Effects of Glucocorticoids and Norepinephrine on the Excitability in the Hippocampus

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The CA1 pyramidal neurons in the hippocampus contain a high density of adrenal corticosteroid receptors. By intracellular recording, CA1 neurons in slices from adrenalectomized rats have been found to display a markedly reduced afterhyperpolarization (that is, the hyperpolarizing phase after a brief depolarizing current pulse) when compared with their sham controls. No differences were found for other tested membrane properties. Brief exposure of hippocampal slices from adrenalectomized rats to glucocorticoid agonists, 30 to 90 minutes before recording, greatly enhanced the afterhyperpolarization. In addition, glucocorticoids attenuated the norepinephrine-induced blockade of action potential accommodation in CA1 neurons. The findings indicate that glucocorticoids can reduce transmitter-evoked excitability in the hippocampus, presumably via a receptor-mediated genomic action.

CORTICOSTEROIDS ARE OF CRITICAL importance for homeostasis (*1*). The steroids coordinate circadian events and restore disturbances in homeostasis induced by stress (*2, 3*). Steroids released into the blood circulation can cross the blood-brain barrier and bind to receptors in neuronal tissue (*4*). In the rat, CA1 hippocampal cells are important target sites for corticosterone (*5, 6*) and express genes that encode for two types of corticosteroid receptors (*7*): (i) the mineralocorticoid receptor, which contributes to control of basal activities throughout the circadian cycle and (ii) the glucocorticoid receptor, which is involved in feedback control of the stress response and which displays a tenfold lower affinity to corticosterone than the mineralocorticoid receptor (*8*).

With in vivo electrophysiological recording, Pfaff and co-workers found that peripherally injected corticosterone decreases unit activity in the dorsal hippocampus (*9*). Subsequent electrophysiological investigations both in vivo and in vitro yielded variable and contrasting data (*10, 11*). The variability can be partly understood in light of the recently recognized receptor heterogeneity. We have used intracellular recording techniques to establish membrane properties of identified CA1 neurons in slices from adrenalectomized rats and have studied the effect of selective glucocorticoid analogs on these neuronal properties.

All experiments were performed in slices of the rat dorsal hippocampus (*12*). The micropipette was inserted into the CA1 pyramidal cell layer, and signals were amplified, filtered, and registered with conventional intracellular recording techniques (*12*). CA1 pyramidal cells were identified as described (*13*). Only neurons exhibiting a stable resting membrane potential (>−55 mV) for more than 30 min and spike amplitudes of at least 80 mV were included. The slices were completely submerged and superfused with standard artificial cerebrospinal fluid of 32°C to which drugs and transmitters could be added. Adrenalectomy (ADX) or sham operation was carried out rapidly in anesthetized rats approximately 7 days before the experiment (*14, 15*).

We established the membrane properties of CA1 neurons in slices obtained from animals 7 days after ADX and compared these with neuronal properties of sham-operated controls. Table 1 shows that no significant differences between the two groups of neurons were observed with respect to resting membrane potential or input resistance. However, the amplitude of the afterhyperpolarization (AHP), which was evoked by a depolarizing current step (50 ms) and is associated with the activation of a slow Ca^{2+}-dependent K^+ conductance (*16–18*), was significantly decreased in cells from ADX animals (Fig. 1A). The CA1 pyramidal neurons from ADX animals yielded consistent data, whereas the variability in the sham controls was considerable, possibly because of variation in plasma corticosteroid concentrations before the slice preparation. The decrease in the AHP was apparent over the whole range (0.2 to 1.0 nA) of tested current steps (Fig. 1B). In contrast, AHPs recorded in slices that were prepared only 1 hour after ADX were not significantly different from their time-matched sham controls.

We next pursued the nature of the corti-

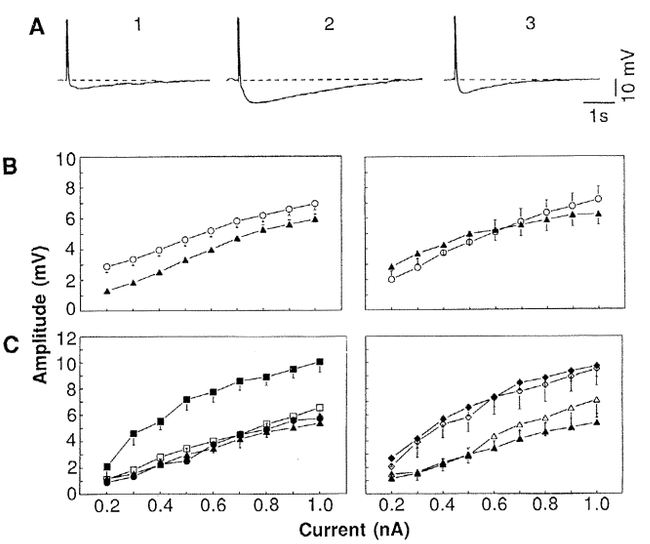

Fig. 1. (**A**) The AHP induced in CA1 pyramidal neurons by a 0.5-nA depolarizing pulse of 50-ms duration. The relatively small AHP in (A_1) is a typical example of the AHP obtained for neurons in slices from rats 7 days after ADX. (A_2) Treatment of a slice with $10^{-7}M$ of the selective glucocorticoid agonist RU 28362 results in a marked increase in the amplitude and duration of the slow AHP. (A_3) Recording of AHP in a slice from a sham-operated rat. In all three records, action potentials are truncated. (**B**) Amplitude of the slow AHP evoked in CA1 pyramidal cells by 50-ms current pulses of increasing intensity. (Left) Neurons recorded in slices from rats 7 days after ADX (▲, $n = 62$) displayed a smaller AHP amplitude when compared with their sham controls (○, $n = 38$). (Right) When the slices were prepared within 1 hour after ADX no differences were observed between the CA1 neurons from ADX rats (▲, $n = 14$) and their time-matched sham controls (○, $n = 11$). (**C**) (Left) A 20-min exposure of slices from ADX rats to $10^{-6}M$ corticosterone (■, $n = 6$) 30 to 90 min before recording resulted in a marked increase in the AHP amplitude when compared to the neurons recorded before the slices were exposed to corticosterone (▲, $n = 18$). If the glucocorticoid antagonist RU 38486 ($10^{-6}M$) was present during the whole experiment, no differences were observed between neurons before (●, $n = 6$) and after (□, $n = 9$) corticosterone treatment. (Right) Perfusion of the slices from ADX rats with $10^{-6}M$ (◆, $n = 9$) or $10^{-8}M$ (◇, $n = 7$) of the selective glucocorticoid agonist RU 28362 (30 to 90 min before recording) increased the amplitude of the AHP significantly when compared with the AHP obtained in CA1 neurons before exposure to the agonist (▲, $n = 18$). The amplitude of the AHPs recorded after slices were exposed to $10^{-9}M$ RU 28362 ($n = 8$) was somewhat smaller but still significantly different from the controls ($P < 0.05$). With $10^{-10}M$ RU 28362 (△, $n = 4$) the increases were not significant. Statistics were done with a multivariate analysis of variance test for repeated measurements.

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Table 1. Membrane properties (mean ± SEM) of CA1 pyramidal cells in hippocampal slices from ADX or sham-operated rats. The amplitude of the AHP induced by a 0.5-nA depolarizing pulse of 50 ms duration was significantly decreased in neurons from rats 7 days after ADX. No differences were observed in slices that were prepared only 1 hour after ADX or sham operation. The means for ADX and sham groups were tested for significance by the *t* test. Numbers in parentheses indicate the number of tested cells.

Time after surgery	Membrane potential (mV)	Input resistance (megohm)	AHP amplitude (mV)
One hour			
ADX (14)	−64.5 ± 1.3	50.8 ± 4.8	4.9 ± 0.7
Sham (11)	−67.4 ± 1.1	51.7 ± 3.8	4.4 ± 0.7
Seven days			
ADX (63)	−65.5 ± 0.4	45.6 ± 1.5	3.2 ± 0.2*
Sham (39)	−64.9 ± 0.8	46.5 ± 2.4	4.6 ± 0.4

*$P < 0.05$.

costeroid receptor involved in the changes in the AHP. A 20-min perfusion with 1 μM corticosterone followed by 30 to 90 min of washout before impailment of the neuron was sufficient to increase the amplitude of the AHP to a level exceeding the values obtained for cells from sham-operated controls (Tables 1 and 2). The increase was suppressed in the presence of the glucocorticoid antagonist (*19*) RU 38486 (Table 2 and Fig. 1C). Correspondingly, a 20-min treatment with the selective (*6, 20*) glucocorticoid agonist RU 28362 (10^{-9} to $10^{-6} M$) 30 to 90 min before recording induced a similar increase in the AHP amplitude as corticosterone itself (Fig 1, A and C). Glucocorticoid treatment also increased the half-decay time of the AHP. These changes occurred in the absence of consistent changes in overall passive membrane properties; we also did not observe differences in the average number of action potentials associated with the 50-ms depolarizing pulse. If the duration of the depolarizing pulse was increased to 500 ms, differences in AHP amplitude between the various groups of cells were somewhat less pronounced, but still significant.

The amplitude of the AHP in CA1 pyramidal cells is under control of many transmitters (*21*). Studies have shown that activation of the β-adrenergic receptor by norepinephrine (NE) results in an adenosine 3′,5′-monophosphate (cAMP)–mediated decrease of the slow AHP (*22*). To evaluate the functional implication of an increase in the AHP by corticosterone, we established how glucocorticoids can affect NE responses in hippocampus. NE increased the total number of action potentials induced by a prolonged (500 ms) depolarizing pulse dose dependently (Fig. 2). The number of action potentials depends (in part) on the degree of activation of the slow Ca^{2+}-dependent K^+ conductance and is inversely related to the amplitude of the slow AHP. The observed increase in total number of action potentials induced by $10^{-7} M$ and $10^{-6} M$ NE was larger for the CA1 cells in slices from ADX animals than for their sham controls. Addition of corticosterone or the agonist RU 28362 reduced the values from ADX cells to or below the level of the sham controls. Similar differences were observed for the reduction in the AHP induced by $10^{-7} M$ NE.

We have shown that the AHP of CA1 neurons in slices from ADX rats is reduced when compared with the sham controls. This finding is in agreement with a study by Kerr and Landfield (*23*). Selective activation of the glucocorticoid receptor in identified CA1 pyramidal cells induces a prolonged increase of the slow AHP. The changes in AHP are apparent 30 to 90 min after a brief (20 min) exposure to the steroids; no changes in AHP occur during or shortly after the steroid application (*24*). Thus, the time delay in our glucocorticoid effects is of the same order as the delay in genomic actions of the steroids described by others (*25*). We also observed that corticosteroids reduce the NE-evoked augmentation of depolarization-induced neuronal activity in CA1 cells. Both effects will result in a reduced hippocampal excitability. In their extracellular study, Pfaff and co-workers observed decreased unit activity in the dorsal hippocampus of hypophysectomized rats after a peripheral injection of corticosterone (0.5 to 1.0 mg/kg) (*9*). Injection of this amount of hormone can cause nearly complete occupation of the glucocorticoid receptor in the hippocampus (*5, 6*). Therefore, the activation of the glucocorticoid receptor may contribute to and underlie the reduced electrical activity in the hippocampus observed by others.

Our experiments have some advantages over previous studies: (i) Most of our experiments were performed in ADX rats, so that all types of corticosteroid receptors are accessible. (ii) With recently developed glucocorticoid analogs we were able to selectively activate or block only one type of corticosteroid receptor in CA1 neurons, in this case the glucocorticoid receptor. We observed effects with concentrations of RU 28362 as low as $10^{-9} M$, which is close to the dissociation constant for the analog (*6, 20*). We studied the dose dependence with RU 28362 rather than with corticosterone, because at low concentrations possible effects of the latter compound on the mineralocorticoid receptor could mask the glucocorticoid receptor–mediated actions. (iii) In previous field potential recordings, changes by corticosterone reflected the over-

Table 2. Membrane properties (mean ± SEM) of CA1 pyramidal cells in hippocampal slices from ADX rats before (ADX, $n = 18$) and after a 20-min superfusion of corticosterone (ADX/CT, $10^{-6} M$, $n = 6$) or the selective glucocorticoid agonist RU 28362 (ADX/RU 28362, $10^{-6} M$, $n = 9$). The amplitude and duration of the AHP after a 0.5-nA depolarizing pulse of 50 ms duration (AHP_s) or 500 ms duration (AHP_l) are increased after treatment with the glucocorticoids. In slices treated with the glucocorticoid antagonist RU 38486 (ADX/RU 38486, $10^{-6} M$, $n = 7$), corticosterone could no longer induce significant changes in the AHP (ADX/CT/RU 38486, $n = 9$). No consistent differences were observed between the groups for membrane potential or input resistance. Statistics were done by one-way analysis of variance with the Student-Newman-Keuls test for multiple comparisons between means.

Treatment	Membrane potential (mV)	Input resistance (megohm)	AHP_s amplitude (mV)	AHP_s half-decay time (s)	AHP_s number of action potentials	AHP_l amplitude (mV)	AHP_l number of action potentials
ADX	−66.0 ± 0.6	47.2 ± 2.7	3.0 ± 0.5	0.85 ± 0.11	3.2 ± 0.2	7.2 ± 0.7	5.9 ± 0.5
ADX/RU 38486	−67.0 ± 0.8	50.9 ± 2.6	2.5 ± 0.3	0.87 ± 0.12	2.9 ± 0.2	9.1 ± 0.6	5.6 ± 0.4
ADX/CT/RU 38486	−65.2 ± 1.1	47.7 ± 2.7	3.4 ± 0.7	0.83 ± 0.13	3.3 ± 0.2	9.5 ± 0.5	7.4 ± 0.5
ADX/CT	−65.8 ± 0.9	57.2 ± 3.5	7.2 ± 0.8*	1.37 ± 0.12*	3.3 ± 0.2	10.7 ± 0.5*	5.0 ± 0.3
ADX/RU 28362	−66.9 ± 1.1	52.1 ± 3.2	6.6 ± 0.7*	1.35 ± 0.08*	3.4 ± 0.2	10.9 ± 0.8*	5.4 ± 0.3

*$P < 0.05$.

all effects of pre- and postsynaptic elements (*11*). Although possible, there is little evidence that presynaptic actions of steroids on the γ-aminobutyric acid A ($GABA_A$) receptor, as reported by others (*26*), will contribute much to the observed changes in the AHP. (iv) In our study all neurons were recorded 30 to 90 min after washout of corticosterone, so that possible acute membrane effects that disappear rapidly after washout of the steroid (*27*) could not interfere with the more prolonged genomic actions of the steroid. The variable results obtained in previous studies with corticosterone might be attributed to differences in one or more of these conditions.

The AHP can be subdivided into three components on the basis of time course, Ca^{2+} dependence, and drug sensitivity (*18*). The time course of the AHP component affected by corticosterone and the fact that corticosterone can change the AHP component that is reduced by NE in the same cell suggest that the steroid influences the slow component of the AHP. The amplitude of the slow AHP depends (among other things) on the resting membrane potential and input resistance (*16–18*). As no consistent differences in these resting membrane properties were observed among the various groups of cells, it is unlikely that they contribute to the observed effects. Also, the total number of action potentials or the action potential frequency during the depolarizing step affects the AHP amplitude (*17*). No changes for these parameters were observed either with a 50- or a 500-ms depolarizing pulse. Finally, the amplitude of the AHP might be affected by voltage-dependent conductances, for example, the anomalous rectifier. We cannot rule out that corticosterone may act on the anomalous rectifier, and thus indirectly on AHP amplitude.

Corticosterone and NE have opposite effects on the AHP. Such reciprocal control of a membrane conductance, also observed for the S-channel in *Aplysia* and the M-current in hippocampal cells (*28, 29*), may be a general principle by which transmitters, peptides, and (on a longer time scale) steroid hormones can modulate neuronal excitability and actions of each other.

The decreased responsiveness to NE due to glucocorticoid treatment may be explained by the opposite actions of these compounds on one membrane property, achieved through independent mechanisms, or may result from a steroid action on the cAMP-dependent cascade linked to the β-adrenergic receptor. The latter is corroborated by the observation that corticosterone suppresses the accumulation of cAMP associated with activation of the β-adrenergic receptor (*30*). Although our experiments only reveal the interaction of steroid hormones with postsynaptic NE receptors, it is conceivable that feedback activation of the glucocorticoid receptor after stress can thus reduce the overall excitability of the tissue evoked by stress-induced release of NE.

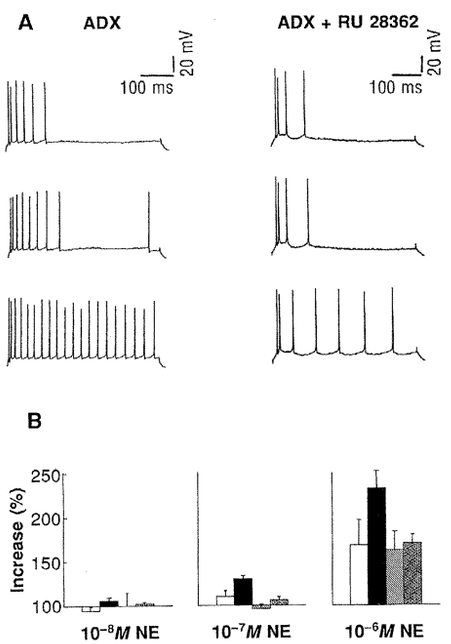

Fig. 2. Interaction of NE and glucocorticoid agonists in CA1 pyramidal cells. (**A**) Action potentials induced by a 0.5-nA depolarizing pulse (500 ms), under control conditions (upper panels), in the presence of $10^{-7}M$ NE (middle), or in the presence of $10^{-6}M$ NE (lower). The CA1 pyramidal cell shown at the left was recorded in a slice obtained from a rat 7 days after ADX. A concentration of $10^{-7}M$ NE induced a small increase in the total number of action potentials in response to the current pulse. Marked increases in the number of action potentials were observed with $10^{-6}M$ NE. The CA1 cell depicted on the right was obtained 7 days after ADX after a slice from a rat was treated with $10^{-7}M$ of the glucocorticoid agonist RU 28362. Exposure to the glucocorticoid was started 80 min before the test with NE. The responses to NE were suppressed when the slice was exposed to the glucocorticoid agonist. We added NE to the perfusion medium for 5 min. The cellular responses to the current pulse were tested once every 3 min. At least 15 min were allowed to pass between the testing of successive NE concentrations or as long as was necessary for the NE-induced effects to normalize. The NE-induced responses in this figure were obtained just before the NE-containing medium was switched back to the standard medium. (**B**) Average increases (+SEM) in the total number of action potentials induced by a depolarizing pulse, as shown in (A), for $10^{-8}M$ NE, $10^{-7}M$ NE, and $10^{-6}M$ NE. CA1 neurons from slices of ADX rats (black bars, $n = 10$) displayed larger responses to $10^{-7}M$ NE or $10^{-6}M$ NE than either cells from slices treated with $10^{-6}M$ corticosterone (light gray bars, $n = 7$) or $10^{-7}M$ RU 28362 (dark gray bars, $n = 7$), or cells from slices of sham-operated rats (open bars, $n = 10$). Statistics were done by one-way analysis of variance with the Student–Newman-Keuls test for multiple comparisons between means ($P < 0.05$).

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12. Transverse hippocampal slices (350 μm thick) from male Wistar rats (120 to 180 g in body weight) were cut on a McIlwain tissue chopper, incubated, and placed in gassed (95% O_2 plus 5% CO_2) artificial cerebrospinal fluid (ACSF) of the following composition: 124 m*M* NaCl, 3.5 m*M* KCl, 1.25 m*M* NaH_2PO_4, 1.5 m*M* $MgSO_4 \cdot 7H_2O$, 2.0 m*M* $CaCl_2$, 25 m*M* $NaHCO_3$, and 10 m*M* glucose. The slices were completely submerged and continuously superfused with warm ACSF (32°C) at a constant rate (2 ml/min). The standard ACSF could at any time be switched to an ACSF medium containing a transmitter or hormone in a known concentration. (−)Arterenol hydrochloride (norepinephrine) was obtained from Sigma. A 1 m*M* NE-containing ACSF stock solution was prepared freshly during the experiment and, immediately before testing, diluted to a lower concentration to prevent oxidation of the NE. Both corticosterone (Organon International, Oss, the Netherlands), RU 28362, and RU 38486 (Roussel-Uclaf, Romainville, France) were dissolved in ethanol (1 m*M*) and kept at −20°C. Just before testing, the steroids were diluted to the intended concentration in oxygenated ACSF. Control experiments with ethanol yielded no significant changes in the amplitude of the AHP. Intracellular recordings of CA1 neurons were made with 4*M* potassium acetate– (or in some cases 3*M* potassium chloride–) filled microelectrodes (impedances, 80 to 150 megohms and 50 to 90 megohms, respectively). The signals were amplified with an Axoclamp 2A amplifier and continuously displayed on a Gould digital storage oscilloscope. The membrane potential and applied current were registered on a Gould 2200 chart recorder and in some cases on a Vetter videocassette instrumentation recorder for later analysis. The membrane resistance was monitored at regular intervals by passing current pulses (0.1 to 0.3 nA, 150 ms) through the record-

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15. Male Wistar rats were housed in an animal room with 12-hour alternating dark:light cycle (8 p.m. to 8 a.m.:8 a.m. to 8 p.m.). Food and water were available ad libitum. The night before ADX, the rats were transferred to the operating room to adapt to the new environment. The next morning (8 to 9 a.m.) the rats were quickly adrenalectomized under ether anesthesia as in (*14*). The sham-operated rats were subjected to the same procedure, except that their adrenals were not removed. After the operation, drinking water was replaced by saline for the animals without adrenals. Both sham-operated and ADX rats were allowed approximately 7 days recovery before the experiment. On the day of the experiment, the rat was placed in a clean cage and put in the decapitation room 30 to 60 min before decapitation. After decapitation the trunk blood of the rat was collected for later analysis of the plasma corticosterone level. All ADX rats used in the present investigation showed corticosterone levels of less than 1 μg per 100 ml of plasma. The sham-operated animals generally exhibited corticosterone levels corresponding to mild stress conditions (8.5 ± 1.9 μg/100 ml, $n = 14$).
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Corticosteroid Modulation of Hippocampal Potentials: Increased Effect with Aging

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Adrenal steroids bind specifically to hippocampal neurons under normal conditions and may contribute to hippocampal cell loss during aging, but little is known about the neurophysiological mechanisms by which they may change hippocampal cell function. In the present studies, adrenal steroids have been shown to modulate a well-defined membrane conductance in hippocampal pyramidal cells. The calcium-dependent slow afterhyperpolarization is reduced in hippocampal slices from adrenalectomized rats, and it is increased after in vivo or in vitro administration of the adrenal steroid, corticosterone. Calcium action potentials are also reduced in adrenalectomized animals, indicating that the primary effect of corticosteroids may be on calcium conductance. The afterhyperpolarization component reduced by adrenalectomy is greater in aged rats than in young rats, suggesting that, with aging, there is an increased effect of corticosteroids on some calcium-mediated brain processes. Because elevated concentrations of intracellular calcium can be cytotoxic, these observations may increase the understanding of glucocorticoid involvement in brain aging as well as of the normal functions of these steroids in the brain.

The finding that some brain regions, particularly the hippocampus and related structures, are rich in specific corticosteroid receptors (*1*) has led to considerable speculation and research on the possible functions of corticosteroids in the brain (*2*). Hippocampal corticosteroid receptors are important for negative feedback regulation of adrenocorticotropic hormone (ACTH), for behavioral functions, and for the synthesis of several neuronal or glial proteins (*2*). Some electrophysiological studies have found that corticosteroids reduce ongoing electrical activity (*3*), whereas others have found apparent strengthening of synaptic function (*4*). In addition, rapid membrane effects (generally hyperpolarization) of steroids on hypothalamic or other neurons (*5*) have been described. However, the specific neurophysiological mechanisms through which corticosteroids modify neuronal function are still not well understood.

Corticosteroids also have been implicated in hippocampal aging. That is, under stressful conditions, corticosterone concentrations are elevated in aging rats (*6*), and long-term corticosteroid exposure may contribute to aspects of hippocampal aging, including neuronal loss and astrocyte reactivity (*7*). In addition, neuronal calcium homeostasis appears to be altered with aging. Calcium-dependent afterhyperpolarizations (AHPs) and Ca^{2+} action potentials are prolonged in aged rat hippocampal neurons, probably because of changes in membrane Ca^{2+} conductance (G_{Ca}) (*8*). There is also evidence that other aspects of Ca^{2+} homeostasis may be disturbed in the aging nervous system (*9*), including evidence of reduced Ca^{2+} clearance from terminals (*10*). Moreover, nimodipine, a Ca^{2+} channel antagonist, counteracts age-related decreases in a number of behavioral and plasticity functions (*11*).

In the present studies, we tested the hypothesis that corticosteroids modulate neuronal G_{Ca} (*12*); we measured the effects of adrenalectomy (ADX) and corticosterone (CORT) on the activation of the slow AHP,

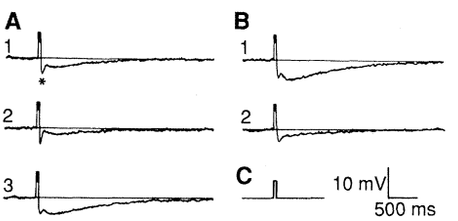

Fig. 1. Representative intracellular recordings showing current-induced bursts of three Na^+ action potentials (cut off at the top for illustration purposes) and subsequent AHPs in CA1 pyramidal cells of hippocampal slices. Traces are the averages of five waveforms for each cell shown. (**A_1**) Young, intact rat cell; (**B_1**) aged, intact rat cell; (**A_2**) young, ADX rat cell; (**B_2**) aged, ADX rat cell; and (A_3) cell from a young, ADX rat that had received CORT replacement injections. (**C**) Current trace showing example of intracellular depolarizing constant current pulse (40 ms, 0.15 to 0.25 nA) used to elicit three action potential bursts in all cells. Asterisk indicates peak of mAHP.

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