REVIEW

The multiple facets of γ-aminobutyric acid dysfunction in epilepsy

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Purpose of review

The polarity of action of γ-aminobutyric acid (GABA) changes from inhibition to excitation in the developing brain and in epilepsies. This review deals with recent observations concerning the mechanisms and clinical implications of the shift in GABA's activity from inhibition to excitation.

Recent findings

GABAergic synapses provide most transmitter-gated inhibition and are the targets of numerous clinically active agents, notably antiepileptic drugs. In a wide range of brain structures and species, GABAergic synapses are excitatory during maturation because of a higher concentration of intracellular chloride. These findings suggest that activation of GABA synapses will excite foetal neurones while inhibiting those of the mother. In epilepsies, recurrent seizures also lead to an accumulation of chloride and an excitatory action of GABA. These observations have major implications for clinical practice and research. They suggest that use of benzodiazepines by pregnant mothers may lead to deleterious consequences when they are taken during the period when GABA is the main excitatory transmitter. Because neuronal activity alters important cell functions, including migration and morphogenesis, aberrant excessive excitation may lead to profound deleterious consequences.

Summary

In several physiological and pathological conditions, activation of GABAergic synapses excites neurones instead of producing classical inhibition. This shift, which is due to an intracellular accumulation of chloride, has major consequences for both the operation of networks and the pathogenic effects of epilepsies. This is particularly important in the immature brain, where the excitatory actions of GABA are particularly prominent.

Keywords

chloride, excitation, GABA, inhibition, maturation

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Abbreviations

[Cl⁻]i	intracellular chloride concentration
GABA	γ-aminobutyric acid
E–I	excitation to inhibition
KCC2	K^+/Cl^- cotransporter

1350-7540

Introduction

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in adult brain. GABA receptors, which are present in all brain regions, are permeable to anions, mostly but not only chloride. Activation of GABA receptors induces a large chloride influx from the extracellular milieu, leading to hyperpolarization of the membrane potential, which will stabilize the resting membrane potential close to chloride equilibrium, inhibit the generation of action potentials and reduce neuronal excitability. It is estimated that only 10–20% of neurones release GABA in cortical circuits, which is far less than the proportion of neurons that release glutamate [1]. However, GABAergic interneurones massively innervate the somata and dendrites of principal cells as well as other inhibitory interneurones. This dense network of synapses enables GABAergic neurones to play a major role in the generation of patterns and oscillations that are instrumental in performing integrative functions. Thus, GABAergic neurones modulate major brain functions, including memory and sleep–wake cycle, and alterations in the efficacy of GABAergic synapses are involved in many neurological disorders and are the targets of widely used drugs, notably benzodiazepines and barbiturates [2]. Therefore, an understanding of the operation of GABA synapses in health and disease is of paramount importance.

Recent studies suggest an unexpected range of alterations in GABAergic synapses and networks in relation to neuronal activity and brain maturation. In addition to the various modulations in synaptic efficacy that operate in GABAergic synapses, as in other transmitter-gated channels, GABAergic systems are endowed with the unique ability to reverse the polarity of GABA action from inhibition to excitation in certain conditions. The key element is the intracellular accumulation of chloride, which may lead to a shift in the gradient and produce an efflux instead of an influx of chloride. This shift from excitation to inhibition (E–I) has best been demonstrated in immature [3–5]

and epileptic [6,7••] neurones in which GABA is depolarizing. The similarity between immature and epileptic neurones is interesting and may provide an explanation for the well established greater incidence of seizures in the developing brain and the greater vulnerability of immature networks to epileptogenic processes [8,9]. The E–I shift also suggests that epileptogenesis may recapitulate ontogenesis and provides an explanation regarding why so many antiepileptic drugs that are GABAergic are ineffective in temporal lobe epilepsy.

Here we review recent studies in the area and speculate on the clinical implications of their findings, notably calling for a re-evaluation of the use of GABA-acting drugs during pregnancy – a time when GABA excites neurones of the foetus and inhibits those of the mother.

γ-Aminobutyric acid excites immature neurones in rodents and primates *in utero*

Studies initiated at the end of the 1980s found that, under physiological conditions, the intracellular chloride concentration ($[Cl^-]_i$) in immature neurones was greater than that in adult neurones [1]. In the rat the action of GABA shifts abruptly from excitation to inhibition in the hippocampus at about the end of the second postnatal week [3–5,10]. Subsequent studies [11–13,14•,15] extended this observation to almost all brain structures, from the spinal chord to brainstem structures and neocortical ones, with the time frame depending on the degree of maturation of the neurones. Parallel studies [16,17] have also confirmed a similar developmental curve in a wide range of species from turtles to subhuman primates, suggesting that this basic property has been conserved throughout evolution. In the hippocampus of primates *in utero*, GABAergic synapses in the hippocampus are excitatory until about the end of the first half of gestation and GABA receptor antagonists fail to generate seizures in slices taken from an early stage, which is in accordance with the actions of GABA (see below) [16]. The exact time course in humans is not yet known, but the E–I shift probably occurs *in utero* in most, if not all, brain structures. Interestingly, GABAergic synapses are operative before glutamate synapses during early maturation, suggesting that GABA also provides the initial excitatory drive during early brain development [18,19].

The mechanisms that underlie the E–I shift were recently subjected to extensive study. The ontogenetic change in GABA responses during brain maturation is coupled to a delayed expression of the neuronal $[Cl^-]_i$ extruding K^+/Cl^- cotransporter (KCC2) [20,21•,22–24]. Thus, in KCC2-knockout mice cortical neurones fail to exhibit a developmental decrease in $[Cl^-]_i$, but they are also unable to regulate $[Cl^-]_i$ upon chloride loading [21]. Another key promoter of this shift is the $[Cl^-]_i$ accumulating $Na^+,K^+/2Cl^-$ cotransporter (NKCC1), which is functional at an early developmental stage and induces an early accumulation of chloride [14•,22].

What, then, are the instructing signals that switch these transporters on or off, leading to the shift? The answer to this question is controversial. One study [4] demonstrated that the expression of KCC2 and the E–I shift in neuronal cultures is prevented by blockade of GABA receptors, suggesting that GABA itself induces the shift. A more recent study conducted in slices [25] suggested that the E–I shift occurs in spite of full blockade of synaptic transmission, suggesting that this is not activity dependent but is more likely genetically programmed to occur at a given developmental stage. However, another study conducted in turtle [17] suggested that visual experience does modulate the GABAergic switch [26].

The excitatory actions of GABA in immature neurones have several major consequences. In particular, developing networks have a unique pattern that is present only as long as GABA excites a significant percentage of synapses – a pattern referred to as the giant depolarizing potential [3,4]. This pattern has been observed in a wide range of preparations, including recently the freely moving pup [3,4,27,28••]. This pattern is most likely equivalent to the delta brush pattern recorded in the electroencephalograms of premature infants [28••]. Of particular importance is the recent demonstration that the intact somatosensory cortex of the newborn rat has spatially confined spindle bursts that represent the first and only network driven pattern [28••]. These spindles are triggered by spontaneous muscle twitches that are analogous to human foetal movements, raising the possibility that this oscillation shapes the formation of cortical connections that are required for sensory motor coordination. The functional significance of this early pattern has also been extensively studied *in vitro*. Giant depolarizing potentials facilitate the synaptic plasticity of immature neurones and contribute to the activity-dependent regulation of neuronal development [29]. In the turtle visual system, in which a similar pattern is present, it has been shown that the E–I shift is necessary and sufficient to cause retinal waves to stop and the system to mature [17]. Therefore, the E–I shift of GABA actions and the earlier function of GABAergic synapses are important events that are developmentally regulated and that signal an important step in the construction of the brain. It has been suggested that they provide a solution to the problem of ensuring throughout development that neither an excessive glutamate excitation (which is neurotoxic) nor a dominating inhibition (which will slow down steps essential to the formation of a cortical network) occurs [5,30].

In yet another significant development, it was recently shown that, *in utero*, electrical stimulation of very

immature neurones that have no synaptic currents generates large currents – several hundred fold greater than synaptic currents – that are also mediated by GABA [31,32]. The release of GABA at that stage is of a non-vesicular type; it does not require calcium influx and the proteins that are essential in packaging GABA into vesicles. The early massive release of GABA may modulate neuronal migration and differentiation, which is in keeping with extensive studies that suggest a trophic role of GABA in early development. Thus, before even having established functional synapses, GABA may play an important role in the construction of cortical networks.

γ-Aminobutyric acid excites many neurones in human and animal epileptic networks

Recent studies have also provided direct evidence that regulation of $[Cl^-]_i$ is also markedly altered in epileptic networks. In human epileptic neurones recorded in hippocampal slices obtained from resections in patients suffering from temporal lobe epilepsy, an interictal pattern is observed that is reminiscent of that recorded *in situ* [6]. This pattern originates in the subicular area before propagating to other hippocampal regions. In many neurones (15–20%) recorded in the focus, the actions of GABA are excitatory because the E_{rev} (reversal potential) of $[Cl^-]_i$ has shifted to more depolarizing levels and the interictal activity is blocked by GABA receptor antagonists, suggesting that GABAergic synapses contribute to ongoing seizures [6]. A more recent study [7••] used a unique triple chamber that accommodates the two intact hippocampi and their connecting commissures in three independent compartments. It is thus possible to apply a convulsive agent to one hippocampus and determine the consequences of the propagation of seizures on the other naïve hippocampus. Using this preparation, the authors first showed that 'seizures beget seizures' for the first time; this demonstration was compelling because interruption of the connexions between the two hemispheres after propagation of 7–10 seizures revealed that the naïve hippocampus had become epileptic – it generated spontaneous seizures [7••]. Furthermore, applications of a GABA receptor antagonist before the formation by seizures of an epileptogenic focus generated seizures, whereas the same procedure blocked ongoing seizures once the mirror focus had been established. Direct measurement of the E_{rev} of $[Cl^-]_i$ showed a I–E shift and excitatory actions of GABA.

Therefore, seizures produce a reduction in the capacity of neurones to deal with their excess chloride accumulation, leading to a quasi permanent accumulation of chloride and excitatory actions of GABA. This mechanism, which has also been observed in other epileptogenic conditions, is suggested to operate in various neurological disorders [33,34•]. It is thought to be mediated by a cascade of intracellular events, including trophic factors and second messenger cascades, that modulate directly the expression or efficacy of KCC2 [23]. It is important to stress that the I–E shift also occurs in more physiological conditions – notably in relation to diurnal rhythm [35]. Therefore, regulation by activity of the polarity of GABA action is an important mechanism that operates in both physiological and pathogenic conditions. It will now be important to unravel the type of patterns that are particularly efficient in downregulating KCC2.

Does epileptogenesis recapitulate ontogenesis?

The observation that GABA excites immature neurones and epileptic ones suggests that seizures may stimulate messenger cascades that lead to an aberrant activation of mechanisms that operate only at an early developmental stage. In keeping with this, the mechanisms that are involved include a downregulation of KCC2 that leads to an accumulation of chloride [34•]. Other events appear to follow a similar trend. Thus, in the immature brain, as in the epileptic one, the percentage of bursting neurones is higher than in naïve adult neurones [36] (Yaari Y, personal communication). Along similar lines, the hippocampal mossy fibres may release GABA in immature and postepileptic neurones but not in controls [37]. Seizures activate a wide range of molecular events; more than 1000 genes are expressed following kainate seizures [38], including some that are normally expressed in immature but not adult neurones. Seizures and ischaemic episodes both activate genes that are involved in cell cycle division – an event that may signal a cascade of events that culminate in cell death. Taken together, these findings provide novel perspectives that better define the events that mediate the deleterious consequences of seizures.

Clinical implications of these elements

The fact that the sequential maturation of GABA and glutamate synapses, and the initially primarily excitatory actions of GABA have been confirmed in all species studied clearly suggests that similar events also occur in humans. The exact correspondence is not known, but the E–I shift most likely occurs *in utero* at some point during the second half of gestation, depending on the brain structure involved. This suggests that GABA-acting drugs – notably benzodiazepines – will excite the immature migrating and differentiating neurones while exerting inhibitory anxiolytic and inhibitory actions on the mother's brain. Because neuronal activity, including that mediated by GABA, modulates several essential functions (migration, differentiation, neuronal growth and synapse formation) [19], a possible deleterious action of GABA-acting drugs must be considered. It is now clear that environmental factors, in addition to genetic ones, exert an important effect on the occurrence of neurological disorders early in life.

The observation that the action of GABA reverses in an epileptic tissue also calls for a re-examination of the development of novel antiepileptic drug strategies. The somewhat simplistic view of inhibitory GABAergic synapses that fail in order for seizures to be generated must be abandoned. The operation of GABAergic synapses is much more multifaceted and includes even shifts in its principal polarity of actions. In adult temporal lobe epilepsies, recent observations clearly indicate that the widely publicized 'interneurone dormancy', which claims that inhibition fails because GABAergic interneurones are disconnected from their glutamatergic drive [39], must be abandoned [40]. It is now clear that seizures induce multiple changes in GABAergic transmission, including a loss of dendritic projecting interneurones, an increase in the GABAergic drive on the somata of principal cells and, in general, an aberrant sprouting of glutamatergic axons [41•]. Epilepsies, like other neurological disorders, are not generated in normal networks in which a single element has been deleted, but rather they are an ensemble of modifications in a wide range of signals, including the expression of molecules that are not expressed in naïve networks. Therefore, the development of novel therapeutic strategies must be based on studies conducted in genuinely 'chronic epileptic' cortical networks, in which all of these rearrangements have taken place, and not in naïve networks, in which seizures are acutely generated by convulsive agents.

Conclusion

The role of GABAergic inhibition in the normal and pathological operation of the brain has been extensively studied because of its major implications in neurological disorders and drug development. The recent discoveries of novel facets to the type of changes that occur during brain development and epilepsies call for a re-evaluation of the concept of inhibition and excitation. The amazing heterogeneity of GABA interneurones and the exquisite regulation of GABA mechanisms by neuronal activity and environmental factors suggest that the 'failure of inhibition in epilepsies' concept must be reassessed. Basic and clinical research into the actions of GABA-acting drugs is warranted, in particular during pregnancy and early brain development.

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