Chapter 10
The Role of Extrasynaptic $GABA_A$ Receptors in Focal Epilepsy

Matthew C. Walker and Ivan Pavlov

Abstract Focal epilepsy can result from both genetic and environmental factors. Acquired focal epilepsy occurs following a specific brain insult such as stroke, head injury or prolonged seizures (status epilepticus). The time from the insult to the development of seizures is termed the epileptogenic period during which there are changes in connectivity, neurotransmission and neuronal excitability.

Epileptogenesis has conventionally been viewed as being associated with increased excitation and a loss of inhibition. This view has been reappraised in recent years due to a better understanding of the multifaceted roles of GABAergic signalling. There is growing evidence that loss of synaptic $GABA_A$ receptor-mediated inhibition observed in animal models of temporal lobe epilepsy is accompanied by compensatory upregulation of tonic currents mediated by extrasynaptic $GABA_A$ receptors.

Here, we provide evidence for such a change in $GABA_A$ receptor-mediated inhibition during epileptogenesis and speculate on the possible functional impact that such a shift in inhibition will have. In particular, we argue that shifts from phasic to tonic inhibition in the hippocampus will lead to a maintenance of "inhibition" of the network but will alter network gain, decreasing network stability. Furthermore, changes in the subunit composition of extrasynaptic $GABA_A$ receptors during epileptogenesis have implications for targeted pharmacotherapy of epilepsy.

Keywords Focal epilepsy · Tonic inhibition · Extrasynaptic $GABA_A$ receptors · Hippocampus · Hippocampal sclerosis

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A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_10

10.1 Introduction

Focal epilepsy can arise as the result of: gene mutations leading to increased neuronal/network excitability (such as mutations in nicotinic acetylcholine receptor genes that result in autosomal dominant nocturnal frontal lobe epilepsy, and mutations in leucine-rich, glioma inactivated 1 resulting in autosomal dominant temporal lobe epilepsy with auditory hallucinations); malformations of cortical development (both genetically and environmentally determined); brain tumours; and acquired lesions following a specific brain insult such as prolonged seizures (status epilepticus), head injury and stroke. The role of extrasynaptic $GABA_A$ receptors has predominantly been determined in the acquired (rather than genetic) focal epilepsies and it is these epilepsies that we concentrate on in this chapter.

The observations that $GABA_A$ receptor antagonists induce seizures, and that drugs that directly potentiate $GABA_A$ receptors (such as benzodiazepines and barbiturates) or that decrease the uptake or breakdown of GABA (tiagabine and vigabatrin, respectively) prevent seizures, indicate the importance of the GABAergic system in seizure generation. Indeed, focal epilepsy has often been viewed as a disruption of the balance between glutamatergic excitation and GABAergic inhibition. The recognition that GABAergic inhibition can take varying forms over different timescales with different effects on neuronal excitability (Mody and Pearce 2004; Farrant and Nusser 2005; Farrant and Kaila 2007) has led to a reappraisal of this simplistic view. A critical observation is that even people or animals with severe epilepsy spend the majority of their time free of seizures. This suggests that the process leading to the development of epilepsy more likely creates networks of neurons that are intrinsically more unstable and in which significant changes in excitability are mostly compensated. However, this compensation either is insufficient or may itself lead to less stable network behaviour.

Most studies in animal models of focal epilepsy have determined changes in fast, synaptic inhibition mediated by the synaptic release of GABA onto postsynaptic $GABA_A$ receptors, and only more recently have changes in tonic currents, mediated by extrasynaptic $GABA_A$ receptors, been investigated. A role for tonic currents in human epilepsy has been suggested by the observation that polymorphisms and mutations in genes encoding extrasynaptic $GABA_A$ receptors, which reduce the magnitude of tonic currents, are associated with several human epilepsies (Dibbens et al. 2004; Feng et al. 2006; Eugène et al. 2007). These, however, are exclusively generalised epilepsies, but emphasise the importance of tonic currents in regulating network excitability. In this chapter, we will argue that, in focal epilepsies, tonic currents are usually increased, perhaps as a compensatory mechanism. This results in a shift from phasic to tonic inhibition, changing network behaviour. Increases in tonic inhibition compensate not just for loss of phasic $GABA_A$ receptor-mediated inhibition but also for loss of other membrane conductances such as the HCN-mediated conductance which is involved in the regulation of cell excitability (Chen et al. 2010), and the loss of which occurs in the development of focal epilepsy.

We also restrict ourselves to considering temporal lobe epilepsy, the most common focal epilepsy syndrome. This has been predominantly studied in an experimental paradigm in which animals are subjected a specific insult (e.g. prolonged seizure induced by a convulsant or traumatic brain injury), after which the animal develops epilepsy over a period of days or months (termed epileptogenesis). It is also worth noting that although we focus on extrasynaptic $GABA_A$ receptors and tonic inhibition, these must be viewed in the context of the panoply of changes in network connectivity, neuronal excitability and synaptic transmission that accompany the development of focal epilepsy.

10.2 Inhibition in Focal Epilepsy

10.2.1 Phasic Inhibition

Early evidence indicated that loss of $GABA_A$ receptor inhibition alone would permit the dominance of excitation and the generation of epileptiform activity (Schwartzkroin and Prince 1977; Gutnick et al. 1982; Connors 1984). Later studies of in vitro models of epileptic activity in acute rodent brain slices have highlighted the importance of a breakdown in feed-forward inhibition for the propagation of seizure-like activity (Trevelyan et al. 2006, 2007). There have been corresponding findings in brain slices from humans, who have previously undergone brain resection to cure their epilepsy, in which there is a loss of synaptic $GABA_A$ receptor-mediated inhibition prior to the occurrence of seizure-like activity (Huberfeld et al. 2011). It was, therefore, expected that epileptogenesis is associated with a loss of interneurons and/or a reduction in the number of or activity of inhibitory synapses on surviving principal neurons (Cossart et al. 2001; Wittner 2001; Buckmaster et al. 2002; Kobayashi and Buckmaster 2003; Shao and Dudek 2005; Hunt et al. 2009; Wyeth et al. 2010). These morphological and functional changes of inhibition occur quite rapidly following the epileptogenic insult, but may continue for a prolonged period of time extending to several months or beyond (Sloviter 1991; Friedman et al. 1994; Leroy et al. 2004; Pavlov et al. 2011). Some studies have suggested that the loss of dendritic-targeting interneurons may be partially compensated by surviving interneurons that project to the soma (Cossart et al. 2001), but the general consensus is that the net outcome of these changes is the loss of phasic (synaptic) inhibition.

10.2.2 Tonic Inhibition

A decrease in synaptic release would be expected to reduce the concentration of GABA present in the extracellular space and so be associated with a reduction in tonic currents mediated by extrasynaptic $GABA_A$ receptors. A correlation between synaptic release and tonic currents has been observed in CA1 pyramidal cells when

GABA uptake is reduced by inhibition of the GABA transporter GAT1 (Glykys and Mody 2007). Surprisingly, experimental evidence indicates that in post-status epilepticus animals, tonic $GABA_A$ receptor-mediated currents are maintained or even increased in many hippocampal neurons including CA1 pyramidal cells, *stratum radiatum* interneurons and dentate granule cells (Scimemi et al. 2005; Naylor et al. 2005; Zhang et al. 2007; Goodkin et al. 2008; Zhan and Nadler 2009; Rajasekaran et al. 2010). Studies in models of post-traumatic epilepsy indicate involvement of the hippocampus in the epileptogenic process and have found similar hippocampal pathology to that observed in post-status epilepticus animals (D'Ambrosio et al. 2005; Kharatishvili et al. 2006; Swartz et al. 2006). These hippocampal changes are also accompanied by maintenance of tonic currents, which have been reported to be preserved in dentate granule cells following lateral fluid-percussion brain injury (Pavlov et al. 2011), or to be enhanced after controlled cortical impact (Mtchedlishvili et al. 2010). Although there is no control comparator, significant tonic $GABA_A$ receptor-mediated currents are also present in neocortical pyramidal cells and dentate granule cells in resected tissue from humans with temporal lobe epilepsy (Scimemi et al. 2006). There are a number of explanations for the above. Recent data indicate that tonic currents in dentate granule cells are largely mediated by spontaneously opening $GABA_A$ receptors, and that extracellular GABA in the hippocampus under baseline conditions is kept at concentrations that are insufficient to activate extrasynaptic $GABA_A$ receptors (Wlodarczyk et al. 2013). Increases in tonic currents may be due to increases or changes in extrasynaptic $GABA_A$ receptors, changes of the sensitivity of extrasynaptic receptors for GABA or an increase in the GABA concentration surroundings neurons.

10.3 Extrasynaptic $GABA_A$ Receptor Plasticity in Focal Epilepsy

Epileptogenesis is associated with changes in the expression of $GABA_A$ receptor subunits in animal models (e.g. Brooks-Kayal et al. (1998) and in humans Loup et al. (2000)). The nature of these changes is partly dependent upon the model of focal epilepsy. Indeed, the heterogeneity of such changes indicates that the down- or upregulation of specific subunits is unlikely to be necessary for the development of epilepsy (Pavlov et al. 2011). In the hippocampus, α5β2/3γ2 and α4β2/3δ receptors generate most of the tonic current. While δ-containing $GABA_A$ receptors are exclusively extrasynaptic or perisynaptic (Wei et al. 2003), α5-containing receptors can also be found at postsynaptic sites (Serwanski et al. 2006). Double knockout mice lacking both δ and α5 subunits have almost no tonic $GABA_A$ receptor-mediated currents in pyramidal cells, dentate granule cells or molecular layer interneurons (Glykys et al. 2008). The relative contribution of different $GABA_A$ receptor subtypes to the generation of tonic currents in the hippocampus varies depending on the cell type. In pyramidal cells, α5-containing receptors contribute to ~50% of the tonic conductance, whilst in dentate granule cells, the majority of the tonic current

(~70%) is mediated by δ-containing receptors (Glykys et al. 2008). In addition, a small proportion (~10%) of extrasynaptic receptors on hippocampal pyramidal neurons lack both γ and δ subunits. These Zn^{2+}-sensitive αβ receptors may also contribute to tonic conductances in hippocampal neurons (Mortensen and Smart 2006).

10.3.1 Changes in α5 and δ Subunit Expression in the Hippocampus

Preservation of tonic $GABA_A$ receptor-mediated currents in epilepsy may be accompanied by their altered pharmacology. For example, despite tonic currents being preserved in CA1 pyramidal cells under baseline conditions and increased upon application of GABA in post-status epilepticus animals, there is a marked reduction in the sensitivity of these currents to an α5-specific inverse agonist L-655,708 (Scimemi et al. 2005). This is consistent with the decrease in α5 subunit expression in these animals (Fritschy et al. 1999; Houser and Esclapez 2003), thus suggesting that there is a substitution of the α5-containing receptors in epileptic tissue by extrasynaptic receptors that are either more numerous or have a greater sensitivity to GABA (Fig. 10.1). The subunit composition of these receptors has yet to be determined.

Changes in the δ subunit expression in dentate granule cells are less clear-cut. It has mostly been found to be reduced following status epilepticus in animal models (Schwarzer et al. 1997; Tsunashima et al. 1997; Peng et al. 2004; Rajasekaran et al. 2010), but see (Brooks-Kayal et al. 1998; Goodkin et al. 2008). Also, studies in post-traumatic epilepsy (Pavlov et al. 2011) and recordings from human epileptic tissue (Scimemi et al. 2006) do not find such a loss.

When the δ-containing $GABA_A$ receptors are lost in dentate granule cells, this is accompanied by an upregulation of α4 subunit expression, leading to increased perisynaptic expression of α4β2/3γ2 receptors (Zhang et al. 2007). However, α4γ2-containing receptors have a fivefold greater EC_{50} for GABA than α4δ-containing receptors (Brown et al. 2002). The observation that tonic currents are preserved can be explained by spontaneous $GABA_A$ receptor openings, greater number of receptors or higher concentrations of extracellular GABA. This latter explanation is supported by the observation of a larger SR95531-sensitive tonic current observed in dentate granule cells (Zhan and Nadler 2009). SR95531 acts as a pure antagonist and has minimal effect on the tonic current in dentate granule cells in control rats (Zhan and Nadler 2009; Wlodarczyk et al. 2013), which is mainly mediated by spontaneously opening receptors. Alternatively, although less likely, a large SR95531-sensitive tonic current in dentate granule cells from epileptic rats may be explained by the increased sensitivity of the receptors to GABA.

It has also been suggested that the contribution of α5-containing $GABA_A$ receptors to tonic currents in dentate granule cells is increased following status epilepticus (Zhan and Nadler 2009). Indeed, a slight increase of α5 mRNA level has been reported in these neurons (Fritschy et al. 1999; Houser and Esclapez 2003). Nonetheless, it is unlikely that such a change can fully compensate for the loss of

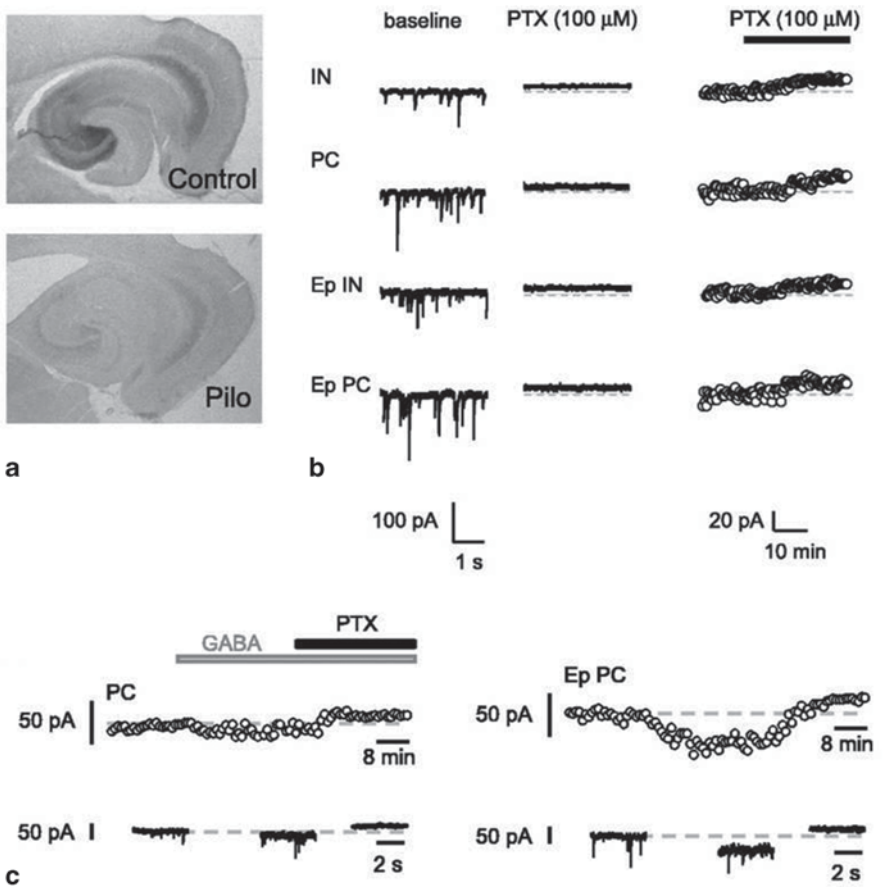

Fig. 10.1 Tonic currents are maintained/increased in CA1 pyramidal cells from epileptic animals. **a** Immunohistochemistry for the α5 subunit, showing decreased expression of this subunit in the hippocampus proper and entorhinal cortex of epileptic rats compared with that of control rats. Pilo, Pilocarpine. **b** Representative traces obtained from one interneuron and one pyramidal cell from a control and an epileptic rat. The tonic current revealed by application of $GABA_A$ receptor antagonist picrotoxin is no different between epilepstic and control neurons. **c** Application of GABA results in larger tonic current in epileptic than in control neurons. (After Scimemi et al. 2005)

the δ subunits and would be sufficient to augment tonic inhibition as reported in electrophysiological studies.

Changes in the subunit composition of extrasynaptic $GABA_A$ receptors during epilepsy have implications for the pharmacological treatment of epilepsy, as more specific drugs are being developed. It should also be noted that changes in the $GABA_A$ receptor subunits may be cell type specific. For example, one study has demonstrated that the reduction of δ subunit expression in dentate granule cells was accompanied by an increase in its expression in dentate interneurons (Peng et al. 2004). This further complicates the prediction of the effect of drugs targeting only specific $GABA_A$ receptors.

10.4 Extracellular GABA in Epilepsy

As detailed above, there is indirect evidence that epileptogenesis results in an increase in extracellular GABA. Such an increase could be due to increased release or decreased uptake. Since most studies show an overall decrease in synaptic inhibition, it is unlikely that increased vesicular release is contributing to increased tonic currents. GABA is also released non-synaptically but the contribution of this pool to extracellular GABA is unclear and the effects of epilepsy on non-synaptic GABA release have not been investigated. An alternative explanation is that there is a change in the number and/or function of GABA transporters (GATs). In addition, GATs are electrogenic such that during periods of depolarisation, GATs can reverse and pump GABA into the extracellular space (Wu et al. 2003, 2007). There are two main cortical GABA transporters, GAT1 and GAT3 (labelled GAT1 and GAT4, respectively, in mice): The former is predominantly neuronal and is located at presynaptic GABAergic terminals, while the latter is expressed in glia (Borden 1996; Ribak et al. 1996; Minelli et al. 1996; Conti et al. 2004). In the rat hippocampus, GAT1 predominantly determines the GABA concentration surrounding neurons, and GAT3 comes into play when extracellular GABA rises, in particular after inhibition of GAT1 (Kersanté et al. 2013).

There is evidence that epilepsy is associated with functional deficits in GABA uptake in human and rat dentate gyrus (Patrylo et al. 2001); other studies, however, have found unaltered GAT1 function in the CA1 region in epileptic rats (Stief et al. 2005). These studies indicate that there may be complex regional-specific changes in GABA transporter function. This has been confirmed in expression studies in resected hippocampi from people with epilepsy in which regional decreases in GAT1 but upregulation of GAT3 have been observed (Mathern et al. 1999; Lee et al. 2006). Under baseline conditions in epileptic tissue, GAT1-mediated transport takes up GABA (rather than acting in reverse), as GAT1 inhibitors significantly increase tonic currents in neurons from chronically epileptic rats (Frahm et al. 2003; Scimemi et al. 2005). However, epilepsy may facilitate reversal of GABA transporters. Interneurons in chronic epilepsy express more GAD and have elevated intracellular GABA concentrations (Esclapez and Houser 1999). Enhanced expression of GATs and an increase in GABA synthesis have also been demonstrated in dentate granule cells in the kainate model of chronic seizures (Sperk et al. 2003). Intracellular accumulation of GABA along with the depolarisation that occurs with seizure-like activity could result in reversal of GABA transporters and elevation of GABA in the extracellular space. However, in the hippocampus in vivo, significant depolarisation induced by raising the extracellular potassium was unable to reverse GAT1 (Kersanté et al. 2013). Nonetheless, such reversal could be facilitated by chronic changes in Cl^- homeostasis in epileptic tissue (Cohen et al. 2002; Palma et al. 2006) and/or gradual build-up of intracellular Cl^- during excessive network activity (Glykys et al. 2009). It is also plausible that increased glutamate uptake by astrocytes in response to massive release of the neurotransmitter during seizure activity may cause intracellular Na^+ accumulation (as a result of cotransport), and thus trigger the reversal of co-localised glial GABA transporters (Héja et al. 2009).

Indeed, there is growing evidence that glia transporters (GAT3) may be reversed during epileptic activity, so that blocking GAT3 increases epileptiform activity (Heja et al. 2012); we have not observed that this is a ubiquitous finding and it may depend upon the in vitro model used (unpublished data).

10.5 Functional Consequences of Enhanced Tonic Inhibition in Epilepsy

There has been scant work on the effects of tonic inhibition on epileptiform activity. Increasing extracellular GABA by inhibiting GABA transporters inhibits (although not universally) seizure-like activity in hippocampal slices and seizures in models of focal epilepsy (Pfeiffer et al. 1996; Sabau et al. 1999; Dalby 2003). It is important to note that this strategy will however increase the activity of both $GABA_A$ and $GABA_B$ receptors and as yet there has been no satisfactory dissection of these separate effects. Moreover, activation of $GABA_B$ receptors may further potentiate tonic $GABA_A$ receptor-mediated conductances (Connelly et al. 2013; Tao et al. 2013). Increasing extracellular GABA may also have cell type-specific actions. Some interneurons, including certain hippocampal subtypes, have $E_{GABA(A)}$ more positive than their resting membrane potential, and therefore GABA depolarises such cells (Martina et al. 2001; Chavas and Marty 2003; Vida et al. 2006; Song et al. 2011); this effect could become even more prominent following increased synaptic activity (Lamsa and Taira 2003). A small rise in extracellular GABA concentrations has an excitatory action on these interneurons through depolarisation, while higher concentrations produces inhibition through mainly a shunting effect (Song et al. 2011). In contrast, the firing of interneurons, in which activation of $GABA_A$ receptors does not produce depolarisation (Verheugen et al. 1999; Martina et al. 2001), will not show this biphasic action of increasing GABA but will solely be suppressed by elevated GABA. This will disinhibit excitatory neurons counteracting reduction of their excitability.

This voltage effect of tonic GABA is likely to be even more complex in principal cells. Generally, the reversal of $GABA_A$ receptors is close to the resting membrane potential such that the voltage effect of tonic inhibition will be minimal. However, in some pyramidal cells during epileptogenesis, the $GABA_A$ receptor reversal potential may become positive with respect to resting membrane potential due to loss of the chloride transporter KCC2 and upregulation of NKCC1 (Rivera et al. 2002; Jin et al. 2005; Muñoz et al. 2007; Huberfeld et al. 2007). It is feasible that tonic current should depolarise such neurons and such a depolarisation would bring the neuron closer to action potential threshold. However, such a depolarisation would also (in)activate other conductances including inactivation of sodium channels, activation of Kv7s and inactivation of HCN, all of which may act to reduce neuronal excitability. It is also worth noting that altered Cl^- homeostasis in epilepsy can itself act as an intracellular signal that controls GABAergic inhibition by modulating the expression of α and δ $GABA_A$ receptor subunits (Succol et al. 2012).

10.5.1 Effects of Tonic $GABA_A$ Receptor Conductance on Neuronal Computation

Perhaps a greater effect of tonic currents on pyramidal cells is the contribution to the input resistance. Persistent activation of $GABA_A$ receptors decreases the membrane input resistance of a neuron and therefore reduces voltage response to incoming excitation (i.e. decreases the amplitude of the excitatory postsynaptic potentials), so decreasing the probability of action potential generation. The relationship between the probability of action potential generation (or the frequency of action potentials in certain cell types) and the magnitude of the input conductance is termed the input–output (I–O) relationship of a neuron and reflects the computation performed by the cell (Silver 2010). The output of hippocampal pyramidal cells is best described by the probability of firing (rather than frequency of firing) in response to temporally correlated synaptic inputs (Azouz 2005; Carvalho and Buonomano 2009). The I–O relationship can be modified through a change in gain (slope) or in offset (threshold), which are equivalent to multiplicative/divisive and additive/subtractive operations, respectively. Since background synaptic noise can summate with the input, then previously subthreshold inputs can on occasions reach threshold, and conversely random inhibitory inputs can decrease the probability of previously suprathreshold inputs from reaching threshold. Therefore, the effect of background synaptic activity is to create voltage variations which reduce the slope of the I–O function, but increase its dynamic range (Wolfart et al. 2005). Decreasing phasic inhibition will decrease voltage fluctuations and so will increase neuronal gain; in addition, decreasing phasic inhibition will increase synaptic summation and so will increase the chance of a specific input reaching threshold, i.e. will offset the I–O function. A fundamental observation is that, partly due to outward rectification, tonic $GABA_A$ receptor-mediated conductances in hippocampal CA1 pyramidal neurons have little influence on subthreshold noise and only affect neurons at spiking threshold. This results in tonic inhibition primarily affecting the offset of the I–O relationship with minimal effect on the slope (Fig. 10.2; Pavlov et al. 2009). Thus, the effect of a shift from phasic to tonic inhibition will be maintenance of neuronal and network excitability when the input is of low magnitude but a change in the gain of the system such that as the input increases there will be a larger increase in output, leading to a potentially more unstable network (Fig. 10.2).

10.6 Pharmacological Implications of Changes in Extrasynaptic Receptors in Focal Epilepsy

As detailed above, decreasing GABA uptake or increasing extracellular GABA by decreasing GABA metabolism will have an action partly through extrasynaptic $GABA_A$ receptors. The enhanced sensitivity of neurons to increases in extracellular GABA that occurs with the development of epilepsy (Scimemi et al. 2005) indicates

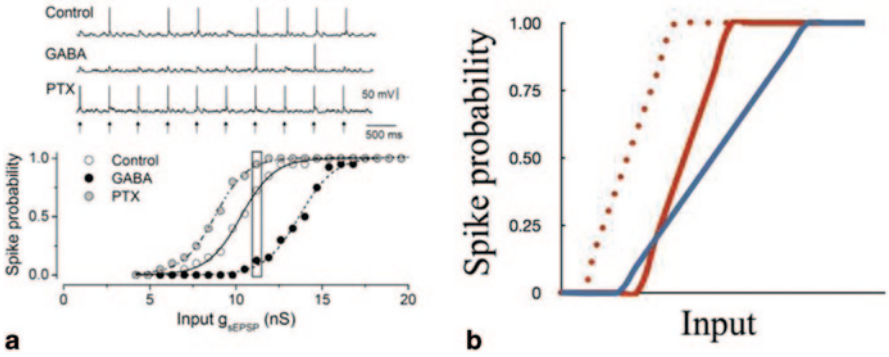

Fig. 10.2 Tonic currents in the hippocampus affect neuronal offset not gain. **a** Application of GABA in the presence of synaptic activity shifts the I–O curve of a neuron to the right without affecting the slope. Subsequent application of the $GABA_A$ receptor antagonist picrotoxin shifts the curve in the opposite direction beyond the control values without a change in slope. **b** In non-epileptic neurons, the I–O relationship as the *blue line*. Loss of synaptic inhibition results in a leftward shift and an increase in slope (*red dotted line*). Increased tonic inhibition compensates for the offset but the slope of the I–O relationship remains increased (*red solid line*). (After Pavlov et al. 2009)

that approaches which increase extracellular GABA are likely to be effective in focal epilepsy (as has been observed clinically), but may have detrimental effects on absence seizures (see Chap. XX). Even within focal epilepsies, such strategies could have complex and paradoxical effects as large increases in tonic currents in interneurons may decrease synaptic inhibition and so paradoxically have a pro-epileptic effect. An alternative strategy would be to target the extrasynaptic receptors directly. However, alterations in subunit composition that occur not only with epileptogenesis but also physiologically, such as during the menstrual cycle (Maguire et al. 2005) or at puberty (Shen et al. 2007, 2010), will alter the sensitivity to such drugs, complicating receptor subtype-targeted pharmacotherapy. Furthermore, the efficacy of different drugs targeting extrasynaptic $GABA_A$ receptors is dependent on the availability and concentration of ambient GABA (Houston et al. 2012).

An important observation has been the loss of benzodiazepine-sensitive synaptic $GABA_A$ receptors during prolonged seizures that contributes to drug resistance (Kapur and Macdonald 1997; Leroy et al. 2004; Feng et al. 2008), but the preservation of extrasynaptic receptors, indicating that therapies aimed at these receptors (either in a non-specific fashion, e.g. barbiturates, or specifically) may be more effective in the late treatment of prolonged seizures.

Conclusion

The development of focal epilepsy seems to be accompanied by a shift from inhibition mediated by synaptic $GABA_A$ receptors to inhibition mediated by extrasynaptic $GABA_A$ receptors. This may result in an increase in the gain of the network. This

would result in only small increases in input leading to a large increase in the probability of neuronal firing, resulting in potentially more unstable networks that would have the propensity to generate seizure activity.

Alterations in the subunit composition of extrasynaptic $GABA_A$ receptors during the development of epilepsy also have significant implications for targeted pharmacotherapy. It is likely that different insults may result in differing degrees and types of subunit alterations, suggesting that more specific therapies may be most useful in epilepsies with distinct and particular aetiologies.

Acknowledgments This work was supported by Pewterer's Fellowship to I.P., funding from Wellcome Trust, Medical Research Council (UK), Epilepsy Research UK and European Integrated Project "EPICURE" (EFP6-037315).

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