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**CHARACTERIZATION OF THE BIOLOGICAL ROLES OF THE ANANDAMIDE-ANALOG, OLEYLETHANOLAMIDE**

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Cannabinoid receptors, the molecular targets of the active principle of cannabis 9-tetrahydrocannabinol, are activated by a small family of naturally occurring lipids (endocannabinoids) that include anandamide (AEA) and 2-arachidonylglycerol. AEA is released upon demand by stimulated neurons and activates cannabinoid receptors with high affinity. It is rapidly eliminated through a two-step process consisting of carrier-mediated transport followed by intracellular hydrolysis. AEA is structurally related to a family of lipids called fatty acid ethanolamides (FAEs) that share common biosynthetic pathways and mechanisms of enzymatic degradation, but do not activate cannabinoid receptors. In this study we show that the anandamide-analog oleylethanolamide (OEA) is a lipid mediator involved in the peripheral regulation of feeding. In rats, food deprivation markedly reduces OEA biosynthesis in the small intestine. Administration of OEA causes a potent and persistent decrease in food intake and gain in body mass. This anorexic effect is behaviorally selective and is peripherally mediated. In fact, it is prevented when peripheral sensory fibers are removed by treatment with capsaicin and is not induced by intraventricular OEA administration. To further elucidate the role of OEA in feeding behavior, we determined the dose-dependent effects of intra-peritoneal (i.p.) administration of OEA on food intake and meal patterns, both in starved and in *ad libitum*-feeding rats. Cumulative food intake was dose dependently inhibited by OEA, with an approximate ID<sub>50</sub> of 5.2 mg/kg. OEA suppressed feeding by increasing the first meal latency in a dose-dependent fashion, by decreasing the amount of food intake in the first hour after the administration, and by decreasing the number of meals. In a separate group of animals, OEA levels were analyzed in plasma by HPLC/MS at different times after administration of exogenous OEA (5 mg/kg). At 15 min we observed an increase of plasma OEA, which remained elevated at 30 min and rapidly decreased at 60 min after administration of exogenous OEA, thus suggesting a very short life span of this molecule. Taken together, these results indicate that the suppressive effect of OEA on food intake is associated with high level of OEA in plasma and that this effect may be mediated by an inhibition of appetite and hunger.

The ability of OEA to regulate feeding behavior led us to hypothesize that OEA might play a role in eating disorders. To test this hypothesis we analyzed FAEs content in plasma and cerebrospinal fluid (CSF) collected from patients suffering from anorexia or bulimia, as well as from normal controls. Both CSF and plasma samples from anorexic/bulimic patients contained higher levels of OEA with respect to controls, thus suggesting a possible participation of OEA in these pathologies. The pharmacological targets of OEA are still missing. This lack of information hinders the possibility to further investigate OEA actions by the pharmacological manipulation of its hypothetical receptors. On the other hand the enzymatic pathway of OEA degradation is well understood. After release, OEA is transported back into cells and broken down to oleic acid and ethanolamine by an intracellular fatty acid amide hydrolase (FAAH). Drugs inhibiting FAAH should cause an increase of endogenous levels of OEA, leading to an enhancement of its biological actions. This prompted us to study the roles of OEA by blocking its enzymatic breakdown and looking at the consequential effects *in vivo*. Potent, selective and systemically active inhibitors of intracellular FAAH activity were necessary to test this idea. Commercially available FAAH inhibitors lack, however, the target selectivity and biological availability needed for *in vivo* studies. Preliminary data from our lab showed that two new aryl-alkyl-carbamates, fenoxbutamate (FB) and carbifenamate (CF), can potently and selectively inhibit FAAH activity *in vitro*. In the second part of this study, we investigate the *in vivo* effects of FB and CF on FAAH activity and on different behavioral paradigms, such as rat emotional reactivity, motor behavior, catalepsy and feeding behavior. I.p. injections of either FB or CF produced a profound, dose-dependent inhibition of brain FAAH activity, accompanied by significant elevations in brain FAEs. FAAH inhibition was also associated with increased sensitivity to exogenously administered anandamide. In fact CF (0.3 mg/kg i.p.) intensified and prolonged the decrease in body temperature elicited by a subthreshold dose of anandamide, whereas it had no effect when injected alone. FB and CF evoked anxiolytic-like responses in two different models for testing rat emotional reactivity, the elevated zero maze and the isolation-induced ultrasonic emission test. The anxiolytic-like effects of FB were attenuated by a

non-anxiogenic dose of the CB1 antagonist rimonabant (2 mg/kg i.p.), suggesting that this response could be, at least in part, mediated by an increase of anandamide levels in the brain. Both FB and CF did not evoke catalepsy. As far as food intake, CF caused a small but significant decrease of the total amount of food eaten by free feeding rats in a 24h-test period. Surprisingly, CF did not potentiate the effects of exogenously administered OEA. Further experiments are currently in progress to elucidate the influence of different possible confounders, such as the central increase of AEA, or the turn over of OEA in peripheral tissues that could differently influence the effects of FAAH inhibitors on feeding behavior.

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