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Effect of ORG 34116, a corticosteroid receptor antagonist, on hippocampal Ca^{2+} currents

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Abstract

ORG 34116, a substituted 11,21-bisarylsteroid compound, binds selectively and with high affinity to human and rat glucocorticoid receptors. At the level of the hypothalamus it attenuates the negative feedback action of corticosterone, suggesting that it acts as an antagonist. In the present study we examined the effect of in vitro and in vivo administered ORG 34116 on cell properties of higher brain areas, i.e. on Ca^{2+} current characteristics of CA1 hippocampal neurons recorded with whole cell techniques in hippocampal slices. We observed that in vitro applied ORG 34116 antagonized corticosterone induced effects on Ca^{2+} currents. Data observed after in vivo application of ORG 34116 corroborate these findings. The results furthermore suggest that pretreatment with the glucocorticoid receptor antagonist ORG 34116 also prevents the development of mineralocorticoid receptor mediated effects on Ca^{2+} currents. If ORG 34116 should indeed prove to be a corticosterone rather than glucocorticoid receptor selective antagonist, this drug may turn out to be an important tool in the treatment of stress-related disorders. © 1997 Elsevier Science B.V.

Keywords: Mineralocorticoid; Glucocorticoid; Hippocampal slice; Patch clamp recording

1. Introduction

Corticosteroid hormones can enter the brain and bind to intracellular receptors (reviews by McEwen et al., 1986; de Kloet, 1991). Two types of receptors have been distinguished, based on their molecular structure and binding properties (Hollenberg et al., 1985; Reul and de Kloet, 1985; Arriza et al., 1987): The high affinity ($K_d \sim 0.5$ nM) mineralocorticoid receptor which is highly enriched in limbic brain structures, and the lower affinity ($K_d \sim 5$ nM) glucocorticoid receptor which is ubiquitous in the brain. Neurons in the hippocampal CA1 area express high amounts of both mineralocorticoid and glucocorticoid receptors (see review de Kloet, 1991). Activation of these receptors leads to changes in gene transcription (Truss and Beato, 1993). This can result in delayed but persistent effects on signal transduction in the brain.

Agonists and antagonists for the mineralocorticoid and glucocorticoid receptor have been extensively used to study the binding characteristics and specific functions mediated by the two receptor types (see reviews de Kloet, 1991; Joëls and de Kloet, 1992, 1994). It was found that mineralocorticoid and glucocorticoid receptors affect cellular processes of CA1 neurons in a different way. In particular properties of voltage gated Ca^{2+} currents were found to be under long term control of corticosteroid hormones. Thus, predominant activation of mineralocorticoid receptors in hippocampal slices prepared from adrenalectomized rats, resulted in small amplitudes of both high and low threshold Ca^{2+} currents (Karst et al., 1994). Voltage sensitivity or kinetic properties were not affected. Additional activation of glucocorticoid receptors in vitro led to an increase in the amplitude of voltage gated Ca^{2+} currents, particularly of the high threshold type (Kerr et al., 1992; Karst et al., 1994). In the absence of corticosteroids, i.e. in untreated slices from adrenalectomized rats, Ca^{2+} current amplitude was also high (Karst et al., 1994, 1997), pointing to a U-shaped dose dependency. The small Ca^{2+} current amplitude observed with predominant mineralocorticoid receptor activation seemed to require some degree of glucocorticoid receptor activation since the 'trough' in the U-shaped curve was not observed in genetically modified mice lacking functional glucocorticoid receptors (Hesen et al., 1996).

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The effects of predominant mineralocorticoid receptor activation on Ca^{2+} currents were mostly studied by applying 30 nM corticosterone — i.e. a concentration high enough to occupy both receptor types — in the presence of the glucocorticoid receptor antagonist RU 38486 (500 nM). However, RU 38486 is not a very selective glucocorticoid receptor antagonist, since it also binds effectively to progesterone receptors (Gagne et al., 1985). Furthermore, it shows some agonistic activity at glucocorticoid receptors. Recently, a potent glucocorticoid receptor antagonist with marginal affinity for the progesterone receptor, ORG 34116, was synthesized (Gebhard et al., 1995). This compound showed no intrinsic agonistic activity in a glucocorticoid receptor transactivation assay. We presently tested the effect of in vivo or in vitro application of ORG 34116 on voltage gated Ca^{2+} currents of CA1 pyramidal cells, recorded in hippocampal slices.

2. Materials and methods

2.1. Animals and drugs

In total, 40 male Wistar rats (Harlan, The Netherlands; 200–300 g) were used for these experiments. Of these, 20 were adrenalectomized 5–7 d before the experiment, as described elsewhere (Ratka et al., 1989). The remaining animals were sham operated or not operated at all. All animals received food and water (saline after adrenalectomy) ad libitum and were group-housed in a standard animal room with lights on between 7.00 am and 7.00 pm. On the day of the experiment, animals were exposed at 9.00 am to a novel environment (clean cage), 45 min before decapitation (Karst et al., 1994). Plasma corticosterone level in trunk blood, collected at the moment of decapitation, was determined with a radio–immuno assay. All adrenalectomized animals included in the present study showed plasma corticosterone levels below 1 μg/100 ml plasma. The present series of experiments is part of a project approved by the local ethic commission and carried out under internationally accepted principles.

Animals were quickly decapitated and the brain was removed from the skull. The brain was dipped into cold (4°C) artificial cerebrospinal fluid (ACSF) of the following composition, in mM: NaCl 120, KCl 3.5, $MgSO_4$ 1.3, NaH_2PO_4 1.25, $CaCl_2$ 2.5, D-glucose 10, $NaHCO_3$ 25. Transversal hippocampal slices (300 μm) were cut on a McIlwain tissue chopper. The slices were stored at room temperature in carbogenated (95% O_2 + 5% CO_2) ACSF. One slice at a time was transferred to a recording chamber, submerged and continuously perfused (2 ml/min) with warm (32°C) carbogenated ACSF.

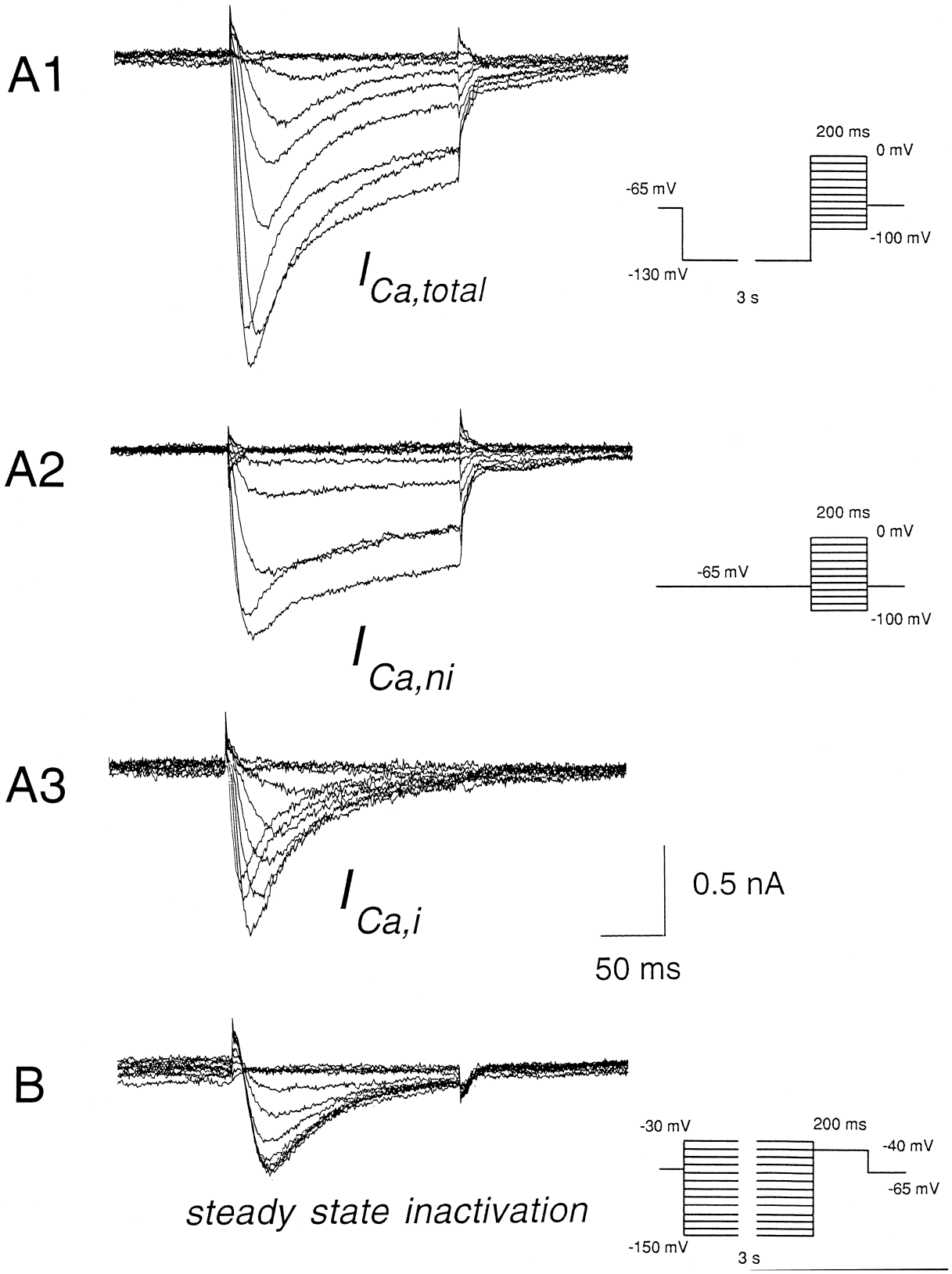
ORG 34116 was applied either in vitro or in vivo. In vitro it was first dissolved to a 10^{-3} M concentration in 95% ethanol, next diluted to either 30 or 500 nM in ACSF and applied to slices from adrenalectomized rats. When tested as an antagonist (500 nM), it was first applied for 20 min in the absence of corticosterone and next for 20 min in the presence of 30 nM corticosterone. In a limited number of experiments ORG 34116 was tested as an agonist, i.e. applied at 30 nM for 20 min in vitro. Due to the delayed effects of the drugs it was impossible to test effects of more than one treatment in individual neurons. Therefore, all neurons were recorded 1–4 h after termination of ORG 34116 administration, and compared with neurons of untreated slices, as described before (Karst et al., 1994). For in vivo application ORG 34116 (2 mg/100 g bodyweight) was dissolved in a small volume of ethanol, which was further diluted with household cream to a final concentration of 2 mg/ml (see Heikinkeimo et al., 1994). This solution was administered with a gastro–oesophageal tube directly into the stomach, either 30 min or 3.5 h before the novelty stress. In some experiments the effect of ORG 34116 was compared with that of corticosterone (1 mg/100 g bodyweight, $n = 5$ rats) or RU 38486 (5 mg/100 g bodyweight, $n = 5$ rats), dissolved and administered in the same way as ORG 34116.

2.2. Ca^{2+} currents in hippocampal slices

Hippocampal pyramidal CA1 neurons were selected for recording with a light microscope (Nikon 104), using a 40 × water immersion objective and 10 × ocular. With a patch-pipette (1.5 mm outer diameter borosilicate glass; 1.5–3.0 MΩ) the selected cell was approached, as described elsewhere (Joëls and Karst, 1995, based on the method of Edwards et al., 1989) and the whole cell recording configuration was established. The pipette solution consisted of (in mM): 100 CsF, 0.5 $CaCl_2$, 2 $MgCl_2$, 2 MgATP, 0.1 NaGTP, 10 Hepes, 10 EGTA, 20 creatinephosphate, 50 U/ml creatine–phosphokinase, 0.1 albumine, 20 tetraethylammonium chloride, pH 7.4, 300

Fig. 1. (A1) application of 200 ms depolarizing voltage steps ranging from −100 to 0 mV in consecutive order, after a hyperpolarizing prepulse of −130 mV (voltage protocol shown as inset) yielded the total Ca^{2+} current, $I_{Ca,total}$ in a CA1 pyramidal cell, recorded with the whole cell patch clamp technique in a hippocampal slice. (A2) when depolarizing voltage steps were applied from holding potential (−65 mV, see inset) only sustained Ca^{2+} currents, $I_{Ca,ni}$, were observed which showed little steady state inactivation. (A3) by subtracting the currents observed in A2 from the $I_{Ca,total}$, transient Ca^{2+} currents which were liable to steady state inactivation, $I_{Ca,i}$, became apparent. (B) by applying a 3 s hyperpolarizing prepulse (voltage protocol shown as inset) steady state inactivation of the voltage dependent Ca^{2+} currents was removed, yielding the total Ca^{2+} currents obtained by subsequently applying a 200 ms depolarizing voltage step to −40 mV. With more depolarized prepulses steady state inactivation became apparent, resulting in smaller amplitudes of the Ca^{2+} currents. All traces were subjected to leak and capacity transient correction as described in Joëls and Karst (1995). These corrections did not affect amplitude or voltage dependency of the Ca^{2+} currents.

mOsm. This solution was frozen and kept on ice during the day of the experiment. To block Na-currents, 0.5 μM tetrodotoxin was added to the extracellular ACSF; K-conductances were blocked by extracellular addition of 10 mM tetraethylammonium chloride, 5 mM 4-aminopyridine and 5 mM CsCl.



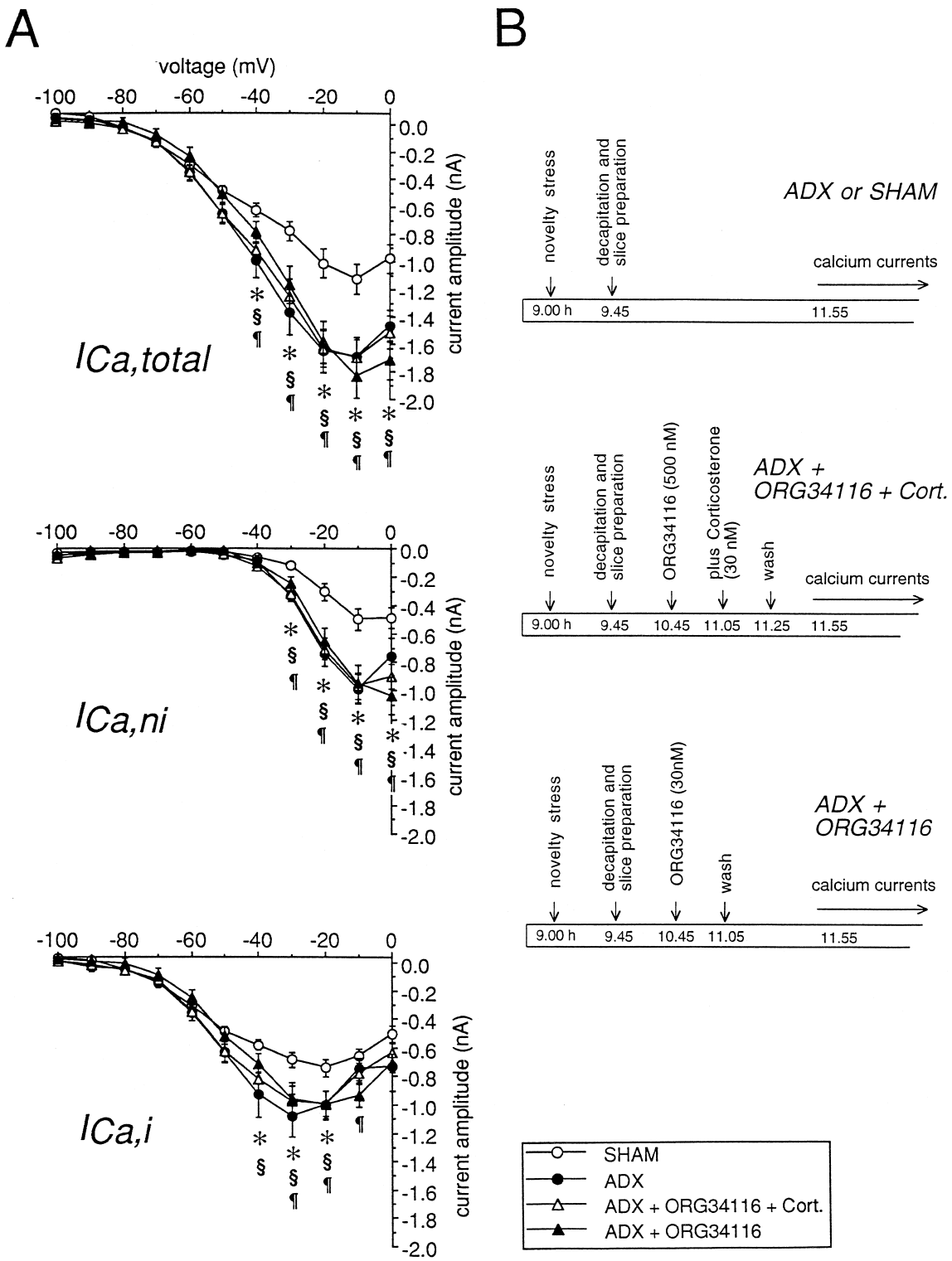


Fig. 2. (A) current voltage relation for the $I_{Ca,total}$ (upper), $I_{Ca,ni}$ (middle) and $I_{Ca,i}$ (lower graph) observed in 4 experimental groups: neurons from adrenally intact controls (open circles), from adrenalectomized rats (closed circles), from slices of adrenalectomized rats treated in vitro with 30 nM corticosterone in the presence of 500 nM ORG 34116 (open triangles) or slices from adrenalectomized rats treated with 30 nM ORG 34116 (closed triangles). The data indicate that Ca^{2+} current amplitude is increased in slices from adrenalectomized rats compared to adrenally intact controls. Treatment with corticosterone in the presence of ORG 34116 or with ORG 34116 alone yielded currents that did not differ from the adrenalectomized group. Number of cells as mentioned in Table 1. * Currents of the adrenalectomized group that are significantly different from the untreated adrenally intact group ($P < 0.05$). § Currents of the ADX/ORG/CT group that are significantly different from the untreated adrenally intact group ($P < 0.05$). ¶ Currents of the ADX/ORG group that are significantly different from the untreated adrenally intact group ($P < 0.05$). (B) experimental protocol used for the in vitro experiments.

Whole cell currents were measured under voltage–clamp conditions using a Biologic RK300 amplifier. Data was collected with an Atari computer, at 1 kHz sampling rate. Voltage step protocols were generated by an in-house developed acquisition program. Each cell was subjected to the same sequence of timed voltage protocols, as described elsewhere (Karst et al., 1994). Ca^{2+} currents showed relatively little rundown over a 20 min period (< 20%). To introduce uniformity in the methods of the data collected all data about Ca^{2+} current activation presented in this paper were obtained at 10 min after the whole cell configuration was established; inactivation properties were established after 15 min.

Single exponential functions were fitted to the inactivation phase of the transient inward Ca^{2+} current. The same least-square minimization algorithm was used to estimate the Boltzmann curves for steady-state inactivation of the transient Ca^{2+} currents. Routinely, correction for linear leak-current and capacity transients was applied as described in detail elsewhere (Joëls and Karst, 1995); current properties were not basically altered by these correction procedures.

2.3. Statistics

The values for the various treatment groups were statistically evaluated with a one-way analysis of variance followed by a post hoc (Student's t) test (level of significance: $P < 0.05$).

3. Results

As shown in Fig. 1, inward currents were evoked by depolarizing pulses of 200 ms duration, preceded by a 3 s prepulse of −130 mV (Fig. 1A). These inward currents are carried by Ca^{2+} ions (Karst et al., 1994; Joëls and Karst, 1995). If the potential of the prepulse was varied from −150 mV to −30 mV, stepping subsequently to a potential of −40 mV (Fig. 1B), it became apparent that part of the total Ca^{2+} current ($I_{Ca,total}$) is liable to steady state inactivation. The currents observed when applying variable prepulse potentials could be normalized to the maximal current and fitted with a Boltzmann equation $I(V)/I_{max} = 1/[1 + \exp\{(V - V_H)/V_C\}]$, where $I(V)/I_{max}$ is the normalized current at membrane potential V, V_H is the voltage of half maximal inactivation and V_C is proportional to the slope of the curve. At −65 mV steady state inactivation was already maximal. Accordingly, when depolarizing voltage steps were applied directly from holding potential (i.e. −65 mV) currents with little inactivation were observed ($I_{Ca,ni}$, see Fig. 1A2). Subtracting these sustained currents from the $I_{Ca,total}$ yielded the transient Ca^{2+} current liable to steady state inactivation, in isolation ($I_{Ca,i}$).

3.1. In vitro ORG 34116 application

As observed in earlier studies (Karst et al., 1994, 1997), Ca^{2+} currents recorded in slices from 5–7 d adrenalectomized rats displayed a larger amplitude than Ca^{2+} currents in adrenally intact control rats (Fig. 2). Current voltage current relations for both the $I_{Ca,ni}$ and $I_{Ca,i}$ showed an increased amplitude in the adrenalectomized group. The voltage dependency of activation and the V_H or V_C for inactivation (Table 1) were not affected by adrenalectomy. Previously it was found that application of 30 nM corticosterone to slices from adrenalectomized rats yielded Ca^{2+} current amplitudes that were similar to those observed in the adrenally intact control group (Karst et al., 1994). This was replicated in a limited number of cells ($n = 11$) in the present study. For a voltage step to −10 mV (peak of the $I_{Ca,total}$) the current amplitude amounted to -1.2 ± 0.1 nA, which was similar to the value observed in the sham operated controls (-1.1 ± 0.1 nA, $n = 18$) and significantly smaller than in the untreated adrenalectomized group

Table 1
Input resistance (R_{in}, in MΩ) and properties of steady state inactivation (V_H in mV and V_C) observed in the various treatment groups indicated above

	In vitro application				In vivo application		
	ADX	ADX/ORG	ADX/ORG/CT	ADX/CT	control	ORG	CT
R_{in}	113 ± 9	113 ± 6	134 ± 10	126 ± 16	111 ± 8	135 ± 9	113 ± 9
V_H	−86 ± 1	−85 ± 1	−83 ± 2	−83 ± 2	−88 ± 1	−90 ± 2	−87 ± 2
V_C	8.0 ± 0.6	7.6 ± 0.5	8.2 ± 0.5	8.1 ± 1.0	9.0 ± 0.5	9.6 ± 0.5	8.0 ± 0.5
N (n)	4 (15)	5 (18)	4 (17)	3 (11)	5 (18)	5 (20)	5 (18)

For statistical comparison, we applied an ANOVA on two sets of experiments: (1) adrenalectomized (ADX), controls, ADX/ORG, ADX/ORG/CT and ADX/CT; (2) ADX, controls, ORG and CT. If the ANOVA indicated that one of the groups differed from the other groups ($P < 0.05$) a post-hoc Student's t-test was performed. For none of the parameters statistically significant differences were observed between the groups. All data represent the mean ± standard error of the mean, for 'n' neurons obtained from 'N' animals.

ADX = slices from adrenalectomized rats; controls = slices from sham operated control animals; ADX/ORG = 20 min administration of 30 nM ORG 34116 to slices from adrenalectomized rats (ORG 34116 tested as an agonist); ADX/ORG/CT = 20 min administration of 30 nM corticosterone in the presence of 500 nM ORG 34116 to slices from adrenalectomized rats (ORG 34116 tested as an antagonist); ADX/CT = 20 min administration of 30 nM corticosterone to slices from adrenalectomized rats (corticosterone tested as an agonist). ORG = ORG 34116 (2 mg/100 g p.o.) administered in vivo to adrenally intact rats; CT = corticosterone (1 mg/100 g p.o.) administered in vivo to adrenally intact rats.

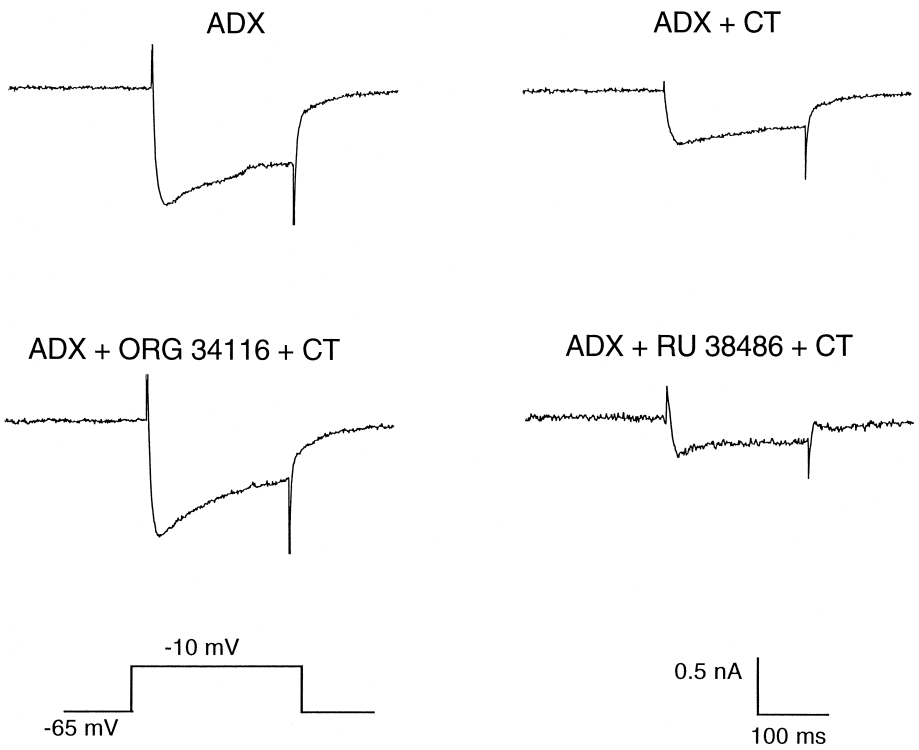


Fig. 3. Typical Ca^{2+} high threshold (sustained) currents evoked in four different hippocampal CA1 neurons by a voltage step to -10 mV, from a holding potential of -65 mV (voltage protocol shown at lower left corner). Ca^{2+} current amplitude was large in untreated tissue from adrenalectomized animals (upper left; $R_{in} = 129$ MΩ). In vitro treatment with 30 nM corticosterone (CT) resulted in smaller Ca^{2+} current amplitudes (upper right; $R_{in} = 131$ MΩ). Pretreatment of the slices with 500 nM of ORG 34116 prevented the effect of corticosterone (lower left; $R_{in} = 105$ MΩ), resulting in Ca^{2+} currents which were comparable to the untreated tissue of adrenalectomized rats. Pretreatment with RU 38486 (500 nM) before corticosterone treatment resulted in a small Ca^{2+} current amplitude (lower right; $R_{in} = 116$ MΩ).

(-1.8 ± 0.1 nA, $n = 15$; typical traces are shown in Fig. 3). Treatment with 500 nM ORG 34116 prevented this effect of corticosterone (-1.8 ± 0.2 nA, $n = 17$; see Figs. 2 and 3). Ca^{2+} current amplitudes after treatment with corticosterone in the presence of ORG 34116 resembled the values observed with untreated slices from adrenalectomized rats (Fig. 2). This is in contrast to earlier studies with the glucocorticoid receptor antagonist RU 38486, where Ca^{2+} currents were found to be very small in amplitude (Karst et al., 1994). In the present study too, Ca^{2+} current amplitudes in slices treated with corticosterone in the presence of RU 38486 (17 cells) were significantly smaller than currents seen when administering corticosterone in the presence of ORG 34116 ($P <$

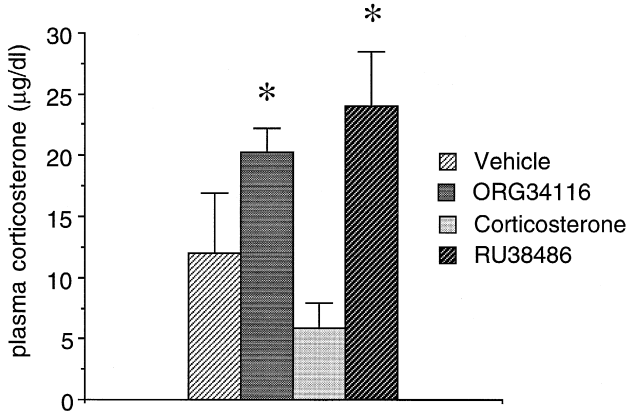


Fig. 4. Plasma corticosterone level (mean + standard error of the mean) observed 45 min after a mild stress in adrenally intact rats treated 3.5 h before the stress with ORG 34116 (2 mg/100 g), with RU 38486 (5 mg/100 g), with corticosterone (1 mg/100 g) or with vehicle. When a GR-antagonist was administered plasma corticosterone level was significantly elevated, indicating an insufficient negative feedback of corticosterone at the level of the pituitary or hypothalamus. The reverse was observed when exogenous corticosterone was administered. * Significantly different from vehicle treated group ($P < 0.05$).

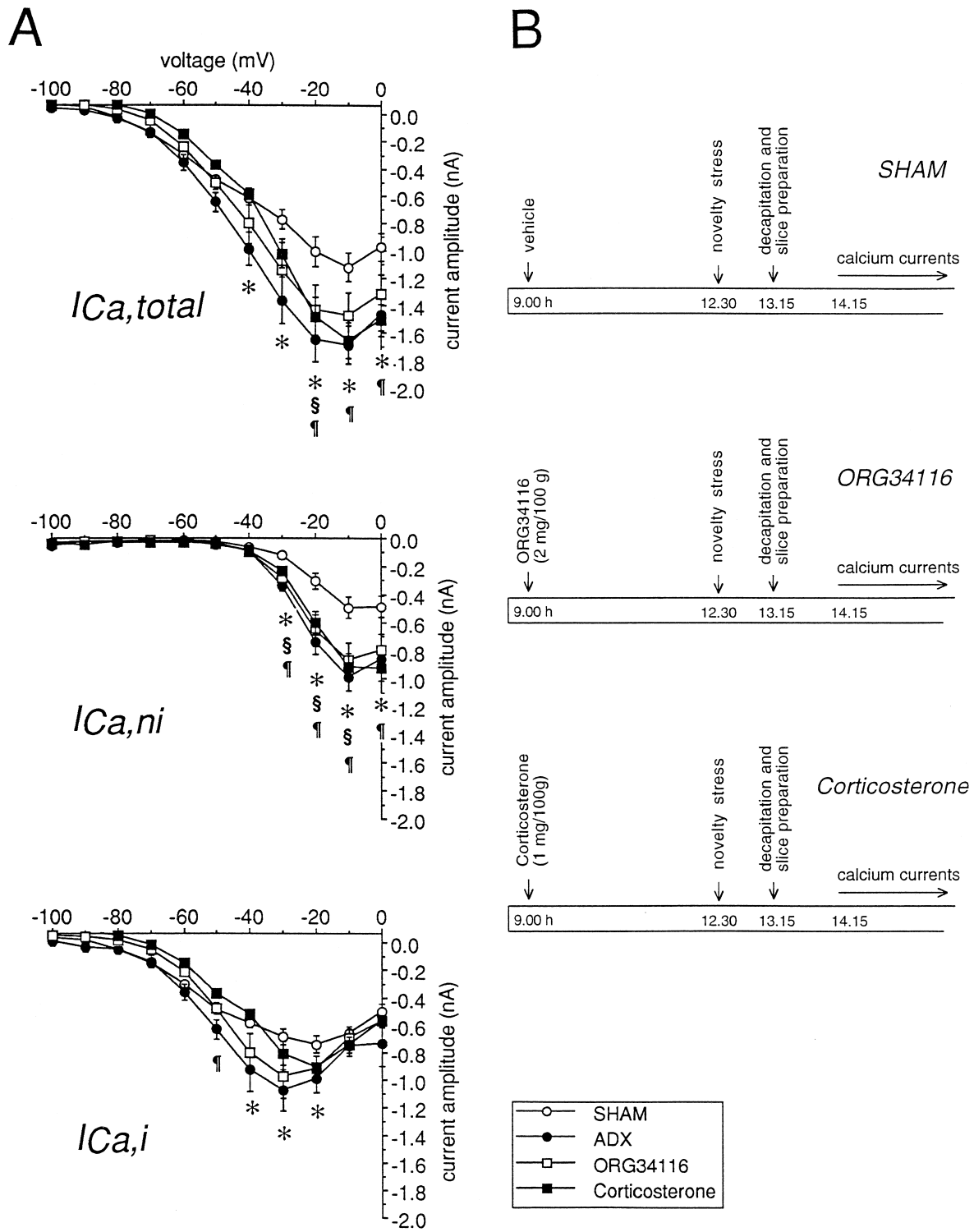


Fig. 5. (A) current voltage relation for the $I_{Ca,total}$ (upper), $I_{Ca,ni}$ (middle) and $I_{Ca,i}$ (lower graph) observed in 4 experimental groups: neurons from adrenally intact controls (open circles), from adrenalectomized rats (closed circles, same data as shown in Fig. 2), from slices of adrenally intact rats treated in vivo with ORG 34116 (open squares) and slices of adrenally intact rats treated in vivo with corticosterone (closed squares). All animals were subjected to a novelty stress, 45 min before decapitation. The steroids were applied 3.5 h before the stress. The data indicate that Ca^{2+} current amplitude is large after treatment with ORG 34116 or with corticosterone. * Currents of the adrenalectomized group that are significantly different from the untreated adrenally intact group ($P < 0.05$). §Currents of the ORG group that are significantly different from the untreated adrenally intact group ($P < 0.05$). ¶Currents of the corticosterone group that are significantly different from the untreated adrenally intact group ($P < 0.05$). (B) experimental protocol used for the in vivo experiments.

0.05; typical example in Fig. 3). In vitro application of ORG 34116 by itself (30 nM, $n = 18$) to slices from adrenalectomized rats resulted in Ca^{2+} current characteristics that were indistinguishable from the untreated adrenalectomized group. As shown in Table 1, input resistance was comparable for all groups tested. The voltage properties for steady state inactivation, V_H and V_C, were also not affected by treatment with ORG 34116.

These data indicate that ORG 34116 blocks the effect of corticosterone on Ca^{2+} currents, in vitro. In contrast to RU 38486, the ORG 34116 compound does not reduce the Ca^{2+} current amplitudes in the presence of corticosterone.

3.2. In vivo ORG 34116 application

In vivo application of ORG 34116 was previously shown to affect the function of the hypothalamo–pituitary–adrenal axis (Gebhard et al., 1995). We presently also tested the effect of in vivo ORG 34116 administration on the negative feedback action of corticosterone, 45 min after a mild (novelty) stress; in control animals plasma corticosterone levels 45 min after the novelty stress amounted to 12.1 ± 4.9 μg/100 ml plasma.

As observed earlier (Gebhard et al., 1995), ORG 34116 (2 mg/100 g p.o.) blunted the negative feedback effect of corticosterone on hypothalamo–pituitary–adrenal activity, taking place at the level of the hypothalamus (Fig. 4). This effect was only significant when ORG 34116 was administered 3.5 h before the novelty stress. When administered 30 min before the stressor, ORG 34116 failed to blunt the negative feedback response. In this respect, ORG 34116 acted in a similar way as RU 38486 (3 mg/100 g; see Fig. 4). By contrast, in vivo administration of corticosterone (1 mg/100 g) 3.5 h before the novelty stress on average reduced the endogenous level of the hormone. These data concerning the activity of the hypothalamo–pituitary–adrenal axis support a role of ORG 34116 as GR antagonist, at the level of the hypothalamus or pituitary.

In the same animals we investigated properties of Ca^{2+} currents, recorded in subsequently prepared hippocampal slices. Administration of ORG 34116, 3.5 h before the mild stress, yielded large amplitudes of the $I_{Ca,ni}$ as recorded in subsequently prepared hippocampal slices (Fig. 5). When ORG 34116 was administered 30 min before the stress, Ca^{2+} current amplitudes were at an intermediate level (data not shown, $n = 12$ cells). On the one hand, these data resembled the Ca^{2+} current properties seen after adrenalectomy ($n = 15$). However, similarly large Ca^{2+} current amplitudes were also seen when a very high dose of corticosterone (1 mg/100 g bodyweight) was administered 3.5 h before the novelty stress to adrenally intact rats ($n = 18$). For voltage steps to -30 and -20 mV, the amplitude of $I_{Ca,ni}$ observed after ORG 34116 ($n = 20$) pretreatment was significantly ($P < 0.05$) larger than amplitudes in animals pretreated with RU 38486 (3 mg/100 g), 3.5 h before the stress ($n = 20$ cells, data not shown). Inactivation properties were not different between the groups tested (Table 1). Similarly, no significant differences were observed for the input resistance, although on average the resistance was slightly elevated in the animals which received ORG 34116 in vivo.

4. Discussion

ORG 34116 binds with high affinity to both human and rat glucocorticoid receptors (Gebhard et al., 1995). It has considerably lower affinity to progesterone receptors, as opposed to RU 38486 which displays almost equal affinity to glucocorticoid and progesterone receptors (Gagne et al., 1985). In a CHO based receptor/reporter transfection system, ORG34116 (up to 100 nM) showed no transactivation agonistic activity. In this assay, RU 38486 (1–100 nM) still exhibited $\sim$20% of the activity shown by dexamethasone (Gebhard et al., 1995). Marked antagonistic activity of ORG 34116 was observed on human glucocorticoid receptors when tested in the presence of 31.6 nM dexamethasone. These data suggest that ORG 34116 is a potent and selective glucocorticoid receptor antagonist, with no transactivation properties.

This was corroborated by studies where ORG 34116 was administered in vivo. ORG 34116 was found to be active in a dexamethasone suppression test, with an ED_{50} of 10 mg/kg p.o. (Gebhard et al., personal communication). No antiprogestagenic activity was observed in a lordosis test. Our present neuroendocrine data support these earlier findings. Thus, administration of ORG 34116 (2 mg/100 g bodyweight) $\pm$3.5 h before a mild stress resulted in significantly higher corticosterone plasma levels 45 min after the stress, similarly to RU 38486. The fact that the glucocorticoid receptor antagonists were less potent when administered 30 min before exposure of the animals to a novelty stress can be explained by the kinetic properties of the hormone and the delayed action via gene transcription. The present data indicate that ORG 34116 potently antagonizes corticosterone mediated effects on the hypothalamo–pituitary–adrenal system.

We next tested if ORG 34116 could also antagonize corticosterone mediated effects in higher brain cells, in vitro. For this, we selected the properties of voltage gated Ca^{2+} currents in hippocampal CA1 neurons, which were previously found to be sensitive to corticosteroid receptor activation (Kerr et al., 1992; Karst et al., 1994). The amplitude of the Ca^{2+} currents displayed a U-shaped dose-dependency on the corticosteroid concentration. Thus, in the absence of corticosterone (in tissue from adrenalectomized rats) Ca^{2+} current amplitude was very large (Karst et al., 1994, 1997). With predominant mineralocorticoid receptor activation, using aldosterone or corticosterone in the presence of RU 38486, Ca^{2+} current amplitude was markedly reduced (Karst et al., 1994). For this effect, the presence of some biologically active glucocorticoid receptors seemed to be essential (Hesen et al., 1996), indicating

that a certain degree of interdependency of mineralocorticoid and glucocorticoid receptor mediated events is important (Arriza et al., 1988; Trapp et al., 1994). Additional activation of glucocorticoid receptors with a moderate dose of corticosterone (30 nM) significantly increased Ca^{2+} current amplitude, although the amplitude was still smaller than seen in the absence of corticosteroids (Karst et al., 1994). Similar doses of the selective glucocorticoid receptor agonist RU 28362 (Philibert and Moguilevski, 1983) did not enhance Ca^{2+} current amplitude, again stressing that both mineralocorticoid and glucocorticoid receptors are involved in corticosterone-dependent modulation of Ca^{2+} currents. When very high doses of RU 28362 were administered Ca^{2+} current amplitudes were very large, comparable to the currents seen in the absence of corticosterone (Kerr et al., 1992).

In the present study we confirmed that Ca^{2+} currents in untreated slices from adrenalectomized rats displayed larger amplitudes than in the adrenally intact control group. Furthermore, corticosterone (30 nM) administration to slices of adrenalectomized rats resulted in Ca^{2+} current amplitudes which were comparable to those seen in the adrenally intact control group. Pretreatment with ORG 34116 reversed these effects of corticosterone, so that Ca^{2+} current amplitude was indistinguishable from the untreated adrenalectomized group. This supports the in vivo neuroendocrine observations, i.e. that ORG 34116 potently antagonizes corticosterone induced effects.

However, in contrast to what was seen before and presently confirmed with RU 38486, ORG 34116 pretreatment did not result in a reduction of Ca^{2+} current amplitude, although according to the binding profile ORG 34116 pretreatment should result in selective occupation of the mineralocorticoid receptors. We considered the possibility that ORG 34116 may have exerted some agonistic activity, so that (combined with the exogenously applied corticosterone) it yielded Ca^{2+} currents seen with (very) high corticosterone concentrations. However, 30 nM of ORG 34116 by itself did not evoke changes in Ca^{2+} current amplitude that were comparable to those seen with 30 nM of corticosterone. An alternative explanation could be that not only the presence (Hesen et al., 1996) but also the activation of some glucocorticoid receptors is necessary for the development of mineralocorticoid receptor mediated effects on Ca^{2+} currents as was previously observed in a transfected cell system (Trapp et al., 1994). Finally, it is possible that the complex of ORG 34116 bound to glucocorticoid receptors has steric effects that hinder a normal function of activated mineralocorticoid receptors.

The results presently obtained with in vivo administration of ORG 34116 were generally in line with the in vitro studies. Administration of a high dose of ORG 34116 (3.5 h before a mild stress) resulted, in subsequently prepared hippocampal slices, in large Ca^{2+} currents, comparable to Ca^{2+} currents seen in slices from adrenalectomized rats. However, a very high dose of corticosterone in vivo also induced large Ca^{2+} currents. Thus, if ORG 34116 would poorly penetrate the blood brain barrier after oral application, the present in vivo data could also be explained by indirect effects of the high endogenous corticosterone level, caused by the action of ORG 34116 in the pituitary or hypothalamus. However, this only seems a remote possibility since preliminary observations (Gebhard et al., personal communication) indicate that ORG 34116 effectively penetrates the blood brain barrier after oral application, within 30 min.

In conclusion: ORG 34116, an 11,21-bisarylsteroid binding selectively and with high affinity to human and rat glucocorticoid receptors, antagonizes corticosterone induced effects on Ca^{2+} currents in vitro. Data observed after in vivo application of ORG 34116 corroborate these findings. The results obtained in the present study suggest that pretreatment with the GR antagonist ORG 34116 also prevents the development of mineralocorticoid receptor mediated effects on Ca^{2+} currents. This idea should be further tested in other preparations where mineralocorticoid and glucocorticoid receptors are colocalized and interact in the modulation of cell characteristics. If ORG 34116 should indeed prove to be a corticosterone rather than glucocorticoid receptor selective antagonist, this drug may turn out to be an important tool in the treatment of stress-related disorders.

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