To Depolarize or Hyperpolarize? At the Axon Initial Segment, E_{GABA} Sets the Stage

GABAergic Depolarization of the Axon Initial Segment in Cortical Principal Neurons Is Caused by the Na-K-2Cl Cotransporter NKCC1. Khirug S, Yamada J, Afzalov R, Voipio J, Khiroug L, Kaila K. *J Neurosci*. 2008;28(18):4635–4639. GABAergic terminals of axo-axonic cells (AACs) are exclusively located on the axon initial segment (AIS) of cortical principal neurons, and they are generally thought to exert a powerful inhibitory action. However, recent work (Szabadics et al., 2006) indicates that this input from AACs can be depolarizing and even excitatory. Here, we used local photolysis of caged GABA to measure reversal potentials (E_{GABA}) of $GABA_A$ receptor-mediated currents and to estimate the local chloride concentration in the AIS compared with other cellular compartments in dentate granule cells and neocortical pyramidal neurons. We found a robust axo-somato-dendritic gradient in which the E_{GABA} values from the AIS to the soma and dendrites become progressively more negative. Data from *NKCC1*$^{-/-}$ and bumetanide-exposed neurons indicated that the depolarizing E_{GABA} at the AIS is set by chloride uptake mediated by the Na–K–2Cl cotransporter NKCC1. Our findings demonstrate that spatially distinct interneuronal inputs can induce postsynaptic voltage responses with different amplitudes and polarities as governed by the subcellular distributions of plasmalemmal chloride transporters.

COMMENTARY

The spatial distribution of ion channels, neurotransmitter receptors and transporters, and even metabolic domains along a neuron's membrane can determine firing properties and contribute to the diversity among cortical neuron firing patterns (1). For example, the density of sodium and potassium channels varies along the axon, giving rise to specific functions at and between nodes of Ranvier (2). Compartmentalization of calcium channels in dendrites versus somata allows generation and propagation of calcium-dependent action potentials to boost depolarization from distal sites (3). Submembrane partition of glycolytic enzymes provides a basis for local energy production that can be modified according to metabolic demand (4).

It has long been recognized that the axon initial segment (AIS) is an integrator of somatic and dendritic activity, specialized for the generation of action potentials (5). The type and density of ion channels at the AIS endow this region with its low threshold for action potential firing (6–8). The fact that the AIS also receives synaptic input directly adds to its functional importance in gating a neuron's output. Specifically, powerful GABAergic input from axo-axonal interneurons onto the AIS of cortical neurons could, at least theoretically, hyperpolarize the region and hence restrict action potential generation. Therefore, it came as a surprise when investigators first reported that GABAergic input onto AIS can be depolarizing rather than hyperpolarizing (9). This effect was explained by a relatively high local concentration of chloride at the AIS (compared to the soma and dendrites), which is due to a relative dearth of the potassium–chloride cotransporter KCC2 at the AIS (9). KCC2 normally transports chloride ions out of the neuron, so that when $GABA_A$ receptors are activated and chloride channels open, chloride ions enter the neuron and hyperpolarize it. With low KCC2 expression at the AIS, chloride accumulates intracellularly, and this region is prone to depolarization when $GABA_A$ receptors are activated.

The growing literature on the depolarizing actions of GABA in neonatal neurons, subicular neurons in temporal lobe epilepsy, and other conditions served as a starting point for hypotheses about a similar role in AIS physiology (10). Khirug et al. compared the GABA reversal potential (E_{GABA}) in response to locally applied GABA at several sites along neurons: AIS, soma, and dendrites. GABA was released at specific anatomic targets along the neuron membrane by laser-induced photolysis of caged GABA molecules. In mouse and rat hippocampal dentate granule cells, as well as in rat neocortical layer 2/3 pyramidal neurons, the authors found that E_{GABA} at the AIS was significantly less negative than E_{GABA} at the soma and dendrites. E_{GABA} at the AIS was about 5 mV more positive than E_{GABA} at the soma, and there was a depolarizing driving force of approximately 13 mV between AIS E_{GABA} and the somatic resting potential. The results suggest that a depolarizing response is mediated by GABA released from presynaptic axo-axonal interneurons terminating on the AIS of cortical principal neurons. This observation implies that the intracellular chloride concentration at the AIS is higher than at other cellular sites, which can be explained only partly by the sparse distribution of KCC2 at the AIS. In addition, an active chloride accumulation mechanism was postulated, as either being due to a persistence of or greater expression of the sodium–potassium–chloride co-transporter NKCC1 at the AIS. NKCC1 expression ordinarily decreases after the neonatal period. In neurons of rodents under 2 weeks of age, GABA-mediated depolarizing responses are thought to account

Epilepsy Currents, Vol. 9, No. 1 (January/February) 2009 pp. 28–29
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for a greater tendency toward hyperexcitability and neonatal seizures (11).

To evaluate the hypothesis that depolarizing GABAergic responses at the AIS are dependent on NKCC1, Khirug et al. compared GABAergic responses in dentate granule cells from wild-type mice and those lacking NKCC1 ($NKCC1^{-/-}$). In $NKCC1^{-/-}$ mice, but not wild types, the E_{GABA} gradient from AIS to soma was abolished, whereas this gradient was maintained between the soma and dendrites. Therefore, chloride accumulation at the AIS, facilitated by NKCC1, subserves a GABA-mediated depolarizing response there. This conclusion is supported by experiments with bumetanide, which blocks NKCC1. When bumetanide is applied, the depolarizing E_{GABA} axo-somatic gradient is also ameliorated.

These intriguing experiments raise the possibility that AIS function can be altered in various pathological conditions, including epilepsy. The findings also expand the potential complexity by which the AIS regulates neuronal excitability and emphasizes the importance of spatial variation in membrane properties. The ability of GABA from interneurons to locally modulate the excitability of principal cortical neurons via AIS chloride concentration represents a novel mechanism for the regulation of excitability. Finally, as the authors state, a given neuron cannot be characterized a single E_{GABA}; rather, E_{GABA} varies spatially over the neuronal surface, creating physiological microdomains throughout the cell.

Several questions arise from these observations. What happens to the E_{GABA} spatial gradient over time—in other words, is there a developmental pattern to the relative expression of NKCC1 and KCC2 at the AIS? How does the proposed mechanism alter the understanding of the more typical hyperpolarizing GABA response at the AIS, conventionally thought to confine and brake excitation at this critical site of action potential generation? What accounts for maintenance of the E_{GABA} gradient between the soma and dendrites, which was unaffected by experimental manipulations of NKCC1? Do acute seizures or the chronic epileptic state affect the E_{GABA} gradient? While these questions await experimental inquiry, it is clear that dysfunction at the AIS must be added to the growing list of mechanisms for hyperexcitability and seizure generation.

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