PHARMACOLOGY

Endogenous cannabinoids

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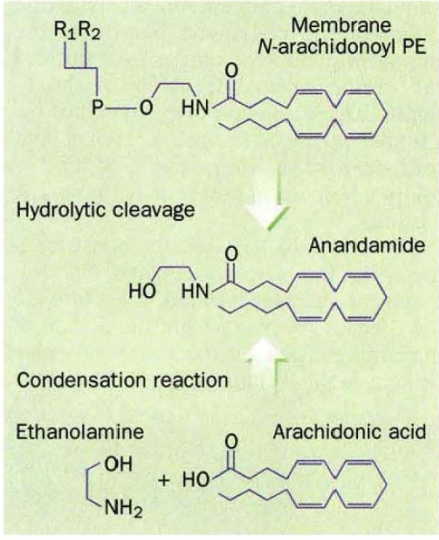
TAKEN together with two other papers[1,2], the report by Di Marzo *et al.* on page 686 of this issue[3] will excite controversy about the mechanisms involved in the biosynthesis and physiological disposition of the brain lipid anandamide (*N*-arachidonoylethanolamine). The reason for the interest in this substance is that it is thought to represent the endogenous cannabis-like substance in brain. Knowledge of the mechanisms involved in the metabolism and inactivation of anandamide could point to fresh approaches to the pharmacological manipulation of these systems in brain, and so to potential therapeutic applications.

The psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC), acts by binding to a specific receptor in brain[4,5]. The brain cannabinoid receptor belongs to the G-protein-coupled superfamily, and when activated inhibits adenylate cyclase activity and voltage-sensitive calcium channels of the N-channel subtype[6,7]. The discovery of a receptor in brain that specifically recognizes Δ^9-THC and related synthetic cannabinoids pointed to the existence of an endogenous ligand, and the most plausible candidate is anandamide[8]. So far, little is known about the mechanisms involved in the synthesis, release and inactivation of this putative new neural signalling molecule. The three new papers[1–3] address some of the relevant questions, although that by Di Marzo *et al.* comes to quite different conclusions to those reached by the others — particularly about the mechanisms involved in the biosynthesis of anandamide in brain.

Devane and Axelrod[1] and Kruszka and Gross[2] propose that anandamide is formed by the enzymically catalysed condensation of arachidonic acid with ethanolamine. Both laboratories demonstrated the formation of anandamide when brain membrane preparations were incubated *in vitro* with ethanolamine and arachidonic acid. Kruszka and Gross suggest that the condensation reaction is unlike others involving arachidonic acid in being independent of coenzyme A and ATP. Instead they propose that arachidonic acid may react with a cysteine at the active centre of the enzyme to form an arachidonoyl–thiol ester enzyme intermediate. Both groups found that arachidonic acid was the preferred substrate among a series of related unsaturated fatty acids tested, and that the reaction was specific for ethanolamine.

Di Marzo and colleagues[3] put forward an alternative biosynthetic mechanism. They propose that anandamide may be synthesized by hydrolytic cleavage from a pre-existing phospholipid precursor, arachidonoyl phosphatidylethanolamine (APE; see figure). Although they identified no specific synthetic enzyme, phospholipase D was shown to be capable of forming anandamide from endogenous brain lipid fractions containing the proposed precursor. In primary cultures of rat brain neurons exposed to radiolabelled ethanolamine, Di Marzo *et al.* showed that stimulation with the calcium ionophore ionomycin (to simulate synaptic activity) led to the formation of radiolabelled anandamide. Furthermore, the authors demonstrated that the newly synthesized anandamide is released into the external medium, and that it can be inactivated by a rapid, saturable uptake mechanism. Incubations using neuron-free astrocyte cultures did not lead to anandamide formation, indicating that its biosynthesis was restricted to neurons. Further experiments, using chromatographic and nuclear magnetic resonance techniques, established the presence of the hypothetical precursor phospholipid APE in brain lipid extracts.



Alternative routes for the biosynthesis of anandamide.

Di Marzo *et al.* argue against the hypothesis that anandamide biosynthesis involves a condensation reaction, on the grounds that the endogenous levels of free arachidonic acid and ethanolamine in brain are very low. In the experiments[1,2] in which anandamide was formed by the condensation route, the reaction may have been driven artificially by the use of very high substrate concentrations (20–100 mM in the case of ethanolamine), and it is suggested that in reality these are enzymic routes for the breakdown of anandamide being driven in reverse.

On the other hand, under the conditions used by Di Marzo *et al.* only very small amounts of radiolabelled ethanolamine were incorporated into anandamide following stimulation of neuronal cultures. Larger amounts of other *N*-acyl-ethanolamines were formed, including *N*-γ-linolenoyl, *N*-linoleoyl, *N*-palmitoyl and *N*-stearoyl, implying that the biosynthetic mechanism proposed is rather unspecific. The authors counter this by arguing that this finding may suggest that in the brain there is a family of *N*-acyl-ethanolamines with differing cellular signalling functions. This is an interesting proposal which deserves further attention. Is it possible, for example, that another *N*-acyl-ethanolamine could be the endogenous ligand for the second cannabinoid receptor described in peripheral tissues[9,10], for which anandamide has only modest affinity?

Regardless of the outcome of the debate on biosynthetic mechanisms, Di Marzo and colleagues have advanced our understanding of anandamide as the putative endogenous cannabinoid receptor ligand. Their findings suggest that anandamide biosynthesis is under physiological regulation, being stimulated by increased neuronal intracellular levels of calcium, and that anandamide can be released into the extracellular medium and recaptured by an uptake mechanism.

In a more cautious vein, it is worth noting that not everyone agrees that anandamide is the endogenous cannabinoid receptor ligand in brain. For example, Evans and colleagues[11] also reported the release of endogenous cannabinoid-receptor-binding activity from rat brain slices *in vitro* in response to depolarizing stimuli or to calcium ionophores. In this case, however, the material released appeared to be an unidentified peptide and not anandamide, so this story may still be far from complete. □

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1. Devane, W. A. & Axelrod, J. *Proc. natn. Acad. Sci. U.S.A.* **91**, 6698–6701 (1994).
2. Kruszka, K. K. & Gross, R. W. *J. biol. Chem.* **268**, 14345–14348 (1994).
3. Di Marzo, V. *et al. Nature* **372**, 686–691 (1994).
4. Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S. & Howlett, A. C. *Molec. Pharmac.* **34**, 605–613 (1988).
5. Matsuda, L. A., Lolait, S. J., Brownstein, M., Young, A. & Bonner, T. I. *Nature* **346**, 561–564 (1990).
6. Howlett, A. C. & Fleming, R. M. *Molec. Pharmac.* **27**, 429–436 (1984).
7. Caulfield, M. P. & Brown, D. A. *Br. J. Pharmac.* **106**, 231–232 (1992).
8. Devane, W. A. *et al. Science* **258**, 1946–1949 (1992).
9. Munro, S., Thomas, K. L. & Abu-Shaar, M. *Nature* **365**, 61–63 (1992).
10. Iversen, L. L. *Nature* **365**, 12–13 (1993).
11. Evans, D. M., Lake, J. T., Johnson, M. R. & Howlett, A. C. *J. Pharmac. exp. Ther.* **268**, 1271–1277 (1994).