We report here that oleamide, a putative sleep factor, and anandamide, an endogenous cannabinoid ligand, cause similar pharmacological effects in mice. Only anandamide binds to the cannabinoid CB₁ receptor but by inhibiting the enzyme which inactivates anandamide (thus increasing its concentration), oleamide potentiates anandamide binding to CB₁, 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 cats1; and we have isolated and characterized anandamide, a lipid from the brain which is an agonist for the cannabinoid receptor2,3. Drowsiness or sleepiness are well-known effects in the later stages of intoxication by marijuana, whose active constituent, Δ9-tetrahydrocannabinol (Δ9-THC), and anandamide have a close biochemical and behavioural profile. Both substances bind to the brain cannabinoid receptor, CB1 (refs 2–4).

The sleep-producing properties of anandamide are not known, but Santucci et al. have found that the CB1 antagonist SR141716A increases the time spent awake at the expense of both slow-wave and rapid-eye-movement sleep. They suggested that “…an endogenous cannabinimetic (anandamergic?) system may regulate the organization of the sleep–waking cycle5.

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) activity6: 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 test, which measures the percentage of time a mouse remains on a hot plate (antinociception). The median effective dose (ED50) values obtained from these assays (Table 1) showed that oleamide potently increased anandamide activity, though it is less potent in vivo and in vitro tests (Table 1) show that oleamide has essentially the same activity profile as anandamide, and oleamide has a close biochemical and behavioural profile. Both substances bind to the brain cannabinoid receptor, CB1 (refs 2–4).

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). ED50 values were calculated by nonlinear regression (Graphpad Prism).

Table 1 Behavioural effects of Δ9-THC, anandamide and oleamide

<table>
<thead>
<tr>
<th>Test</th>
<th>Δ9-THC</th>
<th>Anandamide</th>
<th>Oleamide</th>
<th>Anandamide + oleamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open field (ambulation)</td>
<td>1.4</td>
<td>3.0</td>
<td>5.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Ring immobility</td>
<td>2.3</td>
<td>6.0</td>
<td>18.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>9.5</td>
<td>3.6</td>
<td>17.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Hot plate</td>
<td>10.3</td>
<td>5.0</td>
<td>29.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Ten minutes after intraperitoneal injection, mice were tested in four ways to evaluate cannabinoid-induced effects. 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). ED50 values were calculated by nonlinear regression (Graphpad Prism).

Naturally occurring oleoylethanolamide (the oelic acid analogue of anandamide), the hybrid structure between anandamide and oleamide, also fails to activate CB1 (ref. 12), but has similar effects to oleamide in mice (unpublished results) and inhibits anandamide hydrolysis in rat brain microsomes12.

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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