Mechanisms of Neuronal Hyperexcitability Caused by Partial Inhibition of Na\textsuperscript{+}-K\textsuperscript{+}-ATPases in the Rat CA1 Hippocampal Region

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VAILLEND, Cyrille, Susanne E. Mason, Matthew F. Cuttle, and Bradley E. Alger. Mechanisms of neuronal hyperexcitability caused by partial inhibition of Na\textsuperscript{+}-K\textsuperscript{+}-ATPases (Na\textsuperscript{+}-K\textsuperscript{+} pumps) on neuronal hyperexcitability. Bath application of the low-affinity cardiac glycoside, dihydroouabain (DHO), reversibly induced interictal-like epileptiform bursting activity in the CA1 region. Burst-firing was correlated with inhibition of the pumps, which was assayed by changes in [K\textsuperscript{+}]., uptake rates measured with K\textsuperscript{+}-ion-sensitive microelectrodes. Large increases in resting [K\textsuperscript{+}], did not occur. DHO induced a transient depolarization (5–6 mV) followed by a long-lasting hyperpolarization (~6 mV) in CA1 pyramidal neurons, which was accompanied by a 50% decrease in resting input resistance. Block of an electrogenic pump current could explain the depolarization but not the hyperpolarization of the membrane. Increasing [K\textsuperscript{+}] from 3 to 5.5 mM minimized these transient shifts in passive membrane properties without preventing DHO-induced hyperexcitability. DHO decreased synaptic transmission, but increased the coupling between excitatorypostsynaptic potentials and spike firing (E-S coupling). Monosynaptic inhibitory postsynaptic potential (IPSP) amplitudes declined to ~25% of control at the peak of bursting activity; however, miniature TTX-resistant inhibitory postsynaptic current amplitudes were unaffected. DHO also reduced the initial slope of the intracellular excitatory postsynaptic potential (EPSP) to ~40% of control. The conductances of pharmacologically isolated IPSPs and EPSPs in high-
Ca/high-Mg-containing saline were also reduced by DHO by ~50%. The extracellular fiber volley amplitude was reduced by 15–20%, suggesting that the decrease in neurotransmission was partly due to a reduction in presynaptic fiber excitability. DHO enhanced a late depolarizing potential that was superimposed on the EPSP and could obscure it. This potential was not blocked by antagonists of NMDA receptors, and blockade of NMDA, mGlu, or GABA\textsubscript{A} receptors did not affect burst firing. The late depolarizing component enabled the pyramidal cells to reach spike threshold without changing the actual voltage threshold for firing. We conclude that reduced GABAergic potentials and enhanced E-S coupling are the primary mechanisms underlying the hyperexcitability associated with impaired Na\textsuperscript{+}-K\textsuperscript{+} pump activity.

INTRODUCTION

Na\textsuperscript{+}-K\textsuperscript{+} ATPases (pumps) are membrane-bound enzymes that maintain [Na\textsuperscript{+}] and [K\textsuperscript{+}] gradients across the cell membrane. Na\textsuperscript{+}-K\textsuperscript{+} pumps, which are high-affinity binding sites for cardiac glycosides (CGs), are ubiquitous in neurons (Pietrini et al. 1992) and widely distributed in the mammalian brain (Hauger et al. 1985). At each translocation cycle of operation, the Na\textsuperscript{+}-K\textsuperscript{+} pumps extrude three Na\textsuperscript{+} ions and import two K\textsuperscript{+} ions and are therefore electrogenic. Pump activity contributes to the cell resting membrane potential, returns [Na\textsuperscript{+}] and [K\textsuperscript{+}] to their resting transmembrane levels after bursts of activity, and has secondary effects on processes involving monovalent cation gradients such as the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger (Blaustein 1993). Pump activity can be regulated by a variety of factors. Pump activity is stimulated by [Na\textsuperscript{+}] and [K\textsuperscript{+}], and can be specifically inhibited by CGs (MacGregor and Walker 1993). Major disruption of Na\textsuperscript{+}-K\textsuperscript{+} function causes severe alterations in neuronal function, but very little is known about partial modifications of Na\textsuperscript{+}-K\textsuperscript{+} pump activity. Interest in physiological regulation of these pumps has been considerably increased since the identification of ouabain-like compounds in the brain (Hamlyn et al. 1991). Therefore inhibition of Na\textsuperscript{+}-K\textsuperscript{+} pump activity by endogenous CGs may have profound effects on neuronal function and excitability.

In a previous study (McCarren and Alger 1987), we showed that partial inhibition of the pump could contribute to epileptiform discharges by increasing a voltage-dependent, Ca\textsuperscript{2+} conductance, but effects on synaptic transmission were largely unexplored. The present work was undertaken because of the likelihood that synaptic transmission would also be affected. A role for Na\textsuperscript{+}-K\textsuperscript{+} pumps in seizure onset in the brain was first suggested by in vivo studies that showed that the potencies of CGs or a variety of metal ions in inhibiting the Na\textsuperscript{+}-K\textsuperscript{+} pumps were positively correlated with their potencies in producing convulsions in rats (Donaldson et al. 1971). Decreases in Na\textsuperscript{+}-K\textsuperscript{+} pump activity occur in animal models of epilepsy and in hippocampal tissue from epileptic patients (Brines et al. 1995; Fernandes et al. 1996) and have been proposed as causal factors in myoclonus epilepsy and ragged red fibers (MERRF) disease, a rare inherited form of human epilepsy (McNamara 1994), and could play a role in other neurological disorders, e.g., bipolar illness (Christo and El-Mallakh 1993), spongiform encephalopathy (Renkawek et al. 1992), and Alzheimer’s disease (Rose and Valdes 1994). Disruptions of the genes encoding the Na\textsuperscript{+}-K\textsuperscript{+} pumps are candidate mechanisms for human temporal lobe epilepsy based on their chromosomal locations.
and the importance of ion homeostasis in maintaining normal excitability (Ferraro et al. 1999).

Na\(^+\)-K\(^+\) pump dysfunction could play a role in regulation of seizure susceptibility by elevating [K\(^+\)]\(_o\) and thereby increasing neuronal excitability (McNamara 1994). Application of ouabain can cause a massive efflux of K\(^+\) ions and cell depolarization (Haglund and Schwartzkroin 1990), epileptic activity, and ultimately, cell death (Lees and Leong 1994). However, increased [K\(^+\)]\(_o\) and seizure initiation by CG administration are not always correlated (Cordingley and Somjen 1978), suggesting that additional factors may be involved. Dihydroouabain, DHO, a low-affinity analogue of ouabain, reversibly causes interictal-like epileptiform activity in CA1 hippocampal population spike recordings that is not associated with large increases in [K\(^+\)]\(_o\) (McCarron and Alger 1987). Synaptic potentials have not been examined extensively, and it remains unclear which effects of DHO underlie the epileptiform activity.

The goal of the present study was to determine how partial Na\(^+\)-K\(^+\) pump inhibition in the rat hippocampal slice preparation leads to the onset of burst potential firing. We confirmed that DHO-induced epileptiform activity cannot be accounted for by large increases in [K\(^+\)]\(_o\), or by changes in active or passive membrane properties of CA1 pyramidal neurons. DHO-induced hyperexcitability was characterized by enhanced E-S coupling, which was facilitated by reduced synaptic input and an enhanced late depolarizing potential. Although the mechanisms by which DHO enhanced excitability appeared similar to those underlying the hyperexcitability induced by GABAA\(_x\)-receptor antagonists, they may also involve distinct factors.

Much of the work reported in this report was part of the PhD thesis of S. E. Mason.

METHODS

Hippocampal slice preparation and solutions

Male Sprague-Dawley rats aged 35–70 days (Charles River Laboratories) were deeply sedated with halothane and decapitated in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine. The brain was removed and hippocampi were dissected out and cut into 400-μm-thick transverse slices using a Vibratome Series 1000 (Technical Products International) as previously described (McCarron and Alger 1987). Slices were maintained at room temperature (20–22°C) in a holding chamber at the interface of a physiological saline and a humidified 95% O\(_2\)-5% CO\(_2\) mixture. After ≥1 h of incubation a single slice was transferred to a recording chamber (Nicoll and Alger 1981), where it was held between two nylon nets and continuously perfused with oxygenated saline (29–31°C) at a flow rate of 0.5–1 ml/min.

The extracellular physiological saline (ACSF) comprised (in mM) 120 NaCl, 3 KCl, 2 MgSO\(_4\), 1 Na\(_2\)HPO\(_4\), 25 NaHCO\(_3\), 2.5 CaCl\(_2\), and 10 glucose (pH 7.4 when bubbled with 95% O\(_2\)-5% CO\(_2\)). In some experiments, the slices in the recording chamber were perfused with a saline in which the concentration of KCl was raised to 5.5 mM (see results). Slices were allowed to adapt to this new saline for ~45 min before the start of recordings. Responses were recorded in the same slice in control, experimental, and wash solutions. Drugs were bath applied. Stock solutions DHO (10 mM) were made up in distilled water, and DHO was bath applied at a final concentration of 10–20 μM for 15 min. All other drugs were also prepared as concentrated stock solutions and diluted 1:1,000 in saline. Drugs used included d,l-2-amino-5-phosphonovaleric acid (d,l-APV (50–100 μM), baclofen (5 μM), CGP 35348 (400 μM), CGP 55845 (5 μM), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1 μM), lidocaine (100–300 μM), LY 341495 (100 μM), 2,3-dixo-o-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX, 10 μM), picrotoxin (100 μM), and tetrodotoxin (TTX, 0.5–1 μM). CGP 35348, CGP 55845, and LY 341495 were acquired from Tocris Cookson (Bristol, MO); all other drugs and chemicals were purchased from Sigma (St. Louis, MO).

Electrophysiological recordings

Extracellular recordings were carried out in the CA1 hippocampal area with glass microelectrodes broken to a tip diameter of 4–7 μm and filled with 1 M NaCl or with ACSF (5–15 MΩ). Field population responses were elicited using bipolar concentric stimulating electrodes. Stimulation (100–900 μA; 50–100 μs; 0.05 Hz) was delivered in the stratum radiatum. Presynaptic fiber volleys (FVs) and field excitatory postsynaptic potentials (fEPSPs) were recorded from s. radiatum, and population spikes (PS) were recorded from s. pyramidal. Antidromic CA1 population spikes were evoked by stimulation in the alveus. Input/output (I/O) curves were constructed by taking the slopes of the AMPA- or N-methyl-D-aspartate (NMDA)-receptor-mediated fEPSPs obtained at different intensities of stimulation.

Intracellular current-clamp recordings were made from CA1 pyramidal cells using sharp microelectrodes [80–160 MΩ filled with 1–3 M potassium methyl sulfate (KCH\(_3\)SO\(_4\), ICN, Aurora, OH)]. The pH of the recording solution was adjusted to 7.1 with HEPES. Acceptable cells had stable resting potentials of at least ~50 mV, total neuron input resistances of 35–80 MΩ, and overshooting action potentials. Most of the recordings were stable for ~1 h. Action potentials were elicited either by synaptic stimulation or direct ramp depolarization (~100 to 400 pA). Whole cell voltage-clamp recordings were obtained from CA1 pyramidal neurons using the blind method (Blanton et al. 1989). Whole cell patch electrodes (4–6 MΩ in the bath) were filled with (in mM) 35 KCH\(_3\)SO\(_4\), 120 KC\(_2\), 12, [1,2-bis(2-aminoenoxy)ethane-N,N,N',N'-tetraacetic acid] (BAPTA), 0.2 CaCl\(_2\), 1 MgATP, and 1 HEPES. The intracellular solution was adjusted to pH 7.3 with KOH. Series resistance was <12 MΩ at the beginning of an experiment, and cells were discarded if series resistance increased to >20 MΩ. The cells were voltage clamped at a potential near the membrane resting potential (~55 to ~70 mV). In these experiments, the “liquid junction potential” was part of the electrode “tip potential.” Tip potentials were measured as the voltage difference between the potential measured by the electrode in the bath before and after breaking the electrode tip back 3–5 mm. Measured in this way the tip potentials were ~12 mV, and inasmuch as the liquid junction potentials should be smaller than this, they were considered to be acceptable, and no corrections were made.

[K\(^+\)]\(_o\) was measured with a K\(^+\)-sensitive microelectrode (K+-ISM). The microelectrodes had tip diameters of 4–7 μm and were silverized by exposing the tips to dimethyl dichlorosilane vapor (Sigma) for 15 min, followed by baking for 1 h at 120°C. After cooling, the tips were filled with a liquid ion-exchanger resin made of 1% weight/volume mixture of potassium tetrakis (4-chlorophenyl) borate (Fluka) in 3-nitro-o-xylene (Aldrich), and backfilled with 150–500 mM KCl. The microelectrodes were calibrated at room temperature (~22°C) before and after the experiment by a series of test solutions containing 1–50 mM KCl with the NaCl concentration adjusted so that total Cl concentration was 123 mM. Electrode sensitivity ranged from 48 to 58 mV for a 10-fold change in [K\(^+\)]\(_o\), and the output voltages were linearly proportional to the log of the potassium ion activity >3 mM, as described by others (Lewis and Schuette 1975). The K+-ISM measurements are reported as concentration changes (Voipio et al. 1994). Electrodes with atypical sensitivities or with significant drift (>1 mV/30 min) were discarded. To reject common DC field potentials and isolate potential changes across the column of
ion exchanger due to shifts in potassium ion activity, the K$^+$-ISM recordings in s. pyramidalie of CA1 were differential with respect to a nearby field electrode. No appreciable drift in the extracellular DC potential was recorded by this reference field electrode in control or experimental solutions. The rate of K$^+$ clearance in response to low-frequency trains of afferent stimuli (5 Hz, 10 s) was monitored by measuring the time required for the transient change in [K$^+$], to return to 50% of control levels following the train (t$\text{i}_{1/2}$).

Data acquisition and analysis

Signals were amplified with either Axoclamp 2A, Axoclamp 2B, or Axopatch 1-C amplifiers (Axon Instruments). In cases of two-channel simultaneous recordings, one channel was filtered at 2 kHz with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA), and both channels were digitized at 10 kHz using a Digidata 1200 A/D converter (Axon Instruments). Spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were filtered at 1 kHz and digitized at 5 kHz. Off-line analysis was carried out using the Clampfit program in pCLAMP 7.0 (Axon Instruments).

EXTRACELLULAR RECORDINGS. Fiber volleys were quantified by their amplitude, which was defined as the difference between the initial positive and negative peaks. Amplitudes of antidromic population spikes were determined by taking the difference between baseline potential before the stimulus artifact and the negative peak of the fEPSP to that of the average baseline responses (% of control). The rate of K$^+$ clearance was determined by subtracting the amplitude of the negative peak from the mean amplitude of the two positive peaks. The initial slope of the fEPSP (Vi/s) was measured within the first millisecond of the response. Unless otherwise stated, the time course of field potentials was expressed as the mean percentage change from the average baseline responses (% of control). The paired-pulse ratio was quantified as the ratio of the amplitude of the second fEPSP to that of the first. The changes in basal [K$^+$], the amplitude of [K$^+$], elevation induced by a 5-Hz/10-s train, and the half-time for K$^+$ clearance (t$\text{i}_{1/2}$) from the end of this train were determined from K$^+$-ISM measurements. The voltage deflections recorded with the K$^+$-ISM were converted to [K$^+$], in mM according to the semi-logarithmic calibration curves of the electrodes (Lewis and Schuette 1975).

E-S COUPLING. E-S coupling reflects the ability of an EPSP to discharge an action potential in the postsynaptic cell. Extracellular E-S coupling was expressed as the ratio of the PS amplitude to fEPSP initial slope. Extracellular E-S curves were constructed from a range of stimulation intensities in control and experimental salines, and were expressed as the normalized PS amplitude versus the normalized fEPSP slope (maximal responses were defined as 1). The E-S curves were fitted with a sigmoidal equation using the SigmaPlot 4.01 software (SPSS, Chicago, IL). The E$\text{M}$ was defined as the value of the normalized fEPSP slope at which the PS amplitude was 50% of its maximal response and was calculated from the following built-in function in SigmaPlot

$$E_{M} = \exp\left(\ln|c^*| \times ((a-d)/(0.5-d)) - 1|/b\right)$$

where a = max, b = slope, c = inflection point, and d = min.

Intracellular recordings were used to assess the cellular mechanisms underlying the E-S coupling changes induced by our experimental conditions. The probability of firing was represented by the ratio of spike-containing EPSP/inhibitory postsynaptic potential (IPSP) sequences to the total number of sampled EPSP/IPSP sequences in a 5-min recording period. To measure changes in firing probability, we used a subthreshold stimulus intensity that brought the cell to within 2–5 mV of threshold during baseline stimulation. Change in probability of firing was further quantified by measuring the minimal EPSP slope and the minimal stimulus intensity required to evoke an action potential.

STATISTICS. Data are reported as means ± SE. Most changes induced by the experimental salines are expressed as percent of control value, which is taken to be 100%. Paired comparisons of parameters measured at various time points (2- to 5-min duration) over the course of the experiment were performed on averaged values. Normality and equal-variance tests were performed to select appropriate statistical tests for comparisons (SigmaStat 2.0, SPSS). Data that successfully passed these tests were analyzed using Student’s paired or unpaired t-test for paired versus group comparisons, respectively. Nonparametric Wilcoxon and Mann-Whitney tests based on rank comparisons were performed for paired versus group comparisons, respectively, when data failed the normality and/or equal-variance tests. One-factor ANOVAs followed by post hoc Dunnet or ANOVAs with repeated measures followed by post hoc Tukey tests were run for multiple-group comparisons. Friedman repeated-measures ANOVA on ranks followed by post hoc Dunnett tests was performed for multiple comparisons when the normality test failed. Cumulative amplitude distributions of mIPSCs constructed in control and experimental saline were compared using the Kolmogorov-Smirnov (K-S) test with a significance level of P < 0.005. Correlations were calculated using the Pearson product moment correlation test.

RESULTS

Na$^+$-K$^+$ pump inhibition by dihydroouabain induces epileptiform activity without causing large increases in resting [K$^+$].

In agreement with previous results (McCarren and Alger 1987), we found that a 15-min bath application of DHO, 20 μM, reversibly caused the appearance of robust extracellular epileptiform burst potentials in hippocampal CA1 (Fig. 1A1) in response to single stimuli to s. radiatum. The bursts resembled interictal-like burst potentials recorded in other models of epileptiform bursting. Ictal-like seizure activity—prolonged, spontaneous bursts—was never observed; however, brief spontaneous burst discharges that originated in CA3 and spread into CA1 (Korn et al. 1987) often occurred. Surgical removal of the CA3 subfield blocked these spontaneous events, but it did not prevent evoked burst potentials in CA1 (n = 6, data not shown).

The time course of bursting activity is represented by the presence of a second population spike (PS2) in the burst potential, as shown in Fig. 1A3. This “bursting period” generally spanned the period from the 10th min of the DHO application to about the 20th min of DHO wash, although in some slices a wash period of >30 min was required for complete reversal of burst activity (n = 7; not shown). Epileptiform activity induced by lower doses of DHO (i.e., 10 μM) usually took longer to develop and was often characterized by a burst of only two population spikes. Thus a 15-min bath application of 20 μM DHO induces robust yet reversible bursting activity, and in all experiments DHO was washed off after 15 min.

To verify that DHO inhibits the Na$^+$-K$^+$ pumps and to determine whether the burst potentials generated by DHO were correlated with an impairment of Na$^+$-K$^+$ pump activity, we monitored the change in the rate of K$^+$ uptake using K$^+$-ISMs. Impaired K$^+$ transport by the pumps would be reflected by a decreased rate of recovery of [K$^+$], baseline after a short stimulus train (Krnievic and Morris 1975). Figure 1B1 illustrates a typical K$^+$-ISM response in control, DHO and wash solutions, following a train of afferent stimuli (5 Hz for 10 s). The half-time for [K$^+$], clearance (t$\text{i}_{1/2}$) slowed an average of 403 ± 59% of control during DHO-induced bursting (i.e., n = 4.2 to 17.2 s, n = 10, P < 0.01). Moreover, the amplitude of
the train-induced $[K^+]_o$ transient was larger in DHO (5.0 ± 0.8 mM) than in control (2.8 ± 0.3 mM; $n = 10$, $P < 0.01$). Both the changes in $[K^+]_o$ transient amplitude and clearance rate are signs of reduced Na\(^{+}\)-K\(^{+}\) pump activity. Na\(^{+}\)-K\(^{+}\) pump inhibition by 20 μM DHO did not produce large increases in resting $[K^+]_o$. Indeed, the bursting activity induced by DHO was only accompanied by a modest elevation in resting $[K^+]_o$ (0.8 ± 0.1 mM; $n = 10$, $P < 0.001$), an effect that reversed after 25 min of wash. In the example shown in Fig. 1B1, resting $[K^+]_o$ was only increased by 0.22 mM, while the $t_{1/2}$ was prolonged to 221% of control in agreement with previous results.

Successive stimulus trains were applied over the course of the experiment in five slices to evaluate whether the time course of excitability correlates with inhibition of the Na\(^{+}\)-K\(^{+}\) pumps. Figure 1B2 shows that inhibition of the Na\(^{+}\)-K\(^{+}\) pumps by DHO reverses with time as DHO is washed off. Data recorded during 1 h of wash were fitted with a single exponential, showing that the effect of DHO washed off with a decay-time constant of 12 min. These data suggest that mild inhibition of the Na\(^{+}\)-K\(^{+}\) pumps by DHO is sufficient to increase excitability. In the remainder of this report we examine passive and active properties of CA1 pyramidal cells, synaptically activated potentials, and the coupling between synaptic potentials and burst firing, to understand the mechanisms by which partial Na\(^{+}\)-K\(^{+}\) pump inhibition increases neuronal excitability.

**DHO induces transient shifts in passive membrane properties of CA1 pyramidal neurons**

Time courses of the effects of 20 μM DHO on resting membrane potential ($V_m$), total neuron input resistance ($R_i$), and time constant ($\tau$) are presented in Fig. 2A. $R_i$ was measured as the input “chord resistance” (calculated from the magnitude of membrane deflections from the resting potential caused by single 100-ms-long current steps of −100 pA). Within 5 min of application, DHO caused a mean transient depolarization of 5.4 ± 1.0 mV ($n = 8$, $P < 0.001$; Fig. 2A1). Surprisingly, the DHO-induced depolarization ended just before the onset of bursting activity. The transitory depolarization was followed by a long-lasting membrane hyperpolarization that persisted throughout the bursting period (Fig. 2A1). For the 12 cells that provided the data for Fig. 2, the absolute change in $V_m$ during the hyperpolarization was −3.0 ± 0.9 mV below the resting potential after a 15-min application of DHO ($P < 0.01$), and it further increased to −6.12 ± 1.4 mV 10 min after starting DHO wash ($P < 0.01$). After 20 min of DHO wash, the membrane potential depolarized back toward control values. Changes in input resistance and time constant followed a similar time course to changes in $V_m$. As shown in Fig. 2A2, $R_i$ decreased to 73% of control (i.e., −13.0 ± 2.8 MΩ) at 15 min in DHO ($n = 12$, $P < 0.001$), when bursting was fully established. Likewise, the time constant (determined by fitting a single exponential to the charging phase of the voltage transient caused by the dc step) was decreased by −6.7 ± 1.3 ms at the time of peak bursting ($n = 12$, $P < 0.001$; Fig. 2A3).

When applying long hyperpolarizing current steps (200–400 ms), we noticed that the delayed membrane potential “sag” (Purpura et al. 1968) caused by repolarizing $I_h$ current (originally $I_h$) (Halliwell and Adams 1982) was much reduced in
DHO (see for example, Fig. 4B1). The reduction in membrane potential sag could mean that the \( I_h \) was inhibited or, on the contrary, persistently activated at rest by DHO. To distinguish between these possibilities, we tested the effects of DHO on \( R_i \) in the presence of 2 mM Cs, which blocks \( I_h \) (Halliwell and Adams 1982; Magee 1998), and found that in these conditions, DHO had a significantly reduced effect on \( R_i \) (\( P < 0.05, n = 7 \)). These results suggest, but do not prove, that DHO-induced reduction in \( R_i \) is attributable in part to activation of \( I_h \) at resting membrane potential levels where it is normally inactivated. More work must be done to test this hypothesis, however.

In a previous investigation, it was found that bath application of 10 \( \mu \)M DHO in a saline containing 5.4 mM [K\(^+\)]\({_o}\) was not associated with long-lasting membrane hyperpolarization or a significant decrease in \( R_i \) (McCarren and Alger 1987). The discrepancy between our present observations and those of McCarren and Alger could depend on the dose of DHO and/or [K\(^+\)]\({_o}\). To investigate this issue, we studied the effects of 10–20 \( \mu \)M DHO applications in a saline containing 5.5 mM [K\(^+\)]\({_o}\). As shown in Table 1, the effects of DHO on the Na\(^+\)-K\(^+\) pumps, as represented by the increases in the \( I_{1/2} \) for [K\(^+\)]\({_o}\) recovery, were independent of the concentration of DHO or of [K\(^+\)]\({_o}\). In line with the previous report (McCarren and Alger 1987), DHO applied in a 5.5 mM [K\(^+\)]\({_o}\)-containing saline increased resting [K\(^+\)]\({_o}\) by <1 mM. However, in 5.5 mM [K\(^+\)]\({_o}\), DHO did not hyperpolarize the membrane, but only depolarized it during the bursting period (Fig. 2B1 and Table 1). Nevertheless, epileptiform bursting was observed in all conditions (see e.g., Fig. 2B, 2 and 3). Hence, although the reason for the dramatic difference in results obtained in 3- and 5.5-mM [K\(^+\)]\({_o}\)-containing saline is not known, these data show that onset and recovery of extracellular and intracellular burst activity are independent of changes in \( V_m \) and \( R_i \).

Partial Na\(^+\)-K\(^+\) pump inhibition enhances coupling between fEPSPs and population spikes

We considered that DHO-induced hyperexcitability could be mediated through enhanced coupling of excitatory inputs and firing efficiency (E-S coupling). To assess this, we plotted the E-S coupling ratio (i.e., the amplitude of the first population spike divided by the slope of the fEPSP) in control and DHO-containing saline. Figure 3A shows that the E-S coupling ratio was greater in DHO (1.98 ± 0.3) than in control (0.92 ± 0.03; \( P < 0.001; n = 29 \)). The onset of enhanced E-S coupling was temporally correlated with bursting activity, and DHO induced a leftward shift of the E-S coupling curves (Fig. 3B). The normalized fEPSP slope that generated a PS amplitude at 50% of maximum (i.e., the \( E_{50} \)) was significantly smaller in DHO (0.35 ± 0.03) than in control (0.53 ± 0.03) at the time of maximum bursting activity (\( n = 9; P < 0.01 \)). In principle, enhanced E-S coupling could reflect a decrease in IPSPs, a decrease in the voltage threshold for intracellular spike firing, or an increase in EPSPs, and we investigated each of these possibilities.

DHO-induced hyperexcitability is associated with a decrease in GABAergic inhibition

E-S coupling is known to be affected by several pharmacological agents. Potentiation of E-S coupling can be blocked by
A1 adenosine-receptor antagonists (Fujii et al. 1999) or by the mGlur-selective antagonist (5)-α-methyl-4-carboxyphenylglycine (MCPG) (Breakwell et al. 1996). E-S coupling is enhanced by suppression of GABAergic IPSPs (Chavez-Noriega et al. 1989). We found that DHO-induced enhancement of field potential E-S coupling was not prevented by bath application of the NMDAR antagonist APV (100 μM, n = 3), the mGlur antagonist LY 341495 (100 μM, n = 3), the A1 receptor antagonist DPCPX (1 μM, n = 4), or LY 341495 plus APV (n = 3). Picrotoxin (PTX) induced a large leftward shift in the coupling, which was shown by decrease in the $E_{50}$ from 0.49 ± 0.02 to 0.24 ± 0.06 (Fig. 3C, n = 3, P < 0.05). DHO produced no further shift in E-S coupling ($E_{50} = 0.24 ± 0.01$; n = 3) in the presence of picrotoxin. Figure 3D illustrates that picrotoxin lowered the stimulus intensity threshold for population spike initiation and that DHO did not have a significant effect on spike threshold in the presence of picrotoxin. This suggested that DHO might suppress IPSPs; however, this could not be confirmed at the level of field potential recordings.

We therefore analyzed the effects of DHO on monosynaptic IPSPs recorded in CA1 pyramidal neurons (APV and NBQX present to block ionotropic glutamatergic transmission). As shown in Fig. 4A, DHO greatly suppressed IPSP amplitude [to 24.6 ± 3.2% of control at the end of the 15-min DHO application (n = 6; P < 0.001) following a time course that was temporally correlated with the bursting period (cf. Fig. 1)]. The IPSP amplitude remained depressed in DHO even when the cells were transiently repolarized with DC injection to the control resting membrane potential (not shown), showing that IPSP suppression by DHO was independent of the membrane hyperpolarization. DHO reduced both the early (<100 ms) and late (>100 ms) components of the synaptic response (see traces in Fig. 4A), implying that Na$^+$–K$^+$ pump inhibition altered both GABA$_A$ and GABA$_B$ components of IPSP, respectively, although we did not investigate the GABA$_B$ component extensively.

A reduction in IPSP size by DHO may be caused by partial blockade of the synaptic conductance ($g_{IPSP}$) and/or a shift in the IPSP reversal potential ($E_{IPSP}$). To assess these factors, we constructed $I-V$ relationships for the IPSPs evoked at a range of membrane potentials in seven cells (Fig. 4B). As illustrated in Figs. 4B, 1 and 2, DHO reversibly decreased the slope of the IPSP-V relationship and produced a small depolarizing shift in $E_{IPSP}$ after 15 min of application. We found that $g_{IPSP}$ was significantly smaller in DHO (11.3 ± 3.07 nS) than in control (20.4 ± 3.6 nS), i.e., it was reduced by 49 ± 5.2% (n = 7; P < 0.001). $E_{IPSP}$ shifted by $+4.9 ± 1.06$ mV during the bursting period (n = 7; P < 0.01). To examine DHO effects on IPSPs in the absence of marked changes in passive membrane properties, we also studied monosynaptic IPSPs in a saline containing 5.5 mM [K+]o as DHO has minimal effects on membrane properties in this saline (Fig. 2A and Table 1). The DHO-induced reduction of IPSP amplitude was less dramatic in 5.5 mM [K+]o; i.e., 20 μM DHO only reduced IPSP amplitude to 51.7 ± 16.6% of control (vs. 24.6 ± 3.6 nS). $g_{IPSP}$ was reduced to 30–60% of control in all conditions (see Table 1). Conversely, we found that, in the absence of DHO, exogenously increasing baseline [K+]o from 3 to 5.5 mM shifted $E_{IPSP}$ by $+6.5$ mV (n = 7) but did not produce bursting activity. Because this represents a larger shift in $E_{IPSP}$ than that induced by DHO in 3 mM [K+]o, a shift in $E_{IPSP}$ cannot explain DHO-induced bursting activity. It appears that the decrease in $g_{IPSP}$ is the most significant factor in DHO-induced burst firing.

DHO could reduce $g_{IPSP}$ either by reducing the amount of inhibitory transmitter released or by modifying the properties of postsynaptic GABA$_A$ receptors. Using conventional “blind” whole cell voltage-clamp techniques (Blanton et al. 1989), we found that DHO did not change mIPSC amplitude (42.5 ± 2.8 pA) compared with control (44.4 ± 1.7 pA, n = 5 cells; P > 0.01, K-S test, see Fig. 5C), suggesting that the 50% reduction of IPSP is not caused by postsynaptic factors. DHO did increase spontaneous mIPSC frequency from 2.35 ± 0.19 to 3.17 ± 0.23 Hz during the bursting period (100–500 events per cell, n = 5, P < 0.05, Fig. 5, A and B), showing that it does affect GABAergic nerve terminals directly.

**DHO does not alter action-potential threshold**

DHO might enhance spike firing by decreasing the threshold for action-potential initiation. We measured the action-potential threshold by injecting either rectangular current pulses (“step depolarization,” SD), or current ramps (“ramp depolarization”) into cells in the presence or absence of DHO (Fig. 6).
We also measured spike amplitudes and half-widths in 15 cells and observed that with either method of measurement spike amplitudes were reduced slightly (~10%) but significantly (Fig. 6B, left). Spike properties were measured in 5.5 mM [K+]o to minimize changes in Vm, and when necessary Vm was held constant by small DC injection. Similarly, half-widths were broadened significantly (~20%, Fig. 6B, middle). These alterations in action potential properties probably reflect a slight increase in [Na+]i caused by the partial Na⁺-K⁺ pump inhibition. However, the threshold for spike firing measured with either technique was unchanged in DHO (~48.21 ± 1.95 mV) compared with control (~48.09 ± 1.67 mV; n = 10, P = 0.8, NS, Fig. 6B, right). Therefore we conclude that DHO does not enhance E-S coupling by decreasing the voltage threshold for action-potential generation.

Partial Na⁺-K⁺ pump inhibition also reduces glutamatergic transmission

Suppression of inhibition could explain the DHO-induced hyperexcitability; however, it is also possible that DHO directly increases excitability. Surprisingly, we found that DHO reduced both the intracellular (Fig. 7A) and extracellular (Fig. 7B) EPSPs. The initial slope of the intracellular EPSPs began to decline after ~5 min in DHO and was reduced to 43 ± 4.7% of control (n = 9, P < 0.01) at the end of the DHO application when maximum bursting activity was observed (Fig. 7A2). The fEPSP slope declined to an average of 69 ± 4.9% of control at the time of bursting (n = 29, Fig. 7B). Interestingly, the EPSPs did not recover after ~20 min of DHO wash, whereas the population spike in the same slices, which represents the ability of the cells to fire action potentials, did recover (see Fig. 1A2). This indicates that the slice preparation and principal cells were not damaged by a neurotoxic effect of DHO application. Thus DHO-induced bursting activity was not temporally correlated with enhanced EPSPs. The actual magnitude of intracellular EPSP suppression is unclear with the mixed EPSP-IPSP responses shown in Fig. 7C.

To determine if the DHO effect is exerted on the glutamatergic synapses themselves, we studied isolated EPSPs with intracellular recordings in saline containing 100 μM picrotoxin and a high concentration of divalent cations (4 mM CaCl₂ and 4 mM MgSO₄; “high-Ca/high-Mg saline”) to block epileptiform firing. The CA3 region was removed from these slices to prevent spontaneous epileptiform activity, which originates in CA3, from invading CA1. Synaptic stimulation in the presence

FIG. 3. DHO enhances extracellular field EPSP (fEPSP)-to-spike (E-S) coupling. A: time course of the effects of DHO on extracellular E-S coupling. E-S coupling is represented by the ratio of the amplitude of PS1 (mV) to the fEPSP slope (V/s; n = 29, same slices as in Figs. 1A and 7B. An increase in the ratio indicates a given population spike is triggered by a smaller fEPSP in the presence of DHO than in control. B: the leftward shift of the E-S coupling curves, indicating that a larger PS1 was produced by a given fEPSP slope (n = 9) in DHO. The amplitudes of PS1 normalized to maximal amplitude are plotted against the fEPSP slope normalized to maximal slope in control (○) and DHO (15 min, ●). A leftward shift indicates enhanced E-S coupling. C: picrotoxin mimics and occludes the effect of DHO on the E-S coupling curve. When 100 μM picrotoxin was applied first, the E-S curve was shifted to the left (a) of the control curve (○). Subsequent application of DHO produced only a slight rightward shift of the E-S curve (●). D: group data showing that picrotoxin reduced the stimulus intensity required to trigger an intracellular initial spike (P < 0.05, n = 4).
of picrotoxin usually elicits complex depolarizing waveforms, even with high-Ca/high-Mg saline. To attempt to measure the properties of the monosynaptic EPSP without contamination by other conductances, we determined the time of occurrence of the EPSP peak from the differentiated depolarizing waveform and measured the peak at that time in the original traces (light vertical line in Fig. 8). Somewhat surprisingly, we did not find a consistent effect of DHO on EPSPs in the high-Ca/high-Mg saline: in three cells, \( g_{EPSP} \) was reduced by approximately the same extent as was \( g_{IPSP} \), i.e., to 54.9 \( \pm \) 10.6% of control (\( P < 0.05, n = 3 \)). In three other cells, \( g_{EPSP} \) was apparently unaffected by DHO, decreasing \( \leq 10\% \) in each case. DHO had no effect on membrane properties of either of the groups. We also checked DHO’s effects on IPSPs in high-Ca/high-Mg saline in 5 cells (data not shown) and found that DHO caused the same shift in IPSP reversal potential (of \(+5.4 \pm 1.1\) mV; \( P < 0.01 \)) and decrease in conductance (to 59.7 \( \pm \) 5.4% of control, open circle; \( P < 0.05, n = 5 \)), as it did in normal saline (see Table 1). We conclude that DHO can reduce both glutamatergic and GABAergic transmission but that the reduction in \( g_{EPSP} \) is not always readily apparent.

Conceivably, DHO-induced bursting could arise simply from a failure of polysynaptic inhibition. Because the Schaffer collaterals make monosynaptic contacts onto pyramidal cells, whereas polysynaptic pathways produce both feedforward and feedback IPSPs, a uniform suppression of polysynaptic conduction could cause a greater relative suppression of IPSPs than of EPSPs. However, neither lidocaine (100–300 \( \mu \)M, 10–15 min, \( n = 17 \)), NBQX (0.5–2 \( \mu \)M, 15 min, \( n = 3 \)), the GABA\(_B\)-selective agonist baclofen (5 \( \mu \)M, 2 min, \( n = 2 \)), nor perfusion with a low-calcium/high-magnesium saline (1.25 mM Ca\(^{2+}\)/4 mM Mg\(^{2+}\), \( n = 3 \)) produced epileptiform activity. Hence, uniform suppression of synaptic transmission in the slice does not cause hyperexcitability.

**DHO increases a late depolarizing potential**

A late depolarizing potential (Fig. 8) that is detectable in picrotoxin represents a possible key factor in the development
of hyperexcitability produced by partial $\text{Na}^+-\text{K}^+$ pump inhibition. Picrotoxin greatly reduced the stimulus intensity necessary to trigger an initial spike (104.75 ± 8.78 μA) compared with control (195.25 ± 17.78 μA) and DHO did not decrease action-potential threshold in the presence of picrotoxin. Thus the effects of DHO could be occluded by blocking GABAA-ergic IPSPs with picrotoxin, suggesting again that suppression of IPSPs is the primary mechanism for DHO-induced bursting.

The amplitude of the late depolarizing potential was only decreased to 94.7 ± 11.7% of control by DHO (Fig. 8B, $P > 0.9$, NS). To determine if this late potential is a type of conventional EPSP, we attempted to measure its conductance by evoking it at a subthreshold range of membrane potentials but found that its amplitude did not change significantly over this range (Fig. 8C). This could mean either that the late depolarization is not a conductance-increase phenomenon or that it is produced in an electrotonically distant part of the cell, such that our somatically located microelectrode is incapable of affecting it. In either case, we infer that the early onset of this potential obscured the true EPSP in those cases noted in the preceding text in which DHO appeared not to affect $g_{\text{EPSP}}$.

![Figure 5](http://www.jn.org/)

**Figure 5.** DHO increases miniature IPSC (mIPSC) frequency without affecting mIPSC amplitudes. A: sample traces showing spontaneous mIPSCs recorded under whole cell voltage clamp in control (left) and DHO (right); 10 μM NBQX, 50 μM APV, and 0.5 μM TTX were present in the saline. B: DHO (10 μM) increases mIPSC frequency. Each vertical bar represents the mean number of mIPSCs per min. The horizontal bars indicate the 5-min periods from which data were analyzed. C: Cumulative amplitude distributions for mIPSCs calculated from 100–500 events over 3- to 5-min periods in control (open symbols) and after 15 min of DHO application (filled symbols). Cumulative amplitudes are normalized to the maximal amplitude (100%). Amplitude of mIPSCs was not affected by DHO ($n = 5$), K-S test, $P < 0.005$. 

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J Neurophysiol • VOL 88 • DECEMBER 2002 • www.jn.org
DHO evoked responses in normal saline. Application of 20 \mu M DHO led to an increase in the late depolarizing potential that is measured in high-Ca/high-Mg saline and actually blocked by 100 \mu M M bath-applied APV (n = 15). The latency for action-potential firing was increased in DHO, suggesting that, despite the reduction in EPSP slope, the late depolarization enabled the cell to reach the voltage threshold for firing (Fig. 9C). Indeed, the duration of the depolarizing component of the evoked response was dramatically increased in DHO in normal saline (from 15.23 ± 1.70 to 68.86 ± 8.56 ms; n = 3, P < 0.05). The voltage threshold for action potentials induced by synaptic stimulation was unchanged during DHO-induced bursting activity (n = 10, P > 0.05, NS, Fig. 9C, top traces). We first assumed that the late potential was NMDAR dependent because NMDAR-dependent responses are controlled by GABAergic IPSPs. However, the late depolarization was not blocked by 100 \mu M bath-applied APV (n = 2), suggesting that it is largely non-NMDAR dependent. In any case, we infer that DHO-induced suppression of IPSPs facilitates E-S coupling of CA1 pyramidal neurons by enhancing a late depolarizing potential, which eventually drives the cell past spike threshold despite the smaller size of the EPSP itself. Because of its prolonged duration, the late potential is capable of initiating the multi-action-potential burst.

**DHO-induced decrease in neurotransmission is associated with reduced presynaptic fiber volley**

If, as our results suggest, the primary cause of DHO-induced burst firing is a suppression of synaptic transmission, then it is important to understand how partial inhibition of the Na\(^+\)-K\(^+\) pump suppresses synaptic transmission. It could arise from activation of presynaptic inhibitory receptors (e.g., \(\alpha\)mGlur, GABA\(_B\)) by glutamate or GABA (Davies and Collingridge 1996; Gereau and Conn 1995). However, antagonists of GABA\(_B\) receptors (CGP 35348 at 400 \mu M, n = 4, or CGP 55845 at 5 \mu M, n = 4), metabotropic glutamate receptors (LY 341495 at 100 \mu M, n = 5), or adenosine \(A_1\) receptors (DPCPX at 1 \mu M, n = 4) all failed to prevent DHO-induced bursting activity and reduction of excitatory transmission. Even concomitant application of LY 341495 and APV (100 \mu M) did not prevent DHO-induced bursting (n = 3). The paired-pulse facilitation ratio (interpulse intervals of 50 ms) was not affected in DHO (n = 6), which suggests that Na\(^+\)-K\(^+\) pump inhibition by DHO does not significantly alter the presynaptic mechanisms involved in the Ca\(^{2+}\)-dependent release of glutamate.

Alternatively, because DHO does affect action potential properties (Fig. 6), it could suppress synaptic potentials by impairing axonal conduction in afferent fibers. We found that DHO induced a consistent decrease in the amplitude of the presynaptic fiber volley (FV), the small biphasic response immediately preceding the fEPSP, whether this was measured...
FIG. 7. DHO reduces glutamatergic transmission. A: DHO reduces the slope of the depolarizing component of intracellular EPSP/IPSP sequences. A1: synaptic activation of CA1 pyramidal neurons by a single stimulus delivered to the s. radiatum in control, DHO, and 20 min after switching to the wash. Top and bottom traces show responses evoked by suprathreshold and subthreshold stimuli, respectively. Note again the suppression of IPSPs. A2: time course of changes in EPSP slope (mean % of control). DHO reduced the slope of EPSPs evoked by subthreshold stimulus intensities by 40–50%, suggesting a decrease in glutamatergic transmission (n = 9). B: DHO reduced the initial slope of fEPSPs recorded from s. radiatum simultaneously with the orthodromic PS in the 29 slices shown in Fig. 1A. Top traces show that the fEPSP slope was reduced in DHO and 20 min after starting the wash from DHO. Control record is superimposed on DHO and wash traces. Note that repetitive population spikes are detected after the peak of the fEPSPs in DHO. The plot below shows the time course of DHO effects on the fEPSP slope (mean % of control), and illustrates that fEPSP slope was reduced at peak bursting (15 min of DHO application) and did not recover after 20 min in wash saline.
traces, 50 cell axons by observing that antidromic population spike am-

A

CON DHO WASH

5mV 5ms

B

CON SCALED

DHO

5mV 20ms

C

-66mV

CON DHO

-65mV

10mV 100ms

DISCUSSION

We report that partial inhibition of the Na\(^+\)-K\(^+\) pumps by DHO induces numerous changes in neuronal membrane properties and synaptic function. Increases in [K\(^-\)] and alterations of passive membrane properties of pyramidal neurons are not required for DHO-induced onset of interictal-like burst firing in the CA1 hippocampal area. Rather, DHO application enhances EPSP-to-spike coupling by reducing inhibitory neurotransmission and induction or unmasking a prolonged depolarizing potential. Synaptic potentials are suppressed mainly by presynaptic factors, then both AMPAR-mediated and NMDAR-mediated fEPSPs should be similarly reduced. Surprisingly, DHO induced a greater reduction of the NMDAR component (Fig. 10B2) than of the initial, AMPAR-mediated, component of the fEPSP (Fig. 10B1). The NMDAR component (measured in 0.1 mM [Mg\(^2+\)] and NBQX) of the fEPSP slope induced by a 1-mV amplitude FV was reduced by 65% in DHO compared with control (n = 6, P < 0.05), compared with a reduction of the initial, AMPAR-dependent component of the EPSP of only 26%. Even high stimulus intensities (to 900 μA), which elicited FV amplitude of ~2 mV, were associated with NMDAR fEPSP slopes of only 35–40% of maximal control amplitude (Fig. 10B2). Thus DHO suppressed the NMDAR more than the AMPAR component of the fEPSP, suggesting that DHO may suppress glutamatergic transmission by both pre- and postsynaptic mechanisms. We propose that GABA\(_A\)-ergic IPSPs are also suppressed largely because of interference with conduction in the axonal arborization of interneurons, although because these axons do not form a simple coherent bundle the way that glutamatergic Schaffer collaterals do, we were unable to assess this question directly.
and suggests that disruption of the pump activity is a candidate mechanism in the development of epileptiform activity.

**Specificity and physiological relevance of DHO-induced epileptiform activity**

We attribute the effects produced by DHO to its pump-blocking effects: i.e., it decreased the rate of $K^+$/H$_{11001}$ clearance and increased the amplitude of the $[K^+]_o$ rise triggered by a short stimulus train (Krnjevic and Morris 1975). DHO also decreased the amplitude of $Na^+$/H$_{11001}$-K$_{11001}$ pump inhibition (Blaustein 1993). Finally, the onset and reversal of DHO-induced bursting activity was temporally correlated with
the DHO effects on $K_+^+$ uptake rate. Nevertheless, the cardiac glycosides may have other cellular actions besides $Na^+/K^+$ pump inhibition (Brosemer 1985; Santana et al. 1998; Thieren and Blostein 2000), and we cannot rule out the contribution of other factors to the effects we observed. The high-specificity binding site for CGs is on the catalytic $\alpha_2$ subunit of the $Na^+/K^+$ pump, of which three different isoforms (i.e., $\alpha_1$, $\alpha_2$, and $\alpha_3$) exist in nervous tissue (Sweadner 1989). The $\alpha_2$ and $\alpha_3$ isoforms predominate in neuronal rather than glial cells and have high affinity for CGs in the dose range used in our experiments. Therefore our results can probably be attributed to blockade of the neuronal $Na^+/K^+$ pumps, with little involvement of the glial pump. In emphasizing that partial inhibition of $Na^+/K^+$ ATPases has particularly important effects on GABAergic transmission, our data are in agreement with recent reports. Both fluid percussion injury (Ross and Soltesz 2000) and a brief period of high-frequency stimulation (Ross and Soltesz 2001) produced long-lasting effects on interneurons in the dentate gyrus by causing an inhibition of the interneuronal $Na^+/K^+$ pumps. Their results show that interneurons became significantly depolarized and made hyperexcitable by pump inhibition. Probably the differences in experimental protocols is largely responsible for the differences between their results and ours. The observations of Ross and Soltesz were mimicked and occluded by 100 $\mu$M strophanthidin, which will inhibit the pumps much more profoundly than the lower concentrations of dihydroouabain that we used.

**FIG. 10.** DHO reduces fiber excitability and FV-fEPSP coupling. **A:** DHO reduces fiber excitability. **A1:** Fiber volleys (FV, top) and antidromic population spikes (PS, bottom) recorded simultaneously, in control (a) and after 10 min in wash saline (b). The control trace is superimposed on the other records for comparison (· · ·). Fiber volleys evoked by single stimuli ($400$–$600$ $\mu$A) were isolated with NBQX and APV. The plots below show the time course of DHO effects on the amplitude of FV (**A2**) and antidromic PS (**A3**). Amplitudes are expressed as mean percentages of control ($n = 9$). During the breaks in the time courses, input-output curves were constructed in control (○) and wash salines (●). The curves, insets, show the changes in normalized FV amplitudes plotted against a range of stimulus intensities ($150$–$600$ $\mu$A). **B:** DHO decreases the FV-fEPSP coupling. fEPSPs were evoked by a range of stimulus intensities ($100$–$600$ $\mu$A) in control (○) and after 10 min in wash saline (●). The slopes of the normalized fEPSPs are plotted against the corresponding FV amplitudes. Each symbol represents the mean FV amplitude ± SE and corresponding mean fEPSP slope ± SE for a given stimulus intensity. Sample traces are shown at the top of B, 1 and 2. Traces on the right show initial fEPSP slope in control and DHO. **B1:** DHO reduces the FV-fEPSP coupling for fEPSPs mediated by non-NMDA glutamate receptors. APV was present when sample traces above were obtained but not for the data represented in the graph. **B2:** DHO reduces the FV-fEPSP coupling for fEPSPs mediated by NMDA receptors (NBQX present) to a greater extent.
**Na\(^+\)-K\(^+\) pumps and neuronal hyperexcitability**

**Impaired Na\(^+\)-K\(^+\) pump activity alters passive membrane properties of CA1 pyramidal neurons**

Na\(^+\)-K\(^+\) pump inhibition by DHO application had minor effects on passive membrane properties of pyramidal neurons. DHO induced a transient depolarization of \(\sim 5-6\) mV before the bursting activity began, but the onset of bursting was associated with increased membrane conductance and, unexpectedly, a long-lasting hyperpolarization of \(\sim 3-6\) mV. The Na\(^+\)-K\(^+\) pump exports three Na\(^+\) for each K\(^+\) imported into the cell and therefore produced an electrogenic outward, hyperpolarizing current that contributes to setting the membrane potential. Blocking this current will depolarize the membrane. We calculate that the DHO-induced, transient depolarization (5.4 mV) could be caused by blocking an electrogenic pump current of 87 pA [which would be similar to the pump current measured in other cells (Mitsutoshi et al. 1998; Senatorov et al. 1997; Shimura et al. 1998)], given the input resistance of our cells (62 M\(\Omega\)). The actual electrogenic effect of partial Na\(^+\)-K\(^+\) pump inhibition in our case is unclear, however. The concentrations of DHO that we used only affect the most ouabain-sensitive pump isoforms, \(\alpha2\) and \(\alpha3\), and not the \(\alpha1\) isoform, which plays the major role in “housekeeping” processes of the cell. Nevertheless, block of an electrogenic current could explain a small depolarization but not the DHO-induced hyperpolarization that predominates when the cells become hyperexcitable. The hyperpolarization was dependent on \([K^+]_o\), i.e., it was much reduced in 5.5 mM \([K^+]_o\). The larger changes in passive membrane properties in 3 mM \([K^+]_o\) saline may relate to regulation of Na\(^+\)-K\(^+\) pump activity. Increases in \([K^+]_o\) may antagonize the binding of CGs to the pumps (Gleitz and Peters 1997) or enhance the activity of unblocked pumps (Skou 1991), for example. A detailed study will be required to investigate this issue. DHO did induce burst firing in all experimental conditions, indicating that changes in passive membrane properties of pyramidal neurons were not required for DHO-induced hyperexcitability.

**Partial Na\(^+\)-K\(^+\) pump activity alters synaptic responses**

DHO-induced evoked burst discharges and population-spike burst responses resemble those induced by GABA\(_{A}\) receptor antagonists. We found that DHO reduced \(g_{EPSP}\) and shifted \(E_{EPSP}\) slightly in the depolarizing direction. \(E_{EPSP}\) varies as a function of both Cl\(^-\) and HCO\(_3\)\(^-\) electrochemical gradients, which, in turn, are controlled by various pumps that couple Cl\(^-\) and HCO\(_3\)\(^-\) transport to that of Na\(^+\) or K\(^+\) (Kaila 1994). Hence, DHO-induced changes in \(E_{EPSP}\) probably reflect changes in transmembrane \([Na^+]\) and \([K^+]\) gradients. Decreases in IPSP amplitude did not come about by a reduction in postsynaptic GABA\(_{A}\) receptor sensitivity because TTX-insensitive mIPSC amplitudes were not altered. It therefore appears that the DHO-induced decrease in \(g_{EPSP}\) is the result of a decrease in action-potential-induced GABA release. The increase in action-potential-independent mIPSC frequency may result from a slight increase in nerve terminal \([Ca^{2+}]\), caused by pump inhibition (Blaustein 1993) that can induce spontaneous release of neurotransmitters. An increase in spontaneous transmitter release may contribute to a decrease in action-potential-initiated transmitter release. Note that even the nerve terminal, a very confined region that should have a much higher input resistance than the cell soma, should also only depolarize by a few millivolts when the electrogenic pumps are blocked, unless the density of the Na\(^+\)-K\(^+\) pumps is higher in the terminal than elsewhere. If the density is the same, the magnitude of the pump current will diminish in proportion to the membrane surface area. At present there is no reason to think the terminal pump density is greater, but if it is, then the block of the electrogenic current would play a greater role in influencing synaptic transmission.

DHO also reduced evoked EPSPs. We believe that the generalized reduction in synaptic transmission is caused by DHO-induced reduction in the amplitude of action potentials in afferent fibers of s. radiatum. A reduction in the fiber volley could be due to the DHO-induced decrease in individual action potential amplitudes and/or to changes in fiber excitability, but both factors would lead to a decrease in total neurotransmitter output. In addition DHO could have a more-direct effect on transmitter release because it also affected FV-fEPSP coupling, i.e., a given fiber volley was associated with a smaller fEPSP in DHO. Finally, the greater reduction of the NMDAR component of the fEPSP suggested that postsynaptic mechanisms might also be involved in the DHO reduction of glutamatergic neurotransmission. The major point is that enhancement of the EPSP was not a factor in DHO-induced burst firing. Thus despite the clear epileptogenic effects of IPSP suppression, the approximately equivalent suppression of both EPSPs and IPSPs meant that another mechanism was necessary to explain burst initiation by DHO.

**Cellular mechanisms of initiation of epileptiform activity in DHO**

The occlusion of DHO’s enhancement of E-S coupling by picrotoxin suggested that disinhibition was largely responsible for the enhanced coupling. The induction, or unmasking, of a late depolarizing synaptic component by DHO enabled the cell to reach spike threshold even though the EPSP itself was smaller. Although the ionic mechanisms of the late depolarizing potential in DHO are unknown, the potential itself appears similar to depolarizing potentials induced by synaptic stimulation in the presence of picrotoxin (Dingledine and Gjerstad 1980; Dingledine et al. 1986; Wong and Prince 1978). The depolarizing potential in inhibited neurons (\(-150-250\) ms) is far longer than that of the normal, fast EPSP. It may consist of two synaptic potentials (i.e., AMPA and NMDA) plus a depolarization triggered intrinsically. Activation of mGluRs can also enhance E-S coupling (Breakewell et al. 1996). However, although APV slightly reduced the duration of the depolarizing potential in DHO, it failed to prevent epileptiform burst discharges, and bath application of the mGluR antagonist, LY341495, plus APV, also failed to prevent DHO-induced E-S coupling and bursting activity. Therefore disinhibition of intrinsic conductances may play a role in multiple action potential generation as well. A previous study suggested that a voltage-dependent Ca\(^{2+}\)-dependent conductance participates in DHO-induced burst firing in inhibited cells (McCarron and Alger 1987). If this conductance is activated by evoked synaptic potentials, it could participate in depolarizing the cell and trigger action potentials.

The Na\(^+\)-K\(^+\) pumps regulate many cellular processes directly and indirectly. Which of these constitute the ultimate
cause of the changes in synaptic potentials that we have observed must be investigated in future work. The work was supported by National Institute of Neurological Disorders and Stroke Grants ROI NS-36612 and ROI NS-36612 to B. E. Alger (the 2nd grant also provided support for C. Vaillend and M. F. Cuttle). S. E. Mason was supported by the Membrane Training Program at the University of Maryland School of Medicine. Present addresses: C. Vaillend, Laboratoire de Neurobiologie de l’Apprentissage de la Memoire et de la Communication, CNRS, UMR 8620, Université de Paris-Sud 91405, Orsay, France; and M. F. Cuttle, School of Biological Sciences, University of Southampton, SO16 7PX, UK.

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