Endocannabinoids

D Piomelli, University of California, Irvine, CA, USA

This article is reproduced from the previous edition, volume 2, pp. 13–15, © 2004, Elsevier Inc.

Glossary

Arachidonic acid Common name for eicosatetraenoic acid (eicosatetraenoic acid, 20:4$\Delta^{5,8,11,14}$), an essential fatty acid that serves as metabolic precursor for eicosanoids and endocannabinoids.

Axon terminal Specialized structure of a neuron that secretes neurotransmitters.

G proteins Heterotrimeric proteins with GTPase activity that link the occupation of certain cell surface receptors to cellular responses.

Neurotransmitter Substance secreted by a neuron at a synapse.

Phospholipase A group of enzymes that catalyze the hydrolysis of phospholipids at their glycerol ester (PLA) of phosphodiester (PLC, PLD) bonds.

Protein kinase Enzyme that transfers a phosphate group from adenosine triphosphate to a protein.

Stereospecific numbering (sn) A convention on how to designate the stereochemistry of glycerol-based lipids. When the glycerol moiety is drawn with the secondary hydroxyl to the left, the carbons are numbered 1,2,3 from top to bottom.

Striatum A subcortical brain structure involved in the control of movement, habit learning, and the rewarding properties of drugs of abuse.

Synapse Specialized junction between the ending of the presynaptic neuron and the dendrite, cell body, or axon of a postsynaptic neuron.

Vanilloid receptor A receptor channel permeable to monovalent cations and activated by heat, acid, and capsaicin, the active ingredient of chili peppers.

The endocannabinoids are a family of biologically active lipids that bind to and activate cannabinoid receptors, the G-protein-coupled receptors targeted by Δ^9-tetrahydrocannabinol in marijuana. The term encompasses several derivatives of arachidonic acid, which are generated on demand by neurons and other cells in response to physiological or pathological stimuli. The two best-characterized endocannabinoids are anandamide (arachidonoylethanolamide) and 2-arachidonoylglycerol (2-AG). Others are noladin ether (2-arachidonoyl glyceryl ether) and virodhamine (*O*-arachidonoyl ethanolamine).

Synthesis

Anandamide

Anandamide is produced from the hydrolysis of an N-acylated species of phosphatidylethanolamine (PE), called *N*-arachidonoyl-PE. This reaction is initiated by activating neurotransmitter receptors and/or by elevating intracellular levels of Ca^{2+} ions and is probably catalyzed by phospholipase D. The anandamide precursor, *N*-arachidonoyl-PE, is present at low levels in nonstimulated cells, but its formation can be stimulated by Ca^{2+} and occurs simultaneously with that of anandamide. *N*-Arachidonoyl-PE synthesis is catalyzed by membrane-bound N-acyltransferase, which has been partially purified (see Figures 1 and 2).

2-Arachidonoylglycerol

In brain neurons, 2-AG formation is probably initiated by the activation of phospholipase C, which cleaves membrane phospholipids (e.g., phosphatidylinositol-4,5-bisphosphate) at the proximal phosphate ester bond, producing 1,2-diacylglycerol. This intermediate is broken down by diacylglycerol lipase to yield 2-AG and free fatty acid. Another pathway of 2-AG release might involve the hydrolytic cleavage of a phospholipid at the *sn*-1 position of the glycerol backbone, catalyzed by phospholipase A_1. This reaction yields a *sn*-2 lysophospholipid, which can be further hydrolyzed to produce 2-AG (Figure 3). Finally, 2-AG might be formed by hormone-sensitive lipase acting on triacylglycerols or by lipid phosphatases acting on lysophosphatidic acid. However, as these enzymes preferentially target lipids enriched in saturated or monounsaturated fatty acids, they are unlikely to play a role in the synthesis of a polyunsaturated species such as 2-AG.

Physiological Regulation of Endocannabinoid Synthesis

Anandamide

Anandamide synthesis is initiated by intracellular Ca^{2+} rises and/or by activation of G-protein-coupled receptors. For example, activation of vanilloid receptors elevates intracellular Ca^{2+} levels and stimulates anandamide synthesis in rat sensory neurons in culture. In addition, activation of dopamine D_2-receptors enhances anandamide release in the brain striatum of the rat *in vivo*. The molecular steps involved in these effects have not yet been clarified.

2-Arachidonoylglycerol

2-AG formation is also linked to intracellular Ca^{2+} rises. For example, in freshly dissected slices of rat hippocampus, electrical stimulation of the Schaffer collaterals (a glutamatergic fiber tract that projects from CA3 to CA1 neurons) produces a

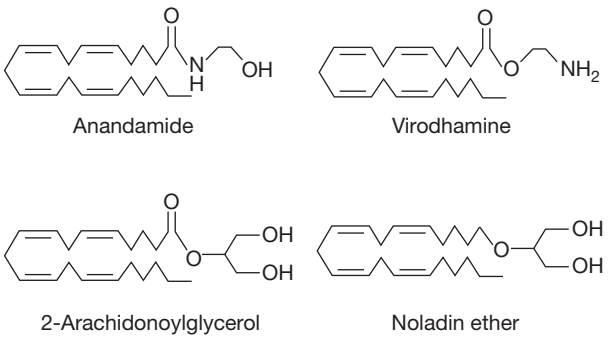

Figure 1 Chemical structures of the endocannabinoids anandamide (arachidonoylethanolamide), 2-arachidonoylglycerol (2-AG), noladin ether, and virodhamine.

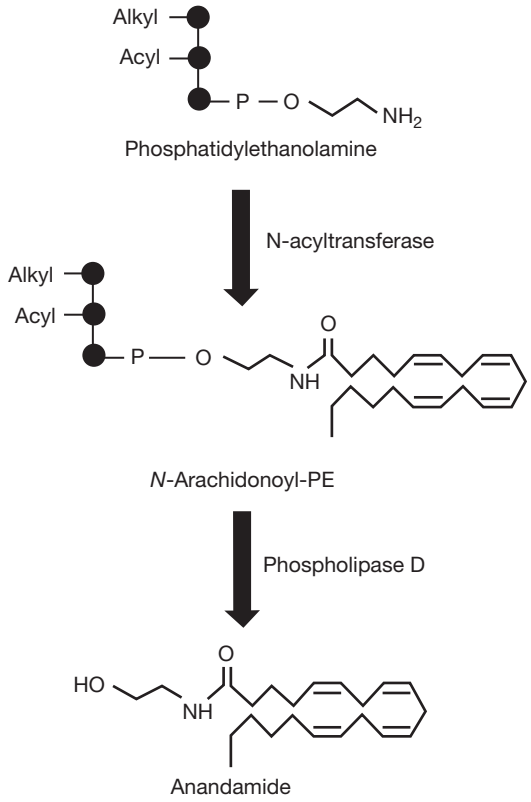

Figure 2 Anandamide biosynthesis. PE, phosphatidylethanol-amine.

fourfold increase in 2-AG levels, which is prevented by the Na^+ channel blocker tetrodotoxin or by removing Ca^{2+} from the medium. It is notable that anandamide levels are not changed by the stimulation, suggesting that the syntheses of 2-AG and anandamide can be independently regulated. This idea is supported by the fact that activation of D_2 receptors, a potent stimulus for anandamide release in the rat striatum, has no effect on striatal 2-AG levels.

Deactivation

In the brain and other tissues, anandamide and 2-AG are rapidly eliminated through a two-step process consisting of

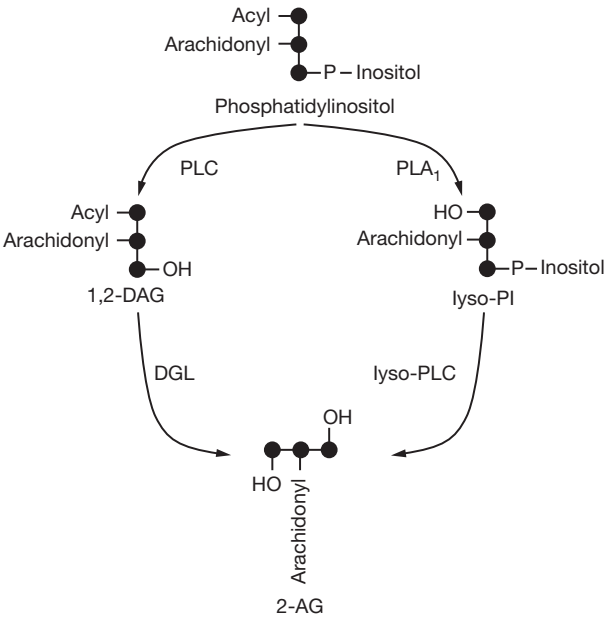

Figure 3 2-AG biosynthesis. 1,2-DAG, 1,2-diacylglycerol; DGL, diacylglycerol lipase; PL, phospholipase.

uptake into cells and enzymatic hydrolysis. The two endocannabinoids share a functionally similar transport mechanism, but they follow distinct routes of intracellular degradation.

Transport into Cells

The transport of anandamide and 2-AG into neurons and astrocytes is structurally specific, displays classical saturation kinetics, and is selectively inhibited by compounds such as *N*-(4-hydroxyphenyl)-arachidonamide (AM404). The putative transporter involved has not yet been identified, but transport has been shown to be Na^+ independent, which is suggestive of a facilitated diffusion mechanism.

Intracellular Hydrolysis

Inside cells, anandamide is metabolized by fatty acid amide hydrolase, a membrane-bound intracellular serine hydrolase that also cleaves oleoylethanolamide, an endogenous satiety factor, and other lipid amides. Hydrolysis of 2-AG is catalyzed instead by monoglyceride lipase, a cytosolic serine hydrolase that converts 2- and 1-monoglycerides into fatty acid and glycerol. The contribution of other lipases to 2-AG degradation cannot be excluded at present.

Cannabinoid Receptors

The endocannabinoids regulate the function of multiple organs and tissues of the body. These regulatory effects are primarily mediated by two G-protein-coupled receptors: CB_1 and CB_2. CB_1 is highly expressed in the central nervous system, but is also present at lower levels in a variety of peripheral

tissues. By contrast, CB_2 is mostly found in immune cells such as lymphocytes. Both subtypes are linked to G_i/G_o proteins and can initiate signaling events typical of these transducing proteins, which include inhibition of adenylyl cyclase activity, opening of K^+ channels, closing of Ca^{2+} channels, and stimulation of protein kinase activities. Nevertheless, CB_1 and CB_2 are structurally different (they have only 44% sequence homology), which has allowed the development of subtype-selective ligands such as the CB_1 antagonist SR141716A (rimonabant) and the CB_2 antagonist SR144528. There is evidence for the existence of at least two additional cannabinoid-sensitive sites in the brain and vasculature, which remain however uncharacterized.

Functions

In broad terms, the endocannabinoids are considered paracrine mediators, substances that act on cells near their sites of synthesis without entering the bloodstream. For example, they are formed by circulating leukocytes and platelets during hypotensive shock and induce the vascular relaxation that accompanies this phenomenon by activating CB_1 receptors on the surface of smooth muscle cells. Similar paracrine actions occur in the brain, where the endocannabinoids mediate a localized signaling mechanism through which neurons modify the strength of incoming inputs. The endocannabinoids are generated by neuronal depolarization and travel backward across the synapse to regulate the release of neurotransmitters from neighboring axon terminals, a process called 'retrograde signaling'.

See also: **Signaling:** Adenylyl Cyclases; G-Protein-Coupled Receptor Kinases and Arrestins; Neurotransmitter Transporters; Phospholipase C.

Further Reading

Devane WA, Hanus L, Breuer A, et al. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949.

Di Marzo V, Fontana A, Cadas H, et al. (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372: 686–691.

Piomelli D (2003) The molecular logic of brain endocannabinoid signaling. *Nature Neuroscience Reviews* 4: 873–884.

Wagner JA, Varga K, and Kunos G (1998) Cardiovascular actions of cannabinoids and their generation during shock. *Journal of Molecular Medicine* 76: 824–836.