Ca$^{2+}$-Dependent Inactivation of High-Threshold Ca$^{2+}$ Currents in Hippocampal Granule Cells of Patients With Chronic Temporal Lobe Epilepsy

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Beck, Heinz, Ralf Steffens, Uwe Heinemann, and Christian E. Elger. Ca$^{2+}$-dependent inactivation of high-threshold Ca$^{2+}$ currents in hippocampal granule cells of patients with chronic temporal lobe epilepsy. J. Neurophysiol. 82: 946–954, 1999. Intracellular Ca$^{2+}$ represents an important trigger for various second-messenger mediated effects. Therefore a stringent control of the intracellular Ca$^{2+}$ concentration is necessary to avoid excessive activation of Ca$^{2+}$-dependent processes. Ca$^{2+}$-dependent inactivation of voltage-dependent calcium currents (VCCs) represents an important negative feedback mechanism to limit the influx of Ca$^{2+}$ that has been shown to be altered in the kindling model of epilepsy. We therefore investigated the Ca$^{2+}$-dependent inactivation of high-threshold VCCs in dentate granule cells (DGCs) isolated from the hippocampus of patients with drug-refractory temporal lobe epilepsy (TLE) using the patch-clamp method. Ca$^{2+}$ currents showed pronounced time-dependent inactivation when no extrinsic Ca$^{2+}$ buffer was present in the patch pipette. In addition, in double-pulse experiments, Ca$^{2+}$ entry during conditioning prepulses caused a reduction of VCC amplitudes elicited during a subsequent test pulse. Recovery from Ca$^{2+}$-dependent inactivation was slow and only complete after 1 s. Ca$^{2+}$-dependent inactivation could be blocked either by using Ba$^{2+}$ as a charge carrier or by including bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA) or EGTA in the intracellular solution. The influence of the cytoskeleton on Ca$^{2+}$-dependent inactivation was investigated with agents that stabilize and destabilize microfilaments or microtubules, respectively. From these experiments, we conclude that Ca$^{2+}$-dependent inactivation in human DGCs involves Ca$^{2+}$-dependent destabilization of both microfilaments and microtubules. In addition, the microtubule-dependent pathway is modulated by the intracellular concentration of GTP, with lower concentrations of guanosine triphosphate (GTP) causing increased Ca$^{2+}$-dependent inactivation. Under low-GTP conditions, the amount of Ca$^{2+}$-dependent inactivation was similar to that observed in the kindling model. In summary, Ca$^{2+}$-dependent inactivation was present in patients with TLE and Amin’s horn sclerosis (AHS) and is mediated by the cytoskeleton similar to rat pyramidal neurons. The similarity to the kindling model of epilepsy may suggest the possibility of altered Ca$^{2+}$-dependent inactivation in patients with AHS.

INTRODUCTION

The application of modern electrophysiological, morphological, and molecular biological tools to tissue removed during epilepsy surgery has permitted the identification of candidate mechanisms that may be responsible for chronically enhanced excitability. Factors controlling levels of intracellular Ca$^{2+}$ are of particular interest because increases in the intracellular Ca$^{2+}$ concentration represent an important trigger for a variety of second-messenger–mediated events that include activity-dependent neuronal plasticity and neuronal cell death. Therefore stringent control of the intracellular calcium concentration is essential to avoid excessive activation of calcium-dependent processes. For this reason, the distribution and functional characterization of ionotropic glutamate receptors and voltage-dependent Ca$^{2+}$ channels (VCCs), two of the main routes for Ca$^{2+}$ entry into neurons, have been at the focus of interest in the investigation of epilepsy-related changes in animal models of epilepsy and in human epilepsy (Beck et al. 1997b; Blümcke et al. 1996; Kamphuis et al. 1992; Köhr and Mody 1991; Vreugdenhil and Wadman 1994). With respect to ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid (AMPA) and N-methyl-d-aspartate (NMDA) receptors, Ca$^{2+}$ influx is controlled by the expression of different receptor isoforms or splice variants as well as by kinase-dependent modification of the receptor protein (e.g., Köhr and Seeberg 1996; Seeberg 1993). Ca$^{2+}$ entry through high-threshold VCCs is controlled through a negative feedback mechanism via the intracellular Ca$^{2+}$ concentration (Eckert and Chad 1984; Imredy and Yue 1994; Köhr and Mody 1991). In L-type channels, this property seems to depend on a short sequence of the C terminus that does not include a nearby sequence with homology to Ca$^{2+}$-binding domains (Zhou et al. 1997). The transduction mechanism of this calcium-dependent inactivation is not well understood, but the signaling pathway seems to involve elements of the cytoskeleton (Johnson and Byerly 1993, 1994). In addition, the amount of Ca$^{2+}$ that can be buffered or sequestered immediately after Ca$^{2+}$ entry into the neuron influences the amount of Ca$^{2+}$-dependent inactivation.

Neurons possess a variety of intracellular Ca$^{2+}$ buffering and sequestration systems including mitochondria, endoplasmic reticulum, and different calcium-binding proteins (Carafoli 1987; McBurney and Neer 1987). High-affinity Ca$^{2+}$ buffering proteins such as calmodulin, parvalbumin, calretinin, and calbindin-D$_{28k}$ especially have been suggested to play an important role in rapidly sequestering Ca$^{2+}$ at the site of Ca$^{2+}$ entry. In dentate granule cells, a reduction of the calcium-binding protein calbindin-D$_{28k}$ has been observed in the kindling model of epilepsy and in human patients with temporal lobe epilepsy (TLE) (Baimbridge et al. 1985; Magloczky et al. 1997; Sloviter et al. 1991). This loss of buffering capacity has been suggested to lead to increased Ca$^{2+}$ levels following
Ca\(^{2+}\) influx and increased calcium-dependent inactivation of voltage-dependent Ca\(^{2+}\) currents in rat dentate granule cells (Köhr et al. 1991; Köhr and Mody 1991). In contrast, reduced Ca\(^{2+}\)-dependent inactivation together with a persistent increase in the amplitude of high-threshold Ca\(^{2+}\) currents has been observed in CA1 pyramidal cells following kindling electrogensis (Vreugdenhil and Wadman 1994). Therefore we attempted to investigate the properties and the mechanism of calcium-dependent inactivation in dentate granule cells from human patients with TLE.

**METHODS**

**Patient data**

Surgical specimens from 28 patients with pharmacoresistant TLE were obtained for electrophysiological analysis (average age at surgery 29.3 ± 6.5 yr). The mean duration of TLE in the adult patients was 19.7 ± 9.0 yr (mean ± SE), and the mean age at the onset of seizures was 11.3 ± 6.1 yr. All adult patients suffered from complex partial seizures (CPS), with additional simple partial seizures (SPS) in 8 patients and additional secondary generalized seizures (SGS) in 14 patients. In all adult patients, the hippocampus was shown to be intimately involved in the generation of temporal lobe seizures by noninvasive and invasive diagnostic procedures as described elsewhere (Engel 1992). The surgical removal of the hippocampus by selective amygdalohippocampectomy was clinically indicated in every case to achieve seizure control. All patients were under a full antiepileptic drug regimen at the time of operation. To reduce interpatient variability, only patients with a histopathological diagnosis of solitary Ammon’s horn sclerosis (AHS) with severe neuronal loss in the CA1, CA3, and CA4 subfield and relative sparing of CA2 (Margerrison and Correllis 1966) were selected for this study. Informed consent was obtained from all patients for additional histopathological and electrophysiological evaluation. All procedures were approved by the ethics committee of the University of Bonn Medical Center and conform to standards set by the Declaration of Helsinki (1989).

**Preparation of acutely isolated dentate granule cells**

Isolated hippocampal granule cells were prepared as described previously (Beck et al. 1997a,b). Human hippocampal specimens were placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 3 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 1.25 NaHPO\(_4\), 10 glucose, and 26 NaHCO\(_3\) (pH 7.4, 95% CO\(_2\)-5% O\(_2\)) immediately following surgical removal. Coronal slices (400 \(µ\)m) were prepared from the corpus of the hippocampus with a vibratome and transferred to a storage chamber with warmed ACSF (95% CO\(_2\)-5% O\(_2\)) and a single dendrite reminiscent of granule cell morphology in situ. The uncompensated series resistance of 2–3 M\(_\Omega\) was estimated by multiplying the maximal Ca\(^{2+}\) current amplitude with the effective series resistance after compensation did not exceed 3 M\(_\Omega\). A liquid junction potential of −9.7 mV was calculated between the intra- and extracellular solution with the gen-eralized Henderson liquid junction potential equation as described by Barry and Lynch 1991. The decay of whole cell Ca\(^{2+}\) currents elicited with 100-ms command pulses was fit with a monoexponential function with a steady-state component

\[
I(t) = A + B \cdot \exp(-t/\tau)
\]

where A and B correspond to the amplitudes of the nonactivating and inactivating component, respectively and \(\tau\) to the decay time constant. All results were expressed as means ± SE. Statistical analyses were performed with the program SPPS version 6.1.2. Differences were proven with the Mann-Whitney U-Wilcoxon Rank test, with the significance level set to 0.05 and denoted with asterisks in the figures.
RESULTS

Recordings from >150 human neurons were obtained between 5 and 90 min after isolation from hippocampal coronal slices of the resected hippocampus of 28 patients with drug-refractory TLE. Between 2 and 11 neurons were studied from each individual patient. As in a previous study (Beck et al. 1997b), slowly activating and inactivating high-threshold calcium currents could be elicited by depolarizing command pulses from a holding potential of −50 mV.

Time-dependent inactivation of high-threshold VCCs in different Ca2+ buffering conditions

Ba2+ currents through VCCs were large and showed a slow inactivation during 100-ms voltage pulses. When the charge-carrying ion was changed to Ca2+ in the superfusion medium, the maximal current obtained at the peak of the current-voltage relation (see Fig. 1B) was markedly reduced. This effect was not influenced significantly by the presence or absence of BAPTA or EGTA, indicating that elevation of intracellular free Ca2+ does not influence the peak amplitude of VCCs.

A markedly faster inactivation of Ca2+ currents during the command pulse became apparent when no extrinsic Ca2+ buffer was present in the recording pipette (Ca2+ currents scaled to Ba2+ current amplitude; Fig. 1A1, top panel, calibration bar applies to Ba2+ current). In contrast, both Ca2+ and Ba2+ currents through VCCs showed a very similar, slow time course of decay when 5 mM BAPTA or 11 mM EGTA (not shown) were included in the patch pipette (Ca2+ currents scaled to Ba2+ current amplitude; Fig. 1A1, bottom panel). This observation was confirmed by calculating the ratio of the current amplitude at the end of the 100-ms command pulse to the peak current at the various command voltages. In the absence of extrinsic buffer, Ca2+ currents showed significantly more inactivation during the command pulse than Ba2+ currents (Fig. 1A2, top panel). In addition, the time constants of inactivation measured by fitting a monoexponential function (Eq. 1) to the decaying phase of the current showed a U-shaped dependence on the command potential (see inset, Fig. 1A2). The inactivation rate could be markedly reduced by including 5 mM BAPTA/0.5 mM Ca2+ in the pipette solution (Fig. 1A2, bottom panel).

Voltage dependence of high-threshold VCCs in different Ca2+ buffering conditions

The peak of the activation curve was shifted to more positive potentials when Ca2+ was used as a charge carrier (Fig. 1B), regardless of the presence of extrinsic intracellular buffer. This suggests that this effect is probably not due to an action of intracellular free Ca2+. Similar to an earlier study in rats (Köhrl and Mody 1991), addition of BAPTA to the intracellular so-
solution shifted the voltage dependence of VCCs toward more positive potentials (Fig. 1B) with either Ca$^{2+}$ or Ba$^{2+}$ as a charge carrier.

### Double-pulse experiments

To further investigate the Ca$^{2+}$ dependence of VCCs, we tested the dependence of VCC amplitudes on prior entry of Ca$^{2+}$. In double-pulse experiments, a varying amount of Ca$^{2+}$ entry was obtained by systematically varying the voltage of a prepulse, and the effect on the peak Ca$^{2+}$ current during a subsequent test pulse was measured (Fig. 2A1; sample traces with prepulse voltages of $-60$ and $0$ mV). The reduction of Ca$^{2+}$ current during the test pulse is indicative of a Ca$^{2+}$-dependent inactivation process, because it directly measures the level of inactivation produced by previous Ca$^{2+}$ entry (Eckert and Chad 1984). The inactivation of the peak Ca$^{2+}$ current elicited during the test pulse was then plotted as a function of the prepulse voltage (Fig. 2A2). The prepulse voltage at which maximal inactivation occurred was usually around $0$ mV, coinciding with voltage at which a peak Ca$^{2+}$ current can be elicited (see Fig. 1B). The degree of test pulse current inactivation shows a U-shaped relation to prepulse potential, being maximal at potentials that produce maximal current inactivation on prior Ca$^{2+}$ entry during the prepulse. In contrast, when the intracellular Ca$^{2+}$ increase during the prepulse was prevented by including $5$ mM BAPTA in the recording pipette, the increase in the inactivation ratio could be markedly reduced (Fig. 3B, ■).

![Fig. 2. Double-pulse experiments. A1: representative traces illustrating the inactivation of Ca$^{2+}$ currents following prior influx of Ca$^{2+}$ during a 100-ms prepulse (top panel, see inset for voltage commands). Bottom panel illustrates a similar experiment carried out with $5$ mM BAPTA/0.5 mM Ca$^{2+}$ in the recording pipette. A2: peak current amplitudes during the test pulse were normalized to their maximal amplitude, averaged (no buffer; $n = 11$; BAPTA: $n = 5$) and plotted vs. the voltage of the 100-ms prepulse ($V_{prep}$). Experiments were again carried out without Ca$^{2+}$ buffer (top panel, □ and ■) or with $5$ mM BAPTA/0.5 mM Ca$^{2+}$ in the recording pipette (○ and □) or Ba$^{2+}$ (● and ○) as a charge carrier. B: the minimal value of the relation shown in A2 was obtained for each individual neuron as an approximate measure of Ca$^{2+}$-dependent inactivation and averaged. In addition to the experiments with BAPTA (●) and without Ca$^{2+}$ buffer (□), experiments with $11$ mM EGTA/$1$ mM Ca$^{2+}$ ($n = 5$) in the intracellular solution (■) are summarized. The inactivation of Ca$^{2+}$ currents without extrinsic buffers was significantly greater than the inactivation in all other recording configurations ($P < 0.05$, asterisk). C: Ca$^{2+}$-dependent inactivation in 5 different patients (A–E). Numbers in brackets refer to the number of neurons that were analyzed from each patient.
Removal of Ca$^{2+}$-dependent inactivation

Next, we investigated the removal of Ca$^{2+}$-dependent inactivation following Ca$^{2+}$ influx into the neuron during a conditioning prepulse. We determined the removal of Ca$^{2+}$-dependent inactivation by gradually increasing the interval between a 50-ms conditioning prepulse and a test pulse in double pulse experiments (Fig. 4A, inset). Recovery from inactivation occurred gradually in the absence of extrinsic Ca$^{2+}$ buffers with Ca$^{2+}$ as a charge carrier within an interpulse interval of 1 s (Fig. 4, A and B). When intracellular Ca$^{2+}$ was strongly buffered with 5 mM BAPTA or 11 mM EGTA (not shown), little inactivation could be observed with the shortest interpulse interval tested (10 ms). This small, putatively Ca$^{2+}$-independent inactivation showed a somewhat more rapid recovery to baseline levels within 300–400 ms interpulse interval (Fig. 4B).

Role of the cytoskeleton in Ca$^{2+}$-dependent inactivation

The role of the cytoskeleton in Ca$^{2+}$-dependent inactivation was investigated by applying substances that stabilize or destabilize microtubules and microfilaments, respectively, via the patch pipette. None of these agents changed the voltage dependence and steady-state inactivation properties of Ba$^{2+}$ currents through VCCs under conditions of strong intracellular Ca$^{2+}$ buffering (not shown). Likewise, neither taxol nor phalloidin altered the Ca$^{2+}$-independent portion of inactivation in double-pulse experiments with 5 mM intracellular BAPTA (see Fig. 5B). The following experiments were then carried out without extrinsic Ca$^{2+}$ buffers and using Ca$^{2+}$ as a charge carrier. Representative current traces in individual neurons following intracellular dialysis of the different cytoskeletal agents are shown in Fig. 5A. Phalloidin (20 μM), a substance that stabilizes actin filaments, slowed the decay of VCCs markedly. This effect could be prevented by additional inclusion of the microfilament-destabilizing agent cytochalasin B (20 μM) into the patch pipette solution. Cytochalasin B administered alone had no significant effects on Ca$^{2+}$-dependent inactivation. In contrast to phalloidin, taxol, which causes most of the tubulin molecules in cells to aggregate into microtubules, had no significant effect in the concentration range tested (2–100 μM). When Ca$^{2+}$-dependent inactivation was investigated using double-pulse protocols (100-ms prepulses, 50-ms prepulses, not shown) as described above, comparable effects could be observed, with 20 μM phalloidin (III) strongly affecting Ca$^{2+}$-dependent inactivation and taxol (II) yielding no significant effects (Fig. 5B). In contrast to the microfilament disruptor cytochalasin B, inclusion of colchicine alone, which disaggregates microtubules, showed a slight increase in Ca$^{2+}$-dependent inactivation in double-pulse experiments.
As the removal of inactivation has been shown to depend on Ca\(^{2+}\)-dependent processes, we hypothesized that the time course of removal of inactivation in the presence of phalloidin should be similar to that observed in the presence of intracellular Ca\(^{2+}\) buffers. We therefore investigated the removal of inactivation following intracellular application of phalloidin. Indeed, the removal of inactivation with phalloidin present in the recording pipette was similar to that observed with strong intracellular Ca\(^{2+}\) buffering (Fig. 6, \(\text{Œ}\), data from Fig. 4 shown for comparison).

**Effects of intracellular GTP**

The present data seemed to suggest that in contrast to rat hippocampal neurons (Johnson and Byerly 1994), it is mainly microfilaments that interact with VCCs, because microtubular stabilizers did not significantly reduce Ca\(^{2+}\)-dependent inactivation. However, this lack of effect might be due to a preexisting high level of polymerization of individual tubulin molecules in our experimental setup. Because GTP is a necessary prerequisite for polymerization of tubulin dimers, we hypothesized that the relatively high concentration of GTP (500 \(\mu\)M) in our intracellular solutions might cause most of the tubulin present in the neuron to aggregate into microtubules with a high degree of stability. In this case, we would not necessarily expect a large effect of taxol in the presence of GTP. Conversely, the absence of GTP should result in 1) increased Ca\(^{2+}\)-dependent inactivation and 2) sensitivity of this increased Ca\(^{2+}\)-dependent inactivation to intracellular application of taxol. Therefore we omitted GTP from our intracellular solutions in a first series of experiments. In addition, a set of experiments with intracellular solutions identical to those used by Köhr and Mody (1991), i.e., lacking GTP and with an ATP regenerating system, was performed. In these recording configurations, Ca\(^{2+}\)-dependent inactivation was significantly increased compared with recordings performed with 500 \(\mu\)M intracellular GTP (Fig. 7). When 20 \(\mu\)M taxol were included in

![FIG. 5. Effects of agents affecting the cytoskeleton. A: representative current traces from neurons dialyzed with different intracellular solutions. Voltage jumps (100 ms) from a holding potential of \(-60\) mV to a test potential of 0 mV were used. Intracellular dialysis with phalloidin reduced the inactivation rate. This effect was prevented by additional inclusion of 20 \(\mu\)M cytochalasin B. B: summary of the effects on Ca\(^{2+}\)-dependent inactivation measured with double-pulse experiments using Ca\(^{2+}\) as a charge carrier as shown in Fig. 2. \(\text{Œ}\) and \(\text{Œ}\), recordings performed without extrinsic Ca\(^{2+}\) buffer in the recording pipette and with Ca\(^{2+}\) as a charge carrier; \(\text{œ}\), recordings performed with 5 mM intracellular BAPTA and Ba\(^{2+}\) as a charge carrier. Recordings performed with intracellular BAPTA and Ba\(^{2+}\) as a charge carrier are shown to demonstrate the amount of Ca\(^{2+}\)-independent inactivation. Neither 20 \(\mu\)M phalloidin nor 20 \(\mu\)M taxol show any effect on putative Ca\(^{2+}\)-independent inactivation in double-pulse experiments (\(\text{œ}\)). Recordings with different intracellular solutions were interleaved to minimize artifacts due to interpatient variability. \(\square\), experiments employing agents acting on microfilaments; \(\triangledown\), those with agents acting on microtubules. Numbers in parentheses denote the number of investigated neurons. tax, taxol; pha, phalloidine; cyt B, cytochalasin B; col, colchizine.

![FIG. 6. Removal of inactivation in the presence of phalloidin. Measurements were performed as demonstrated in Fig. 4. For comparison, data from Fig. 4 are shown superimposed. \(\text{œ}\), recordings performed with Ca\(^{2+}\) as a charge carrier and without extrinsic Ca\(^{2+}\) buffer \((n = 7)\); \(\text{œ}\), recordings performed with Ca\(^{2+}\) as a charge carrier with 5 mM intracellular BAPTA \((n = 5)\); \(\text{œ}\), recordings performed with Ca\(^{2+}\) as a charge carrier without extrinsic Ca\(^{2+}\) buffer, but with 20 \(\mu\)M phalloidin present in the intracellular solution \((n = 7)\).](http://jn.physiology.org/doi/10.1152/jn.1995.273.7.951)
the intracellular solution in the absence of GTP, the Ca\(^{2+}\)-dependent inactivation could now be reduced significantly. Additional inclusion of 20 \(\mu\)M colchicine in the recording pipette reduced the effects of taxol (Fig. 7). Similar experiments in the absence of ATP could not be performed, because omitting ATP accelerated rundown of VCCs.

**DISCUSSION**

The electrophysiological properties of human (Isokawa et al. 1991, 1993; Williamson et al. 1993) and rat dentate granule cells (see Fricke and Prince 1984; Stanton et al. 1989) have been investigated in a number of studies employing intracellular recording with sharp microelectrodes. In addition, some groups have analyzed voltage-dependent ionic currents in isolated human hippocampal (Beck et al. 1997a,b; Reckziegel et al. 1998) and cortical neurons (Sayer et al. 1993). In this study, we have taken advantage of the possibility to investigate dentate granule cells isolated from the hippocampus of patients with drug-refractory TLE using the whole cell patch-clamp method, enabling us both to record VCCs in the voltage-clamp mode and to control the composition of the intracellular solution via the patch pipette. We have used these techniques to characterize Ca\(^{2+}\)-dependent inactivation of VCCs and the involvement of the cytoskeleton in its mechanism.

Voltage- and Ca\(^{2+}\)-dependent processes of inactivation are not easily dissected in most neurons, because many manipulations that alter Ca\(^{2+}\)-dependent inactivation also alter voltage-dependent inactivation and vice versa. However, a number of criteria have been proposed by Eckert and Chad (1984) to demonstrate the presence of Ca\(^{2+}\)-dependent inactivation in addition to voltage-dependent inactivation of Ca\(^{2+}\) currents. First, Ca\(^{2+}\)-dependent inactivation should depend on the species of ion carrying current through the membrane, with Ca\(^{2+}\) being more effective than Sr\(^{2+}\) or Ba\(^{2+}\). Indeed, the inactivation rate as well as inactivation measured in double-pulse experiments were considerably enhanced when Ca\(^{2+}\) as opposed to Ba\(^{2+}\) was used as a charge carrier. Second, Ca\(^{2+}\)-dependent inactivation should be reduced by any means diminishing the intracellular Ca\(^{2+}\) increase following influx of Ca\(^{2+}\). Indeed, introduction of different Ca\(^{2+}\) buffers (BAPTA or EGTA) into dentate granule cells via the patch pipette resulted in markedly slowed inactivation and reduced inactivation measured in double-pulse experiments. Finally, we find that, in double-pulse experiments, the degree of inactivation during the test pulse has a U-shaped relation to prepulse potential, being maximal at potentials that produce maximal Ca\(^{2+}\) entry, and showing a pronounced reduction as the prepulse potential approaches the Ca\(^{2+}\) equilibrium potential (see Fig. 2). In addition, plotting inactivation versus the time integral of Ca\(^{2+}\) entry during pre pulses of varying duration and voltage yields a tight, approximately linear relationship (see Eckert and Chad 1984).

These experiments show that an inactivation that is dependent on Ca\(^{2+}\) entry inducing an increase in [Ca\(^{2+}\)], is present in these neurons in addition to voltage-dependent inactivation. Voltage-dependent inactivation is probably most accurately reflected by the recordings performed with high concentrations of Ca\(^{2+}\) buffers included in the recording pipette.

**Mechanism of Ca\(^{2+}\)-dependent inactivation: involvement of the cytoskeleton**

In control experiments, we have shown that this putative voltage-dependent inactivation component is not influenced by agents that affect the cytoskeleton. In contrast, the mechanism of Ca\(^{2+}\)-dependent inactivation in human dentate granule cells seems to be similar to that described in hippocampal pyramidal neurons in the rat (Johnson and Byerly 1994), involving microfilaments as well as microtubular elements of the cytoskeleton. The mechanism of inactivation presumably involves Ca\(^{2+}\)-dependent destabilization of cytoskeletal elements that have a structural relationship with the channel. This destabilization possibly leads to channel inactivation. Including microfilament stabilizers in the recording pipette would, according to this model, reduce the effects of Ca\(^{2+}\) on microfilaments, thereby also reducing Ca\(^{2+}\)-dependent inactivation. Agents that stabilize microtubules were effective only in the absence of intracellular GTP. Because aggregation of tubulin dimers into microtubular structures requires GTP, one possible explanation for these results may be that high (500 \(\mu\)M) concentrations of GTP present intracellularly cause most of the tubulin dimers in the neuron to aggregate into functionally stable microtubules. In this case, taxol would not be expected to have an additional effect. In addition, omitting GTP from the intracellular solution would be predicted to have the two effects that were experimentally observed, namely that Ca\(^{2+}\)-dependent inactivation should be increased and that taxol should then be effective in reducing the increased Ca\(^{2+}\)-dependent inactivation. Thus lowered concentrations of GTP during periods of energy depletion might cause increased Ca\(^{2+}\)-dependent inactivation of Ca\(^{2+}\) currents. Whether the actual free GTP concentration in the cytosol of vertebrate CNS neurons is in a range where it can contribute to a regulation of VCCs is presently unknown. Nevertheless, this mechanism may be considered potentially neuroprotective by reducing the Ca\(^{2+}\) influx into these neurons.

The data presented here are of considerable interest in relationship to data on the Ca\(^{2+}\)-dependent inactivation of currents through the pore-forming \(\alpha_{IC}\) subunit, in which Ca\(^{2+}\)-dependent inactivation has been shown to depend critically on a short
sequence in the C-terminal end of the channel (Zhou et al. 1997). Interestingly, this sequence is not identical to an immediately adjacent Ca$^{2+}$-binding EF hand motif, and disruption of the Ca$^{2+}$-binding ability of the EF-hand domain does not interfere with Ca$^{2+}$-dependent inactivation. Whether this critical sequence might possibly interact with proteins that are related to the cytoskeleton is hitherto unknown.

Comparison to animal models of epilepsy

The maximal Ca$^{2+}$-dependent inactivation we could observe, i.e., using low concentrations of GTP, were very similar to data obtained in the kindling model of epilepsy (Köhre and Mody 1991). This group used intracellular solutions lacking GTP. In our own control experiments performed with solutions identical to those employed by Köhr and Mody, we have obtained results quantitatively very similar to our measurements without GTP. This may be due to the substantial loss of the Ca$^{2+}$-binding protein Calbindin-D$_{28K}$ from dentate granule cells that is present both in kindled rats (Sloviter 1989) and human hippocampus from patients with TLE (Magloczky et al. 1997). The high variability observed between neurons obtained from an individual patient may correlate with the finding that Calbindin-D$_{28K}$ is present at considerable levels in some individual dentate gyrus granule cells in the specimens studied electrophysiologically in this study (unpublished observations). However, caution must be exercised in the interpretation of electrophysiological data from human specimens, because human control tissue is not available for comparison with TLE tissue. Nevertheless, the similarity between the kindling model and human TLE specimens may suggest a pathophysiological relevant increase in Ca$^{2+}$-dependent inactivation that is associated with kindling in rats and TLE in humans.

In summary, we demonstrate that Ca$^{2+}$-dependent inactivation in granule cells isolated from patients with TLE shows properties similar to the kindling model in rats. The mechanism of Ca$^{2+}$-dependent inactivation involves microfilaments as well as microtubules, with the microtubule-dependent pathway being modulated by intracellular GTP. Interesting questions to be clarified by future studies include the nature of the transduction mechanism involved in Ca$^{2+}$-dependent destabilization of cytoskeletal elements and the functional consequences of Ca$^{2+}$-dependent inactivation for Ca$^{2+}$ influx into dendrites following synaptic stimulation.

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