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67

Neuronal Circuitry of Thalamocortical Epilepsy and Mechanisms of Antiabsence Drug Action

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Powerful mechanisms exist within the thalamus that lead to the promotion of synchronous and phasic 3 Hz neuronal activity. These mechanisms include robust burst-firing capability of thalamic neurons, recurrent excitatory and inhibitory synaptic connectivity, and long-lasting and powerful inhibitory synaptic responses arising from activity in thalamic reticular neurons and mediated by γ-aminobutyric acid (GABA) receptors. The 3 Hz thalamic synchronization appears to arise from a perturbation of a physiologic, higher frequency spindle oscillation. Two currently available antiabsence medications interact with this circuitry with the net result of decreased synchronization, largely through reduction in inhibitory output from the thalamic reticular nucleus. Ethosuximide blocks T-type calcium channels and thus reduces the ability of thalamic neurons to fire bursts of spikes, thereby reducing inhibitory (and excitatory) output within the circuit. By contrast, clonazepam enhances recurrent inhibitory strength within the reticular nucleus. This results in a decreased ability of neighboring inhibitory neurons to fire synchronously and produce the powerful inhibitory synaptic responses that are required for network synchronization.

INTRODUCTION

Typical absence epilepsy of childhood is a nonconvulsive form of epilepsy that is characterized by frequent staring spells or "absences" and bilaterally synchronous three-per-second spike and wave electroencephalographic (EEG) features (1–4). The disease appears to have a genetic component, and occurs predominately in girls. Age of onset is most commonly around 6 to 7 years, and the seizures spontaneously resolve in most cases by the time of pubescence. Juvenile onset absence epilepsy has a somewhat later onset, and is a more severe disorder in that it is persistent and somewhat more resistant to pharmacotherapy (5). Although pure absences are generally not convulsive, they can occur hundreds of times per day; they are associated with significant cognitive affects (6,7) and may lead to increased risk of injury (8). Some antiepileptic drugs demonstrate specific efficacy in the treatment of this disorder (e.g., ethosuximide) (9), especially the childhood onset form. Others are broad spectrum, showing efficacy in both absence and other epilepsies (e.g. valproate), whereas still others either show no effect or actually exacerbate the absences (10). These data suggest a wide variety of seizure causes and antiepileptic drug mechanisms (11). Although simple absences are well controlled by currently available medications, more severe forms of the disease are resistant to therapy (2,4), indicating the need for continued development of improved antiabsence drugs.

Recent work from several laboratories has built upon a large body of evidence obtained in the feline penicillin model (12,13) and provides overwhelming evidence that a reverberant thalamocortical discharge underlies the seizures. In this chapter, we review thalamocortical involvement in absence epilepsy, aspects of the intrathalamic circuitry that seem to be critical for the generation of 3 Hz synchronous network activity, and discuss how this intrathalamic circuit might interact with the global corticothalamic system to produce the spike-wave discharge (SWD) that is the hallmark of absences. In addition, we will describe the putative mechanisms of action for two antiabsence drugs. These compounds, ethosuximide and

it has even been suggested that spike wave discharge can arise from an aberration of the neuronal networks that generate sleep spindles, which originate in the thalamus (61). Evidence for a connections between spindles and SWD arises from both human and animal studies. In absence patients the highest incidence of spike wave activity can occur at the transition between wakefulness and sleep (62,63) periods when spindle activity is high, but not during rapid eye movement (REM) sleep when spindles are largely absent (63). Evoked single spindles in cats are transformed in a progressive fashion to longer lasting epileptiform neocortical rhythms following systemic injection of penicillin (50,64). In addition, several rodent models have an increased incidence of seizure discharge on sleep or during quiet wakefulness or immobility (19,65,66).

In summary, thalamic and cortical activity are central to the genesis of SWD. Although other brain structures can influence the seizure activity, little evidence is seen for direct participation of these areas in the phasing or synchronization of the seizures. The critical role of the thalamus as a pacemaker in absence epilepsy is suggest by the finding that SWD appears to be an aberrant manifestation of sleep spindles, and these have been shown to originate in the thalamus.

IN VITRO ANALYSIS OF THALAMOCORTICAL SYNCHRONIZING MECHANISMS

Recent experimental work has proved the powerful utility of *in vitro* models of spike wave activity. These thalamic or thalamocortical slice preparations have been developed specifically to retain critical circuit connectivities in reduced preparations where quantitative physiologic and pharmacologic studies are to be performed (67–69). It is clear that these preparations, by their very nature, do not retain comprehensive connectivity and, therefore, only provide incomplete information. For example, little yet has been learned from slice preparation regarding the critical contribution of midline thalamic structures (43) to thalamocortical synchronization. Nevertheless, results from *in vitro* studies have provided clear evidence regarding basic mechanisms of intrathalamic and thalamocortical synchronization.

Three major factors appear critical for the synchronization of slow thalamic network activity—reciprocal connectivity, specific synaptic mechanisms, and intrinsic burst-firing ability. The anatomic basis for synchronized thalamic discharge is the topographically organized (70) reciprocal connections between thalamic reticular nucleus (or nucleus reticularis thalami [nRt]) and thalamic relay nuclei (71). nRt consists of a shell-like nucleus that surrounds mainly lateral and anterior aspects of dorsal thalamus, is composed entirely of GABA-containing neurons (72), and is a critical site for generation of sleep spindles (73) and shaping sensory receptive fields (74). nRt receives a collateral projection from the major thalamocortical radiation (71). In turn, nRt neurons send an inhibitory projection back to the appropriate dorsal thalamic sector. Recent evidence suggests a heterogeneity of nRt cell axonal projection patterns, ranging from extremely focal to very diffuse (75). Thus, a heterogeneous reciprocal excitatory-inhibitory connectivity exists within the thalamic circuit. In the case of focal nRt projections, the reciprocal connectivity would lead to regionally restricted recurrent activity, whereas the diffuse projections would lead to more global activity. The divergence of the intrathalamic circuit provided by the diffuse output nRt cells is postulated to promote the eventual spread or synchronization of synchronous activities such as SWD (75,76,76a).

A second factor underlying the ability of the thalamic circuit to become self-synchronizing is the essential capacity of nearly all relay and nRt neurons to fire phasic, Ca^{2+}-dependent bursts of action potentials (61). This feature was first clearly demonstrated by Jahnsen and Llinás (77) in an *in vitro* slice preparation of guinea pig thalamus. They showed that on appropriate conditioning, which amounted to membrane potential hyperpolarization, thalamic neurons would fire in a burst pattern, which was dependent on extracellular Ca^{2+}. Subsequent voltage-clamp studies (78,79) have identified the ion channel responsible for burst firing in these neurons as the T-type calcium channel. These biophysical studies have provided a complete characterization of kinetic properties of the T channel, such that burst firing behavior can be accurately reconstructed via computer simulations (80,81). As a result of a high level of T-channel expression, thalamic neurons fire action potentials in high-frequency, short-duration bursts after membrane hyperpolarizations, as opposed to the regular firing pattern obtained in the absence of hyperpolarization. Thus, because burst-firing depends on membrane potential and bursting is critical for synchronous network discharge (see below), membrane polarization is a powerful means to regulate the network. A number of neuromodulators (including, for example, acetylcholine, norepinephrine, and serotonin) have been shown to depolarize thalamic neurons mainly via reductions in K^+ channel activity (82) and, thus, reduce their burst-firing capacity. Consequently, al-

the T current (95,96). By contrast, the unsubstituted ring structure succinimide (which lacks therapeutic actions), the convulsant tetramethylsuccinimde, and the antiepileptic drug phenytoin (which lacks antiabsence activity) all had no effect (94,96). The blockade of Ca^{2+} currents by ethosuximide was specific for the T current; however, metabolites of tridione and methsuximide also blocked other voltage-gated Ca^{2+} currents (95,96). The mechanism of channel antagonism appears to be open channel block (97). It should be noted that valproic acid is one of the primary medications used in the treatment of absence and other forms of epilepsy, yet it has little, if any, effect on the T-type calcium current (94,98). Thus, specific antiabsence compounds seem to be effective blockers of this current, whereas other compounds exert their antiepileptic actions through different mechanisms (11). In other words, T-channel blockade is not likely to be the sole antiabsence drug mechanism.

Within the thalamic circuit, T-channel blockade would be expected to have profound effects. As the intrathalamic oscillations depend on Ca^{2+}-dependent burst firing in both nRt and relay neurons, ethosuximide would be a powerful down-regulator of the seizurelike activity. Indeed, ethosuximide causes dramatic reductions in the probability of obtaining burst responses in thalamic neurons. Clinically effective concentrations of ethosuximide (600 to 700 μM) do not alter the basic excitability of neurons or even the basic morphology of a burst response. Instead, for a given stimulus the likelihood of obtaining a burst is decreased (68). Thus, the network oscillation, which depends on the ability of the recurrent circuitry to continue to evoke burst output at each temporal phase of the reentrant activity, is powerfully and reversibly dampened by the drug (68) as it progressively reduces the late recurrent IPSPs arising from nRt activity. In support of the hypothesis that T-channel blockade is the mechanism of antiabsence actions is the finding that an experimental compound, U-92032, also blocks thalamic T currents and has effects on thalamic network activity that are equivalent to those of ethosuximide (99).

Another therapeutic agent for which we have gained insight regarding its mechanism of action on absence epilepsy is clonazepam. This benzodiazepine drug has the seemingly paradoxical ability to ameliorate absence epilepsy (100). Other compounds that enhance inhibition can have proconvulsant activity in humans and in animal absence models (29,45,47, 101). Yet clonazepam was able to dampen intrathalamic oscillations (102). Intracellular analysis of synaptic responses during the network activity revealed that clonazepam had little effect on the $GABA_A$ component of the IPSP recorded in relay neurons, but it diminished the $GABA_B$ component (102). The $GABA_A$ antagonist, bicuculline, caused an increase in the $GABA_B$ IPSP, an effect opposite from that obtained with the pro-$GABA_A$ benzodiazepine compound. This suggests that the effects of clonazepam are network related, and not a result of direct interaction with the $GABA_B$ receptor. It was postulated that the recurrent intranuclear inhibitory fibers within nRt normally provide an "antioscillatory" braking mechanism on the thalamic circuit. Local perfusion of bicuculline into nRt supported this finding. Recurrent network responses were more closely synchronized and longer lasting after disinhibition of nRt (102, 102a). Similarly, spindlelike activity in ferret thalamic slices was transformed into hypersynchronous absencelike activity by bath application of bicuculline (69). Thus, modulation of GABAergic receptors, especially within nRt, seems to be a particularly effective means of regulating synchronous thalamic activity, although GABA-modulatory effects in cortex may also prove to be important (103).

The approach of indirectly modulating thalamic and thalamocortical circuits will prove to be useful in future antiepileptic drug development. This may come about through a number of different approaches. For example, given the heterogeneity of GABA receptors throughout the brain (104), it may be possible to target specific brain nuclei with neuromodulatory agents to produce a desired final network modification. Synaptic responses in thalamic relay and reticular neurons are differentially modulated by the broad spectrum benzodiazepine compound midazolam (105) and nucleus specific differences are found in benzodiazepine potency within the thalamus (106), suggesting the potential utility of such an approach. Another possible target is the synaptic release machinery. A number of neurotransmitters or neuromodulators are known to alter synaptic release through interaction with presynaptic receptors (107), and activation of receptors for endogenous neuromodulators in the thalamus has been shown to alter synaptic release. Adenosine, acting at purinergic A1 receptors (108), and baclofen acting at $GABA_B$ receptors (109) both dramatically reduce IPSPs and EPSPs and dampen thalamic network activities. If presynaptic receptors can be selectively targeted it may be possible to exploit a use-dependent down-regulation of synaptic release. Thus, endogenous hypersynchronous activity such as that occurring during the onset of SWD might be rapidly stopped and the seizure averted.

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