A Thalamic Sleep Tonic

$GABA_A$ Receptor-Mediated Tonic Inhibition in Thalamic Neurons

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J Neurosci 2005;25:11553–11563

Tonic $GABA_A$ receptor-mediated inhibition is typically generated by δ subunit-containing extrasynaptic receptors. Because the δ subunit is highly expressed in the thalamus, we tested whether thalamocortical (TC) neurons of the dorsal lateral geniculate nucleus (dLGN) and ventrobasal complex exhibit tonic inhibition. Focal application of gabazine (GBZ) (50 μM) revealed the presence of a 20 pA tonic current in 75 and 63% of TC neurons from both nuclei, respectively. No tonic current was observed in GABAergic neurons of the nucleus reticularis thalami (NRT). Bath application of 1 μM GABA increased tonic current amplitude to 70 pA in 100% of TC neurons, but it was still not observed in NRT neurons. In dLGN TC neurons, the tonic current was sensitive to low concentrations of the δ subunit-specific receptor agonists allotetrahydrodeoxycorticosterone (100 nM) and 4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridin-3-ol (THIP) (100 nM) but insensitive to the benzodiazepine flurazepam (5 μM). Bath application of low concentrations of GBZ (25–200 nM) preferentially blocked the tonic current, whereas phasic synaptic inhibition was primarily maintained. Under intracellular current-clamp conditions, the preferential block of the tonic current with GBZ led to a small depolarization and increase in input resistance. Using extracellular single-unit recordings, block of the tonic current caused the cessation of low-threshold burst firing and promoted tonic firing. Enhancement of the tonic current by THIP hyperpolarized TC neurons and promoted burst firing. Thus, tonic current in TC neurons generates an inhibitory tone. Its modulation contributes to the shift between different firing modes, promotes the transition between different behavioral states, and predisposes to absence seizures.

COMMENTARY

Tonic inhibition is a remarkable form of signaling in the CNS, which contrasts in both form and function from classic phasic inhibitory signaling. The latter, as described in any neurobiology text, is the result of neural activity of inhibitory interneurons, fusion of GABA filled vesicles at synapses, transient (millisecond) increases in synaptic GABA concentration to millimolar levels, gating (opening) of $GABA_A$ receptors, and chloride ion flux into postsynaptic neurons. These phasic responses, which have effects whose durations are on the order of milliseconds to tens of milliseconds, influence the timing, synchrony, and number of spikes produced in the neuron(s) receiving the inhibition. Phasic inhibition has surgical-like precision in terms of timing and focality of inhibitory signaling—it is localized to the synapse at which GABA is released. If phasic inhibition is a scalpel, designed to sculpt inputs by selectively eliminating brief periods of input from specific dendrites, then tonic inhibition is, pardon the expression, somewhat of a sledgehammer.

Tonic inhibition is the steady activation of extrasynaptic $GABA_A$ receptors, which produces a corresponding steady $GABA_A$ conductance. Tonic inhibition, thus, increases the electrical conductivity of the neuronal membrane and serves as a short circuit, such that electrical signals traveling down the membrane are shunted to the extracellular fluid "ground" and lose their efficacy. Tonic inhibition can be considered a time-invariant "veto" of synaptic and intrinsic signals. The veto exerted by tonic inhibition is not absolute; that is, the degree of tonic inhibition and the efficacy of the shunt will vary depending on the resting level of GABA in the extracellular fluid. Changes in the efficacy of tonic inhibition have been reported as a consequence of modulation of the extrasynaptic $GABA_A$ receptors by neuromodulators, such as alcohol and neurosteroids (1), which alter the sensitivity of extrasynaptic GABA receptors and thus, increase their openings in response to ambient GABA concentrations.

As far as is known, tonic inhibition does not distinguish between one dendrite and another and certainly does not change much over time. However, to date, tonic inhibition has only been studied in recordings from the soma; it might be that in different dendrites, tonic inhibition increases or decreases as GABA released from localized synapses spreads to extrasynaptic receptors on that particular dendrite. GABA might even be

Epilepsy Currents, Vol. 6, No. 5 (September/October) 2006 pp. 164–166
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released to specific locations in the extracellular space by the reversed action of GABA transporters on neuronal and axonal membranes (2). Recordings from the soma would reveal only the average GABA conductance from all the dendrites, and so at this time, there is no specific evidence that tonic inhibition has any time or location specificity.

What is the point of this sledgehammer approach to inhibition? Certainly, anesthesiologists and epileptologists can provide a ready answer: tonic inhibition, by reducing or blocking excitatory synaptic input, is a great way to put neurons to sleep. Recently, Belelli and colleagues (3) as well as Cope et al., reviewed here, have examined thalamic neurons in vitro to determine whether tonic GABA inhibition might play a role in the sleep/wake cycle and in the generation of absence seizures (3).

The investigators used a standard method to evaluate tonic inhibition, which is to evaluate the overall conductance of a neuron before and after $GABA_A$ receptors are blocked. Unfortunately, there are no drugs that perfectly select between phasic and tonic inhibition, so blocking tonic inhibition usually reduces phasic inhibition as well. However, because tonic inhibition is generated by a low concentration of GABA reaching the extrasynaptic receptors, whereas phasic inhibition is generated by a very high concentration of GABA in the synaptic cleft, low concentrations of competitive GABA antagonists are relatively selective for tonic versus phasic inhibition.

When tonic inhibition was compared with phasic inhibition, Cope et al. confirmed a finding that is perhaps surprising but previously had been observed in the cerebellar slices: more than 90% of the total GABA conductance recorded in vitro arises from tonic inhibition, leaving less than 10% that arises from phasic inhibition. While the conductance change underlying tonic inhibition is not large compared with the peak conductance changes that occur during synaptic GABA release, tonic inhibition is always present. Thus, the 90% fraction reflects the steady presence of a small conductance change compared with the intermittent presence of a much larger conductance change. The relative contributions of these two types of inhibition to physiological activity in vivo, where both the intensity of synaptic activity as well as the resting levels of extracellular GABA are likely to be different than in isolated brain slices, remains to be seen.

Tonic inhibition is not observed in all types of neurons (4), and both Cope et al. and Belelli et al. established that this finding also is true in the thalamus. Thus, GABA-releasing neurons in the nucleus reticularis thalami (nRT) do not exhibit tonic inhibition, whereas thalamocortical relay neurons in both the dorsal lateral geniculate nucleus and ventrobasal complex, exhibit tonic inhibition. nRT neurons inhibit the thalamocortical neurons, so agents that increase tonic inhibition will selectively affect the thalamocortical principal neurons. For example, benzodiazepines modulate synaptic receptors containing a $\gamma 2$ subunit but do not affect nonsynaptic receptors that contain a δ subunit. These δ-subunit–containing $GABA_A$ receptors are ideal for subserving tonic inhibition, because they are sensitive to low concentrations of GABA and are resistant to desensitization (5). Another study showed that membrane noise, presumably an index of tonic-receptor activation, was decreased in mice relay neurons of deficient in the $GABA_A$ receptor δ subunit (6). Both Cope et al. and Belelli et al. studies demonstrated that while benzodiazepines do not alter tonic inhibition (consistent with the presence of a δ subunit in the $GABA_A$ receptors that subserve tonic inhibition), they do enhance phasic inhibition. Furthermore, the direct GABA agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridin-3-ol (THIP), to which such δ-subunit–containing receptors appear to be selectively sensitive, preferentially increased tonic inhibition in the thalamocortical neurons.

Because thalamocortical neurons express high levels of T-type calcium channels, membrane hyperpolarization enables subsequent calcium-dependent burst firing. Thus, enhancement of tonic inhibition by THIP causes the thalamocortical neurons to begin bursting. As burst firing in thalamocortical cells underlies sleep (7), the finding that GABA agonists induce sleep-related behavior in the thalamic slices is interesting and plausible. This finding by Cope and colleagues was augmented by the finding of Belelli et al. that THIP administration in vivo enhances slow-wave sleep activity. In absence epilepsy, thalamocortical neuron burst firing also is prominent; therefore, the efficacy of benzodiazepines in treatment of absence seizures makes sense, as the tonic inhibition that promotes bursting is not sensitive to benzodiazepines. Benzodiazepine efficacy in absence seizures likely is related to selective enhancement of inhibitory signaling in the nRT, which decreases inhibitory output of this nucleus and suppresses bursting of thalamocortical relay neurons (8,9).

These new results from Cope et al. suggest one possible function of tonic inhibition: rapid switching of neurons from one state (tonic firing that subserves wakefulness) to another state (burst firing that subserves sleep. . .and absence seizures). Changes in ambient GABA concentration would then trigger the switch. Why this effect might occur via GABA, rather than a neuromodulator (e.g., acting on potassium conductances), remains unknown. Perhaps, the ability to quickly exit sleep states by rapidly altering GABA release or uptake provides a more robust mechanism to become "instantly awake" than would be possible using neuromodulators acting through second messenger systems.

What do these findings mean for epilepsy treatment? If benzodiazepines are effective therapy for absence seizures by virtue of their lack of effect on tonic inhibition in thalamocortical neurons, in addition to the specificity of their effect on intrathalamic phasic signaling, one would predict that less

selective GABA agents should be similarly less effective therapies for absence. This hypothesis seems to hold up well; for example, barbiturates are effective but nonselective modulators of $GABA_A$ receptors, and are not effective in the treatment of absence. Tiagabine is a GABA reuptake inhibitor that increases the concentration of extracellular GABA. Tiagabine would be expected to enhance tonic inhibition in the thalamocortical neurons and, thus, would not be expected to be an effective treatment for absence. Accordingly, tiagabine has been reported to induce absence status in children (10) and enhance spike wave activity experimentally (11). Thus, the differential action of selected allosteric GABA modulators on phasic versus tonic inhibition may have important consequences not only for the physiology and pharmacology of sleep, but also for the treatment of epilepsy.

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