## Pharmacology of endogenous cannabinoid substances

Anandamide (*N*-arachidonoylethanolamine) is a brain chemical that activates the same cell membrane receptors that are targeted by tetrahydrocannabinol, the active ingredient in marijuana and hashish. The pharmacological effects of anandamide suggest that it may play important roles in the regulation of mood, memory, appetite, and pain perception. It may act as the chief component of a novel system involved in the control of cognition and emotion. Physiological experiments show, in fact, that anandamide may be as important in regulating our brain functions in health and disease as other better-understood neurotransmitters, such as dopamine and serotonin. The research objective is to understand the physiological roles of anandamide and the biochemical mechanisms of its synthesis and inactivation.

Anandamide is released from rat brain neurons by a unique mechanism: it is stored in the cell membrane in the form of a phospholipid precursor, which is cleaved by a calcium- and activity-dependent enzymatic reaction. *N*-arachidonoyl phosphatidylethanolamine (NAPE) has been identified as a precursor for anandamide, which is formed by a phosphodiesterase-mediated cleavage of NAPE. The biosynthesis of NAPE is catalyzed by an *N*-acyltransferase enzyme, which has been characterized and partially purified from rat brain extracts. The formation of NAPE and its cleavage to yield anandamide are highly regulated processes, which take place in select regions of the brain.

The inactivation of anandamide, necessary to terminate its biological effects, occurs in two steps. It is first removed from the extracellular space by a selective carrier protein that transports it into cells, where it is then broken down by hydrolysis, catalyzed by the enzyme anandamide amidohydrolase, into biologically inactive compounds. A potent inhibitor of this enzyme has been identified (a bromoenol lactone, BTNP), and its availability will facilitate pharmacological analysis of anandamide action. A high-affinity anandamide transporter has been characterized in rat cortical neurons and in astrocytes. A compound (N-(4-hydroxyphenyl)arachidonylamide) has been found that selectively and potently inhibits such transport, without binding to cannabinoid receptors or affecting anandamide hydrolysis. This transport system appears to constitute a novel lipid uptake system analogous to, but distinct from, the prostaglandin uptake system. Also, the use of these inhibitors allowed the demonstration that anandamide transport constitutes the rate-limiting step in the biological inactivation of anandamide, both *in vitro* and *in vivo*. It is important to understand how anandamide levels are regulated, because a deregulation may lead to brain dysfunction.

Fellows have shown that anandamide is present in cocoa powder and in chocolate, along with two other *N*-acylethanolamines that could act as cannabinoid mimics, either by directly activating cannabinoid receptors or by increasing anandamide levels. The relationship of this finding to the subjective feelings associated with eating chocolate remains to be determined.

A second compound, 2-arachidonylglycerol, has been identified as an endogenous cannabinoid ligand in the central nervous system. This compound is present in brain tissue in amounts 170 times greater than anandamide. Hippocampal slice preparations were used to show that neural activity increases the production of 2-arachidonylglycerol; its formation is calcium-dependent and is mediated by the enzymes phospholipase C and diacylglycerol lipase. In the presence of 2-arachidonylglycerol, long-term potentiation in hippocampal slices was completely inhibited, although synaptic transmission itself was normal.

Taken together, the results indicate that both anandamide and 2-arachidonylglycerol serve an endogenous cannabinoid role in the central nervous system. However, the two compounds may be produced under distinct physiological conditions or in distinct brain regions. So, despite their common ability to activate cannabinoid receptors, the physiopathological implications of these signaling molecules may be different. Thus it may be possible to identify pharmacological agents that selectively interfere with discrete components of the endogenous cannabinoid system. (See Publications 85, 97, 101, 124, 134, 135, 150, 151, 161, 171, and 172.)