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Endogenous cannabinoid ligands—chemical and biological studies

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Abstract

Arachidonic acid ethanolamide (anandamide) is a brain constituent that binds to the brain cannabinoid receptor (CB_1). It produces many of the pharmacological effects caused by Δ^9-tetrahydrocannabinol (Δ^9-THC) in mice. Anandamide parallels Δ^9-THC in its specific interaction with the cannabinoid receptor and in inhibition of adenylate cyclase. Two additional fatty acid ethanolamides that bind to the cannabinoid receptor, homo-γ-linolenylethanolamide and docostetraenylethanolamide, have been identified in the brain. We believe that the anandamides are involved in the coordination of movement and short term memory. Depression of ambulation in an open field and the analgetic response to anandamide are not fully developed until adulthood, possibly due to an age-related increase in the CB_1 receptor concentration. This observation has clinical implications in pediatrics. A second cannabinoid receptor (CB_2) is present in the spleen. A monoglyceride, 2-arachidonyl-glycerol which binds to both CB_1 and CB_2 in transfected cells and inhibits andenylate cyclase in spleen cells was found in the gut. Its role is apparently associated with the immune system. These fatty acids amides and esters represent a new family of chemical modulators in the body.

Keywords: Anandamides; Cannabinoid receptor; Docostetraenylethanolamide; Homo-γ-linolenylethanolamide; Pediatrics

Arachidonic acid ethanolamide (anandamide) was recently identified by us as a brain constituent that binds to the brain cannabinoid receptor (CB_1) (Devane et al.,

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1992). We have shown that anandamide produces many of the pharmacological effects caused by Δ^9-tetrahydrocannabinol (Δ^9-THC) in mice, such as hypothermia, analgesia and reduced activity in an open field test (Fride and Mechoulam, 1993). Anandamide parallels Δ^9-THC in its specific interaction with the cannabinoid receptor and inhibition of adenylate cyclase (Vogel et al., 1993). These observations indicate that anandamide is an endogenous ligand agonist that may serve as a genuine mediator for the cannabinoid receptor. We have identified in the brain two additional fatty acid ethanolamides that bind to the cannabinoid receptor, homo-γ-linolenylethanolamide and docosatetraenylethanolamide (Hanuš et al., 1993).[1] The biological roles of the anandamides have not been established. We believe that they are involved in the coordination of movement, short term memory and emotions, in view of the known physiological activities of the cannabinoids and the localization of CB_1 receptors in high concentrations in brain areas associated with such activities (Herkenham et al., 1991). However, there is not a complete overlap in the activities of THC and anandamide. The anandamide time course is shorter than that of Δ^9-THC (Smith et al., 1994) and in most assays anandamide is less potent than Δ^9-THC (Mechoulam et al., 1994; Howlett, 1995). We have recently observed that anandamide and anandamide (22:6, *n*-3) under certain experimental conditions, antagonize the effects of Δ^9-THC both in vivo and in vitro (Fride et al., 1995). A significant decrease in the potency of Δ^9-THC-induced inhibition of adenylate cyclase was observed in N18TG2 neuroblastoma cells that were pretreated with low concentrations of anandamides. At these low concentrations, anandamides had no effect when applied alone. In parallel, mice were subjected to tests, designed to detect cannabinoid-induced effects. Mice pretreated (i.p.) with 10 mg/kg of Δ^9-THC received injections with anandamides. Only low doses (0.0001–0.1 mg/kg) of the anandamides, which had no effects when administered alone, partially or fully inhibited the THC-induced effects. These inhibitory effects are specific for the anandamides and are not caused by THC itself under the same experimental conditions. It is possible that low doses of the anandamides are capable of activating a G_s protein mediated signaling pathway (Fride et al., 1995).

Mackie et al. (1993) have found inhibition by anandamide of N-type calcium channels in N-18 neuroblastoma cells. This inhibition is voltage-dependent. However, anandamide differs from other cannabinoid agonists as an inhibitor of N-type calcium channels in that it behaves as a partial agonist. We have also observed partial agonism with the endogenous anandamides (22:4, *n*-6) and (20:3, *n*-6). With both compounds (and to a lesser extent with anandamide) the maximal effects obtained in various tests are smaller than the maximal effects obtained with Δ^9-THC. The assays used were a tetrad of behavioral tests (ambulation, rearing in open field, immobility on the ring, and analgesia on a hot plate) known to reflect cannabinoid-induced effects (Compton et al., 1993).

[1] We suggest naming this group of compounds 'anandamides' with each individual member identified with the parent fatty acid indicated (in parentheses) using the generally accepted fatty acid shorthand designation. Thus the anandamide derived from arachidonic acid will be anandamide (20:4, *n*-6). When only the term 'anandamide' is used it is meant to refer to the 20:4, *n*-6 homolog.

Nearly 25 years ago Paton and Pertwee (1973) pointed out that Δ^9-THC apparently shows a biphasic type of activity with low doses being stimulant and "depressant action becoming increasingly important as dose increases". However, so far this biphasic type of activity has not been methodically investigated, possibly because with THC this type of activity is not robustly expressed. We have now found that anandamide at the lowest dose tested (0.01 mg/kg) elicited stimulatory effects on behavioural activities in the open field locomotion assay and in a catalepsy test and stimulated aggressive behaviour in timid mice. The higher doses tested (10–100 mg/kg) produced inhibitory effects in all of the above mentioned parameters (Sulcova et al., 1996). The results with Δ^9-THC were less clear cut. However, HU-210, a potent cannabinoid agonist, at 0.01 mg/kg increased aggressivity in timid mice and inhibited locomotion and sociability at higher doses. These biphasic effects can be explained, for example, by differential involvement of a G_s and G_i protein activated by low and high doses, respectively.

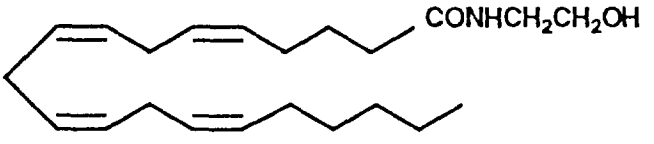
Several groups have reported that in rats CB_1 receptors are present from the first days after birth but only reach adult level after the first weeks of life (McLaughlin and Abood, 1993, Belue et al., 1994). We have found that depression of ambulation in an open field caused by anandamide and the analgetic response to this compound are not fully developed until adulthood (Fride and Mechoulam, 1996). These results may have clinical relevance. We have reported that Δ^8-THC at relatively high doses (18 mg/m^2) prevented vomiting caused by anti-cancer chemotherapy in young children, without producing undesirable cannabimimetic CNS effects (Abrahamov et al., 1995). At such doses one would normally expect very significant cannabimimetic effects, as seen in adults. A tentative explanation could be that in young children the cannabinoid receptor system is not fully developed (hence the lack of cannabimimetic effects) but, as the antiemetic effects are presumably not transmitted through the cannabinoid receptors but may be nonspecific, this effect is seen at an early age. The existence of such presumably nonspecific effects caused by cannabinoids has been shown previously (Martin, 1986). Nonpsychotropic cannabinoids with antiemetic properties are indeed known (Feigenbaum et al., 1989). The above suggestion is supported by the report that the chemoreceptor trigger zone in the area postrema (a vomiting center in the brain) is poor in cannabinoid receptors (Herkenham et al., 1991).

A second cannabinoid receptor, CB_2, has been identified in rat spleen (Munro et al., 1993). We have found that the signal transduction mechanism utilized by CB_2 receptors resembles that of CB_1 receptors, namely that activation of the CB_2 receptor, like the CB_1 receptor, inhibits adenylate cyclase activity and that this inhibition is pertussis toxin sensitive indicating that the CB_2 receptor is coupled to the G_i/G_o binding proteins (Bayewitch et al., 1995).

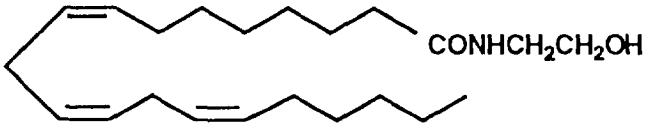
We are at present looking for endogenous ligands for the CB_2 receptor. We have identified in the gut a monoglyceride, 2-arachidonyl glycerol, that binds to membranes from cells transiently transfected with expression plasmids carrying DNA of either CB_1 or CB_2 (Mechoulam et al., 1995). Its K_i values in this system are 472 ± 55 nM and 1400 ± 172 nM for CB_1 and CB_2 respectively. These values are somewhat higher than those recorded for anandamide, namely 252 ± 47 nM for

CB_1 and 581 ± 111 nM for CB_2. 2-Arachidonyl glycerol, in the presence of forskolin, inhibited adenylate cyclase in isolated mouse spleen cells at the potency level of Δ^9-THC and caused the typical tetrad of effects produced by THC. Its potency was at the level of anandamide but less potent than Δ^9-THC (Mechoulam et al., 1995).

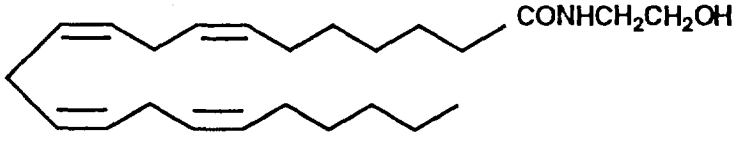
Our work and that of numerous other groups (e.g. Di Marzo et al., 1994) strongly point towards the conclusion that the anandamides and related compounds, e.g. 2-arachidonyl glycerol, may represent novel endogenous messengers (Scheme 1).



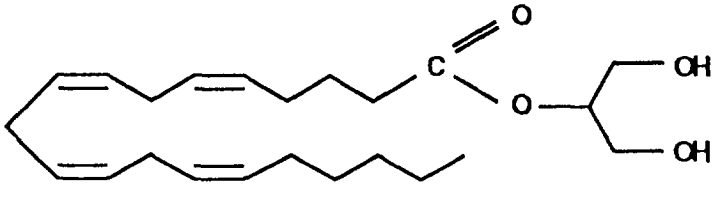
anandamide



homo-γ-linolenylethanolamide



7,10,13,16-docosatetraenylethanolamide



2-arachidonyl glycerol

Scheme 1.

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