Endocannabinoids and Their Implications for Epilepsy

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This review covers the main features of a newly discovered intercellular signaling system in which endogenous ligands of the brain's cannabinoid receptors, or endocannabinoids, serve as retrograde messengers that enable a cell to control the strength of its own synaptic inputs. Endocannabinoids are released by bursts of action potentials, including events resembling interictal spikes, and probably by seizures as well. Activation of cannabinoid receptors has been implicated in neuroprotection against excitotoxicity and can help explain the anticonvulsant properties of cannabinoids that have been known since antiquity.

Cannabis in its various forms, including marijuana and hashish, is produced from the flowers and leaves of the hemp plant, *Cannabis sativa*. Through their primary psychoactive ingredient, Δ^9-tetrahydrocannabinol (THC), these drugs affect the central nervous system by activating specific membrane-bound receptors (1). The primary brain receptors, cannabinoid receptors type 1 (CB1), are G protein–coupled, seven-transmembrane domain proteins that share numerous similarities with heterotrimeric G protein–coupled receptors for conventional neurotransmitters such as γ-aminobutyric acid (GABA) and glutamate. The CB1s bind THC with a high degree of selectivity and are heterogeneously distributed throughout the brain. Inasmuch as THC is a plant-derived compound not produced in mammals, endogenous ligands must exist for the cannabinoid receptor, that is, endocannabinoids. Indeed, several endogenous ligands for CB1 have been discovered (2,3), with anandamide being the first (4). Anandamide and 2-arachidonoyl glycerol (2-AG), are thought to be the major brain endocannabinoids, with regional differences in which

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one or the other predominates. Endocannabinoids have been strongly implicated in a growing variety of physiologic phenomena, including regulation of eating (5), anxiety (6), pain (7), extinction of aversive memories (8), and neuroprotection (9). Potent agonists and antagonists (10) for CB1 exist and may serve as the foundation of new therapeutic strategies for treating pathologies. The voluminous work summarized here has been extensively covered in recent reviews on cannabinoid neurochemistry and pharmacology (3,11–14) as well as neurophysiology (15–19). This review focuses on the neurophysiology of the endocannabinoid systems.

Neurophysiological Properties of the Endocannabinoids Systems

Anandamide and 2-AG are small fatty acid derivatives of arachidonic acid that are synthesized primarily by cleavage from membrane phospholipids by lipases. Unlike conventional neurotransmitters, they are not stored in or released from vesicles but rather are produced inside cells when neuronal activity triggers the enzymes. How they gain access to the extracellular environment is not understood, yet clearly, they get out and reach their target CB1 receptors on other cells. They may diffuse through the membranes of the originating cells or be transported across them.

Synthesis and release of anandamide and 2-AG can be initiated by an increase in intracellular neuronal calcium concentration. Important variables, therefore, include the factors that cause intracellular calcium to increase and the magnitude and kinetics of the related processes. A single action potential does not admit enough calcium for endocannabinoid production, but action-potential bursts do. The duration of endocannabinoid actions is limited by cellular uptake and enzymatic degradation. A transporter operating by facilitated diffusion returns both anandamide and 2-AG to the interior of cells where the degradative enzymes, fatty-acid amide hydrolase (FAAH) and monoglyceride lipase (MGL), degrade them. Whereas neither FAAH (3) nor MGL (20) is strongly selective in cell-free systems, in intact cells, FAAH selectively degrades anandamide, and MGL preferentially affects 2-AG. In addition, FAAH is found predominantly in postsynaptic cell somata and dendrites (21), whereas MGL is in presynaptic nerve terminals (20). These factors may make possible therapeutic strategies for targeting one or the other ligand. For example, FAAH knockout mice are more susceptible to endocannabinoid-induced seizures (22), implying an involvement of anandamide that could be exploited.

Endocannabinoids as Retrograde Messengers

When do cells release endocannabinoids and how is this detected? Much of the information reviewed here was gathered during electrophysiological studies of synaptic transmission using in vitro brain slices. In the early 1990s, it was discovered that depolarization, for a second or so, of a single pyramidal cell or Purkinje cell in rodent hippocampal (23) and cerebellar slices (24) was followed by transient suppression of the incoming GABA-mediated inhibitory synaptic currents. This phenomenon was named depolarization-induced suppression of inhibition (DSI). Although DSI, in principle, could have been caused by a reduced sensitivity of the postsynaptic GABA receptors, detailed quantal analyses showed that GABA-receptor sensitivity was unchanged. Instead, DSI caused a decrease in GABA release from the interneurons. As it is induced in the pyramidal cell, yet expressed as a decrease in GABA release, DSI represents a retrograde form of synaptic signaling (see Fig. 1 for
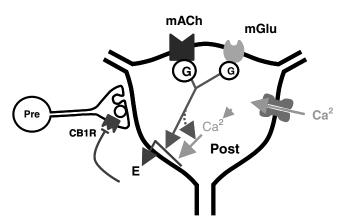

Figure 1. Summary of endocannabinoid synthesis and actions. Endocannabinoids are synthesized from membrane phospholipids in the postsynaptic cell via at least two distinct pathways in postsynaptic (Post) principal cells: a calcium-dependent pathway and a pathway that is independent of calcium but dependent on the activation of G proteins. The calcium-dependent release is caused by entry of calcium into the cell through voltage-gated calcium channels. G protein–dependent synthesis is set into motion by activation of either a muscarinic acetylcholine receptor (mAChR) or a metabotropic glutamate receptor (mGluR). These G protein pathways are independent at the receptor level and probably interact in some way that is not yet fully defined. In addition, the G protein activation can enhance the synthesis and release of endocannabinoids via calcium. Once released, either via diffusion through the postsynaptic cell membrane or by transport out of the cell, the endocannabinoids gain access to the CB1 cannabinoid receptors that are located on the nerve terminals of either GABAergic interneurons (e.g., in hippocampus, neocortex, and amygdala) or on excitatory glutamatergic axons (e.g., cerebellum). The calcium-dependent pathway produces depolarization-induced suppression of inhibition (DSI) or DSE, depending on whether the target CB1 receptors are located on inhibitory or excitatory nerve terminals. (Figure prepared with the assistance of Dr. J. Kim.)

a schematic illustration). Numerous features of DSI and the entirely analogous depolarization-induced suppression of excitation (DSE) were unraveled, but the retrograde messenger between the pyramidal cell and the interneurons remained elusive. In 2001, the problem was solved when two groups showed that an endocannabinoid is the messenger in DSI (25,26), and a third showed that, in the cerebellum, it is the messenger in DSE (27). Agonists of CB1 mimicked or occluded and antagonists of CB1 blocked these phenomena, whereas mice lacking CB1 had no DSI (28,29).

The Inhibitory Actions of Endocannabinoids

What do endocannabinoids do? They inhibit neurotransmitter release from nerve terminals that synapse on the endocannabinoid-generating cell. The localization of the CB1s is obviously the key to this interpretation and to understanding the functions of endocannabinoid systems. In neocortex, hippocampus, and amygdala, for instance, immunocytochemical methods show that CB1s are found overwhelmingly on the nerve terminals of a distinct group of GABAergic interneurons, which besides GABA, also contain the neuropeptide cholecystokinin (CCK) (19). In the cerebellum and striatum, in contrast, CB1s are found in high concentration on the terminals of the excitatory glutamatergic fiber systems. Thus depending on the brain region, endocannabinoids can regulate the release of GABA (and CCK) or glutamate.

Activation of presynaptic CB1s causes presynaptic inhibition of transmitter release. Generally, a major mechanism of synaptic inhibition is the suppression of presynaptic voltage-gated calcium channels, and exogenous cannabinoids do block calcium currents (30–32). In hippocampus and cerebellum, endocannabinoids appear to reduce the calcium influx necessary for release. Imaging of calcium concentrations in cerebellar climbing fiber terminals directly reveals the reduction in calcium influx associated with DSE (27). Additional mechanisms for DSI and DSE expression include an increase in presynaptic potassium channel activity (33), which would impede action-potential invasion into synaptic boutons and interference with the vesicular release machinery (34,35). In all cases, CB1 activation reduces transmitter release.

During DSI and DSE, the suppression of synaptic transmission is transient, lasting seconds. What neurophysiologic functions are served? DSI can facilitate induction of long-term potentiation (LTP). The strength of GABAergic inhibition usually regulates the ability of excitatory synapses to induce LTP—strong inhibition prevents opening of the *N*-methyl-D-aspartate (NMDA) receptors that is required for LTP induction. Synaptic potentials normally too weak to induce LTP can do so when inhibition is pharmacologically blocked. DSI is a transient form of disinhibition, and indeed, weak excitatory

potentials that occur during DSI induce LTP (36). LTP did not occur if CB1s were blocked. Hence, endocannabinoids can regulate synaptic plasticity. They also can modulate rhythmic firing patterns. High frequency (~40 Hz, gamma) rhythms, such as detected by EEG, are strongly reduced by exogenous cannabinoids (37). The slower (4–14 Hz) theta rhythms prevalent in the hippocampus during various behavioral states also are affected by cannabinoids. Recently, a novel form of theta rhythm, generated by an interneuronal network within the hippocampus, was found to be transiently interrupted by DSI (Reich, Carson, and Alger, unpublished data, 2004). DSE probably serves analogous functions.

Calcium-dependent Production of Endocannabinoids

It might appear that endocannabinoid release is a restricted phenomenon, tied to specific conditions of intracellular calcium increase. Actually, endocannabinoids can be released under a wide variety of circumstances because their production does not require activation of voltage-gated calcium channels and, evidently, is not even calcium dependent. Additional endocannabinoid actions first were suggested by the observation that activation of striatal D2 dopamine receptors selectively elevated anandamide levels (2-AG was not affected). Anandamide mitigated the behavioral hyperactivity that was induced by the direct actions of dopamine (38).

Two other classes of G protein–coupled receptors can induce endocannabinoid production and release: (a) the group I class of metabotropic glutamate receptors (mGluRs) in principal cells in the cerebellum (39) and hippocampus (28), and (b) activation of muscarinic acetylcholine receptors (mAChRs) in the hippocampus (40). The mGluR and mAChR pathways seem distinct from the calcium-dependent pathway of endocannabinoid production. Preventing increases in intracellular calcium with high concentrations of calcium chelators has no effect on G protein–induced endocannabinoid production, and measurements of intracellular calcium reveal no increase in calcium concentration associated with the activation of the mGluR and mAChR receptors. Injection of GTPγS, a strong activator of G proteins, causes persistent release of endocannabinoid in the absence of any other form of activation of the cell (40). Two major reasons exist for emphasizing endocannabinoid release by mGluRs and mAChRs. First, they are components of prominent neurotransmitter systems that mediate numerous neurophysiological and behavioral effects. A new understanding of these transmitter systems will result if endocannabinoids are the proximate mediators of these effects. Second, it implies that endocannabinoids have a much broader scope of action than initially imagined.

Different Modes of Endocannabinoid Release Have Different Functions

Are the endocannabinoids released by the G protein–coupled and calcium-dependent pathways the same; and do they subserve the same neurophysiologic functions? As noted, in areas of the brain studied thus far, 2-AG and anandamide are favored as the major endocannabinoid candidates. Nevertheless, the evidence is not conclusive, and questions, such as whether different biosynthetic pathways release different endocannabinoids, currently cannot be answered. Data increasingly point to more than one synthetic pathway producing more than one type of physiologic effect. The G protein–dependent pathway can enhance DSI (i.e., calcium-dependent endocannabinoid release) (28,40), although an effect of DSI on the G protein–dependent pathway has not been described. Inhibitors of phospholipase C and diacylglycerol lipase inhibit some G protein pathways without affecting DSI, implying further distinctions between the two modes of endocannabinoid biosynthesis.

Persistent release of endocannabinoids will not occur during brief periods of synaptic transmission, and stronger neuronal stimulation, such as epileptic seizure, is probably required. The consequences of prolonged endocannabinoid release will undoubtedly be different from brief, transient release. DSI and DSE are transient phenomena—the effects of the endocannabinoids are readily reversed when CB1 activation ceases. However, G protein–dependent endocannabinoid release can lead to lasting synaptic depression of inhibitory synapses (41). LTD of inhibitory synapses has been called iLTD and is caused by minutes-long activation of mGluRs. Blocking either mGluRs or CB1s does not alter iLTD maintenance, even though earlier blockade of either receptor prevents iLTD initiation. Several short bursts of synaptic glutamate stimulation can release endocannabinoids for several minutes, and it was proposed that the duration of endocannabinoid release was sufficient to convert the normally reversible synaptic suppression into iLTD (41). A caveat is that these results were obtained at room temperature and the duration of endocannabinoid actions are very temperature sensitive. It is not yet clear if synaptic stimulation would cause such prolonged release at physiologic temperatures. In any event, iLTD induction requires more than simply prolonged activation of CB1, because an equivalent release of endocannabinoids caused by mAChR activation does not induce iLTD (Kim and Alger, unpublished data, 2004).

Pathologic long-term remodeling of the endocannabinoid system also may occur. Developmental febrile seizures can increase endocannabinoid-mediated suppression of synaptic GABA release (42) by upregulating the number of presynaptic CB1 receptors on the GABAergic interneurons. The ultimate effect on the young brain is not clear, although greater susceptibility to disinhibition could be a destabilizing influence.

Conclusion

From what is known about their synthesis and release, endocannabinoids should be produced under many conditions of increased neuronal excitability and specific intercellular signaling. For example, an epileptic seizure, with its large swings in transmembrane voltage, increases in intracellular calcium, and marked release of neurotransmitters, such as acetylcholine and glutamate, should prominently release endocannabinoids. Indeed, seizures induced by kainic acid (a glutamate agonist) increase hippocampal levels of anandamide in normal and wild-type mice (9). Intriguingly, CB1 knockout mice and normal mice treated with a CB1 antagonist had more pronounced seizures and more severe excitotoxic cell death than untreated normal mice. Although the detailed mechanisms of neuroprotection have not been worked out, the rapid increases in expression of the immediate early genes, *c-fos* and *zipf268*, and subsequent increase in brain-derived neurotrophic factor (BDNF) normally induced by kainic acid, were absent in the CB1 knockout mice. The results complement previous evidence that exogenous cannabinoids can be neuroprotective and show that CB1 activation by seizure-induced release of endocannabinoids also is normally neuroprotective.

The important new directions being opened by investigations of endocannabinoids underscore the prescient opinion of Robert Christison (43), who, in 1848, noting its various beneficial effects, argued that cannabis "is a remedy which deserves a more extensive inquiry . . ."

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