

ENDOCANNABINOIDS: A CRITICAL REGULATOR OF ACTIVITY IN THE DEVELOPING BRAIN

Altering Cannabinoid Signaling During Development Disrupts Neuronal Activity

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In adult cortical tissue, recruitment of GABAergic inhibition prevents the progression of synchronous population discharges to epileptic activity. However, at early developmental stages, GABA is excitatory and thus unable to fulfill this role. Here, we report that retrograde signaling involving endocannabinoids is responsible for the homeostatic control of synaptic transmission and the resulting network patterns in the immature hippocampus. Blockade of cannabinoid type 1 (CB1) receptor led to epileptic discharges, whereas overactivation of CB1 reduced network

activity *in vivo*. Endocannabinoid signaling thus is able to keep population discharge patterns within a narrow physiological time window, balancing between epilepsy on one side and sparse activity on the other, which may result in impaired developmental plasticity. Disturbing this delicate balance during pregnancy in either direction, for example, with marijuana as a CB1 agonist or with an antagonist marketed as an antiobesity drug, can have profound consequences for brain maturation even in human embryos.

COMMENTARY

One of the most powerful factors regulating the efficacy of subsets of inhibitory synapses in the mature CNS is the cannabinoid system. Periods of sustained activity trigger postsynaptic elevations in intracellular calcium, which in turn can activate synthesis of endocannabinoids in many neurons, including hippocampal pyramidal neurons. These endocannabinoids, which are lipid soluble, can diffuse within the membrane as well as across the synaptic cleft in a retrograde manner to influence neighboring presynaptic terminals. In the limbic system, the receptors that respond to endocannabinoids, the cannabinoid type 1 (CB1) receptors, are exclusively localized to a subset of GABA- and cholecystinin (CCK)-containing presynaptic terminals. Activation of G-protein-coupled CB1 receptors in these terminals suppresses release of the neurotransmitter, GABA (1,2).

In the mature brain, activity of the cannabinoid system (and transient, activity-dependent suppression of inhibition mediated by GABA/CCK interneurons) may be important in modifying the emotional and motivational state of the brain (3) as well as in regulating plasticity of excitatory synapses, thereby regulating learning and memory formation (4,5). In the developing brain, GABA subserves novel functions, because it primarily excites postsynaptic targets. GABA's excitatory ca-

capacity stems from altered transmembrane chloride gradients in postsynaptic neurons, which occur as a result of reduced expression of the chloride extruder, KCC2, during early development. The depolarizing, excitatory response to GABA triggers calcium entry in postsynaptic neurons, which in turn promotes growth and synapse formation in the developing brain (6). If the cannabinoid system is active in early brain development, it would have significantly different effects from those seen in the mature brain, since it primarily would suppress excitatory actions of GABA, which may subsequently alter circuit formation and disrupt brain development. In the Bernard et al. study, the functional status of the cannabinoid system and the consequences of activation and blockade of cannabinoid signaling are assessed in early postnatal rats.

Using pre-embedding immunohistochemical electron microscopic staining techniques to visualize CB1 receptors and postembedding anti-GABA labeling procedures, the authors first established that CB1 receptors are present on inhibitory (i.e., GABA-positive) presynaptic terminals in the hippocampus early in postnatal development (postnatal day 4). They then demonstrated, using patch clamp recordings, that these receptors were functional. Standard depolarization-induced suppression of inhibition protocols involving sustained depolarization of postsynaptic neurons resulted in robust suppression of inhibitory postsynaptic currents, and these effects were blocked by CB1 receptor antagonists (1). CB1 receptor agonists could mimic the inhibitory effect of depolarization and occlude depolarization-induced suppression of inhibition mediated by release of endocannabinoids.

Interestingly and in contrast to studies in the mature CNS, depolarization-induced suppression of inhibition could also be induced at GABAergic synapses onto inhibitory neurons, demonstrating that hippocampal GABAergic interneurons were capable of synthesizing endocannabinoids early in development—a capability that is lost in mature hippocampal (but not neocortical) interneurons. Another major difference in endocannabinoid signaling was evident in the early postnatal hippocampus, in which significant tonic activation of CB1 receptors was evident, reflecting tonic release of endocannabinoids. This finding was demonstrated in studies examining the effects of a CB1 receptor antagonist and involving an endocannabinoid uptake inhibitor (WB 404). The antagonist enhanced both evoked inhibitory postsynaptic currents and spontaneous GABAergic network oscillations, while the uptake inhibitor suppressed these events. These agents have little effect on activity in the mature hippocampus, suggesting that tonic activation of CB1 receptors does not occur (1).

In a final set of studies, Bernard and colleagues recorded electrical activity from the frontal and occipital cortex in 5-day-old rat pups *in vivo* and examined the effects of CB1 receptor antagonists, agonists, and endocannabinoid uptake inhibitors. Unlike responses in the mature CNS and consistent with the depolarizing actions of GABA in the immature brain, blockade of CB1 receptors enhanced spontaneous GABA-mediated activity in the cortex and induced the appearance of epileptic, ictal activity in 5 of 9 animals. Conversely, enhancing endocannabinoid actions by blocking uptake significantly reduced GABA-mediated activity. Similarly, activation of CB1 receptors by systemic injection of a CB1 agonist blocked GABA-mediated activity. Thus, in the immature CNS, endocannabinoid signaling appears to be critical in maintaining spontaneous activity within a relatively narrow range. This degree of activity is presumably favorable to ensure optimal trophic signaling by GABA and to allow appropriate synapse formation. Under conditions in which GABA is excitatory, the endocannabinoid system appears to function as an activity-dependent brake, preventing over-activation of this excitatory system.

What are the implications of the findings of Bernard et al. regarding the endocannabinoid system and its regulation of normal and potentially pathophysiological development of the CNS? One significant aspect of this study is that it was able to demonstrate that endocannabinoid signaling is a critical regulator of early developmental activity and is important

in CNS circuit development. Since cannabinoids are drugs of abuse, there is the potential for these agents to interfere with normal brain development. It has been suggested that the first postnatal week in rats corresponds to late fetal stages of development in humans. Maternal use of cannabinoids has the potential to suppress important neuronal activity critical to establishing appropriate CNS networks in the fetus and, thus, has the potential to induce long-term cognitive deficits. CB1 receptor pharmacology also has begun to be exploited by the pharmaceutical industry, with CB1 receptor antagonists under development for the treatment of obesity. Maternal use of CB1 antagonists has the potential to over-activate the fetal CNS to the point of seizure induction, which could be a life-threatening event, and also has the potential to induce long-term pathophysiological sequelae. Interestingly, among the possible downstream outcomes of fetal seizures induced by CB1 receptor antagonists are long-term alterations in endocannabinoid signaling. A recent publication describes activity-dependent plasticity of the cannabinoid system in which febrile seizures in postnatal day-10 rats (corresponding to term infants) can induce prolonged up-regulation in CB1 receptor expression, depolarization-induced suppression of inhibition, as well as lifelong hyperexcitability (7).

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