Prolonged Epileptic Seizures: identification and treatment

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Treating acute seizures with benzodiazepines: does seizure duration matter?

David E. Naylor
Harbor-UCLA Medical Center & Los Angeles Biomedical Research Institute, Department of Neurology, California, USA

ABSTRACT – Several clinical trials have shown improved seizure control and outcome by early initiation of treatment with benzodiazepines, before arrival in the emergency department and before intravenous access can be established. Here, evidence is provided and reviewed for rapid treatment of acute seizures in order to avoid the development of benzodiazepine pharmacoresistance and the emergence of self-sustaining status epilepticus. Alterations in the physiology, pharmacology, and postsynaptic level of GABA-A receptors can develop within minutes to an hour and hinder the ability of synaptic inhibition to stop seizures while also impairing the efficacy of GABAergic agents, such as benzodiazepines, to boost impaired inhibition. In addition, heightened excitatory transmission further exacerbates the inhibitory/excitatory balance and makes seizure control even more resistant to treatment. The acute increase in the surface expression of NMDA receptors during prolonged seizures also may cause excitotoxic injury, cell death, and other pathological expressions and rearrangements of receptor subunits that all contribute to long-term sequelae such as cognitive impairment and chronic epilepsy. In conclusion, a short window of opportunity exists when seizures are maximally controlled by first-line benzodiazepine treatment. After that, multiple pathological mechanisms quickly become engaged that make seizures increasingly more difficult to control with high risk for long-term harm.

Key words: GABA-A receptor trafficking, NMDA receptor trafficking, seizure, status epilepticus, epilepsy, hippocampus

Background: clinical perspective

Acute repetitive or prolonged seizures are some of the most common neurological emergencies presenting to the emergency department and can rapidly progress to status epilepticus (SE), with a mortality that approaches 23% (DeLorenzo et al., 1996). Prolonged seizures themselves also may be harmful (Berg et al., 1996; Dube et al., 2010). Many factors contribute to the high morbidity and mortality and include aetiology (especially anoxia) and seizure duration, though it is often difficult to separate the effects of each (Maytal et al., 1989; Lowenstein and Alldredge, 1993; Towne et al., 1994; DeLorenzo et al., 1996; Treiman et al., 1998). However, several studies have shown that seizure duration
is an independent adverse predictor of outcome (Lowenstein and Alldredge, 1993; Towne et al., 1994). In addition, the longer the duration of a seizure, the more likely it is to continue (Scott et al., 1999; Shinnar et al., 2008), with an increasing likelihood of a poor outcome. In particular, 80% of seizures with a duration of less than 30 minutes respond to treatment while less than 40% seizures with a duration of greater than two hours respond well (Mayer et al., 2002). Also, seizures with a duration of greater than one hour are predictive of poor outcomes in all aetiological categories (Towne et al., 1994). After seizing for two hours, much damage may have already been done because no significant worsening of outcome is noted beyond that (Mayer et al., 2002).

Several long-term sequelae may result from prolonged seizure activity. These include neuronal death by 30-60 minutes of seizures (Meldrum and Horton, 1973), hippocampal injury (Ben-Ari, 1985; Cavalheiro et al., 1991; Fountain and Lothman, 1995; Cavalheiro et al., 1996), as well as a liability for chronic epileptic seizures. For example, status epilepticus increases the risk of spontaneous seizures in patients by 2.9 times (Walker, 1998), and seizure duration correlates with the development of spontaneous seizures in animal models (Dube et al., 2010). In addition, epilepsy occurs in 88% of cases of refractory SE but only 22% of cases of SE that respond to therapy (Mayer et al., 2002). Persistent disabling cognitive dysfunction also occurs in 11% of patients (Claassen et al., 2002), and animal studies reveal cognitive dysfunction associated with MRI hippocampal signal abnormalities in adults after prolonged febrile seizures as pups (Dube et al., 2009). Seizures rapidly evolve (over minutes) through several stages as they become increasingly refractory to pharmacotherapy (Walton and Treiman, 1988; Treiman et al., 1998). They become self-sustaining by 20 minutes, paralleling the emergence of benzodiazepine resistance. While all seizures at early stages stop within the first two minutes of benzodiazepine treatment, the response rate rapidly decreases to less than 50% for more prolonged seizures. The results suggest an evolution of seizures that occurs over minutes.

Given the potential harm of prolonged seizure activity and the need for early recognition and treatment, an operational definition of status epilepticus (SE) as seizure activity greater than five minutes has been adopted by many for adult patients (Lowenstein and Alldredge, 1998; Lowenstein, 1999; Lowenstein et al., 1999). Activity that persists for greater than five minutes greatly exceeds (by more than two s.d.) the duration of a typical seizure and is unlikely to spontaneously arrest. Early treatment would shorten duration and help avoid adverse effects from prolonged seizure activity.

Some differences may exist for paediatric populations. First, although the majority of seizures that spontaneously abort will do so by five to ten minutes, up to 20% of both afebrile and particularly febrile seizures can spontaneously arrest after much longer durations, often exceeding 30 minutes (Hesdorffer et al., 2011). Lower morbidity also may be associated with these prolonged seizures compared to adults (Dunn, 1988; Maytal et al., 1989) and, unlike adults, rat pups that experience SE do not develop spontaneous recurrent seizures later (Zhang et al., 2004). In addition, SE is not associated with a rise in neuron-specific enolase (NSE) (a marker of neuronal injury) in pups while large elevations are noted in adults, and this correlates with histological evidence of damage (Sankar et al., 1997). Developmental differences in GABA-AR (and/or glutamatergic) subunit expression may contribute to these age-deterministic effects (Kapur and Macdonald, 1999; Zhang et al., 2004; Brooks-Kayal, 2005).

Despite possible age-related differences, the benefit of treating early (when treatment is most effective), in order to ensure seizure duration is short and avoid adverse outcomes, appears to outweigh potential risks such as respiratory depression, somnolence, and ataxia (Dreifuss et al., 1998; Kutlu et al., 2003). In fact, respiratory depression occurs more commonly in untreated patients who continue to seize (23%) compared to those who receive benzodiazepines in the field (10%) (Alldredge et al., 2001), and is not described as an adverse event in other studies (Knudsen, 1979; Dreifuss et al., 1998; McIntyre et al., 2005).

Benzodiazepines are the preferred initial therapy by neurologists and neuro-intensivists (Brophy et al., 2012; Riviello et al., 2013) for many reasons that include: rapid attainment of peak serum concentrations and onset of action/efficacy (within minutes), good CNS penetration, long duration of action (Leppik et al., 1983; Working Group on Status Epilepticus, 1993; Treiman et al., 1998), safety, and ease of use that includes the choice of multiple formulations and routes of access with high bioavailability, especially for water-soluble agents such as midazolam (Schwagmeier et al., 1998; Scott et al., 1999; Kutlu et al., 2003; Mahmoudian and Zadeh, 2004; McIntyre et al., 2005; Scott, 2005). However, clinical and animal studies show a rapid reduction in the potency of benzodiazepines with increasing seizure duration. While more than 60% of patients who present earlier with overt motor SE respond to lorazepam, less than 20% of those who present later with subtle SE achieve control of acute seizures (Treiman et al., 1998; Mayer et al., 2002). Buccal midazolam has a 100% response rate in children whose seizures are treated within 30 minutes, though only 50% respond beyond that time point (Kutlu et al., 2003). Also, the efficacy of rectal diazepam is significantly
Seizure-induced trafficking of GABA-ARs with loss of synaptic inhibition and available sites for benzodiazepine action

Within one hour of lithium-pilocarpine-induced seizures in rats, a reduction of miniature inhibitory postsynaptic current (mIPSC) amplitude by 27% and area-under the curve (AUC) by 16% indicates a loss of synaptic inhibition mediated by postsynaptic GABA-AR in dentate gyrus (DG) granule cells (Naylor et al., 2005) (figure 1A). Receptor kinetic models and mean-variance fluctuation analysis estimate that the number of postsynaptic GABA-ARs is decreased by 50% (Naylor et al., 2005; Naylor, 2010), consistent with the correlation between mIPSC amplitude and number of synaptic receptors (Nusser et al., 1997). Kinetic changes also occur that primarily involve an increase of mIPSC decay time (Naylor et al., 2005; Goodkin et al., 2005; Feng et al., 2008) and suggest alterations of GABA-AR

Attenuated after 15 minutes of seizures (Knudsen, 1979; Scott, 2005).

For several different animal models, the efficacy of benzodiazepines to stop seizures drops by 50% or more by 10-15 minutes (Walton and Treiman, 1988; Jones et al., 2002), with most anticonvulsant drugs including GABAergic agents becoming completely ineffective by 35 minutes (Morrisett et al., 1987). A 10-20 fold decrease in benzodiazepine potency is noted at this time (Kapur and Macdonald, 1997; Mazarati et al., 1998a), and this is associated directly with alterations in the pharmacology of GABA-ARs that effectively render them unresponsive to benzodiazepines (Kapur and Macdonald, 1997).

Because the transit time to the emergency department (ED) often exceeds 15 minutes with a mean seizure duration exceeding 30 minutes upon arrival to the ED in one study (Alldredge et al., 2001) and closer to 1.3 hours in another (Mayer et al., 2002), several clinical trials have explored ways to avoid unnecessary delays and initiate treatment before arrival in the ED. These have included out-of-hospital delivery of benzodiazepines by paramedics or carers instructed on the use of rescue therapy in high-risk patients (Alldredge et al., 1999; Lowenstein and Alldredge, 1998; Scott et al., 1999; Alldredge et al., 2001; Silbergleit et al., 2012). Other trials attempt to reduce time-to-treat further by avoiding delays associated with obtaining intravenous access (McIntyre et al., 2005; Silbergleit et al., 2012). Minimizing delays due to patient transfer or access improves seizure control and outcome. Out-of-hospital lorazepam in adults shortens seizure duration prior to treatment and terminates seizures in 59.1% before ED arrival compared to spontaneous seizure arrest in 21.1%, and decreases intensive care unit (ICU) admissions by more than 50% (an effect that was independent of aetiology) (Alldredge et al., 2001). In children, pre-hospital treatment with intravenous or rectal diazepam shortens seizure duration to 32 minutes compared to 60 minutes for those initially treated in the ED and also decreases the likelihood of recurrent seizures or intubation (Alldredge et al., 1995).

In addition, compared to the intravenous route, the intramuscular route shortened time-to-treat by more than three minutes and stopped seizures in 73.4% vs. 63.4% of patients by the time of ED arrival, raising the possibility that shortening the delivery time of benzodiazepines by even a few minutes may improve outcome (Silbergleit et al., 2012).

Here, basic mechanisms are considered for the rapid development of self-sustaining seizures associated with an erosion of GABAergic inhibition and the development of benzodiazepine pharmaco-resistance. The argument is supported, that early intervention is the most effective treatment to prevent prolonged seizures and their harmful effects.

**Figure 1.** mIPSCs and NMDA-mEPSCs from dentate gyrus granule cells of SE and controls. (A) mIPSC mean traces from a typical granule cell from a control (black) and a SE animal (red), demonstrating smaller amplitude and prolonged decay in the latter. The mIPSCs were obtained by visualized whole-cell patch-clamp techniques with CsCl in the recording electrode and $V_{\text{clamp}}$ at -70 mV. (B) NMDA-mEPSC mean traces from a typical granule cell from a control (black) and a SE animal (red), demonstrating larger amplitude in the latter. The NMDA-mEPSCs were obtained by visualized whole-cell patch-clamp techniques with Cs gluconate in the recording electrode and $V_{\text{clamp}}$ at -60 mV.
functional properties, in addition to the decrease in postsynaptic receptor numbers.

Immunocytochemical labelling of the gamma 2 subunit, used to synthetically locate GABA-ARs (Nusser et al., 1998) and associated with the synaptic clustering molecule gephyrin (Essrich et al., 1998), confirms the decrease in the expression of synaptic receptors predicted by physiological measurements (Naylor et al., 2005). The gamma 2 subunit also confers benzodiazepine sensitivity of synaptic GABA-ARs (Saxena and Macdonald, 1996); benzodiazepines bind at the pocket between alpha and gamma 2 subunits (Nusser et al., 1998; Venkatachalan and Czajkowski, 2012), and gamma 2 is essential for benzodiazepine sensitivity (Pritchett et al., 1989; Sigel et al., 1990). Consequently, the loss of synaptic gamma 2 subunit-containing GABA-ARs would be expected to decrease the number of available receptors for benzodiazepine binding and action.

The remaining synaptic GABA-ARs have a similar response compared with controls to maximal doses of diazepam, with a prolongation of mIPSC decay time and increase in AUC. But, the augmentation of synaptic inhibition by the benzodiazepine still remains insufficient to counter the initial loss by GABA-ARs (Saxena and Macdonald, 1996) as well as insufficient to proportionally increase the number of GABA-ARs in the postsynaptic density (Naylor et al., 2005; Venkatachalan and Czajkowski, 2012). A similar study in juvenile rats shows diazepam responsiveness early and 30 minutes after acute seizures, though some blunting of the benzodiazepine response is noted at 30 minutes (Feng et al., 2008). Whether or not direct alterations of GABA-AR function and pharmacology are contributory (Kapur and Lothman, 1990) to synaptic inhibition remains an open question. However, the loss of synaptic inhibition of GABA-ARs predicted by physiological measurements (Naylor et al., 2005; Venkatachalan and Czajkowski, 2012) appears to precede the onset of seizures and the trafficking of GABA-AR associated with SE (Naylor et al., 2005). This augmented excitation in the background of degraded synaptic inhibition will further upset the balance between inhibition and excitation and greatly diminish the prospect for seizure control by benzodiazepines and other anticonvulsants.

In addition, NMDARs contribute to the downregulation of GABA-ARs, either as the result of circuit hyperactivity or direct NMDAR activation (Bannai et al., 2009; Muir et al., 2010), via calcineurin phosphatase action on gamma 2 subunits (Wang et al., 2003; Muir et al., 2010) and lateral diffusion of GABA-ARs away from synapses and potentially towards endocytic sites (Wang et al., 2003; Bannai et al., 2009). These changes affect the synaptic, gamma 2 subunit-containing, and benzodiazepine sensitive, GABA-ARs. Pretreatment with NMDAR antagonists prevents the acute loss of synaptic inhibition (Kapur and Lothman, 1990) and the loss of benzodiazepine sensitivity, even after 60 minutes of seizures (Rice and DeLorenzo, 1999). Similarly, seizure-related AMPAR activation also down-regulates synaptic inhibition via calcineurin activation (Sanchez et al., 2005).

**NMDAR trafficking to synapses rapidly increases excitation**

Unlike synaptic GABAergic inhibition, glutamatergic excitation increases in DG granule cells by one hour of lithium-pilocarpine-induced seizures. An increase of NMDA-mEPSC amplitude and AUC to 123 and 132%, respectively, of controls (figure 1B) is estimated (by mean-variance fluctuation analysis) to involve a 38% increase in the number of postsynaptic NMDARs (Naylor et al., 2013). NR2B subunit-containing NMDARs account for the increase, and immunocytochemical labelling of NMDAR subunits confirms trafficking of receptors to synapses.

An increased contribution of non-NMDARs to mEPSCs also occurs by one hour with an increase of amplitude to 120% of controls and estimated increase of 22% in the number of non-NMDARs at synapses (unpublished results). AMPAR potentiation is noted after hypoxic seizures as well (Rakhade et al., 2008; Rakhade et al., 2012), and seizure-induced switches of AMPAR subunit composition to Ca++ permeant, GluA2-lacking, variants also sustains seizure activity (Rajasekaran et al., 2012). This augmented excitation in the background of degraded synaptic inhibition will further upset the balance between inhibition and excitation and greatly diminish the prospect for seizure control by benzodiazepines and other anticonvulsants.

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**Activity-dependent and immediate functional losses of synaptic inhibition**

Prolonged decay times of mIPSCs suggest functional alterations of postsynaptic GABA-ARs after one hour of seizures (figure 1A), but extracellular field recordings in the DG show that loss of evoked paired-pulse inhibition (PPI), another metric of synaptic inhibition, occurs after as little as one minute of perforant path electrical stimulation in vivo, and persists for greater than 20 minutes before recovery (Naylor and Wasterlain, 2005) (figure 2A). A similar loss of PPI for evoked postsynaptic GABA-AR responses recorded in DG granule cells occurs immediately after five minutes of stimulation in vivo (figure 2B). Because the loss of inhibition with electrical stimulation in vivo occurs well before the occurrence of isolated seizures and certainly before the 30 minutes of perforant path electrical stimulation necessary for self-sustaining seizures (Mazarati et al., 1998b), diminished synaptic inhibition appears to precede the onset of seizures and the trafficking of GABA-AR associated with SE (Naylor et al., 2005).

In addition, GABA-AR trafficking decreases of postsynaptic receptors would be expected to proportion-
and Macdonald, 1996; Knoflach (tors with delta subunits have much less desensitization Unlike gamma 2 subunit-containing GABA-ARs, recep-

subunit of synaptic receptors. include a delta subunit as opposed to the gamma 2

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ming the co-assembly of three subunits: two alphas,

GABA-ARs are pentomeric structures primarily invol-

of sustained loss of inhibition and ongoing seizures

Increase in the number of extrasynaptic GABA-ARs also could explain the increased tonic currents, and increased delta subunit expression has been described with SE by some (Terunuma et al., 2008), but not others (Goodkin et al., 2008). Because the addition of 100 μM GABA occludes the difference in tonic currents between SE and control DG granule cells (Naylor et al., 2005), the increase with SE is attributed to an increase in extracellular GABA more than to an increase in the number of extrasynaptic receptors. Regardless of whether some change in extrasynaptic delta subunit surface expression occurs during SE, our results support micromolar increases in extracellular GABA (Naylor et al., 2005), and steady increases have been observed more directly with assay measurements of GABA at various time points after the onset of seizures (Walton et al., 1990; Wasterlain et al., 1993). Also, even brief stimulation may increase tonic currents. DG granule cell tonic currents increase 18.9±4.8 pA (p<0.05) after five minutes of hyperstimu-

Figure 2. Loss of paired-pulse inhibition (PPI) measured in the DG after brief perforant path stimulation in vivo and in vitro. (A) Loss of PPI measured by field recordings in vivo with excitatory population-spikes on both P1 and P2 appearing within minutes after stimulation for one minute of 2-Hz continuous and 20 Hz for 10 sec/min. (B) Evoked IPSC responses recorded from the same DG granule cell before (top) and after (bottom) five minutes of repetitive perforant path stimulation (PPS). Note greater inhibition with P2 before stimulation, but less after. Cs gluconate was in the recording electrode with Vclamp at 0 mV.

Seizures increase extracellular GABA

Along with the reduction of mIPSC currents, an increase of tonic GABA-AR currents occurs in DG granule cells after seizing for one hour (figure 4A). GABA-ARs are pentomeric structures primarily involving the co-assembly of three subunits: two alphas, two betas, and a gamma or delta subunit (McKernan and Whiting, 1996). The tonic currents are mediated by extrasynaptic GABA-ARs (Nusser et al., 1998) that, in DG, contain subunit subtype combinations that include a delta subunit as opposed to the gamma 2 subunit of synaptic receptors.

Unlike gamma 2 subunit-containing GABA-ARs, receptors with delta subunits have much less desensitization (figure 5C) and lack benzodiazepine sensitivity (Saxena and Macdonald, 1996; Knoflach et al., 1996; Haas and Macdonald, 1999; Brown et al., 2002). In addition, extrasynaptic receptors are much more sensitive to low levels of ambient GABA in the extracellular space. As a result of high sensitivity and low desen-

stization to GABA, extrasynaptic receptors can be reliable indicators of GABA levels. The mean and vari-

ance of extrasynaptic tonic currents correlates with GABA levels and can be used to generate a dose-

response curve for extracellular GABA (figure 4B).

Based on this curve, GABA-AR tonic currents predict that GABA levels can exceed 5 μM after one hour of seizing (Naylor et al., 2005). In fact, tonic currents after seizures are similar to those after added GABA (figure 4A).

Increase in the number of extrasynaptic GABA-ARs also could explain the increased tonic currents, and increased delta subunit expression has been described with SE by some (Terunuma et al., 2008), but not others (Goodkin et al., 2008). Because the addition of 100 μM GABA occludes the difference in tonic currents between SE and control DG granule cells (Naylor et al., 2005), the increase with SE is attributed to an increase in extracellular GABA more than to an increase in the number of extrasynaptic receptors. Regardless of whether some change in extrasynaptic delta subunit surface expression occurs during SE, our results support micromolar increases in extracellular GABA (Naylor et al., 2005), and steady increases have been observed more directly with assay measurements of GABA at various time points after the onset of seizures (Walton et al., 1990; Wasterlain et al., 1993). Also, even brief stimulation may increase tonic currents. DG granule cell tonic currents increase 18.9±4.8 pA (p<0.05) after five minutes of hyperstimu-

Figure 3. Schematic relating loss of inhibition to self-sustaining seizures and unbalanced glutamatergic excitation. Initial perturbation at any point in the cycle can lead to increase circuit activity with activation of NMDARs that trigger decreases of post-

synaptic GABA-AR level and function. The erosion of inhibition further upssets the balance and supports persistent over-activity and seizures.
curve consistent with up to a micromolar elevation in the extracellular GABA (figure 4B), qualitatively similar, but less than is observed after one hour of SE (figure 4A). Tonic extrasynaptic GABA currents in the DG appear to parallel levels of circuit activity, which occurs in the cerebellum (Brickley et al., 1996).

Sources of GABA may derive from synaptic release (Glykys and Mody, 2007), but also may occur from reversals of GABA transport by glia (Wu et al., 2007). Certainly, an increase in synaptic release with circuit hyperactivity is expected during prolonged seizures, and blockade of GABA uptake after SE causes an increase, not a decrease, of GABA (as indicated by the increase in tonic currents) (Naylor et al., 2005).

**GABA exposure (tonic or phasic) desensitizes and functionally alters synaptic GABA-ARs with early loss of paired-pulse inhibition**

It is estimated that activity-dependent increases in extracellular GABA can exceed a few micromolar, especially after prolonged seizures. Moreover, adding micromolar amounts of GABA is sufficient to cause significant, rapid, and reversible desensitization of postsynaptic GABA-ARs (figure 5A) (Naylor, 2010), especially if uptake mechanisms are blocked and extracellular GABA can readily invade synapses and affect...
IPSCs. Exposure of desensitizing synaptic receptors to elevated levels of GABA may explain some reports of paradoxical worsening of seizures after treatment with tiagabine (Walton et al., 1994; Shinnar et al., 2001; Fitzek et al., 2001), as well as instances of benzodiazepine ineffectiveness as treatment for these precipitated seizures (de Borchgrave et al., 2003).

In addition, physiologically based synaptic models indicate that desensitization of postsynaptic GABA-ARs also occurs with brief high-frequency pulsatile exposure that simulates direct synaptic release under overactive conditions (using GABA-AR receptor kinetic parameters defined previously [Naylor et al., 2005]). At 40 Hz, predicted desensitization of postsynaptic GABA-ARs will degrade IPSCs by more than 50% and this occurs after only 100-200 msec (figure 5B), raising the possibility that even very brief hyperactivity such as “fast ripples” could have an impact on GABA-AR properties.

Such rapid desensitization of gamma 2 containing receptors to high-frequency pulses of GABA has been observed with in vitro expression systems (Bianchi and Macdonald, 2002). In fact, exaggerated pulsatile release of GABA at synapses may desensitize postsynaptic GABA-ARs more potently than extracellular elevations of transmitter, especially when GABA uptake mechanisms are intact. GABA transporters, though numerous, have low turnover rates and regulate low (micromolar) levels of extracellular tonic (Jensen et al., 2003; Hu and Quick, 2008) and spillover (Wei et al., 2003) of GABA better than they can shape and control the high phasic concentrations (millimolar) of transmitter inside the synaptic cleft. Extrasynaptic delta subunit-containing receptors are more likely to be influenced by this type of transporter control, while synaptic gamma 2 subunit-containing GABA-ARs may remain vulnerable to circuit hyperactivity and desensitization. Desensitization from tonic GABA exposure or pulsatile release can contribute not only to an effective loss of available GABA-ARs (with decrease mIPSC amplitude; figure 5A), but also can alter postsynaptic receptor kinetic properties, including a loss of evoked PPI (figure 6C). Based on computational models of evoked IPSCs as a filtered sum of individual mIPSCs (with synaptic mIPSC representations and GABA-AR receptor kinetic parameters defined previously; Naylor et al.,...
(2005)), simulations of evoked paired-pulse responses that apply parameters for GABA as either 5 μM of tonic or brief 40 Hz pulsatile GABA exposure show similar losses of PPI for either condition (figure 6C). A similar loss of PPI is observed experimentally after perforant path stimulation in vitro (figure 2B). Because desensitization occurs rapidly, it may cause very early losses of inhibition that occur during, or even precede, seizures (figure 2).

In addition, gamma 2 subunit-containing GABA-ARs at synapses have properties that include not only receptor desensitization but also sensitivity to benzodiazepines (Saxena and Macdonald, 1996; Haas and Macdonald, 1999). Therefore, desensitization
affects GABA-ARs that overlap with those that are benzodiazepine sensitive and could contribute to the development of benzodiazepine insensitivity even earlier than would occur from receptor trafficking. In addition, although GABA exposure and receptor desensitization is insufficient itself to induce synaptic GABA-AR trafficking (Goodkin et al., 2008), it may be sufficient to trigger losses of inhibition with increases of DG circuit activity and stimulation of excitatory synaptic receptors. Activation of NMDARs (Bannai et al., 2009) and AMPARs (Sanchez et al., 2005) then can down-regulate synaptic GABA-ARs.

Discussion

Both clinical and animal studies note a rapid loss of benzodiazepine potency as seizures persist (Kapur and Macdonald, 1997; Mazarati et al., 1998a; Treiman et al., 1998; Jones et al., 2002), and this parallels the emergence of self-sustaining seizures with pronounced losses of synaptic inhibition. Several factors may be important. First, a decreased number of postsynaptic gamma 2 subunit-containing GABA-ARs occurs by one hour of seizures and is associated with reduced mIPSC amplitudes and selective trafficking of benzodiazepine-sensitive receptors (Naylor et al., 2005; Terunuma et al., 2008). Conversely, a rapid increase in the delivery of NMDARs containing NR2B subunits to the cell surface also occurs by one hour and contributes to increases in both synaptic and extrasynaptic excitation (Frasca et al., 2011; Naylor et al., 2013). This combination of effects causes an imbalance between inhibition and excitation that can sustain seizures and make them increasingly more difficult to treat (figure 3).

In addition, functional losses of synaptic inhibition in the DG occur within minutes of hyper-active perforant path stimulation and may involve rapid alterations of GABA-AR kinetic properties, such as desensitization (Naylor et al., 2005; Naylor and Wasterlain, 2005; Naylor, 2010). Over-exposure to excess synaptic release and/or rising tonic levels of GABA may be sufficient to desensitize the gamma 2 subunit-containing and benzodiazepine-sensitive postsynaptic GABA-ARs.

The activation of NMDAR and Ca++ entry simultaneously may trigger pathways that shift GABA-AR surface expression away from synapses, further aggravating acute losses of synaptic inhibition (Wang et al., 2003; Bannai et al., 2009), while also contributing to long-term effects that include epilepsy (Rice and De Lorenzo, 1998), cognitive dysfunction (Dube et al., 2009) and neuronal injury and cell death (Fujikawa, 1995; Deshpande et al., 2008; Frasca et al., 2011).

The multiple effects of seizures and circuit hyperactivity on GABA-ARs include some that change receptor functional properties (Kapur and Coulter, 1995; Kapur and Macdonald, 1997). Others, such as lateral diffusion of receptors away from synapses (Bannai et al., 2009; Muir et al., 2010) and trafficking of receptors to the cell interior (Naylor et al., 2005; Goodkin et al., 2005; Terunuma et al., 2008), primarily change the number of receptors available at synapses and on the cell surface, although selective trafficking of particular subtypes of GABA-ARs could skew physiological and pharmacological properties based on changes in the proportion of receptor subtypes. In addition, the lateral diffusion of GABA-ARs away from synapses and closer to endocytotic zones may herald receptor trafficking.

These effects occur by one hour and some within minutes. For example, activity-dependent lateral diffusion of GABA-ARs decreases mIPSC amplitude by five to ten minutes, with recovery over a similar time course (Bannai et al., 2009; Muir et al., 2010). Functional alteration of synaptic inhibition associated with a loss of PPI, that initially may result from postsynaptic GABA-AR desensitization among other possibilities, also occurs within minutes and may recover within minutes or can persist longer, depending on the duration of seizure activity (Kapur and Lothman, 1989; Naylor et al., 2002; Naylor et al., 2005; Naylor and Wasterlain, 2005; Holtkamp et al., 2005).

Many routes are available to initiate acute losses of synaptic inhibition. Heightened circuit activity, either by increases of excitation or by decreases of inhibition (Bannai et al., 2009), leads to stimulation of NMDARs (Bannai et al., 2009; Muir et al., 2010) or AMPARs (Sanchez et al., 2005; Rakhade et al., 2008; Rakhade et al., 2012). Calcineurin is a target of such activation with dephosphorylation of GABA-AR subunits and unmasking of AP2 binding sites for GABA-AR endocytosis (Bannai et al., 2009). Alternatively, the activity of kinases including isoforms of PKC may be decreased (or increased) with similar results (Terunuma et al., 2008). Changes in the phophorylation state of GABA-AR subunits may not only alter the synaptic and cell surface numbers of receptors, but also can alter receptor physiological and pharmacological properties, including a loss of benzodiazepine sensitivity (Gao and Greenfield, 2005).

In addition, seizure-induced expression and potentiation of excitatory NMDARs (Frasca et al., 2011; Naylor et al., 2013) and AMPARs (Sanchez et al., 2005; Rakhade et al., 2008) will not only facilitate excitatory transmission and circuit hyperactivity, but also will further engage the same kinase and phosphatase transduction pathways that are responsible for GABA-AR down-regulation in the first place. The interaction
between NMDAR activation and GABA-AR regulation may explain why NMDA blockade prevents loss of benzodiazepine sensitivity as seizures progress (Kapur and Lothman, 1990; Rice and DeLorenzo, 1999). Based on this scheme, a perturbation of inhibition or excitation could trigger circuit over-activity with movement of GABA-ARs away from synapses, loss of synaptic inhibition, and greater activity and stimulation of NMDARs (which is also increased by seizures). A “vicious cycle” of self-sustaining seizure activity could be established that preferentially drives the removal of postsynaptic gamma 2 subunit-containing GABA-ARs that are benzodiazepine sensitive.

Many anticonvulsants, including non-GABAergic drugs such as phenytoin, lose potency with prolonged seizures (Morrisett et al., 1987; Treiman et al., 1998; Jones et al., 2002), but benzodiazepines appear to be particularly affected (Walton and Treiman, 1988; Kapur and Macdonald, 1997). Effects on Cl- gradients that diminish GABA-AR-mediated hyperpolarisation occur during SE (Kapur and Coulter, 1995; Rivera et al., 2004; Lee et al., 2010) and certainly should diminish and possibly reverse the efficacy of benzodiazepines (Staley, 1992). However, such an effect should generalise to all drugs that act on GABA-ARs and to all GABA-ARs, including those at both synaptic and extrasynaptic sites.

However, even though the efficacy of GABAergic drugs as a class may be diminished with seizure progression, this is not uniform and differential effects have been noted between benzodiazepines, barbiturates, and propofol (Kapur and Macdonald, 1997; Treiman et al., 1998; Mayer et al., 2002; Rossetti et al., 2002; Shorvon, 2011). Prolonged seizures respond less well to benzodiazepines than barbiturates and propofol (Mayer et al., 2002; Rossetti et al., 2002), and GABA-ARs can become completely insensitive to benzodiazepines (Kapur and Coulter, 1995) while barbiturate sensitivity is preserved (Kapur and Macdonald, 1997). While treatment failure approaches 45% for midazolam in refractory SE, failure is indicated as 13 and 25% for barbiturates and propofol, respectively (Rossetti et al., 2002). A significant proportion of refractory SE responds to barbiturates after benzodiazepines have failed (Mayer et al., 2002).

A potential mechanism for differential loss of potency between benzodiazepines and barbiturates may relate to the prefered GABA-AR subunit binding sites for these agents and selective trafficking of GABA-ARs of particular subtypes during seizures. In particular, GABA-ARs with a gamma 2 subunit are synaptic (Nusser et al., 1998) and necessary for benzodiazepine sensitivity (Pritchett et al., 1989; Saxena and Macdonald, 1996). Benzodiazepines bind at the pocket between alpha and gamma subunits (Nusser et al., 1998; Venkatachalan and Czajkowski, 2012), while barbiturates and propofol bind the beta subunit that is ubiquitous for all GABA-ARs (Amin and Weiss, 1993; Serafini et al., 2000). Therefore, the movement of gamma 2 subunit-containing GABA-ARs away from synapses by lateral diffusion (Bannai et al., 2009; Muir et al., 2010) and/or receptor trafficking (Naylor et al., 2005; Terunuma et al., 2008) would preferentially affect the receptors with the greatest benzodiazepine sensitivity. Desensitization of the susceptible gamma 2-containing receptors (Haas and Macdonald, 1999; Bianchi and Macdonald, 2002) also may selectively restrict the availability of receptors with benzodiazepine sensitivity.

Pharmacoresistance may evolve from a combination of effects, both general and specific. The establishment, late into seizures, of self-sustaining hyperactive circuits, now characterised by alterations of both GABAergic and glutamatergic synapses, may be resistant to any intervention. However, there may be more specific effects of seizures, especially early, which focus on particular subtypes of GABA-ARs.

The subunit combinations of GABA-ARs evolve through brain development and may impact seizure characteristics, pharmacosensitivity, and long-term effects. In particular, alpha 1 and gamma 2 subunits, which combine to make up to 55% of GABA-ARs in mature synapses (Benke et al., 1994; McKernan and Whiting, 1996; Jacob et al., 2008), are at a low level at birth and increase two to three fold through adulthood (Brooks-Kayal et al., 1998; Brooks-Kayal, 2005). Receptors that contain this combination of alpha 1 and gamma 2 subunits are among the most benzodiazepine sensitive, with a seven fold increase in GABA efficacy (Pritchett et al., 1989), which explains the lack of benzodiazepine sensitivity in newborn rats (Kapur and Macdonald, 1999). In addition, the alpha 1 subunit is protective of seizures (Poulter et al., 1999; McIntyre et al., 2005; Raol et al., 2006), and mutations of alpha 1 and gamma 2 are associated with familial epilepsy (Bouthour et al., 2012). These developmental effects on GABA-AR subtypes may play a role in the longer duration of seizures in children compared to adults (Hesdorffer et al., 2011) as well as the high incidence of convulsive SE in children (Walker, 1998; Scott et al., 1999). Presumably, a lower expression level of GABA-ARs with combinations of alpha 1 and gamma 2 subunits could alter seizure responsiveness to particular pharmacological agents in an age-dependent manner as well.

Similarly, atypical GABA-AR subtypes with alpha 4 gamma 2 subunit combinations, that occur with epileptogenesis (Peng et al., 2004; Joshi and Kapur, 2013) and are benzodiazepine insensitive (Knoflach et al., 1996; Wafford et al., 1996; Brown et al., 2002), potentially could alter benzodiazepine responses in chronic epileptic patients, compared to new presentations.
When synaptic inhibition is lost and seizures become self-sustaining and benzodiazepine resistant, alternate therapies that might help restore the balance of inhibition and excitation include antagonists of NMDARs. NMDA blockade with ketamine or MK-801 is successful in several animal models of SE long after the development of benzodiazepine pharmacoresistance (Fariello et al., 1989; Walton and Treiman, 1991; Mazarati and Wasterlain, 1999; Borris et al., 2000).

Interestingly, NMDA antagonists may not be effective early or may even worsen seizures (Fariello et al., 1989; Bertram and Lothman, 1990), but may provide 100% control at 60 minutes (Borris et al., 2000). Perhaps inhibition from interneurons remains relatively intact early during seizures and NMDAR blockade not only decreases excitation of pyramidal and granule cells, but also decreases excitation of inhibitory interneurons, with disinhibition of pyramidal and granule cells. However, later after synaptic inhibition from interneurons has failed, the primary effect of NMDAR antagonism would be on excitatory cells. Combinations of NMDAR blockers and benzodiazepines may be much more effective than either agent alone (Walton and Treiman, 1991).

Clinically, success rates for treatment of SE by ketamine have been reported as high as 60-70% in epileptics (Rosati et al., 2012; Synowiec et al., 2013), but may be lower for refractory SE in patients with other aetiologies (Gaspard et al., 2013). An added benefit of treatment with NMDA blockers such as ketamine may not only be immediate seizure control, but also the prevention of long-term sequelae such as chronic epilepsy (Rice and DeLorenzo, 1998) and other adverse effects of excitotoxicity (Fujikawa, 1995; Deshpande et al., 2008; Frasca et al., 2011).

In conclusion, seizures rapidly become self-sustaining and pharmacoresistant secondary to multiple mechanisms that include: alterations of GABA-AR physiology and pharmacology, losses of synaptic GABA-ARs that mediate benzodiazepine action, and increases in the surface expression of excitatory NMDARs that make the task of restoring the balance between inhibition and excitation even more daunting. Very early treatment with a safe, fast, and effective drug, such as a benzodiazepine, before this intractable and deleterious sequence of events has opportunity to take hold, appears to be the best strategy. □

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