CURRENT AWARENESS

New dawn of cannabinoid pharmacology

The initial demonstration of the existence of a cannabinoid receptor[1] in 1988 suggested the presence of endogenous cannabimimetic ligands. The cloning of a cannabinoid receptor[2] in 1990 gave hope that the identification of receptor subtypes would follow, thus enabling medicinal chemists to develop drugs with selective actions. The recent discovery of anandamide[3] (Fig. 1), an endogenous cannabimimetic eicosanoid, and the cloning of a peripheral cannabinoid receptor[4] have opened the door to a new era in cannabinoid pharmacology.

Anandamide

Anandamide is the ethanolamide of arachidonic acid and its name was derived from *ānanda*, the Sanskrit word for bliss. It was initially purified from porcine brain using a ligand-binding assay to screen for endogenous cannabimimetic compounds[3]; organic soluble brain fractions were tested for their ability to inhibit the specific binding of [^{3}H]HU243 (Fig. 1), a newly developed cannabinoid receptor probe[5]. In addition, anandamide has been recently purified from bovine brain while screening for endogenous modulators of calcium channels, since it inhibited binding of 1,4-dihydropyridine to L-type calcium channels in brain and cardiac membranes[6].

Anandamide behaves as a typical cannabimimetic compound in several *in vitro* and *in vivo* tests. For example, cannabinoids inhibit adenylate cyclase[7,8] via both brain[2,9] and peripheral[10] G protein-coupled cannabinoid receptors, and anandamide was observed to inhibit forskolin-stimulated cAMP production (IC_{50} = 160–200 nM) in Chinese hamster ovary (CHO) cells transfected with either the rat[11] or the human[12] cannabinoid receptor, but not in cells lacking the receptor. Cannabinoids also inhibit the N-type calcium channel current through cannabinoid receptors[13,14]. Although this effect is mediated via a pertussis toxin-sensitive G protein, it does not appear to involve the inhibition of cAMP production[13]. Patch-clamp studies with N18 neuroblastoma cells indicate that anandamide is quite potent at inhibiting N-type calcium channel currents (IC_{50} ~ 20 nM)[15]. It acts as a partial agonist, relative to the cannabimimetic compound WIN55212-2 (Fig. 1). The effect on N-type calcium channels suggests a physiological role of anandamide involving the regulation of neurotransmitter release[15].

Cannabinoids also produce several biochemical effects *in vitro* that are not mediated via cannabinoid receptors. Micromolar concentrations of cannabinoids activate phospholipase A_2 and mobilize intracellular calcium in CHO cells that lack the cannabinoid receptor[16]. Anandamide also produces similar receptor-independent effects[12]. For example, anandamide inhibits binding of dihydropyridine to L-type calcium channels in both brain and heart membranes with an IC_{50} of ~15 μM (Ref. 6). This inhibition is noncompetitive[6], and does not appear to be mediated via cannabinoid receptors, since these receptors are absent in heart tissue[17]. Receptor-independent effects of cannabimimetic compounds occur at relatively high concentrations and may have little relevance *in vivo*.

Tests of the cannabimimetic activity of anandamide *in vivo* have so far been limited to rodents. When administered intraperitoneally, anandamide reduces spontaneous motor activity[18,19], produces hypothermia[18,19] and also exhibits antinociception[18]. The effects of anandamide have a rapid onset but are of shorter duration than other cannabinoids. An anandamide amidase activity has been demonstrated in rat brain membranes[20] and hence exogenously administered anandamide is probably hydrolysed to arachidonic acid and ethanolamine *in vivo*.

At present, anandamide is the only endogenous candidate ligand for the cannabinoid receptor that has been identified, but others may soon follow. While screening for anandamide, two other brain constituents were detected that demonstrated cannabimimetic activities[3]. The partially purified compounds were observed to inhibit both the binding of the cannabinoid probe [^{3}H]HU243 to synaptosomal membranes and the stimulated twitch response of the mouse vas deferens[3], another property of psychotropic cannabinoids[21]. Until these compounds are purified and their structures are identified, their potencies relative to anandamide cannot be ascertained.

Peripheral cannabinoid receptors

A peripheral cannabinoid receptor has recently been cloned following an effort to identify novel G protein-coupled receptors expressed in myeloid cells[4]. The human peripheral cannabinoid receptor protein[4] shares only 44% identity with the human brain receptor[9], but the homology rises to 58% when comparing the 162 amino acid residues in the putative transmembrane domains. Northern blot analysis of RNA from various tissues indicates that this cannabinoid receptor subtype is present in the macrophage/monocyte population of the spleen but not in the brain[4]. *In situ* hybridization studies using labelled oligonucleotides demonstrated that the peripheral cannabinoid receptor mRNA is concentrated in the marginal zones of the spleen[4]. A similar distribution of peripheral cannabinoid receptors has been found using the technique of autoradiography. In a study of over 60 different non-neuronal tissue types in the rat, the highest levels of specific binding of the cannabinoid receptor probe [^{3}H]CP55940 were observed in the marginal zone of the spleen[17]. Cannabinoid receptors were also found to be present in the cortex of the lymph nodes, the corona of the Peyer's patches and leukocyte-enriched blood smears[17]. Thus, the peripheral cannabinoid receptor appears to be confined to the immune system. However, a more detailed investigation is needed to determine exactly which immune cell types in different species express

Therapeutic implications of a cannabinoid receptor subtype localized to the immune system include the development of anti-inflammatory[22] and immunosuppressive compounds[23,24]. The fact that the brain and the peripheral receptor have such low homology suggests the possible development of subtype-selective drugs, and it has been noted that the nonpsychotropic compound cannabinol may have a preference for the peripheral over the brain receptor[4]. Another nonpsychotropic cannabinoid, the 3-dimethylheptyl, 11-carboxylic acid homologue of Δ^8-tetrahydrocannabinol, has been shown to have potent anti-inflammatory and leukocyte anti-adhesion activities[22]. These effects are possibly mediated through the peripheral cannabinoid receptor.

□ □ □

The discovery of anandamide, a candidate ligand for cannabinoid receptors, and the cloning of a peripheral cannabinoid receptor have many implications. The regulation of anandamide synthesis and its role as a neurotransmitter or possibly an intracellular messenger has yet to be determined. Furthermore, a method for the quantitation of anandamine needs to be developed to study its localization and its possible role in pathological states. The structure of anandamide is different from other classes of cannabimimetic compounds, and thus provides a new starting point for developing cannabinoid receptor antagonists. Likewise, the identification of cannabinoid receptor subtypes should facilitate the development of subtype-selective ligands. The low degree of homology between the brain and the peripheral receptor suggests the possible existence of other cannabinoid receptor subtypes that have low sequence identity with the cloned receptors, and have thus not been detected using conventional screening methods. It is possible that the other cannabimimetic compounds detected in brain bind to different receptor subtypes. As little as five years ago, most articles concerning the molecular pharmacology of cannabinoid drugs began with the standard refrain 'the cellular bases of cannabinoid actions are unknown'. The identification of brain and peripheral cannabinoid receptors and the discovery of a candidate endogenous ligand provide a good foundation for future investigations.

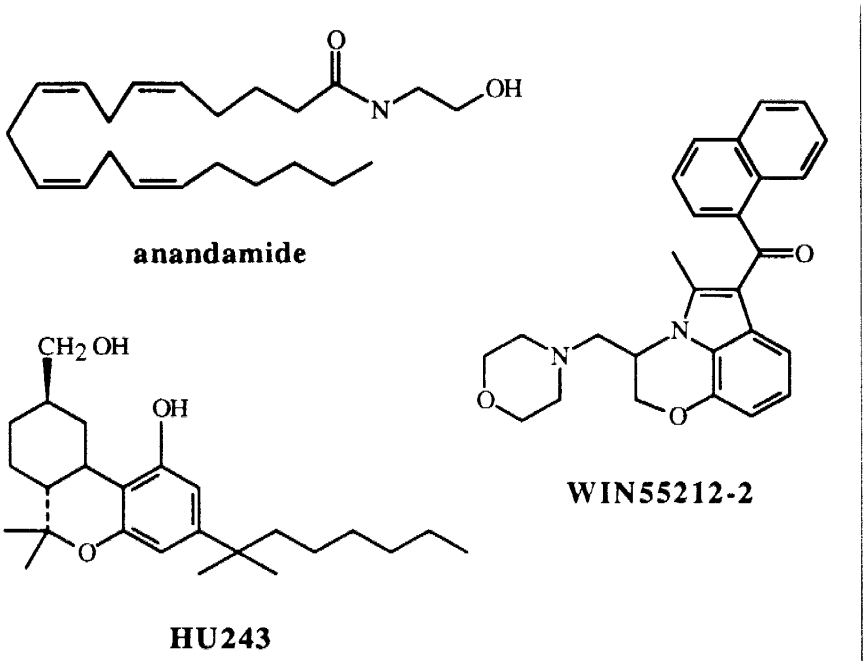

Fig. 1. Chemical structures of anandamide and two recently developed cannabimimetic compounds.

WILLIAM A. DEVANE

Laboratory of Cell Biology, National Institute of Mental Health, Bldg 36/Rm 3A-17, Bethesda, MD 20892, USA.

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CP55940: (*cis*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-(*trans*)-4-(3-hydroxypropyl)cyclohexanol
HU243: 3-(1,1-dimethylheptyl)-β-11-hydroxyhexahydrocannabinol
WIN55212-2: R-(+){2,3-dihydro-5-methyl-3-[(4-morphonolinyl)methyl]pyrrollo[1,2,3-*d,e*]-1,4-benzoaxin-6-yl}(1-napthalenyl methanone monomethanesulphonate