly when the extracellular Ca-level is high [47]. More direct evidence was obtained by Kerr et al. [25] who reported that high doses (7 μ M) of the GR agonist RU 28362 increase high-threshold Ca conductances in CA1 neurons. While our present findings support the idea that Ca currents are large when GRs are occupied additionally to MRs, they are only partly in line with the findings of Kerr et al. since we did not observe large Ca currents after occupation of GRs only, with 30 nM of RU 28362. It should be noted though that the R_{in} in the RU 28362-treated group was variable and relatively high. This may have consequences when the data are analysed in terms of changes in current density rather than amplitude. The idea, however, that exclusive occupation of GRs induces effects that differ from those seen with concurrent MR and GR occupation is not unprecedented. Similar observations were made with respect to a voltage-dependent K conductance [20].

In summary, the present data suggest that corticosteroid hormones exert a persistent, slow control over voltage-gated Ca influx in the CA1 area, the nature of which is determined by the relative degree of MR and GR occupation. Under conditions of low adrenocortical activity, Ca currents will be small while large currents may occur at the peak of the circadian steroid release and particularly after stress. Apart from the changes in Ca influx associated with physiological variations in corticosteroid levels, the presently described effects could also have implications for situations in which the MR and GR balance is chronically disturbed. Thus, conditions resulting in an insufficient activation of MRs or in continuous 'over-activation' of GRs (additionally to MRs), as occurs after chronic stress, could expose the cells to large Ca influxes, a situation that potentially affects the functioning of neuronal networks.

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