Anandamide may mediate sleep induction

We report here that oleamide, a putative sleep factor[1], and anandamide, an endogenous cannabinoid ligand[2,3], cause similar pharmacological effects in mice. Only anandamide binds to the cannabinoid CB_1 receptor but by inhibiting the enzyme which inactivates anandamide (thus increasing its concentration), oleamide potentiates anandamide binding to CB_1 enhancing anandamide effects in mice. Our observations raise the possibility that some oleamide effects (including induction of sleep) may be mediated by anandamide.

Cravatt *et al.* identified and reported the sleep-inducing properties of oleamide, a lipid found in the cerebrospinal fluid of

sleep-deprived cats[1]; and we have isolated and characterized anandamide, a lipid from the brain which is an agonist for the cannabinoid receptor[2,3]. Drowsiness or sleepiness are well-known effects in the later stages of intoxication by marijuana, whose active constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), and anandamide have a close biochemical and behavioural profile. Both substances bind to the brain cannabinoid receptor, CB_1 (refs 2–4).

The sleep-producing properties of anandamide are not known, but Santucci *et al.*[5] have found that the CB_1 antagonist SR141716A increases the time spent awake at the expense of both slow-wave and rapid-eye-movement sleep. They suggested that "…an endogenous cannabimimetic (anandamidergic?) system may regulate the organization of the sleep–waking cycle".

To establish whether there is any relationship between anandamide and oleamide we compared their *in vivo* and *in vitro* effects. We examined oleamide in four assays commonly used together for testing cannabinoid (including anandamide) activity[3,6]: the ring immobility (catalepsy) test, which measures the percentage of time mice remain motionless; the open-field test, which measures locomotor activity; hypothermia; and response to a hot plate (antinociception). The median effective doses (ED_{50} values) obtained from these tests (Table 1) show that oleamide has essentially the same activity profile as anandamide, though it is less potent in most tests. Testing anandamide in the presence of 7.5 mg oleamide per kg body mass (Table 1) showed that oleamide potently increased anandamide activity. But oleamide is not a cannabinoid, as even at 10 μM, it did not bind to CB_1, confirming a previous report[7].

The enzyme responsible for anandamide inactivation, fatty-acid amide hydrolase (FAAH), recognizes oleamide as a substrate[8], and its molecular characterization, cloning and expression have been reported recently[9]. Using the assays described in refs 8 and 10, we found that oleamide inhibited FAAH-mediated hydrolysis of [^{14}C]anandamide by mouse neuroblastoma $N_{18}TG_2$ cells in a dose-dependent manner (Fig. 1a). With the 10,000*g* particulate fraction, which contains most of the cell FAAH[8], the effect was significant at an oleamide concentration of 5 μM, with 48% inhibition with 100 μM oleamide. This effect was smaller than that obtained with anandamide (90% inhibition with 100 μM), in agreement with previous observations that FAAH catalyses the hydrolysis of anandamide more rapidly than that of oleamide[8,9].

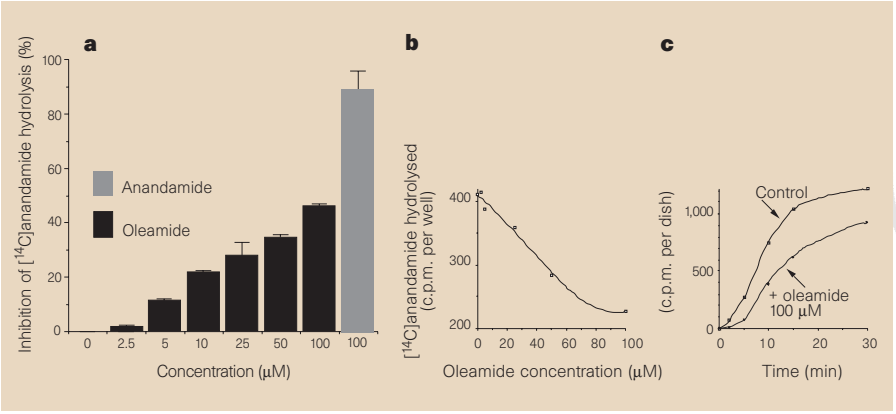

Figure 1 a, Dose-dependent inhibition of [^{14}C]anandamide hydrolysis by N18TG2 cell 10,000*g* particulate fractions. **b,** Dose-dependent inhibition of [^{14}C]-anandamide hydrolysis by intact confluent N18TG2 cells. **c,** Effect of 100 μM oleamide on time-dependent [^{14}C]anandamide hydrolysis by intact confluent N18TG2 cells. The breakdown of [^{14}C]anandamide was measured from the [^{14}C]ethanolamine released by hydrolysis of the amide bond in anandamide as described previously[10]. Data in **a** are means ± s.e.m of three separate experiments. Data in **b** and **c** are means of duplicates, representative of three experiments.

With intact cells, the inhibitory effect of oleamide was dose-dependent, producing marked elevations in [^{14}C]anandamide levels (Fig. 1b). When [^{14}C]anandamide (50 μM) and oleamide (100 μM) were co-incubated, there was a 75–100% inhibition of [^{14}C]anandamide hydrolysis with short incubation times (2–5 min; Fig. 1c), under which conditions the physiological activation of CB_1 receptors by anandamide is likely to occur.

We also tested the ability of oleamide to affect the K_i of anandamide in competition binding experiments (against [^{3}H]HU-243, a high-affinity cannabinoid ligand[2]) to CB_1 in transfected COS-7 cells[11]. Anandamide alone yielded a K_i of 350 ± 7 nM. In the presence of the amidase inhibitor phenylmethylsulphonyl fluoride (PMSF; 200 μM) the observed K_i decreased by roughly one order of magnitude. A similar enhancement in affinity was seen on addition of oleamide (50 μM). With PMSF and oleamide together, the affinity did not increase any further.

Naturally occurring oleoylethanolamide (the oleic acid analogue of anandamide), the hybrid structure between anandamide and oleamide, also fails to activate CB_1 (ref. 12), but has similar effects to oleamide in mice (unpublished results) and inhibits anandamide hydrolysis in rat brain microsomes[12].

Our observations, together with previous results, raise the possibility that some oleamide effects (including induction of sleep) may be mediated by anandamide.

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Table 1 Behavioural effects of Δ^9-THC, anandamide and oleamide

	ED_{50} values			
Test	Δ^9-THC	Anandamide	Oleamide	Anandamide + oleamide
Open field (ambulation)	7.0	3.4	5.7	0.8
Ring immobility	2.3	5.0	18.9	2.9
Hypothermia	9.5	3.6	17.5	1.5
Hot plate	10.3	28.0	29.2	3.0

Ten minutes after intraperitoneal injection, mice were tested in four ways to evaluate cannabinoid-induced effects[6]. For each drug, dose-response curves were obtained using 5–7 doses in the range 1–10 mg per kg body mass (n = 4–7 mice for each dose). ED_{50} values were calculated by nonlinear regression (Graphpad Prism). Anandamide + oleamide denotes anandamide in the presence of 7.5 mg oleamide per kg body mass.

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