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Research report

2-Arachidonoylglycerol as a possible treatment for anorexia nervosa in animal model in mice

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ABSTRACT

We have investigated the effects of 0.001 mg/kg 2-arachidonoylglycerol (2-AG) administered in combination with compounds present in the body alongside 2-AG like 2-palmitoylglycerol and 2-linoleylglycerol (also termed "entourage"), on cognitive function,food intake, and neurotransmitter levels in the hippocampus and hypothalamus of mice under diet restriction. Young female Sabra mice were treated with vehicle, 2-AG, 2-AG + entourage, 2-AG + entourage + 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)- 4-methyl-*N*-(piperidin-1-yl)-1 H-pyrazole-3-carboxamide (SR141716A, a CB_1 antagonist) and SR141716A. The mice were fed for 2.5 h a day for 14 days. Cognitive function was evaluated by the eight arm maze test, and neurotransmitter (norepinephrine, dopamine, L-DOPA and serotonin) levels were measured in the hippocampus and hypothalamus by high-performance liquid chromatography-electrochemical detection. Food intake was increased by 2-AG and, to an even greater extent, by 2-AG + entourage. SR141716A reversed the effect of 2-AG + entourage. The administration of 2-AG + entourage improved cognitive function compared to the vehicle mice, and this improvement was blocked by SR141716A. 2-AG + entourage-treated mice showed an increase in norepinephrine (NE), dopamine and L-DOPA levels in the hippocampus. SR141716A normalized NE and L-DOPA levels. There were no significant changes in hypothalamic neurotransmitter levels. The use of very low doses of the endocannabinoid 2-AG + entourage can improve cognitive function by elevating norepinephrine and L-DOPA levels in the hippocampus, without cannabinomimetic side effects. These findings may have implications for cognitive enhancement in anorexia nervosa.

1. Introduction

2-Arachidonoyl glycerol (2-AG) is the most abundant endogenous cannabinoid present in the mammalian body. It was first found in canine gut (Mechoulam et al., 1995) and later in rat brain (Sugiura et al., 1995). 2-AG binds the CB_1 cannabinoid receptor with low affinity, but exhibits full efficacy (Sugiura et al., 2000; Sugiura et al., 2002). It is present in the brain, gut and immune system, as well as in human and cow milk. The level of its activity is enhanced by compounds present in the body alongside 2-AG: 2-palmitoylglycerol and 2-linoleylglycerol, which protect it by delaying its hydrolysis and by lowering the uptake rate into the cells (producing a so called "entourage" effect). These compounds were thus named "entourage" (Ben-Shabat et al., 1998).

Two kinds of cannabinoid receptors, termed CB_1 and CB_2, have been characterized and cloned (Devane et al., 1988; Matsuda et al., 1990; Munro et al., 1993). 2-AG binds to both cannabinoid receptors. The CB_1 receptor is mostly distributed in several brain regions such as the cortex, hippocampus, basal ganglia and cerebellum (Herkenham et al., 1990) while the CB_2 receptor is found in immune tissues as the spleen, thymus and tonsils (Pertwee, 1997) but also in the brain in disease states, such as in the microglia in postmortem brains from Alzheimer's disease patients (Benito et al., 2003). Specific antagonists for the CB_1 receptor and for the CB_2 receptor are SR141716A (Rinaldi-Carmona et al., 1994) and SR144528 (Rinaldi-Carmona et al., 1998) respectively.

There is much documentation of the effects of cannabinoid agonists on food intake and the importance of endocannabinoids in general, and specifically 2-AG, in suckling, growth, development and anti-inflammatory effect. We have shown that 2-AG can be used to reverse the symptoms of hepatic encephalopathy induced

Abbreviations: AUC, area under the curve; CB_1/CB_2, cannabinoid receptor 1/2; CBD, cannabidiol; HE, hepatic encephalopathy; NE, norepinephrine; 2-AG, 2-arachidonoylglycerol; 5-(4-Chlorophenyl)-1-(2, 4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide, SR141716A; 5-HIAA, 5-hydroxyindoleacetic acid.

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by TAA, and that 2-AG levels are very significantly enhanced in mouse brain after hepatic encephalopathy (Avraham et al., 2006).

Research which has been done thus far in the area has been performed in rats, and the levels of 2-AG involved were relatively high and produced cannabinomimetic side effects. Our goals were to investigate the effects of very low doses (0.001 mg/kg) of 2-AG, which do not produce cannabinomimetic side effects (Sulcova et al., 1998) on cognitive function and food consumption, which were improved following administration of low doses (0.001 mg/kg i.p.) of the cannabinoids ANA, THC and noladin and fish oil in diet-restricted mice (Hao et al., 2000; Avraham et al., 2005, 2004, 2011). Food consumption was also shown to be increased by low dose of 2-AG in rats (Kirkham et al., 2002). Since the former compounds were also shown to reverse some of the changes in catecholamine levels and turnover rates caused by diet restriction in the hippocampus, a brain area associated with learning, and the hypothalamus which is associated with feeding behavior, and as CB_1 receptors were found to regulate bone formation via modulation of adrenergic signaling (Tam et al., 2008) we aimed to investigate the effect of 2-AG on these biochemical indices, as a possible explanation for the effects on cognitive function and food intake. We also wished to determine whether the effects of 2-AG are mediated by the CB_1 receptors, using the selective antagonist SR141716A.

2. Results

2.1. Dose response experiment for the effects of 2-AG on food intake

The effects of doses of 0.001, 0.01 and 0.1 mg/kg 2-AG on food intake were tested. Of these doses, only 0.001 mg/kg displayed a significant effect (Fig. 1; *t*-test with multiple comparisons: $t_{16} = 2.522$, $p < 0.05$ vs. control). Therefore, we decided to use this dose for the next experiments.

2.2. Food intake

Food intake, calculated as AUC, increased in the 2-AG and the 2-AG + entourage groups, compared to the vehicle group (Fig. 2; ANOVA: $F_{(4,45)}=4.929$, $p = 0.002$; planned comparisons: $p < 0.01$ and $p < 0.001$, respectively, vs. vehicle). SR141716A reversed the effect of 2-AG + entourage on food intake ($p < 0.05$), but the 2-AG + entourage + SR141716A group still displayed a higher food intake than the vehicle group ($p < 0.05$).

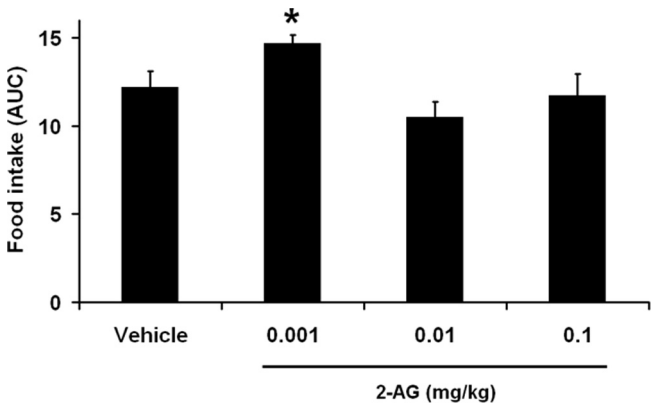

Fig. 1. Dose response experiment to determine the most effective dose of 2-AG on food intake. Only 0.001 mg/kg increased food intake. *−p < 0.05 vs. vehicle.

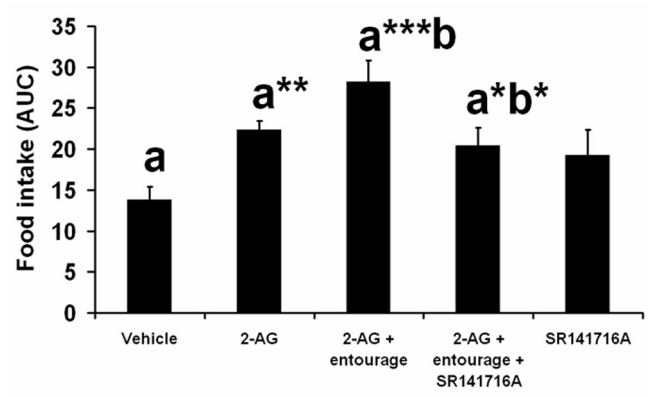

Fig. 2. Food intake in AUC. 2-AG and 2-AG + entourage increased food intake compared to vehicle, and SR141716A reversed the effect of 2-AG + entourage. *−p < 0.05, **−p < 0.01, ***−p < 0.001, respectively, vs. corresponding letter.

2.3. Body weight

The animals received food for 2.5 h a day, as a model for anorexia nervosa. The initial body weight was about 36 grams. During 9 days the control group lost 19%, the 2AG + entourage 14%, 2-AG + entourage + antagonist 34%, and vehicle + SR141716A 32% of initial body weight. The animals were not hyperactive.

2.4. The effect of 2-AG on cognitive function

Cognitive function in the eight arm maze test improved following 2-AG + entourage treatment (Fig. 3; ANOVA: $F_{(2,83)} = 4.76$; planned comparisons: $p < 0.05$ vs. vehicle). Administration of 2-AG alone did not improve performance compared to vehicle (data not shown). Administration of SR141716A reversed the effect of 2-AG + entourage on cognitive function ($p < 0.05$ vs. 2-AG + entourage).

2.5. Levels of NE, DA, L-DOPA and serotonin in the hippocampus

Mice treated with 2-AG + entourage showed an increase in the levels of NE (Fig. 4A; *t*-test with multiple comparisons: $t_{60} = 2.465$, $p < 0.05$) compared to vehicle. SR141716A administration to the 2-AG + entourage group normalized the levels compared with the 2-AG + entourage ($t_{49} = 2.49$, $p < 0.05$). SR141716A did not affect NE levels in the vehicle group. Dopamine levels

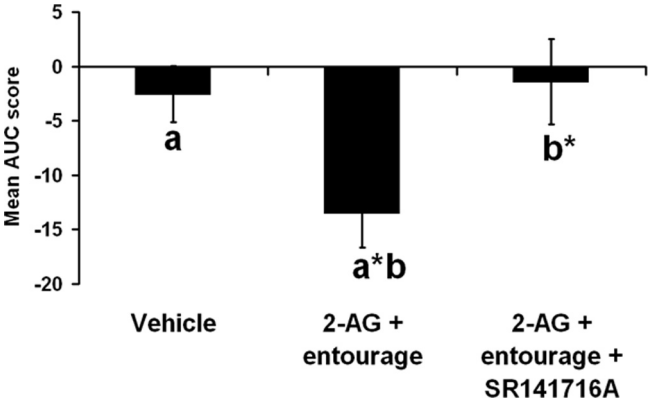

Fig. 3. Cognitive function of the mice in the eight arm maze was calculated in relation to their first day in the maze (see methods for explanation). 2-AG + entourage improved learning ability while SR141716A reversed this improvement. *−p < 0.05 vs. corresponding letter.