RAPID COMMUNICATION

Epileptogenesis Following Neocortical Trauma From Two Sources of Disinhibition

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Yang, Lie and Larry S. Benardo. Epileptogenesis following neocortical trauma from two sources of disinhibition. J. Neurophysiol. 78: 2804–2810, 1997. Intracellular and field potential recordings were obtained from superficial and deep neurons from both intact coronal rat somatosensory slices, and from slices which had been acutely divided into a superficial strip of cortex (~450 μm from the pia) and a deep segment. Membrane properties for cells in the traumatized slices were similar to those of their counterparts in intact slices. However, synaptic hyperexcitability developed in the deep segments in which a majority of cells likely underwent dendrotomy. This hyperexcitability was manifested by epileptiform activity in 54% of traumatized slices. Measurements of fast GABAergic inhibitory strength showed these slices were disinhibited. Superficial delivery of tetrodotoxin to the upper 450 μm of intact slices led to disinhibition of fast GABAergic transmission as well as an attendant increase in excitative postsynaptic potential strength but not epileptogenesis. Pharmacological maneuvers aimed at preventing glutamate-triggered increases in intracellular calcium [glutamate ionotropic antagonists, dantrolene, and bis-(o-aminophenoxy)-N,N,N*,N*-tetraacetic acid (BAPTA)-AM] showed that a 1 h treatment in these agents conferred protection against epileptogenesis. These results demonstrate that the seizure-like activity developing in deep dendrotomized cortical segments resulted from two sources of GABAergic disinhibition: the physical removal of important superficial inhibitory circuits and glutamate-triggered increases in intracellular calcium.

METHODS

Slice preparation

Somatosensory cortical slices (450 μm) prepared from Sprague-Dawley rats (60–150 g) as previously described (Ling and Benardo 1995) were placed in an interface recording chamber (Fine Science Tools, Foster City, CA), gradually warmed, and maintained at 30 ± 1°C (mean ± SD) in a humidified atmosphere of 5% CO2-95% O2. Slices were superfused (~1 ml/min) with physiological solution containing (in mM) 124 NaCl, 5 KCl, 26 NaHCO3, 1 ± 1.6 MgCl2, 2 CaCl2, and 10 glucose, pH 7.35–7.4. Given the flow rates used and the volume of the experimental chamber (one-time), turnover of the chamber volume occurs within 3–5 min, and complete replacement of the extracellular medium is effected within 10–15 min (Benardo 1994; Ling and Benardo 1995).

Preparation of traumatized slices was initially identical to control slices. Randomly selected slices (~50% of the slices) were positioned under a dissecting microscope while in ice-cold saline, or after transfer to the recording chamber. Micrornives consisting of a razor blade fragment held by a hemostat were used to make a cut parallel to the pial surface and running the length of the slice, at a subpial depth of ~450–500 μm (diagrammed in Fig. 1A). The cut superficial strip was then separated from the deep segment. The cut, which was placed in layers II-III, dendrotomized most, if not all the layer V pyramidal cells. Slices were allowed to equilibrate in the experimental chamber for 1–2 h before recording from them.

Electrophysiological recording and intracellular staining

Intracellular recordings with microelectrodes pulled from 1 mm thin-walled, fiber-filled capillaries (20–60 MΩ) were routinely filled with 2 M potassium acetate. For intracellular staining the

INTRODUCTION

One hallmark of cerebral cortical trauma is the development of seizure activity. Not surprisingly, such abnormal activity is seen in a majority of patients sustaining moderate to severe head injury (Dinner 1993). Within 24 h following head injury, up to 80% of patients experience clinical seizures (Dinner 1993; Kollevold 1976). Several processes must contribute to increase excitation following cerebral cortical injury, manifested as seizure activity. Traumatic insults are known to result in excess accumulation of extracellular glutamate which subsequently activates glutamate receptors (Lipton and Rosenberg 1994). As a consequence, intracellular calcium rises via transmitter-gated channels (Weiss et al. 1990) and perhaps through voltage-dependent channels as well (Murphy and Miller 1989). Calcium may also be released from intracellular stores (Frandsen and Shousboe 1991), or calcium influx may be enhanced directly as a result of dendrotomy at the site of injury (Soltesz and Mody 1995). Increased intracellular calcium, in turn, enhances the response to glutamate (Mayer et al. 1984; Nowak et al. 1984), further feeding the process, and spreading the excitotoxic damage (through recurrent excitatory afferents) to neurons not directly injured by the mechanical insult. What is the status of GABAergic inhibition in the wake of the storm of excitation that follows trauma? Is fast inhibition simply overwhelmed by excitation due to the inherent (Ling and Benardo 1995) or spatial (Barkai et al. 1995; Van Brederode and Spain 1995) limitations of inhibition, or could γ-aminobutyric acid-A (GABA_A) receptor function be depressed by the resulting increased intracellular calcium (Stelzer et al. 1988)? We have developed an in vitro model of traumatic neuronal injury in order to investigate the mechanisms of excitotoxicity and epileptogenesis without complicating systemic alterations. We show here that a pure mechanical insult in neocortex can induce seizure activity perhaps as a consequence of disinhibition owing to physical removal of superficial inhibitory circuits and glutamate-triggered increases in intracellular calcium.

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tips of the recording electrodes were filled with 2% Neurobiotin (Vector Laboratories, Burlington, CA) in 2 M potassium acetate, and backfilled with 2 M potassium acetate. For experiments in which isolated inhibitory postsynaptic potentials (IPSPs) were recorded, electrodes contained 1 M cesium acetate to block potassium currents [including γ-aminobutyric acid-B (GABA B) IPSPs], increasing cell input resistance, allowing significant neuronal depolarization using injected current. Membrane potentials were measured using a high impedance amplifier (Axoclamp 2A, Axon Instruments, Foster City, CA) in current-clamp mode. Slices were stimulated using cathodal shocks (200 μs) via a sharpened bipolar, coated tungsten electrode placed lateral to the recording site. Voltage and current signals were displayed on a digital oscilloscope and stored digitally on videotape for later off-line analysis. Field potentials were recorded through glass micropipettes “broken back” to resistances of 2–5 MΩ filled with 2 M NaCl.

Neurobiotin was injected into cells using depolarizing pulses (150 ms at 3.3 Hz) of 1–3 nA for 5–15 min. Slices containing filled cells were processed as previously described (Benardo 1997) using the avidin-biotin-peroxidase complex (ABC solution, Vector Laboratories). The dendrites, axonal processes, and somata of the labeled neurons were drawn at ×200 using ×20 objective and a camera lucida.

Data analysis

To avoid some of the problems of overlap of synaptic potentials inherent in current-clamp recordings we attempted to isolate recordings of inhibitory from excitatory postsynaptic events [i.e., IPSPs and excitatory postsynaptic potentials (EPSPs)]. Thus IPSPs were recorded at EPSP reversal potential (Erev), Erev, was determined empirically by slowly depolarizing the membrane. Erev was taken as the point when the synaptic potential just became completely negative (~0 mV), and the synaptic potential thus recorded was the relatively isolated IPSP. The validity of Erev, determined in this way was tested in the following manner. In control experiments cells were recorded with electrodes containing 1–2 mM picrotoxin in 1 M cesium acetate. Initially a large IPSP was recorded at the empiric Erev. After several minutes, picrotoxin leached out of the electrode tip sufficiently to completely block the IPSP (Akaike et al. 1985). Erev was then redetermined; in all cases giving an identical value to that obtained when the IPSP had been present (see Fig. 1A). To estimate the level of contamination of the IPSP by the opposing EPSP, the IPSP was initially recorded at Erev, noting the membrane potential attained at the peak of the IPSP. (These experiments were performed in traumatized deep segments so as to address the overlap problem specifically in those preparations where the process should be most prominent.) Once picrotoxin became fully effective (usually 15–20 min after impalement) the isolated EPSP was recorded at the membrane voltage attained at the IPSP peak. The percent increase in the IPSP that would potentially be recorded in the absence of the EPSP was then calculated as

\[
\frac{\text{integral of the EPSP}}{\text{integral of the EPSP + integral of IPSP}}} \times 100\%
\]

Therefore these IPSPs may be understated by at most 8.5 ± 2.2% (n = 6). It should be noted that this correction factor likely overestimates the actual influence of the EPSP on the IPSP. Because the EPSP was obtained at steady-state voltage, the magnitude of the EPSP actually opposing the IPSP during the transient voltage change of the IPSP must be substantially less. Moreover, control IPSPs likewise have a correction factor; given this, and the magnitude of changes observed, corrections for overlap would not significantly influence our comparisons.

EPSPs were relatively isolated by recording these potentials at IPSP reversal potential. IPSP reversal was determined empirically and verified using plots of IPSP amplitude versus membrane potential. To estimate the level of contamination of the IPSP by potentials were recorded through glass micropipettes “broken back” and stored digitally on videotape for later off-line analysis. Field age and current signals were displayed on a digital oscilloscope.

A traumatic injury was inflicted by making a horizontal cut through the recording electrode sufficiently to completely block the IPSP (PTX). Lack of a significant residual response indicated that the potential was indeed recorded at the true EPSP reversal potential. B: EPSP recorded at the empiric or calculated fast IPSP equilibrium potential (control). After exposure to the ionotropic glutamate receptor antagonists (CNQX + CPP) no appreciable residual response remained indicating this membrane level was the true IPSP reversal potential.

In some experiments, this potential was reconfirmed by adding the glutamate blockers CNQX and CPP and again checking the reversal potential for the fast IPSP (see Fig. 1B). Values obtained by each of these methods were nearly identical. Recorded EPSPs would underestimate completely isolated EPSPs. But in experiments examining EPSPs we show that EPSPs increase significantly without accounting for this underestimate (e.g., Fig. 3). If corrected for IPSP overlap, the findings regarding EPSPs would be even more marked.

Postsynaptic potential strength was determined by first constructing an input-output curve for each event (as in Fig. 2E), plotted as the integral bounded by each potential and the baseline (in volts/s) against stimulus intensity. The strength of the potential is defined by the slope of the resultant regression line for the input-output relationship, using a semilog scale for the x-axis [cf. Fig. 2 in Salin and Prince (1996)]. For pooling data, stimulus intensities for each cell were normalized to the peak or maximum stimulus utilized. All intensity values less than the maximum were expressed as a percentage of the peak stimulus, ranging down to the threshold stimulus. Statistical significance was determined by Student’s t-test, except where noted.

RESULTS

Control recordings were made from coronal slices of rat somatosensory cortex maintained in vitro. We empirically separated physiological data obtained from superficial neurons (present in the outer 450 μm of slices), from those acquired in more deeply residing cells (i.e., in the remaining inner 900–1,000 μm); presented in Table 1. The values for the parameters listed here are consistent with those previously reported for neocortical neurons (Mason and Larkman 1990; Pena and Geijo-Barrientos 1996). In other slices a traumatic injury was inflicted by making a horizontal cut...
isolating the superficial (pial) one-third of the slice (450–500 μm) from the deeper layers (Fig. 2). In practical terms the division was made within layers II/III. This procedure would axotomize many neurons in the resultant superficial segment and interrupt their extracortical and ascending intracortical efferents. For deep segments, most (~85%) of the pyramidal cells present would be dendrotonized (e.g., Fig. 2A, bottom) with descending intracortical projections to them, severed.

The intrinsic properties of both superficial and deep neurons from traumatized slices were remarkably similar to those obtained under control conditions (Table 1). The evoked field potential activity from superficial segments also appeared similar to that of control cells. However, in deep segments we detected the development of abnormal discharges within 1–2 h in 25 of 46 preparations. As the stimulus intensity was increased in these slices, a threshold was reached at which the early synaptic potential was followed by an all-or-none multiphasic field potential having a delayed and variable latency that could last several hundred milliseconds (Fig. 2A). Such activity was mirrored in simultaneous intracellular records obtained in these same slices (Fig. 2A). Multiphasic field potentials were associated with prolonged intracellular depolarizations that could last up to a few hundred milliseconds and were capable of evoking bursts of spikes (Fig. 2, A–D). With increasing stimulus intensity, the onset latency of delayed multiphasic potentials shortened (Fig. 2B). The triggering of late events was also dependent on stimulus frequency, with these all-or-none responses alternating with the short latency synaptic potential at low frequencies (i.e., up to 0.1 Hz; Fig. 2C). These events had a number of characteristics previously described for epileptiform events in neocortical slices treated with convulsant agents (Schwartzkroin 1995), although in some preparations the activity was somewhat less robust than the activity in those slices treated with high concentrations of convulsants; better resembling those of chronic epileptic foci (Prince and Tseng 1993) or low-dose bicuculline (Chagnac-Amitai and Connors 1989). Depolarizing components of the paroxysmal event had the appearance of a large EPSP in its response to

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**TABLE 1. Cellular properties of intact and traumatized neurons**

<table>
<thead>
<tr>
<th>Cellular Property</th>
<th>Intact Superficial</th>
<th>Intact Deep</th>
<th>Traumatized Superficial</th>
<th>Traumatized Deep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mV</td>
<td>72.7 ± 1.6 (19)</td>
<td>67.0 ± 0.7 (72)</td>
<td>73.6 ± 1.8 (19)</td>
<td>66.6 ± 0.5 (110)</td>
</tr>
<tr>
<td>Time constant, ms&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2 ± 0.4 (14)</td>
<td>7.1 ± 0.28 (65)</td>
<td>7.2 ± 0.4 (19)</td>
<td>7.2 ± 0.2 (110)</td>
</tr>
<tr>
<td>Input resistance, MΩ&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.3 ± 2.8 (19)</td>
<td>34.4 ± 1.4 (72)</td>
<td>33.4 ± 2.2 (19)</td>
<td>33.7 ± 1.4 (66)</td>
</tr>
<tr>
<td>% Overshoot</td>
<td>9.7 ± 1.7 (7)</td>
<td>24.2 ± 2.5 (17)</td>
<td>6.5 ± 1.2 (8)</td>
<td>25.4 ± 2.2 (17)</td>
</tr>
<tr>
<td>AP duration, ms&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.26 ± 0.04 (32)</td>
<td>1.43 ± 0.04 (15)</td>
<td>1.23 ± 0.04 (35)</td>
<td></td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>-48.5 ± 1.3 (18)</td>
<td>-49.6 ± 0.7 (65)</td>
<td>-50.2 ± 1.2 (24)</td>
<td>-50.5 ± 0.6 (108)</td>
</tr>
<tr>
<td>F-I relation, Hz/nA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>38.5 ± 2.0 (6)</td>
<td>43.5 ± 2.5 (26)</td>
<td>38.3 ± 3.9 (10)</td>
<td>42.3 ± 1.9 (31)</td>
</tr>
</tbody>
</table>

Values are means ± SE with number of neurons in parentheses. AP, action potential; F-I, frequency-current. *Time constant obtained by fitting the membrane potential response to a −0.3 nA, 250 ms current injected into the cell at resting membrane potential using a single exponential function (y = A<sub>0</sub> + A<sub>e</sub> e<sup>−t/τ</sup>, A<sub>0</sub> and A<sub>e</sub>; offsets, τ; time constant). †Apparent steady-state input resistance calculated from the slope of the linear portion of the current-voltage relationship. ‡Percentage overshoot, i.e., the amount by which the membrane potential became transiently more positive than resting potential following a hyperpolarizing pulse (usually −0.5 nA), expressed as a percentage of the steady-state voltage deviation during the pulse (V<sub>peak−steady state</sub> × 100). The percentage overshoot was relatively constant regardless of the size of the current pulse. Data obtained limited to cells whose resting potentials ranged from −69 to −71 mV. ∗Action potential (AP) duration was obtained by measuring the width of the 1st spike at action potential half-amplitude.

Because the intrinsic properties of neurons we measured were not significantly different following trauma (Table 1), we focused our examination on whether cells from traumatized deep segments did in fact demonstrate altered synaptic inhibition. Electrically evoked, relatively isolated fast GABAergic IPSPs were recorded at holding potentials equal to the empirically determined EPSP reversal potential (voltage level at which the depolarizing component of the synaptic potential just disappears; ~0 mV). Under control conditions the strength of inhibitory postsynaptic potentials (IPSPs) increased with increasing stimulus intensity (up to ~3 times the threshold stimulus; Fig. 2E, △, n = 8). In neurons from traumatized deep segments, IPSP strength (Fig. 2E, ●, n = 8) was significantly decreased (P < 0.025). The source of this disinhibition was unclear. It is known that 95–100% of layer I cells are GABAergic (Jones 1993; Peters and Kara 1985), and projections from these neurons (as well as those in layers II/III) apparently constitute an important source of inhibition for deep (Salin and Prince 1996), as well as local (superficial) (Barkai et al. 1995; Van Brederode and Spalding 1995) pyramidal neurons. Thus disinhibition in traumatized slices could simply result from removal of superficial, inhibition-rich cortical laminae. To explore this issue more directly we attempted to simulate the removal of the superficial portion of slices by exposing the distal third of intact preparations to tetrodotoxin (TTX, 1 μM; Fig. 3, left panel). This blocks sodium spikes in that zone, thereby blocking evoked synaptic transmission originating from cells located there or from axons that traverse or terminate in the affected area (Fig. 3, left panel). We then recorded IPSPs and EPSPs in layer V pyramidal neurons. To ensure that TTX had not diffused into the deep layers only data from cells capable of fast spike firing were used (data from one cell were discarded).

Figure 3, A and D, shows the synaptic potentials recorded before and after TTX addition (n = 8). The IPSP recorded following TTX exposure appeared smaller (Fig. 3A). Al-
FIG. 2. Results from intact and traumatized neocortical slices. A: diagram showing intact (left, top) and traumatized (left, bottom) slices. Half the neurons included in this study (i.e., 110 of 220) were stained by Neurobiotin injection. Of these, 95% of deep cells were pyramidal neurons (vs. 64% of superficial cells). About 85% of deep pyramidal neurons had dendrites that reached layer I. Middle: typical camera lucida drawings of Neurobiotin-filled neurons from intact and traumatized (dendrotomized) preparations. Right: intracellular records from cells shown in middle panel with simultaneously recorded field potentials. Stimuli (here and throughout) were delivered in layer VI at the indicated times. Cells from intact slices showed activity characteristic for neocortical neurons, i.e., an EPSP giving rise to a single action potential followed by a 2-component IPSP. The majority of cells from traumatized slices showed epileptiform activity characterized by a large, long-lasting depolarization on which a burst of spikes was superimposed, which could be followed by afterdischarge (right, bottom). Calibration for middle panel, 100 µm. Calibration for right panel: voltage, 10 mV for intracellular recordings, 1 mV for field recordings and time, 250 ms. Full amplitude of spikes were attenuated at slower sweep speeds.

B: response to increasing stimulus intensity. At low intensity a small EPSP is triggered. A small increment in stimulus intensity triggered an all-or-none giant depolarizing event that shifted its latency with increasing stimulus strength. C: at low stimulus frequencies (i.e., ~0.1 Hz) alternation of the small synaptic potential, with the all-or-none event, was observed. D: the large depolarization grew with hyperpolarization. Calibration in D for B–D, 20 mV, 200 ms. E: plot of IPSP strength against %peak intensity recorded in cells from intact (n = 8, ●) and traumatized slices (n = 8, ▲). IPSP strength was obtained from the integral of the IPSP recorded at EPSP reversal potential (~0 mV, inset; calibration, 10 mV, 200 ms). Data were pooled by normalizing the stimulus intensity delivered to the peak intensity utilized in each cell, expressed as a percentage of the peak intensity. IPSP strength was significantly depressed following trauma (P < 0.025). Here and throughout, error bars indicate standard error (SE).

Though the time constant of decay (τ) of the IPSP was unchanged (Fig. 3B, top), IPSP duration at half-amplitude was significantly decreased (Fig. 3B, bottom, P < 0.025). IPSP strength was also significantly decreased (P < 0.05). We attempted to record EPSPs in relative isolation by holding cells at the presumed IPSP reversal potential determined from plots of IPSP amplitude versus membrane potential (about −70 to −75 mV) (Benardo 1997), minimizing IPSPs, analogous to the method described by Ling and Benardo (1995). As demonstrated in Fig. 3D, EPSPs appeared larger and more prolonged following TTX exposure, perhaps as a consequence of relative disinhibition of distal inhibitory output. This was manifested by a significant increase in the time constant (τ) of decay of the EPSP and its duration at half-amplitude (P < 0.005 and P < 0.01, respectively).

Moreover, EPSP strength (Fig. 3F) was found to be significantly increased after superficial TTX application (P < 0.005). Apparently, blockade of superficial inhibitory circuits by TTX induced sufficient disinhibition to uncover latent excitatory components, likely through release of recurrent excitation (Ling and Benardo 1995; Schwartzkroin 1995). Nonetheless, this disinhibition was not of the magnitude seen following traumatic removal of the superficial cortex, and epileptiform activity was not observed. Thus disconnection of superficial inhibition could not entirely explain the disinhibition and epileptogenesis encountered in traumatized slices.

Therefore we explored the possibility that the disinhibition and epileptiform activity recorded in traumatized slices were also sequels to the neuronal injury itself, likely secondary...
FIG. 3. Effects of tetrodotoxin (TTX) delivery to the distal third of the slice on evoked synaptic potentials. A: experimental setup. TTX was delivered to the outer (pial) third of the slice while recording intracellularly from deep layer V pyramidal neurons. Delivery was made via a separately manipulated pipette by pressure injection. Drug application was confirmed visually and did not appear to extend across the imaginary boundary represented by the dotted line in the figure. B: IPSP recorded in the same neuron at EPSP reversal (0 mV), triggered by the same stimulus intensity, before and after TTX application. Calibrations in A and D for A and D. Inset: response to intracellular depolarizing current pulse following TTX delivery to the superficial region of the slice. Somaically triggered action potentials retained their full amplitude (calibration, 20 mV, 20 ms). C: relationship of IPSP strength to % peak stimulus intensity in the same population of cells before (●) and following (▲) TTX exposure. D: EPSP recorded in the same neuron at IPSP reversal (~75 mV), triggered by the same stimulus intensity, before and after TTX application. E: EPSP decay (top) and duration at half-amplitude (bottom) before and after TTX application. F: relationship of EPSP strength to % peak stimulus intensity in the same population of cells (n = 8) before (●) and after (▲) TTX exposure.

to associated increases in [Ca\textsuperscript{2+}]. As mentioned, increased [Ca\textsuperscript{2+}], following physical insult can result from glutamate receptor-mediated influx (Weiss et al. 1990), calcium influx through voltage-gated channels (Murphy and Miller 1989), calcium release from intracellular stores (Frandsen and Shousboe 1991), or dendrotoxin itself (Soltesz and Mody 1995). Therefore, a set of experiments was directed at preventing the probable increase in [Ca\textsuperscript{2+}]. By assessing whether pharmacological maneuvers directed at different stages of the process prevented the development of epileptiform activity, we hoped to gain information about the mechanisms underlying the epileptogenesis seen in this model of neurotrauma. To accomplish this, slices were exposed to four different drug treatments: 1) ionotropic glutamate antagonists CNQX (10 μM), an AMPA/kainate blocker plus CPP (10 μM), an NMDA blocker; 2) dantrolene [blocks ryanodine receptor-dependent Ca\textsuperscript{2+} release and may suppress the maximum rate of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release without affecting the sensitivity of the release mechanism to Ca\textsuperscript{2+} (Mody and MacDonald 1995); 40 μM]; may be effective against alterations in GABA\textsubscript{A} receptor (Tong and Jahr 1994) and NMDA receptor (Soltesz and Mody 1995) produced by mechanical injury to central neurons; 3) a cocktail of CNQX, CPP, and dantrolene; or 4) BAPTA-AM (30 μM), a cell permeant calcium chelator. Drugs were added to the slice media just after making the traumatic horizontal cut. One hour later slices were returned to control solution and their physiology assessed. The results of these experiments are shown graphically in Fig. 4A. Preparations exposed to either CNQX+CPP, dantrolene, dantrolene+CNQX+CPP, or BAPTA-AM following trauma were all significantly protected from developing seizure activity (Fig. 4A) for up to 6 h, suggesting glutamate triggers increased [Ca\textsuperscript{2+}], which leads to epileptogenesis.

To examine the actions of dantrolene and BAPTA-AM on neocortical neuron intrinsic properties and synaptic responses, cells were assessed in a control set of neocortical slices. Although neither drug had a significant effect on resting potential, time constant, input resistance, action potential duration, and spike threshold, both agents decreased the slow afterhyperpolarization that follows a train of spikes (Fig. 4, B and C) and spike adaptation. Dantrolene had no significant effect on the EPSP, fast IPSP or slow IPSP (Fig. 4D), but BAPTA-AM depressed all three synaptic events (Fig. 4E). These actions are consistent with previous reports (Mody and Macdonald 1995). Of note, the effects of these agents persisted for up to 1 h (duration of the recording) following drug washout.
tetraacetic acid (BAPTA-AM) (variable latency, which occurred all-or-none, and consisted of large depolar-
ization). We thank Drs. R.K.S. Wong and D.S.F. Ling for critically reading the manuscript. This project was supported by National Institute of Mental Health Grant MH-51677. Address for reprint requests: L. S. Benardo, Dept. of Pharmacology, State University of New York, Health Science Center, 450 Clarkson Ave., Box 29, Brooklyn, NY 11203.

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DISCUSSION

In clinical neurotrauma, several processes ensue that can quickly lead to excitotoxicity manifested as epileptogenesis (Dinner 1993). Resolving the contribution of the acute changes to the abnormal physiology that results is difficult in vivo. We report here the development of a novel in vitro trauma model that appears promising as a complement to in vivo studies aimed at elucidating the basic mechanisms of traumatic neocortical injury, in particular mechanisms relevant to penetrating head injuries. In this model, significant disinhibition of GABA\(_A\)-mediated transmission occurred after removal of the superficial third of the neocortex, often resulting in epileptogenesis. The source of disinhibition appears to be twofold. The first derives from physical removal of inhibition-rich superficial cortical laminae, which must normally function to hold deeper excitatory circuits in check. The second results from the downstream events that follow the traumatic insult, in which increased [Ca\(^{2+}\)], (triggered by glutamate release) must play the primary role.

Nonetheless, other explanations may be proffered to account for what may have only appeared to be smaller amplitude IPSPs (when recorded at \(\sim 0\) mV). One is that increased intracellular chloride, with an attendant altered chloride gradient, led to a shift in chloride equilibrium potential. Against this possibility is the observation that, although fast IPSPs were smaller in traumatized preparations, the reversal potentials obtained in dendrotomized neurons were similar to those obtained in control cells. Another explanation is that the input resistance at depolarized levels in dendrotomized cells is somehow decreased (compared with control neurons). However, resistance measurements made at depolarized membrane voltages in dendrotomized neurons were no different than those from control cells.

Thus disinhibition as a consequence of removal of superficial cortical circuitry and glutamate-triggered increases in intracellular calcium would seem to be a parsimonious explanation for our results. The demonstration of superficial inhibitory gating of activity in deep pyramidal neurons has important implications for normal cortical signal processing. Moreover, findings that show that significant neuroprotection can be achieved using a variety of pharmacological agents has significant therapeutic implications, especially given the rapid onset of neurotoxicity in acute head injury, and the chronic morbidity (e.g., epilepsy and focal neurological disorder) experienced by most of the victims who survive significant head injury (Salazar 1992).

Dantrolene and BAPTA-AM depressed the afterhyperpolarization after a train of spikes induced by intracellular current injection and on synaptic transmission. B–D: both dantrolene and BAPTA-AM depressed the afterhyperpolarization, 24 and 30%, respectively. D–E: synaptic potentials (IPSP and 2-component IPSP) were not significantly affected by dantrolene \((n = 10)\), but BAPTA-AM had a generalized depressant effect on synaptic potentials, reducing all 3 components by 30–45% \((n = 10)\).
nized activity in neocortex, and its control by GABA-mediated inhibition. 


