Molecule of the Month

Anandamide, a Brain Constituent that Functions as a Natural Ligand to the Cannabinoid Receptors

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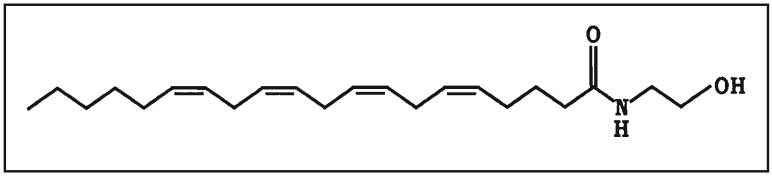
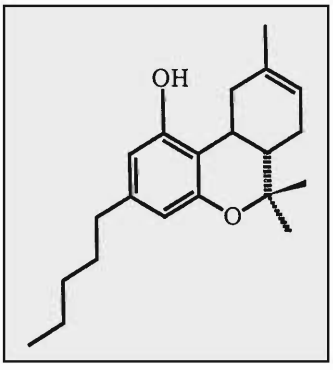
Anandamide is the trivial name given to the molecule *N*-arachidonylethanolamine or arachidonylethanolamide (*Structure* 1). It is the amide formed by the condensation reaction of arachidonic acid – a 20-carbon linear-chain fatty acid containing 4 unconjugated double bonds – with the amino group of 2-ethanolamine. Anandamide belongs to a class of molecules, called *N*-acylethanolamines (NAEs for short), which are present in the membranes of most organisms. As the title of this article indicates anandamide is present in the brain and binds to specific receptor molecules, known as the *cannabinoid receptors*. In this article we shall learn about anandamide and its various interesting properties.

Most of you would have heard of the psychoactive and intoxicating drugs, marijuana and hashish, which are prepared from extracts of the plants, *Cannabis sativa* and *Cannabis indica*. One such intoxicating preparation is known in the Indian subcontinent as *bhang*. These drugs have been used for therapeutic purposes for a long time, but in the recent past have made headlines for their abuse. The most abundant active principle of these drugs is (–)Δ^9-tetrahydrocannabinol (THC, *Structure* 2), though certain other structurally related compounds, present in smaller quantities are also known to mimic THC in this respect. Because they are isolated from the *Cannabis* plants, these compounds are called *cannabinoids*.



Structure 1. Anandamide

THC is a highly nonpolar molecule with just one polar hydroxy group and therefore can easily be dissolved in the hydrophobic interior of lipid membranes. Because of this lipophilic nature, initially it was thought that THC exerts its effects by interacting with membrane lipids and perturbing the membrane structure. However, in 1988 it was discovered by William A Devane and his colleagues that THC binds to specific receptors on the cell membranes [1]. This has raised the possibility that there might be endogenous ligands for these receptors, because it would be surprising if specific receptors existed in human beings for molecules that most of us would never have encountered in our lifetime (see *Box* 1 to learn more about ligands and endogenous ligands). Thus began the search for endogenous molecules that may act as ligands to the cannabinoid receptor. In the year 1992, Devane and his collaborators reported the isolation and characterization of arachidonylethanolamide (or *N*-arachidonylethanolamine) from porcine brain, as an endogenous ligand of the cannabinoid receptors [2]. They christened the compound 'anandamide', after the Sanskrit word for bliss, *ananda*. This is because the use of psychoactive drugs such as hashish and marijuana gives a short-lived sense of happiness (or well being) and *N*-arachidonylethanolamine mimics their action. For this reason, anandamide and other similar endogenous molecules are referred to as *endocannabinoids*, although structurally they do not have any resemblance to the cannabinoids such as THC and similar molecules isolated from the *Cannabis* plant. Since they mimic the activity of THC, which is a cannabinoid, they are said to exhibit *cannabimimetic activity*. However, they are highly addictive and once someone gets used to them, it will not be easy



Structure 2. (-) Δ^9-Tetrahydrocannabinol (THC).

Box 1: What is a Ligand? What are Endogenous and Exogenous Ligands?

In chemistry, especially co-ordination chemistry, the term ligand is used to refer to small molecules or ions that complex with metal ions through coordinate covalent bonds. On the other hand, in biochemistry and biophysics (and other biological sciences as well), the term ligand is used to refer to small molecules that bind to macromolecules or macromolecular assemblies such as antibodies, receptors, enzymes, lectins, ribozymes, DNA and RNA. An endogenous ligand is one that is synthesized in the organism. An exogenous ligand is one that is added externally; it does not otherwise exist in the system.

Gap-junctions are channels connecting adjacent cells in animals and plants that mediate communication between them.

to give up their use. In this sense they are similar to alcohol, but much more addictive.

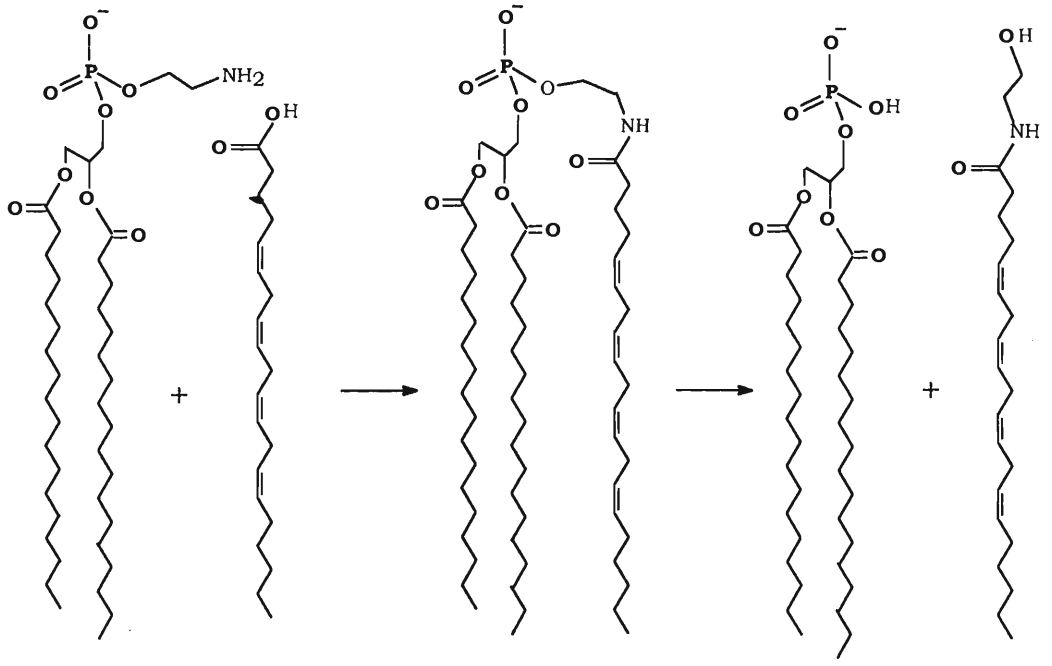
Anandamide competitively inhibited the binding of a radiolabeled THC analogue to the cannabinoid receptor with an inhibition constant (K_i) of 52 nM, whereas under similar conditions the K_i estimated for THC was 46nM (2). This shows that anandamide is comparable to THC in its ability to bind to the cannabinoid receptor.

Since the discovery of its ability to act as an endogenous cannabinoid receptor agonist, a great deal of attention has been focused on anandamide and it has been shown that besides binding to the cannabinoid receptor, this simple single-chain lipid molecule exhibits a variety of highly interesting biological properties. For example, in a paper published in *Nature*, a group of scientists led by Christian Giaume of the INSERM in Paris and Daniele Piomelli of the Neurosciences Institute in La Jolla, USA have demonstrated that anandamide inhibits gap-junction conductance and prevents intracellular calcium signaling [3]. Gap-junctions are channels connecting adjacent cells in animals and plants that mediate communication between them. Ions and small molecules can pass through them while macromolecules such as proteins and polysaccharides cannot. Ca^{2+} is a well-known second messenger that can convey messages across cell membranes by binding to proteins such as calmodulin. Prevention of its transport across the gap-junctions will inhibit its ability to perform this role. Another important property exhibited by anandamide is that it reduces the fertilizing capacity of spermatozoa by inhibiting the acrosome reaction [4].

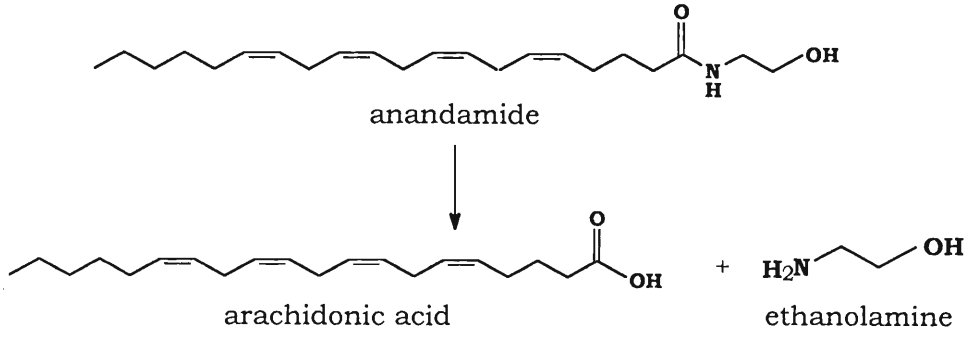
Anandamide is synthesized *in vivo* by the following mechanism (*Box 2*). In the first step, the zwitterionic membrane lipid phosphatidylethanolamine (PE) is derivatized by attaching arachidonyl moiety to the amino functionality of the lipid to give *N*-arachidonyl phosphatidylethanolamine. This is then cleaved by a specific phospholipase to yield anandamide and phosphatidic acid (PA), a negatively charged membrane phos-

Box 2: Biosynthesis and Catabolism of Anandamide

A: Biosynthesis



B: Catabolism (degradation)



Despite its linear structure, anandamide adopts a folded conformation that resembles THC, the active component in some psychoactive drugs.

pholipid [5, 6]. In biological tissues, anandamide is degraded by the action of a specific enzyme (anandamide amidohydrolase) to yield arachidonic acid and ethanolamine. Fine-tuning these two opposing reactions therefore, regulates the *in vivo* concentration of anandamide. The concentration of most biomolecules is modulated by such opposing reactions, which are responsible for their biosynthesis and degradation.

It is very interesting to note that anandamide, which is a linear molecule can bind to the same receptor binding site as THC which has three six-membered rings fused to one another. Though its structure is linear, anandamide has a lot of conformational flexibility around the carbon-carbon single bonds and conformational energy calculations have shown that it adopts a folded structure, which resembles the alphabet U, and hence the structure of THC more closely [7]. Minor conformational changes can take place in the receptor binding site also, thus accommodating the ligand better. In most macromolecule-ligand systems such small conformational changes take place to accommodate the ligand in the binding site.

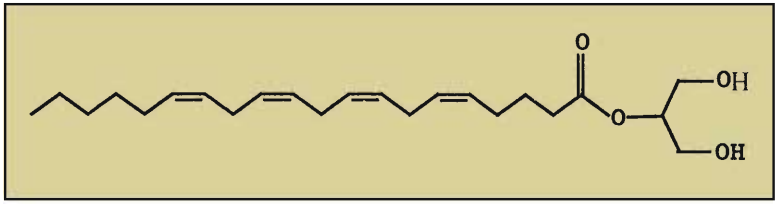
Why has nature chosen to use anandamide, a linear chain molecule as an endogenous ligand for the cannabinoid receptor, while it could have made something similar to THC which has a conformationally more rigid structure, and hence already in a form that fits into the receptor binding site? One possible reason is that anandamide is catabolized by a single-step reaction to give arachidonic acid and ethanolamine, both of which can be utilized in the synthesis of lipids. Therefore the concentrations of anandamide can be regulated by a rather simple and rapid mechanism and degradation products could be utilized in other metabolic processes. Also, one of the precursors required for anandamide biosynthesis, PE is already present in the biomembrane, whereas arachidonic acid could be generated by the hydrolysis of phospholipids or diacylglycerols, in a single-step reaction. On the other hand, THC has a rigid framework of three six-membered rings that are fused and it would require

several enzymes to degrade it into smaller units. As a result, the degradation could be rather slow, thus making it an inefficient process for modulating the ligand concentration.

Increased production of N-acylethanolamines may be a part of the stress-fighting mechanism of life forms.

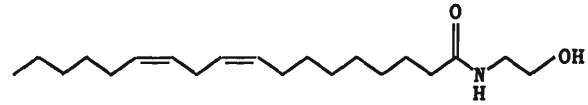
In addition to anandamide, the concentrations of several other *N*-acylethanolamines (NAEs), such as *N*-palmitoylethanolamine and *N*-stearoylethanolamine are also regulated *in vivo* by biosynthetic and degradative processes similar to those described above. Among these, *N*-palmitoylethanolamine has been shown to act as an endogenous ligand of the cannabinoid receptor, type 2 (CB-2) [8]. Studies conducted over the past three decades show that the content of NAEs increases dramatically when the parent tissue is subjected to a condition of stress, such as wounding in animals and dehydration in plants. This has suggested that increased production of NAEs may be part of a stress-fighting mechanism of the system. It appears that NAEs stabilize the bilayer structure of the membrane, and are thus involved in the stress-fighting mechanism [9]. Indeed, single-crystal X-ray diffraction studies on *N*-myristoylethanolamine clearly show that molecules of this single chain lipid are organized in the bilayer format [10].

The identification and characterization of anandamide gave a strong impetus for the search of other endocannabinoids and these studies have already led to the identification of *sn*-2 arachidonylglycerol (*Structure* 3) as a second endogenous cannabinoid receptor ligand in the central nervous system [11]. The concentration of this molecule in the brain was found to be about 170 times higher than that of anandamide. Additionally, several of the *N*-acylethanolamines are also shown to bind to the cannabinoid receptors. The structures of a number of molecules, which exhibit cannabimimetic activity, are given in *Box* 3.

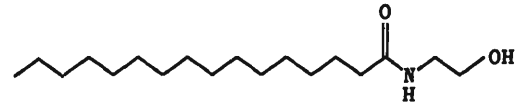


Structure 3. 2-Arachidonylglycerol.

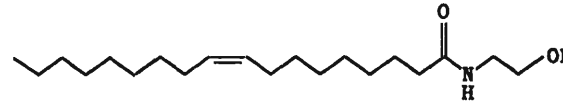
Box 3. Structures of some Endocannabinoids.



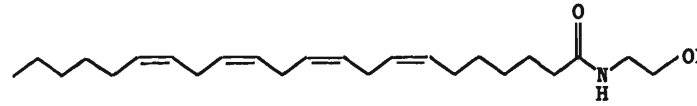
N-linoleoylethanolamine



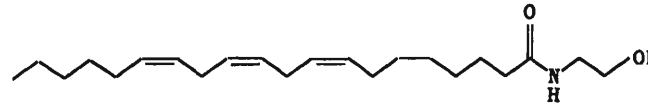
N-palmitoylethanolamine



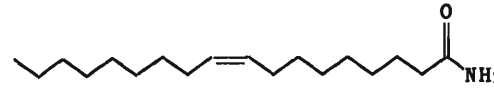
N-oleoylethanolamine



N-docosatetraenoylethanolamine



N-di-homo-γ-linolenoylethanolamine



oleamide

In the western societies, chocolates are consumed in relatively large quantities and it has been observed that many people develop a craving for chocolate, in much the same way as a smoker craves for a cigarette, or an alcoholic craves for a swig of alcohol. This craving for chocolate has not been clearly under-

stood, though caffeine, which is present in chocolate, has been considered as a possible candidate for inducing the craving. Recent studies indicate that commercial chocolate contains anandamide, in addition to two other NAEs, namely *N*-oleoylethanolamine and *N*-lineolylethanolamine and suggest that they may also be responsible for chocolate-addiction [12]. Further research is required to clarify this; the role of anandamide is certainly worth exploring.

In less than 8 years since its identification as an endogenous ligand of the cannabinoid receptor, a very large amount of work has been done on anandamide. A search through the PubMed database during May 2000 gave close to 500 references on anandamide and the list is growing every month. It would be interesting to see what new properties would be identified in the coming years for the innocuous looking molecule that has already proved the adage 'appearances are deceptive'.

Suggested Reading

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